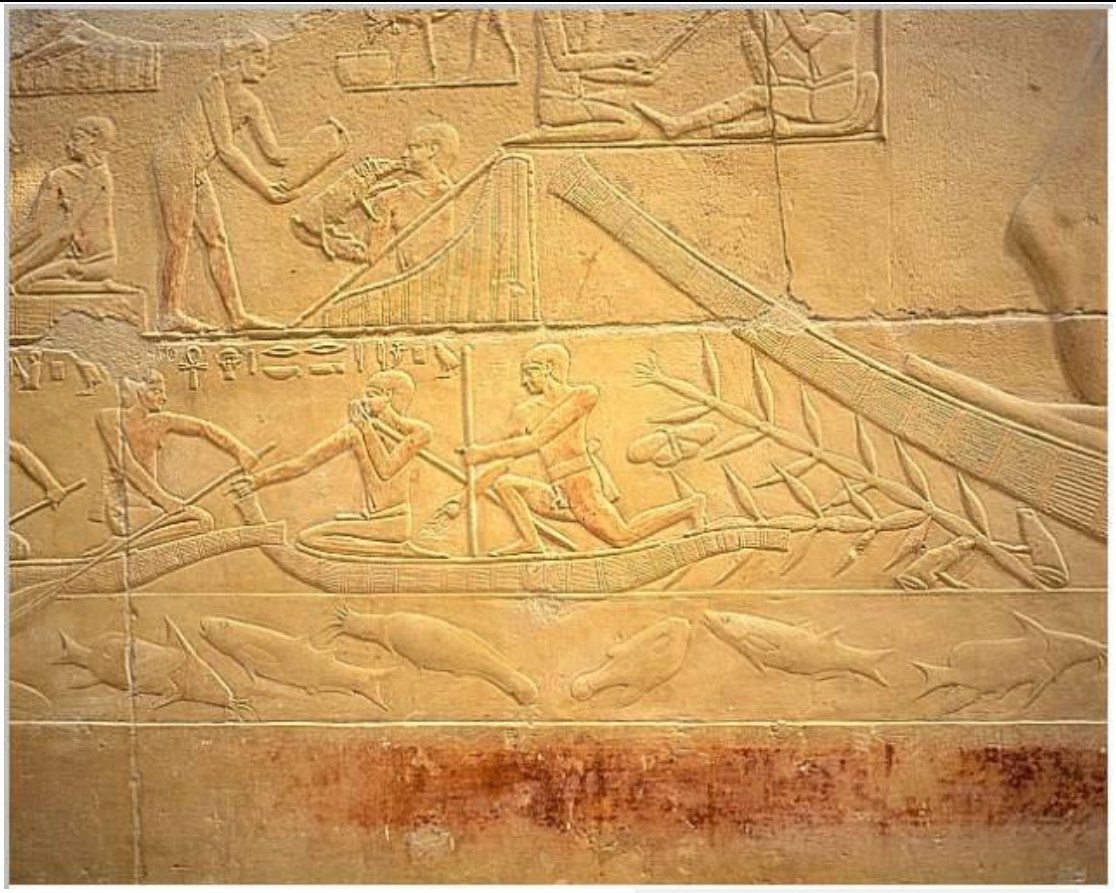
Monograph on Fungal Diseases of Fish

A guide for postgraduate students

PART 2



One of the scenes showing livestock and fishing activities, DIANABUJA'S BLOG - WordPress.com

By

Mohamed Kamal Refai

Sherif Marouf, Nermeen Abuelala, Rasha Hamza Sayed El-Ahl

November, 2016

Refai *et al.* (2016). Monograph on Fungal Diseases of Fish. A guide for postgraduate students

https://www.academia.edu/21679188/ or
http://scholar.cu.edu.eg/?q=hanem/book/ or
https://www.researchgate.net/publication


Prof. Dr. Mohamed K Refai, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt


Ass. Prof. Dr. Sherif Marouf, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt


Ass. Prof. Dr. Nermeen Abuelala, Department of Fish Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt


Ass. Prof. Dr. Rasha Hamza Sayed El-Ahl, Department of Mycology and Mycotoxins, Animal Health Research Institute, Dokki

Preface

The first paper I published on fungi in fish was concerned with the isolation of **Aspergillus niger and Penicillium funiculosum from imported smoked herring**, which was published in Mykosen. 1968 Jan 1;11(1):83-6, and the last paper was a part of MS thesis of my student Sheimaa, entitled: [Refai, M.K., Laila, A. Mohamed, Amany, M. Kenawy, Shimaa, El-S.M.A. **The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt**. Journal of American Science 2010;6(11):823-831]. (ISSN: 1545-1003).

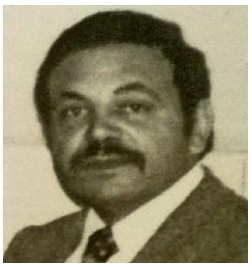
I was asked several times by my postgraduate students to prepare a review on fungal infections of fish, but I was postponing this act, because I realized the great efforts I have to do to fulfil this job, the

uncertain classification of most fungi affecting fish and my limited expertise with the Oomycetes, however, I felt it is my duty to invade this field and try to get the basic knowledges as guide for the post-graduate students.

This monograph is dedicated to the Egyptian pioneers in the field of fish diseases, who were the first to teach fish diseases in faculties of Veterinary Medicine, Cairo (**late Professors Mohy Elsaied Eissa& Mahmoud Essam Hatem and Prof. Mohamed Marzouk)**, Zagazig (late prof. Noor Eldeen Amin), Alexandria **(Prof. Mohamed Fai**sal) and Suez Canal (**Prof. Ismail Eissa**) Universities.

I was lucky to join them as a co-author in some publication:

- Easa, M., Hatem, M., Sakr, E. and Refai, M. : **Phoma herbarum as a mycotic fish pathogen in Clarias lazera.** Vet. Med. J. 92, 257-267 (1984)
- Faisal, M., Refai, M. and Peter, G. : **Augenmykosen bei Zuchtfischen. Pilzdialog** 3, 56 (1986
- Faisal, M., Popp, W. and Refai, M. : **Hohe Mortalitaet der Nil-Tilapia Oreochromis niloticus verursacht durch Providencia rettgeri**. Berl. Muench. Tieraerztl. Wschr. 100, 238-240 (1987),
- Refai M, Abdel halim MM, Afify MMH, Youssef H, Marzouk KM. **Studies on aspergillomycosis in catfish (*Clarias Lazera*)**. Allgemeine Pathologic and pathologische Anatomic. Tagung der Deutachen Veterinar - Medizinischen Gesellschaft& der Europaeischen Gesellschaft fur. Vet Pathol 1987; 63:1-12
- Salem AA, Refai MK, Eissa IAM, Marzouk M, Bakir A, Moustafa M, Manal Adel. **Some studies on aspergillomycosis in Tilapia nilotica.** Zagazig Vet J 1989; 17(3):315-328.


Noor El Deen Amin


Mohy Elsaied Eissa


Mahmoud Essam Hatem


Mohamed Marzouk


Ismail Eissa


Mohamed Faisal

Prof. Dr. Mohamed Refai
Cairo, November 15, 2016

Contents

PART 2

5. Dermocystidium
6. Sphaerothecum
7. Ichthyophonus
8. Lagendium
9. *Haliphthoros*
10. Halioticida
11. Halocrusticida
12. Atkinsiella
18. *LCD*
19. *Ochroconis*
20. Purpureocillium
21. Phoma
22. Miscellaneous fungi
23. Yeasts
24. Mycotoxins

5. Dermocystidium

Definition

Dermocystidium is the cause of a disease that affects the skin of fish and can be found on the gills, fins or body. It causes raised swellings varying in size from 1-2 cms to large lesions up to 10 cm. The lesions are pinkish to red and vary in shape from circular to long elongated ovals. There is minimal inflammation around the lesion. Because of the presence of both spores and hyphae it is currently believed to be a fungal infection, although some authorities class it with the protozoa. As the lesion swells, the epidermis covering the swelling gets progressively thinner, at which stage it is sometime possible to see the white hyphae inside. These give the swelling a whitish, cloudy look. When the lesion matures, it ruptures spreading thousands of spores into the water.

Historical

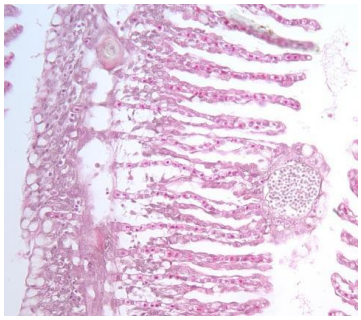
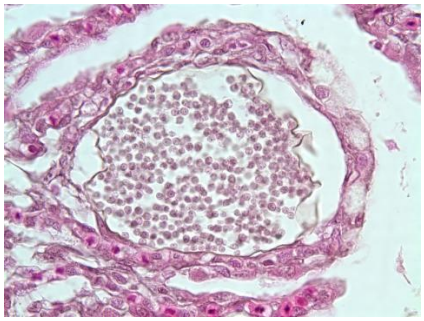
- **Perez, 1907**, was the first to erect a genus under the name Dermocystis, he reported the type species D. pusula from the skin of various salamanders (Triturus marmoratus, T. cristatus, and T. palmata) and from the obstetrical toad (Alytes obstetricans).
- **Perez, 1908** proposed the new name Dermocystidium for the genus.
- **Leger, 1914** described the infection *Dermocystidium* in rainbow trout (*Oncorhynchus mykiss*)
- **Guyenot and Naville, 1921-22**, reported D. ranae in the skin of Rana temporaria and R. esculenta.
- **Jirovec, 1939**, found D. vejdovskyi on the gills of the pike (Esox lucius). and daphniae in the body cavity of Daphnia magna and placed it in genus Dermocystidium
- **Weiser, 1943** removed D. daphniae from this genus and erected the genus Iymphocystldium (microsporidian?) for it.
- **Davis, 1947**, reported D. salmonis from a single adult Chinook salmon (Oncorhynchus Tschawytscha) from the Sacramento River, California.

- **Hoshina & Sahara, 1950** incriminated *Dermocystidium koi* as the aetiology of cysts measuring up to 10 mm in the skin of koi carps (*Cyprinus carpio*) and goldfish (*Carassius auratus*) with the spores ranging from 6.3 to 14.4 μm in diameter
- **Mackin *et al.* (1950)** described the typical morphological appearances of D. marinum as seen in stained histological sections of oyster tissue
- **Mackin (1951)** presented an extensive paper dealing with the histopathology of infections of the oyster by D. marinum and various aspects of the disease produced by this parasite. The small spherical organisms as seen in or among host tissue cells possess a single, large, partially eccentric vacuole which frequently contains a large polymorphic "vacuoplast". The presence of such a vacuole, occupying the greater part of the body of the organism is the distinguishing feature of the genus Dermocystidium, and its presence was the chief reason for the assignment of the oyster parasite to this genus.
- **Ray, 1952** made cultural studies which definitely established the fungus nature of D. marinum and supported Mackin's (1951a) later idea that D. marinum might be related to certain mycotic disease-producing organisms such as Cryptococcus, Blastomyces, Coccldloides, and others—all of which produce a yeast-like cell in the host
- **Cervinka & Lom, 1974** described cysts in the gills of the common carp (*Cyprinus carpio*) caused by *Dermocystidium cyprini*
- **Garkavi *et al.*, 1980** described *D. erschowi* in the common carp. This species was seen localized in the subcutis of the lateral and ventral parts of body, where they formed dark red structures filled with the thread like cysts, which were convoluted in the skin.
- **Regan *et al.*, 1996** assigned the genus in a group called the DRIPs clade (*Dermocystidium*, rosette agent, *Ichthyophonus*, *Psorospermium*), near the dichotomy of animals and fungi.
- **Herr *et al.*, 1999** established under phylogenetic analysis, with the 18S small-subunit ribosomal DNA, for the DRIPs clade a new clade Mesomycetozoa.

Classification:

1. Index Fungorum:

- **Dermocystidium Pérez 1908**
  - *Dermocystidium branchiale* L. Léger 1914
  - *Dermocystidium cochliopodii* Valkanov 1967
  - *Dermocystidium cyprini* Cervinka & Lom 1974
  - *Dermocystidium daphniae* Jírovec 1939
  - *Dermocystidium gasterostei* Elkan 1962
  - *Dermocystidium granulosum* Sterba & W. Naumann 1970
  - *Dermocystidium guyenotii* Thélin{?} 1955
  - *Dermocystidium koi* Hoshina & Sahara 1950
  - *Dermocystidium marinum* Mackin, H. M. Owen & Collier 1950
  - *Dermocystidium percae* Rchb. -Klinke 1950
  - *Dermocystidium pusula* Pérez 1908
  - *Dermocystidium ranae* Guyénot & Naville 1922
  - *Dermocystidium salmonis* H. S. Davis 1947
  - *Dermocystidium vejdovskyi* Jírovec 1939


Dermocystidium cysts in gills https://fishpathogens.net/, Dermocystidium in gills of Fall Chinook

Life cycle

The life cycle is maintained from year to year by overwintering cysts. Young perch acquire first infections in their first summer of life, and fish over 3 years possibly develop some immunity. Cysts of *D. percae* occurred in the skin of abdominal fins and rarely elsewhere. Cysts develop from thin-walled, round plasmodium mother cells, about 10 mm in diameter, by elongating and increasing in size. The nucleus of the plasmodium mother cell degenerates, and a reticular chromatin-containing structure with dense centra spreads out between conspicuous lipid droplets in the plasmodium. Nuclei reappear during fragmentation of the plasmodium. The sporonts thus formed divide to form sporoblasts, which in addition to small lipid droplets acquire a non-lipid 'central' inclusion. The inclusion grows in size to the typical inclusion of the ultimate Dermocystidium spore. Sporogenesis takes place mostly in summer, and can continue until autumn.

Clinical signs

During outbreaks, *Dermocystidium spp* cysts initially become visible breaking through the skin they are usually around 1mm. They grow in size until ultimately they rupture releasing infectious spores they into the water to then seek a new host fish. The size of each cyst can vary but they seldom rupture at less than 6mm in size however few remain intact to reach 10mm and cysts over that size are extremely rare and usually due to two individual lesions that appear as one.

Treatment

There is no known treatment.

Fish infected can be housed in malachite green or acriflavine which should be used in conjunction with salt in order to reduce the secondary risks of fungus and bacteria into the lesions and this can also lower the level of cross-infection. Topical treatment can be applied after the cyst has ruptured but this can be discontinued once the crater or hole the cyst creates develops a slight gloss which indicates that healing has begun. Secondary bacterial infection may require antibiotics. The recovery period is temperature related and therefore it is not possible to be accurate about how long this will take but weeks rather than days should be anticipated.

Infectivity and re-infection

It is infective and likely to affect very large numbers of fish. When the lesion ruptures the lesion left, which can be substantial, seems to heal fairly well and quickly, leaving little sign of the infection. It seems to be a spring-time disease, lasting some 6-8 weeks. It has been reported that re-occurrences can sometimes happen in previously infected ponds.

Although *Dermocystidium* does not seem to be fatal in most cases, it does bring with it a very real threat of secondary infections. Antibiotic treatments and regular cleaning of the wounds will help prevent secondary infections and aid recovery. It is also important, as with all diseases, to maintain optimum conditions to prevent stress causing additional complications.

Reports:

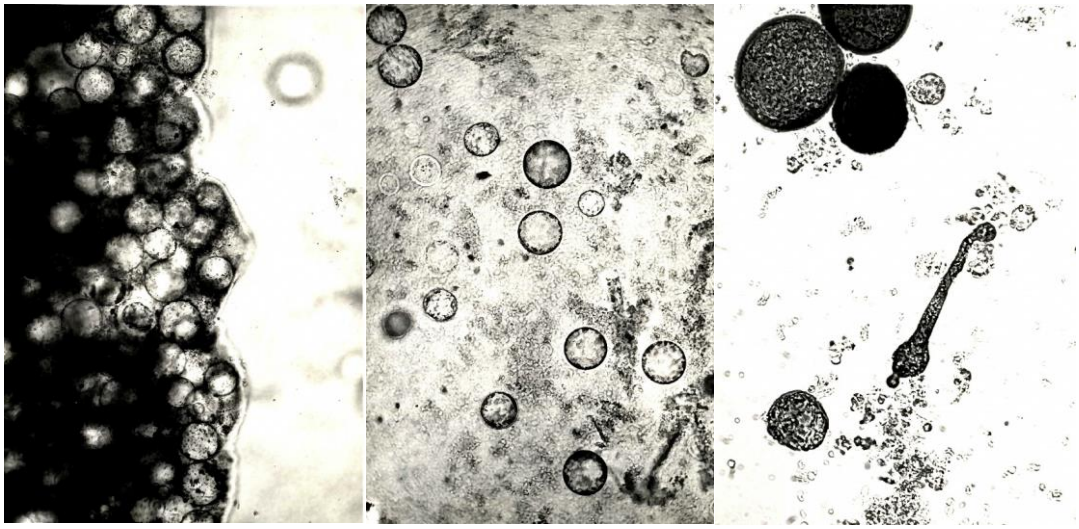
HOSHINA and SAHARA (1950) found a parasite in one case parasiting to the integument and in another to the muscle of each separate Cyprinuscarpis L. The specimen of the former was captured at the Yamada Fish Culture Farm (LocalityYamada Village, Hyogo Pref., Date-July 23, 1940), and that of the latter obtained from the Seto Fish Culture Farm. (Locality-Vicinity of Odawara City, Kanagawa Pref., Date-May 11, 1959). In the case of integumentary parasitim, the parasites grow between the epidermis and the cutis in uarious places of the body surface without scales ; and the characteristic lesionsa e formed arising from the integument, due to the increase of the parasites and host'stissues such as fibrous connective tissueand blood vessels. The muscular parasitism occurs when the parasites are parasitic to the scaley host ; and in this case the parasites grow in the muscle nearthe body surface; on the infected parts, the large and hard tumor like inflammatory swelling arise from the increase of the parasites and the same host's tissues asthe former. The parasite is filiform in external character;its transuerse section is round; the diameter 0.04-0.30 mm., and it contains innumerable spares. Spare subspherical, 6•`14/t in diameter ; the diameter of the large characteristic spherical enclosure is 4.5•`10.0,a. The details are compiled in the table 1. The spares are figure in text-fig. 3, NOS. 1-9. The present species is distinguishable from allother known species, in external form of th parasiteor the cyst, the modus parasitic, andthe dimension of the spare etc. The lesions may be ruptured naturally in a certa'n period ; and the contents fall out from them ; and thenthey seem to be healed gradually. Therefore, the host should not suffer great influence from the parasite. But the histological reactions of the host are marked, which are chiefly proliferocis inflammation, rarely accompanied with phagocytosis.

Mackin *et al.* (1950) mentioned that cells of D. marinum in stained sections normally measure from 2 JI to 20 JI, occasionally even 30 ji in diameter, the average being approximately 10 p. The cytoplasm occurs in a rather thin layer around the periphery of the cell membrane, being thickened somewhat in the region containing the nucleus. When stained with Heidenhain’s iron hemotoxylin in sectioned tissue, the extremely eccentric nucleus consists of a relatively large, compact, deeply stained endosome, surrounded hy a clear zone* The size of the endosome varies with the size of the nucleus. the "vacuoplast” stains shades of gray to black vrith Heidenhain’s iron hemotoxylin, and a very light rose or diffuse pink with Delafield's hemotoxylin and

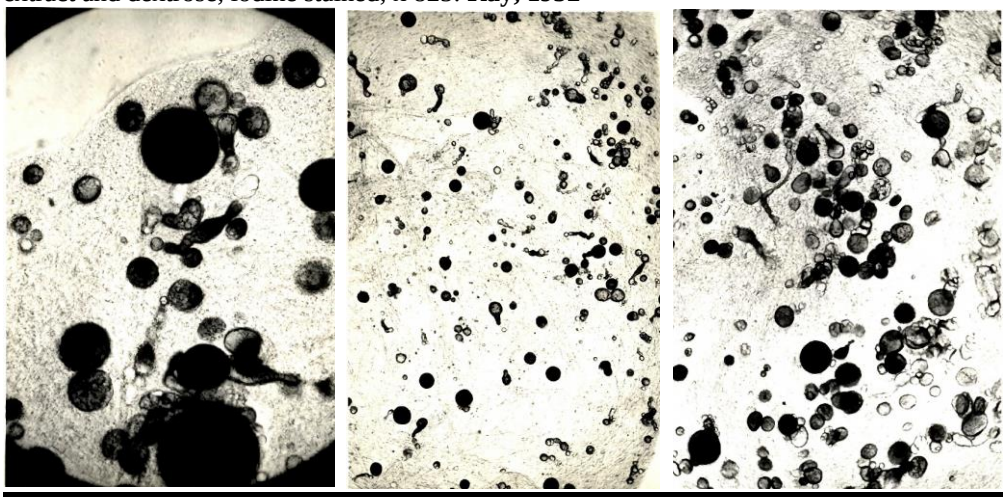
eosin. In cultures the organisms developed thick walls which stain blue when treated with iodine, but the thin cell membranes of the organisms, as they occur in living oysters, do not give this reaction^ The parasites may frequently be observed in amebocytes (phagocytic cells) and connective tissue cells, with a resulting displacement of the host-cell nucleus. The parasites may also be observed in the intercellular spaces of the tissue. This organism may invade any of the host tissues, although

Mackin (1951) reported that it rarely invades the external epithelia or peripheral nerves. The reproduction of the parasite in the host takes place by multiple fission. This method of reproduction has not been reported as occurring in any of the other species of Dermocystidium. The size and the number of the daughter cells produced by a single mother cell may vary greatly, from 3 to to as many as 25 or 30. The daughter cells are liberated by the rupture of the thin retaining cell membrane. At the time of liberation their size varies considerably, some being as small as 2 µ. Depending on the state of development, the daughter cells within the mother cell may or may not show the eccentric vacuole; however, they usually possess a deep-staining endosome. When liberated the vacuole of the daughter cells is usually well developed. The size of the mother cells is within the range of the single organisms.

Ray (1952) demonstrated by the culture technique in oysters from Pensacola, Florida; Wadmalaw River, South Carolina; the presence of D. marinum Biloxi, Mississippi; and numerous areas in Louisiana. The method has advantages over the histological techniques usually employed in economy of time and labor, in amount of equipment and skill required for a reliable diagnosis, and in accuracy in the detection of light infections. It was recently used for the first time in the field to investigate the incidence of D. marinum in a sudden outbreak of oyster mortality in Louisiana. This test of the technique was considered to be successful, for within 48 hours after the oyster bed was sampled the incidence of D. marinum, hased on the examination of 30 oysters, was known. A germination of the cyst-like bodies, with the production of short thick hyphae several times as long as the diameter of the body producing it, has been observed. Infrequently, bodies that appear to be undergoing binary fission have been observed. The hyphae and bud-like processes give a blue reaction when treated with iodine, although the trails are thin. The production of hyphae and budding forms definitely establishes D. marinum as a fungus and, as previously mentioned, supports Mackin's (1951) idea that D. marinum might be related to such organisms as Blastomyces and Cryptococcus. In addition to the two instances of hypha formation and budding observed in tissues incubated in fluid thioglycollate medium, such forms were consistently obtained when infected oyster tissues were incubated in sterile sea water containing yeast extract (5 per cent) and dextrose (5 per cent). In all cases where hyphal and budding forms have been observed in culture, the cytoplasm of many of the cyst-like bodies was very granular and non-vacuolated.

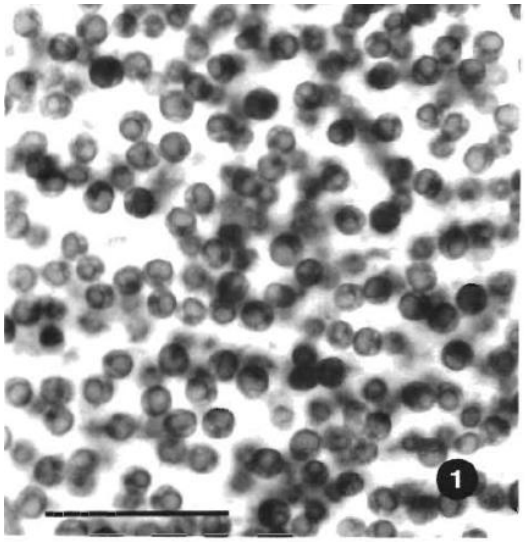


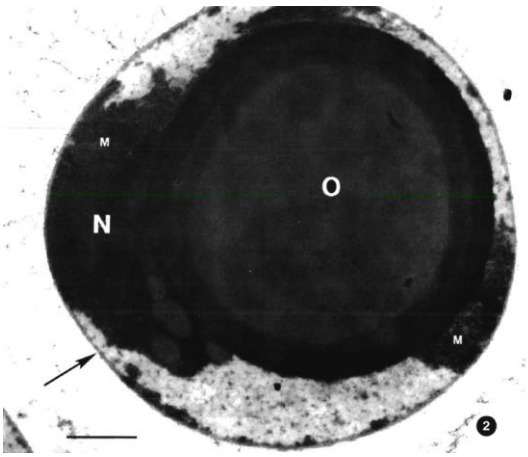
D. marinum in oyster gill tissue after 35 days of incubation in fluid thioglycollate medium; x 205.
D. marinum left on slide after removal of piece of tissue shown in Figure 1; x 205.
D. marlnum from oyster mantle tissue after 1^ days of incubation in sterile sea water containing yeast extract and dextrose, iodine stained; x 825. **Ray, 1952**



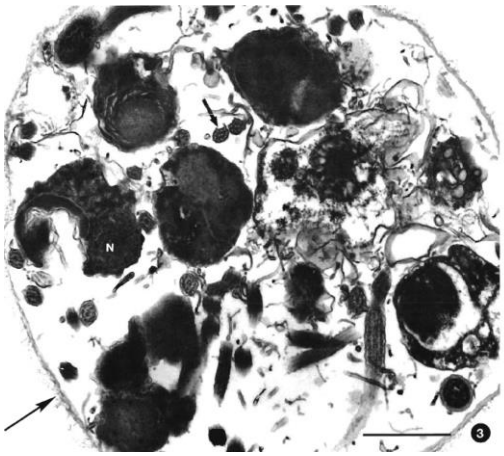
D. marinum in oyster heart tissue after 23 days of incubation in fluid thioglyeollate medium, iodine stained; x 750. Hyphae and buds showing evidence of degeneration with accumulation of "fatty material". D. marinum in oyster heart tissue after 23 days of incubation in fluid thioglycollate medium, iodine stained; x 240. D. marinum in oyster heart tissue after 23 days of incubation in fluid thioglycollate medium, iodine stained; x 315. **Ray, 1952**

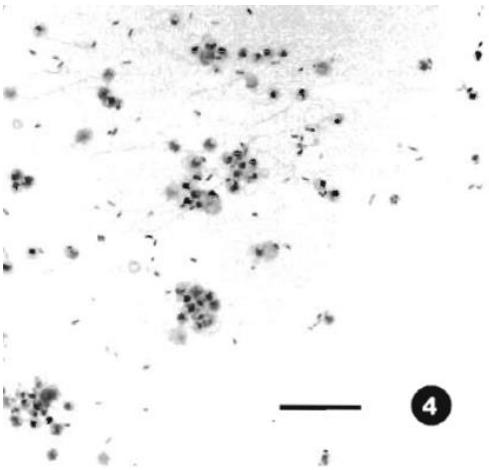
Olson *et al.* (1991) mentioned that Cystidjum salmonis is a gill pathogen of salmonid fishes in the U.S. Pacific Northwest where it has been associated with mortality of adult and juvenile chinook salmon Oncorhynchus tshawytscha. The previously unknown mode of D. salmonis transmission was determined and demonstrated in the laboratory. Uniflagellated zoospores developed within spores obtained from gill cysts and produced infections in pink salmon 0. gorbuscha fry. These infections were lethal, and histological examination of infected gill tissue revealed large numbers of D. salmonis cysts in gill epithelia. Electron microscopic examination of immature spores from experimental infections showed that they were identical to immature spores in naturally infected juvenile chinook salmon.



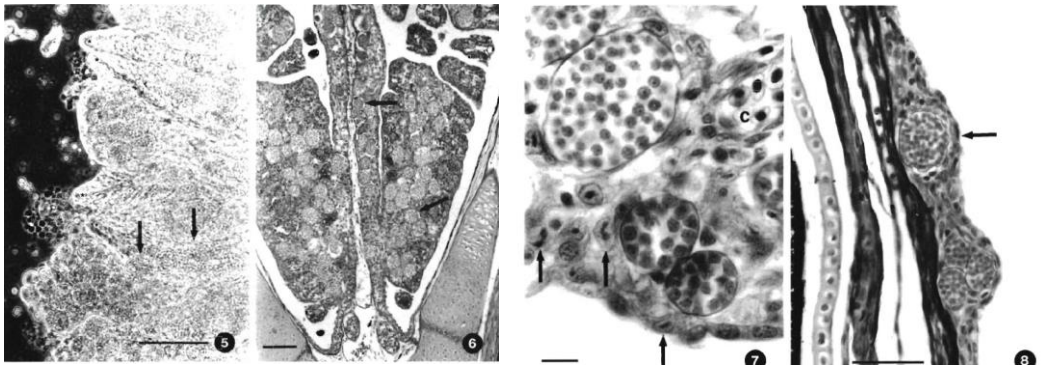


Dermocystidium salmonis in *Oncorhynchus tshawytscha.* Mature spores in histological section of a cyst from the gill of a naturally infected adult chinook salmon (H&E). Bar = 25 pm Dermocystidium salmonis. Transmission electron micrograph of mature spore from a naturally infected adult chinook salmon. N: nucleus; M: mitochondrion; 0: osmiophilic inclusion; arrow: spore wall. Bar = 1.0 pm **Olson *et al.* (1991)**

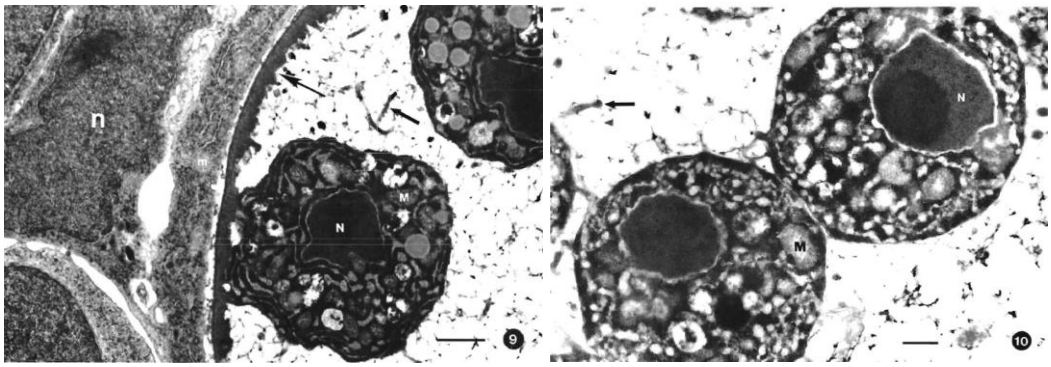




Derrnocystidiurn salmonis. Transmission electron micrograph of several developing zoospores within a single mature spore, after incubation at 4°C for 14 d. Long arrow: spore wall; short arrow: cross-sectioned flagellum showing typical microtubule arrangement; N. nucleus. Bar = 1.0 km g. 4. Dermocystidiurn salmonis. Smear of zoospores that were free-swimming in water incubated at 4'C (Glemsa). Arrow- zoospore. Bar = 10 **Olson *et al.* (1991)**



Figs. 5 to 8. Dermocystidium salmonisin Oncorhynchus gorbuscha. Fig. Wet mount of experimentally infected pink salmon gill, 15 d post-exposure to D salmonis zoospores (phase contrast). '. tips of 2" gill lamellae; arrows: small developing cysts containing immature, prollierating spores. Bar = 100 km. Flg. H~lstological section of experimentally infected pink salmon gill, 15 d postexposure to D. salmonis zoospores (H&E). Small cysts containing immature, proliferating spores are seen associated with 1" and 2" g111 lamellae (arrows). Destruction of normal gill architecture resulting from parasite growth and epithelial hyperplasia can be seen. Bar = 100 pm. Fig Histological section of experimentally infected pink salmon gill, 15 d post-exposure to zoospores (H&E). Several walled cysts contalning immature, proliferating spores are shown, as well as a blood capillary (C). Note mitotic figures in hyperplastic epithelial cells associated with cysts (arrows), and thickened gas exchange barrier which results. Bar = 10 km. Flg. Histological section of head of experimentally infected pink salmon, 15 d post-exposure to D. salmonis zoospores (H&E). Several small cysts containing immature, proliferating spores are seen within the dermal epithelium (arrow). Bar = 50 **Olson *et al.* (1991)**



Dermocystidiurn salmonis in Oncorhynchus gorbuscha. Transmission ,.,:tron micrograph of a developing spore within a cyst on the gill of an experimentally infected pink salmon fry, 15 d after exposure to zoospores. n: host cell nucleus; m: host cell mitochondrion; N: spore nucleus; M: spore mitochondrion; long arrow: cyst wall; short arrow: pseudopodial spore projection. Bar = 1.0 µm
Dermocystidium salmonis in Oncorhynchus tshawytscha. Transmission electron micrograph of developing spores within a cyst on the gill of a juvenile chinook salmon with a natural D, salmonis infection. N: spore nucleus; M: spore mitochondrion; arrow: pseudopodial spore projection. Bar = 1.0 µm **Olson *et al.* (1991)**

Dyková, J. Lom *(1992)* *mentioned that Dermocystidium koi* Hoshina and Sahara, 1950 is characterized by formation of a web of aseptate hyplye which pervade the subcutaneous tissue of the host, *Cyprinus carpio var. koi*. Within the hyphae, multinucleate cytoplasmic contents eventually produce a large number of spores with a typical central refractile inclusion. They are of extremely variable size (diameter from 6.5 to 15 µm); there is some indication that the size range may reflect tle gradual growth of spores. Hyphae formation confirms the assumption of the fungal nature of this organism, yet its precise osition remains unsolved. The host reaction is first reflected in oedema formation, later with cellular infiltrate leading to proliferative inflammation followed by the formation of granulation tissues.

Dykova and Lom (1992) mentioned that *Dermocystidium koi* was characterized by formation of a web of aseptate hyphae, which pervaded the subcutaneous tissue of the host, *Cyprinus carpio var. koi* . Within the hyphae, multinucleate cytoplasmic contents eventually produced a large number of spores with a typical central refractile inclusion. They were of extremely variable size (diameter from 6.5 to 15 mu m); there was some indication that the size range may reflect the gradual growth of spores. Hyphae formation confirmed the assumption of the fungal nature of this organism, yet its precise position remains unsolved. The host reaction was first reflected in oedema formation, later with cellular infiltrate leading to proliferative inflammation followed by the formation of granulation tissue.

Olson and Holt (1995) reported that intense infections of the gill pathogen Dermocystidium salmonis were associated with mortality of prespawning chinook salmon Oncorhynchus tshawytscha in several Oregon rivers in 1988. The occurrence of the pathogen in returning adult chinook salmon was monitored in several coastal Oregon stocks from 1989 to 1993. Although the prevalence of the pathogen was high in these fish (up to 66.6%), infection intensities were generally low, and no mortality attributable to D. salmonis was observed. In 1988, the pathogen was associated with a lethal epizootic among juvenile chinook salmon smolts at the Trask State Fish Hatchery near Tillamook, Oregon. Histological examination of gills from heavily infected fish revealed hyperplasia of gill epithelium and fusion of gill lamellae. When naturally infected smolts were transferred from fresh to salt water, the most heavily infected fish died within 10 d, and the number of D. salmonis cysts declined and disappeared from previously infected salmon after 21-42 d.

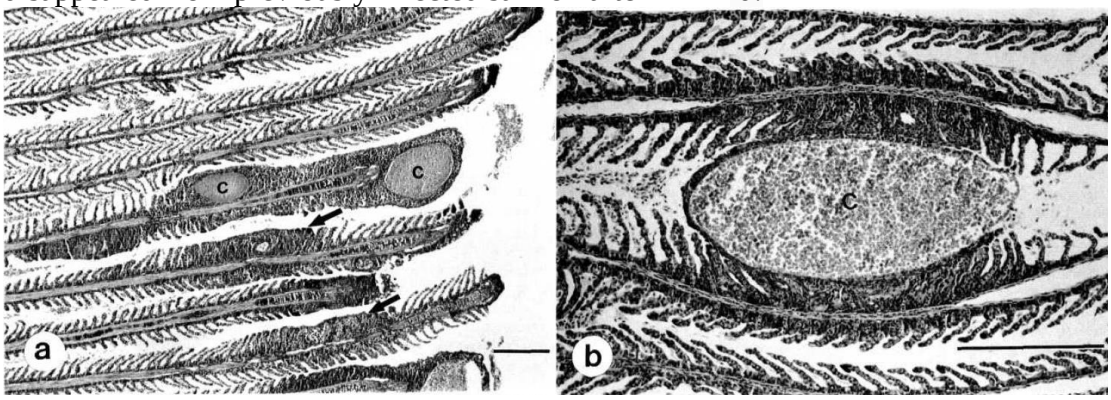
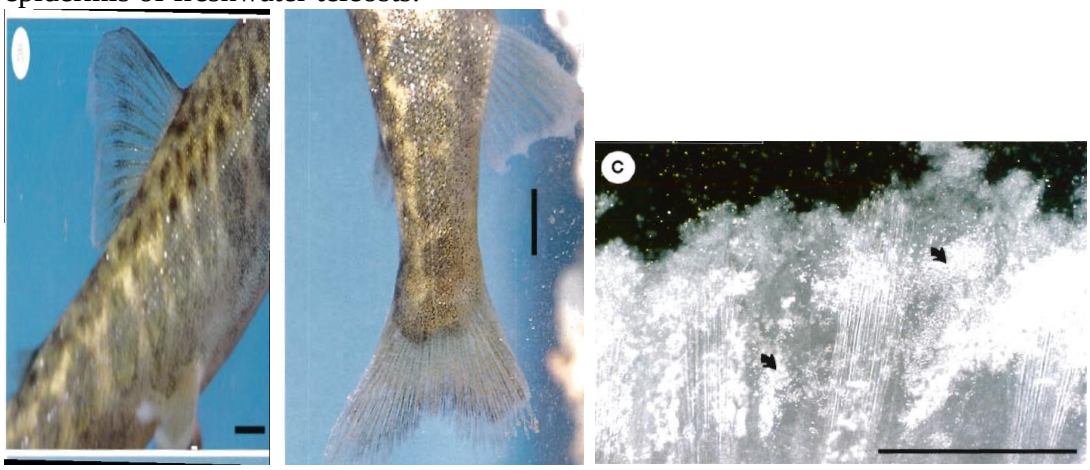


FIGURE 1.—Histological sections of juvenile chinook salmon gills infected with *Dermocystidium salmonis*. **(a)** Low magnification showing hyperplastic response to cysts (c), leading to fusion of lamellae. Fusion of lamellae in apparent absence of cysts (arrows) is the result of reaction to cysts that are out of the plane of the section (bar = 250 μm). **(b)** Higher magnification of a cyst loosely attached to gill tissue that shows fusion of lamellae and filaments (bar = 250 μm).

Olson and Holt (1995)

Huglund *et al.* (1997) recovered free spores of a Derrnocystidiurn-like organism from the epidermis and covering mucus of gills and fins of moribund farmed salmon Salmo salar. The parasite appeared in juvenile fish only and at low water temperatures (15°C). The most prominent external macroscopical clinical signs of disease were thickened fins that gave the tips a pronounced greyish opaque appearance often in combination with signs of fin rot/fin erosion. The gills were swollen and pale and could also be necrotic. Examination of fresh mounts and tissues prepared for light and electron microscopy showed vacuolated spherical spores typical for parasites of the genus Dermocystidium. Irregularly vacuolated spores with 1 or multiple nuclel were also observed. Histological examination of infected salmon indicated concurrent Flexibacter sp. infection that was venfied in Gram-negative stained imprints. The

present findmg is the first observation of Dermocystidlum in Sweden. In addition, this is the first record of a Dermocystidiurn-hke agent that occurs freely in the mucus and epidermis of freshwater teleosts.



Salmo salar. Gross pathological signs caused by the Dermocystidiurn-like organism in: (A) the dorsal fin of juvenile salmon, note the thickened tip of the fin; (B) the caudal fin of salmon concurrently infected with Flexibacter sp. showing signs of progressive erosion and &sintegration of the soft tissues between the rays; (C) the distal tip of the pectoral fin (close up). Arrows: clusters of spores. Bars in A and B = 1 cm, in C = 1 m **Huglund *et al.* (1997)**

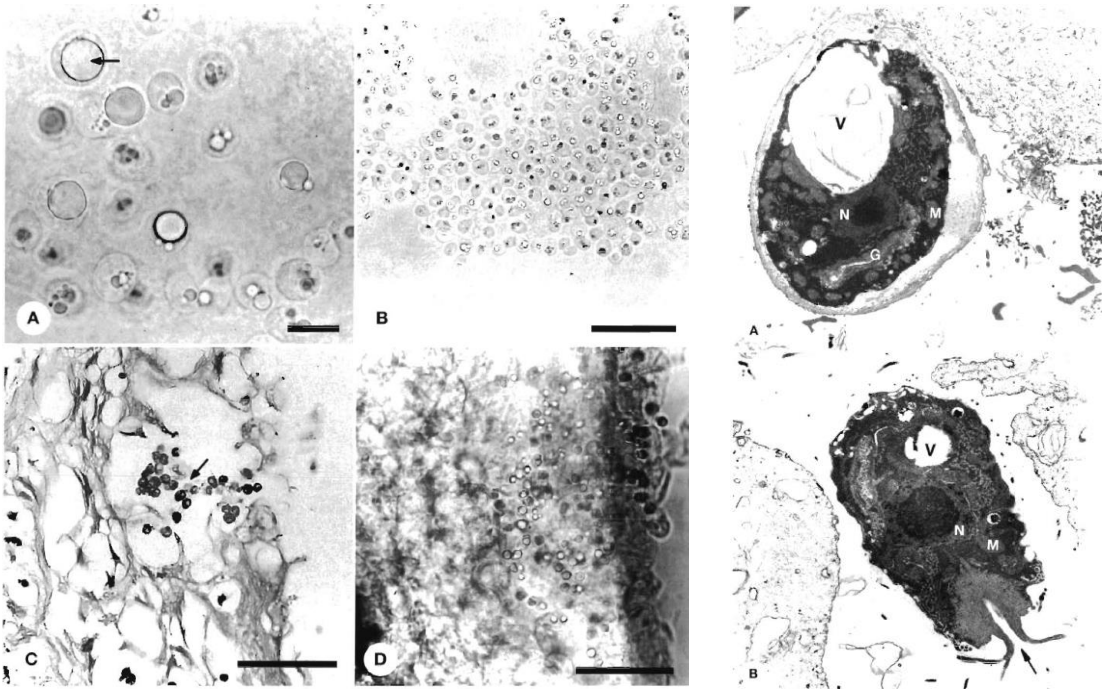


Fig. 2. signet (A, B) Wet mounts of the Dermocystidium-like organism in the epidermis and covering mucus of the fins in salmon. Arrow: ring cell. (C) Histological appearance of the paraslte in the epidermis of salmon; (D) wet mount of the same piece as sectioned in C. Arrow: spores. Bars in A. B = 10 pm. in C, D = 50 pm **Huglund *et al.* (1997)**

g. 3. Transmission electron micrograph of Dermocysti&um-like species from the epiderms and covenng mucus of a naturally infected salmon: (A) Signet ring cell or hypnospore; (B) spore with filamentous projechons (arrow). Magnification in A and B = x2500. V: vacuole, N: nucleus, M: mitochondna, G: Golgi-complex **Huglund *et al.* (1997)**

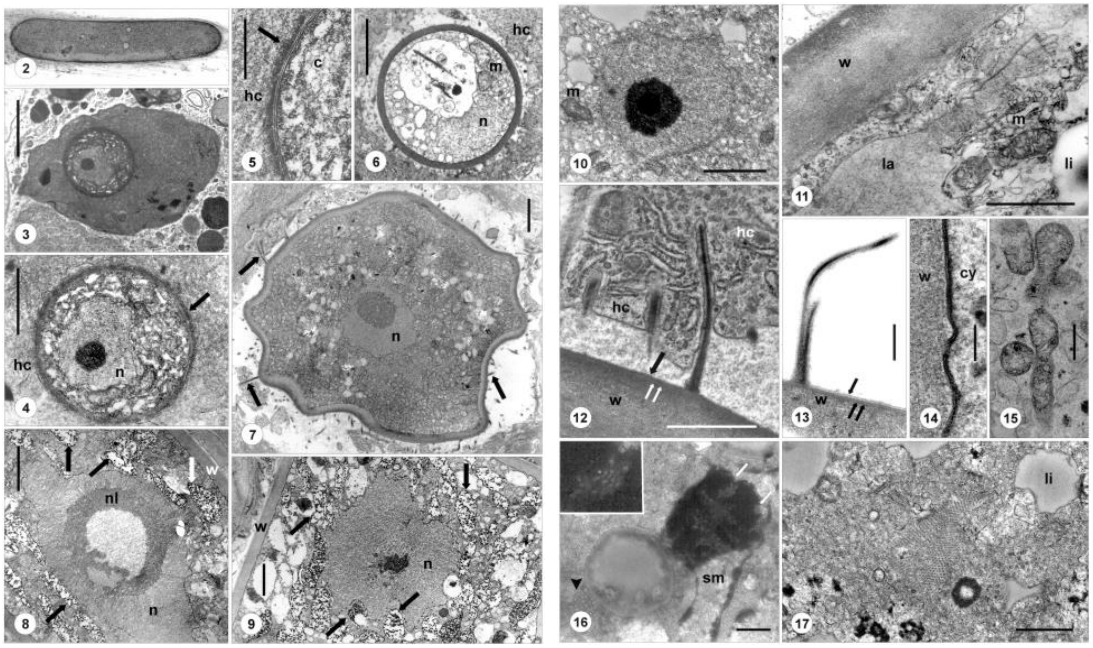
Pekkarinen and Lotman K. (2003) mentioned that in Finland Dermocystidium percae Reichenbach-Klinke was first recorded by Pekkarinen in the fins of a perch in

1988. Because it is a poorly known parasite, its occurrence and life stages are studied here. In occasional sampling during 1995-1998 and 2001, and more systematic sampling during 1999 and 2000 in two different lakes (one oligotrophic, the other slightly eutrophic), it was found to occur almost continuously, although sometimes very sparsely, in both lakes. The life cycle is maintained from year to year by overwintering cysts. Young perch acquire first infections in their first summer of life, and fish over 3 years possibly develop some immunity. In Estonia, in the Kasari River and the Matsalu Bay, the parasite seems to be very rare. Cysts of D. percae, capable of sporogenesis, can occasionally occur in the ruff, too. In perch in Finland, cysts were found differing from the longish and thin-walled cysts typical of D. percae. These roundish to dumbbell-shaped cysts with thicker walls are here suggested to belong to a different Dermocystidium species, called D. sp. Cysts of D. percae occurred in the skin of all fins, but most often in abdominal fins and rarely elsewhere. In ruff, cysts of D. percae were also found in the gills. Of the fins D. sp. favoured the first dorsal fin, but also occurred elsewhere, e.g. in the head region of perch. Cysts of D. percae develop from thin-walled, round plasmodium mother cells, about 10 µm in diameter, by elongating and increasing in size. The nucleus of the plasmodium mother cell degenerates, and a reticular chromatin-containing structure with dense centra spreads out between conspicuous lipid droplets in the plasmodium. Nuclei reappear during fragmentation of the plasmodium. The sporonts thus formed divide to form sporoblasts, which in addition to small lipid droplets acquire a non-lipid 'central' inclusion. The inclusion grows in size to the typical inclusion of the ultimate Dermocystidium spore. Sporogenesis both in D. percae and D. sp. takes place mostly in summer, and at least in D. percae can continue until autumn. In addition, both species can produce numerous zoospores from their spores within 2 days in water at 25°C and at slower rates at lower temperatures. The body of the zoospore is about 1.2-2.2 µm in length and the flagellum is about six times the body length. The zoospores may then slightly grow in size and transform into amoebae. Small cysts, which possibly originated from an experimental infection by zoospores of D. sp., developed in 0-group perch kept in an aquarium at 17°C. The two Dermocystidium species here discussed can be grouped together with some other species, in which nuclei reappear and the plasmodium divides late in development and in which sporogenesis, unlike that in D. vejdovskyi Jírovec and D. cyprini Červinka and Lom, does not take place in compartments.

Feist *et al*. (2003) mentioned that in Finland Dermocystidium percae Reichenbach-Klinke was first recorded by Pekkarinen in the fins of a perch in 1988. Because it is a poorly known parasite, its occurrence and life stages were studied here. In occasional sampling during 1995-1998 and 2001, and more systematic sampling during 1999 and 2000 in two different lakes (one oligotrophic, the other slightly eutrophic), it was found to occur almost continuously, although sometimes very sparsely, in both lakes. The life cycle is maintained from year to year by overwintering cysts. Young perch acquire first infections in their first summer of life, and fish over 3 years possibly develop some immunity. In Estonia, in the Kasari River and the Matsalu Bay, the parasite seems to be very rare. Cysts of D. percae, capable of sporogenesis, can occasionally occur in the ruff, too. In perch in Finland, cysts were found differing from the longish and thin-walled cysts typical of D. percae. These roundish to dumbbell-shaped cysts with thicker walls are here suggested to belong to a different Dermocystidium species, called D. sp. Cysts of D. percae occurred in the skin of all fins, but most often in abdominal fins and rarely elsewhere. In ruff, cysts of D. percae

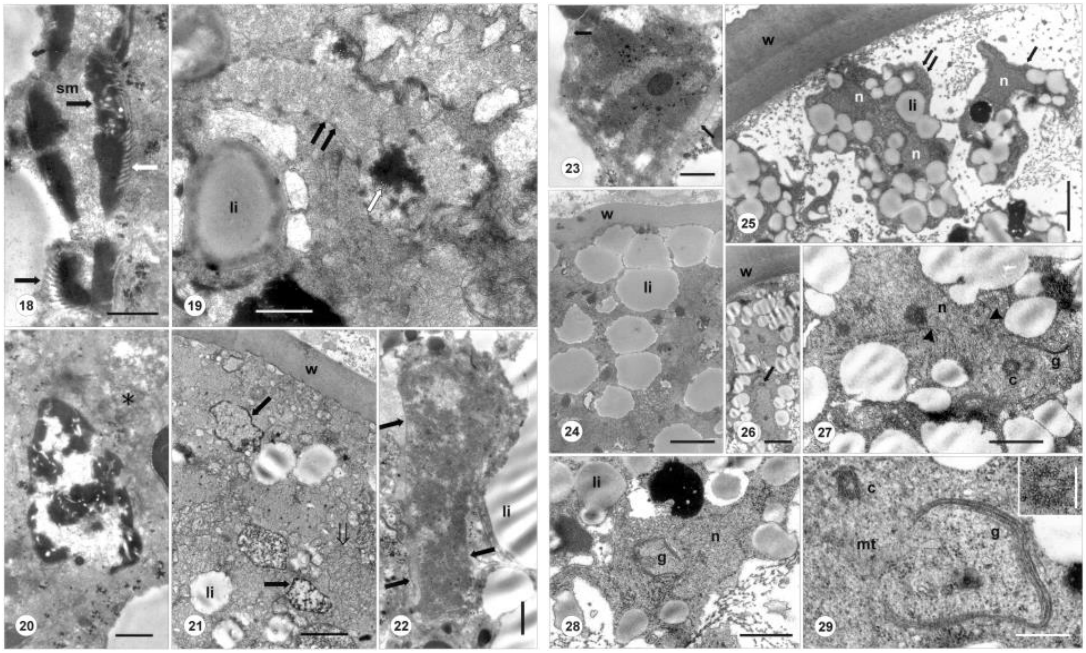
were also found in the gills. Of the fins D. sp. favoured the first dorsal fin, but also occurred elsewhere, e.g. in the head region of perch. Cysts of D. percae develop from thin-walled, round plasmodium mother cells, about 10 µm in diameter, by elongating and increasing in size. The nucleus of the plasmodium mother cell degenerates, and a reticular chromatin-containing structure with dense centra spreads out between conspicuous lipid droplets in the plasmodium. Nuclei reappear during fragmentation of the plasmodium. The sporonts thus formed divide to form sporoblasts, which in addition to small lipid droplets acquire a non-lipid 'central' inclusion. The inclusion grows in size to the typical inclusion of the ultimate Dermocystidium spore. Sporogenesis both in D. percae and D. sp. takes place mostly in summer, and at least in D. percae can continue until autumn. In addition, both species can produce numerous zoospores from their spores within 2 days in water at 25°C and at slower rates at lower temperatures. The body of the zoospore is about 1.2-2.2 µm in length and the flagellum is about six times the body length. The zoospores may then slightly grow in size and transform into amoebae. Small cysts, which possibly originated from an experimental infection by zoospores of D. sp., developed in 0-group perch kept in an aquarium at 17°C. The two Dermocystidium species here discussed can be grouped together with some other species, in which nuclei reappear and the plasmodium divides late in development and in which sporogenesis, unlike that in D. vejdovskyi Jírovec and D. cyprini Červinka and Lom, does not take place in compartments.

Pekkarinen *et al.* (2003) determined sequences of small-subunit rRNA genes for Dermocystidium percae and a new Dermocystidium species established as D. fennicum sp. n. from perch in Finland. On the basis of alignment and phylogenetic analysis both species were placed in the Dermocystidium-Rhinosporidium clade within Ichthyosporea, D. fennicum as a specific sister taxon to D. salmonis, and D. percae in a clade different from D. fennicum. The ultrastructures of both species well agree with the characteristics approved within Ichthyosporea: walled spores produce uniflagellate zoospores lacking a collar or cortical alveoli. The two Dermocystidium species resemble Rhinosporidium seeberi (as described by light microscope), a member of the nearest relative genus, but differ in that in R. seeberi plasmodia have thousands of nuclei discernible, endospores are discharged through a pore in the wall of the sporangium, and zoospores have not been revealed. The plasmodial stages of both Dermocystidium species have a most unusual behaviour of nuclei, although we do not actually know how the nuclei transform during the development. Early stages have an ordinary nucleus with double, fenestrated envelope. In middle-aged plasmodia ordinary nuclei seem to be totally absent or are only seldom discernible until prior to sporogony, when rather numerous nuclei again reappear. Meanwhile single-membrane vacuoles with coarsely granular content, or complicated membranous systems were discernible. Ordinary nuclei may be re-formed within these vacuoles or systems. In D. percae small canaliculi and in D. fennicum minute vesicles may aid the nucleuscytoplasm interchange of matter before formation of double-membrane-enveloped nuclei. Dermocystidium represents a unique case when a stage of the life cycle of an eukaryote lacks a typical nucleus.



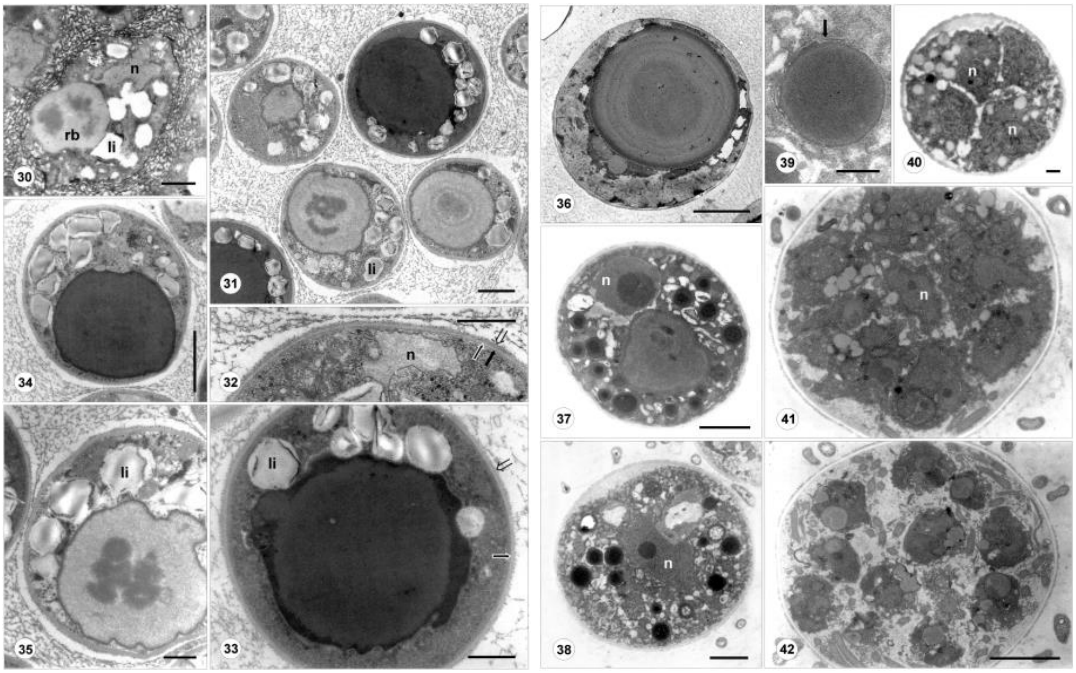
Dermocystidium percae. 2 - a cyst from perch fin, actual length is 580 µm; 3 - intracellularly located early stage; 4 - the stage from Fig. 3, enlarged, arrow indicates the cell envelope; 5 - cross section of the cell envelope of the preceding stage; arrow indicates the fuzzy cell coat; 6 - a slightly more advanced stage encased with a thick wall; 7 - growing young plasmodium; arrows indicates the fine projections of the wall; 8 - part of a growing plasmodium with a huge nucleus and a hollow, large nucleolus, arrows indicates vacuoles with dense granules; 9 - part of a growing plasmodium with a nucleus with short projections; some of the vacuoles with dense granules (arrows) are lodged between them. c - parasite cytoplasm, hc - host cell cytoplasm, m - mitochondria, n - nucleus, nl - nucleolus, w - plasmodium wall. Scale bars - 0.5 µm (5); 1 µm (4); 2 µm (3, 6-9). **Pekkarinen *et al.* (2003)**

Dermocystidium percae. 10 - a normal nucleus in a young plasmodium; 11 - a branched, single membrane-bound lacuna with finely granular content; 12 - plasmodium wall with its homogeneous outer layer (white double arrows) covered by fine surface coat (arrow); 13 - a villus branched near the surface of the wall; arrow - surface coat, double arrow - outer homogeneous layer of the wall ; 14 - the dense lamina lining the inner face of the plasmodium wall; 15 - mitochondria in the cytoplasm; 16 - smooth-membrane envelope (sm) containing dense substance with small tubules (arrows, enlarged in the inset); at left, a multivesicular body-like structure (arrowhead); 17 - bundle of small fibrils in the plasmodia. cy - plasmodium cytoplasm, hc - host cell, la - membrane-bound lacuna, li - lipid inclusion, m - mitochondrion, w - plasmodium wall. Scale bars - 100 nm (14); 0.4 µm (13), 0.5 µm (15, 16); 1 µm (11, 12, 17); 2 µm (10). **Pekkarinen *et al.* (2003)**



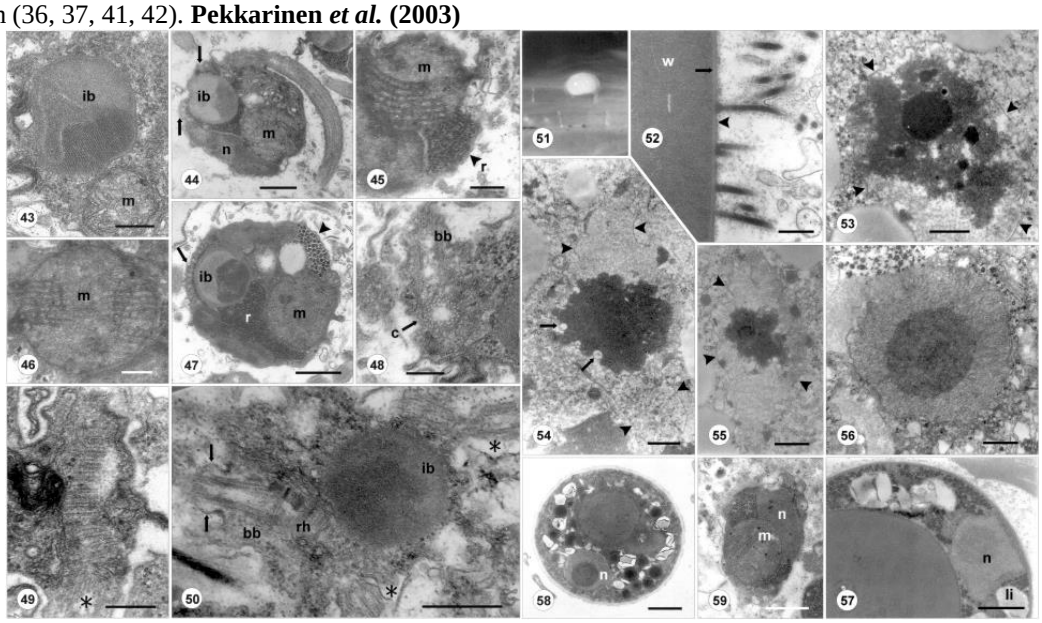
18-22. Dermocystidium percae, grown plasmodia. 18 - dense matter within a space delimited by a smooth membrane with small tubules (arrows); 19, 20 - smooth membrane envelopes (double arrow in Fig. 19) containing dense, chromatin-like material with small tubuli (white arrow in Fig. 19) in a lucent or finely granular matrix, asterisk - granular matter; 21 - single membrane vacuoles with coarsely (arrows) or finely granular substance (hollow arrow); 22 - a single membrane-bound envelope, probably the precursor of the new nucleus, filled with moderately dense granulation; arrows indicates the envelope wall. li - lipid inclusion, sm - matter within a space, w - plasmodium wall. Scale bars - 0.4 µm (18); 0.5 µm (19, 20, 22); 2 µm (21). **Pekkarinen *et al.* (2003)**

Figs. 23-29. Dermocystidium percae, mature and presporogonic plasmodia. 23 - a single membrane-bound structure (arrows indicate the envelope) with granular content of variable density; a possible precursor of the new nucleus; 24 - part of the periphery of the presporogonic plasmodium with a mass of large lipid droplets; 25 - the plasmodium fragmenting to form sporoblast mother cells with two nuclei (double arrow) which divide to produce sporoblasts (arrow); the wall is now two-layered; 26 - part of the periphery of the fragmenting plasmodium which is seen in more detail in Fig. 27; arrow points at the centriole; 27 - formation of sporoblasts, with a new nucleus with envelope marked by arrowheads; 28 - sporoblast formation with nucleus and Golgi; 29 - cells forming sporoblasts: centriole next to Golgi; inset, upper right - transverse section through a centriole, bar - 0.2 µm. c - centriole, g - Golgi, li - lipid droplets, mt - microtubules, n - nucleus, w - plasmodium wall. Scale bars - 0.5 µm (23, 29); 1 µm (27, 28); 2 µm (25, 26); 4 µm (24). **Pekkarinen *et al.* (2003)**



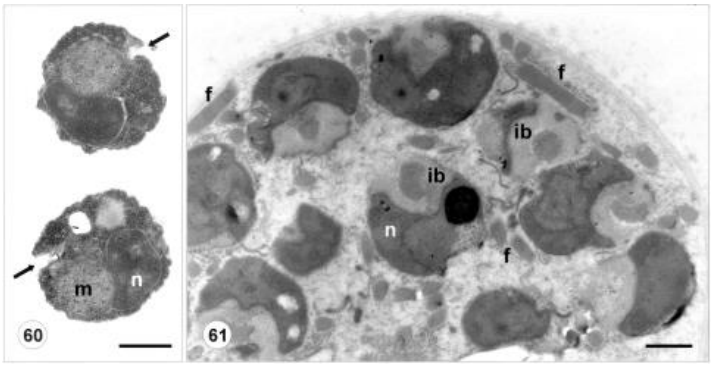
Figs 30-35. Dermocystidium percae, sporogenesis. 30 - sporoblast separated from the others by a foamy matrix; rounded body, possibly a precursor of the large spore inclusion; 31 - a group of one mature (upper right) and several young spores with the large central inclusions; all are embedded in a foamy matrix; 32 - section through the periphery of a young spore; black arrow marks the cell membrane, white-lined arrow the lucent spore wall, hollow arrow marks the surface coat. 33 - spore, note the irregular rim of the central inclusion, denser than the inclusion itself; white-lined arrow points at the wall of the spore, hollow arrow at the surface coat; 34 - another mature spore with distinctly eccentric inclusion; 35 - young spore with immature inclusion, reminiscent of the rounded body of Fig. 30, with lipid vacuoles displaying crumpled lamellae. The mitochondrion at left of the lipid inclusion is exceptional in having tubular cristae. n - nucleus, li - lipid vacuole or inclusion, rb - rounded body. Scale bars - 1 µm (30-33, 35); 2 µm (31); 3 µm (34). **Pekkarinen *et al.* (2003)**

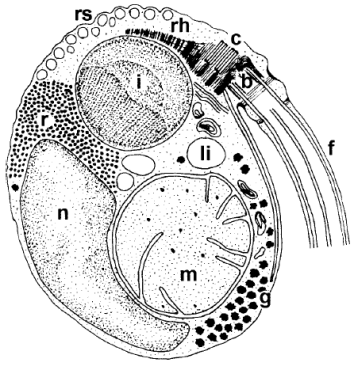
Figs 36-42. Dermocystidium percae, spores and zoosporogenesis. 36 - an old spore in which lipid and glycogen reserves became scarce, with distinct concentric layers in the inclusion; 37 - first phase of zoosporogenesis with enlarged nucleus of the spore; note the appearance of dense globules in the cytoplasm; 38 - next step of sporogenesis; the central inclusion has almost vanished; 39 - one of the dense globules from Fig. 38 enlarged; arrow points at the tubuli at the periphery of the globule; 40 - the spore has divided into four cells; 41 - a rosette of daughter cells within the old spore wall; 42 - old spore wall with developing zoospores inside it. n - nucleus. Scale bars - 0.5 µm (39, 40); 1 µm (38); 2 µm (36, 37, 41, 42). **Pekkarinen *et al.* (2003)**



Figs 43-50. Details of zoospore structures in **Dermocystidium percae**. 43 - inclusion body of the zoospore, part of it revealing a densely striated structure; 44 - the zoospore with its single, posteriorly curved flagellum; arrows point at the rumposome-like structure; 45, 46 - mitochondria with diverse structures of cristae. 47 - section through the zoospore; next to the inclusion there is the rumposome-like structure (arrow); arrowhead points at glycogen rosettes. 48 - basal body of the zoospore flagellum; beneath it is the barren (non-functional) centriole; 49 - transversely striated rhizoplast, associated with the basal body of the flagellum (asterisk); 50 - basal body with the flagellum extending through the cell membrane (arrows) and its associated rhizoplast; asterisks - flagella of neighbouring zoospores. bb - basal body, c - centriole, ib - inclusion body, m - mitochondrion, n - nucleus, r - ribosomes, rh - rhizoplast. Scale bars - 0.2 µm (43, 45, 46, 49); 0.4 µm (44, 48, 50); 0.5 µm (47) **Pekkarinen *et al.* (2003)**

Figs 51-59. **Dermocystidium fennicum** sp. n. 51 - a cyst on the surface of the gills, actual size is 360 µm; 52 - the cyst wall and the villi; arrow points at the outer homogeneous layer of the wall; arrowhead points at the surface coat; 53-55 - single membrane-bound structures with (chromatin-like) concentrates of dense matter, possible precursors of (presporogonic) nuclei; arrowheads point at the delimiting membranes, arrows at vesicles wedged in the margin of the dense substance; 56 - a presporogonic nucleus with double envelope. 57 - a sector of mature spore with the inclusion, mass of glycogen, nucleus and lipid inclusion; 58 - spore in the early phase of zoosporogenesis with reduced inclusion, dense globules, lipid globules and growing nucleus; 59 - a mitochondrion of the preceding stage. li - lipid inclusion, m - mitochondrion, n - nucleus, w - cyst wall. Scale bars - 0.4 µm (54); 0.5 µm (52, 53, 55, 59); 1 µm (57, 58); 2 µm (58). **Pekkarinen *et al.* (2003)**





Figs 60, 61. **Dermocystidium fennicum** sp. n. 60 - zoospores escaped from spore walls with a pit (arrow) in their cell; 61 - zoospores discharging their inclusion bodies. ib - inclusion bodies, f - flagellum, m - mitochondrion, n - nucleus. Scale bars - 0.5 µm (60, 61). **Pekkarinen *et al.* (2003)**

Fig. 62. A drawing of the site of the flagellar basal body and associated structures in Dermocystidium percae zoospore. b - basal body, c - non-functional centriole, f - flagellum, g - glycogen rosettes, i - inclusion body, li - lipid inclusion, m - mitochondrium, n - nucleus, r -ribosomes, rh - rhizoplast, rs - rumposome. **Pekkarinen *et al.* (2003)**

.

Feist *et al*. (2004) detected Bullheads, Cottus gobio, with macroscopic external cysts on the skin and fins measuring up to 3 mm in diameter in the River Allen and its tributaries in southern England between 1992 and 1998. The prevalence of these cysts was up to 50% at some sites. Examination of cyst contents revealed the presence of numerous spores, typical of the genus Dermocystidium, measuring 8 microm in diameter. The parasite developed within well-defined cysts, which were located in the hypodermal connective tissues of the host. No cysts were present on the fins of any of the fish examined. Histological examination revealed a cyst wall consisting of an inner layer of dense eosinophilic material similar to that reported for Dermocystidium spp. forming coenocytic hyphae. No evidence was found of systemic infection or hyphal formation. Spores contained a prominent refractile body, which gave a weakly positive reaction for polysaccharides with the periodic-acid Schiff reaction and was positively stained with acidic dyes. Several examples of ruptured cysts were seen in histological sections and in some of these cases the host epithelial layer was breached,

allowing release of the spores to the environment. Morphological features of, and host response towards, the Dermocystidium sp. in bullheads are compared with similar infections in salmonids and other freshwater fish species.

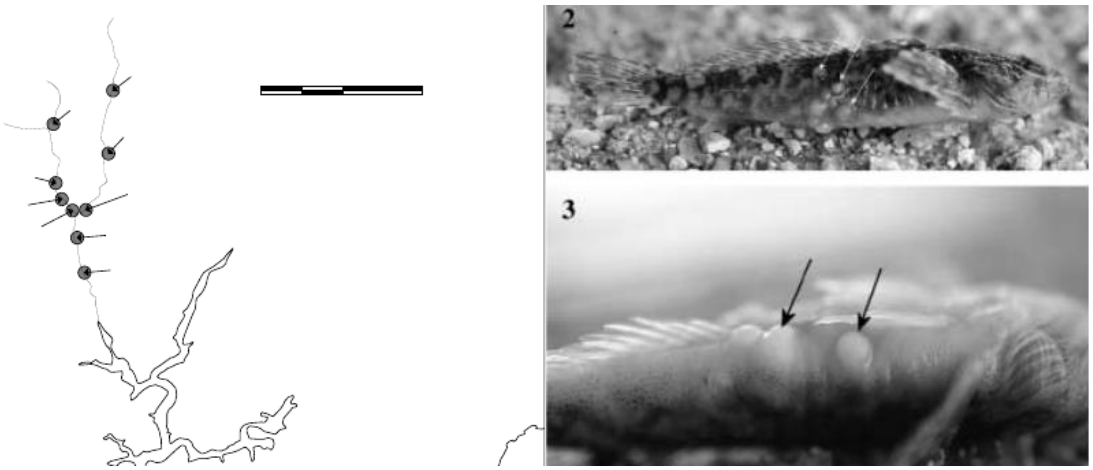


Fig. 1.Detail of River Allen in Cornwall and of sites sampled for bullheads: 1, Trenerry; 2, Lanner Mill; 3, Ladys Wood; 4, Scawswater Mill; 5, Dabuz's Moor; 6, Ventontrissick; 7, Garras; 8, Higher Lamerton; 9, Lords Wood.Figure 2: 3. Ventral and lateral surfaces of Cottus gobio, showing the presence of several Dermocystidium sp. cysts (arrowed). These are raised with a smooth intact epithelial surface Feist *et al*. (2004)

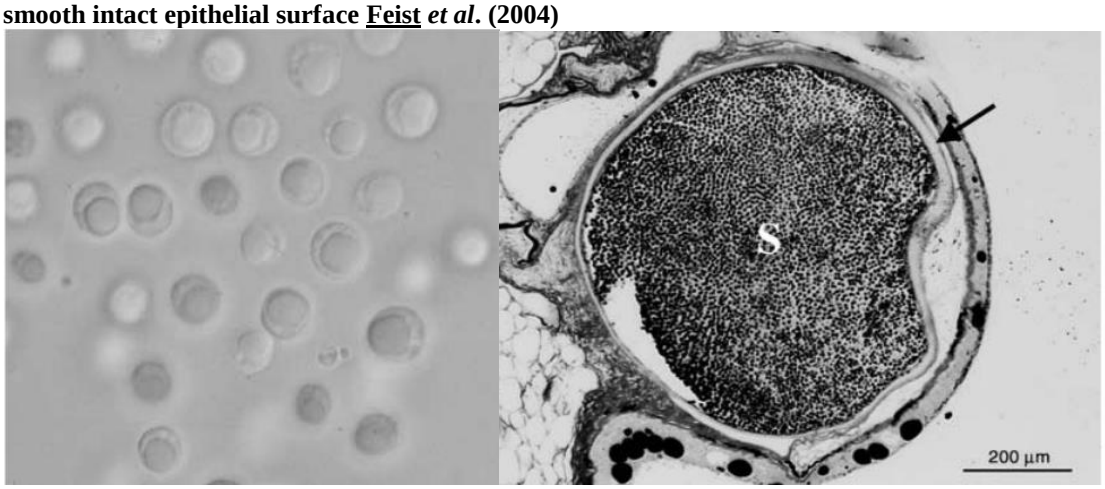


Figure 3: Spores of Dermocystidium sp. released from an epidermal cyst. The spherical 'spores' measuring approximately 8 µm in diameter are characterized by a large central vacuole or refractile.
Figure 4: Section through Dermocystidium sp. cyst in the hypodermal connective tissues. These cystic lesions (1–3 mm diameter) were present at various sites on the body surface. **Feist *et al*. (2004)**

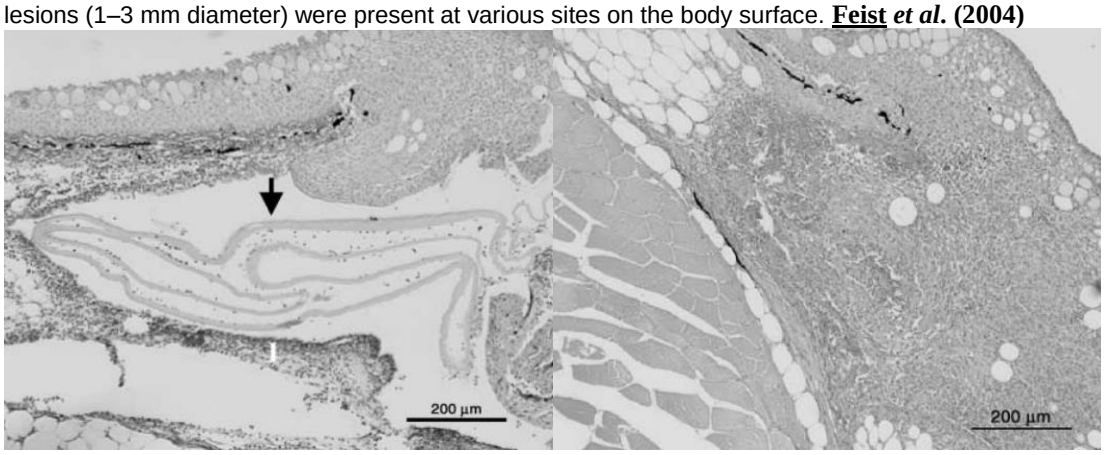
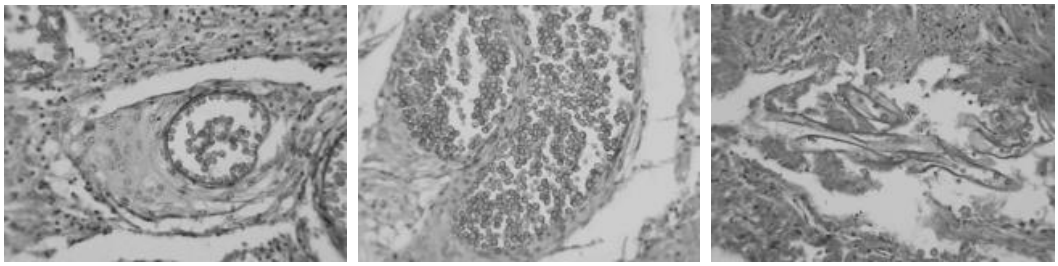


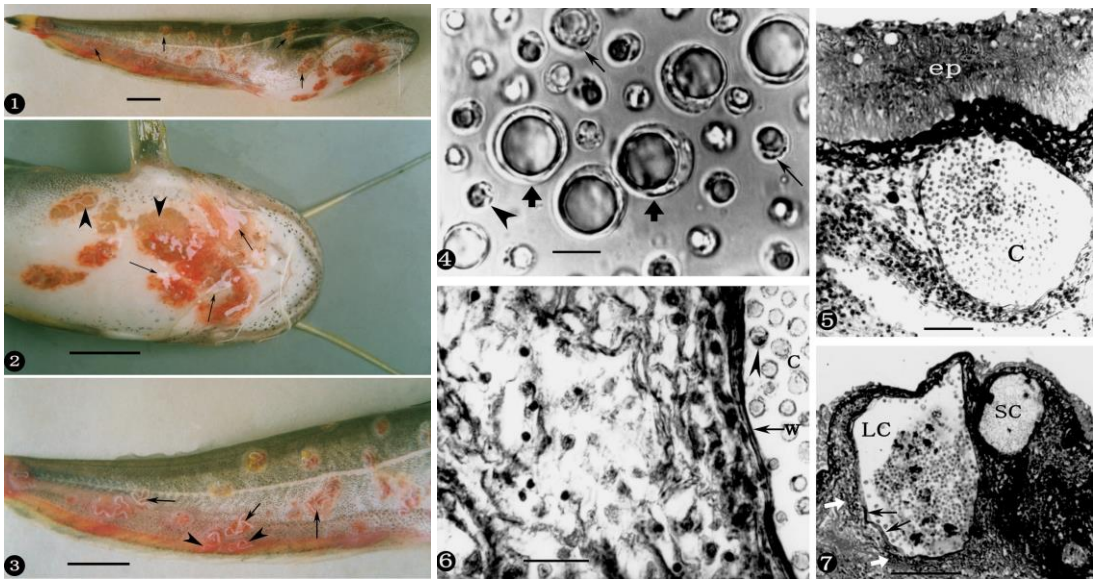
Figure 5: Section of ruptured cyst showing collapsed cyst wall (arrowed) and mild infiltration with host lymphocytes and phagocytes in the surrounding tissues (I) (Gomori trichrome, bar = 200 µm).
Figure 6: Marked inflammation involving the epidermis and dermal tissues following rupture of a Dermocystidium sp. cyst (H & E, bar = 200 µm). **Feist *et al*. (2004)**

Novotny and Smolova (2006) described the infection of a *Dermocystidium* sp. with unusual skin localization and morphology in common carp in the Czech Republic. Clinical examination revealed red coloured lenticular lesions, with a diameter of 3 – 5 mm on the bases of the ventral and anal fins. Inflammation was observed in the tissues surrounding the lesions. A tangle of vermiform structures was seen in wet mounts from the lesions. Histopathology revealed the presence of cysts of elliptic and spherical shape with globoid PAS-positive spores. The size of these spherical cysts varied from 50 to 110 µm in diameter and elliptic cysts had length of 550 µm and width 150 µm. The one layer cyst wall consisting of fibrocytes was surrounded by macrophages with progress to the granuloma. Hyperplasia of epidermis with abundant goblet cells and lymphocyte infiltration was seen near the cysts. Cysts with mature and also with immature spores were present. Mature spores measured 5 to 6 µm in diameter; immature spores were 3 to 4 µm in diameter. Hyphae-like structures were noted.

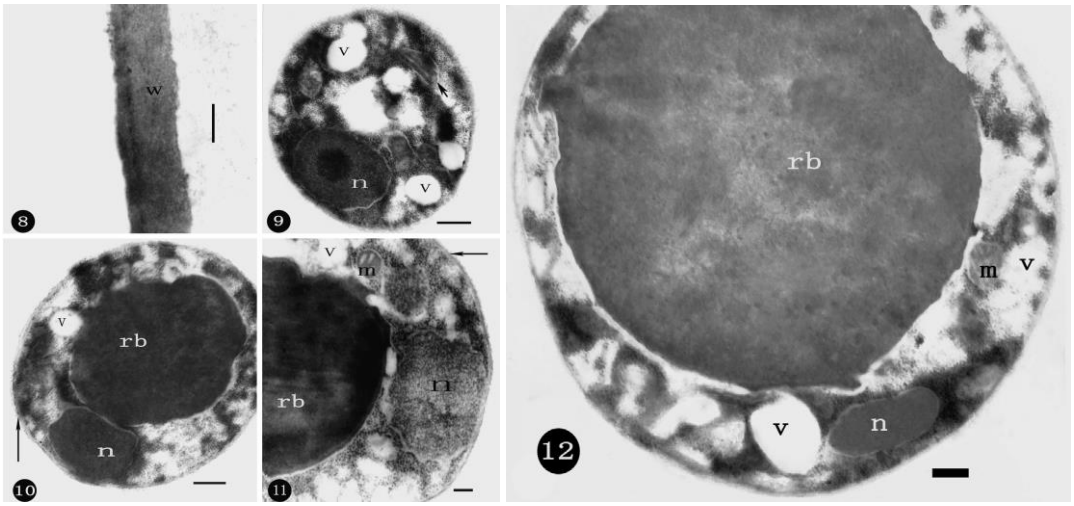


Histopathology of the skin of the common carp (*Cyprinus carpio*) with spherical cysts and globoid spores. The cyst is surrounded by macrophages, which form the granuloma.Haematoxylin and Eosin, x 400 Mature spores with PAS positive content. PAS reaction, x 400.Hyphae-like structures in the skin of the carp. In these structures were also noted the spores. Haematoxylin and Eosin, x 400 **Novotny and Smolova (2006)**

Zhang and Wang (2005) discovered a species of Dermocystidium on the skin and fins of reared southern catfish Silurus meridionalisChen. The parasite only appeared and caused disease in juvenile catfish at a water temperature of 18 to 23 degrees C. Marked external macroscopical clinical signs of the disease were sluggish movement of the fish, and the appearance of white filiform dermal cysts varying in size (3-20 mm in length and 0.15-0.35 mm in width). Examination of both fixed and fresh mounts for light microscopy and of samples for transmission electronmicroscopy (TEM) showed spherical spores (3.2-15 microm in diameter) with a peripheral nucleus (1.1-1.8 x 0.5-1.6 microm in diameter) and a prominent refractile body (2.08-10.83 microm in diameter) which occupied most of the volume of a mature spore. Three types of spore were identified, and are presumed to represent various developmental stages. Meanwhile, TEM showed the remnant nuclei in the residual plasmodium of a cyst, revealing its degenerative process. This paper represents the first observation and description of Dermocystidium sp. parasitizing catfishes.



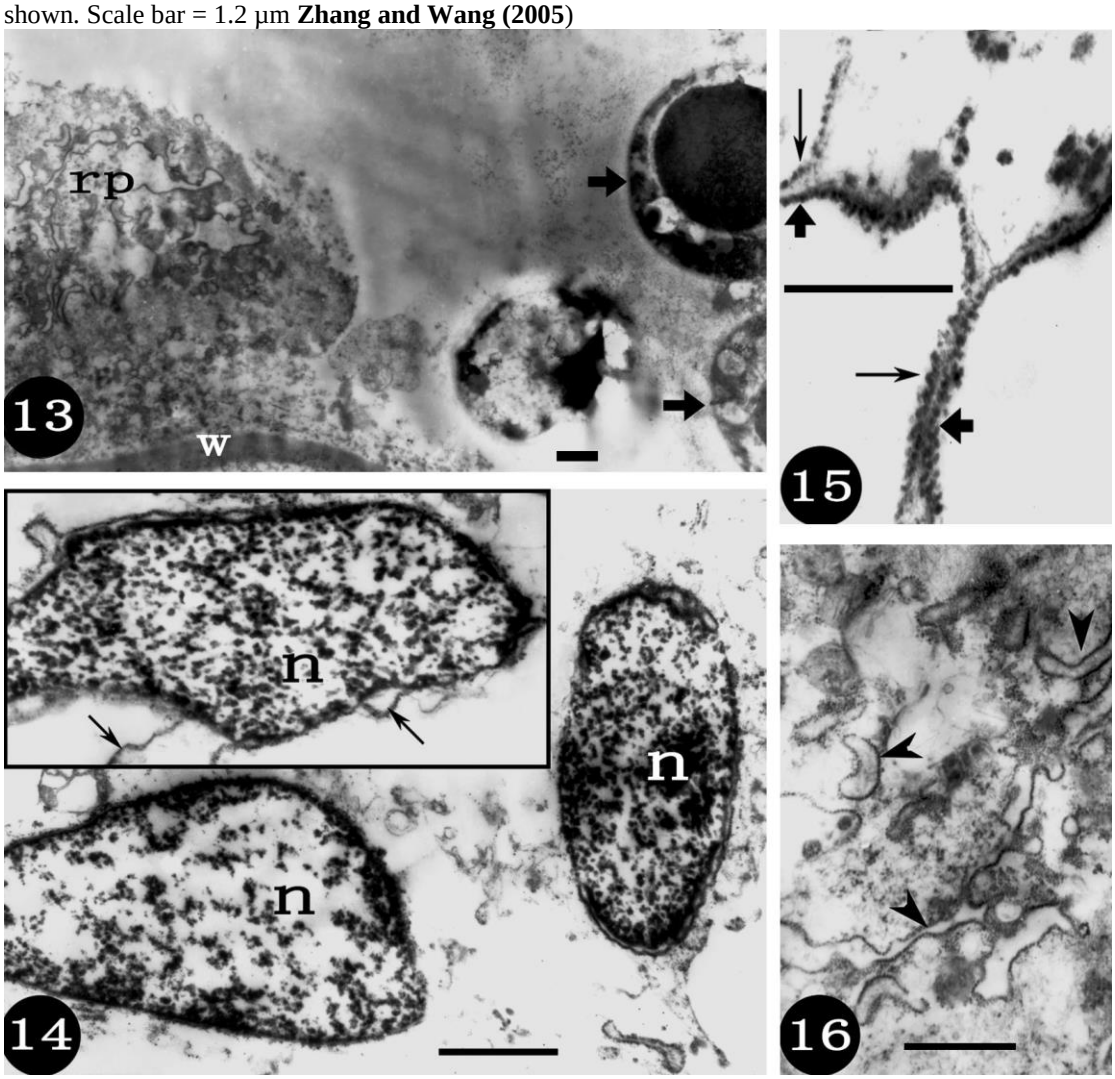
Dermocystidium sp. infecting Silurus meridionalis. Infected juvenile southern catfish cultured in a net cage in Jialing River, Chongqing, China. Fig. 1. Individual juvenile catfish infected by Dermocystidium sp. Note ca. 42 pink or red parasitic sites; cysts of Dermocystidium sp. marked with arrows. Scale bar = 1 cm. Fig. 2. Ventral view of the infected catfish. Note the parasitic sites displaying hyperaemia and oedema; ruptured epidermis marked with arrows, cysts of the parasite marked with arrowheads. Scale bar = 1 cm. Fig. 3. Lateral view of the infected partial body; whitish cysts marked with arrows, cysts in the anal fin marked with arrowheads. Scale bar = 1 cm **Zhang and Wang (2005**) Figs. 4 to 7. Dermocystidium sp. infecting Silurus meridionalis. Photomicrographs of both a wet mount preparation of the Dermocystidium sp. and H&E-stained sections of infected skin. Fig. 4. Wet mount of Dermocystidium sp. parasitizing the juvenile southern catfish; largest spores marked with bold arrows, medium-sized spores marked with thin arrows, smaller spore marked with an arrowhead. Scale bar = 10 µm. Fig. 5. A parasite cyst (C) within the catfish dermis containing numerous spores. Note the intact epidermis layer (ep) overlaying the cyst. Scale bar = 50 µm. Fig. 6. Higher magnification of a cyst (c) and tissues around it. Note cyst wall (w), spores (arrowhead) and a slight infiltrate of mixed inflammatory cells to the left of the cyst. Scale bar = 20 µm. Fig. 7. Photomicrograph of 2-cyst cross section. Note the larger cyst (LC) enclosing larger spores than those in smaller cyst (SC), hyperplastic fibrous connective tissues (bold white arrows) around the cyst wall (thin arrows), and the epidermis layer overlaying the cysts has disappeared. Scale bar = 200 µm **Zhang and Wang (2005**)



Dermocystidium sp. infecting Silurus meridionalis. Transmission electron micrographs of cyst wall, small and medium-sized spores. Fig. 8. Cyst wall (w) consisting of an aggregation of amorphous material. Scale bar = 0.2 µm. Fig. 9. Small spore with a nucleus (n), mitochondrion (short arrow) and vacuoles (v), but without a refractile body. Scale bar = 0.5 µm. Fig. 10. Medium-sized spore bearing a nucleus (n), a medium-sized refractile body (rb) and vacuole (v); thin arrow: thin spore membrane. Scale bar = 0.7 µm. Fig. 11. Partial medium-sized spore with a clear nucleus (n), mitochondrion (m),

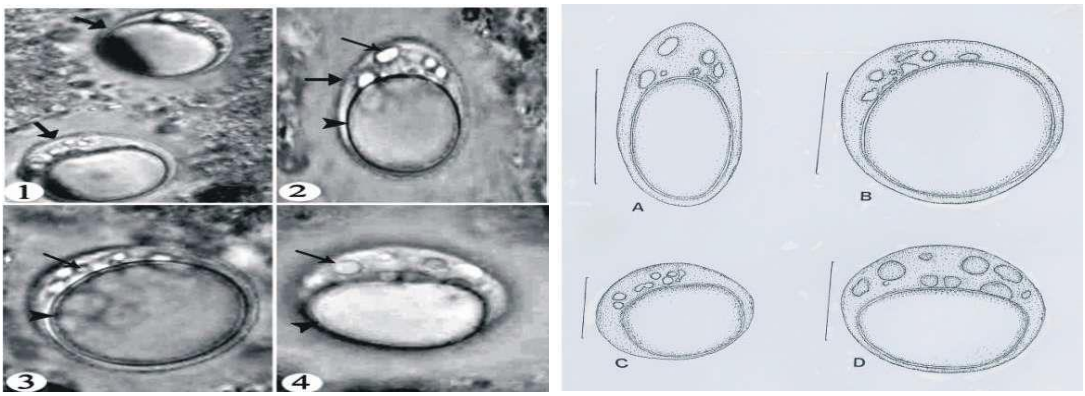
vacuoles (v) and a partial refractile body (rb); thin arrow: thin spore membrane. Scale bar = 0.2 µm **Zhang and Wang (2005**)

Fig. 12. Dermocystidium sp. infecting Silurus meridionalis. Transmission electron micrographs of a larger spore. Large refractile body (rb), a small nucleus (n), a mitochondrion (m) and a vacuole (v) are shown. Scale bar = 1.2 µm **Zhang and Wang (2005**)



Dermocystidium sp. infecting Silurus meridionalis. Transmission electron micrographs of the degenerative residual plasmodium in a mature cyst. Fig. 13. Residual plasmodium (rp) and spores marked with bold arrows in the cyst. w: cyst wall. Scale bar = 1 µm. Fig. 14. Residual nuclei (n) in the plasmodium. Insert: note the protruded outer membrane (arrows) of the remaining nucleus. Scale bar = 1 µm. Fig. 15. Higher magnification of a partial residual nucleus. Note the protruded outer membrane (bold arrows) with ribosomes (thin arrows). Scale bar = 0.5 µm. Fig. 16. Partial residual plasmodium. Note various vesicles (arrowheads) with ribosomes, probably resulting from the disintegration of residual nuclei. Scale bar = 1 µm **Zhang and Wang (2005**)

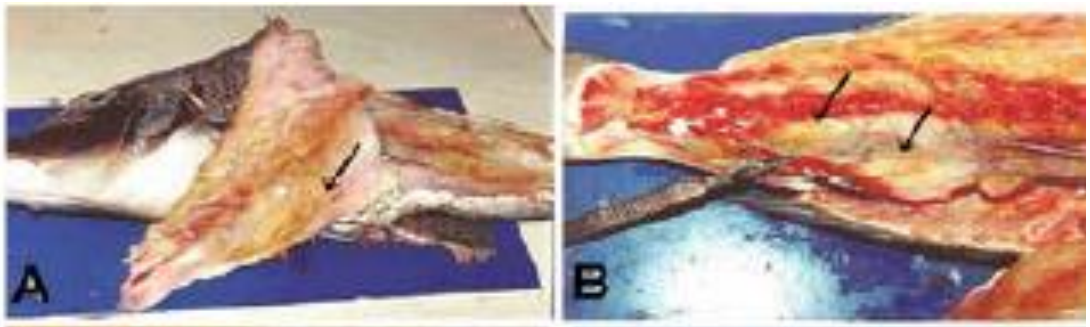
El-Mansy (2008) described a new species of the genus Dermocystidium that was isolated from the intestine of cultured Oreochromis niloticus inhabiting some aquatic resources particularly Lake Burullus, Egypt. This species lackes a typical nucleus and has a large central vacuole with a diameter 11.4±0.9×11.6±3.3 µm and vacuolated peripheral bodies embedded within tipped cytoplasmic area with dimensions 1.7±0.7×1.0±0.8 µm. They are surrounded by double and single membrane respectively. The reported spores are investigated microscopically, photographed, sketched, measured and compared with previous related ones.



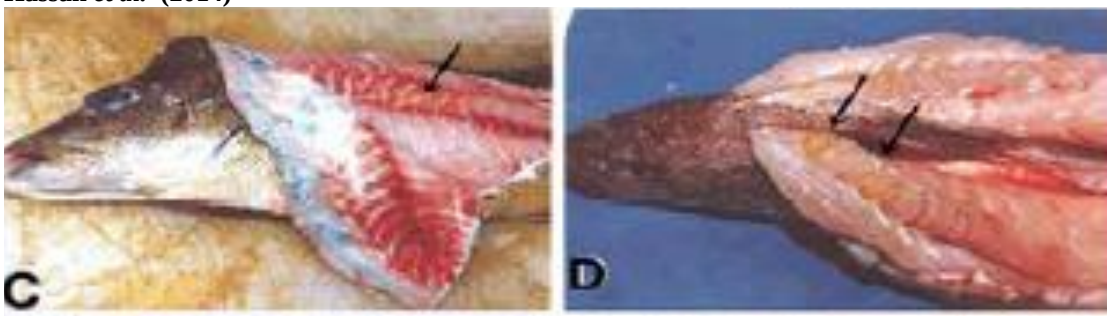
Fresh preparations of intestine of Oreochromis niloticus show spores of Dermocystidium aegyptiacus at different microscopically views. Note the spherical spore (thick arrow) with a large central vacuole (arrowhead), x1500. (1) arrows refer to thick-wall of typical spores. (3, 4) show peripheral vacuolated bodies with different sizes in cytoplasmic area (arrows) and internal wall of the central vacuole is surrounded by thick membranous like-shape (arrowheads). **El-Mansy (2008)**
D. aegyptiacus of a typical stage found in the intestine of O. niloticus. Scale bar-10 µm. **El-Mansy (2008)**

Gjurcevic *et al.* (2008) presented the first finding of Dermocystidium sp. in Croatia. The species was found on a fish farm, in broodstock of common carp (Cyprinus carpio L.). The localisation of the hyphae and morphological characteristics of spores confirms that the species is identical with Dermocystidium sp. described by Novotny & Smolova (2006). Hyphae were 109 ± 44 µm (mean ± SD) wide, with 4.8 ±1.3 µm thick homogeneous walls. Mature and immature spores measured 8.3 ± 1.5 µ and 5.1 ± 0.2 µm, respectively. The refractile body was 4.7 ± 0.9 µm in diameter. Despite small variations in size, the authors tentatively identified these species as Dermocystidium koi Hoshina & Sahara, 1950

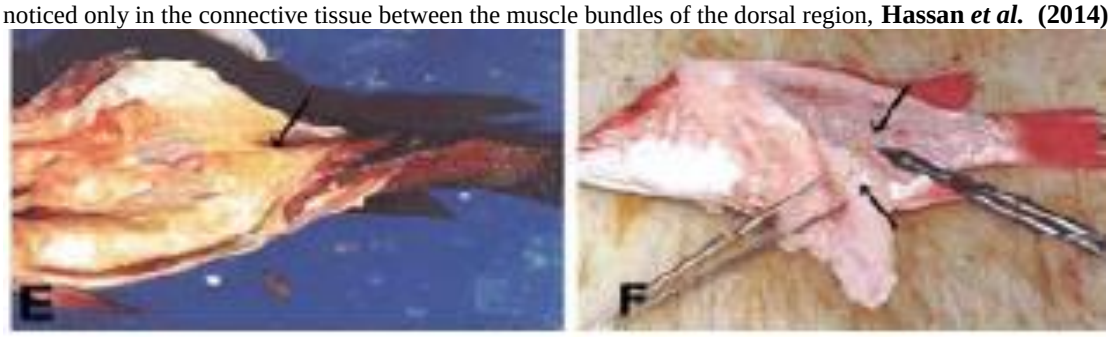
Hassan *et al.* (2014) examined freshly caught marine fish from the coast of Arabian Gulf at Qatef, Eastern province and Red Sea coast, Jeddah of Saudi Arabia were examined as a routine fish health survey for Dermocystidium infections. Altogether, 1500 specimens from 45 species at Qatef and 116 specimens of grouper *Epinephelus polyphekadion* at Jeddah were sampled. The prevalence of Dermocystidiosis in Qatef was 7.66% while prevalence in Jeddah was 18.96 %. The following fish species were infected with Dermocystidiosis at the given prevalence; *Johinus maculatus* (37.5 %) *Lethrinus nebulosus* (11.53 %), *Lutjanus ehrenbergi* (28.75 %), *Lutjanus malabaricus* (22.5 %) & *Cephalopholis hemistiktos* (20%) at Qatef while one species *Epinephelus polyphekadion* (18.96 %) only infested at Jeddah. *Lutjanus ehrenbergi & Lutjanus malabaricus* showed low intensity of infestation. *Johinus maculatus* & *Cephalopholis hemistiktos* with medium intensity of infestation and appeared to be perfectly normal, while *Lethrinus nebulosus* was highest intensity of infestation and showed detached scales, dull opaque body color with turbidity on various parts of the body and emaciation with sunken belly. Diseased fish showed grossly visible yellow blotches or spots within the musculature at sections cut from musculature of fishes suspected infection with Dermocystidiosis. Fresh sample preparations and histopathological examination for gills and musculature of naturally infected fish, revealed various spore stages of a new Dermocystidium species, it established as *D. Arabica* sp. n. Scan Electron Microscope (SEM) was carried out for isolated hyphae and spores to confirm the diagnosis in this study, because of the yellow color of the muscle was a characteristic sign for the all infected fish and the available articles not previously dealt with this phenomenon, the disease is suggested to be named as “Yellow Muscle Disease “.



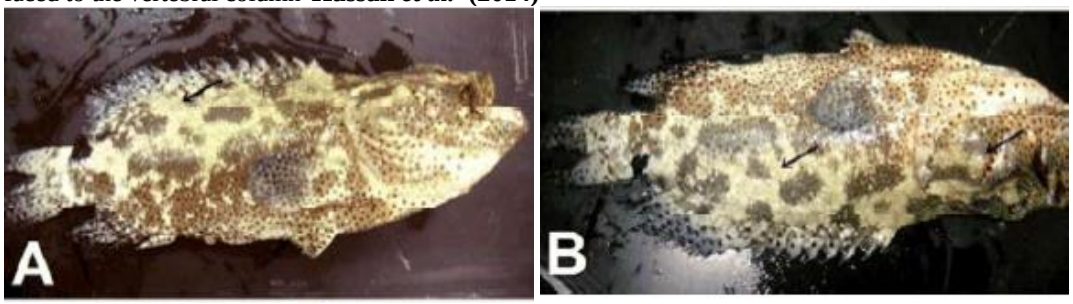
Dermocystidium hyphae (Threads) embedded in musculature of infested fish species in Qatef (A) *Lethrinus nebulosus* fish the yellow patches were observed deeply in the musculature that faced to the vertebral column. (B) *Lethrinus nebulosus* fish the yellow patches were observed in the entire muscles under the skin. **Hassan *et al.* (2014)**



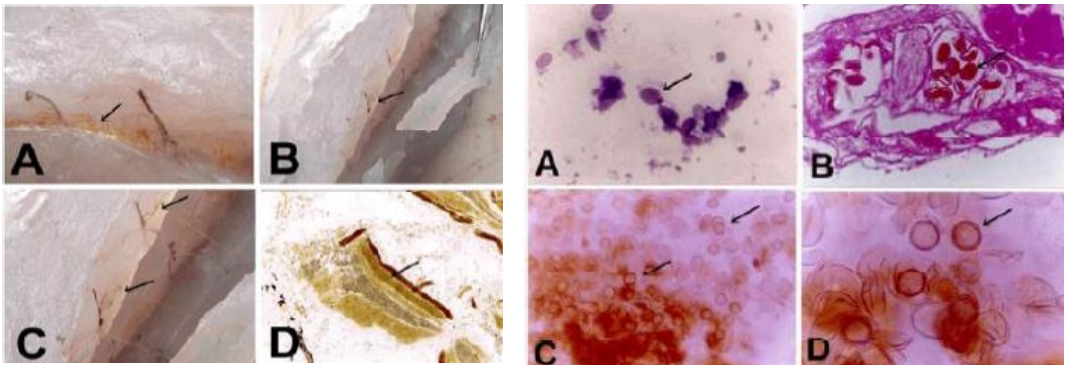
Brown dots or sticks were also seen within the musculature (C) In *Lutjanus ehrenbergi*, small yellow patches area were observed in muscle layer under the skin. (D) In *Johinus maculatus*, the yellow threads were noticed only in the connective tissue between the muscle bundles of the dorsal region, **Hassan *et al.* (2014)**



(E) *Cephalopholis hemistiktos* had a web of yellow threads (Hyphae) in the abdominal muscles. (F) *Lutjanus malabaricus* fish, small yellow patches (Hyphae) were observed deeply in the musculature that faced to the vertebral column **Hassan *et al.* (2014)**

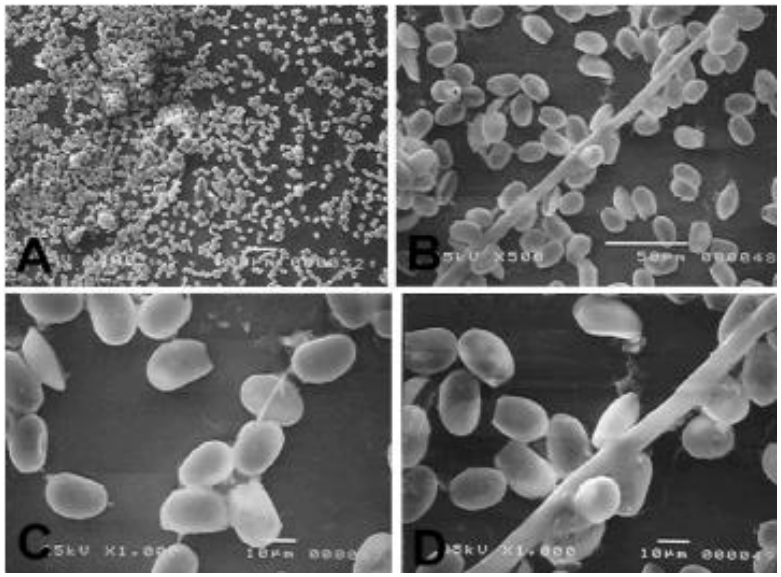


Grouper, *Epinephelus polyphekadion* fish in Jeddah appearing with opaque surface covered with blue mucous especially at the upper surface with eroded caudal fins (Arrows) **Hassan *et al.* (2014)**

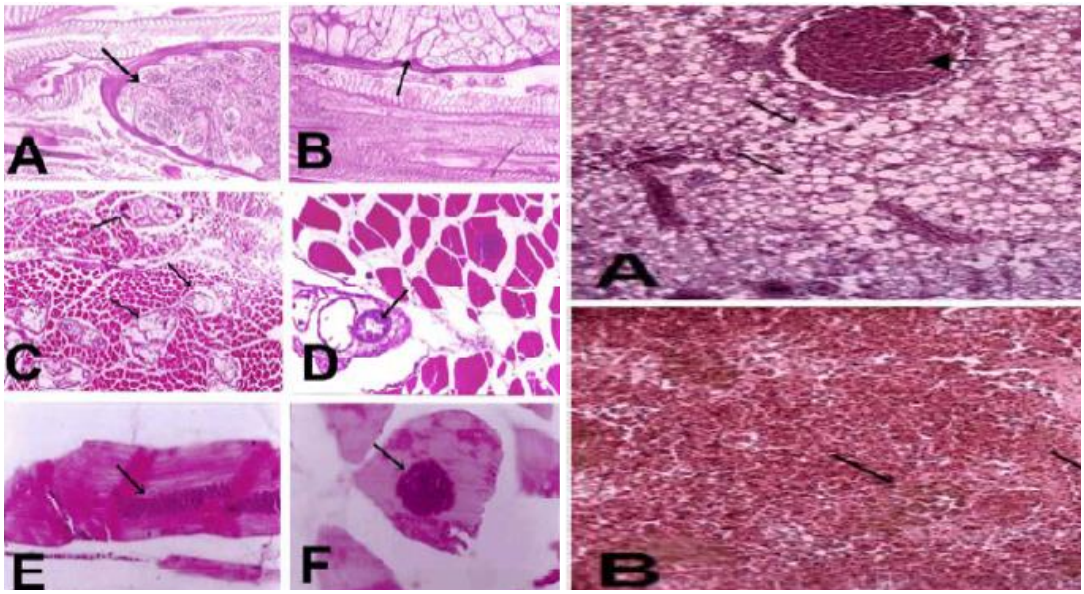


In *Johinus maculatus*, yellow spots consists of large numbers of threads [filament or hyphal like structure] (Diameter: Less than 0.1 mm) which easily seen in the infected musculature (Arrows) (D) Fresh preparations from the infected musculature revealed lots of spores of different developmental stages (Arrows).

Spores stained with Giemsa stain, a large inclusion body nearly fills the spore (Arrow) (B) The mature spore has a large, PAS [Periodic acid-Schiff stain] positive inclusion body surrounded by a thin rim of hostcytoplasm (Arrow) (C.&D) Fresh preparation, the mature spores appeared spherical in shape, variable in size with a large inclusion body with a narrow rim size with a large inclusion body with a narrow rim of cytoplasm and internal wall of the central inclusion body is surrounded by thick membranous like-shape (Arrows). **Hassan *et al.* (2014)**



Scan electron microscopy for hyphae as well as the spores of *Dermocystidium Arabica* **Hassan *et al.* (2014)**

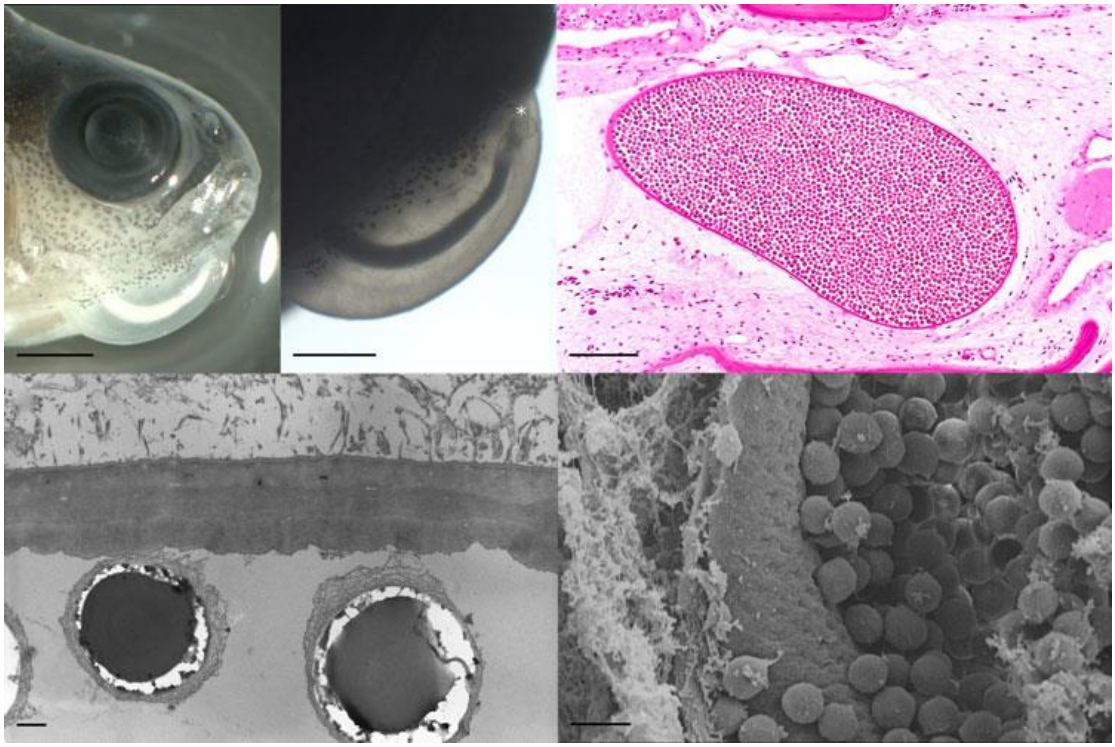


Many hyphae of Dermocystidium Arabica, contain a large number of unicellular spores (Arrows), embedded along the secondary gill lamellae with hyperplasia of secondary gill filaments, the hyphae and spores substitutes the branchial tissues, (C&D) Cross sections of infected musculature showing many hyphal-like structures filled with a massive numbers of oval spores between the muscle bundles (Arrows) (E) Longitudinal section of skeletal muscle fiber of Johinus maculatus showed penetrated spores in the muscle fibers (Arrows) (F) Cross section of skeletal muscle fiber of Johinus maculatus showed central area of sporulation (Arrows). **Hassan *et al.* (2014)**

Liver of Epinephelus polyphekadion suffered from vacuolar degeneration with deposition of dermocystidium spores (Arrows), (B) spleen of Epinephelus polyphekadion showing deposition of hemosiderin pigments degeneration with deposition of dermocystidium spores (Arrows) X100 H&E. **Hassan *et al.* (2014)**

Langenmayer *et al.* (2015) mentioned that in January 2013, increased mortalities of cardinal tetra, P. axelrodi of a 350-L aquarium were reported, occurring after purchase of additional fish. During the clinical examination, most of the fish were swimming in normal active condition, but some were lethargic or displayed a transparent mass on the skin of the head or body. The masses were up to 5 mm in diameter and contained a central, white tubular structure. Similar masses were located on the fins of some fish, but were considerably smaller in these locations. A parasitic infestation of the skin of these fish was excluded via microscopic examination, and normal results were obtained after analysis of the water values and inspection of the pump and filter system. Six cardinal tetra, P. axelrodi and two firehead tetra, Hemigrammus bleheri were submitted for pathological examination in formalin and for molecular genetic examination in ethanol. Only two cardinal tetra displayed skin lesions. Before embedding, one tetra was post-fixed in Davidson's fixative and sections of glycolmethacrylate/methylmethacrylate- embedded samples were routinely processed for histological examination and stained with haematoxylin and eosin (HE), Giemsa, silver impregnation and periodic acid Schiff (PAS) reaction according to standard protocols. Samples of the mass were also routinely processed for transmission and scanning electron microscopy on a transmission electron microscope (Zeiss EM 10) or on a digital scanning electron microscope (Zeiss DSM 950), respectively. The macroscopic examination of the cardinal tetra revealed a focally extensive, bulging, hemispherical, transparent oedema (4 mm in diameter) of the ventral skin on the head. Central, within the oedematous skin, was an elongated, white, opaque tube (about 400 lm in diameter), which extended beneath the right side's scales with its posterior end. In transmitted light, the rostral end of the tube appeared empty and displayed a

minute, dermal, covering cap. Microscopically, the epidermis was intact and the dermis was deep, focally extended oedematous with marked separation of dermal cells and extracellular matrix elements. Within the oedematous tissue, a cystic structure was situated, which showed prominent features of Dermocystidium sp. cysts. The homogenous, eosinophilic, PAS-negative cyst wall was surrounded by a concentric monolayer of elongated spindle cells with scant eosinophilic cytoplasm and flattened basophilic nuclei. Numerous spherical spores with eosinophilic inclusions and clear cytoplasm were located within the cyst. The cytoplasm stained partially positive in PAS reaction and with the silver impregnation, whereas the inclusion showed no specific staining. Lesions that could explain the increased mortality in the aquarium were not found histologically. Ultrastructurally, the cyst wall was bilayered with an up to 100-nm-thin homogenous outer and a 2.5–3-lm-thick granular inner layer. Multiple, villous protrusions of up to 60 nm in width were projecting from the outer layer into the surrounding tissue. The inner layer displayed multiple indentations on the inner side. The spores were 5–7 lm in diameter and contained a single, large, osmiophilic inclusion. The cytoplasm was often fragmented (most probably a tissue processing were not observed. The spores and the inside of the cyst wall were covered by a fibrillary meshwork. Flagellated zoospores were not observed. Scanning electron microscopy further illustrated the cyst wall lying within a loosely arranged fibrillar sparsely cellular matrix, containing numerous spherical spores. The surface of the spores was slightly granular and irregularly covered by a proteinaceous material, forming connections between single spores. The inside of the cyst wall showed multiple indentations of the Dermocystidium spores. Based on the macroscopical, histological and ultrastructural findings, a cutaneous dermocystidiosis was diagnosed. To confirm the pathomorphologic diagnosis and to identify the Dermocystidium species, DNA was extracted from separated skin mass of one tetra using a QIAamp DNA Mini Kit tissue protocol (QIAGEN). The sample was vortexed in 180 ml of the kit lysis buffer. Subsequently, it was incubated with proteinase K and lysis buffer at 56°C until complete lysis. DNA extraction was then completed according to the manufacturer’s instructions and eluted in 50 lL elution buffer. A negative extraction control was performed.



Dermocystidium salmonis infection in cardinal tetra, Paracheirodon axelrodi. (a) White, opaque, tubular cyst on the ventral head. Bar = 1 mm. (b) The cyst tube appears empty and displays a dermal cap on the rostral end (asterisk) in transmitted light. Bar = 1 mm. (c) Typical Dermocystidium cyst lying in oedematous dermis with eosinophilic cyst wall and numerous intracystic spores. HE. Bar = 100 lm. (d) Double-layered cyst wall of D. salmonis with multiple villous protrusions from the outer layer and intracystic spores with a prominent osmiophilic inclusion covered by fibrillary meshwork. Transmission electron microscopy. Bar = 1 lm. (e) D. salmonis cyst within loosely arranged fibrillary matrix (left side), there are multiple indentations on the inner side of the cyst wall of the spherical spores, which are sometimes connected by proteinaceous material (right side). Scanning electron microscopy. Bar = 10 lm. **Langenmayer *et al.* (2015)**

Newly developed PCR primers for detection of Dermocystidium salmonis 18S rDNA

Type	Sequence	Melting temperature (Tm)[a]
Forward primer	CCTATCAACTTTCGATGGTAAGGTATTGGC	66.7 °C
Reverse primer	CCGAAGACCTCACTAAACCATTCAATCG	67.9 °C

[a]Tm was calculated using applied bio system Tm calculator at 50 mM salt concentration http://www6.appliedbiosystems.com/support/techtools/calc.

Central, within Correspondence M C Langenmayer, Institute of Veterinary Pathology at the Centre for Clinical Veterinary Medicine, LMU Munich, Veterinarstrasse 13, 80539 Munich, Germany (e-mail: € langenmayer@patho.vetmed.uni-muenchen.de) Langenmayer and Lewisch contributed equally to this work. 503 2014 John Wiley & Sons Ltd J

Blazer *et al.* (2016) observed raised pale cysts on Blue Ridge Sculpin *Cottus caeruleomentum* during stream fish community surveys in Catoctin Mountain Park, Maryland. When examined histologically, preserved sculpin exhibited multiple cysts containing spherical endospores with a refractile central body characteristic of *Dermocystidium* spp. Cysts were not observed on the gills or internally. The portion of the watershed in which affected sculpin were observed contained lower than expected numbers of sculpin, raising concerns about the population effects of this infection. A nearby stream lacked sculpin even though they are common in this region, further suggesting the possibility of regional effects. This is the first report of a *Dermocystidium* infecting any fish species in the eastern United States.

Release Date: JULY 26, 2016, https://www.usgs.gov/news/west-coast-fish-pathogen-now-

found-east

.

A fish pathogen similar to one previously found in the United States only in Pacific salmonids -- salmon and trout species -- has been identified for the first time in the eastern United States and in a non-salmon species, according to new research by the U.S. Geological Survey.

A 2015 sampling effort found the pathogen, *Dermocystidium*, within cysts on the bodies of Blue Ridge sculpin, although it can cause cysts in the gills and internal organs of infected fish as well, and sometimes death. Before this new study, infections of this pathogen in the U.S. were restricted to Pacific salmonid species in west coast rivers, although it has been reported in other species in Europe.

"The infection was found on Blue Ridge sculpin during fish community surveys in Catoctin Mountain Park, Maryland, in 2015," said Vicki Blazer, a research fish biologist and lead author of the study. "In portions of the watershed where the infection was observed, sculpin numbers were lower than expected. And a nearby stream lacked sculpin altogether, though they are typically common in the region. The lack of sculpin indicates the possibility of regional effects."

Dermocystidium is a protist belonging to the phylum Mesomycetozoea, which are mostly found on fish and amphibians. Mesomycetozoea is considered an emerging threat to aquatic and terrestrial animals because they are not species specific, are opportunistic and can be carried as chronic sub-lethal infections.

"Sculpin can be found in many watersheds throughout the Chesapeake drainage and are an important part of the fish community," said Blazer. "It is currently not known if other fish species may be affected, or if the pathogen will have significant effects on sculpin populations."

More fieldwork, in collaboration with the National Park Service and Maryland Department of Natural Resources, is planned to monitor fish populations in the affected stream as well as nearby watersheds.

The study, "*Dermocystidiumsp.* Infection in Blue Ridge Sculpin *Cottus caerulomentum* Captured in Maryland, USA," is available online in the Journal of Aquatic Animal Health.

References:

1. **Blazer, V. S. , Nathaniel P. Hitt, Craig D. Snyder, Erin L. Snook & Cynthia R. Adams. *Dermocystidium* sp. Infection in Blue Ridge Sculpin Captured in Maryland Journal of Aquatic Animal Health ,28, 2016 - 3**
2. **Davis, H.S. 1947 Studies of the protozoan parasites of freshwater 29. fishes. U.S. Fish and Wildlife Fish. Bull. 51:1-29**
3. **Dyková, J. Lom. New evidence of fungal nature of *Dermocystidium koi*Hoshina and Sahara, 1950.J. Appl. Ichthyol.**
   Volume 8, Issue 1-4 August 1992 Pages 180–185
4. El-Mansy, A. A New Finding of Dermocystidium - like Spores in the Gut of Cultured Oreochromis niloticus. Global Veterinaria 2 (6): 369-371, 2008
5. Feist, S., Matt Longshaw, R H Hurrell, B Mander Occurrence and life cycles of Dermocystidium species (Mesomycetozoa) in the perch (Perca fluviatilis) and ruff

(Gymnocephalus cernuus) (Pisces: Perciformes) in Finland and Estonia Journal of Natural History 37(10):1155-1172 · May 2003

6. Feist,S., Matt Longshaw, R H Hurrell, B Mander. Observations of Dermocystidium sp. infections in bullheads, Cottus gobio L., from a river in southern England. Journal of Fish Diseases 27(4):225-31, 2004.
7. Gjurcevic E., Bambir S., Kozaric Z., Kuzir S., Gavrilovic A., Pasalic I. (2008). Dermocystidium infection in common carp broodstock (Cyprinus carpio L.) from Croatia. Bull. Eur. Assoc. Fish Pathol.28, 222–229
8. Hassan, M. A. Hussien A., M. Osman and Mahmoud A. Mahmoud. Studies on Dermocystidiosis (Yellow Muscle Disease) among Some Marine Fishes of Arabian Gulf and Red Sea Coast, Jeddah, Saudi Arabia. Middle-East Journal of Scientific Research 22 (4): 478-487, 2014
9. **Hoshina, T. and Y. Sahara 1950 A new species of the genus Dermocystidium, D. koi sp. nov., parasitic in Cyprinus carpio L. Bull. Jap. Soc. Scientific Fisheries. 15: 825-829**
10. Huglund, J., Anders lfjorden, Tapio Nikkila. Infection of juvenile salmon Salmo salar with a Dermocystidium-like organism in Sweden. Dis Aquat Org. 30, 181-176, 1997
11. **Jirovec, Otto 1939 Dermocystidium vejdovskyi n. sp. ein neuer Parasit des Hechtes nebst einer Bemerkung ueber Dermocystidium daphniae (Ruhberg). Arch f. Protistenk. 92:137-146.**
12. Langenmayer, M C , E Lewisch , M Gotesman , W Hoedt , M Schneider , M El-Matbouli and W Hermanns. Cutaneous infection with Dermocystidium salmonis in cardinal tetra, Paracheirodon axelrodi (Schultz, 1956). Journal of Fish Diseases 2015, 38, 503–506
13. **Leger, L. 1914 Sur un nouveau protiste du genre Dermo¬ cystidium parasite de la truite. C. R. Acad. Sci. Paris. 158:807-809.**
14. **Mackin, J.G. 1951a Histopathology of infections of Crassostrea virginica (Gmelln) by Dermocystidium marinum Maclcin, Owen, and Collier. Bull. of Marine Science of Gulf and Carribean. JL: 72-87.**
15. **Mackin, J.G. 1951b Incidence of infection of oysters by Dermocystidium in Barataria Bay area of Louisiana. Paper presented at meeting of National Shellfisheries Assoc., August, 1951.**
16. **Mackin, J.G., H.M. Owen, and Albert Collier 1950 Preliminary note on the occurrence of a new protistan parasite, Dermocystidium marinum n. sp. in Crassostrea virginica (Gmelin). Science. 111:328-329**
17. Novotny, L. and J. Smolova. *Dermocystidium* sp. in the skin of the common carp (*Cyprinus carpio*) in the Czech Republic- a case report. Bull. Eur. Ass. Fish Pathol., 26(3) 2006, 125
18. Olson R.E. & Holt R.A. (1995) The gill pathogen Dermocystidium salmonis in Oregon salmonids. Journal of Aquatic Animal Health 7, 111–117.
19. Olson, R. E., Christopher F. Dungan, Richard A. Holt. ater-borne transmission of Dermocystidium salmonis in the laboratory. Dis.quat. Org.: 41-48, 1991
20. Pekkarinen M. & Lotman K. (2003) Occurence and life cycles of Dermocystidium species (Mesomycetozoa) in the perch (Perca fluviatilis) and ruff (Gymnocephalus cernuus) (Pisces: Perciformes) in Finland and Estonia. Journal of Natural History 47, 1155–1172.
21. Pekkarinen, M., Lom J., Murphy C.A., Ragan M.A. & Dykov_a I. (2003) Phylogenetic position and ultrastructure of two Dermocystidium species (Ichthyosporea) from the common perch (Perca fluviatilis). Acta Protozoologica 42, 287–307.
22. **Perez, Charles 1907 Dermocystis pusula, organisme nouveau de la peau des Tritons. ST R. Soc. Biol. Paris. 63; 445-446.**
23. **Perez, Charles 1908 Rectification de nomencalture a propos de Dermocystis pusula. C. R. Soc. Biol. Paris. 63:738.**
24. **Perez, Charles 1913 Dermocystidium pusula, parasite de la peau des Tritons. Arch. Zool. Gen. et Exp. 52:343-357.**
25. Ray, S. M. CULTURAL STUDIES OP DERMOCYSTIDIUM MARINUM WITH SPECIAL REFERENCES TO DIAGNOSIS OP THIS PARASITE IN OYSTERS. Thesis, Texas Univ. 1952
26. **Weiser, J. 1943 Beitrage zur Entwicklungsgeschichte von Dermocystidium daphniae Jirovec. Zool. Anz. 142: 200-205**

27. **Zhang Q. & Wang Z. (2005) Dermocystidium sp. infection in cultured juvenile southern catfish Silurus meridionalis inChina. Diseases of Aquatic Organisms 65, 245–250**

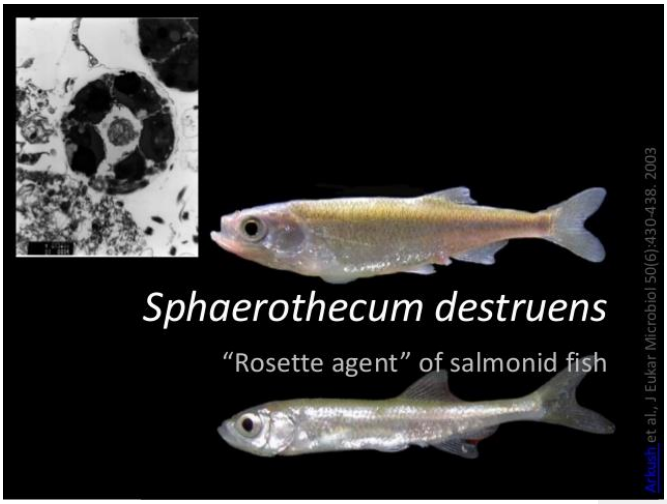
6. Sphaerothecum destruens

- **Sphaerothecum destruens**: (the **rosette agent**) is an obligate intracellular parasite
- **Sphaerothecum destruens** was first observed in Washington, USA, in net-pen reared O. tshawytscha where it caused 80% mortality in three year old fish (Harrell et al., 1986).
- **Sphaerothecum destruens** was later detected in subadult Atlantic salmon Salmo salar (Linnaeus) in a Northern Californian farm where it had caused chronic deaths (Hedrick et al., 1989).
- **Sphaerothecum destruens** was then detected in winter run O. tshawytscha held at the Bodega Marine Laboratory in California, where 40.1% o f dead fish were found to be heavily parasitized with S. destruens (Arkush et al., 1998).
- ***Sphaerothecum destruens*** is thought to pose more of a risk in Europe than in the USA as native species there are more susceptible to the parasite.
- ***Sphaerothecum destruens*** is the agent of a disease that causes high rates of morbidity and mortality in a number of different salmonid species and can also infect other freshwater fish such as bream, carp and roach.
- The genus *Sphaerothecum* is closely related to the genera *Dermocystidium* and *Rhinosporidium*.

Classification:

NCBI Taxonomy

- Eukaryota +
  - Opisthokonta +
    - Opisthokonta incertae sedis +
      - Ichthyosporea +
        - Dermocystida +
          - Sphaerothecum +
            - ***Sphaerothecum destruens***



SlideShare Publicdomain; 40. Sphaerothecum destruens "Taxonomy pics

Sphaerothecum destruens in the press

In early October 2015, the press has widely reported the results of a study by the IRD (Institute of Research for Development) also called for Pseudorasbora Asian stud '. The information comes from a communication from the IRD, recovery in the large scale by the press (http://www.ird.fr/la-mediatheque/fiches-d-actualite-scientifique/486-le-goujon - asiatique-new-fear-of-rivers).

The concern mainly concerns the pathogen whose Pseudorasbora is healthy carrier (a type of fungus called Sphaerothecum destruens) likely to cause, in some cases, very significant fish mortality (80%). The scientific publication which is based on the alert concerns a Turkish basin with significant potential consequences for bar farms. (Http://www.nature.com/emi/journal/v4/n9/full/emi201552a.html).

Aujourd'hui en France Lundi 12 octobre 2015

C'est la nouvelle terreur des rivières

ENVIRONNEMENT. Le goujon asiatique risque de décimer les poissons d'eau douce à cause du parasite, particulièrement redoutable, qu'il transporte malgré lui.

IL MESURE À PEINE 10 CM mais est en train de semer une belle panique dans nos cours d'eau. Au risque de vider nos assiettes ? En soi, le goujon asiatique, poisson d'eau douce au dos couleur jaune olive que les pêcheurs utilisent plus volontiers comme appât que comme plat de résistance, n'est pas dangereux pour l'homme. En revanche, il l'est pour ses congénères. « Le problème est qu'il est porteur sain d'un parasite, qui, lui, est mortel pour de nombreuses espèces de poissons comme les truites, les carpes, les perches ou encore les saumons et les bars d'élevage », explique Rodolphe Gozlan, chercheur à l'IRD (Institut de recherche pour le développement) qui vient de publier une étude sur le caractère « invasif de cette espèce » dans la revue « Emerging Microbes & Infections - Nature ».

Pour ce spécialiste des écosystèmes aquatiques, « il faut agir vite », car ce pirate d'eau douce est en train de coloniser nos rivières à toute allure. La première fois que ce goujon, originaire de Chine, a été repéré en France, c'était en 1980, dans la Sarthe, affluent de la Loire. Des années auparavant, il avait été sans doute importé en Europe accidentellement, au milieu de carpes chinoises commandées par une ferme aquacole. « Depuis, il s'est installé dans la Loire, le Rhône, le Rhin et dernièrement la Garonne, car c'est une espèce qui se reproduit vite explique le chercheur. Ce poisson a une autre caractéristique : c'est un athlète hors pair capable de s'adapter à toutes les températures, en eau douce mais aussi en eau salée.

Un athlète hors pair capable de s'adapter à toutes les températures

« En Turquie, il a décimé, en l'espace de trois ans, des espèces marines comme le bar jusqu'à quasi-extinction. C'est inquiétant pour le saumon breton », souligne l'expert. Son parasite, appelé *Sphaerothecum* ou agent rosette, qui se faufile dans le système vasculaire des poissons pour empoisonner leurs organes, est presque aussi robuste, résistant à des températures allant de + 4 °C à + 35 °C. Particulièrement tenace, il libère dans l'eau, une fois sa proie morte, des spores qui iront en coloniser d'autres victimes.

Au Royaume-Uni, où la pêche en eau douce est le sport national, cette redoutable association de malfaiteurs a provoqué l'émoi. Remontant même jusqu'au 10 Downing Street et au Parlement dès 2005. « Là-bas, ils ont opté pour l'éradication : ils ferment le lac, abaissent le niveau, mettent à l'abri les autres espèces, puis empoisonnent l'eau avec un insecticide naturel, puis remettent en eau une fois le goujon et son parasite disparus », explique Rodolphe Gozlan. En France, alerté par son étude, l'Onema (l'Office national de l'eau et des milieux aquatiques) vient de décider se pencher sur le cas de ce flibustier hors normes.

ALINE GÉRARD

Originaire de Chine, le goujon asiatique, qui ne mesure que 10 cm, a été repéré pour la première fois en France dans la Sarthe en 1980.

Le Goujon Asiatique: Attention Danger ***Publié le 3 octobre 2010 par APASMC***
The Asian Stud: Danger Warning ***Published on October 3, 2010 by APASMC***

Asian gudgeon bring new terror to rivers

February 9, 2016

Pseudorasbora parva. Credit: IRD / R. Gozlan

Pseudorasbora parva

- **Pseudorasbora parva** are Small in size but significant in terms of the ecological and economic damage they cause, Asian gudgeon are invading a great number of water courses across the world, particularly in Europe.
- Three years after the arrival from China more than 50 years ago, **Pseudorasbora parva** caused devastation in the rivers of Europe and North Africa.
- **Pseudorasbora parva** has successfully colonised various aquatic environments due to its highly efficient strategy for reproduction.
- **Pseudorasbora parva** *is* a healthy carrier of the fungal parasite, **Sphaerothecum destruens** which has very likely been present in China for millions of years and which is fatal to most other fish species.
- **Pseudorasbora parva** is propagating a devastating mycosis, caused by **Sphaerothecum destruens**, a cousin of the well-known chytrid fungus, which has decimated frogs and toads throughout the world over the last few decades.

Sphaerothecum destruens

- ***Sphaerothecum destruens*** is a unicellular eukaryotic parasite of fish which has caused disease and mortalities up to 80% in north American Chinook salmon Oncorhynchus tshawytscha (Walbaum) and chronic mortalities in cultured salmon Salmo salar (L.) (Elston et al. 1986, Harrell et al. 1986, Hedrick et al. 1989, Arkush et al. 1998).
- **Sphaerothecum destruens** is a multi-host parasite which can infect and cause mortality in a number of fish species including
    - Chinook salmon Oncorhynchus tshawytscha,
    - Atlantic salmon S. salar

- Coho salmon O. kisutch (Walbaum),
- Rainbow trout O. mykiss (Walbaum),
- Brown trout Salmo trutta and
- Brook trout Salvelinus fontinalis (Mitchill) (Arkush et al. 1998).
- Sunbleak Leucaspius delineatus (Heckel),
- Fathead minnow Pimephales promelas (Rafinesque)
- Bream Abramis brama (L.) (Andreou 2010).

- **Sphaerothecum destruens** is able to infect multiple organs (kidney, liver, gill, gonad and intestine).

- **The pathology**
  was described in detail for Oncorhynchus tshawytscha and Salmo salar (Arkush et al. 1998), includes 2 forms of host response:
  - **disseminated form**
    - S. destruens spores and developmental stages are widely dispersed throughout the host with little apparent host cell response (Elston et al. 1986).
  - **nodular form**
    - is characterised by a chronic inflammatory response with the formation of distinct granulomas in visceral organs (Hedrick et al. 1989, Arkush et al. 1998).
- Pathology can differ in hosts belonging to different families (Arkush et al. 1998) and the potential for misdiagnosis exists.

- **Diagnosis** is based on
  - pathogen identification
  - thorough descriptions of histopathology
  - A quantitative polymerase chain reaction was developed in order to quantify S. destruens' infection levels.

- **Experimental infections**
  - are a powerful tool for determining whether new hostparasite associations are plausible (Poulin, 2007) and can be used to predict possible parasite impacts on naive hosts:
  - Following intraperitoneal injection with S. destruens spores, infection was achieved in coho salmon O. kisutch (Walbaum), rainbow O. mykiss, (Walbaum), brown S. trutta (Linnaeus) and brook trout Salvelinus fontinalis (Mitchill) Arkush et al., 1998).
  - Infection severity varied, with O. kisutch becoming heavily infected whilst the role of O. mykiss, S. trutta, and S. fontinalis as potential healthy carriers of the disease was highlighted (Arkush et al., 1998).

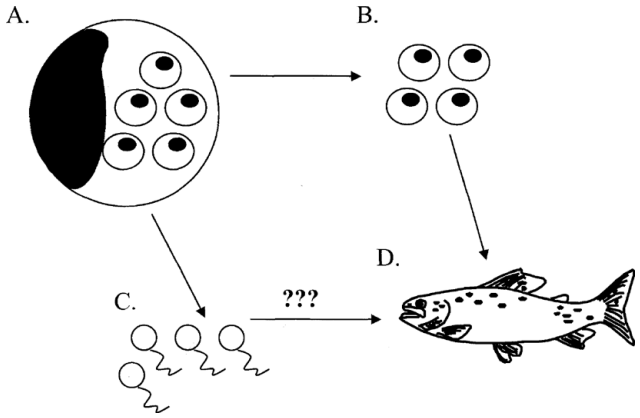
- **Natural infections**
  - Occurred in two salmonid species, O. tshawytscha and S. salar support the low host specificity of S. destruens.

- The wide range o f host susceptibility revealed by the experimental infection using salmonids (Arkush et al., 1998) suggests that in addition to L. delineatus other cyprinids could be potential hosts.
- S. destruens prevalence was 98 % for O. kisutch, 42.5 % for O. mykiss, 43.3 % for S. trutta and 2.6 for S. fontinalis (Arkush et al., 1998).

- **Sphaerothecum destruens' life stages** include
  - Spherical intra-cytoplasmic spore stages of two distinct morphotypes, 2-4 pm and 4-6 pm in diameter
  - Spores replicate asexually through fission and can infect epithelial, mesenchymal, and hematopoietic cells, eventually causing cell death (Arkush et al., 1998).
  - S. destruens can infect the gills, heart, brain, kidney, liver, spleen, swim bladder, ovary, testis and the hind gut (Arkush et al., 1998).
  - It is most often detected in the kidney and upon release; the spores can infect further tissues or be excreted through the bile, urine, gut epithelium, and seminal and ovarian fluids (Arkush et a!., 2003).
  - Fish infection is believed to occur through either ingestion and gut penetration, or attachment to the gills or skin, or both (Arkush et al., 2003).
  - When incubated in freshwater, spores undergo zoosporulation and release a minimum of five motile uniflagellate zoospores.
    - Zoospores have an average body diameter and flagellum length o f 2 pm and 10 pm, respectively (Arkush et al., 2003). Although the spore stage of S. destruens is directly infectious, the zoospore stage has not yet been shown to be directly infectious (Arkush et al

Proposed life cycle of Sphaerothecum destruens adapted from Arkush et al. (2003).

- **S. destruens spores** infect cells and replicate sequentially through asexual division, eventually causing the host cell to erupt releasing spores
- Released spores can release flagellated zoospores when incubated in sterile distilled water
- Released spores can directly infect new host fish
- Infection of new host fish by zoospores has not been demonstrated.
- Infected hosts can release spores via urine, bile, gut epithelium or reproductive fluids



Proposed life cycle of the mesomycetozoean *Sphaerothecum destruens* n. g., n. sp. (**A**) Spores infect cells of CHSE-214 cell cultures or fish tissues, replicating intracytoplasmically by sequential asexual division until host cell erupts. (**B**) If transferred to cell culture media, phosphate-buffered saline (PBS) or sterile artificial seawater, spores may remain temporarily viable but do not divide or zoosporulate. (**C**) However, when infected cells of CHSE-214 cell cultures or fish tissues are placed in sterile distilled water, the spores undergo zoosporulation. (**D**) Spores are released from infected fish via urine, bile, gut epithelium or reproductive fluids and infect new host fish. Transmission of the zoospore stage has not been demonstrated. **Arkush *et al*. (2004)**

The cell wall of S. destruens is comprised of three well defined layers;

- an outer layer having a membraneous structure, has fibrogranular material adherent to its surface ((Arkush et al.,
- a middle electron dense layer and
- an inner electro lucent layer (Elston et al., 1986).

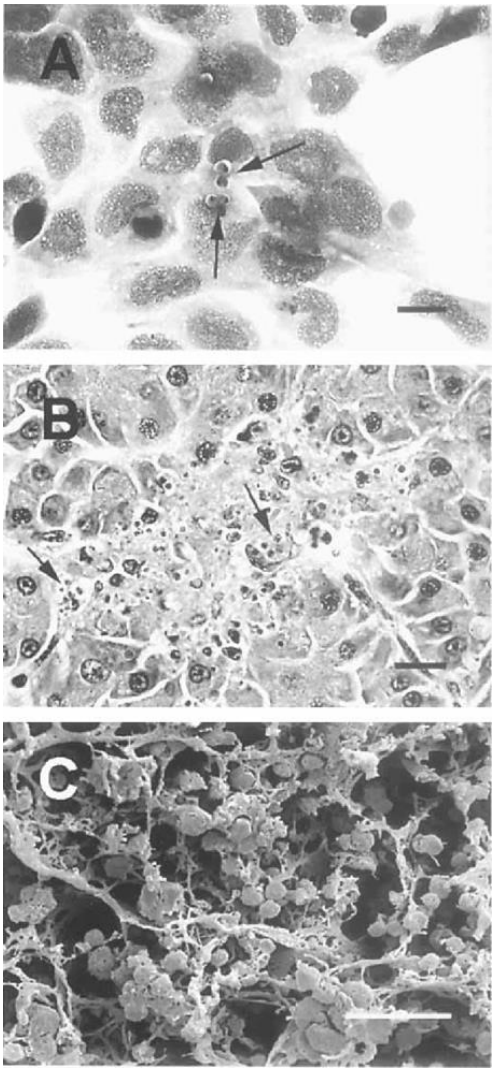
Reports:

Arkush *et al.* (1998) observed mortality and morbidity among 1–5-year-old captive broodstock of Sacramento River winter-run chinook salmon *Oncorhynchus tshawytscha* that had been reared in seawater and were infected with the systemic protist termed the "rosette agent." Two types of lesions were found in naturally occurring infections. The first was disseminated and was characterized by systemic dispersion of parasites accompanied by minimal host inflammatory cell response, whereas the second was limited and nodular with parasites restricted to granulomas in the kidney, spleen, and liver. In the disseminated form of the disease, the parasite was detected within hematopoietic, epithelial, and mesenchymal cell types. Aggregates of the organism and associated cellular debris were found in the kidney, liver, spleen, heart, gill, brain, ovary, testis, and hindgut. Renal tubular necrosis, membranous glomerulonephritis, necrotizing interstitial nephritis, multifocal hepatocellular necrosis, and necrotizing vasculitis were evident. In the nodular form of the disease, multifocal granulomas were identified in the kidney, liver, and spleen. Parasites ranged 2–6 μm in diameter in both disease presentations and were strongly periodic acid–Schiff (PAS) positive, argyrophilic, basophilic following Giemsa staining, and acid-fast negative. Transmission electron microscopy revealed that the parasite was surrounded by a trilaminar cell wall and had a ribosome-laden cytoplasm with scattered segments of rough endoplasmic reticulum, vesicular mitochondria, and a single nucleus. Variable numbers of electron-dense granules and lipid droplets were present in the cytoplasm, and solitary concentric bodies were identified in some of the organisms. The agent was isolated from kidney tissue of a naturally infected chinook salmon and was propagated in the chinook salmon embryo cell line (CHSE-214). Parasites from these cultures were injected at a dose of 1.6×10^7 organisms per fish into chinook salmon, coho salmon *O. kisutch*, rainbow trout *O. mykiss*, brown trout *Salmo trutta*, and brook trout *Salvelinus fontinalis*. At 3 and 6 months postinfection, chinook salmon and coho salmon were most heavily infected, followed by rainbow trout and brown trout. Few parasites were detected in brook trout. Evidence from natural outbreaks and experimental infections of chinook salmon and coho salmon suggests that the rosette agent is a significant pathogen of at least two

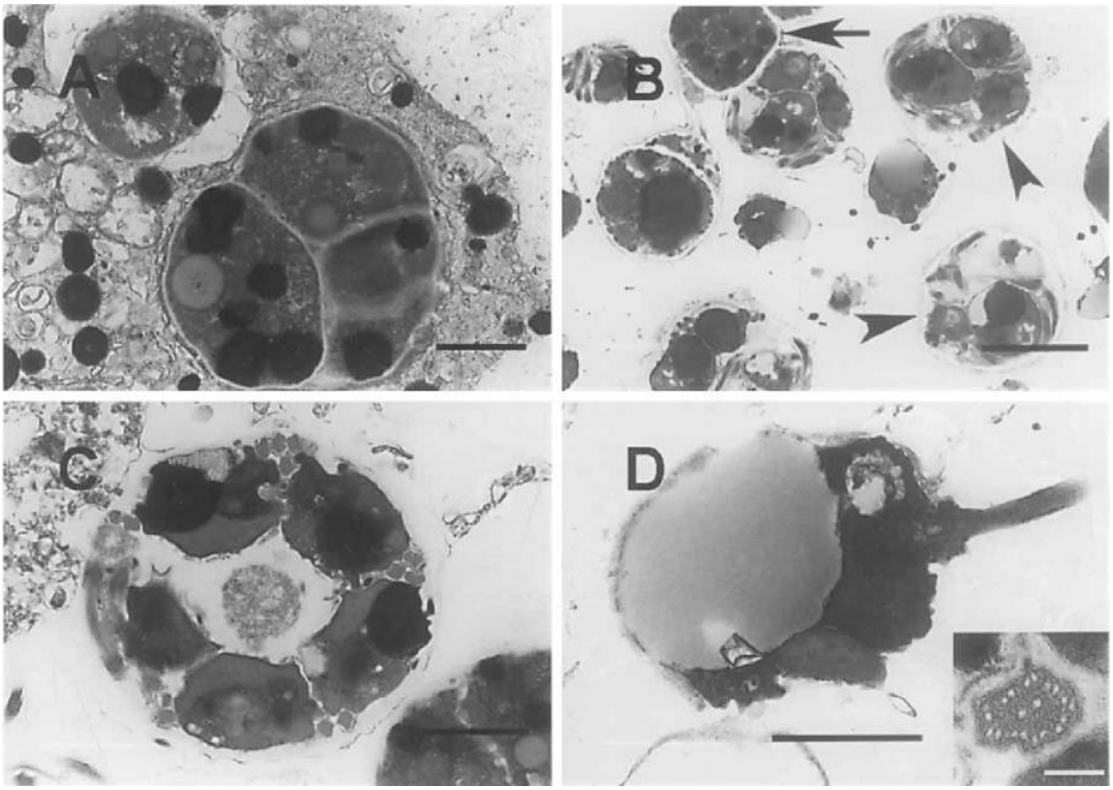
salmon species. Trout appear to be more resistant, but their potential role as carriers of the pathogen remains unknown.

Gozlan et al. (2000) showed that the emerging rosette-like agent was Sphaerothecum destruens, originally found to be responsible for disease outbreaks in salmon in the United States. Sequencing of the ribosomal internal transcribed spacer (ITS) DNA highlights some level of geographical isolation. Unlike the situation in the United States, its occurrence in invasive fishes presented a risk of spread from wild invasive populations to sympatric populations of susceptible native fish and as such represented a risk for fisheries, as movement of fish for stocking purposes is common practice.

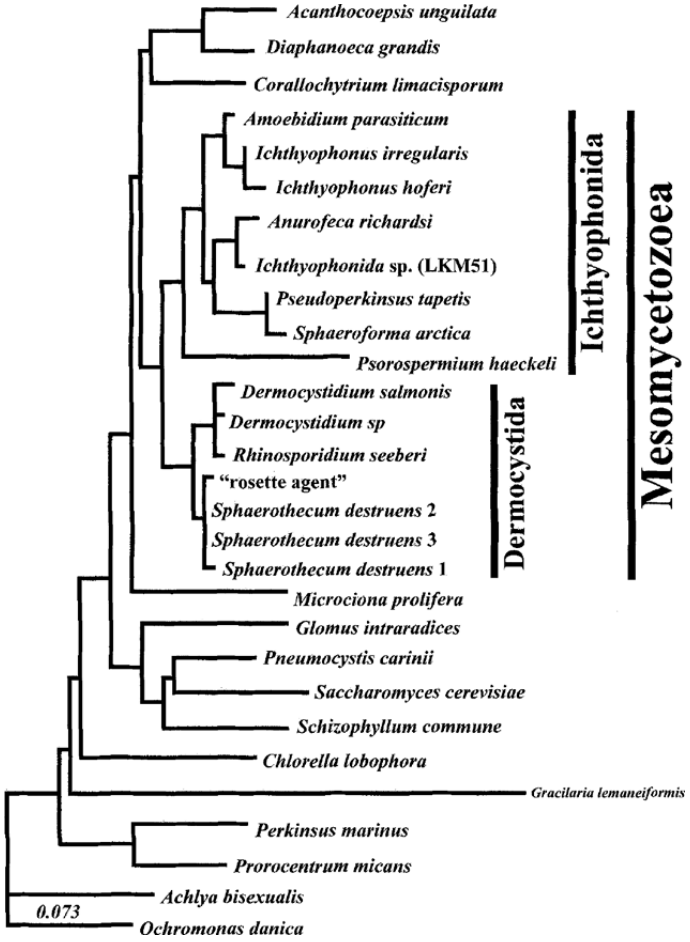
Arkush *et al*. (2004) stated that the rosette agent is an obligate intracellular parasite that causes morbidity and mortality in salmonid fish. In laboratory cultures, the spore stage (2-6 microm diam.) replicates in a salmonid cell line by sequential asexual division, giving rise to daughter cells. If infected cell cultures are transferred to distilled water, the spore stage undergoes internal division to give rise to at least 5 cells each of which develops into a uniflagellated zoospore with a body of approximately 2 microm and a flagellum approximately 10 microm long. Zoosporulation does not occur in cell culture medium alone, artificial seawater, or phosphate-buffered saline. This parasite is currently classified as a member of the Class Mesomycetozoea (formerly Ichthyosporea) based on phylogenetic analyses of the small subunit ribosomal DNA of three different isolates from fish. Given these new morphological observations combined with the available molecular phylogenetic data on other mesomycetozoeans, It was proposed to classify the rosette agent as Sphaerothecum destruens, n. g., n. sp. This new genus has unique features including (1) intracellular development of spore stages in various organs eliciting a host granulomatous response; and (2) the differentiation of mature spores into multiple, flagellated zoospores. Taken together, these characteristics clearly distinguish it from the closely related genera Dermocystidium and Rhinosporidium.



Light micrographs of cells and tissues infected by the me-somycetozoean*Sphaerothecum destruens* n. g., n. sp. (RA-3) depicting (**A**) May-Grünwald Giemsa-stained CHSE-214 culture infected with *Sphaerothecum destruens*spores (arrows). Bar =10 μm; (**B**) *S. destruens* spores (arrows) in liver of naturally infected winter-run Chinook salmon. Bar = 20 μm, and (**C**) scanning electron micrograph depicting masses of *S. destruens* spores in the tissue of a naturally infected Chinook salmon. Bar = 10 μm. **Arkush *et al*. (2004)**



Transmission electron micrographs depicting various stages of the mesomycetozoean *Sphaerothecum destruens* n. g.,n. sp. (RA-3). (**A**) Divisional stage of *S. destruens* in tissue of naturally infected winter-run Chinook salmon. Bar = 2 μm; (b)-(d) *S. destruens* from in vitro cultures placed in distilled water; (**B**) both spore stage (arrow) and spore containing zoospores (arrowheads) evident. Bar = 2 μm; (**C**) 5 zoospores seen within a spore. Bar = 1 μm; (**D**) zoospore stage. Bar = lμm, inset demonstrates 9 + 2 microtubular pattern in flagellum. Bar = 50 nm. **Arkush *et al*. (2004)**



Phylogenetic relationships between several eukaryotic SSU-rDNA sequences including all meso-mycetozoeans and three isolates of*Sphaerothecum destruens* (1 = AY267344, 2 = AY267346, 3 = AY267345). The tree was made by neighbor joining in PAUP using distance estimated by maximum likelihood. The scale for the percent nu-cleotide substitution per nucleotide is given on the branch of *Ochromonas danica* used in this study as an out-group along with *Achlya bisexualis*. **Arkush *et al.* (2004)**

Mendonca and Arkush (2004) developed single-round and nested polymerase chain reaction (PCR) tests for amplification of a 434 bp fragment of the small subunit ribosomal RNA (18S rRNA) gene from Sphaerothecum destruens, previously known as the rosette agent, an intracellular parasite of salmonid fishes. Both tests have successfully amplified S. destruens-specific DNA from different isolates of S. destruens but not from related organisms. The limits of detection using the nested PCR test were 1 pg for purified S. destruens genomic DNA and 0.1 fg for plasmid DNA. We conducted 2 experimental transmission studies, consisting of injection or waterborne exposure of juvenile winter-run Chinook salmon Oncorhynchus tshawytscha to spore stages of the parasite. In the injection study, parasite DNA was detected in 100% of kidney samples from exposed fish (n = 83) at 1 and 3 mo post-exposure using nested PCR, versus 98% using microscopic analysis of Gram-stained impression smears made from the kidney. Following waterborne exposure, fish were sampled over the course of a year. From each fish, samples of gill, liver, posterior intestine and kidney were analyzed. S. destruens-specific DNA was detected most often in gill and kidney over the course of the experiment, and 71% (64/90) of the exposed fish were identified as positive for S. destruens using the nested PCR test, versus 16% (14/90) using microscopic analysis of Gram-stained kidney smears. Natural infections in captive broodstock of adult winter-run Chinook salmon,

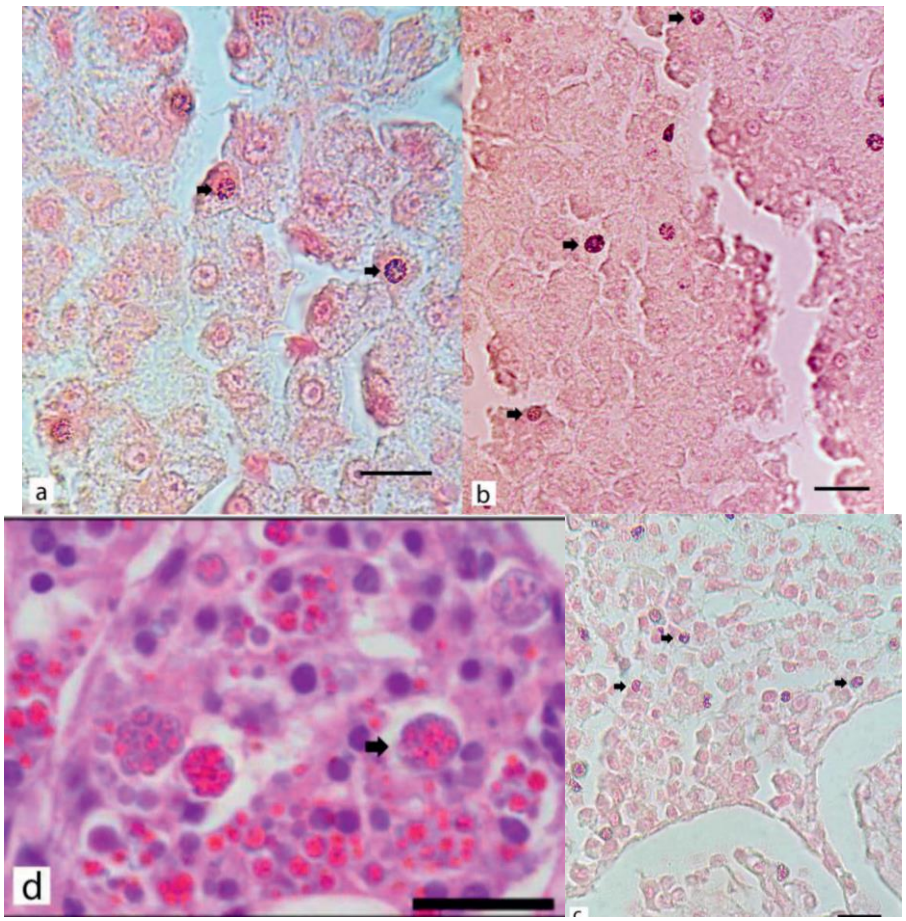
originally diagnosed by examination of Gram-stained kidney smears, were confirmed using the nested PCR test in all fish examined (15/15). Further, the nested test amplified parasite-specific DNA from other tissues in these fish with varying frequencies. This report introduces the first DNA-based detection method for S. destruens, to be used alone as a diagnostic tool or in conjunction with histologic tests for confirmatory identification of the parasit

Gozlan *et al.* (2009) showed that the emerging rosette-like agent is Sphaerothecum destruens, originally found to be responsible for disease outbreaks in salmon in the United States. Sequencing of the ribosomal internal transcribed spacer (ITS) DNA highlights some level of geographical isolation. Unlike the situation in the United States, its occurrence in invasive fishes presents a risk of spread from wild invasive populations to sympatric populations of susceptible native fish and as such represents a risk for fisheries, as movement of fish for stocking purposes is common practice.

Paley *et al.* (2010) stated that Sunbleak (*Leucaspius delineatus*), a cyprinid fish native to continental Europe is experiencing population decline which appeared to be linked to the spread of the invasive Asian cyprinid (*Pseudorasbora parva*). Species interaction studies showed inhibition of spawning, wasting then death in *L. delineatus* cohabited with *P. parva*, or exposed to their holding water (Gozlan *et al*. 2005). Histological examination lead to the identification of an intracellular parasite, similar to the freshwater Mesomycetozoean parasite, Rosette agent (S*phaerothecum destruens*) that infects salmonids in the USA. Subsequent PCR and sequence analysis of a partial 18S rRNA gene demonstrated 100% homology. *S. destruens* is capable of survival in fish in the marine environment and has been associated with sporadic severe infectious disease (occasionally mortalities up to 90%) of cage-reared Chinook salmon (*Oncorhynchus tshawytscha*) in North America (Elston *et al.* 1986; Arkush *et al.* 1998) and in farmed Atlantic salmon (*Salmo salar*) in freshwater in California (Hedrick *et al.* 1989). In the US the disease is usually chronic and does not appear to impair spawning of infected fish. Information on the impact on wild stocks is extremely limited. This is the first identification of this parasite in the UK and from a cyprinid. Given the potential for causing severe disease we have developed cell-culture of the sunbleak rosette agent for use in pathogenicity studies. Sunbleak rosette agent spores are infective to EPC, CHSE and FHM cells replicating most rapidly in EPC cells. Spores can be induced to zoosporulate in water forming motile uni-flagellated zoospores in a temperature dependant manner. Challenge experiments indicated the spores, when injected intraperitonealy, are able to replicate and disperse in Atlantic salmon and sunbleak and contribute to significant mortality.

Spikmans *et al.* (2013) studied the prevalence of Sphaerothecum destruens, a pathogenic parasite, in two wild populations of topmouth gudgeon (Pseudorasbora parva), an invasive freshwater fish non-native to the Netherlands. Using genetic markers and sequencing of the 18S rRNA gene, we showed the prevalence of this parasite to be 67 to 74%. Phylogenetic analysis demonstrated a high similarity with known sequences of S. destruens. The topmouth gudgeon, which functions as a healthy carrier of the pathogen, is rapidly colonizing the Netherlands, its expansion showing no signs of saturation yet. Both the presence of S. destruens and the rapid dispersal of the topmouth gudgeon are considered to constitute a high risk for native freshwater fish.

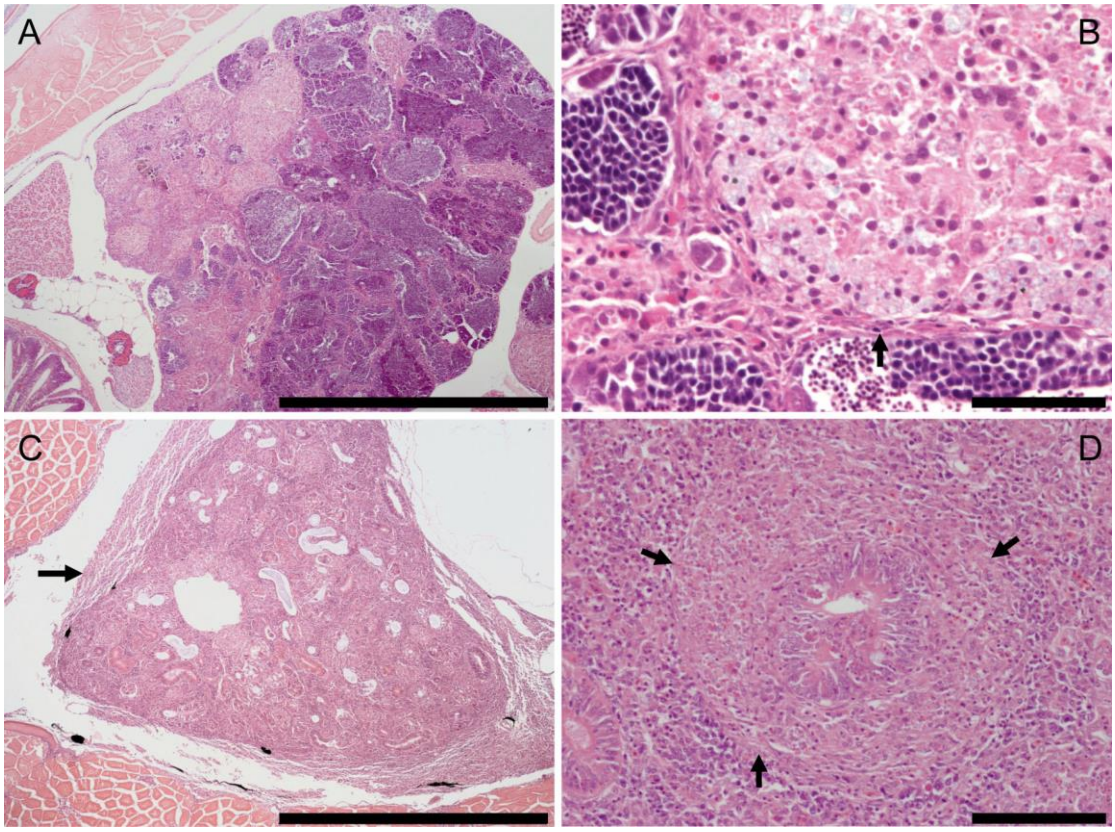
Ercan (2015) reported that recent years have seen a global and rapid resurgence of fungal diseases with direct impact on biodiversity and local extinctions of amphibian, coral, or bat populations. Despite similar evidence of population extinction in European fish populations and the associated risk of food aquaculture due to the emerging rosette agent *Sphaerothecum destruens*, an emerging infectious eukaryotic intracellular pathogen on the fungal–animal boundary, our understanding of current threats remained limited. Long-term monitoring of population decline for the 8-year post-introduction of the fungal pathogen was coupled with seasonal molecular analyses of the 18S rDNA and histological work of native fish species organs. A phylogenetic relationship between the existing EU and US strains using the ribosomal internal transcribed spacer sequences was also carried out. Here, we provide evidence that this emerging parasite has now been introduced via *Pseudorasbora parva* to sea bass farms, an industry that represents over 400 M€ annually in a Mediterranean region that is already economically vulnerable. Evidence was also provided for the first time linking *S. destruens*to disease and severe declines in International Union for Conservation of Nature threatened European endemic freshwater fishes (i.e. 80% to 90 %mortalities). Our findings are thus of major economic and conservation importance.



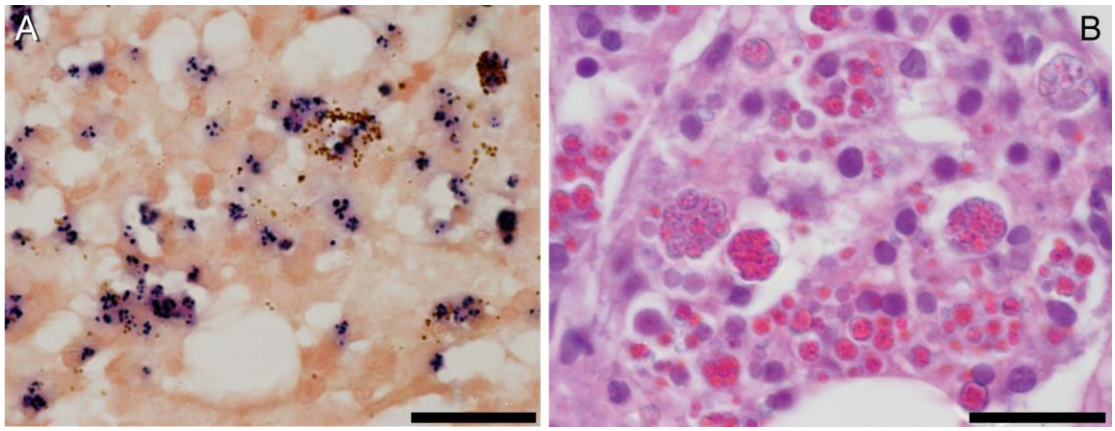
High power micrograph of a section of a) P. parva liver, b) D. labrax liver, c) S. felowesi kidney and d) L. delineatus liver for reference (Andreou 2010). Arrow indicates Sphareothecum destruens spores. Slides stained with haematoxylin and eosin. Scale bars 20 mm. Reference: Andreou D. (2010) Sphareothecum destruens: Life history and host range. Thesis Cardif University **Ercan (2015)**

Andreou *et al.* (2011) described the associated histopathology of S. destruens infection along with its pathogenesis in the endangered cyprinid sunbleak Leucaspius

delineatus. Histological examination of 100 L. delineatus in a wild population in the south of England revealed the presence of S. destruens infections, with a prevalence of 5% with S. destruens, suggesting an over-dispersed distribution within the L. delineatus sample. Clinical signs of the infection were absent, but histological examination revealed the presence of both disseminated and nodular lesions in several organs.

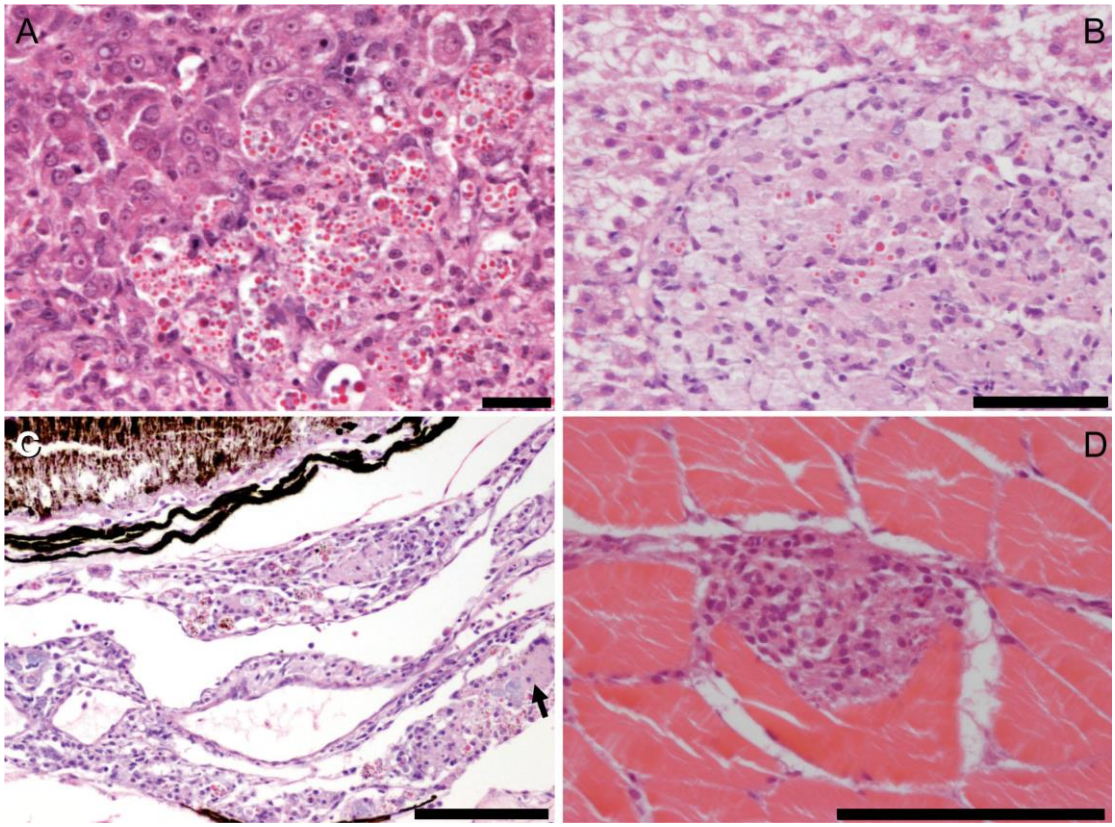


Leucaspius delineatus. Light micrographs of tissue sections stained with haematoxylin and eosin from L. delineatus naturally infected with Sphaerothecum destruens. (A) Low magnification view of testis showing localised, multiple granulomas of different sizes. Scale bar = 1 mm. (B) High magnification view of a granuloma in the testis. The granuloma is surrounded by a thin fibroblast layer (arrow). Within the granuloma there are numerous stages of S. destruens, cell necrosis, numerous 'ghost' (unstained dead) parasites, and macrophages. Scale bar = 50 µm. (C) Low magnification view of kidney. Note inflammation around the organ periphery (arrow). Scale bar = 1 mm. (D) Intense inflammation surrounding a kidney tubule (arrows). Clusters of S. destruens are present within tubular epithelial cells. Scale bar = 100 µm **Andreou *et al.* (2011)**

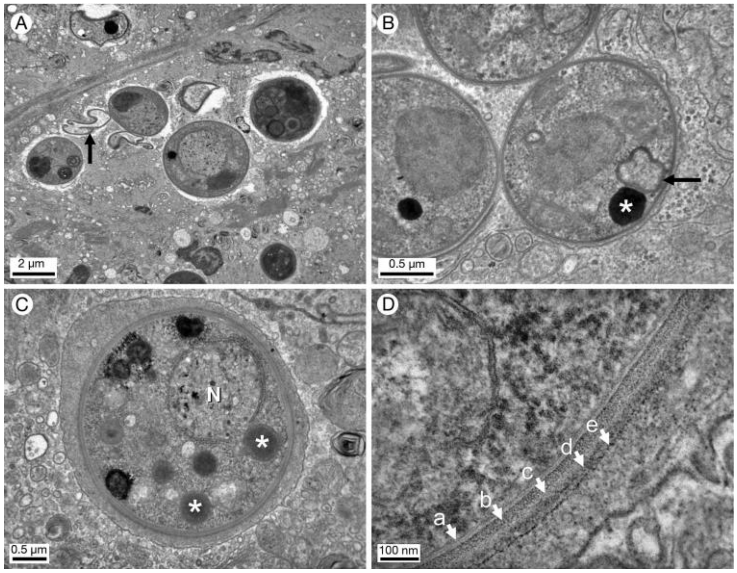


Leucaspius delineatus. Light micrographs of tissue sections stained with (A) Gram's stain and (B)

haematoxylin and eosin. (A) Liver tissue showing numerous Gram-positive granules within Sphaerothecum destruens cells. Scale bar = 20 µm. (B) High magnification view showing intracellular and extracellular S. destruens rosettes of different sizes. Scale bar = 20 µm **Andreou *et al.* (2011)**



Leucaspius delineatus. Light micrographs of tissue sections stained with haematoxylin and eosin from L. delineatus naturally infected with Sphaerothecum destruens. (A) Hepatic lesion associated with numerous S. destruens spores. Host response involving phagocytic cells infiltrating into the hepatic parenchyma and frequently containing spores. Scale bar = 50 µm. (B) Enlarged macrophage aggregation in the liver containing moderate numbers of S. destruens. Scale bar = 50 µm. (C) S. destruens in the connective tissue and vessels posterior to the retina. S. destruens cells associated with melanomacrophages and giant cells (arrow). Scale bar = 100 µm. (D) Small focus of inflammatory tissue associated with S. destruens cells between muscle fibres. Scale bar = 100 µm **Andreou *et al.* (2011)**



Leucaspius delineatus. Electron micrographs of tissue infected with Sphaerothecum destruens. (A)

Intracellular stages of S. destruens in the granulomatous tissue of sunbleak kidney. Note the presence of necrotic S. destruens with loss of cellular contents and folding of the cell wall (arrow). Scale bar = 2 µm. (B) Cluster of 3 S. destruens spores showing the characteristic granular cytoplasm with densely osmiophilic structures (*) and vesicular structure (arrow). Scale bar = 0.5 µm. (C) An isolated S. destruens spore located intracellularly within a phagocyte. The nucleus (N) in this case is pale-staining with conspicuous electron-dense granules. Note the presence of multiple lipoid inclusions (*) and membrane-bound vesicular structures. Scale bar = 0.5 µm. (D) High-power view of the spore wall of S. destruens. Inner trilaminar plasma membrane (a) coated by a dense finely granular layer (b) and separated from the host cell's cytoplasm by an intermediate amorphous region (c) and another electron-dense layer (d) with a further membrane that appears to be of host cell origin (e). Scale bar = 100 nm **Andreou *et al.* (2011)**

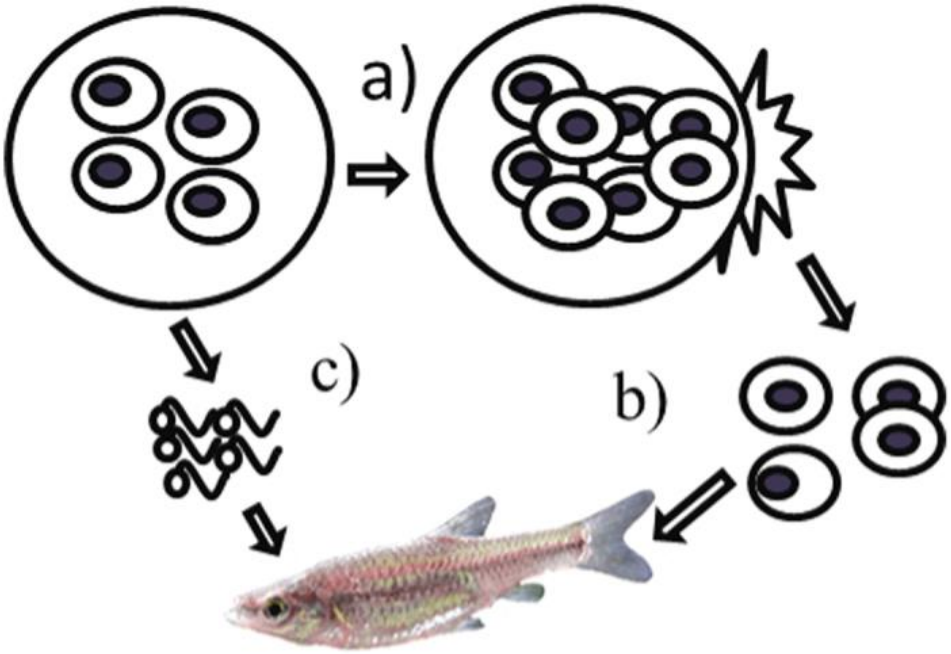
Andreou *et al.* (2011b) investigated the influence of L. delineatus's reproductive state on the prevalence and infection level of S. destruens. A novel real time quantitative polymerarse chain reaction (qPCR) was developed to determine S. destruens' prevalence and infection level. These parameters were quantified and compared in reproductive and non-reproductive L. delineatus. The detection limit of the S. destruens specific qPCR was determined to be 1 pg of purified S. destruens genomic DNA. Following cohabitation in the lab, reproductive L. delineatus had a significantly higher S. destruens prevalence ($P<0.05$) and infection levels ($P<0.01$) compared to non-reproductive L. delineatus. S. destruens prevalence was 19% (n=40) in non-reproductive L. delineatus and 41% (n=32) in reproductive L. delineatus. However, there was no difference in S. destruens prevalence in reproductive and non-reproductive fish under field conditions. Mean infection levels were 18 and 99 pg S. destruens DNA per 250 ng L. delineatus DNA for non-reproductive and reproductive L. delineatus respectively. The present work indicates that S. destruens infection in L. delineatus can be influenced by the latter's reproductive state and provides further support for the potential adverse impact of S. destruens on the conservation of L. delineatus populations.

Andreou *et al.* (2012) showed that the emerging S. destruens is also a threat to a wider range of freshwater fish than originally suspected such as bream, common carp, and roach. This is a true generalist as an analysis of susceptible hosts shows that S. destruens is not limited to a phylogenetically narrow host spectrum. This disease agent is a threat to fish biodiversity as it can amplify within multiple hosts and cause high mortalities.

Paley *et al.* (2012) established laboratory cultures of S. destruens from sunbleak in the UK and used these cultures in challenge experiments to determine if the UK isolate of S. destruens from cyprinid species is a potential threat to Atlantic salmon (Salmo salar). The first isolation and culture of S. destruens in the UK and from a cyprinid species was described. Cultured S. destruens spores from sunbleak were infective to EPC, CHSE and FHM cells, replicating most rapidly in FHM and EPC cells. Spores could be induced to zoosporulate in water forming motile, uni-flagellated zoospores. Challenge experiments indicated the spores were able to replicate and disperse in Atlantic salmon and were associated with increased mortality (up to 90%) when injected intraperitonealy.

Al-Shorbaji *et al.* (2015) obtained tractable data on infectivity and pathogen life cycle for the first time. Here, based on the outcomes of a set of infectious trials and combined with an epidemiological model, they showed a high level of dependence on direct transmission in crowded, confined environments and establish that incubation rate and length of infection dictate the epidemic dynamics of fungal disease. The

spread of Mesomycetozoea in the wild raise ecological concerns for a range of susceptible species including birds, amphibians and mammals.Their results shed light on the risks associated with farming conditions and highlight the additional risk posed by invasive species that are highly abundant and can act as infectious reservoir hosts.



Lifecycle of *Sphaerothecum destruens*

a) Spores multiply within host cells until cell death; **b**) Spores spread within the host and are released into the water through urine, bile, or gut epithelium; **c**) In freshwater, each spore can divide into up to 5 uniflagellate zoospores and survive for several days depending on the water temperature. Infection occurs directly or indirectly by ingesting the spores, attachment to the gills or skin, or gut penetration. **Photo R. E. Gozlan**

Ercan et al. (2015) reported that recent years have seen a global and rapid resurgence of fungal diseases with direct impact on biodiversity and local extinctions of amphibian, coral, or bat populations. Despite similar evidence of population extinction in European fish populations and the associated risk of food aquaculture due to the emerging rosette agent Sphaerothecum destruens, an emerging infectious eukaryotic intracellular pathogen on the fungal-animal boundary, our understanding of current threats remained limited. Long-term monitoring of population decline for the 8-year post-introduction of the fungal pathogen was coupled with seasonal molecular analyses of the 18S rDNA and histological work of native fish species organs. A phylogenetic relationship between the existing EU and US strains using the ribosomal internal transcribed spacer sequences was also carried out. They provided evidence that this emerging parasite has now been introduced via Pseudorasbora parva to sea bass farms, an industry that represents over 400 M□€ annually in a Mediterranean region that is already economically vulnerable. They also provided for the first time evidence linking S. destruens to disease and severe declines in International Union for Conservation of Nature threatened European endemic freshwater fishes (i.e. 80% to 90 % mortalities). These findings are thus of major economic and conservation importance

Andreou and Gozlan (2016) mentioned that the rosette agent *Sphaerothecum destruens* is a novel pathogen, which is currently believed to have been introduced into Europe along with the introduction of the invasive fish topmouth

gudgeon *Pseudorasbora parva* (Temminck & Schlegel, 1846). Its close association with *P. parva* and its wide host species range and associated host mortalities, highlight this parasite as a potential source of disease emergence in European fish species. Here, using a meta-analysis of the reported *S. destruens* prevalence across all reported susceptible hosts species; they calculated host-specificity providing support that *S. destruens* is a true generalist. They have applied all the available information on *S. destruens* and host-range to an established framework for risk-assessing non-native parasites to evaluate the risks posed by *S. destruens* and discuss the next steps to manage and prevent disease emergence of this generalist parasite.

References:

1. Al-Shorbaji FN, Gozlan RE, Roche B, Robert Britton J, Andreou D. The alternate role of direct and environmental transmission in fungal infectious disease in wildlife: threats for biodiversity conservation. *Scientific Reports*. 2015;5:10368. doi:10.1038/srep10368.
2. Andreou, D., Gozlan, R. E., and Paley, R. (2009). Temperature influence on production and longevity of *Sphaerothecum destruens*zoospores. *J. Parasitol*. 95, 1539–1541. doi: 10.1645/GE-2124.1
3. Andreou D (2010) Sphaerothecum destruens: life history traits and host range. PhD dissertation, Cardiff University, Cardiff Andreou D, Gozlan RE, Paley R (2009)
4. Andreou, D., R. E. Gozlan , D. Stone , P. Martin, K. Bateman , S. W. Feist. Sphaerothecum destruens pathology in cyprinids.Dis. Aquat Org. Vol. 95: 145–151, 2011
5. Andreou D, Hussey M, Griffiths SW, Gozlan RE. Influence of host reproductive state on Sphaerothecum destruens prevalence and infection level. Parasitology. 2011b Jan;138(1):26-34.
6. Andreou, D., Arkush, K. D., Guégan, J.-F., and Gozlan, R. E. (2012). Introduced pathogens and native freshwater biodiversity: a case study of *Sphaerothecum destruens*. *PLoS ONE* 7:e36998. doi: 10.1371/journal.pone.0036998
7. Andreou, D, Gozlan, R. E. Associated disease risk from the introduced generalist pathogen *Sphaerothecum destruens*: management and policy implications. *Parasitology*. 2016;143(9):1204-1210. doi:10.1017/S003118201600072X.
8. Arkush, K. D., Frasca, S., and Hedrick, R. P. (1998). Pathology associated with the Rosette Agent, a systemic protist infecting salmonid fishes. *J. Aquat. Anim. Health* 10, 1–11.
9. Arkush KD, Mendoza L, Adkison MA, Hedrick RP. Observations on the life stages of Sphaerothecum destruens n. g., n. sp., a mesomycetozoean fish pathogen formerly referred to as the rosette agent [correction]. J Eukaryot Microbiol. 2004 Mar-Apr;51(2):259
10. Ercan D , Andreou D, Sana S, Öntaş C, Baba E, Top N, Karakuş U, Tarkan AS, Gozlan RE. Evidence of threat to European economy and biodiversity following the introduction of an alien pathogen on the fungal-animal boundary. Emerg Microbes Infect. 2015 Sep 2;4:e52.
11. Ercan, Didem, Demetra Andreou, Salma Sana, Canan Öntaş, Esin Baba, Nildeniz Top, Uğur Karakuş, Ali Serhan Tarkan and Rodolphe Elie Gozlan Evidence of threat to European economy and biodiversity following the introduction of an alien pathogen on the fungal–animal boundary, *Emerging Microbes & Infections* (2015). DOI: 10.1038/emi.2015.52
12. Gozlan RE, Whipps CM, Andreou D, Arkush KD. Identification of a rosette-like agent as Sphaerothecum destruens, a multi-host fish pathogen. Int J Parasitol. 2009 Aug;39(10):1055-8.
13. Mendonca HL, Arkush KD. Development of PCR-based methods for detection of Sphaerothecum destruens in fish tissues. Dis Aquat Organ. 2004 Nov 4;61(3):187-97.
14. Paley R., D. Andreou, P.Martin, D. Stone, K. Bateman, S. Irving1 and S. Feist. 14th Annual Meeting of the National Reference Laboratories for Fish Diseases and Workshop on Use of Diagnostic kits for the Detection of Fish Diseases Aarhus, Denmark May 26-28, 2010
15. Paley RK, Andreou D, Bateman KS, Feist SW. Isolation and culture of Sphaerothecum destruens from Sunbleak (Leucaspius delineatus) in the UK and pathogenicity experiments in Atlantic salmon (Salmo salar). Parasitology. 2012 Jun;139(7):904-14.

16. Spikmans, F, Tomas van Tongeren, Theo A. van Alen, Gerard van der Velde and Huub J.M. *Op den Camp*. High prevalence of the parasite *Sphaerothecum destruens* in the invasive topmouth gudgeon*Pseudorasbora parva* in the Netherlands, a potential threat to native freshwater fish. Aquatic Invasions (2013) Volume 8, Issue 3: 319–332

7. Ichthyophonus

Ichthyophonus is a genus of unicellular parasites of fish. They were once considered to be fungi, but phylogenetic evidence suggests they are protists related to both fungi and animals.
Ichthyophonus has been placed taxonomically in the newly proposed **class Mesomycetozoea**, Kingdom Protista (Protoctista). Members of the Mesomycetozoea are believed to link fungi and animals evolutionarily.

Historical:

- Hofer, 1893, described *Ichthyophonus* sp. from brown trout *Salmo trutta* L., 1758, and brook trout *Salvelinus fontinalis* Mitchill, 1815, in Germany.
- Caullery and Mesnil (1905) included the fungus in the genus *Ichthyosporidium*
- Plehn & Mulsow (1911) identified it as a fungus and named it *Ichthyophonus hoferi*.
- Sindermann & Scatergood,1954; reported epizootics of *Ichthyophonus hoferi*. accompanied by economically important losses in *Clupea harengus* L., 1758
- Rucker & Gustafson, 1953, reported epizootics of *Ichthyophonus hoferi*.in *Oncorhynchus mykiss*

NCBI Taxonomy
Cellular organisms +

- Eukaryota +
  - Opisthokonta +
    - Opisthokonta incertae sedis +
      - Ichthyosporea +
        - Ichthyophonida +
          - Ichthyophonus +
            - ***Ichthyophonus hoferi***
            - *Ichthyophonus irregularis*
            - Ichthyophonus sp. A3
            - Ichthyophonus sp. D5
            - Ichthyophonus sp. ex Theragra chalcogramma
            - Ichthyophonus sp. JLG-2013a
            - Ichthyophonus sp. JLG-2013b
            - Ichthyophonus sp. JLG-2013c
            - Ichthyophonus sp. JLG-2013d
            - Ichthyophonus sp. JLG-2013e

Ichthyophonus Disease (**Paul K. Hershberger, 2012**).

Synonyms:

- Ichthyophoniasis
- Ichthyosporidium disease
- Ichthyosporidiosis

- Ichthyophonus infection is a systemic granulomatous disease caused by Ichthyophonus spp. A lack of distinguishing morphological characteristics and incomplete species descriptions of the causative agent have resulted in nomenclature inconsistencies within the genus; to avoid further confusion, the organism(s) should be referred to generically as Ichthyophonus until phylogenetic studies provide an objective basis for speciation (**Paul K. Hershberger, 2012**).
- Ichthyophonus infections is one of the most widespread diseases of fish (McVicar 1999, Kocan et al 2004, Marty et al 2010, Hershberger et al 2010)
- Ichthyophonus infections has been reported from cultured marine and freshwater species (Gustafson and Rucker 1956, Doriere and Degrange 1960, Erickson 1965, Miyazaki and Kubota 1977, Anonymous 1991, Athanassopoulu 1992, Franco-Sierra 1997, Gavryuseva 2007)
- Ichthyophonus infection has been periodically documented in free-ranging freshwater fishes (Schmidt-Posthaus & Wahli 2002).

Host Species

The host range of Ichthyophonus

- encompasses more than 80 fish hosts (Spanggaard et al 1994)
- includes 35 marine and 48 freshwater fishes (ReichenbachKlinke & Elkan 1965).
- low parasite-host specificity in fish (McVicar 1999).

Transmission

- a natural route of infection has not been demonstrated
- in piscivorous and scavenger hosts likely occurs through consumption of infected prey (Kocan et al 1999).
- Horizontal transmission through cohabitation occurs in some species, including cultured rainbow trout
- The route of transmission for planktivorous hosts, including Clupeids, remains unclear;
- laboratory studies have repeatedly failed to establish infections through cohabitation, feeding with food containing Ichthyophonus schizonts, or by direct intubation of Ichthyophonus schizonts into the stomach of Pacific herring (Hershberger & Gregg).
- repeated feeding of captive, Atlantic herring with Ichthyophonus-spiked mussel and liver tissues resulted in low prevalence of infection.
- schizonts released from the skin of infected herring were found infectious when injected into the body cavity of Pacific herring but not when administered orally (Kocan et al 2010).
- Infection can result in one of three outcomes:
    - acute disease and mortality,

    - chronic disease associated with decreased condition and performance, or
    - subclinical infection
        - infected Atlantic herring typically have lower condition factor and gonad weight (Kramer-Schadt et al 2010),
        - infected Pacific herring demonstrate a reduction in total energy content and energy density relative to uninfected cohorts (Vollenweider et al 2011).
- The prevalence of infection often increases with host size and age (Hershberger et al 2002, Marty et al 2003, Kramer-Schadt et al 2010).

Disease Signs

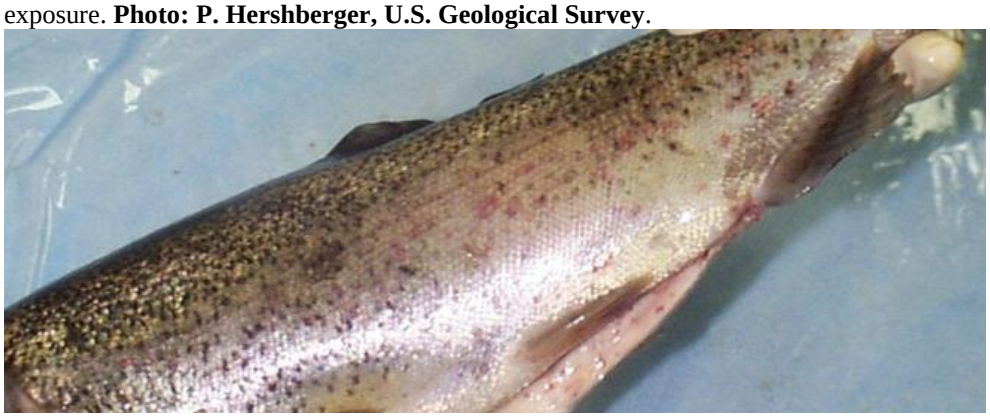
- decreased swimming performance, more pronounced at warmer temperatures (Kocan et al 2009).
- in hatchery conditions, diseased individuals may appear lethargic and consume less food than uninfected cohorts.
- infected wild herring may aggregate around the periphery of highly dense schools (Holst 1996).
- Gross signs externally:
    - few if any gross signs typically appear on most affected hosts
    - 'sandpaper skin' on clinically diseased Atlantic and Pacific herring.
        - often most pronounced on the caudal third of the body surface
        - caused by large numbers of raised papules under the skin surface.
        - The parasite is eventually released from these papules, leaving pigmented ulcers that resemble flakes of pepper on the skin surface
    - Heavily infected rainbow trout may demonstrate petechial hemorrhages on the skin and pigmented ulcers on the ventral surface.
- **Gross signs Internally**
    - white or cream-colored nodular lesions throughout the blood-rich organs, including heart, liver, kidney, and spleen
    - Pigmented lesions occur in the skeletal muscle of heavily infected fishes,
- **Microscopic signs**
    - Developmental stages of Ichthyophonus occurs within well-defined host cellular granulomas and consists of a large (10-250µm), thick-walled, multi-nucleate, spherical body (referred to as spore, macrospore, resting spore, multinucleate resting spore, cyst, a schizont, or multinucleate stage that reproduces asexually and produces a number of daughter cells).
    - Germination tubes (hyphae and pseudohyphae) are typically observed after the infected host has been dead for a period of time.
    - A small, motile mono-nucleate stage (referred to as endospore, microspore, amoeboblast, and plasmodium).

Diagnosis:

Presumptive Diagnosis

- **Internal and external signs**
  - high intensities of the disease are accompanied by gross signs on internal organs
  - subclinical infections can be easily overlooked
- **Tissue squash preparations**
  - Spherical schizonts (10-250 µm diameter) can be observed in fresh squash preparations
  - Schizonts are often surrounded by host granulomatous tissues.
- **Culture of Ichthyophonus** from infected tissues
  - Ichthyophonus schizonts and pseudohyphae grow readily in common broth media including Tris or Hepes-buffered Eagles Minimum Essential Medium (MEM) and Leibovitch-15 (L-15) supplemented with 5% fetal bovine serum and 100 IU ml-1 penicillin, 100 µg ml-1 streptomycin, 100 µg ml-1 gentamycin, incubated at 15°C
- schizont germination,
- histopathology.

Juvenile Pacific herring demonstrating external signs of ichthyophoniasis including pigmented skin ulcers and general emaciation. This fish died from ichthyophoniasis after experimental laboratory exposure. **Photo: P. Hershberger, U.S. Geological Survey**.

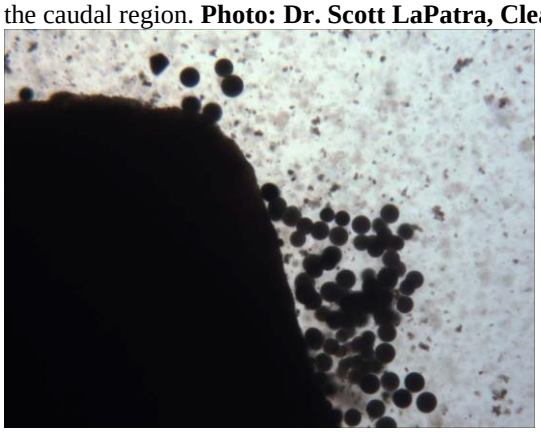


Rainbow trout with ichthyophoniasis demonstrating petechial hemorrhages on the skin surface. **Photo: Dr. Scott LaPatra, Clear Springs Foods, Inc. P. Hershberger**

Cultured rainbow trout with ichthyophoniasis demonstrating open ulcers and pigmented spots on the ventral surface..Rainbow trout with ichthyophoniasis, demonstrating white nodular lesions throughout all internal organs. **Photo: Dr. George Savvidis, Vet. Res. Institute of Thessaloniki, Greece**.


Macroscopic signs of ichthyophoniasis, including white nodular lesions, throughout the heart of a diseased Chinook salmon. **Photo: Stan Zuray, Yukon River Rapids Research Center**.

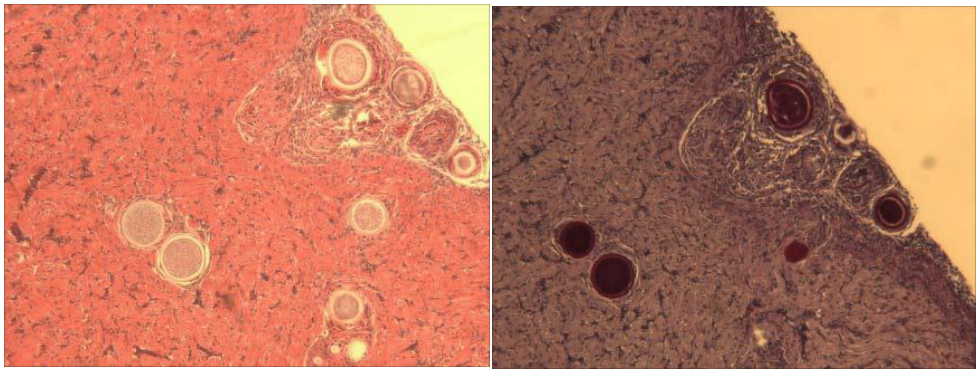
Unmarketable fillets from rainbow trout with heavy ichthyophoniasis infections. Note the pigmented lesions and focal hemorrhages throughout the fillets, with signs becoming more pronounced towards the caudal region. **Photo: Dr. Scott LaPatra, Clear Springs Foods, Inc**.




Typical Ichthyophonus schizonts in liver culture from an infected Pacific herring (40X magnification). **Photo: P. Hershberger, U.S. Geological Survey.** Wet mount of cultured Ichthyophonus isolated from Pacific herring. Note the nonseptate germination tubes originating from a parent schizont and terminating at clubshaped daughter cells (200X magnification). **Photo: P. Hershberger, U.S. Geological Survey**

Histopathology.

- The parasite often occurs as single or multiple schizonts inside well-defined host cellular granulomas; although un-encapsulated schizonts are also common throughout infected tissues during various stages of infection.
- The host granulomatous reaction is easily observed in hematoxalyn and eosin (H&E) stained tissue sections. Polysaccharides on the surface of the parasite stain strongly positive with periodic acid-Schiff (PAS); however, other spherical organisms in the 50-250 µm size range also stain PAS-positive and superficially resemble Ichthyophonus schizonts in histological sections. As such, detection of PAS-positive spherical bodies in tissue sections should not be considered confirmatory.



Stained histological sections (100X magnification) of Ichthyophonus in the heart of Pacific staghorn sculpin, stained with H&E (A) and PAS (B). **Photos: P. Hershberger, U. S. Geological Survey**.

Molecualr biological tests:

- Polymerase-chain reaction (PCR) using Ichthyophonus-specific primers.
- Genomic DNA from Ichthyophonus schizonts, pseudohyphae or infected fish tissue can be isolated using standard methods;
- PCR amplification of a 371 bp segment of the small subunit (SSU) rDNA is achieved using primers and PCR conditions (Whipps et al. 2006)

Reports:

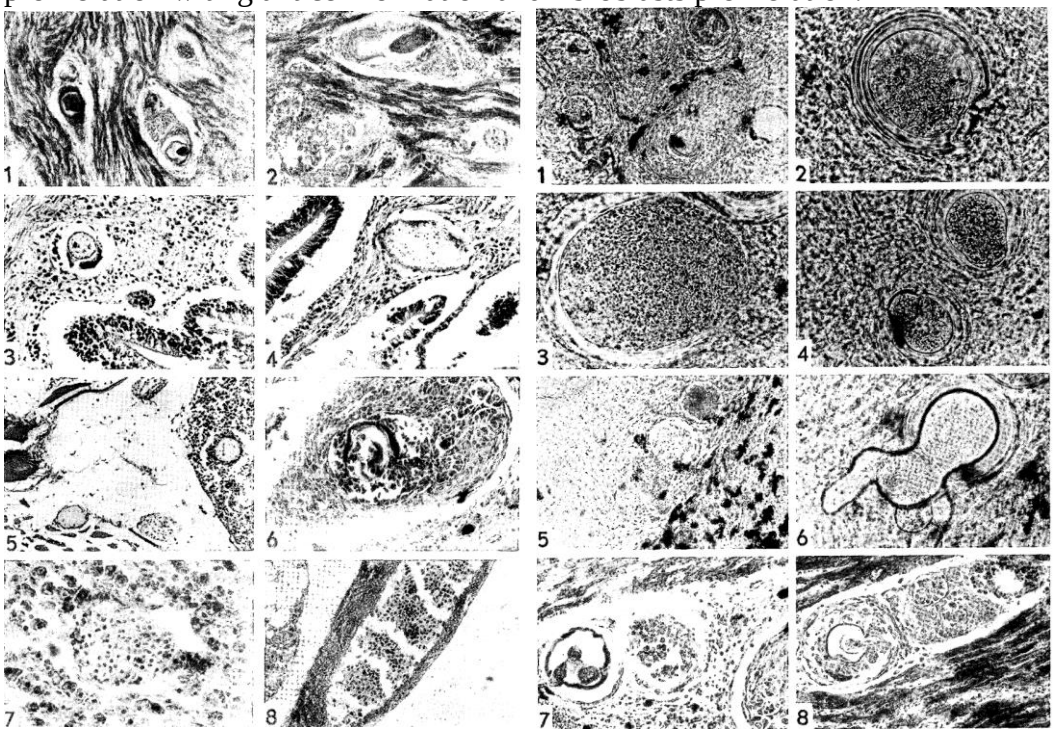
Fish (1934) found a fungus disease of epidemic proportions in the common sea herring (*Clupea harengus*) throughout the Gulf of Maine. The fungus was also found to infect the common winter flounder (*Pseudopleuronectes americanus*) and the alewife (*Pomobolus pseudoharengus*). The causative agent was found to be a species of fungus belonging to the genus *Ichthyosporidium* Caullery and Mesnil (1905). The specific name is tentatively accepted as *hoferi* Plehn and Muslow (1911). The organism is believed to be a normal parasite to the herring and reaches epidemic proportions only when certain unknown factors are operative. The causative organism was found in herring preserved in 1926, and it is believed that the epidemic has been increasing in severity since that time. It was believed that such an epidermic, once initiated, increased in severity, reached a peak, and subsided to a subpatent level. The peak was believed to have been reached in 1931. The herring was believed to acquire the infection by the ingestion of parasites liberated from fish in the same school. The flounder was believed to acquire the infection by the consumption of infected herring. 10. The alewife is believed to acquire the infection by ingestion of the parasite during its infrequent association with the herring. Infection was believed to be established by way of the alimentary canal and, once established, to spread throughout the host by way of the blood stream or the lymphatics. Direct cross infection from the herring to the flounder established the theory that the parasites in these two hosts were one and the same organism. Direct cross infection experiments from the herring to the flounder eliminated the necessity of an intermediate host. There was no reason to believe that this parasite is capable of infecting warm-blooded animals.

Rucker and Gustafson (1953) noted that as the disease developed trout showed clear signs of agitation, and the skin along the lateral line and then in other areas of the body became darkened, and the belly was observed to protrude because of the increased size of the internal organs. However, the brain was seldom found to be affected.

Fijan and Maron (1977) in Yugoslavia succeeded to isolate *Ichthyophonus hoferi* from an outbreak of ichthyosporidiosis in a fish farm with low mortalities. The affected fishes were lethergic, emaciated and some showed exophthalmia with distended abdomen. Postmortem examination showed miliary lesions in the myocardium, spleen, liver and kidneys which proved to be granuloma in nature.

Chien and Yu (1978) reported that A. japonica was a new host for Ichthyophonus. The morphology of this fungus in eel was similar to *Ichthyophonus hoferi* reported in other fishes. The infected eels had swollen liver and kidney externally, some eels were dotted with many transparent cysts on their abdomenal wall. Large or small cysts appeared in gills, liver, kidney, spleen, alimentary duct, pancreas and musculature etc. The mortality of the diseased fish was very high.

Chun and Kim (1981) mentioned that Ichthyophonus disease broke out among rainbow trout (*S. gairdneri*) fry in November, l979, and after that a fish group containing diseased ones was kept for one year. The histopathological examination of the diseased fish was carried out 3 times, at 6 months intervals. Diseased fish showed markedly stunted growth, darkish colouration, the liver with small white nodules, the heart with red nodules, the spleen with granular nodules and the markedly tumefied kidney. Ichthyophonus invaded various tissues in the host and took the shape of multinucleate spherical or hyphal bodies. Histopathologically, systemic dissemination and systemic proliferation by *Ichthyophonus* sp. were identified. The inflammatory response against Ichthyophonus was characterized by mononuclear-cellular proliferation with giant cell formation and fibroblasts proliferation.



Faisal *et al.* (1985) recorded *Ichthyophonos hoferi* infection among the labyrinth catfish "*Clarias lazera*" in Egypt. The fungal nodules were found mostly in the liver and kidneys of the infected fish. Squash preparations of the fungal nodules revealed

the presence of small mononuclear endospores, large multinucleated double-walled cysts and germinating flask shaped cysts. Healthy *Clarias lazera* were infected experimentally by feeding infected organs.

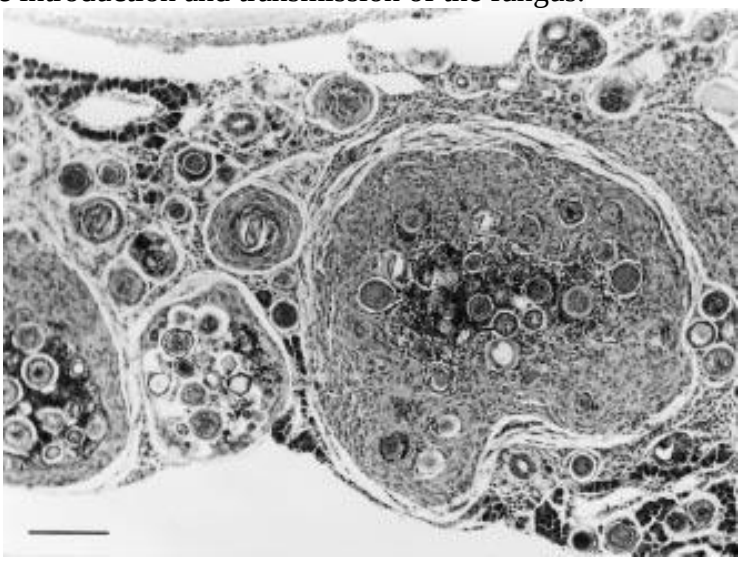
Miyazaki (1985) stated that *Ichthyophonos hoferi* infection occurred in cultured ayu (*Plecoglossus, altivelis*) in Tokushima prefecture during the Spring and Summer of 1979. Diseased fish had pale body colouration, small open ulcers on the body surface, swollen abdomen due to accumulation of ascitic fluid, and the production of small nodules in visceral organs. Histological lesions were disseminated multinucleate spherical bodies of Ichthyophonus and a reaction against the spherical bodies by macrophages, multinucleated giant cells and granulomas in the affected visceral organs of diseased fish.

Okamoto *et al.* (1987) carried out experimental oral infection of *rainbow trout Salmo gairdneri* with cultivated multinucleate spherical bodies of *Ichthyophorus hoferi* . *Rainbow trout* orally injected with thick-walled multinucleate spherical bodies cultivated in MEM containing 10% fetal bovine serum showed 100% infection and 90% cumulative mortality at 16°C water temperature for 25 days. However, those orally injected with spherical multinucleate hyphal terminal bodies cultivated in TGC containing 1% *rainbow trout* serum showed no infection and no cumulative mortality. The former showed the same symptoms such as darkness of the skin colour, perforation in the body surface and/or nodular white spots in several internal organs as in the naturally infected fish. Amoeboblasts which underwent endogenous cytoplasmic cleavage were also observed in the liver of the rainbow trout infected artificially.

Okamoto *et al.* (**1988)** studied the relationships between water temperature, fish size, infective dose and degree of Ichthyophonus infection of rainbow trout. They found that mortality after 35 days was 100% at 20ºC and 15C, 10% at 10ºC and 0% at 4ºC. Mortality was greater in larger size fish. Size of infective dose also affected mortality, which was, 100%, 44%, 4% and 0.0% in fish receiving orally an infective dose of 3000, 300, 30 and 3 spherical bodies of *Ichthyophonus hoferi*, respectively.

Ragan *et al.* (1996) determined sequences of nuclear-encoded small-subunit rRNA genes for representatives of the enigmatic genera Dermocystidium, Ichthyophonus, and Psorospermium, protistan parasites of fish and crustaceans. The small-subunit rRNA genes from these parasites and from the "rosette agent" (also a parasite of fish) together form a novel, statistically supported clade. Phylogenetic analyses demonstrate this clade to diverge near the animal-fungal dichotomy, although more precise resolution is problematic. In the most parsimonious and maximally likely phylogenetic frameworks inferred from the most stably aligned sequence regions, the clade constitutes the most basal branch of the metazoa; but within a limited range of model parameters, and in some analyses that incorporate less well-aligned sequence regions, an alternative topology in which it diverges immediately before the animal-fungal dichotomy was recovered. Mitochondrial cristae of Dermocystidium spp. are flat, whereas those of Ichthyophonus hoferi appear tubulovesiculate. These results extend our understanding of the types of organisms from which metazoa and fungi may have evolved.

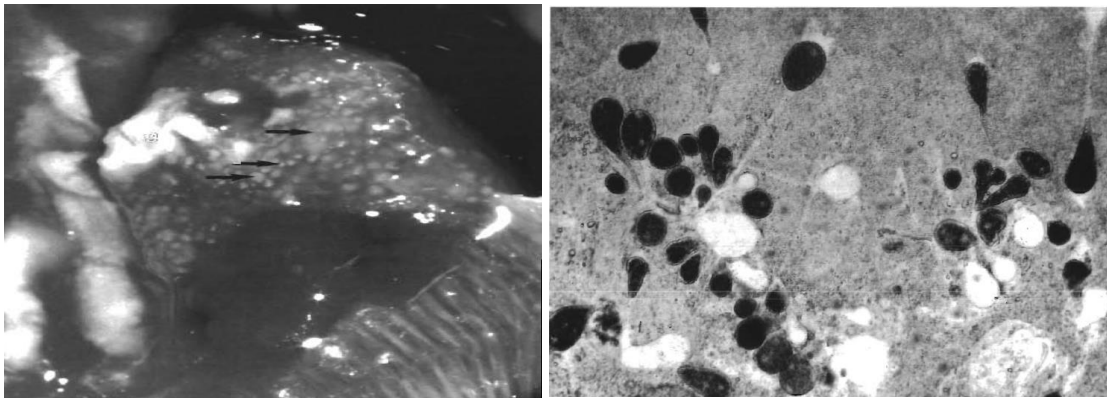
Franco-Sierra *et al (1997)* *mentioned that Ichthyophonus* sp. is reported for the first time in *Mugil capito* (thinlip grey mullet) and *Li a saliens*(leaping grey mullet). The fungus was also found in *L. aurata* (golden grey mullet), *Dicentrarchus labrax*(sea bass), *Sparus aurata* (gilthead sea bream) and *Scophthalmus maximus* (turbot), whereas *Mugil cephalus* (grey mullet) was not parasitized. In fish sampled periodically, the highest prevalences were observed in sea bass and the lowest in turbot. Among the fish sampled occasionally, the fungus was found associated to an epizootic in thinlip grey mullet. *Ichthyophonus* was never found in fish weighing <0·5 g. An increase in the prevalence of infection with the age of turbot and gilthead sea bream was observed. Gilthead sea bream and sea bass showed higher prevalences in a closed system than in open and semi-intensive systems. Multinucleate spherical spores, hyphae and endospores of *Ichthyophonus* sp. parasitized different organs of thinlip and leaping grey mullets, though infection intensity was maximal in the spleen. In the remaining fish, the fungus was found mainly in the trunk kidney, where it appeared frequently in a necrotic form. *Ichthyophonus* sp. can be considered a potential threat for marine fish aquaculture, especially in culture conditions which may favour the introduction and transmission of the fungus.



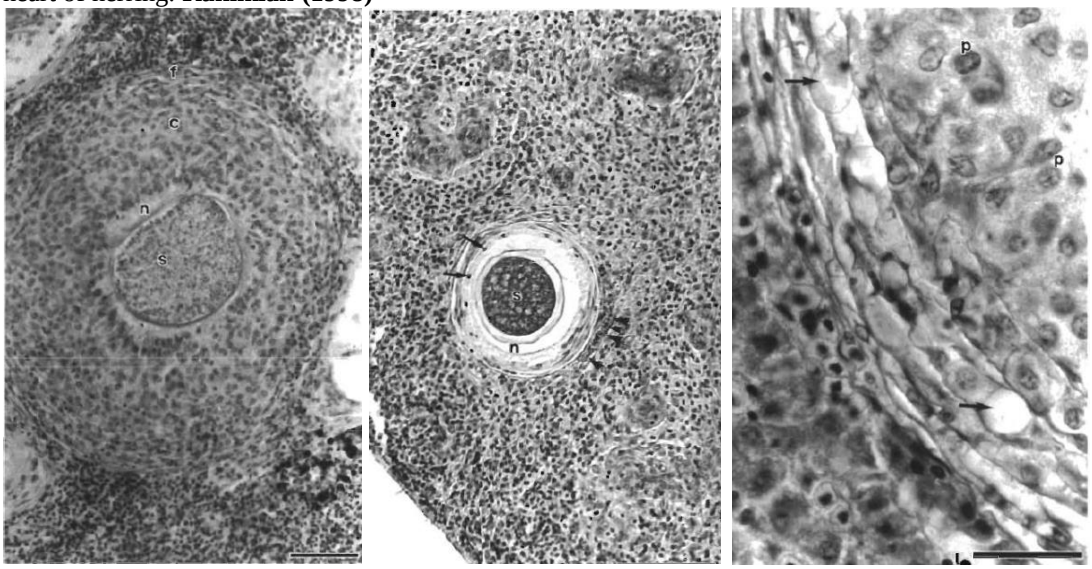
Section of the pancreatic tissue of *Mugil capito* infected by *Ichthyophonus* sp. Note the strong granulomatous reaction. H & E. **Franco-Sierra *et al (1997)***

Rahimian (1998) studied the pathology and morphology of Ichthyophonus hoferi in naturally infected Atlantic herring Clupea harengus, in sprat Sprattus sprattus, and in flounder Pleuronectes flesus from the west coast of Sweden. The pathogen was found in all organs examined, with the intensity of infection varying in different organs of the different fish species. Two main phases in the life of infecting parasites were identified, 'active' and 'passive', the latter being able to switch to active. The active phase of the infection in herring was usually accompanied by a lean and slender appearance of the body, a drastic decrease in intestinal fat, emaciation of the somatic muscles, swelling of the visceral organs. poor quality of flesh texture and a distinctive off-odour. The most characteristic macroscopic sign of ichthyophonosis in herring and flounder was the occurrence of creamy white nodules on the heart. The infection causes a chronic systemic granulomatous inflammation The nature of the granulomatous inflammation was host- and tissue-dependent. The pathogenlcity of the parasite in its active form and the side effects of host defence cells were also reflected in dramatic tissue damage and loss of structure and function of the infected organs. Three kinds of spores were identified: 'un-developing spore', 'developing spore' and

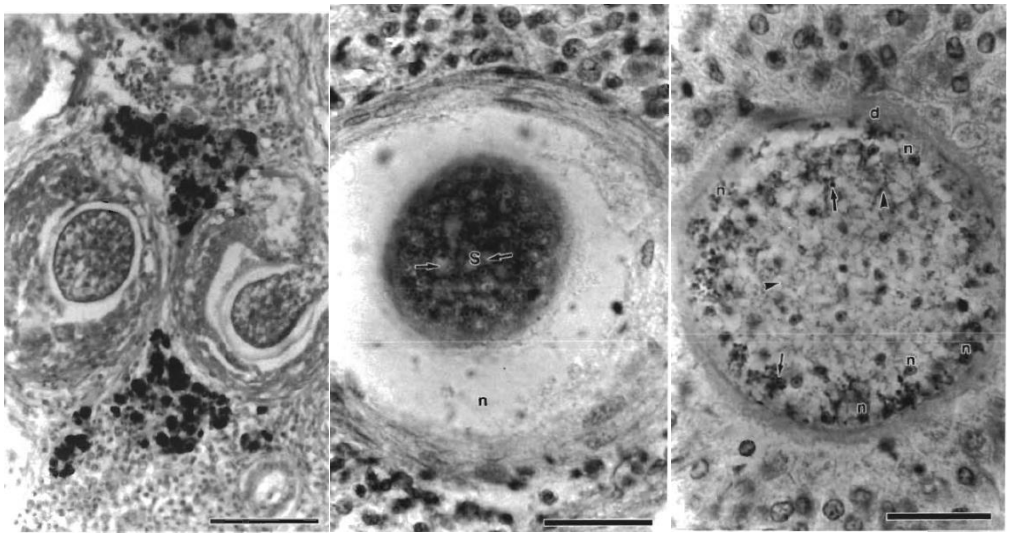
'plasmodio-spore' The formation and spread of 'plasmodia', from plasmodiospores, as a secondary infection agent is documented. Transmission electron microscopy revealed I. hoferi to be multirrucleated, containing different organelles and structures These included a cell wall, an undulating cell membrane, a thin paramural endoplasm, an endoplasmic reticulum, polymorphic but usually spherical mitochondria with short tubulo-vesicular cristae, dictyosomes w~th plate-like cristernae, large electron-dense lipid droplets and electron-lucid vacuoles, probably containing glycogen.



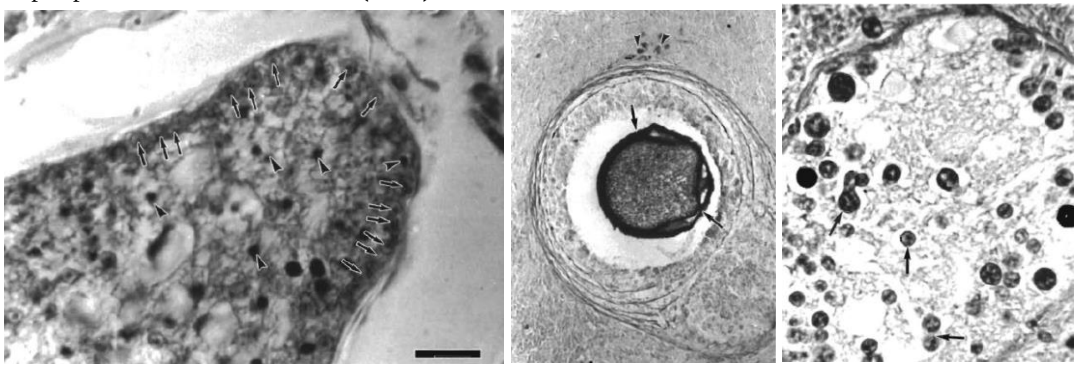
Gross sign of ichthyophonosis on the heart of heavily infected hernng. The heart is covered by small (arrows) and giant (g) nodules. Growth of pseudohypha from spores of Ichthyophonus hoferi in the heart of herring. **Rahimian (1998)**



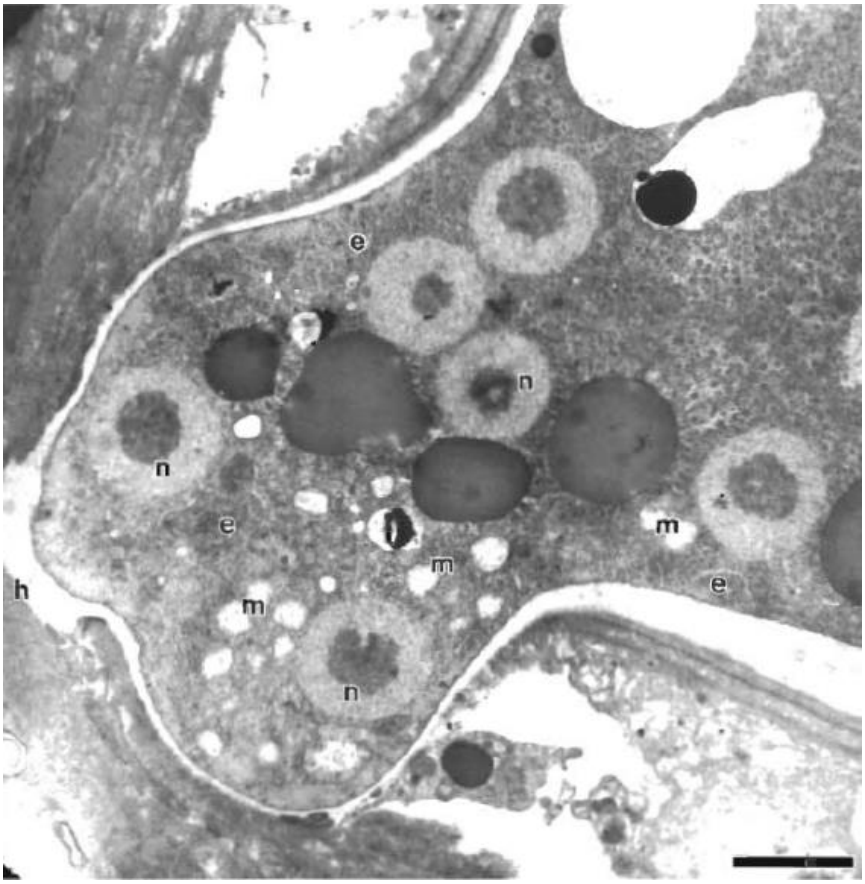
The passive spore of Ichthyophonus hoferi (s) surrounded by the granuloma phase 1. f: Fibrocytic layer; c: cellular layer; n: necrotic layer. H&E, flounder, spleen. Spore of Ichthyophonus hoferi (s) encapsulated in the last phase of the granulomatous process,i.e. phase 3. Arrows: pyknosis degeneration of host cells; arrowheads: melanomacrophages; n: necrotic layer. H&E, herring, spleen. Outer most part of a granuloma. Arrows: hydropic degeneration; 1: lymphocytes; p: plasma cells. H&E, **Rahimian (1998)**



Black melanomacrophage centres in close association Ichthyophonus hoferi. H&E, flounder, spleen.
Early stage of degeneration of passive spore of Ichthyophonus hoferi (S). Note the nuclei distribution, vacuolation of the cytoplasm (arrows), thickness of the necrotic layer (n) and absence of any live host cell in the capsule, an indication of the relative age of the spore. H&E, herring kidney.. Multi-nucleated active spore of Ichthyophonus hoferi surrounded by relatively thin necrotic deposits (d). Ground cytoplasm with dense granular bodies (arrows), containing a network of fibre-like cords (arrowheads); n: peripheral nucleus. **Rahimian (1998)**



Tip of a pseudohypha. Note dense granular bodies (arrowheads) and the dominant peripheral distribution of nuclei (arrows). Scale bar = 15 pm. ng PAS reaction towards the bilaminated wall [round the developing spore of Ichthyophonus hoferi, the extension of the inner section as pseudohypha growth (arrows) and patchy distribution of reaction inside the spore. Melanomacrophages (arrowheads) applied to the fibrocytic capsule. PAS, herring, heart. Scale bar = 100 pm. modia (arrows) formation within the plasmodiospore of Ichthyophonus hoferi. H&E, herring, heart. **Rahimian (1998)**

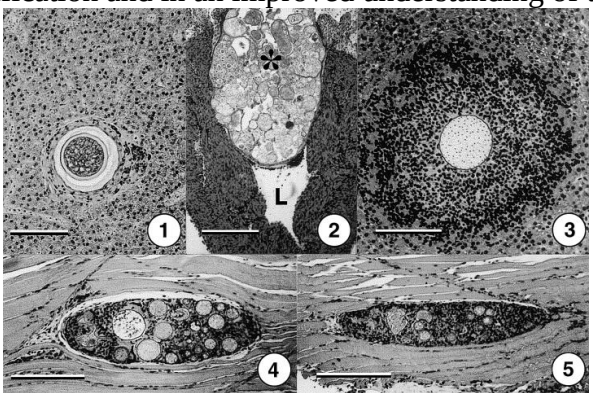


Pseudohyphae of Ichthyophonus hoferi penetrating and lysing the host issue (h) by means of lytic activities. Note the well-developed endoplasmic reti culurn (e), mitochondria (m); also note flow of nuclei (n) and other cytoplasmic material in the newly formed pseudohyphae. Scale bar = 2.5 pm **Rahimian (1998)**

Hershberger *et al.* (2002) reported a decrease in the mean age of adult Pacific **herring *Clupea pallasi* in Puget Sound associated with a high prevalence** of *Ichthyophonus hoferi*, a protistan parasite that can be highly pathogenic to Pacific herring. In Puget Sound, high intensities of *I. hoferi* infection may be maintained in older cohorts of Pacific herring because the prevalence of *I. hoferi* increased with age from 12% among juveniles to 58% among the oldest, age-6 and older cohorts. Low intensities of *I. hoferi* infection in the region may be maintained in alternative fish hosts, such as surf smelt *Hypomesus pretiosus*, Puget Sound rockfish *Sebastes emphaeus*, Pacific tomcod *Microgadus proximus*, and speckled sanddab *Cithanichthys stigmaeus*.

Jones and Dawe (2002) screened Pacific herring, *Clupea pallasi* Valenciennes, collected from three areas of coastal British Columbia for *Ichthyophonus* by histological examination. The infectivity of *Ichthyophonus* to juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), was examined in laboratory studies.*Ichthyophonus* was detected in a total of 82 of 356 herring from all three areas. Prevalence in 2000 and 2001 ranged from 10.5 to 52.5% and was significantly lower in more northern (Hecate Strait) samples. *Ichthyophonus* was detected by histological examination in chinook salmon following oral or intraperitoneal (i.p.) exposure to homogenates of infected herring tissue. Infections in Yukon stock chinook salmon were occasionally associated with mortality and with inflammation in all tissues examined. Infections were detected significantly more frequently in the caecal

mesenteries of i.p.-infected compared with oral-infected chinook salmon. The distribution and prevalence of*Ichthyophonus* isolates among diverse host species may assist in stock identification and in an improved understanding of trophic interactions.

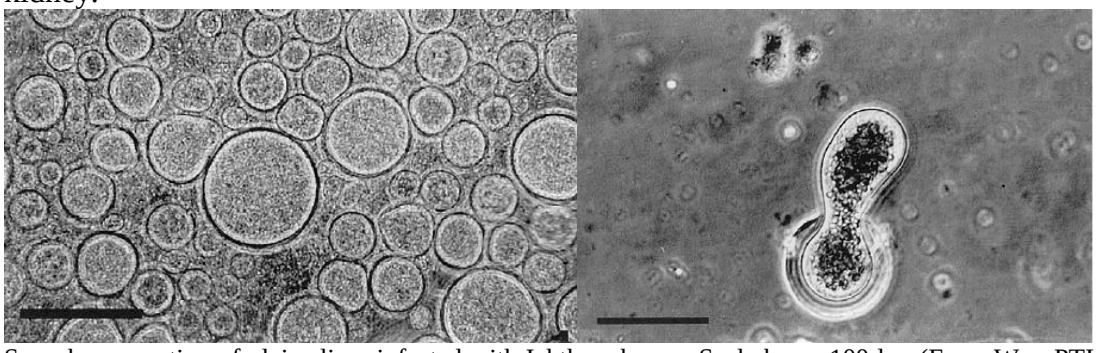


Histological sections of Pacific herring and juvenile chinook salmon. H&E stain. (1) Herring liver showing *Ichthyophonus* within a multilaminate, fibrous capsule (bar=100 μm). (2) Anterior heart from a chinook that died 35 days after feeding on infected herring heart. The lumen of the bulbus arteriosus (L) is occluded with a spore-like structure (*) containing several endospores (bar=250 μm). (3) Zonal, diffuse granulomatous inflammatory reaction surrounding a multinucleate spore in chinook salmon liver, sampled 35 days following oral exposure. The reaction is comprised of an inner area of monocytes surrounded by a ring of lymphocytes (bar=100 μm). (4, 5) Pseudocysts of intact and degenerate *Ichthyophonus*spores and inflammatory infiltrate in chinook salmon skeletal muscle. Samples were collected 35 days after oral and intraperitoneal exposure, respectively (bars=250 μm). **Jones and Dawe (2002)**

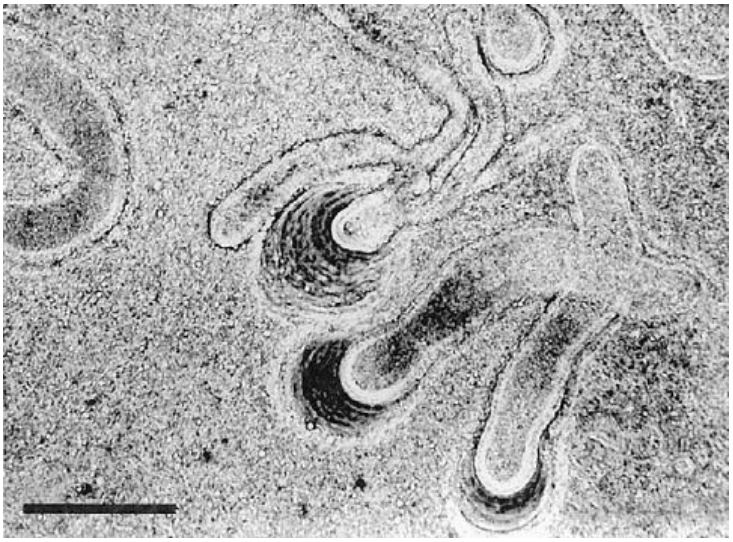
Mendoza *et al.* (2002) mentioned that when the enigmatic fish pathogen, the rosette agent, was first found to be closely related to the choanoflagellates, no one anticipated finding a new group of organisms. Subsequently, a new group of microorganisms at the boundary between animals and fungi was reported. Several microbes with similar phylogenetic backgrounds were soon added to the group. Interestingly, these microbes had been considered to be fungi or protists. This novel phylogenetic group has been referred to as the DRIP clade (an acronym of the original members: Dermocystidium, rosette agent, Ichthyophonus, and Psorospermium), as the class Ichthyosporea, and more recently as the class Mesomycetozoea. Two orders have been described in the mesomycetozoeans: the Dermocystida and the Ichthyophonida. So far, all members in the order Dermocystida have been pathogens either of fish (Dermocystidium spp. and the rosette agent) or of mammals and birds (Rhinosporidium seeberi), and most produce uniflagellated zoospores. Fish pathogens also are found in the order Ichthyophonida, but so are saprotrophic microbes. The Ichthyophonida species do not produce flagellated cells, but many produce amoeba-like cells. This review provides descriptions of the genera that comprise the class Mesomycetozoea and highlights their morphological features, pathogenic roles, and phylogenetic relationships.

Ragan *et al.* (2003) sequenced the EF-1alpha gene from the ichthyosporean parasite Ichthyophonus irregularis and determined its phylogenetic position using neighbor-joining, parsimony and Bayesian methods. They also sequenced EF-1alpha genes from four chytrids to provide broader representation within fungi. Sequence analyses and the presence of a characteristic 12 amino acid insertion strongly indicated that I. irregularis is a member of Opisthokonta, but do not resolve whether I. irregularis is a specific relative of animals or of fungi. However, the EF-1alpha of I. irregularis exhibits a two amino acid deletion heretofore reported only among fungi.

Yanong (2003) mentioned that Ichthyophonis hoferi has been placed taxonomically in the newly proposed class Mesomycetozoea, Kingdom Protista (Protoctista). Members of the Mesomycetozoea are believed to link fungi and animals evolutionarily. This class also includes the ‘‘fungal’’ fish pathogens known as the rosette agent (proposed, Sphaerothecum destruens gen. and species nov.) and Dermocystidium. Ichthyophonis hoferi is primarily a disease of marine and estuarine fish Pathogen–host specificity is low, with infections in over 80 marine fish species reported worldwide. Clinically, ichthyophoniasis can mimic mycobacteriosis. Signs vary based on species, location, and severity of infection and include darkening, behavioral abnormalities (abnormal swimming), lethargy, emaciation, ascites, increased mortality, skin roughening, and ulceration. Internal lesions include white or cream-colored nodules (granulomatous inflammation), although species differences in affected tissues have been noted. Infected tissues include muscle, heart, liver, and kidney.

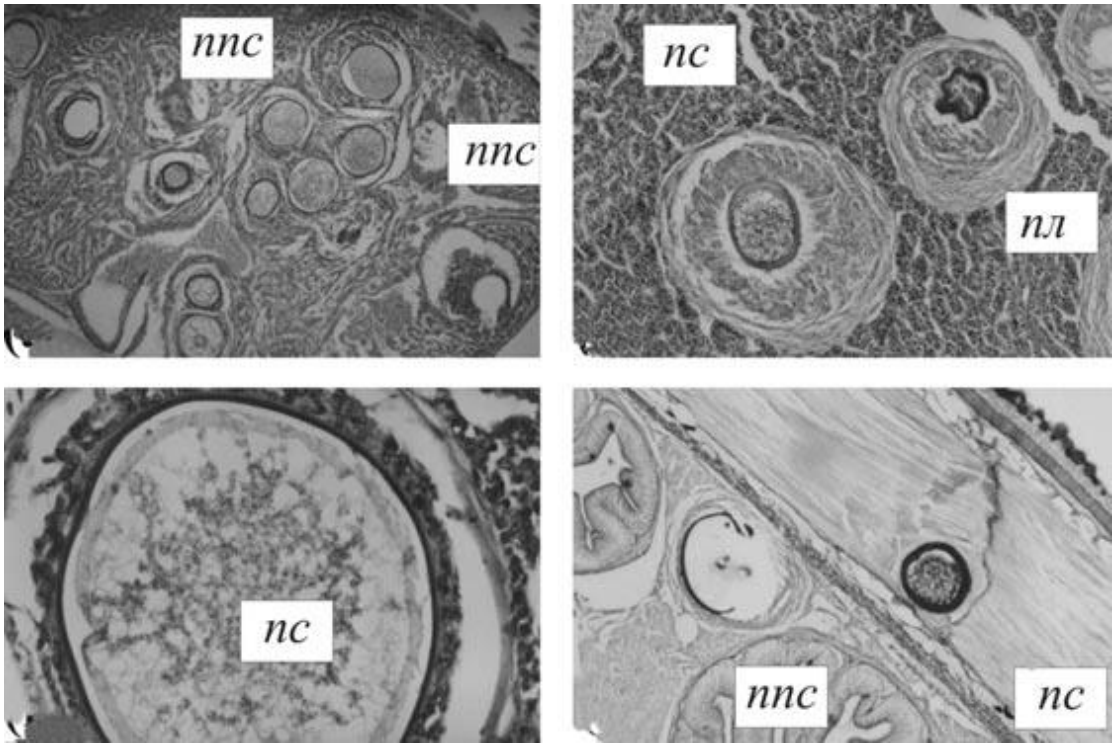


Squash preparation of plaice liver infected with Ichthyophonus. Scale bar = 100 lm. (From Woo PTK, Bruno DW, editors. Fish diseases and disorders.. **Yanong (2003)**

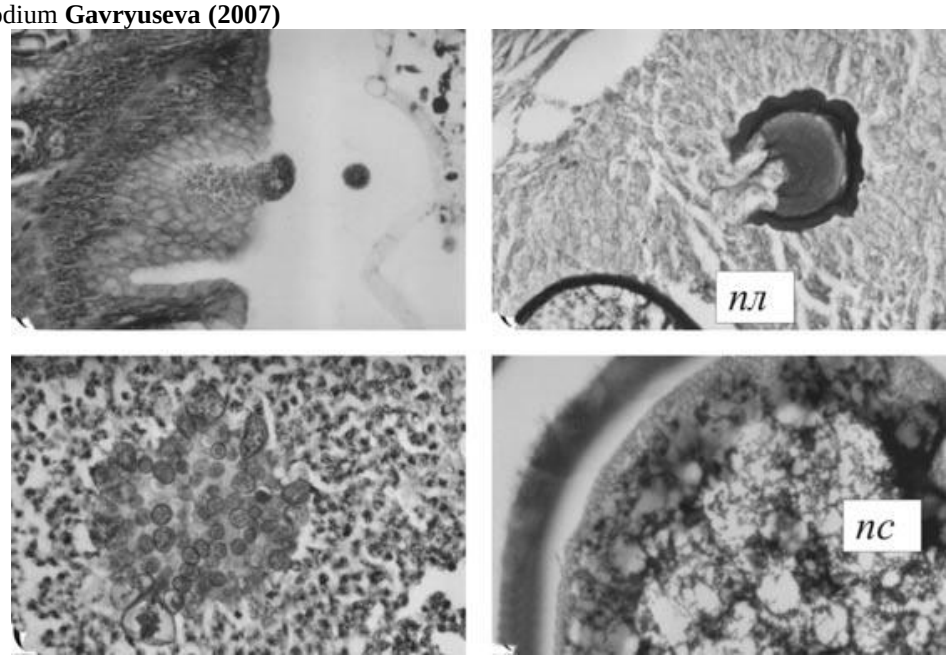


Development of branched germination tubes (‘‘hyphae’’) from spherical bodies in theintestine of an experimentally challenged rainbow trout. Scale bar = 100 lm. (From Woo PTK, Bruno DW, editors. Fish diseases and disorders. **Yanong (2003)**

Gavryuseva (2007) found Resting spores of *Ichthyophonus hoferi* (50–230 μm in diameter) in the kidney, heart, liver, skeletalal muscles, exocrine pancreas, and connective and fatty tissues of young coho salmon *Oncorhynchus kisutch* from the Vilyuisky fish hatchery. In 10% of the fish, there were granulomas and giant cells in these organs. This is the first report of *Ichthyophonus*infection in Kamchatka.



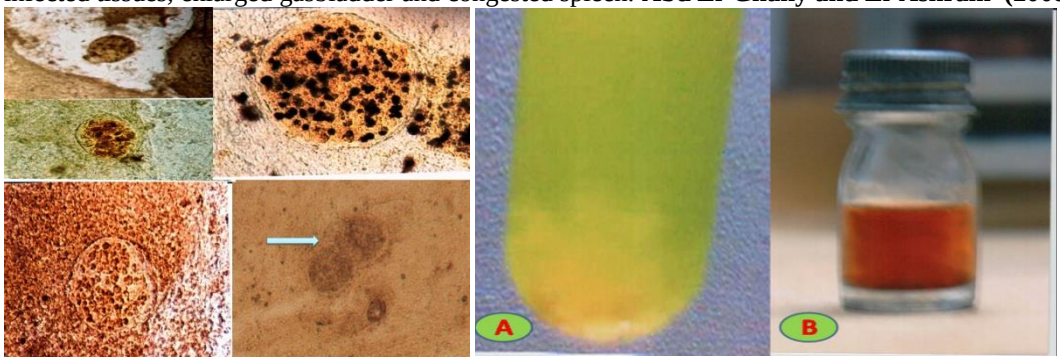
Histopathological changes in the organs and tissues of hatchery-reared one-year-old coho salmon infected with *Ichthyophonus*. a—multiple cluster of resting spores of *Ichthyophonus hoferi* (arrows) in the ventricle of the heart (□50, H-E); b—granulomas around a resting spore and plasmodium (□100, Romanovsky-Giemsa technique); c—melanocytes, macrophages and necrotized cells (arrowed) around a resting spore (400, H-E); d—resting spores of *I. hoferi* in skeletal musculature and pancreas with signs of degeneration) (100, PAS light green). Designations: rs—resting spore, ers—empty spore, pl—plasmodium **Gavryuseva (2007)**



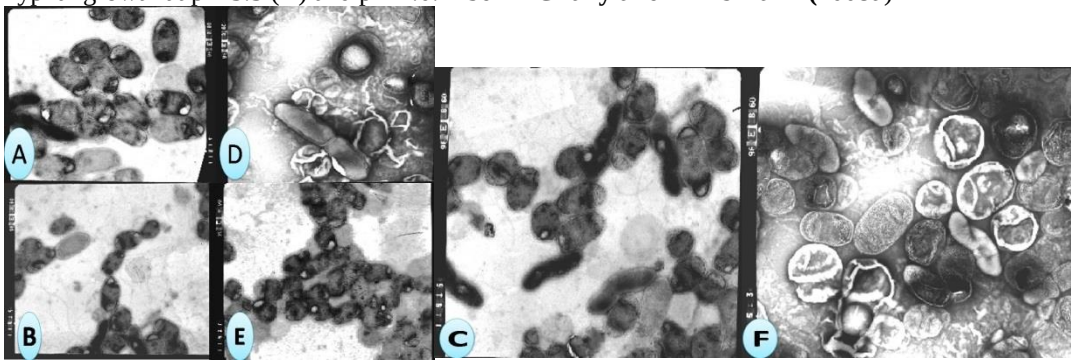
Life history stages of *Ichthyophonus hoferi* in organs and tissues of one-year-old coho salmon. a—division of amoeboblasts into amoeboid embryos (arrowed) in the cardiac region of the stomach (400, H-E); b—plasmodium of *I. hoferi* in the liver (400, PAS light green); c—budding of daughter spores (arrowed) in hemopoietic tissue of the kidney (400, H-E); d—multiple cluster of endospores (arrowed) in the resting spore of *I. hoferi* (1000, Romanovsky-Giemsa technique). Designations: pl—plasmodium, rs—resting spore. **Gavryuseva (2007)**

Abd El-Ghany and El-Ashram (2008a) collected and screened 200 *Oreochromis niloticus* (100 from each wild and cultured) for *Ichthyophonus* infection. The prevalence of infection was 32%. Prevalence was higher for cultured (40%) and females fish (44.7%) than for wild (24%) and males (22.6%). The morphology of *Ichthyophonus hoferi* was described by electron microscopy at pH 3.5 and 7.0. Kidney is the target organ of isolation of *I. hoferi*. Clinical signs were lacked in low or moderate infection rates. While, in heavy infected one showed dark coloration and rough skin, nervous disorders and occasionally scales lose and ulceration of the skin. Also, slight abdominal swelling was noticed. Internally, the infected fish showed grossly visible white to creamy fusiform raised nodules or cysts on the internal organs. The infectivity of *Ichthyophonus* to *O. niloticus* was examined in laboratory studies. The use of polymerase chain reaction test as diagnostic test was discussed. Histopathological changes associated with *I. hoferi* infection were described with the aid of light and transmission electron microscope.

O. niloticus showing black coloration of the skin. *O. niloticus* showing congestion of gills, nodules on infected tissues, enlarged gasbladder and congested spleen. **Abd El-Ghany and El-Ashram (2008a**)

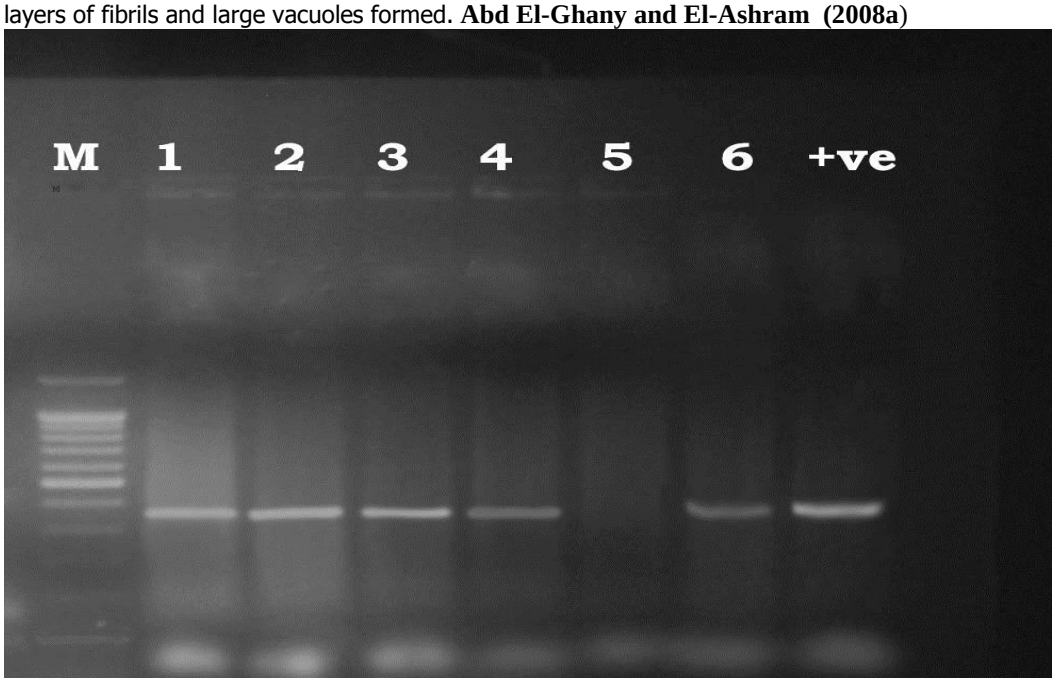


Squash preparation from nodules showing double walled resting spore. Wet preparation showing budding of the cyst (postmortem germination) (arrow). Culture of *I. hoferi* on MEM-10 showing hyphal growth at pH 3.5 (A) and pH 7.0. **Abd El-Ghany and El-Ashram (2008a**)

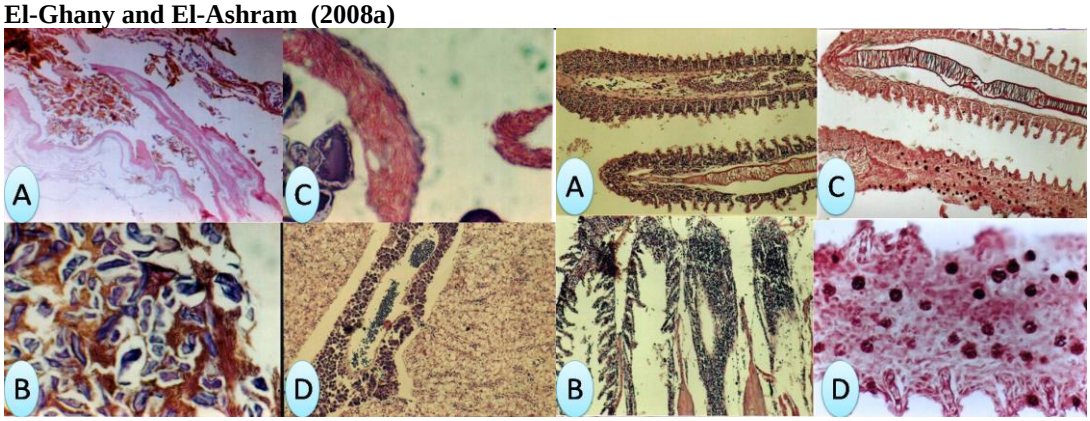


EM image of Ichthyophonus stages. **(A)** Multinucleate spore from culture in SDA with 1% bovine serum. Note the thick wall, several nuclei with peripheral nucleoli, abundant glycogen granules and the

reticulum among the nuclei. **(B)** Showing the multinucleate spores of Ichthyophonus with positive glycogen granules and the contents of some membrane bounded vesicles. Spore constriction leading to new spores by division to from germinating hypha consisted of inner part of spore wall. **(C)** Showing budding yeast like germination in SDA 1% bovine serum. Mitochondria with scarce tubulovesicular cristae were abundant near the plasmalemma. **(D)** Showing the spores detail and mitochondria with scarce tubulovesicular cristae and rough endoplasmic reticulum. **(E)** Groups of small, thin walled spores have arisen from hyphae or from large spores. One to two nuclei were noted in the sections as well as glycogen rosettes and lipid droplets. **(F)** Showing the wall of larger spores was organized concentric layers of fibrils and large vacuoles formed. **Abd El-Ghany and El-Ashram (2008a**)



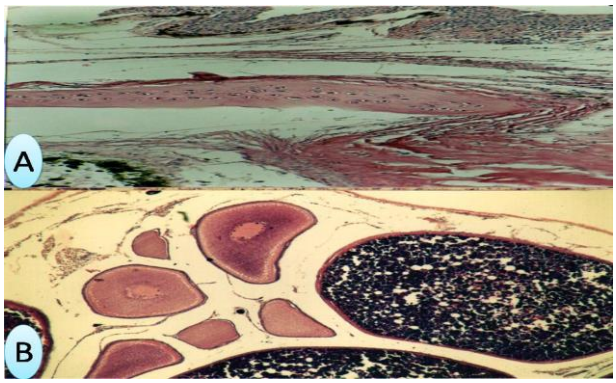
Electrophoretic pattern of small subunit ribosomal DNA (SSU) r DNA of *I. hoferi*. **(1)** pure isolate of *Ichthyophonus* on MEM-10 adjusted at pH 3.5. **(2)** pure isolate of *Ichthyophonus* on MEM-10 adjusted at pH 7 **(3)** kidney of *O. niloticus* heavily infected with *Ichthyophonus* **(4)** kidney of *O. niloticus* with moderate infection of *Ichthyophonus* **(5)** non-infected kidney. **(6)** pure isolate of Ichthyophonus on SDA. **(7)** showing the positive amplification of SSU rDNA gene of the *I. hoferi* reference strain. **Abd El-Ghany and El-Ashram (2008a)**



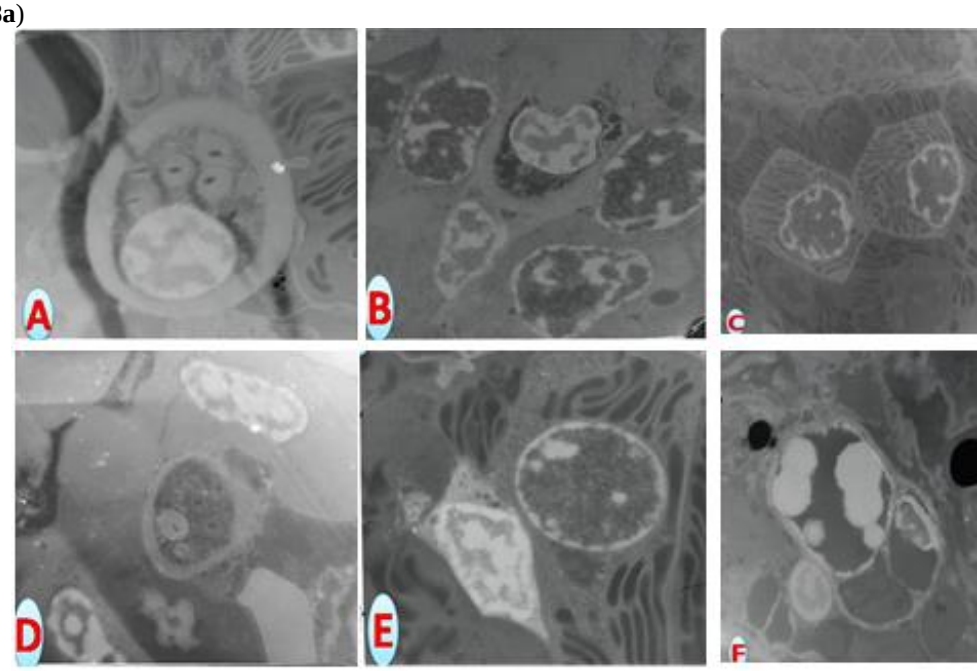
(A) Kidney of *O. niloticus* showing granuloma surrounded by inflammatory cells, necrosis of the tubules and haemorrahages in the parenchyma. The normal architecture of kidney was lost. H&EX60. (B) Showing the magnification of (A). H&EX300. (C) Showing the magnification of (A) to identify the degenerative changes. H&EX600. (D) Liver showing sever dilatation in the portal vein and sinusoids associated with sever degeneration in the hepatocytes. H&EX40. **Abd El-Ghany and El-Ashram (2008a**)

(A) Gill of *O. niloticus* showing sever hyperemic filament with hyperplastic adhesive lamellae. H&EX40. **(B)** Gill of *O. niloticus* showing massive number of inflammatory cells infiltration in the base of the filament. H&EX40. **(C)** Gill of *O. niloticus* showing PAS positive reaction for the spores in the gill filament. PASX40. **(D)** Gill of O. niloticus showing the magnification of (Fig. C) to identify the PAS positive reaction for the spores in the filament. PASX160. **Abd El-Ghany and El-Ashram**

(2008a)



(A) Eye of *O. niloticus* showing melanin pigment cells and edema in the iris. H&EX160. **(B)** Ovary of *O. niloticus* showing no histopathological alteration. H&EX40 **Abd El-Ghany and El-Ashram (2008a**)



Transmission electron micrographs showing ultrastructure of *Ichthyophonus* spore in heavy infected tilapia with the chronic inflammatory changes. **Abd El-Ghany and El-Ashram (2008a**)

Abd El-Ghany and Abd (2008b) carried out the first trial in Egypt on the treatment of Ichthyophonosis in *Oreochromis niloticus*. Clinically naturally infected *O niloticus* with *Ichthyophonus spp*. was observed as spherical multinucleate spores white and creamy in colour were very variable in size, found in liver, spleen and kidney and presence of spores freely with mucus in gills, the germinating stages, hyphae and endospores were also found. Different cultures were used in growth of *Ichthyophonus hoferi* spores as Eagles minimum essential medium (MEM), thioglycollate medium and Sabourauds dextrose broth. In addition, solid media as Sabourauds dextrose agar, all media were supplemented by different concentration of fetal bovine serum. Healthy *O.niloticus* was infected experimentally by spores of *Ichthyophonus spp* MEM-10 pH 3.5 culture as 1ml/ fish. Mortality, clinical signs and postmortem changes in experimentally infected fish were recorded. The results of biochemical analysis and hematology showed increased plasma levels of cortisol, eosinophils and monocytes while decreased total protein, albumin, total globulin and lymphocytes in group infected with *Ichthyophonosis*. In the present study, we investigated the effect of crude extract of *Azadirachta indica* (neem) leaves at dose 5ppm /kg ration and *Fucus vesiculosus* extract at dose 2g/kg ration in controlling of

such disease in fish. Approximately, most of these parameters increased in infected fish treated with neem and fucus .The present data concluded that neem is more effective than fucus and qualifies as a safe and efficient in the prevention of *Ichthyophonosis* in fish.

Rasmussen *et al.* (2010) reported a major genetic division between west coast freshwater and marine isolates of Ichthyophonus hoferi. Sequence differences were not detected in 2 regions of the highly conserved small subunit (18S) rDNA gene; however, nucleotide variation was seen in internal transcribed spacer loci (ITS1 and ITS2), both within and among the isolates. Intra-isolate variation ranged from 2.4 to 7.6 nucleotides over a region consisting of approximately 740 bp. Majority consensus sequences from marine/anadromous hosts differed in only 0 to 3 nucleotides (99.6 to 100% nucleotide identity), while those derived from freshwater rainbow trout had no nucleotide substitutions relative to each other. However, the consensus sequences between isolates from freshwater rainbow trout and those from marine/anadromous hosts differed in 13 to 16 nucleotides (97.8 to 98.2% nucleotide identity).

Kocan *et al.* (2011) mentioned that several different techniques have been employed to detect and identify *Ichthyophonus* spp. in infected fish hosts; these include macroscopic observation, microscopic examination of tissue squashes, histological evaluation, in vitro culture, and molecular techniques. Examination of the peer-reviewed literature revealed that when more than 1 diagnostic method is used, they often result in significantly different results; for example, when in vitro culture was used to identify infected trout in an experimentally exposed population, 98.7% of infected trout were detected, but when standard histology was used to confirm known infected tissues from wild salmon, it detected ~50% of low-intensity infections and ~85% of high-intensity infections. Other studies on different species reported similar differences. When they examined a possible mechanism to explain the disparity between different diagnostic techniques, they observed non-random distribution of the parasite in 3-dimensionally visualized tissue sections from infected hosts, thus providing a possible explanation for the different sensitivities of commonly used diagnostic techniques. Based on experimental evidence and a review of the peer-reviewed literature, they have concluded that in vitro culture is currently the most accurate diagnostic technique for determining infection prevalence of *Ichthyophonus*, particularly when the exposure history of the population is not known

Óskarsson *et al.* (2011) recorded an outbreak of Ichthyophonus infection in the Icelandic summer-spawning herring was first observed in November 2008, consequently a comprehensive research program was launched to estimate its magnitude. These researches continued the two following autumns and winters. The infection rate in the adult part of the stock was estimated to be 32%, 43% and 37% during the autumns 2008-2010, respectively. All existing information from the literature indicate that the infection causes a dead within at maximum six months, while preliminary results for the Icelandic stock indicate that this could take some longer time. Estimates of infection in herring juveniles on the nursery grounds off the NW and N coast over the same period indicated further how widely distributed the infection and the source of the infection was. The consequences of the infection on the development of the stock size are apparent. The increase in the natural mortality has been estimated directly from the infection rate and the estimates should be used in

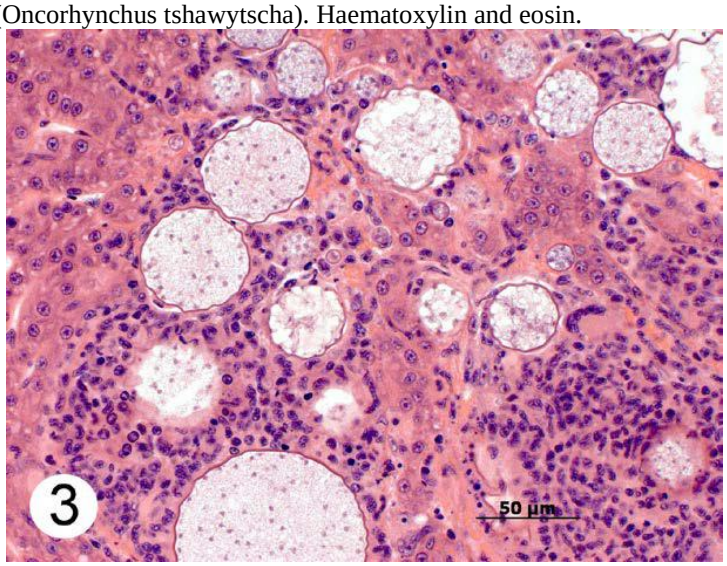
analytical assessment of the stock, at least until the estimates can be verified with some stock assessment software packages some years after the outbreak ceases.

Hamazaki *et al*. (2013) evaluated the comparability of culture and PCR tests for detecting *Ichthyophonus* in Yukon River Chinook salmon *Oncorhynchus tshawytscha* from field samples collected at 3 locations (Emmonak, Chena, and Salcha, Alaska, USA) in 2004, 2005, and 2006. Assuming diagnosis by culture as the 'true' infection status, we calculated the sensitivity (correctly identifying fish positive for *Ichthyophonus*), specificity (correctly identifying fish negative for*Ichthyophonus*), and accuracy (correctly identifying both positive and negative fish) of PCR. Regardless of sampling locations and years, sensitivity, specificity, and accuracy exceeded 90%. Estimates of infection prevalence by PCR were similar to those by culture, except for Salcha 2005, where prevalence by PCR was significantly higher than that by culture ($p < 0.0001$). These results show that the PCR test is comparable to the culture test for diagnosing *Ichthyophonus*infection.

Kocan (2013) mentioned that much of the terminology describing *Ichthyophonus* sp. life stages and structures can be traced to the mistaken classification of this organism as a fungus. This misidentification led early investigators to use mycological terms for the structures they observed; while some terminology is not so easily explained, it appears to have been co-opted from the fields of botany and bacteriology. The purpose of this exercise is to attempt to standardize the terminology associated with *Ichthyophonus* and to bring it into agreement with terminology currently used to define similar life stages of other protists. The proposed changes are (1) spore/macrospore/mother spore to "schizont," (2) microspore/endospore to "merozoite," and (3) pseudohyphae to "hyphae" or "germ tube."

McVicar and Jones (2013) mentioned that a maximum prevalence of Ichthyophonus infection of 78% was reported in adult herring from the Gulf of St Lawrence between 1954 and 1955, with an estimated mortality of 50%. Between 1991 and 1994, an outbreak in herring encompassing the Baltic Sea, the Skagerrak, the Kattegat and the North Sea caused mortality ranging from 1.9% to 8.9% in Swedish waters and between 12.8% and 36% of fish in Danish waters. The latter outbreak was associated with declines of 10% to 20% in catch or population size. The infection is common in Pacific herring, Clupea pallasi Valenciennes in Puget Sound, Washington (70% prevalence) and Prince William Sound, Alaska (27%); An outbreak in plaice may have caused an annual mortality of 50%. However, mortality in demersal fish may be less obvious than in pelagic species such as herring because dead fish are rarely observed. Ichthyophoniasis in adult Chinook salmon, Oncorhynchus tshawytscha (Walbaum) during freshwater migration in the Yukon River is associated with mortality and reduced fillet quality. Gross clinical signs Non-specific signs may include swimming abnormalities, lethargy, emaciation, colour abnormalities, abdominal distension, exophthalmos, and elevated mortality. The appearance and texture of the skin may be altered due to ulcers and a sandpaper roughness. Internally, white or cream-coloured nodules 1 to 5 mm in size may occur in the skeletal or cardiac muscle and in most well-vascularised organs and are most evident in heavily infected fish.

Hyphae-like structures emerging from tissues of Pacific herring (Clupea pallasi) infected with Ichthyophonus. Fresh preparation. Ichthyophonus spherical bodies in skeletal muscle beneath skin of Chinook salmon (Oncorhynchus tshawytscha). Haematoxylin and eosin.



Ichthyophonus spherical bodies in liver of Chinook salmon (Oncorhynchus tshawytscha), with infiltration of inflammatory cells. Haematoxylin and eosin.

White *et al.* (2013) developed a quantitative PCR (qPCR) assay specific for genus Ichthyophonus 18S ribosomal DNA for parasite detection and surveillance. The new assay was tested for precision, repeatability, reproducibility, and both analytical sensitivity and specificity. Diagnostic sensitivity and specificity were estimated using tissue samples from a wild population of walleye pollock Theragra chalcogramma. Ichthyophonus sp. presence in tissue samples was determined by qPCR, conventional PCR (cPCR), and histology. Parasite prevalence estimates varied depending upon the detection method employed and tissue type tested. qPCR identified the greatest number of Ichthyophonus sp.-positive cases when applied to walleye pollock skeletal muscle. The qPCR assay proved sensitive and specific for Ichthyophonus spp. DNA, but like cPCR, is only a proxy for infection. When compared to cPCR, qPCR possesses added benefits of parasite DNA quantification and a 100-fold increase in analytical sensitivity. Because this novel assay is specific for known members of the genus, it is likely appropriate for detecting Ichthyophonus spp. DNA in various hosts from multiple regions. However, species-level identification and isotype variability would require DNA sequencing. In addition to distribution and prevalence applications, this assay could be modified and adapted for use with zooplankton or environmental samples. Such applications could aid in investigating alternate routes of transmission and life history strategies typical to members of the genus Ichthyophonus.

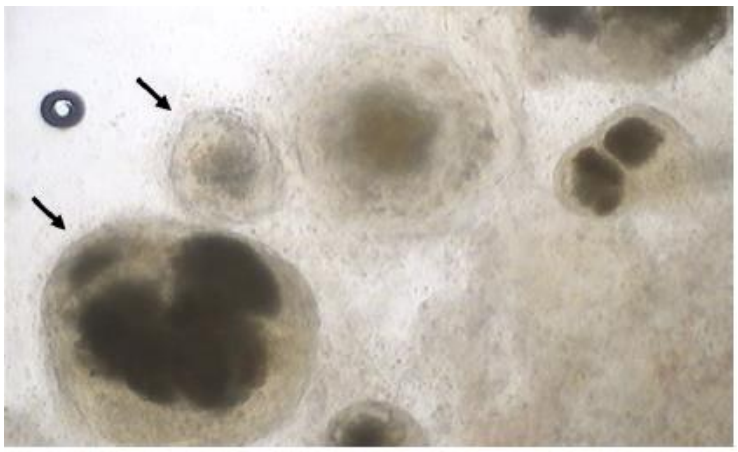
Gregg *et al.* (2014) used a combination of field surveys, molecular typing, and laboratory experiments to improve our 37 understanding of the distribution and transmission mechanisms of fish parasites in the genus Ichthyophonus. Ichthyophonus spp. infections were detected from the Bering Sea to the coast of Oregon in 10 of 13 host species surveyed. Sequences of rDNA extracted from these isolates indicated that an ubiquitous Ichthyophonus type occurs in the NE Pacific Ocean and Bering Sea and accounts for nearly all the infections encountered. Among NE Pacific isolates, only parasites from yellowtail rockfish and Puget Sound rockfish varied at the DNA locus examined. These data suggest that a single source population of these parasites is available to fishes in diverse niches across a wide geographic range. A direct life cycle within a common forage species could account for the relatively low parasite diversity. In the laboratory, the hypothesis that waterborne transmission occurs among Pacific herring , a common NE Pacific forage species. No horizontal transmission occurred during a four- month cohabitation experiment involving infected herring and conspecific sentinels. The complete life cycle of Ichthyophonus spp. is not known, but these results suggest that system-wide processes maintain a relatively homogenous parasite population.

Kocan *et al.* (2014) allowed *Ichthyophonus*-infected Pacific herring, *Clupea pallasii*, to decompose in ambient seawater then serially sampled for 29 days to evaluate parasite viability and infectivity for Pacific staghorn sculpin, *Leptocottus armatus*. *Ichthyophonus* sp. was viable in decomposing herring tissues for at least 29 days post-mortem and could be transmitted via ingestion to sculpin for up to 5 days. The parasite underwent morphologic changes during the first 48 hr following death of the host that were similar to those previously reported, but as host tissue decomposition progressed, several previously un-described forms of the parasite were observed. The significance of long-term survival and continued morphologic transformation in the post-mortem host is unknown, but it could represent a saprozoic phase of the parasite life cycle that has survival value for *Ichthyophonus* sp.

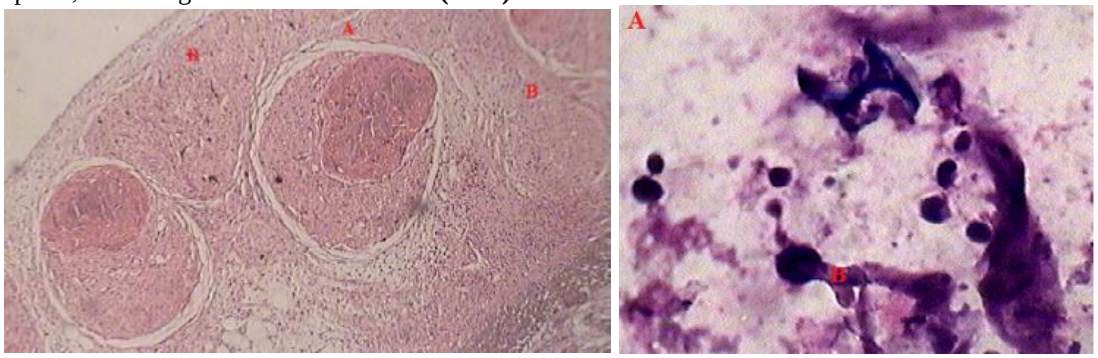
White *et al.* (2014) identified an unspecified parasite in fish muscle in Bering Sea pollock using molecular and histological methods as Ichthyophonus. Infected pollock were identified throughout the study area, and prevalence was greater in adults than in juveniles. This study not only provided the first documented report of Ichthyophonus in any fish species captured in the Bering Sea, but also revealed that the parasite has been present in this region for nearly 20 years and was not a recent introduction. Sequence analysis of 18S rDNA from Ichthyophonus in pollock revealed that consensus sequences were identical to published parasite sequences from Pacific herring and Yukon River Chinook salmon. Results from this study suggested potential for Ichthyophonus exposures from infected pollock via two trophic pathways; feeding on whole fish as prey and scavenging on industry-discharged offal. Considering the notable Ichthyophonus levels in pollock, the low host specificity of the parasite and the role of this host as a central prey item in the Bering Sea, pollock likely serve as a key Ichthyophonus reservoir for other susceptible hosts in the North Pacific.

Zadeh *et al.* (2014) reported Ichthyophonus hoferi from two species of ornamental

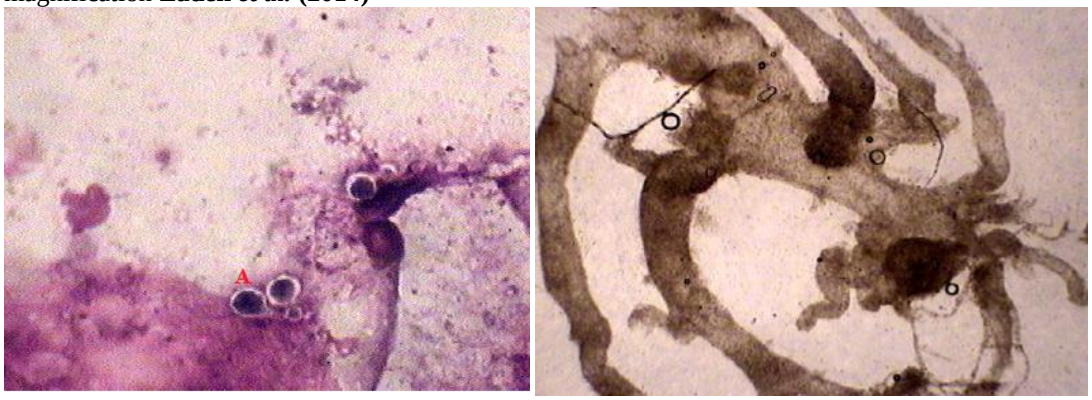
fish, black tetra (Gymnocorymbus ternetzi) and tiger barb (Pentius tetrazona) in Ahvaz-Iran. Examined fishes had marked signs such as abnormal swimming, lethargy, swelling abdominal and low rate mortality. In this study, the two phases of life cycle of I. hoferi involving active and passive detected. The obvious internal sign was white cysts and nodules, which embedded in infected spleens. The cysts were full of schizonts that were surrounded by collagen fibers and many eosinophilic cells. Plasmodium spherical bodies with variable sizes were detected by microscopic examination of wet mount squash from the infected organs. In addition, histopathology studies showed that there were many granulation tissues surrounded by multilayer connective tissues in the infected tissues. Tissue samples were also isolated and put in to Minimum Essential Medium (MEM) to detect the germination of Ichthyophonus hoferi for distinguish Ichthyophoniasis from Mycobacterial infections



Squash preparation from nodules showing thick walled resting schizont in naturally infected tiger barb, spleen, x100 magnification **Zadeh *et al.* (2014)**



A. Encapsulated schizonts. B. Un-encapsulated schizonts, inside well-defined host cellular granulomas in naturally infected tiger barb, spleen, x40 magnification. A. Plasmodium. B. Existing a collapsed Ichthyophonus hoferi schizonts with two plasmodia. Culture media (MEM). Black tetra, spleen, x400 magnification **Zadeh *et al.* (2014)**



Terminating club-shaped cells. Culture media (MEM). Black tetra, spleen, x400 magnification. Non septate germinating tubes from a schizont in culture media (MEM). Black tetra, spleen, x40 magnification. **Zadeh *et al.* (2014)**

References:

1. Abd **El-Ghany, N. A.**; **El-Ashram, A. M. M.** Diagnosis of ichthyophoniasis in *Oreochromis niloticus* in Egypt by polymerase chain reaction (PCR). **From the pharaohs to the future. Eighth International Symposium on Tilapia in Aquaculture. Proceedings. Cairo, Egypt, 12-14 October, 2008** 2008 pp.1307-1328 ref.32
2. ABD EL-GHANY. N., AND HODA M. L. ABD. A TRIAL FOR TREATMENT OF ICHTHYOPHONOSIS IN CULTURED *OREOCHROMIS NILOTICUS* USING FUCUS AND NEEM PLANTS, 8th *int. Symposium on Tilapia in Aquaculture, 2008*
3. Anonymous 1991. Result of Fish Health Surveys: *Ichthyophonus hoferi*. The Ichthyogram Newsletter of the Fisheries Experiment Station Utah Division of Wildlife Resources 2(1): 2-3.
4. Athanassopoulu, F. 1992. Ichtyophoniasis in sea bream, *Sparus aurata* (L.), and rainbow trout, *Oncorhynchus mykiss* (Walbaum), from Greece. Journal of Fish Diseases 15:437-441.
5. Chien,C. H. and Yu,T. C. (1978):Infection of *Ichthyophonus* sp. (fungus) in Japanese eel (*Anguilla japonica*). JCRR.Fish.-Ser., 34, 83-88.
6. Chun, S.K. and Kim, Y.G.(1981) Infection by an *Ichthyophonus* sp. fungus in rainbow trout *Salmo gairdneri* . Bull. Korean Fish. Soc.14, (1) 37-42
7. Dorier, A., and C. Degrange. 1961. L'évolution de l'Ichthyosporidium (Ichthyophonus) hoferi (Plehn et Mulsow) chez les Salmonides d'élevage (Truite arc en ciel et Saumon de fontaine). Trav. Lab. Hydrobiol. Piscicult. Univ. Grenoble, 1960/1961: 7–44.
8. Erickson J. D. 1965. Report on the problem of *Ichthyosporidium* in rainbow trout. Prog. Fish Cult. 27:179-184.
9. Faisal,M.; Torky,H. and Reichenbach-Klinke,H.H (1985): A note on swimming disease among the Labyrinth catfish (*Clarias lazera*).J. Egypt. Assiut Vet. Med., 45, 53-60.
10. Fijan,N. and Maron, B. (1977):A case of ichthyosporidiosis in rainbow trout.Veterinarski Arhiv., 46, 65-67
11. Fish, F.F. (1934). **A fungus disease in fishes of the Gulf of Maine.** Parasitology 26(1):1-16
12. Franco-Sierra A, Sitjà-Bobadilla A, Alvarez-Pellitero P (1997) Ichthyophonus infections in cultured marine fish from Spain. J Fish Biol 51: 830-839
13. Gavryuseva T. V. (2007). First report of Ichthyophonus hoferi infection in young coho salmon Oncorhynchus kisutch (Walbaum) at a fish hatchery in Kamchatka. Russ. J. Marine Biol. 33, 43–48
14. Gregg, J.L., C.A. Grady, R.L. Thompson, M.K. Purcell, C.S. Friedman, and P.K. Hershberger. 2014. 57 Distribution and transmission of the highly pathogenic parasite Ichthyophonus in marine fishes of Alaska. 58 North Pacific Research Board Project #1015, Final Report, 46pp.
15. Hamazaki T., Kahler E., Borba B. M., Burton T. (2013). PCR testing can be as accurate as culture for diagnosis of *Ichthyophonus hoferi* in Yukon River Chinook salmon *Oncorhynchus tshawytscha*. Dis. Aquat. Organ. 105, 21–25
16. Hassan Rahimian. Pathology and morphology of Ichthyophonus hoferi in naturally infected fishes of the Swedish west coast. Dis Aquat. Org.34, 109-123,1998
17. Hershberger P. K., Stick K., Bui B., Carroll C., Fall B., Mork C., et al. (2002). Incidence of*Icthyophonus hoferi* in Puget Sound fishes and its increase with age of Pacific Herring. J. Aquat. Anim. Health 14, 50–56
18. Hershberger. P. K. 3.2.18 Ichthyophonus Disease (Ichthyophoniasis) – 1, http://afs-fhs.org/perch/resources/14069249443.2.18ichthyophonus201
19. Hofer, B. (1893). Eine Salmoniden-Erkrankung. *Allgemeine Fishchereizeitung* 18, 168–171.
20. ICES IDENTIFICATION LEAFLETS FOR DISEASES AND PARASITES OF FISH AND SHELLFISH Leaflet No. 3 Ichthyophonus, a systemic mesomycetozoan pathogen of fish Original by A. H. McVicar Revised and updated by S. R. M. Jones, 2013
21. Jones SRM, Dawe SC (2002) Ichthyophonus hoferi Plehn & Mulsow in British Columbia stocks of Pacific herring, Clupea pallasi Valenciennes, and its infectivity to chinook salmon, Oncorhynchus tshawytscha (Walbaum). J Fish Dis 25: 415-421.

22. Kocan, R., H. Dolan and P. Hershberger (2011). **Diagnostic methodology is critical for accurately determining the prevalence of** *Ichthyophonus* **infections in wild fish populations.** J Parasitol **97**(2): 344-348
23. Kocan RM (2013) Proposed Changes to the Nomenclature of Ichthyophonus sp. Life-Stages and Structures. J Parasitol 99: 906-909.
24. Kocan, R., Lucas Hart, Naomi Lewandowski, and Paul Hershberger, Viability and Infectivity of *Ichthyophonus* sp. in Post-Mortem Pacific Herring, *Clupea pallasii*. Journal of Parasitology 100(6):790-796. 2014
25. McVicar A. H. 1999. *Ichthyophonus* and related organisms. *In* P.T.K. Woo and D.W. Bruno, editors. Fish Diseases and Disorders. Viral, Bacterial and Fungal Infections, Vol. 3. CABI Publishing, New York. pp 661-687.
26. Miyazaki, T., S. S. Kubota. 1977. Studies on *Ichthyophonus* disease of fishes – I. Rainbow trout fry. Bulletin of the Faculty of Fisheries, Mie University 4:45-56.
27. Mendoza L, Taylor JW, Ajello L. The class mesomycetozoea: a heterogeneous group of microorganisms at the animal-fungal boundary. Annu Rev Microbiol. 2002;56:315-44. Epub 2002 Jan 30.
28. Óskarsson, G. J. and J. Pálsson (2011). **The** *Ichthyophonus hoferi* **outbreak in the Icelandic summer-spawning herring stock during the autumns 2008 to 2010.** Int Counc Explor Sea. WKBENCH 2011, WD Her-Vasu No. 2: 17 pp.
29. Plehn, M. & Mulsow, K. (1911). Der Erreger der ‘‘ Taumelkrankheit ’’ der Salmoniden. *Zentralblatt fu¨r Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* 59, 63–68.
30. Ragan MA, Goggin CL, Cawthorn RJ, et al. A novel clade of protistan parasites near the animal-fungal divergence. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;93(21):11907-11912.
31. Ragan MA, Murphy CA, Rand TG. Are Ichthyosporea animals or fungi? Bayesian phylogenetic analysis of elongation factor 1alpha of Ichthyophonus irregularis. Mol Phylogenet Evol. 2003 Dec;29(3):550-62.
32. Rasmussen C, Purcell MK, Gregg JL, LaPatra SE, Winton JR, Hershberger PK. Sequence analysis of the internal transcribed spacer (ITS) region reveals a novel clade of Ichthyophonus sp. from rainbow trout. Dis Aquat Organ. 2010 Mar 9;89(2):179-83.
33. Rucker, R.R., and P.V. Gustafson. 1953. An epizootic among rainbow trout. Prog. Fish-Cult. 15: 179–181.
34. Schmidt-Posthaus, H., T. Wahli. 2002. First report of *Ichthyophonus hoferi* infection in wild brown trout (*Salmo trutta*) in Switzerland. Bull Eur. Ass. Fish Pathol. 22:225-228.
35. Sinderman, C. J. & Scatergood, L. W. (1954). *Icthyosporidium* disease of the sea herring (*Clupea harengus*). *Maine Department of Sea Shore Fisheries Research Bulletin* 18,
36. Spanggaard, B., Gram, L., Okamoto, N. & Huss, H. (1994). Growth of the fish-pathogenic fungus, *Ichthyophonus hoferi*, measured by conductimetry and microscopy. *Journal of Fish Diseases* 17, 145–153.
37. White VC, Morado JF, Crosson LM, Vadopalas B, Friedman CS. Development and validation of a quantitative PCR assay for Ichthyophonus spp. Dis Aquat Organ. 2013 Apr 29;104(1):69-81..
38. White VC, Morado JF, Friedman CS. Ichthyophonus-infected walleye pollock Theragra chalcogramma (Pallas) in the eastern Bering Sea: a potential reservoir of infections in the North Pacific. J Fish Dis. 2014 Jul;37(7):641-55.
39. Zadeh MJ, Peyghan R, Manavi SE (2014) The Detection of Ichthyophonus hoferi in Naturally Infected Fresh Water Ornamental Fishes. J Aquac Res Development 5: 289. doi:10.4172/2155-9546.1000289

8. Lagenidium

Historical :

- **Couch (1942)** described Lagenidium callinectes as being parasitic in ova of the blue crab, Callinectes sapidus.

John Nathaniel Couch (1896-1986)

- **Rogers-Talbert (1948)** described the fungus as a peripheral parasite of the egg masses. She noted that the eggs were susceptible to infection in all stages of development.
- **Johnson and Bonner (1960)** reported the occurrence of the same fungus on lamellae of the barnacle, Chelonibia patula.
- **Scott (1962),** in a survey of the phycomycetous fungi of marine and brackish waters in the vicinity of Gloucester Point, Va., reported that 40 percent ofthe blue crab egg masses collected were infected with Lagenidium callinectes.
- **Bland and Amerson (1973)** surveyed over 2,000 ovigerous crabs during the summer of 1971 and obtained isolates of L. callinectes with which they performed a detailed morphological study.
- **Lightner and Fontaine (1973)** observed that a Lagenidium sp. was infective to larval white shrimp, Penaeus seti/erus, and a brown shrimp, Penaeus aztecus, reared under laboratory conditions. Natural mortality occurred in 12.4 percent of the shrimp after the fungal mycelium had invaded and replaced nearly all the internal tissues, while 20.0 percent of the larval shrimp died after experimental exposure to the fungus
- **Hatai and Lawhavinit (1988**) found ***Lagenidium myophilum*** **Hatai & Lawhavinit** in the abdominal muscle of adult northern shrimp, *Pandalus borealis* Kroyer, has only been reported in Japan
- **Hatai (1991)** reported *L. myophilum* infection in larvae of coonstripe shrimps, artificially produced at Hokkaido Institute of Mariculture, Hokkaido. Mortality was 100% (, unpublished).
- In 1993, a fungal infection occurred in juvenile coonstripe shrimps which had been reared in tanks after seed production. Mortality was about 70%.

A NEW FUNGUS ON CRAB EGGS

By John N. Couch

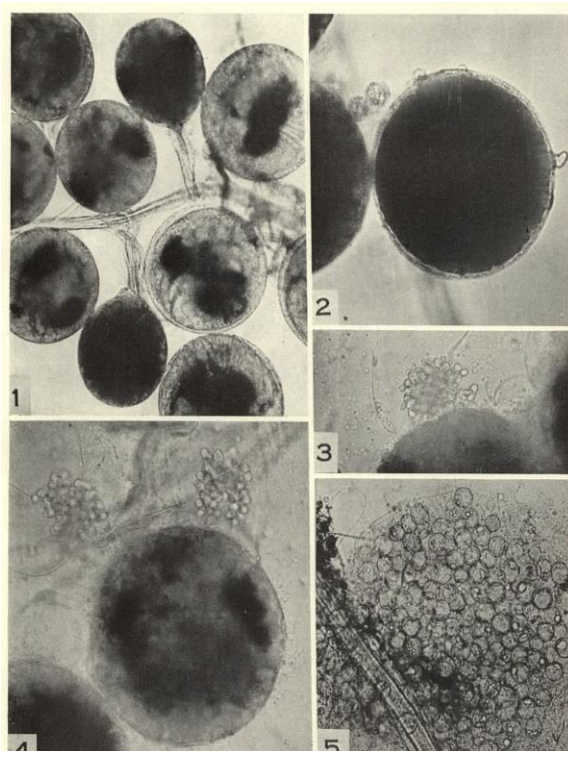
Plates 18 and 19

For two successive seasons Dr. C. L. Newcombe, Director of the Virginia Fisheries Laboratory, has sent me eggs of the blue crab (*Callinectes sapidus*) parasitized by fungi. The first season (fall, 1941) the eggs had been kept several weeks in sea water before they were sent and no indications of a parasitic fungus could be detected, except that on some of the disintegrated eggs the empty spherical zoosporangia of an organism resembling *Rhizophidium* were found. Again this past summer Dr. Newcombe and his associates noticed that some of the crab eggs failed to hatch and again, suspecting a parasitic fungus, material was sent to me for examination. This time a considerable number (2–5%) of the eggs were infected by an internal fungus which apparently belongs in the genus *Lagenidium*. Also a considerable number of the newly hatched living crabs had the mycelium in their bodies. With living material at hand it was possible to work out most of the details in the life history of the fungus and the stages as observed in the living but rather stale material were corroborated for the most part by material preserved in formalin immediately after removing the eggs from the crab. This fungus so far as I have been able to ascertain is the first member of the Lagenidiales which has been described from a marine habitat.

Lagenidium callinectes n. sp.

Mycelium developing entirely within the egg and eventually pretty well filling the egg, or body of the young crab, consisting of branched irregular hyphae which are sparingly septate, 5.4–12.6μ thick. Protoplasm with a pale whitish gleam and numerous oil bodies, becoming coarsely granular just before spore formation. In the process of spore formation and discharge the end of a thread becomes applied to the inner egg wall and swells to form a clavate structure up to 30μ thick. The part in contact with the wall forms a narrow tube which grows through the wall and immediately thickens and elongates to form a large tube 11–29 × 25–70μ. The tip of the tube gelatinizes and protoplasm flows out from the mycelium to collect into a spherical or subspherical undifferentiated mass surrounded by a thick gelatinous envelope. Protoplasmic mass up to 100μ thick. Spores formed as in *Pythium* from this mass of protoplasm, and becoming very active within the gelatinous layer before discharge. When mature breaking through the outer gelatinous membrane or vesicle and swimming sluggishly away. Vesicle persistent. Spores pointed at the front end and rounded behind with a diagonal groove which arises near the front end and extends backward over the rounded end. Cilia arising from this groove and one extending forward, the other backward while swimming. Zoospores about 9.6 × 12.6μ, with several oil globules one of which is distinctly larger than the others. Encysted spores oblong or subglobose, 10 × 11.3μ; monoplanetic. Resting bodies formed only after the crab eggs begin to disintegrate, apparently formed asexually in the threads, spherical, subspherical or oval, 18–30μ, thick, usually about 25μ thick, wall up to 3μ thick, containing pale whitish protoplasm and an eccentric mass of oil bodies; germination not observed.

Found growing parasitically on eggs and newly hatched young of Callinectes (common blue crab of eastern seaboard) collected by Mrs. Mildred Sandoz, July 7, 1942, East Lynnhaven, Virginia, and sent to me through the courtesy of Dr. C. L. Newcombe.



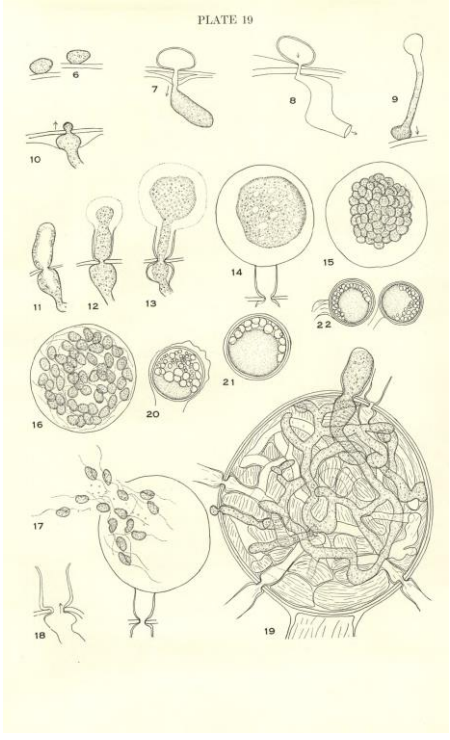


PLATE 18

Fig. 1. Part of a cluster of eggs of *Callinectes*, showing central stalk to which eggs are attached, the two smallest ones parasitized by the fungus *Lagenidium*. × 100.

Fig. 2. One parasitized egg showing exit tubes of sporangium to right and empty cyst of infecting spore above. × 200.

Fig. 3. Cluster of zoospores in vesicle which is indistinctly visible. × 200.

Fig. 4. Parasitized egg showing two clusters of zoospores forming within vesicle; vesicle not visible. × 200.

Fig. 5. Resting bodies in egg, the membrane of which is visible above. × 200.

PLATE 19

(Figures were inked by Else R. Couch)

Fig. 6. Spores encysted on egg membrane. × 585.

Fig. 7. Spore cyst empty, germ tube elongating. × 1000. Fig. 8. Later stage of same.

Fig. 9. Spore settled not in contact with egg membrane, sprouted long tube which formed appressorium against egg membrane. × 585.

Fig. 10. Early stage in formation of exit tube. × 635.

Figs. 11–17. Stages in discharge of protoplasm into vesicle and formation and discharge of spores. ×290.

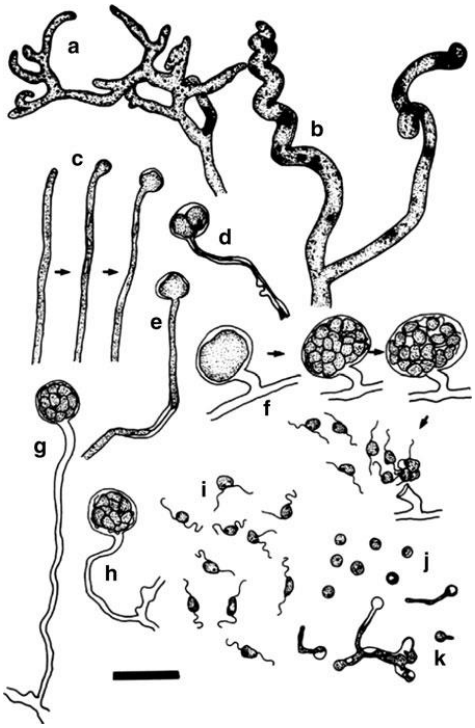
Fig. 18. Empty exit tube. × 635.

Fig. 19. Crab egg heavily infected with fungus. Note branched rarely septate mycelium; one sporangium at top about to discharge its contents; and four empty exit tubes. Semi-diagrammatic. × 290.

Figs. 20–22. Resting bodies formed after seven days. × 635.

Description:

Lagenidium callinectes **Couch, 1942**



L. callinectes isolated from an egg of P. pelagicus. Scale ¼ 50 mm. (a) Irregularly branched hyphae with numerous shiny rod granules; (b) Coiled hyphae in PYGS broth; (c) Vesicle formation; (d, e) Protoplasmic masses flow into the vesicle with a protoplasmic thread; (f) Division into initial zoospores and zoospores liberation; (g, h) Mature vesicles; (i) Zoospores; (j) Encysted zoospores; (k) Germination (**Nakamura and Hatai 1995a)**

Classification

NCBI Taxonomy

Cellular organisms +

- Eukaryota +
  - Stramenopiles +
    - Oomycetes +
      - Lagenidiales +
        - Lagenidiaceae +
          - Lagenidium +
            - ***Lagenidium callinectes***
            - Lagenidium aff. deciduum strain LEV5864
            - *Lagenidium caudatum*
            - *Lagenidium chthamalophilum*
            - *Lagenidium deciduum*
            - *Lagenidium giganteum* +
            - *Lagenidium humanum*
            - *Lagenidium myophilum*
            - *Lagenidium thermophilum*
            - Unclassified Lagenidium +

Index Fungorum

Lagenidium Schenk, 1857

- *Lagenidium americanum* G.F. Atk. 1909
- Lagenidium astrum S. N. Dasgupta & R. John 1990

- *Lagenidium brachystomum* Scherff. 1925
- *Lagenidium callinectes* Couch, 1942
- *Lagenidium canterae* Karling 1981
- *Lagenidium caudatum* G. L. Barron 1976
- *Lagenidium chthamalophilum* T. W. Johnson 1958
- Lagenidium clavatum S. N. Dasgupta & R. John 1990
- *Lagenidium closterii* De Wild. 1893
- Lagenidium coenocyticum S. N. Dasgupta & R. John 1990
- Lagenidium contortum S. N. Dasgupta & R. John 1990
- *Lagenidium cyclotellae* Scherff. 1925
- Lagenidium cylindriforme S. N. Dasgupta & R. John 1990
- *Lagenidium destruens* Sparrow 1950
- *Lagenidium distylae* Karling 1945
- *Lagenidium elegans* (Perronc.) Cif. 1962
- *Lagenidium ellipticum* De Wild. 1893
- *Lagenidium enecans* Zopf 1884
- *Lagenidium entophytum* (Pringsh.) Zopf 1878
- Lagenidium entosphaericum S. N. Dasgupta & R. John 1990
- *Lagenidium giganteum* Couch 1935
- *Lagenidium globosum* Lindst. 1872
- *Lagenidium gracile* Zopf 1884
- *Lagenidium humanum* Karling 1947
- *Lagenidium intermedium* De Wild. 1895
- *Lagenidium lundiae* Karling 1981
- *Lagenidium lundii* Karling 1981
- *Lagenidium marchalianum* De Wild. 1897
- *Lagenidium microsporum* Karling 1945
- *Lagenidium muenscheri* Cutter 1943
- *Lagenidium myophilum* Hatai & Lawhav. 1988
- Lagenidium netrii C. E. Mill. 1965
- Lagenidium nodosum (P. A. Dang.) Ingold 1949
- Lagenidium obovatum S. N. Dasgupta & R. John 1990
- *Lagenidium oedogonii* Scherff. 1902
- *Lagenidium oophilum* Sparrow 1939
- *Lagenidium oviparasiticum* G. L. Barron 1989
- *Lagenidium papillosum* Cocc. 1894
- *Lagenidium parthenosporum* Karling 1945
- Lagenidium podbielkowskii A. Batko 1973
- *Lagenidium proliferum* (Schenk) Lindst. 1872
- *Lagenidium pygmaeum* var. *pygmaeoides* Karling 1981
- *Lagenidium pygmaeum* var. *pygmaeum* Zopf 1888
- *Lagenidium pygmaeum* Zopf 1888
- Lagenidium pyriforme S. N. Dasgupta & R. John 1990
- *Lagenidium pythii* Whiffen 1946
- *Lagenidium rabenhorstii* Zopf 1878
- *Lagenidium reductum* (De Wild.) Karling 1942
- *Lagenidium sacculoides* Serbinow 1925
- *Lagenidium scyllae* Bian, Hatai, Po & Egusa 1979
- *Lagenidium septatum* Karling 1969
- *Lagenidium syncytiorum* Kleb. 1892
- *Lagenidium thermophilum* K. Nakam., Miho Nakam., Hatai & Zafran 1995
- Lagenidium tortum S. N. Dasgupta & R. John 1990
- *Lagenidium zopfii* De Wild. 1890

Diagnostic techniques

Gross Observations: Appendages or body filled with white mycelia, vegetative fruiting structures visible under dissecting microscope.

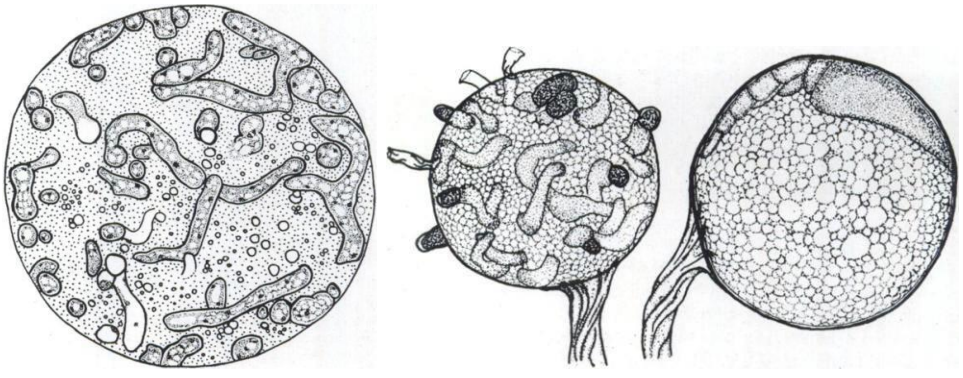
Culture: On saline mycological media. Microscopy may be necessary for specific indentification.

Methods of control

Disease probably related to poor husbandry and can be prevented by enhanced cleanliness.

Reports:

ROGERS-TALBERT (1948) carried out laboratory and field studies: (1) to ascertain the conditions of existence of Lagendium parasite in the individual crab eggs as well as on and in the egg mass; (2) to show how readily infection may be trans mitted under certain conditions; (3) to indicate the effects of salinity and temperature on the survival and development of the fungus; and (4) to show the areas of Chesapeake Bay in which it occurs and the approximate degree of infection.The description of the life history of Lagenidium callinectes Couch (1942) has been a valuable aid in this study. In his observations of the organism Couch found that when germination of the zoospore begins, a delicate germ tube is sent through the egg membranes. This tube grows rapidly into a network of branched mycelium that soon fills the entire egg. From the mycelium, stumpy, thumb-like projections, or hyphae, pass through the egg membranes to the outside. These hyphae quickly mature into sporangia which rupture and discharge new spores to continue the cycle of infection. When the nutrient material of the egg has been exhausted by the fungus, the mycelium appears to break up into heavy walled, rest ing cells that seem to be resistant to adverse conditions. However, neither germi nation of these cells nor a sexual phase of reproduction has yet been observed. In fected eggs soon give definite indication of being abnormal; they are opaque and dwarfed, the diameter becoming reduced from about 290 micra to approximately 231 micra.



Cross section of a blue crab egg parasitized by Lagenidium callinectes, showing extensive enternal mycelium (400X). Two blue crab eggs from a single pleopod filament (200 X). The parasitized egg (left) demonstrates 8 external hyphae and 3 empty exit tubes. Internal mycelium is seen through the transparent egg membranes. Parasitized egg shows reduction in size. **ROGERS-TALBERT (1948)**

Lightner and Fontaine (1973) described a primary mycosis of larvae of the white shrimp, *Penaeus setiferus*. The disease first became apparent in larvae in the second

protozoeal stage and disappeared as the shrimp reached the first mysis stage. Affected shrimp became immobilized by near complete tissue destruction and replacement by the expanding mycelium. The fungus was found to be *Lagenidium* sp. and was infective to larval brown shrimp, *Penaeus aztecus*.

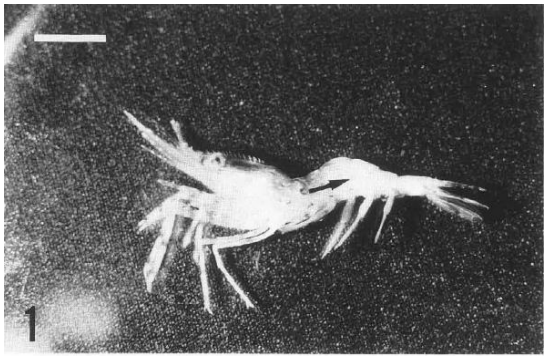
Bian *et al.* (1979) described and illustrated *Lagenidium scyllae* , a marine mastigomycete from the ova and larvae of the mangrove crab, as new. The fungus grew at a temperature range of 16-42 C, with an optimum at 22.5-31.8 C. It grew well in peptone-yeast-glucose (PYG) broth containing 2-3% NaCl. In PYG-sea water medium, it grew at a pH range of 5-11.

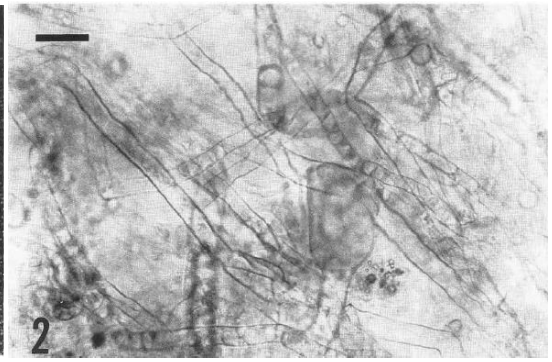
Hatai and Lawhavinit (1988) reported a fungal infection that occurred in juvenile coonstripe shrimps,*Pandalus hypsinotus*, cultured at Hokkaido Institute of Mariculture, Hokkaido, Japan. The fungus was identified as*Lagenidium myophilum*, the same fungus that had previously been isolated from the abdominal muscle of adult northern shrimps,*Pandalus borealis*, and larvae of the coonstripe shrimp. Histopathologically, numerous nonseptate hyphae were observed in the lesions, and melanized hemocytes were present within the blackened areas. The optimum temperature for growth of the present strain was 25–30°C, and the optimum NaCl concentration for growth was 0.5–1.0%. Its biological characteristics were compared with those of*Lagenidium myophilum* isolated from diseased larval coonstripe shrimp and adult northern shrimp. The fungus was pathogenic toward shrimps of the genus*Pandalus*, which live in deep sea areas. The fungus could infect shrimps at various stages, from larva to adult.

Crisp *et al.* (1989) described quantification and analysis of differences among eight isolates of L. callinectes grown under standardized conditions. The study included morphological comparisons (spore size, vesicle size, and eleven hyphal growth characters) of the isolates during major points in their life cycles, as well as an analysis of physiological characteristics of isolates of L. callinectes and, for comparison, Haliphthoros milfordensis. Although results of the morphological evaluation were inconclusive, computerized clustering analysis of physiological characters grouped the isolates in four distinct subgroups, with the isolates in each subgroup occurring in similar geographic regions. Because of overall morphological similarity among the isolates, previously reported physiological differences were not deemed sufficient for recognition of separate species within the L. callinectes "complex." For this reason, the original description of L. callinectes is modified to reflect observed variation among isolates.

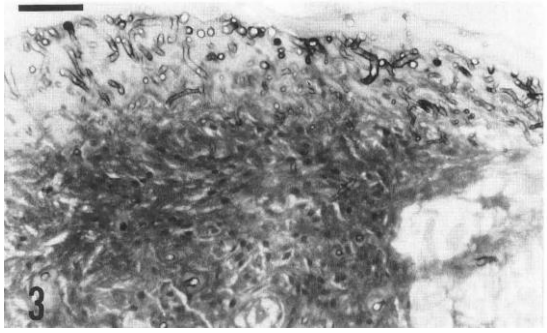
Nakamura *et al.* (1994) reported a fungal infection that occurred in juvenile coonstripe shrimps, *Pandalus hypsinotus*, cultured at Hokkaido Institute of Mariculture, Hokkaido, Japan. The fungus was identified as *Lagenidium myophilum*, the same fungus that had previously been isolated from the abdominal muscle of adult northern shrimps, *Pandaius borealis*, and larvae of the coonstripe shrimp. Histopathologically, numerous nonseptate hyphae were observed in the lesions, and melanized hemocytes were present within the blackened areas. The optimum temperature for growth of the present strain was 25–30°C, and the optimum NaCI concentration for growth was 0.5–1.0%. Its biological characteristics were compared with those of *Lagenidium myophilum* isolated from diseased larval coonstripe shrimp and adult northern shrimp. The fungus was pathogenic toward shrimps of the

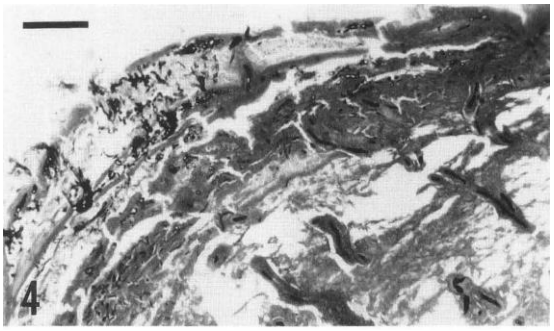
genus *Pandalus*, which live in deep sea areas. The fungus could infect shrimps at various stages, from larva to adult.



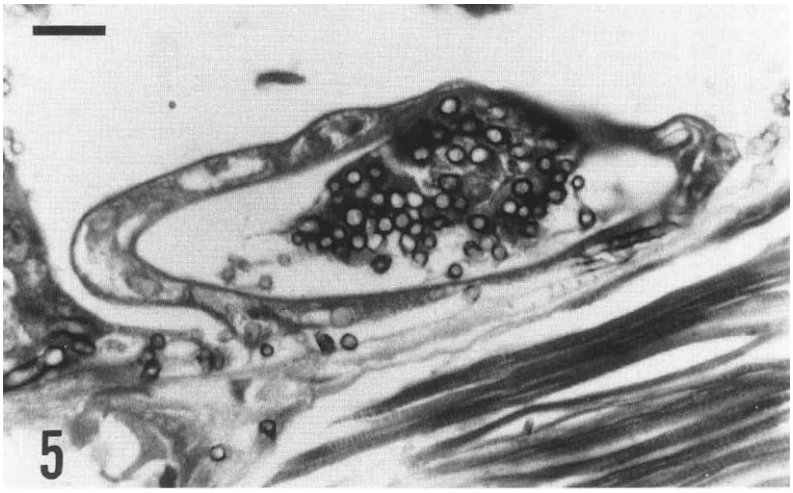


Gross appearance of the diseased coonstripe shrimp, *Panda/us hypsinotus.* Note the muscle with whitish color (arrowl. Scale: 42 mm. Hyphae observed in the whitened muscle of the infected shrimp, **Nakamura *et al.* (1994)**

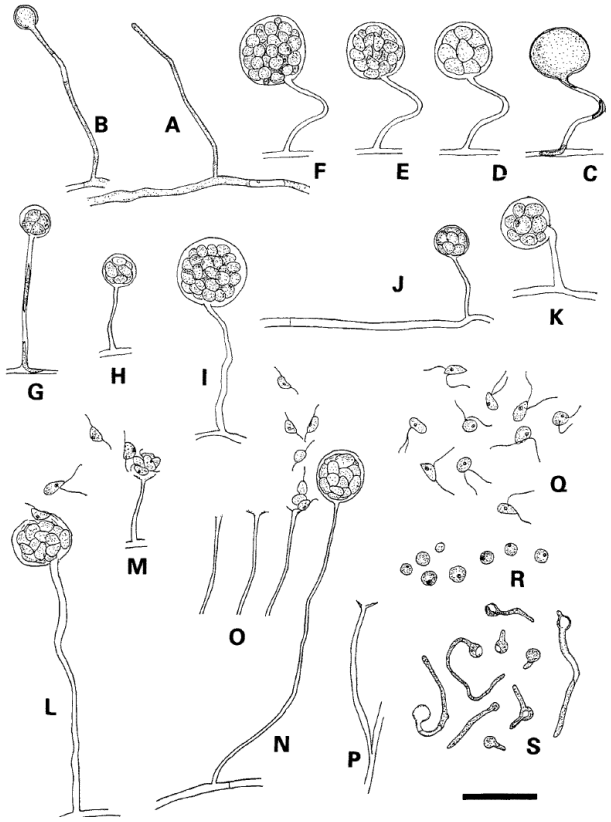




Many hyphae observed in the whitened muscle. Grocott stain. Many hyphae observed in the blackened area. Grocott-H & E stains. **Nakamura *et al.* (1994)**



Hyphae observed in the blood vessels. Grocott stain Nakamura *et al.* (1994)



Zoospore formation of *L. myophilum* NJM 9331 isolated from juvenile coonstripe shrimp. A. Discharge tube formation from hypha; B. Vesicle formation; C-F. Zoospore formation in a vesicle; G. Vesicle formation; H-K. Matured vesicles; L-P. Releace of zoospores; Q. Swimming zoospores, laterally biflagellate; R. Encysted zoospores; S. Germination. Scale: 50 µm. Nakamura *et al.* (1994)

Nestrud and Anderson (1994) exposed 11 fresh water species to a zoosporeproducing fungus, Lagenidium giganteum, with the goal of determining species sensitivity with standard and new test procedures. The tests included standard,

4-day acute exposures of cladocerans (Ceriodaphnia dubia, Daphnia pulex, and D. magna) and the fathead minnow (Pimephales promelas). Standard 7-day chronic exposures of C. dubia and a 7-day embryo-larval exposure of P. promelas were also conducted. New, 4-day acute, methods were developed for mosquitos (Aedes aegypti), chironomids (Chironomus sp.), oligochaetes (Lumbriculus sp.), cyclopoid copepods, snails (Physa sp.), hydrozoans (Hydra sp.), and ostracods. To assess L. giganteum zoospore (z) infectivity, each test included daily bioassays with the mosquito (A. aegypti), a target organism. Four-day A. aegypti LC50s ranged from 81 to 516 z/ml. Ceriodaphnia dubia acute test LC50s were as low as 6700 z/ml and the 96-hr LC50 from the chronic test was near 6250 z/ml with reproductive impairment at 12,500 z/ml. Daphnia sp. were also susceptible, with LC50s near 7700 z/ml for D. pulex and 9400 z/ml for D. magna. Chironomus tentans was infected at concentrations of > or = 5000 z/ml, but mortality was low and an LC50 could not be calculated even after exposures to 50,000 z/ml. The 7-day, early life stage test with P. promelas produced reduced larva growth in most treatments. Several species (Hydra sp., L. variegatus, ostracoda, copepoda, Physa sp., and P. promelas) were not affected in acute tests at exposures of 50,000 z/ml. The data show, contrary to many reports, that L. giganteum may affect some nontarget aquatic species. The key to successful laboratory tests is monitoring and maintaining the zoospores infection capacity.

Nakamura *et al.* (1995) classified a fungal infection occurred in the eggs and larvae of mangrove crab (*Scylla serrata*) in seed production in Bali, Indonesia. The causative fungus as a member of the genus*Lagenidium* (Oomycetes, Lagenidiales). After comparison of its biological and physiological characteristics with those of*L. callinectes* ATCC 24973, a known parasite of various crustaceans, was concluded that the isolate is a new species of*Lagenidium, L. thermophilum*, because of its rapid and thermotolerant growth and unique discharge process. Fungal growth was observed on PYG agar containing 0–5.0% (w/v) NaCl and 0–2.5% (w/v) KCI. Similar pathogenicity toward the zoeae of swimming crab (*Portunus trituberculatus*) was demonstrated

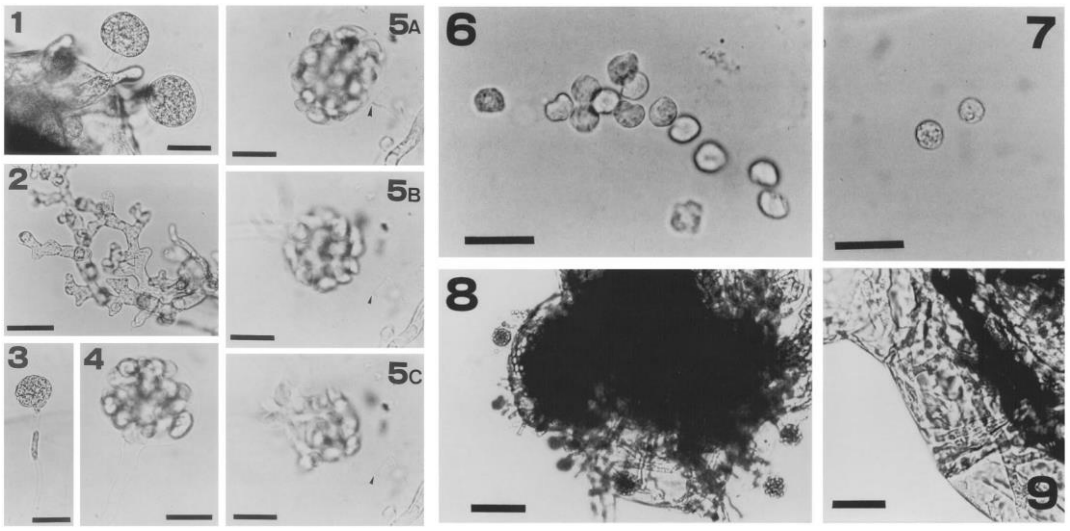
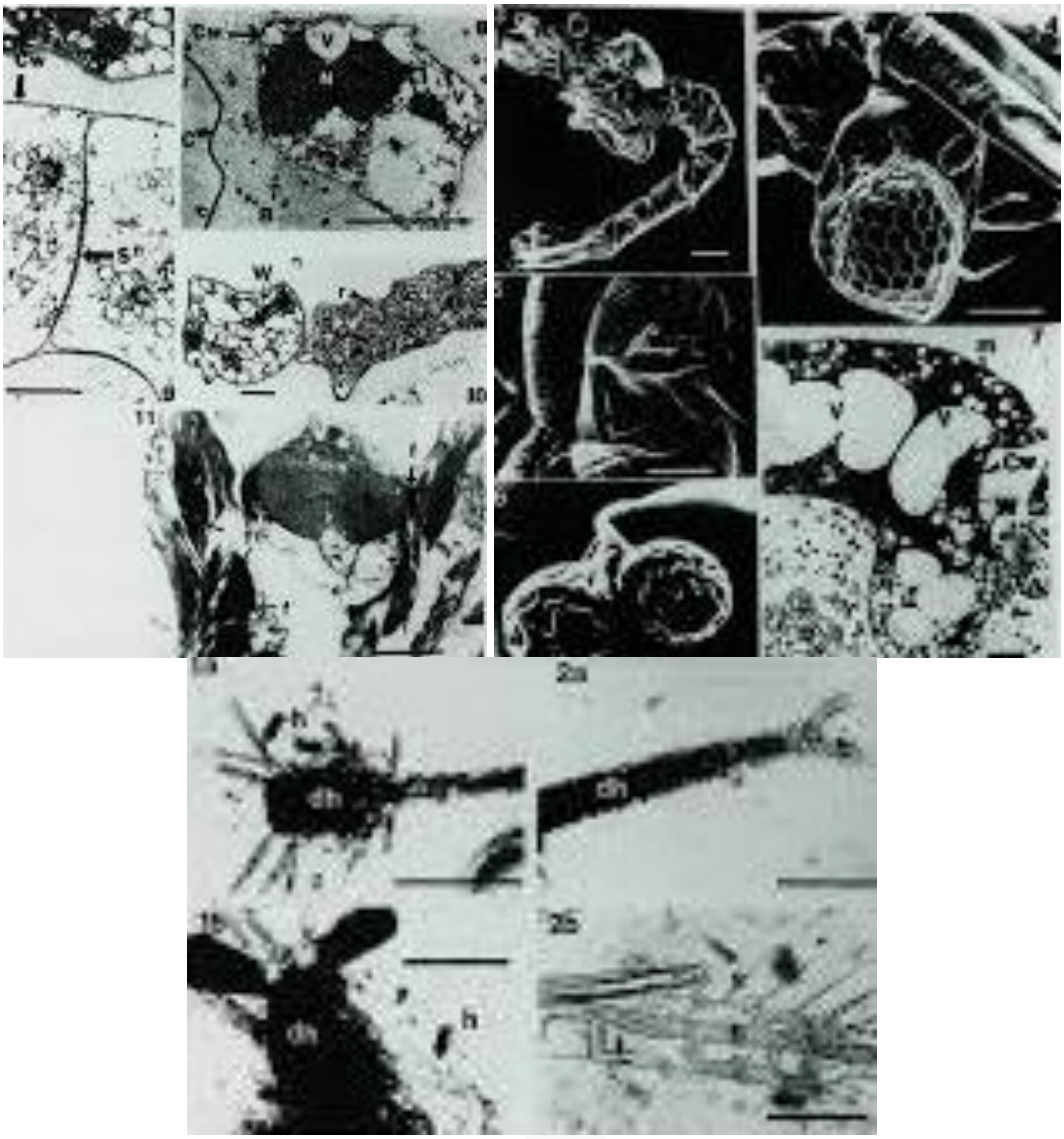


Fig. 1. A spontaneously infected zoea of mangrove crab, *Scylla serrata*. Vesicles formed on the orifices of the discharge tubes.

Figs. 2–5. Morphology of *Lagenidium thermophilum* NJM 9338.
2. Vegetative hyphae in PYGS broth. 3. Vesicle formation. 4. Mature vesicle. 5. Zoospore liberation. A. Mature vesicle. B. Vesicle separated from the discharge tube (arrow head). C. Zoospores released from a vesicle. Scales: 30 μm in Figs. 2, 4 and

Nakamura *et al.* (1995)

Ramasamy *et al.* (1996) described mycosis in larval tiger prawn, *Penaeus monodon* Fabricius, for the first time from India. The hyphae of *Lagenidium callinectes* are contorted, irregularly branched, sparingly septate, and contain a cell wall and membrane, vacuoles, mito chondria, ribosomes, small and large vesicles, and Woronin bodies. The spores occur singly or in pairs. The fungal mycelium may either invade and embed itself in the tissues, or alternatively, replace all the muscle tissues of the infected larval *P. monodon.* Fungus infected, untreated populations of nauplii, zoea and myksis exhibited mortalities of 5.33 ± 0.55%, 24.68 ± 4.58% and 47.89 ± 0.27%, respectively. A 0.5 ppm treatment with trifluralin significantly reduced the mortality of infected larval populations (i.e. 1.1% nauplii, 3.28% zoea and 5.21% myksis mortality). *Lagenidium* sp. exhibited growth in potato dextrose agar medium and in Sabouraud's agar at 28 °C.

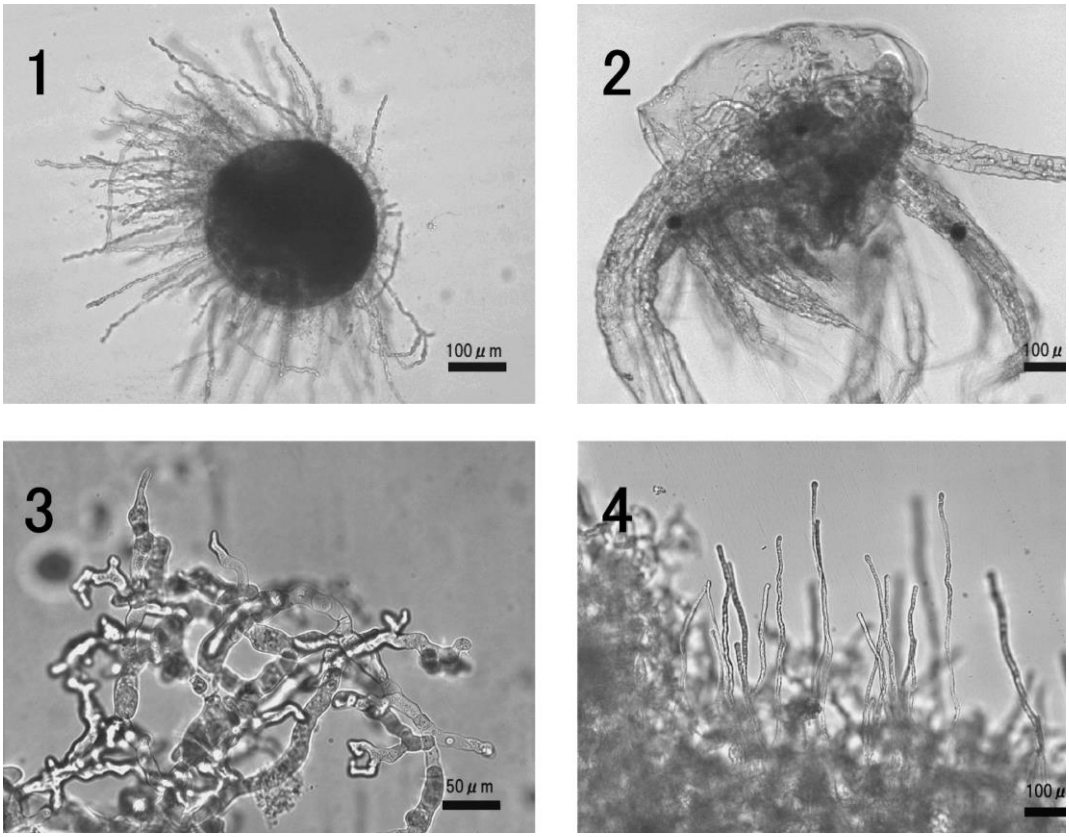


Ramasamy *et al.* (1996)

Hatai *et al.* (2000) mentioned that Since 1992, seed production of mangrove

crab,*Scylla serrata*, has been attempted at the Gondol Research Station for Coastal Fisheries, Bali, Indonesia. During the production process, almost all of the larvae have died due to fungal infection. Fungi isolated from the larvae with fungal infection were classified into three species in the order Lagenidiales:*Lagenidium callinectes, Haliphthoros milfordensis* and*Halocrusticida baliensis* sp. nov. based on detailed morphological characteristics. The effects of temperature, pH and mineral content of the water on their growth were also examined.

Muraosa *et al.* (2006) found a fungal infection was in eggs and larvae of black tiger shrimp Penaeus monodon at a hatchery in Chachensao Province, Thailand in August 2000. Fungi were isolated from eggs and larvae with fungal infection, and studied on the morphological and biological characteristics. When it was transferred from PYGS broth to artificial seawater, discharge tubes developed from the mycelia, and a vesicle for zoospore formation was produced at the top of each discharge tube. The characteristic feature of an asexual reproduction of the fungus was that zoospores swam away in seawater after the vesicle separated from the discharge tube. Based on these morphological characteristics, the fungus was identified as Lagenidium thermophilum. Some biological characteristics of the selected isolate NJM 0031 were compared with the other species in the genus Lagenidium isolated from some crustaceans. As a result, the isolate NJM 0031 showed similar characteristics to those of L. thermophilum ATCC 200318 isolated from mangrove crab Scylla serrata. The isolate was demonstrated to be pathogenic to larvae of black tiger shrimp by artificial infection. This is the first report of L. thermophilum infection in black tiger shrimp in Thailand.

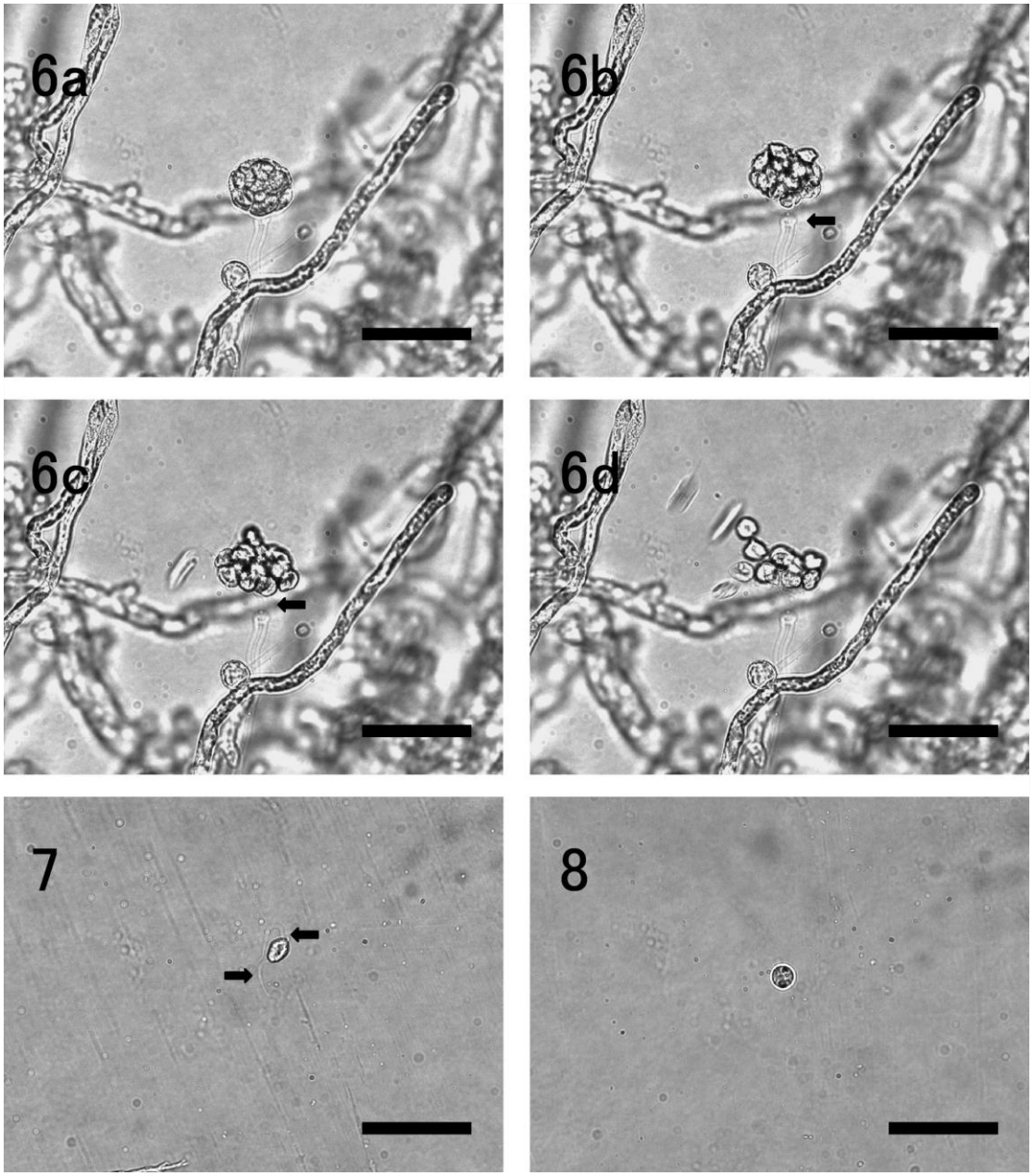


1. An egg of black tiger shrimp P. monodon infected with a fungus. Bar = 100 μm. **2.** A zoea of black tiger shrimp infected with a fungus. Bar = 100 μm. **3.** Vegetative hyphae growing in PYGS broth. Bar = 50 μm. **4.** Discharge tubes developed from mycelia. Bar 100 μm. **Muraosa *et al.* (2006)**

Table 1. Source of *Lagenidium* strains used in this study

Species	Strains	Locality	Host	Year
L. thermophilum	NJM [a] 0031	Chachensao, Thailand	*Penaeus monodon*	2000
L. thermophilum	NJM 9338 (ATCC [b] 200318)	Bali, Indonesia	*Scylla serrata*	1993
L. callinectes	ATCC 24973	North Carolina, U. S. A.	*Callinectes sapidus*	1973
L. callinectes	NJM 8989	Okayama, Japan	*Portunus trituberculatus*	1986
L. myophilum	NJM 8601 (ATCC 66280)	Ishikawa, Japan	*Pandalus borealis*	1988

[a] Culture collection in the Division of Fish Diseases, Nippon Veterinary and Animal Science University, Musashino, Tokyo, Japan.
[b] American Type Culture of Collection, Manassas, VA, U. S. A.

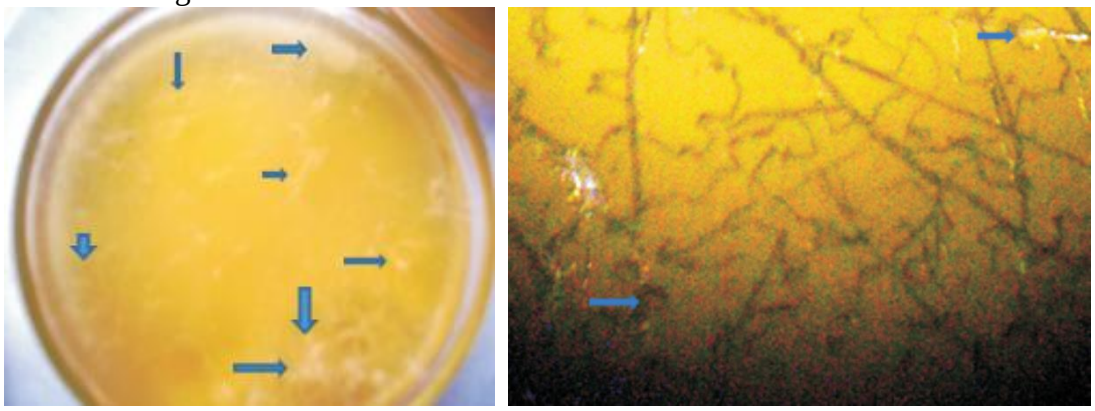


Zoospores liberation of L. thermophilum NJM 0031. a, vesicle formed at the top of the discharge tube; b, zoospore liberation occurred after the vesicle was separated from the discharge tube (arrow); c–d, all zoospores swum away simultaneously when the vesicle was burst (arrow); d, vesicle was not persistent. Bar = 50 μm. **7.** A swimming zoospore. Arrows show lateral biflagellates. Bar = 50 μm.. **8.** An encysted zoospore. Bar = 50 μm. **Muraosa *et al.* (2006)**

Table 5. Fungi in the genus *Lagenidium* previously reported as pathogen of aquatic animals

Species	Reference	Host	Stage
1. *L. giganteum*	Couch (1935)	mosquito	larvae
		daphnia	–
		copepods	–
2. *L. callinectes*	Couch (1942)	blue crab *Callinectes sapidus*	eggs
3. *L. scyllae*	Bian *et al.* (1979)	mangrove crab *Scylla serrata*	eggs and larvae
4. *L. myophilum*	Hatai and Lawhavinit (1988)	northern shrimp *Pandalus borealis*	adults
	Nakamura *et al.* (1994)	coonstripe shrimp *Pandalus hypsinotus*	adults
5. *L. thermophilum*	Nakamura *et al.* (1995)	mangrove crab *Scylla serrata*	eggs and larvae
6. *L.* sp.	Lightner and Fontaine (1973)	white shrimp *Penaeus setiferus*	larvae

Aftab-Uddin *et al.* (2013) conducted a study between February to August 2012, concerning the fungal diseases of P. monodon larvae reared in a commercial shrimp hatchery in Cox's Bazar, Bangladesh. The causative fungus was identified as a member of the genus Lagenidium (Oomycetes, Lagenidiales). High mortalities up to 50% was observed soon after infection. The affected larvae were whitish and filled with numerous aseptate hyphae and larvae lost equilibrium and exhibited respiratory difficulties. The fungal growth was observed on PYGS agar medium at 25°C. Infected untreated populations of nauplii, zoea and mysis stages showed mortalities of 15.75±0.76%, 31.25±3.12%, and 49.5±3.9% respectively. A 0.75 ppm treatment with trifluralin significantly reduced the mortality of the infected larval population. The pathogenicity tests of the infected fungi against the larvae of P. monodon by immersion method showed that the isolates were pathogenic causing 50%, 80% and 82% mortality in nauplii, zoea and mysis stage respectively in 96 hours post exposure at 104 zoospores/mL. This is the first report of Lagenidium sp. infection in shrimp larvae in Bangladesh.



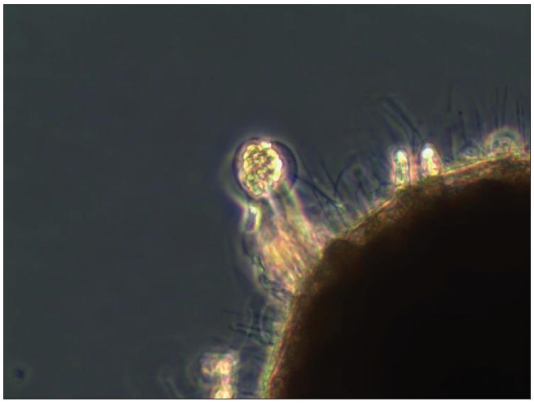
Whitish flat and filamentous fungus on PYGS agar. Hyphae of the fungus, **Aftab-Uddin *et al.* (2013)**

P. monodon larvae infected by Lagenidium sp. , Swimming zoospores with biflagella **Aftab-Uddin *et al.* (2013)**

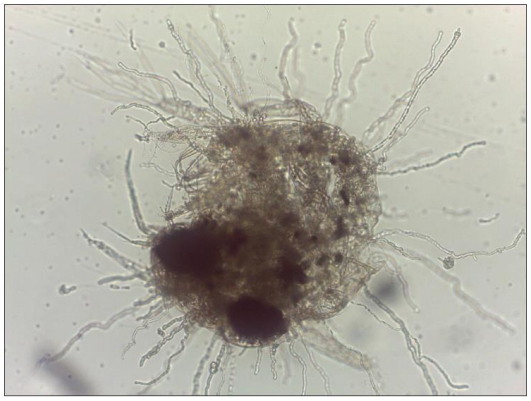
Krishnika and Ramasamy (2014) investigated the occurrence, infectivity and pathogenecity of Lagenidium sp. in the hatchery of Macro brachium rosenbergii. Microscopic examination revealed that the Lagenidium sp. infected zoea appeared white in colour. The presence of non-septate fungal hyphae was observed within the body of the exposed larvae replacing nearly all the muscles. Light and scanning electron microscopy demonstrated that 24 h post-exposure, the infection began to appear externally. After 32 h, zoospores appeared on external hyphae of infected larvae. The fungus Lagenidium sp. exhibited optimum growth at 30 degrees C, 0-2% NaCl and slow growth at 5% NaCl on potato dextrose agar (PDA). Antimicrobials, clotrimazole and griseofulvin were found to be more effective than miconazole, itraconazole and fluconazole in inhibiting the growth of Lagenidium sp. Under experimental conditions, Lagenidium sp. causes 100% mortality within two days of infection, producing 10(3) spores ml(-1).

Lee *et al.* (2016) reported the first isolation of *Lagenidium thermophilum* from eggs *and larvae of mud crap* (*Scylla tranquebarica*) in Sabah, Malaysia.

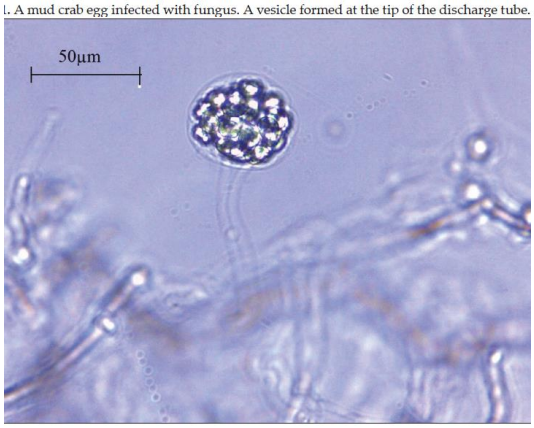
In April 2014, marine Oomycetes were first isolated from mud crab *Scylla tranquebarica* eggs and larvae at the University Malaysia Sabah shrimp hatchery. A fungus was isolated from infected eggs and larvae using PYGS agar. It was thought that the same fungus infected both eggs and larvae; therefore, strain IPMB 1401 was randomly selected for further characterization in this study. The isolated fungus produced a discharge tube from the mycelium, and a vesicle was formed at the tip. The zoospores swam away after the vesicle separated from the discharge tube. The strain IPMB 1401 was classified as a *Lagenidium* sp., closely related to *L. thermophilum* based on the mode of zoospore release. The differences between the strains IPMB 1401 and pathogenic *Lagenidium* spp. isolated from marine crustaceans were compared in nucleotide sequence of ITS 1 region. As a result, the IPMB 1401 showed high similarity of 99-100% and belonged to the same cluster with *L. thermophilum*. Therefore, the strain IPMB 1401 was identified as *L. thermophilum*. This is the first report of *Lagenidium* infection in Malaysia.



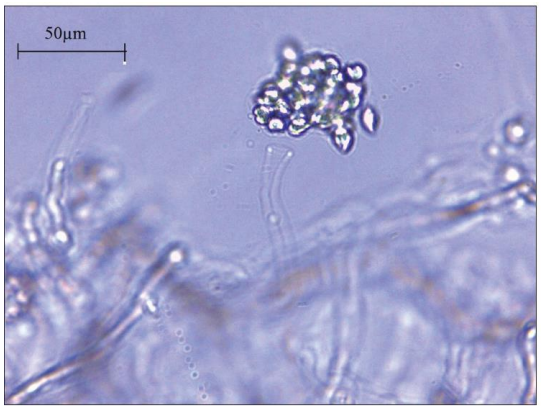
l. A mud crab egg infected with fungus. A vesicle formed at the tip of the discharge tube.



. Larva infected with fungus. Many discharge tubes developed from the body.



he mass of protoplasm inside the vesicles divided into zoospores.



. Vesicle separated from the tip of a discharge tube before zoospores swam away.

References:

1. Aftab Uddin, S., Chowdhury Hasan. Mohammad Nurul Azim Sikder, Miah Ali Hossain. A Fungal Infection Caused by Lagenidium sp. and its Control Measures in Hatchery Reared Shrimp Larvae Penaeus monodon in Bangladesh Journal of Pure and Applied Microbiology 7(4):3137-3142 · August 2013 *with* 170
2. Bian, B. Z., K. Hatai, G. L. Po and S. Egusa (1979): Studies on the fungal diseases in crustacean. 1. Lagenidium scyllae sp. nov. isolated from cultiva. Trans. Mycol. Soc. Japan, **20**, 115–124.
3. Couch, J. N. (1942): A new fungus on crab eggs. J. Elisha Mitchell Sci. Soc, **58**, 158–162.
4. Crisp, L. M., Charles E. Bland, Gunther Bahnweg Biosystematics and Distribution of Lagenidium callinectes, a Fungal Pathogen of Marine Crustacea. Mycologia 81(5):709 · September 1989 *with* 9 Reads
5. Hatai, K. and O. Lawhavinit (1988): Lagenidium myophilum sp. nov., a new parasite on adult northern shrimp (Pandalus borealis Kryøer). Trans. Mycol. Soc. Japan, **29**, 175–184.
6. Hatai, K., D. Roza and T. Nakayama (2000): Identification of lower fungi isolated from larvae of mangrove crab, Scylla serrata, in Indonesia. Mycoscience, **41**, 565–572.
7. Krishnika, A. and Palaniappan Ramasamy. Lagenidium sp infection in the larval stages of the freshwater prawn Macrobrachium rosenbergii (De Man). Indian Journal of Fisheries 61(2):90-96 · April 2014
8. Lee , Y. N. , K. Hatai and O. Kurata. First report of *Lagenidium thermophilum isolated from eggs and larvae of mud crap* (*Scylla tranquebarica*) in Sabah, Malaysia. Bull. Eur. Ass. Fish Pathol., 36(3) 2016, 111

9. Lightner, D.V. and C.T. Fontaine **A new fungus disease of the white shrimp *Penaeus setiferus***. Journal of Invertebrate Pathology Volume 22, Issue 1, July 1973, Pages 94-9
10. Muraosa, Y. Lawhavinit, O. A.Hatai, K. Lagenidium thermophilum isolated from eggs and larvae of black tiger shrimp Penaeus monodon in Thailand. Fish Pathology **.**Vol. 41 No. 1 Pages 35-40, 2006
11. Nakamura, K., S. Wada, K. Hatai and T. Sugimoto (1994): Lagenidium myophilum infection in the coonstripe shrimp, Pandalus hypsinotus. Mycoscience, **35**, 99–104.
12. Nakamura, K., Nakamura, M., Hatai, K. et al. Lagenidium infection in eggs and larvae of mangrove crab (Scylla serrata) produced in Indonesia. Mycosci. 36. 399-404-1995
13. Nestrud LB, Anderson RL. **Aquatic safety of Lagenidium giganteum: effects on freshwater fish and invertebrates.** J Invertebr Pathol. 1994 Nov;64(3):228-33.
14. Ramasamy, P R, Rajan,R Jayakumar,S Rani,G P Brennan. *Lagenidium callinectes* (Couch, 1942) infection and its control in cultured larval Indian tiger prawn, Penaeus monodon Fabricius. J. Fish Dis. View issue TOC Volume 19, Issue 1January 1996 Pages 75–82
15. ROGERS-TALBERT, R. THE FUNGUS LAGENIDIUM CALLINECTES COUCH (1942) ON EGGS OF THE BLUE CRAB IN CHESAPEAKE . *Biological Bulletin* Vol. 95, No. 2 (Oct., 1948), pp. 214-228

9. Haliphthoros

- The genus *Haliphthoros* Vishniac was first described as a filamentous, holocarpic parasite on eggs of the oyster drill *Urosalpinx cinerea* Say, and the family Haliphthoraceae was established to accommodate *Haliphthoros* (type genus) and *Atkinsiella* Vishniac in the order Saprolegniales (Vishniac 1958).
- The genus *Haliphthoros* contains two species, *H. milfordensis* Vishniac (type species; Vishniac 1958) and *H. phillippinensis* Hatai et al., that were distinguished on the basis of morphological differences associated with zoosporogenesis and zoospore release (Hatai et al. 1980).
- *Haliphthoros* species are known as parasites of a wide range of marine crustaceans and some other marine animals (Vishniac 1958; Lightner 1981; Alderman 1982; Hatai 1989; Hatai et al. 1992; Diggles 2001).
- *Haliphthoros milfordensis* opportunistically infected juvenile*Homarus americanus*, causing red-brown necrotic lesions in the gills at the base of walking legs and mortalities up to 46% in some rearing facilities (Fisher, Nilson & Shleser 1975; Fisher *et al*. 1978).
- Oomycetes of the genus *Haliphthoros* also cause disease in penaeid shrimp (Tharp & Bland 1977; Hatai, Bian, Baticados & Egusa 1980; Hatai, Rhoobunjongde & Wada 1992) and abalone (Hatai 1982).
- *Haliphthoros* species have been frequently isolated from diseased organisms and are considered to be serious pathogens of economically important marine crustaceans,
- *Haliphthoros* has been isolated from all over the world, and nearly all the isolates have been identified as *H. milfordensis.*
- Haliphthoros milfordensis has been reported as a parasite of various marine crustaceans (Nakamura and Hatai, 1995).

Classification:

Species 2000 & ITIS Catalogue of Life: April 2013

Chromista +

- Oomycota +
  - Oomycetes +
    - Not assigned +
      - Not assigned +
        - Haliphthoros +
          - ***Haliphthoros milfordensis* Vishniac 1958**
          - *Haliphthoros philippinensis* Hatai, Bian, Batic. & Egusa 1980
          - *Haliphthoros zoophthorum* (Vishniac) M. W. Dick 2001

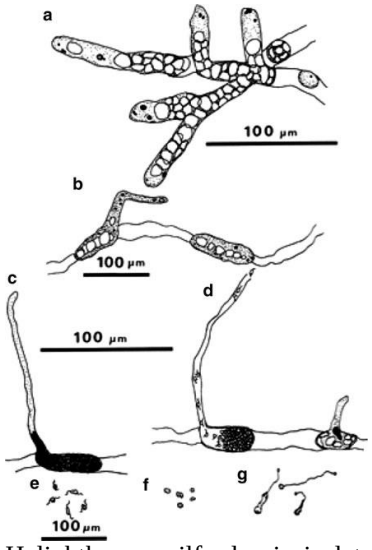
NCBI Taxonomy

- Eukaryota +
  - Stramenopiles +
    - Oomycetes +
      - Lagenidiales +
        - Haliphthoraceae +
          - Haliphthoros +
            - ***Haliphthoros milfordensis***
            - *Haliphthoros philippinensis*
            - Haliphthoros sp. NJM 0034
            - Haliphthoros sp. NJM 0143
            - Haliphthoros sp. NJM 0440
            - Haliphthoros sp. NJM 0443
            - Haliphthoros sp. NJM 0449
            - Haliphthoros sp. NJM 0535

Description of *Haliphthoros* species:

1. Haliphthoros milfordensis Vishniac (type species; Vishniac (1958)

Colonies on PYGS agar were whitish and reached a diameter of 20-25 mm after 5 d at 25~ The centers were damp. Hyphae in PYGS broth were stout, aseptate, branched with numerous shiny spherical granules, and sometimes concentrated masses of protoplasm were observed in the hyphae. The width of the hyphae was 7.5-30 pm. In artificial seawater, fungal fragments were clearly observed to be concentrated masses of protoplasm in the hyphae. Fragments were tuberculate, saccate or irregular, and quite variable in size and shape. They changed into zoosporangia producing discharge tubes. Many vacuoles appeared in the sporangia and extending discharge tubes, and were also observed in the active mycelia. Zoospore formation was observed about 8-12 h after the mycelia were transferred into sterilized artificial seawater and continued for one week. One discharge tube was usually formed on the lateral side of each zoosporangium. The tubes were 5-10 pm in diam and 15-300 pm in length, and usually straight or slightly curved. Division of the protoplasm started in the sporangia and continued in the discharge tubes just before zoospore liberation. Zoospores were elongate, reniform and slipper-shaped, laterally biflagellate, isokont, monoplanetic, 6-7.5 • 7-12/~m, 6.5 • 8.5 pm on average. Encysted zoospores were globose or subglobose, 3-7 Fm in diam, 5 Fm on average. Spores germinated with a hair-like filament measuring 15-150 pm in length about 4-5 h after encystment. Sexual reproduction was not observed.



Morphological characteristics of Haliphthoros milfordensis isolated from a zoea of S. serrata. (a) Hyphae in PYGS broth; (b) Fragments. Discharge tube formation on the left fragment; (c) Zoospore formation; (d) Zoospore liberation; (e) Zoospores; (f) Encysted zoospores; (g) Germination(Hatai et al. 2000)

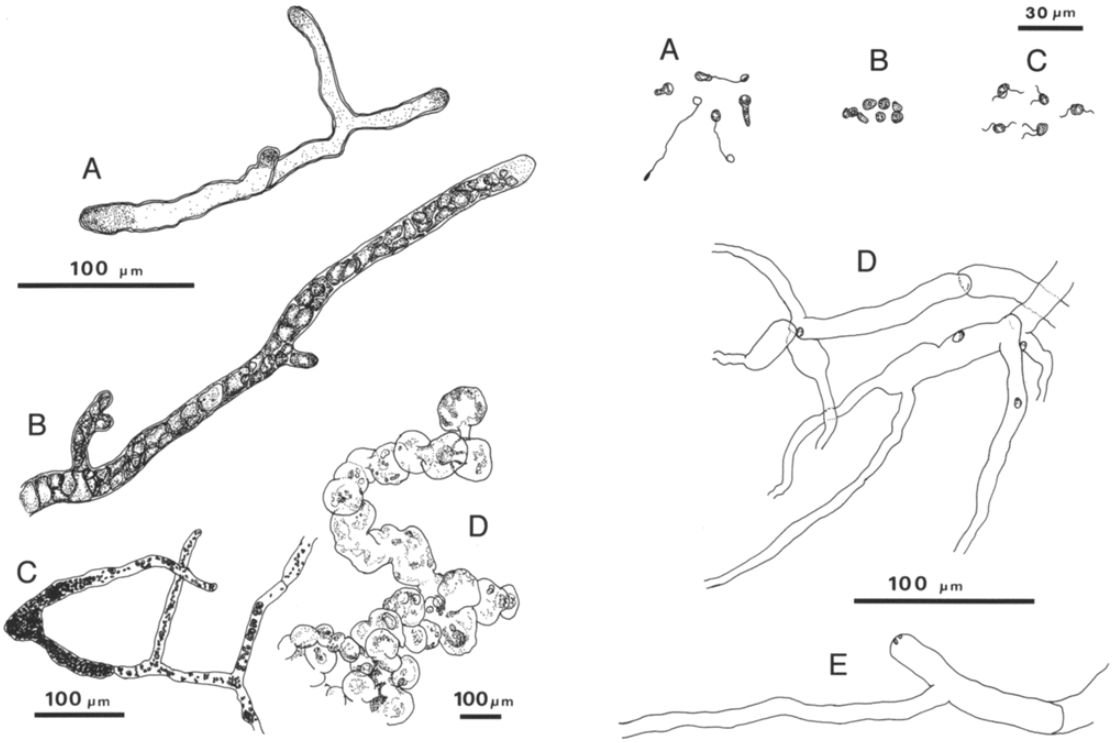
2. *Haliphthoros philippinensis* Hatai, Bian, Batic. & Egusa 1980

H. philippinensis, was isolated from larvae of the jumbo tiger prawn, Penaeus monodon in Philippines (Hatai et al. 1980). The hyphae were stout, branched, irregular, non-septate, developing within the bodies of larvae of the prawn, and it was holocarpic. In pure cultures, the hyphae were homotrichous, at first somewhat uniform, sometimes highly vacuolated, 10–37.5 mm in diameter, becoming fragmentary by means of cytoplasmic constriction with age. Fragments with a dense cytoplasm were variable in size and shape, globose, elongate or tubular, often with protuberances, up to 190 _ 100 mm, not disarticulated, connected in bead-like chains, functioning as sporangia and developing discharge tubes which were straight, wavy or coiled, up to 7.5–12.5 mm. Zoospores were polyplanetic. Encysted spores were spherical, 5–7.5(12.5) mm in diameter, producing a delicate germ tube. Germ tube was simple, sometimes once branched and up to 250 mm in length. Sexual reproduction was not observed. When the fragment with protuberance on the medium was transferred into sea water, the protuberance might again constrict and transform into another sporangium, or might extend and serve as a part of the discharge tube.

3. *Halocrusticida baliensis* Hatai, Roza et Nakayama, sp. nov. Hatai et al., 2000

Colonies on PYGS agar were yellowish and 17 mm indiam at one month after incubation at 25~ Hyphae in PYGS broth were stout, irregularly branched, aseptate, saccate-lobed, and 15-40 #m in width. In PYGS broth, the thallus was aseptate at first, and then became seprate as it divided into subthalli. Vacuoles were observed during the process of zoosporogenesis with numerous shiny granules. Gemmae present, saccate-lobed, thickwalled with shiny globules, 20-60 f~m diam, developed in zoosporangia in seawater. Subthalli cylindrical, saccate, irregular, tuberculate, variable in size and shape. Zoosporangia were the same size and shape as subthalli. Each sporangium extended one to several discharge tubes. In the discharge tubes, zoospores were lined more than two deep. Discharge tubes were produced laterally or

terminally from the sporangia, straight or wavy, usually with a broad cone-shaped base, tapering or equal, measuring 7.6-25 #m in width and 30-450 #m in length. Branches of the discharge tubes were rarely observed near the zoosporangia. Zoospore formation was observed 22-24 h after the vegetative hyphae were transferred into sterilized artificial seawater and incubated at 25~ and it continued for 10 d. Zoospores were laterally biflagellate, monoplanetic, 7.2(5.6-8.5) x6.1(4.9-7.4) #m size, pyriform, slipper-shaped, oblong, and spherical. In zoosporangia with several discharge tubes, zoospores were usually released from one of them, but sometimes all the zoospores were released at the same time. The encysted spores were 5.9(5.3-6.8)Fm in diam, spherical to subglobose, with or without oil droplets. The encysted spores in sterilized artificial seawater developed a hair-like filament, 7.5-210 ~m in length. The tip of the filament enlarged and developed in 10-12 h into a hyphal bud, 12.2x50 #m, after the zoospores became encysted. Sexual reproduction was not observed.



Morphological characteristics of Halocrusticida baliensis GSM 9703 isolated from a zoea of S. serrata. A, B. Hyphae in PYGS broth; C. Zoosporangia; D.Gemma**.** Morphological characteristics of Halocrusticida baliensis GSM 9703 isolated from a zoea of S. serrata. A. Germination; B. Encysted zoospores; C. Zoosopres; D, E. Empty zoosporangia with discharge tubes.

Reports:

Hatai *et al.* (1980) reported Haliphthoros philippinensis to be associated with fungal infection of cultured larvae of the shrimp, Penaeus monodon, in the Philippines. the pathogenicity of Haliphthoros philippinensis on Penaeus monodon larvae was not established, and it was suggested that it is not severely pathogenic as this species is rarely found in shrimp hatcheries.

LIO-PO *et al.* (1985) exposed pure cultures of the fungus *Haliphthoros*

philippinensis isolated from infected *Penaeus monodon* larvae for 24 h to varying concentrations of the antifungal agents Benlate, calcium hypochlorite, clotrimazole, copper sulphate, Daconil, formalin, Fungitox, Furanace, griseofulvin, hydrogen peroxide, malachite green, Mysteclin C, phenol, potassium permanganate, Resiguard, Tide, tolnaftate and Treflan. The efficiency of each compound in inhibiting sporulation and mycelial growth of the fungus was measured. The results establish mycostatic and mycocidal levels for each fungicide.

Hataik *et al.* (1992) isolated Haliphthoros milfordensis from gill lesions of juvenile kuruma prawns, Penaeus japonicus, with black gill disease at a private farm in Nagasaki Prefecture in 1989. This report described the first case of H. mifordensis infection in Crustacea in Japan.

HATAI, K. (1992) mentioned that rom June to August in 1981, an epizootic of mycosis occurred among the abalone (Haliotis sieboldii), which were temporarily held in aquaria with circulating sea water adjusted to 15 ^{o}C by a cooling system, at an abalone storage facility in Sasebo, Nagasaki Prefecture. The typical external symptom of diseased abalones was flat or tubercle-like swelling formed on mantle, epipode and dorsal surface of foot. The mycelium was always observed in the lesions. A fungus was isolated by inoculating materi als taken from lesions of diseased abalone onto AMES agar and incubating at 15 ^{o}C. For the observa tion of spore discharge, fragments of vegetative hyphae were washed several times with sterile sea water, and then placed in a Petri dish containing sterile sea water. As a result, zoospores formed within the fragment were liberated through the orifice of discharge tube. Encysted spores were spherical, usually 7µm (range of 6 to 10µm) in diameter. The fungus was identified as Haliphthoros milfordensis. The fungus was identified as Haliphthoros milfordensis. The fungus grew at a temperature range of 4.9 to 26.5 ^{o}C, with optimum of 11.9-24.2 ^{o}C.

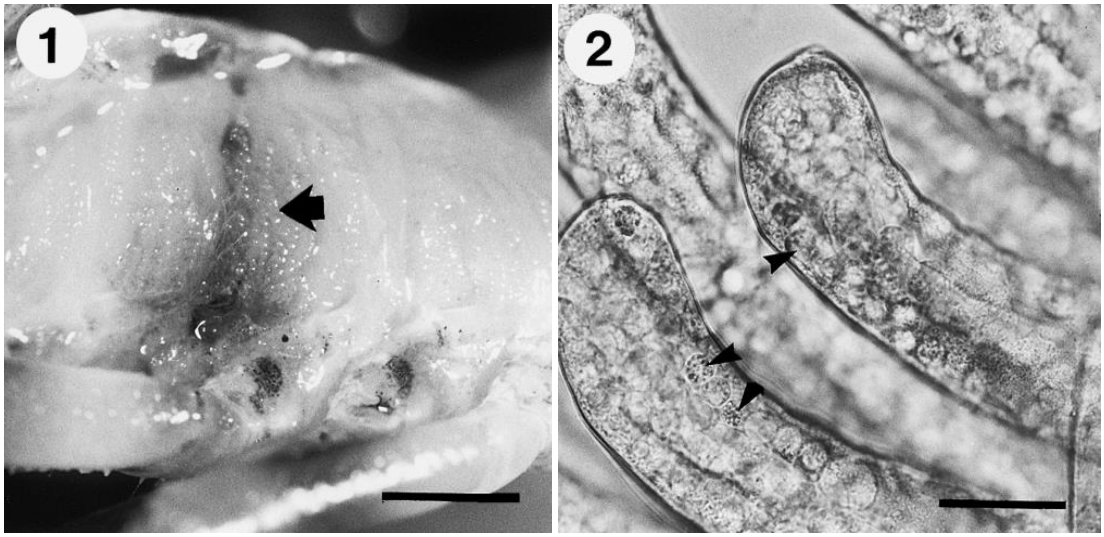
Hamasaki and Hatai (1993) reported that infection in eggs and larvae of S. serrata occurred at 102_103 zoospores mr! inoculum (using Haliphthoros, Lagenidium and Sirolpidium). Furthermore, infection was evident starting at day 2 post-inoculation, when infected Scylla serrata eggs showed the presence of discharge tubes emerging from the egg surface.

Hatai *et al.* (2000) mentioned that, Since 1992, seed production of mangrove crab, Scylla serrate, has been attempted at the Gondol Research Station for Coastal Fisheries, Bali, Indonesia. During the production process, almost all of the larvae have died due to fungal infection. Fungi isolated from the larvae with fungal infection were classified into three species in the order Lagenidiales: Lagenidium callinectes, Haliphthoros milfordensis and Halocrusticida baliensis sp. nov. based on detailed morphological characteristics. The effects of temperature, pH and mineral content of the water on their growth were also examined.

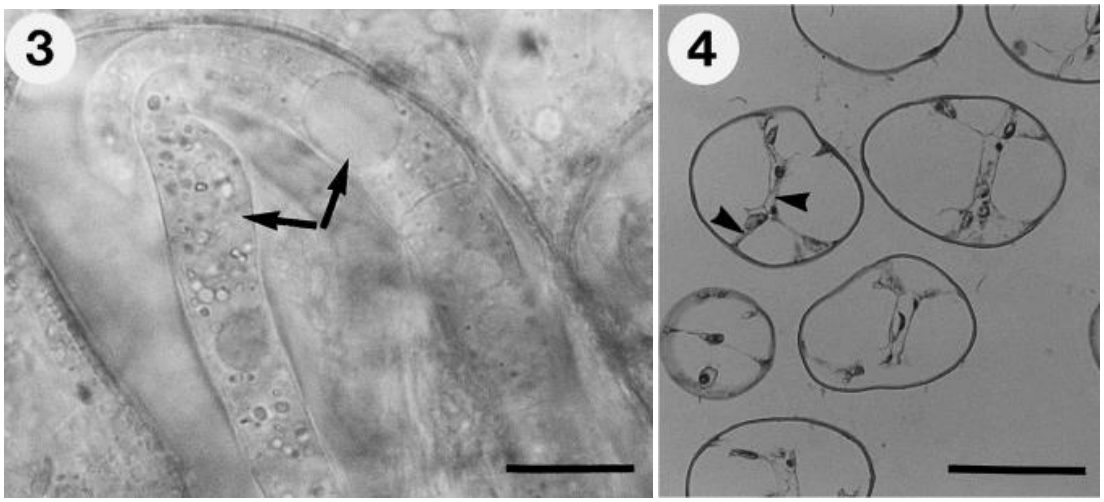
Cook et al. (2001) sequenced the gene for mitochondrialencoded cytochrome *c* oxidase subunit II (cox2) for two *Haliphthoros philippinensis* and *H.milfordensis*. The resulting molecular phylogenetic trees showed that both *Haliphthoros* isolates form a monophyletic clade, which rather surprisingly clustered with another marine parasite genus, *Halocrusticida* Nakamura et Hatai, at the base of the oomycete clade,

diverging before separation of the main saprolegnialian and peronosporalean clades.

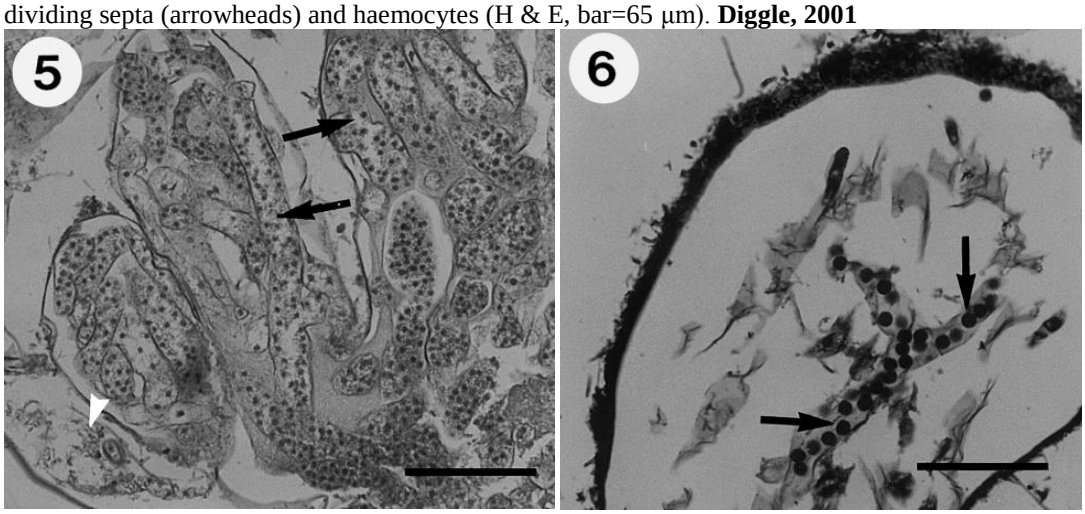
Diggles (2001) associated mortalities of puerulus and juvenile rock lobsters, *Jasus edwardsii* (Hutton, 1875), held in shore-based ongrowing facilities at water temperatures between 10 and 18 °C with infection by an invasive oomycete identified as *Haliphthoros* sp. Gross signs of disease included loss of appetite, lethargy and 1–3 brown/black focal necrotic lesions in the gills near insertion of the walking legs. Hyphae were observed in wet preparations of gill filaments excised from lesions. Histology of gill lesions showed hyphae inside the gill cuticle, invasion and lysis of the skeletal muscle and massive haemocyte infiltration and melanization at the base of walking legs adjacent to infected gill filaments. Lobsters over approximately 30 mm carapace length appeared resistant to infection. Death of affected lobsters usually occurred prior to or during the moult and in some cases may have been associated with secondary bacterial infection. *Haliphthoros* sp. was isolated in pure culture and marine agar 2216 was the medium that produced best growth *in vitro*. Two isolates were exposed *in vitro* to five chemicals to determine if an effective treatment could be found. Chemicals that interrupted the life cycle by killing zoospores or preventing sporulation included malachite green, trifuralin, formalin and copper sulphate. The appearance of the disease was associated with poor hygiene and its elimination may be achievable by improving husbandry practices.



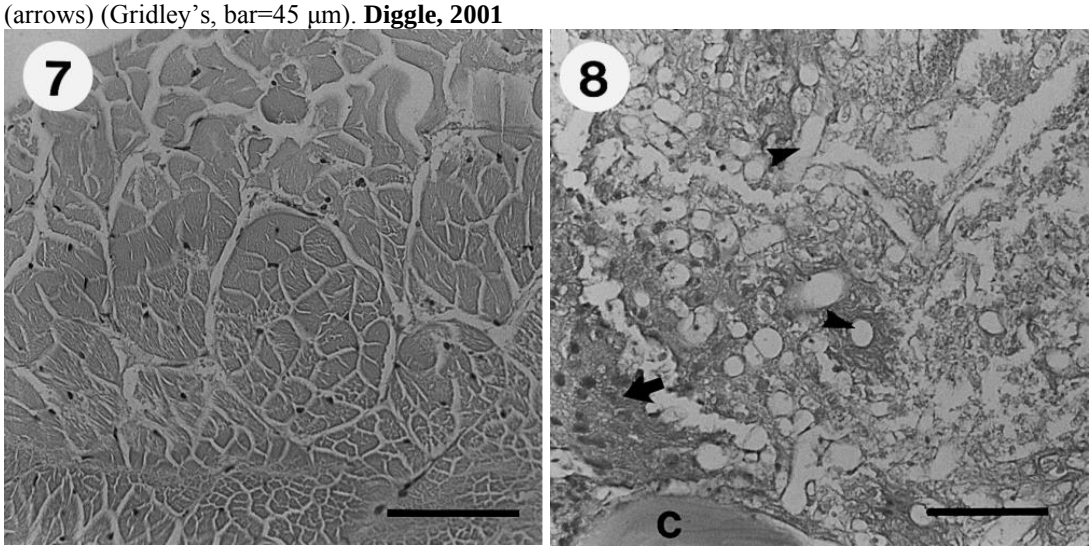
Gross appearance of lesion (arrow) in gills of juvenile *J. edwardsii* caused by infection with *Haliphthoros* sp. Note blackening caused by host reaction at base of walking leg (bar=5 mm). Wet preparation of normal gill filaments containing numerous haemocytes (arrowheads) (bar=65 μm). **Diggle, 2001**



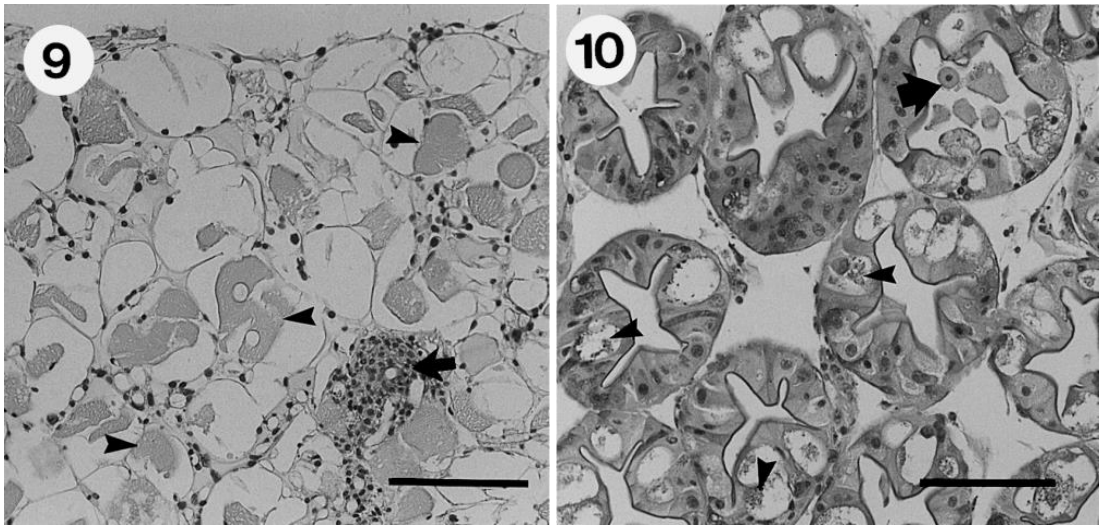
Wet preparation of gill from lesion showing fungal hyphae (arrows) inside gill cuticle and absence of haemocytes (bar=16 μm). Histological transverse section of normal gill filaments showing presence of dividing septa (arrowheads) and haemocytes (H & E, bar=65 μm). **Diggle, 2001**



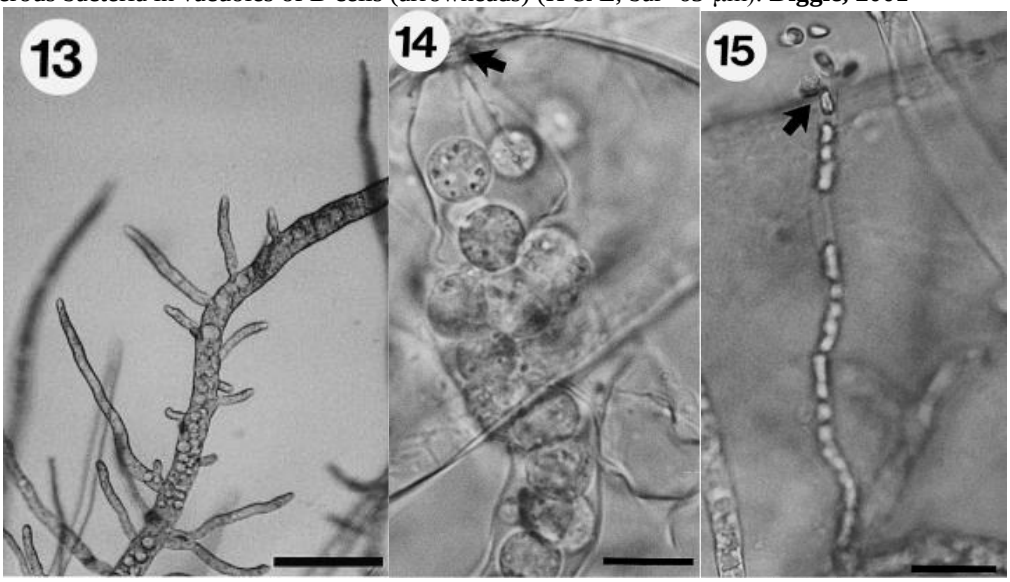
Histological longitudinal section of gill filaments packed with multinucleate fungal hyphae (arrows). Various filamentous fouling organisms are also evident on the outside of the cuticle (white arrowhead) (PAS, bar=65 μm). Histological section of gill filament with hyphae which have produced zoospores (arrows) (Gridley's, bar=45 μm). **Diggle, 2001**



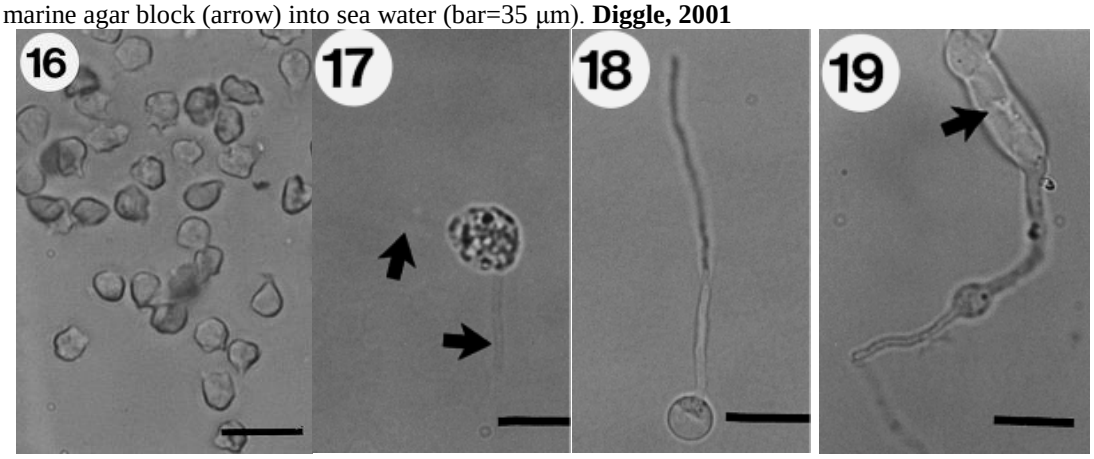
Histological section of normal muscle at the base of a walking leg (H & E, bar=95 μm). Histological section of muscle at the base of a walking leg adjacent to gill filaments infected with *Haliphthoros* sp. Note melanized region (arrow) and haemocyte infiltration surrounding necrotic area containing hyphae (arrowheads), C=cuticle (H & E, bar=65 μm). **Diggle, 2001**



Histological section of muscle further into the base of walking leg showing deep penetration of hyphae with associated haemocyte infiltration (arrow), and apparent lysis of muscle bundles (arrowheads) (H & E, bar=95 µm). Histological section of hepatopancreas of moribund juvenile *J. edwardsii*infected with *Haliphthoros* sp. Note sloughing of necrotic hepatopancreocytes into tubule lumen (arrow) and numerous bacteria in vacuoles of B cells (arrowheads) (H & E, bar=65 µm). **Diggle, 2001**



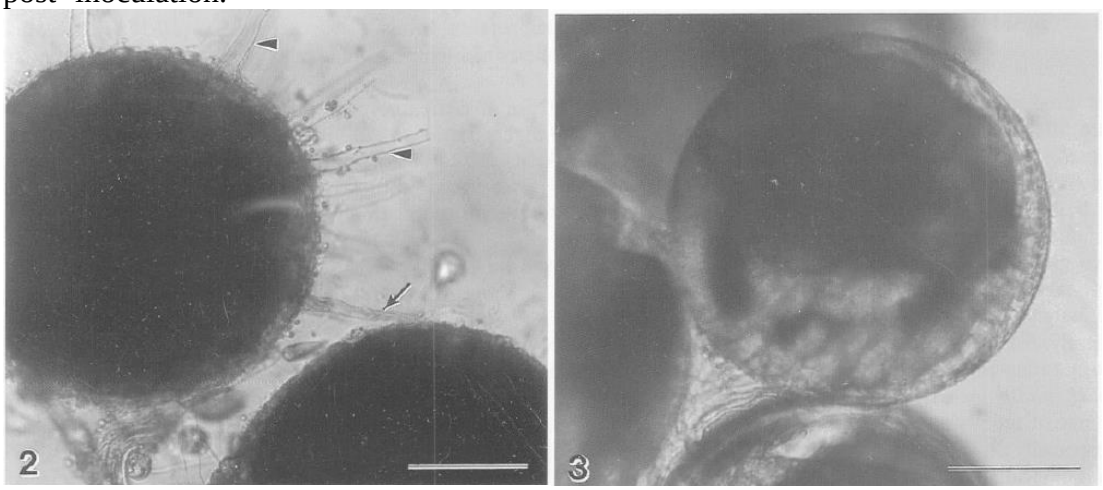
Typical appearance of branched, vacuolate vegetative hyphae of*Haliphthoros* sp. grown in marine agar (bar=200 µm). Zoospores inside exit tube which is seen penetrating the gill cuticle (arrow) (bar=9.25 µm). Zoospores exiting singly through a mycelium which protrudes from the edge of a marine agar block (arrow) into sea water (bar=35 µm). **Diggle, 2001**



Irregularly shaped zoospores after exiting into sea water from a marine agar block (bar=17 µm).High power of a free swimming zoospore showing two flagella (arrows) (bar=7 µm).Rounded up zoospore

post-encystment with hair like germ tube (bar=9 µm). Growth of hyphae (arrow) from encysted zoospore (bar=9 µm). **Diggle, 2001**

Leafio (2002) conducted monitoring of the fungal flora of spawned eggs of captive mud crab, Scylla serrata in Philippines. Quantification of the egg mycoflora revealed the dominance of oomycetes, particularly Haliphthoros spp. among spawners which aborted their eggs prior to hatching. Two species of Haliphthoros (H. philippinensis and H. milfordensis) were identified from the 24 isolates collected. Haliphthoros milfordensis was the dominant species. Physiological studies on vegetative growth and sporulation of the two species show that H. philippinensis have wider optimal range for salinity and temperature requirements than H. milfordensis, especially in sporulation. The pathogenicity study snowed that only H. mi/fordensis was pathogenic to spawned eggs of S. serrata, while H. philippinensis was not. Infection of S. serrata eggs by H. milfordensis was observed starting at two days after inoculation of zoospores with 2-5% infection rate, reaching up to 10% at five days post- inoculation.



Figs. 2-3. *Scylla serrata* eggs. **2.** Infected with *Haliphthoros milfordensis;* note the presence of discharge tubes (arrowheads) releasing the zoospores (arrow). **3.** Un infected eggs (control treatment). Bars = 100 */µm.* **Leafio (2002)**

Chukanhom **et al. (2003)** isolated a marine fungus from the black tiger prawn Penaeus monodon at Nha Trang, Vietnam, on March 20, 2001 and named isolate NJM 0131. The fungus was identified as Haliphthoros milfordensis from the characteristics of asexual reproduction, and its physiological characteristics were investigated. Although the optimum temperature for growth of the isolate was 25 deg-30 deg C, the fungus grew at a wide range of temperatures (15 deg-40 deg C). H. milfordensis grew well in 50%-100% seawater, but poorly in PYG agar containing 1.0%-5.0% NaCl and KCl. The fungus grew at a wide range of pH (4.0-11.0) with the optimum pH value of 7.0-9.0. The isolate also showed pathogenicity to swimming crab larvae (Portunus trituberculatus) by artificial infection, but mortality was not high. This is the first report of disease in the black tiger prawn P. monodon in Vietnam caused by H. milfordensis.

Sekimoto ***et al.*** **(2007)** sequenced the partial nuclear- encoded small-subunit ribosomal RNA (SSU rRNA) gene, the partial large-subunit ribosomal RNA (LSU rRNA) gene, and the cox2 gene of the isolate of NJM0034, and analyzed these to investigate the molecular phylogenetic position of NJM0034, to verify its affinity with the genus

Haliphthoros, and to further confirm the monophyly of the genus *Haliphthoros*. They also used another isolate of *Haliphthoros* (NJM0131) for this study, which was originally isolated from a prawn in Vietnam in 2001 and described as the typical *Haliphthoros milfordensis* based upon morphological observations (Chukanhom et al. 2003). They performed this multigene approach using new sequence data of the SSU and LSU rRNA genes of *Haliphthoros* isolates to contribute to the overall understanding of the phylogenetic position of this economically important genus. All phylogenetic trees showed that NJM0034 and NJM0131 were branched before separation of the two main saprolegnian and peronosporalean clades. These data suggest that the clear phylogenetic separation of those marine oomycete endoparasites from the two main oomycete clades. Excepting the LSU rRNA gene tree, NJM0034 and *Haliphthoros* spp. did not form a monophyletic group. On the other hand, *H. milfordensis* NJM0131 clustered with *H. philippinensis* SANK 15178, not with *H. milfordensis* NJM9434 in the cox2 amino acid sequence (COII) tree. This result strongly suggests that a taxonomic reinvestigation of the genus *Haliphthoros* should be considered.

References:

1. Bian, B.l. and Egusa, S. (1980). Atkinsiella hamanaensis sp. novo isolated from cultivated ova of the mangrove crab, Scylla serrata (Forssktll). Journal of Fish Diseases 3: 373-385
2. ***Chukanhom, K., Borisutpeth, P. Khoa, L.V.Hatai, K.*** **Haliphthoros milfordensis isolated from black tiger prawn larvae (Penaeus monodon) in Vietnam [2003]**
3. Diggles, B K. A mycosis of juvenile spiny rock lobster, *Jasus edwardsii* (Hutton, 1875) caused by *Haliphthoros* sp., and possible methods of chemical control J. Fish Dis. Volume 24, Issue 2 February 2001 Pages 99–110
4. Hamasaki, K and Hatai, K. (1993). Experimental infection in the eggs and larvae of the swimming crab Portunus trituberculatus and the mud crab Scylla serrata with seven fungal strains belonging to Lagenidiales. Nippon Suisan Gakkaishi 59: 1059-1066.
5. Hatai, K. (1982). On the fungus Haliphthoros milfordensis isolated from temporarily held abalone Haliotis sieboldii. Fish Pathology 17: 199-204
6. HATAI, K. (1992) On the Fungus Haliphthoros milfordensis isolated from Temporarily held Abalone (Haliotis sieboldii) . Fish Pathology 17(3)199-204,1982.12
7. **Hataik K.; Rhoobunjongde W.; Wada S., 1992: Haliphthoros milfordensis isolated from gills of juvenile kuruma prawn penaeus japonicus with black gill disease. Nippon Kingakukai Kaiho 33(2): 185-192**
8. Hatai ,K, Des Roza and Takane Nakayama. Identification of lower fungi isolated from larvae of mangrove crab, Scylla serrate, in Indonesia. Mycoscience 41: 565-572, 2000
9. Leafio, E. M. Haliphthoros spp. from spawned eggs of captive mud crab, Scylla serrata, broodstocks. Fungal Diversity 9: 93-103. 2002
10. LIO-PO, G. D.,M. C. L. BATICADOS,C. R. LAVILLA,M. E. G. SANVICTORES. *In vitro* effects of fungicides on *Haliphthoros philippinensis. J. Fish Dis.* View issue TOC. Volume 8, Issue 4,July 1985 , Pages 359–365
11. Sekimoto, S., K. Hatai, D. Honda. Molecular phylogeny of an unidentified Haliphthoros-like marine oomycete and Haliphthoros milfordensis inferred from nuclear-encoded small- and large-subunit rRNA genes and mitochondrial-encoded cox2 gene. Mycoscience (2007) 48:212–221

10. Halioticida

- Halioticida infection was reported from abalone, Haliotis spp. in Japan (Muraosa et al. 2009).
- The genus was classified in Peronosporomycetes (formerly Oomycetes) as a new genus.
- The class Peronosporomycetes contains species that are pathogens of many commercially important plants, fish, and crustaceans (Kamoun 2003).
- Among the marine invertebrates, infections resulting from some members of the Peronosporomycetes cause problematic diseases, especially in the seed production of marine crustaceans such as shrimp and crabs.

NCBI Taxonomy \

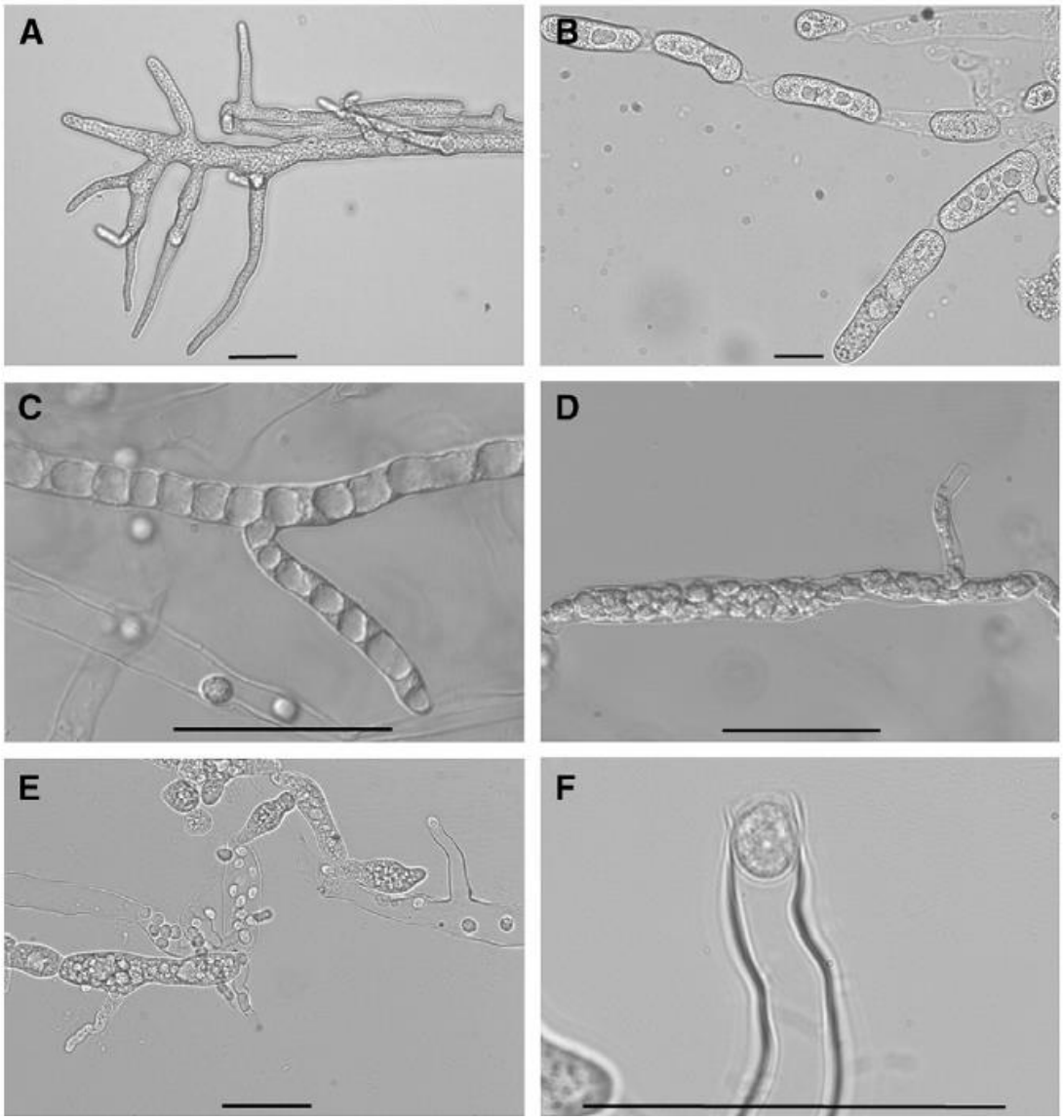
Cellular organisms +

- Eukaryota +
  - Stramenopiles +
    - Oomycetes +
      - Lagenidiales +
        - Haliphthoraceae +
          - **Halioticida**
            - ***Halioticida noduliformans***
          - Atkinsiella +
          - Environmental samples +
          - Haliphthoros +
          - Halocrusticida +
          - Halodaphnea +

Description:

Halioticida noduliformans Muraosa, Y., Morimoto, K., Sano, A. et al. Mycoscience (2009) 50: 106.

Key morphological characteristics of isolate AF08527 in PYGS broth culture included stout (11–20 μm in diameter), aseptate and highly branched hyphae, particularly at the terminal ends, withnumerous protoplasmic oil droplets. Fragmentation of the hyphae by protoplasmic constrictions occurred in isolate AF08527 when the hyphae were transferred into sterile natural seawater. Fragment length was 36–113 μm and the spaces between fragments were 4–53 μm. Each of the fragments developed into a zoosporangium and produced large numbers of zoospores approximately 24–30 h following transfer to sterile seawater. Zoospores were released into the seawater following the formation of one or more discharge tubes from each zoosporangium. The discharge tubes were approximately 5–9 μmin diameter, 36–64 μm in length and were either straight or coiled. The released zoospores were biflagellate, globose and measured 6–8 μm in diameter. The isolate AF08527 was identified as H. noduliformans based on the mode of protoplasmic constriction and zoospore formation (asexual reproduction)

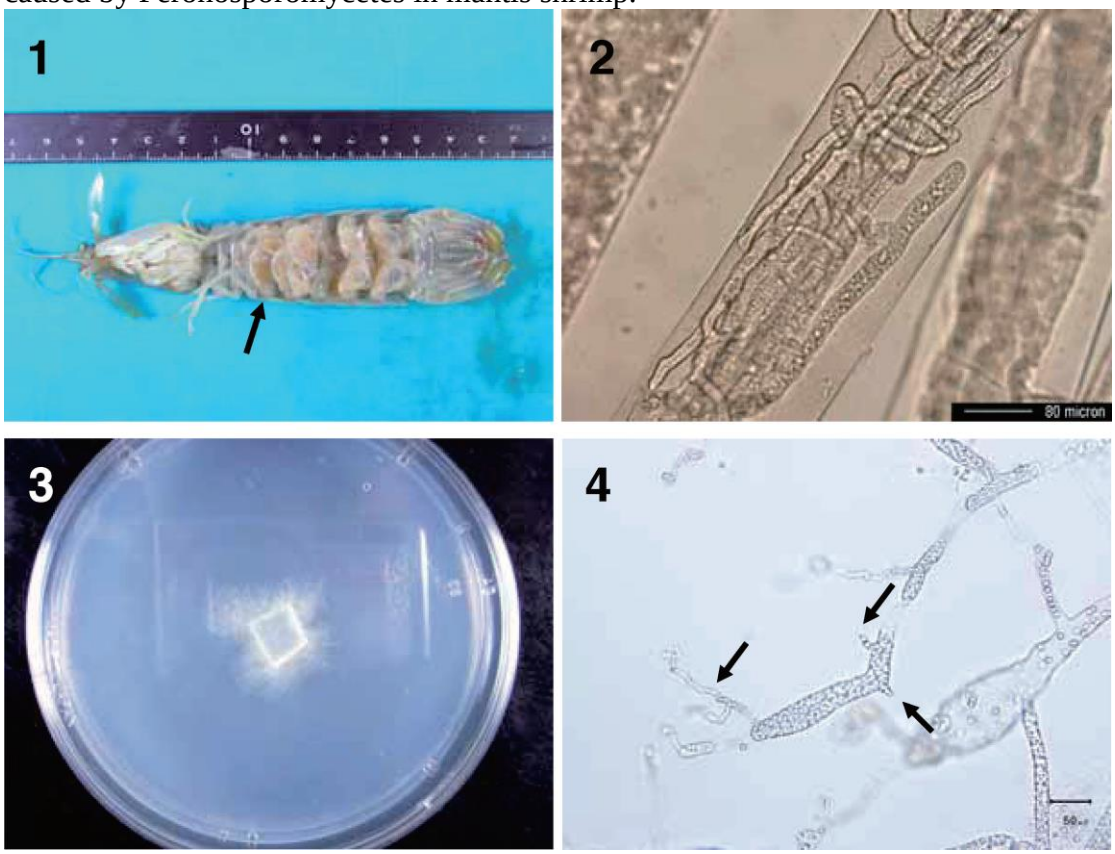


Light micrographs of Halioticida noduliformans isolate AF08527 showing various stages of zoosporogenesis. (A) Aseptate, branched hyphae. (B) Hyphae showing cytoplasmic fragmentation. (C) Developing zoosporangium. (D) Developed zoosporangium with discharge tube. (E) Sporulation of zoospores. (F) Discharge tube releasing zoospore. Scale=50 μm. **Muraosa et al., 2009**

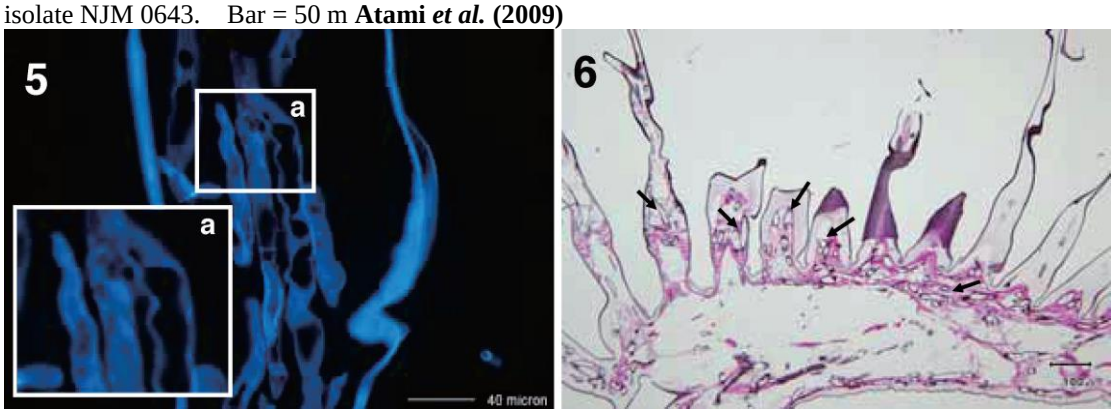
Reports:

Atami *et al.* (2009) reported a Halioticida infection in wild mantis shrimp Oratosquilla oratoria in Tokyo Bay, Japan. Fungi were found in the gills of mantis shrimp, isolated from lesions using PYGS agar, and identified by morphological observation and molecular analysis. The fungi formed fragments in the hyphae and several discharge tubes developed from each fragment. Zoospores were formed within the fragments and released into the seawater through the tops of discharge tubes. Based on the characteristics of zoospore production mode, the fungi were classified into the genus Halioticida. Fungal isolates NJM 0642 and NJM 0643, isolated from mantis shrimp, were compared by molecular analysis of the D1/D2 region of the large subunit ribosomal RNA gene (LSU rDNA) with other fungi belonging to Peronosporomycetes, isolated from various marine crustaceans and abalones Haliotis spp. As a result, both isolates were identified as Halioticida noduliformans, which has been isolated from abalone. Moreover, experimental infection demonstrated that the

fungus was pathogenic to mantis shrimp. This is the first report of fungal disease caused by Peronosporomycetes in mantis shrimp.



1. A mantis shrimp with fungal infection. The color of gills changes to brown (arrow).**2.** Gill filaments of mantis shrimp naturally infected with fungus. Bar = 80 m m.**3.** A colony of the fungus isolated from mantis shrimp grown on PYGS agar **4.** Fragment with discharge tubes (arrow) of the isolate NJM 0643. Bar = 50 m **Atami *et al.* (2009)**

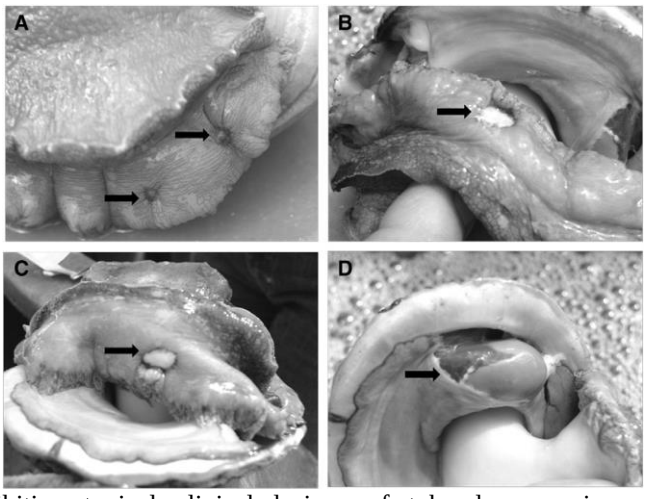


Histopathology of gill filaments in mantis shrimp naturally infected with fungus (panel a). Stained with H&E and Uvitex 2B (UV light). Bar = 40 m m. **6.** Histopathology of base of gills in mantis shrimp naturally infected with fungus (black arrow). Stained with Grocott H&E. Bar = 100 m m. **Atami *et al.* (2009)**

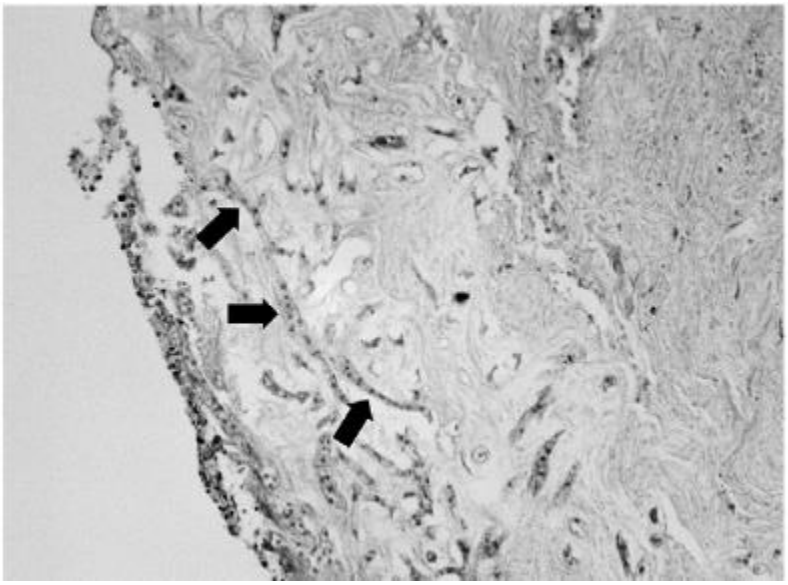
Muraosa *et al.* (2009) isolated 4 strains belonging to the Peronosporomycetes (formerly Oomycetes) from white nodules found on the mantle of three species of abalone. In artificial seawater, the four isolates formed fragments such as in the genus *Haliphthoros*, but the protoplasm constriction was weaker, and fragments were longer, with smaller spaces between them, than those of *Haliphthoros*. The four strains form one or more discharge tubes from each zoosporangium. The four strains were similar, but not identical, to the genus *Haliphthoros* based on morphological characteristics. As a result, the four isolates were classified in a new genus and species, *Halioticida noduliformans* gen. et sp. nov. Phylogenetic

analysis of the D1/D2 region of the large subunit ribosomal RNA gene (LSU rDNA) was performed, and the four isolates showed 100%–99.8% concordance. In the phylogenetic tree, the four isolates were not classified in the subclass Peronosporomycetidae, Saprolegniomycetidae, or Rhipidiomycetidae. However, the four isolates formed a new clade with genera *Haliphthoros* and *Halocrusticida* in. Within this new clade, the four isolates, *Haliphthoros* spp. And *Halocrusticida* spp., were grouped in their respective independent subclades. These results showed that these were the new genus and species from the morphological characteristics.

Macey *et al.* (2011) discovered an outbreak of mycosis in abalone culture facilities in South Africa. Infected abalone are characterised by multifocal areas of necrosis of the epithelium, underlying muscle fibres and connective tissues of the foot, epipodium and mantle. The lesions were typically 2–3 mm in diameter and contained numerous hyphae. Affected aquaculture facilities have suffered significant production losses, with up to 90% mortality among spat and up to 30% mortality among older animals. The pathogen has been identified as Halioticida noduliformans Muraosa, Morimoto, Sano, Nishimura and Hatai, 2009 from the morphological characteristics, the physiological characteristics that were investigated and molecular analysis of the large subunit ribosomal RNA (LSU rRNA) gene. Although the optimum temperature for growth of the fungus was 20–25 °C, it grew at a wide range of temperatures (10–25 °C). No growth occurred at 5 and 30 °C. The fungus grew well in peptone yeast glucose saline (PYGS) agar containing 50–100% seawater, with optimum growth occurring in full strength seawater (~35 ppt salinity). No vegetative growth was observed on PYG agar without seawater or supplemented exclusively with varying concentrations (0–5%) of NaCl. The isolate grew at a wide range of pH (4.0–10.0) with the optimum pH value of 7.0–8.0. The disease was reproduced in juvenile abalone (30–50 mm shell length) by artificial infection and the fungus was re-isolated from moribund abalone, demonstrating that the isolated H. noduliformans fungus is the cause of abalone tubercle mycosis disease that has been occurring in South Africa since 2006.



Haliotis midae exhibiting typical clinical lesions of tubercle mycosis caused by Halioticida noduliformans. (A) Epithelial defect. (B–C) Epithelial defect covered in loosely adherent off-white material and surrounded by a thin black reaction zone. (D) Enlarged lesion affecting a large area of tissue. **Macey *et al.* (2011)**



Histological section of epipodium showing numerous hyphae (arrows) in naturally infected Haliotis midae (200× magnification).

Greeff *et al.* (2012) discovered Abalone Haliotis midae exhibiting typical clinical signs of tubercle mycosis in South African culture facilities in 2006, posing a significant threat to the industry. The fungus responsible for the outbreak was identified as a Peronosporomycete, Halioticida noduliformans. Currently, histopathology and gross observation are used to diagnose this disease, but these 2 methods are neither rapid nor sensitive enough to provide accurate and reliable diagnosis. Real-time quantitative PCR (qPCR) is a rapid and reliable method for the detection and quantification of a variety of pathogens, so therefore we aimed to develop a qPCR assay for species-specific detection and quantification of H. noduliformans. Effective extraction of H. noduliformans genomic DNA from laboratory grown cultures, as well as from spiked abalone tissues, was accomplished by grinding samples using a pellet pestle followed by heat lysis in the presence of Chelax-100 beads. A set of oligonucleotide primers was designed to specifically amplify H. noduliformans DNA in the large subunit (LSU) rRNA gene, and tested for cross-reactivity to DNA extracted from related and non-related fungi isolated from seaweeds, crustaceans and healthy abalone; no cross-amplification was detected. When performing PCR assays in an abalone tissue matrix, an environment designed to be a non-sterile simulation of environmental conditions, no amplification occurred in the negative controls. The qPCR assay sensitivity was determined to be approximately 0.28 pg of fungal DNA (~2.3 spores) in a 25 µl reaction volume. Our qPCR technique will be useful for monitoring and quantifying H. noduliformans for the surveillance and management of abalone tubercle mycosis in South Africa.

References:

1. Atami, H., Yasunori Muraosa and Kishio HataiHalioticida Infection Found in Wild Mantis Shrimp Oratosquilla oratoria in Japan. Fish Pathology 44(3):145-150. 2009 ·
2. Greeff MR, Christison KW, Macey BM. Development and preliminary evaluation of a real-time PCR assay for Halioticida noduliformans in abalone tissues. Dis Aquat Organ. 2012 Jun 13;99(2):103-17.
3. Macey. B.M. , K.W. Christison , A. Mouton. Halioticida noduliformans isolated from cultured abalone (Haliotis midae) in South Africa. Aquaculture 315 (2011) 187–195

4. Muraosa, Y., Morimoto, K., Sano, A. et al. A new peronosporomycete, *Halioticida noduliformans* gen. et sp. nov., isolated from white nodules in the abalone *Haliotis* spp. from Japan. Mycoscience (2009) 50: 106. doi:10.1007/s10267-008-0462-0

11. Halocrusticida

- A new genus Halocrusticida gen. nov. (Lagenidiales, Haliphthoraceae) was proposed for the six species formerly reported as the fungi in the genus Atkinsiella except A. dubia (Nakamura and Hatai 1995b).
- The six species of Atkinsiella were reported from various aquatic animals (Martin 1977; Bian and Egusa 1980; Nakamura and Hatai 1994, 1995a; Kitancharoen et al. 1994; Kitancharoen and Hatai 1995)
- Mycelia contained granular clusters without oil droplets and vacuoleson A. dubia, but many vacuoles and numerous shiny granules were found on the others.
- The most apparent difference between A. dubia and the other six species of Atkinsiella was the behavior of zoospores in the first motile stage.
- Zoospores encysted within zoosporangia and discharge tubes following the first motile stage in A. dubia, while zoospores in the first motile stage were released from zoosporangia in the other six species.
- The genus is characterized by: Thallus is endobiotic, holocarpic, stout, and branched. Zoosporangia are the same in size and shape as thalli. Discharge tubes develop one to several per sporangium. Zoospores in the first motile stage emerge from the zoosporangia. Zoospores are monoplanetic or diplanetic, isokont, laterally biflagellate. Germinating zoospore has a slender germ tube. Sexual reproduction is absent. It is parasitic on aquatic animals, especially marine crustaceans.

NCBI Taxonomy

Cellular organisms +

- Eukaryota +
  - Stramenopiles +
    - Oomycetes +
      - Lagenidiales +
        - Haliphthoraceae +
          - Halocrusticida +
            - *Halocrusticida baliensis*
            - *Halocrusticida okinawaensis*
            - *Halocrusticida parasitica*

Index Fungorum

Halocrusticida baliensis Hatai, Roza & T. Nakay. 2000

- *Halocrusticida awabi* (Kitanch., K. Nakam., S. Wada & Hatai) K. Nakam. & Hatai 1995
- *Halocrusticida entomophaga* (W. W. Martin) K. Nakam. & Hatai 1995
- *Halocrusticida hamanaensis* (Bian & Egusa) K. Nakam. & Hatai 1995
- *Halocrusticida okinawaensis* (K. Nakam. & Hatai) K. Nakam. & Hatai 1995

- *Halocrusticida panuliri* (Kitanch. & Hatai) K. Nakam. & Hatai 1995
- *Halocrusticida parasitica* (K. Nakam. & Hatai) K. Nakam. & Hatai 1995

Reports:

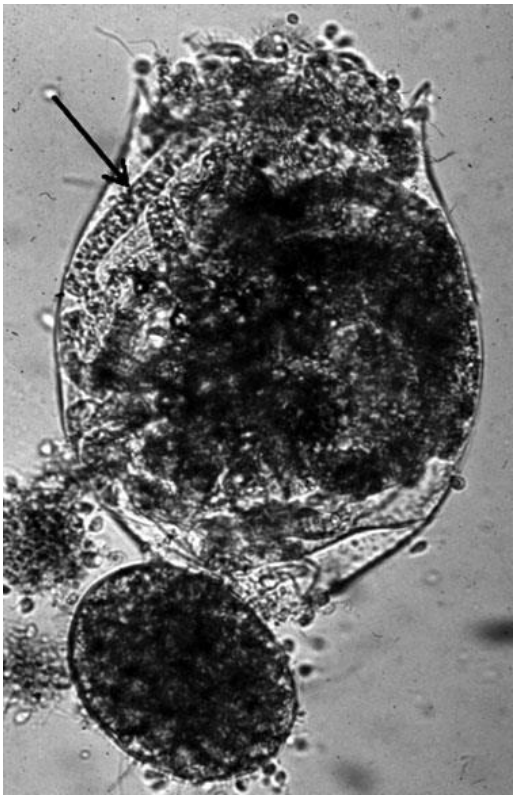
Halocrusticida awabi was originally reported as Atkinsiella awabi (Kitancharoen et al. 1994). The fungus was isolated from diseased abalone, Haliotis sieboldii in Japan. It showed external signs of infection of tubercle-like swelling on the mantle and melanized lesions on the peduncle.

Halocrusticida hamanaensis was originally reported as Atkinsiella hamanaensis (Bian and Egusa 1980). The fungus was isolated from ova of mangrove crab, Scylla serrata in Japan. The swollen hyphal tips up to 150 mm in diameter contained dense cytoplasm. Each sporangium was formed through the formation of septa and several lateral or terminal discharge tubes. The discharge tubes were straight or wavy, measuring 40–1,150 _ 5–15 mm. Zoospores measured 6.3 (5–10) _ 4.5 (3.8–5) mm in size, were pyriform or slipper-shaped, with two lateral flagella. The encysted spores were 5 (4.5–7.5) mm in diameter, spherical, subglobose, or angular. The encysted spore in sterile sea water developed a hair-like filament, 10–270 mm in length.

Halocrusticida okinawaensis was originally reported as Atkinsiella okinawaensis (Nakamura and Hatai 1995a). The new fungus was isolated from infected eggs and zoeae of the marine crab, Portunus pelagicus.

Halocrusticida panulirata was originally reported as Atkinsiella panulirata (Kitancharoen and Hatai 1995). This species was isolated from philozoma of the diseased spiny lobster, Panulirus japonicus in Japan.

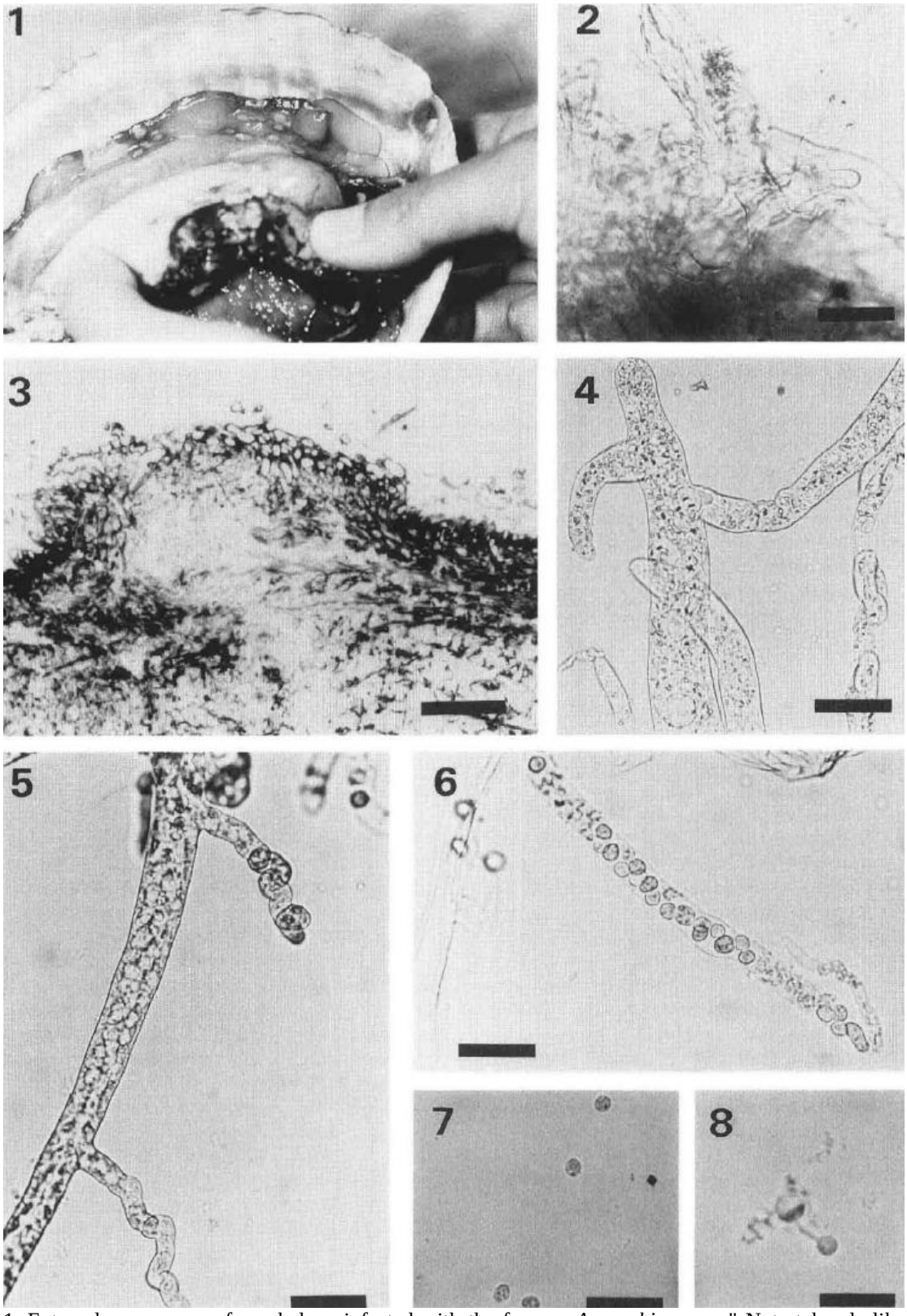
Halocrusticida parasitica was originally reported as Atkinsiella parasitica (Nakamura and Hatai 1994). In May 1992 the rotifer, Brachionus plicatilis did not increase in number when it was bred in a concrete tank as food supply for seed production of crustaceans and fishes. Because protozoa were observed microscopically on the surface of rotifers, a bath treatment with 25 ppm formalin was first conducted to solve the problem in the tank. However, no increase in the number of rotifers in the tank was found following the treatment. Further detailed microscopical observation revealed thick, non-septate hyphae measuring about 10 mm diameter in the eggs and bodies of many rotifers examined. Discharge tubes were extended outside the bodies, and zoospores with lateral biflagella were released into the seawater through the tubes. Vesicles were not formed at the tip of discharge tubes (Nakamura and Hatai 1994; Nakamura et al. 1994a). The fungus isolated from the rotifer was characterized by producing monoplanetic, lateral biflagellate zoospores, and infrequently branched discharge tubes.



Hyphae in bodies of a rotifer (arrow)

BIAN and. EGUSA (1980) described and illustrated *Atkinsiella hamanaensis* sp. nov. a marine mastigomycete isolated from ova of the mangrove crab, *Scylla serrata* (Forsskål). The fungus grew over a temperature range of 15–32°C, with an optimum of 29–32°C. Its growth was observed in peptone-yeast extract glucose broth containing 1–5% NaCl, with optimum growth at 2–3% NaCl concentration. At 6% or more NaCl concentration, growth was inhibited. Its pH tolerance ranged from 4 to 9.

Kitancharoen *et al*. (1994) reported a fungal disease in the abalone,Haliotis sieboldii, stocked in Yamaguchi Prefecture, Japan, which showed external signs of infection of tubercle-like swelling on the mantle and melanized lesions on the peduncle. The fungus responsible was isolated by inoculating materials taken from the lesions onto PYGS agar with streptomycin sulphate and ampicillin, and incubation at 20C. For morphological observation and spore formation study, the fungus was transferred respectively into PYGS broth and sterilized artificial seawater and incubated at 20C. Resulting, hyphae were stout, irregular, branched, 16–140m diam, sporadically consisting of dense cytoplasmic swollen hyphae. Sporangia were formed through the formation of septa and lateral or terminal discharge tubes which were wavy or coiled. Zoospores were pyriform, biflagellate and diplanetic. The encysted spore generally developed a hairlike filament with globular enlarged tip in PYGS broth. Direct germination without filament formation also occurred occasionally. This fungus was identified as belonging to the genusAtkinsiella, and was designatedAtkinsiella awabi sp. nov. The fungus was exclusively a marine fungus and grew best in shrimp extract medium at 20C. Five chemicals were tested for their effects against fungal zoospores.



1. External appearance of an abalone infected with the fungus. *A. awabi* sp. nov" Note tubercle-like swelling on the mantle and melanized lesions on the peduncle. 2. Hyphae of *A. awabi* sp. nov. taken from the lesions of abalone. 3. Histopathological section through a lesion of an infected abalone illustrating many fungal hyphae (Grocott-Giemsa) . (Scale bar= 600 I'm.) 4 . *Vegetative* hyphae grown in PYGS broth after three days of incubation at 15° C. Kitancharoen *et al*. (1994)

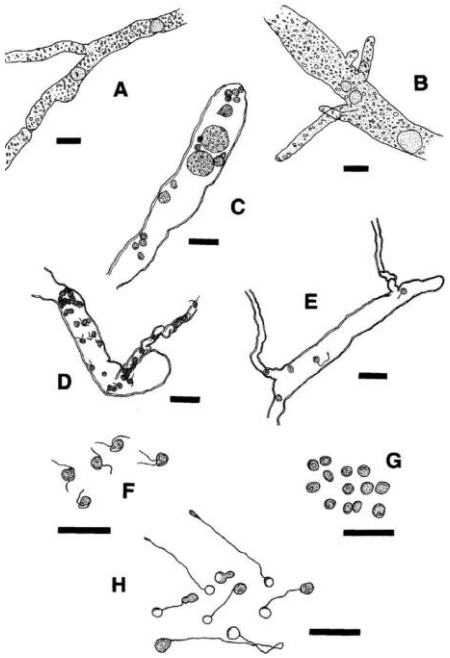
Nakamura and Hatai (1994) described and illustrated a new species of *Atkinsiella* (Lagenidiales, Oomycetes), *Atkinsiella parasitica* . It was isolated from the eggs and bodies of a rotifer, *Brachionus plicatilis.* with fungal infection in 1992. The rotifer was reared in a hatchery as the first food supply for seed production of crustaceans and fishes. The fungus is characterized by producing monoplanetic. lateral biflagellate zoospores, and infrequently branched discharge tubes. Optimum temperature for the fungus was 25°C. The fungus was considered an obligate marine fungus, because its growth was observed onlyon PYGS medium including seawater.

Description:

1. Halocrusticida awabi (Kitanch., K. Nakam., S. Wada & Hatai) K. Nakam. & Hatai, Mycoscience 36 (4): 437 (1995)

≡Atkinsiella awabi Kitanch., K. Nakam., S. Wada & Hatai, Mycoscience 35 (3): 267 (1994)
≡Halodaphnea awabi (Kitanch., K. Nakam., S. Wada & Hatai) M.W. Dick, Mycological Research 102 (9): 1065 (1998)

Hyphae are stout, irregular, branched, 16-140μm diam, sporadically consisting of dense cytoplasmic swollen hyphae. Sporangia are formed through the formation of septa and lateral or terminal discharge tubes which are wavy or coiled. Zoospores were pyriform, biflagellate and diplanetic. The encysted spore generally develop a hairlike filament with globular enlarged tip in PYGS broth. Direct germination without filament formation also occurrs occasionally. This fungus was identified as belonging to the genus*Atkinsiella*, and was designated *Atkinsiella awabi sp.* nov. The fungus was exclusively a marine fungus and grew best in shrimp extract medium at 20°C. Five chemicals were tested for their effects against fungal zoospores.



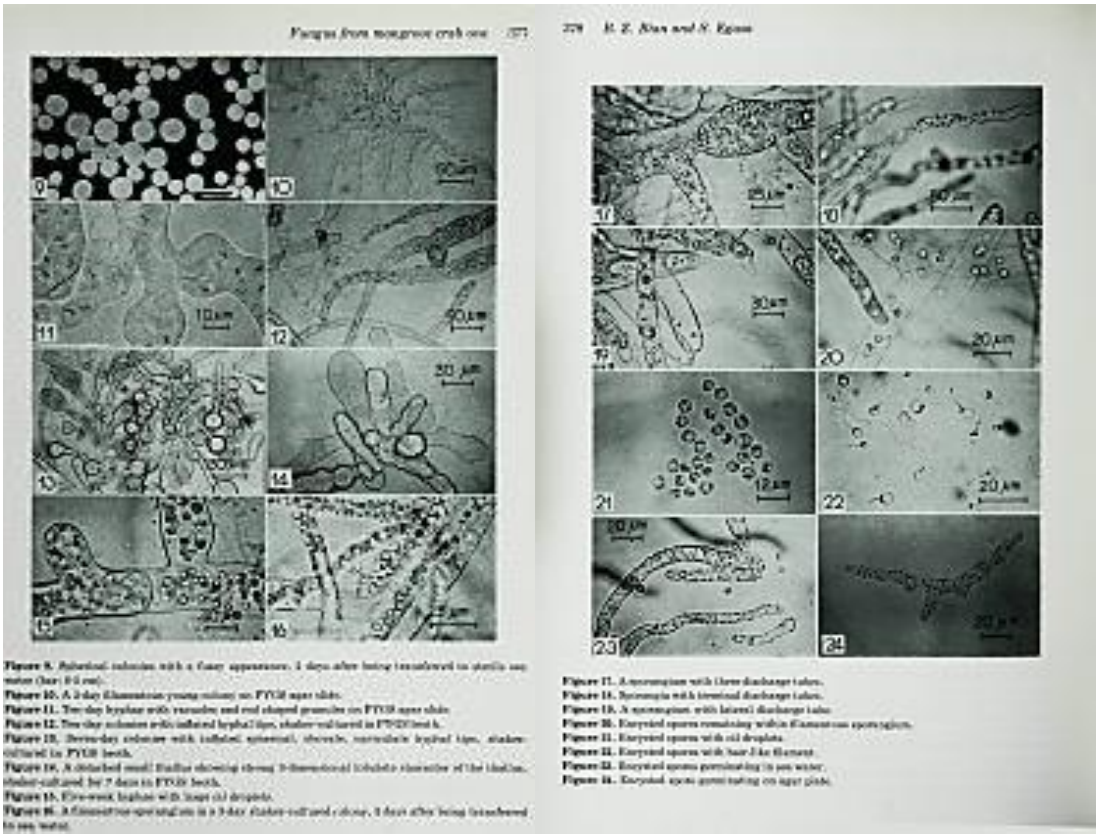
Halocrusticida awabi sp. nov. isolated from abalone. A, B. Vegetative hyphae grown in PYGS broth; C. Zoosporangium during zoospore formation, protoplasm cleaved and formed zoospores; D. Release

of zoospores; E. Some zoospores encysted and germinated within zoosporangium; F. Swimming zoospores; G. Encysted zoospores; H. Germination. (Scale bars= 50 *I,m.)*

2. *Halocrusticida hamanaensis* (Bian & Egusa) K. Nakam. & Hatai, Mycoscience 36 (4): 437 (1995)

≡Atkinsiella hamanaensis Bian & Egusa, Journal of Fish Diseases 3 (5): 379 (1980)
≡Halodaphnea hamanaensis (Bian & Egusa) M.W. Dick, Mycol. Res.102 (9): 1065 (1998)
≡Halocrusticida okinawaensis (K. Nakam. & Hatai) K. Nakam. & Hatai, Mycosci. 36 (4): 437 (1995)
≡Atkinsiella okinawaensis K. Nakam. & Hatai, Mycoscience 36 (1): 89 (1995)
≡Halodaphnea okinawaensis (K. Nakam. & Hatai) M.W. Dick, Mycological Research 102 (9): 1065

Hyphae are stout, nonseptate at first, irregularly branched with numerous shiny rod granules, 10–38 mm width. In seawater, hyphae are divided into subthalli with septa. Gemmae are present with thick walls, 22–190 mm in diameter. Zoosporangia are the same size and shapes as subthalli and gemmae. Discharge tubes are produced laterally or terminally from the sporangia, usually coiled or wavy. Each sporangium extendsone to several discharge tubes. In the discharge tubes, zoospores are produced in more than two rows. The discharge tubes are 6–10 mm diameter and 40–510 mm length. Zoospores are laterally biflagellate, diplanetic, 4.7 _ 6.3 mm on average. Germination is observed about 3 h after spores had encysted, with a hair-like filament measuring 5–190 mm length.

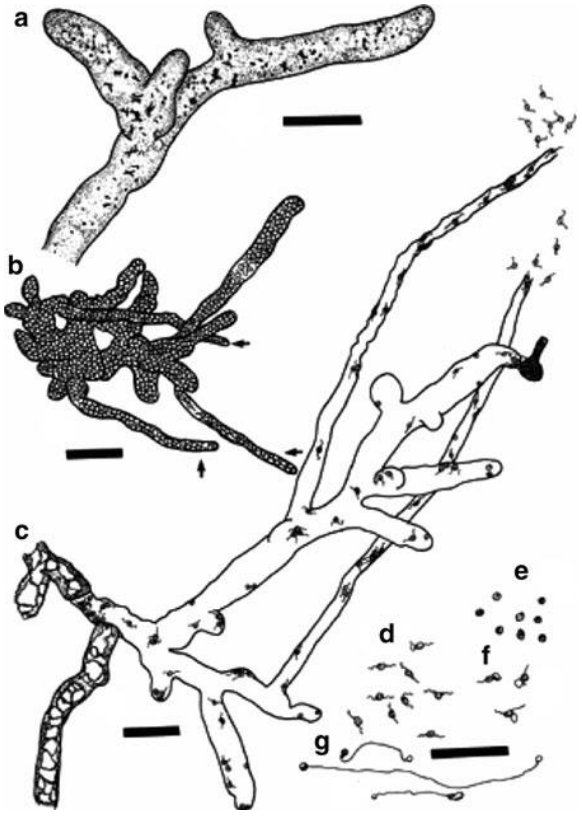


BIAN and EGUSA, 1980

3.Halocrusticida okinawaensis (K. Nakam. & Hatai) K. Nakam. & Hatai, Mycoscience 36 (4): 437 (1995)

≡Atkinsiella okinawaensis K. Nakam. & Hatai, Mycoscience 36 (1): 89 (1995)
≡Halodaphnea okinawaensis (K. Nakam. & Hatai) M.W. Dick, Mycological Research 102 (9): 1065 (1998)

Hyphae are stout, nonseptate at first, irregularly branched with numerous shiny rod granules, 10–38 mm width. In seawater, hyphae are divided into subthalli with septa. Gemmae are present with thick walls, 22–190 mm in diameter. Zoosporangia are the same size and shapes as subthalli and gemmae. Discharge tubes are produced laterally or terminally from the sporangia, usually coiled or wavy. Each sporangium extends one to several discharge tubes. In the discharge tubes, zoospores are produced in more than two rows. The discharge tubes were 6–10 mm diameter and 40–510 mm length. Zoospores are laterally biflagellate, diplanetic, 4.7 _ 6.3 mm on average. Germination is observed about 3 h after spores had encysted, with a hair-like filament measuring 5–190 mm length.

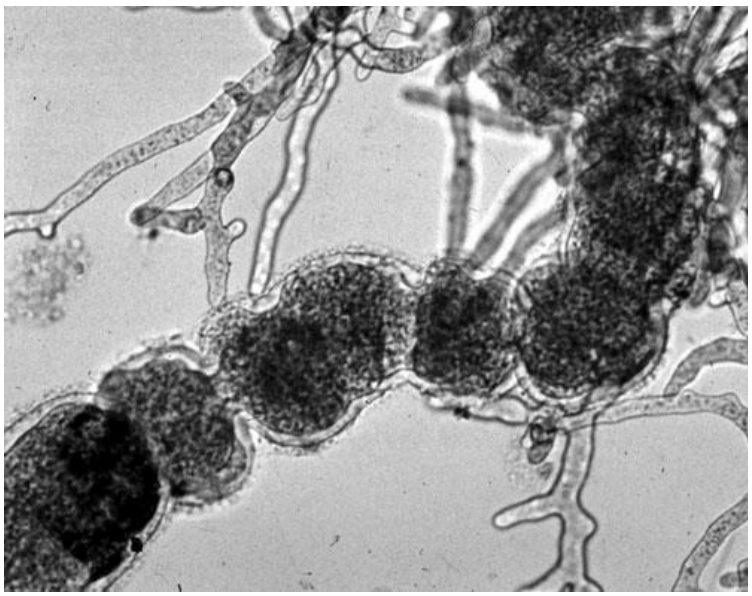


Morphological characteristics of *Halocrusticida okinawaensis* isolated from a zoea of P. pelagicus. Scale ¼ 50 mm. (a) Hyphae in PYGS broth; (b) A zoosporangium with three discharge tubes (arrows); (c) Zoospores released from the orifices of two discharge tubes. Another zoosporangium with one discharge tube is on the right; (d) Zoospores; (e) Encysted zoospores; (f) Secondary zoospores released from cysts; (g) Germination (Nakamura and Hatai 1995a)

4. Halocrusticida panulirata (Kitanch. & Hatai) K. Nakam. & Hatai, Mycoscience 36 (4): 437 (1995)

≡Atkinsiella panulirata Kitanch. & Hatai, Mycoscience 36 (1): 100 (1995)
≡Halocrusticida panuliri (Kitanch. & Hatai) K. Nakam. & Hatai (1995)
≡Halodaphnea panulirata (Kitanch. & Hatai) M.W. Dick, Mycol Res 102 (9): 1065 (1998)

The fungus exhibits slow growth, occasionally submerged, with a creamy white, raised moist colony. Hyphae are stout, arranged in radiating pattern, irregularly branched, 10–22 mm diameter, occasionally separated by cross walls into subthalli. Thalli occasionally consist of swollen features. Sporangia are formed from the subthalli had one to three or partly coiled discharge tubes at the terminal or subterminal area. Zoospores are pyriform or reniform, biflagellate, isokont, and diplanetic. Encysted spores germinate as a hair-like filament with a globular enlarged tip in sterilized synthetic seawater, and directly as stout initial hyphae in PYGS broth. Gemmae spontaneously occurre in 3-day-old culture in PYGS broth at 25^{o}C. They are characterized by saccate-lobed-chained, thick-walled dense cytoplasmic and non-vacuolate features, width of 179–270 mm and various lengths up to 18 mm. Gemmae not only developed new thalli on PYGS agar or in PYGS broth, but also in sterilized synthetic seawater.



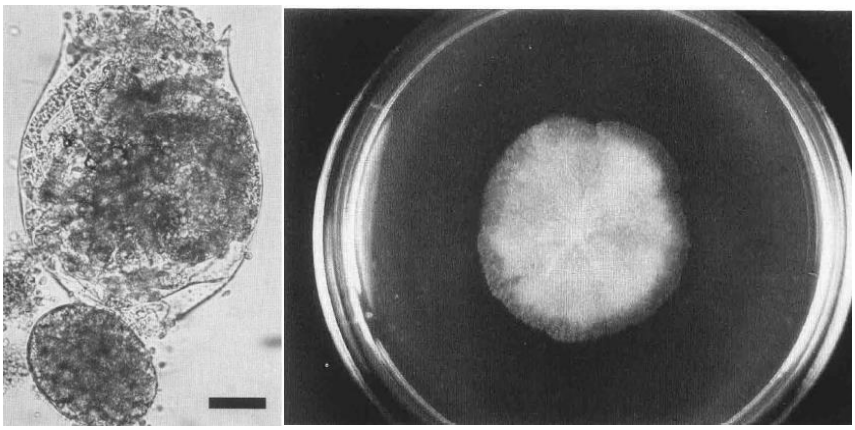
Gemmae (arrow) spontaneously occurred in Halocrusticida panulirata culture in PYGS broth at 25^{o}C

5.Halocrusticida parasitica (K. Nakam. & Hatai) K. Nakam. & Hatai, Mycoscience 36 (4): 437 (1995)

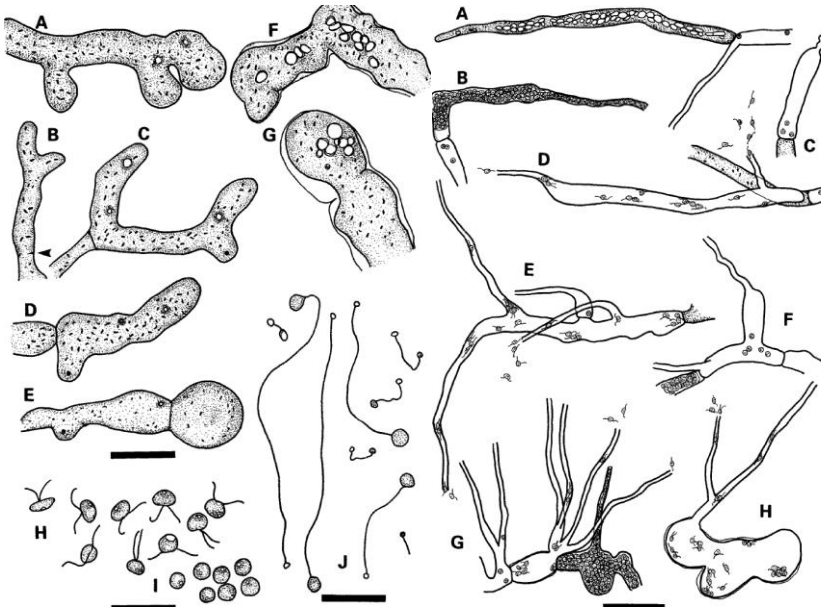
≡Atkinsiella parasitica K. Nakam. & Hatai, Mycoscience 35 (4): 387 (1994)
≡Atkinsiella parasitica K. Nakam. & Hatai, Mycoscience 35 (4): 387 (1994)

Thalli endobiotic, holocarpic, partly eucarpic in age or at lower temperatures, stout, branched, non-septate, saccate-lobed, *15-50,um* diam, swollen hyphal tips up to 110 pm diam. In PYGS broth at 25°C, the thallus at first was non-septate, generally vacuolate, with numerous shiny granules, septate in age dividing into subthalli. Gemmae present, saccate-lobed, thick-walled, with shiny globules, 40-200 *pm* diam, developing zoosporangia in seawater. Subthalli cylindrical, saccate, irregular, tuberculate, very variable in size and shape. Zoosporangia of same size and same shape as subthalli, extending one to several simple or infrequently branched discharge tubes. Discharge tubes straight, wavy or coiled, usually with a broad cone-shaped base, tapering or equal diameter, 6-14 x 20-780 pm, formed laterally or terminally from a zoosporangium. Zoospore production occurred within the zoosporangium and discharge tubes, 18-21 h at 25°C after vegetative hyphae were transferred into

sterilized seawater. In the course of zoospore formation, flagellae appeared around the mass of protoplasm, and then protoplasm divided into initial zoospores. This behavior occurred first in the zoosporangium, then in the discharge tubes. The sequential zoospore production of subthalli separated from a single thallus with septa was observed. Proliferation was not observed. Zoospores pyriform, oblong, slipper-shaped, spherical, monoplanetic, laterally biflagellate, isokont, 4.8-7.4 x 4.0-5.6 pm, 6.0 x *4.6 um* on average. Zoospores were discharged within 30 min after beginning to move in the zoosporangium and discharge tubes, by rupture of the orifice of discharge tubes. In zoosporangia with several discharge tubes, zoospores were generally released first from one of the discharge tubes, then from others; but sometimes other discharge tubes did not open, because the movement of zoospores at their orifices was too weak.



A rotifer, *Brachionus plicatilis,* with fungal infection. (Scale: 50 I'm.) Yellowish moist colony incubated on PYGS agar plate at 25°C for 20 days.



Halocrusticida parasitica isolated from the rotifer *Brachionus plicatilis.* (Scales: A-G=50 *pm;* H-I=20 *,um;* J=50 *pm.)* A. Thallus with numerous shiny granules. B. Septum appeared in thallus (arrow head). C-D. Subthallus separated with a septum.E. Swollen hyphal tip. F-G. Gemmae. H. Zoospores, laterally biflagellate. I. Encysted zoospores. J. Germination. Zoosporogenesis of *Atkinsiella parasitica* sp. nov. (Scale: A-H = 50 *,urn.)* A. Numerous large vacuoles appeared at an early stage of zoosporogenesis, and later discharge tubes developed. B. Zoospore formation in a zoosporangium and a discharge tube at the final stage of zooaporogenesis. C-F. One to three discharge tubes formed from a zoosporangium. G-H. Branched discharge tubes.

References:

1. BIAN, B. Z., S. EGUSA. *Atkinsiella hamanaensis* sp. nov. isolated from cultivated ova of the mangrove crab, *Scylla serrata*(Forsskål)˙ J. Fish Dis. Volume 3, Issue 5September 1980 Pages 373–385
2. Nakamura, K. and Kishio Hatai. *Atkinsiella parasitica* sp. nov. isolated from a rotifer,*Brachionus plicatilis*. Mycoscience 35: 383-389, 1994
3. Kitancharoen, N., Kazuyo Nakamura, Shinpei Wada, Kishio Hatai. *Atkinsiella awabi* sp. nov. isolated from stocked abalone,*Haliotis sieboldii*. Mycoscience Volume 35, Issue 3, October 1994, Pages 265-270

12. Atkinsiella

- **Atkins (1954)** isolated a fungus from eggs of pea crab, Pinnotheres pisum in England, and assigned it to the genus Plectospira. Atkins observed the same species on the eggs of Gonoplax rhomboids and succeeded in experimentally infecting the eggs of some species of crustaceans.
- **Vishniac (1958**) renamed Atkins' fungus as Atkinsiella dubia.
- **Fuller et al. (1964)** described the morphology and development of Atkinsiella dubia isolated in pure culture from marine algae
- **Sparrow (1973)** isolated Atkinsiella dubia from marine algae and the eggs of various crabs
- **Dick (2001)** classified Atkinsiella dubia and Haliphthoros spp. into Saprolegniomycetidae, but at present the genus Haliphthoros is classified into different clade, Haliphthoros/Halocrusticida clade, because they constructed different clades from phylogenetic analysis.
- **Nakamura and Hatai *(1995)*** described and illustrated *Atkinsiella dubia*, isolated from the mantle of abalone (*Haliotis sieboldii*), as a new record from Japan.
- **Roza and Hatai (1999)** reported that heavy mortalities reaching 100% among larvae of the Japanese mitten crab, Eriocheir japonicus, occurred in Yamaguchi Prefecture, Japan.

Classification:

Integrated Taxonomic Information System (ITIS)
Myxomycota +
- Phycomycota +
  - Saprolegniales +
    - Haliphthoraceae +
      - Atkinsiella H. S. Vishniac, 1958
        - *Atkinsiella dubia* (Atkins) Vishniac
      - Haliphthoros Vishniac, 1958 +

Index Fungorum
Atkinsiella Vishniac 1958
- *Atkinsiella awabi* Kitanch., K. Nakam., S. Wada & Hatai 1994
- Atkinsiella dubia (D. Atkins) Vishniac 1958
- *Atkinsiella entomophaga* W. W. Martin 1977

- *Atkinsiella hamanaensis* Bian & Egusa 1980
- *Atkinsiella okinawaensis* K. Nakam. & Hatai 1995
- *Atkinsiella panulirata* Kitanch. & Hatai 1995
- *Atkinsiella parasitica* K. Nakam. & Hatai 1994

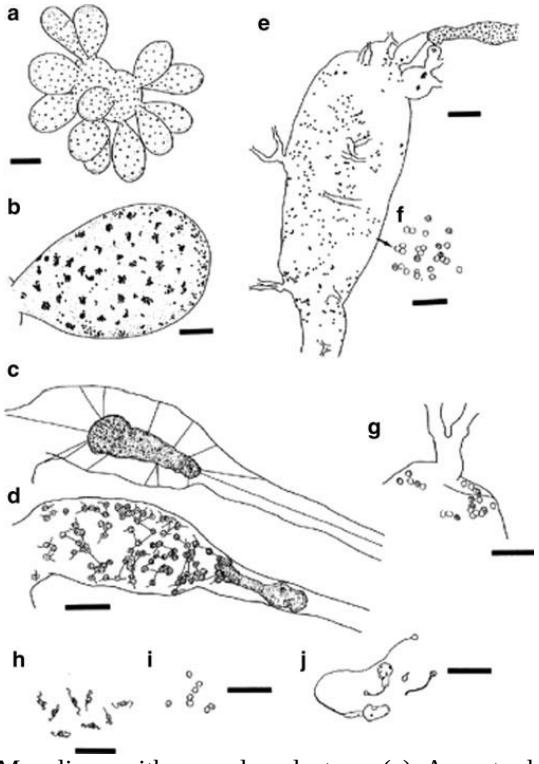
NCBI Taxonomy
Cellular organisms +
- Eukaryota +
  - Stramenopiles +
    - Oomycetes +
      - Lagenidiales +
        - Haliphthoraceae +
          - Atkinsiella
            - *Atkinsiella dubia*
          - Environmental samples +
          - Halioticida +
          - Haliphthoros +
          - Halocrusticida +
          - Halodaphnea +

Description:

Atkinsiella dubia (D. Atkins) Vishniac, Mycologia 50: 75 (1958)

≡Plectospira dubia D. Atkins, Journal of the Marine Biological Association of the United Kingdom 33 (3): 731 (1954)

Colonies on PYGS agar attaining a diameter of about 25 mm in 15 d at 25~ crystalline, tuberculate, and moist; moderately heaped at the center. Mycelia in the broth aseptate, radially branched, stout, swollen up to 150 µm in diam, with clusters of shiny spherical granules, without oil droplets and vacuoles. Granularclusters evenly distributed inside mycelia, generally consisting of several decades of granules. Mycelia in seawater developing narrow branches (discharge tubes), followed by zoospore production. Gemmae present. Zoospores in the first motile stage produced after 30h at 25 ^{o}C. Protoplasmic masses due to gathering of granular clusters on zoosporogenesis, supported at the center of zoosporangia by several protoplasmic threads; differentiated into loose networks of zoospores, then into free individual zoospores in the first motile stage. Zoosporangia the same in size and shape as the mycelia, with several discharge tubes extending from each zoosporangium. Zoospores in the first motile stage swimming dully and encysting within zoosporangia and discharge tubes, biflagellate, subglobose to globose, 3-6µm in size. Zoospores in the second motile stage releasing one by one from encysted zoospores within zoosporangia and discharge tubes, swimming freely for a long time; laterally biflagellate, pyriform, slipper-shaped, isokont, 2-7 µm. Zoospores dimorphic and diplanetic. Encysted spores globose to subglobose, 3-7 µm in the first motile stage and 3.5-6µm in the second motile stage. Discharge tubes unbranched or occasionally branched, straight or tapering with flared openings, rarely with a central swelling, 4-9 µm in width, 5-16 µm inlength. Germination produced after 6-8 h after spores transferred to broth with a germ tube.



Atkinsiella dubia. (a, b) Mycelium with granular clusters. (c) A protoplasmic mass supported by several protoplasmic thread. (d) Loose net-works of zoospores. These differentiated into free individual zoospores in the first motile stage. (e) A zoosporangium with branched discharge tubes. (f) Empty encysted zoospores, and encysted zoospores with protoplasm from which zoospores in the second motile stage will emerge. (g) A branched discharge tube with flared openings. (h) Zoospores in the second motile stage. (i) Encysted zoospores after the second motile stage. (j) Germination. Scales: (a, e) 150 mm; (b) 70 mm; (c, d, g–j) 50 mm; (f) ¼ 40 mm

Reports:

Atkins (1954) isolated a fungus from eggs of pea crab, Pinnotheres pisum in England, and assigned it to the genus Plectospira. Atkins observed the same species on the eggs of Gonoplax rhomboids and succeeded in experimentally infecting the eggs of some species of crustaceans.

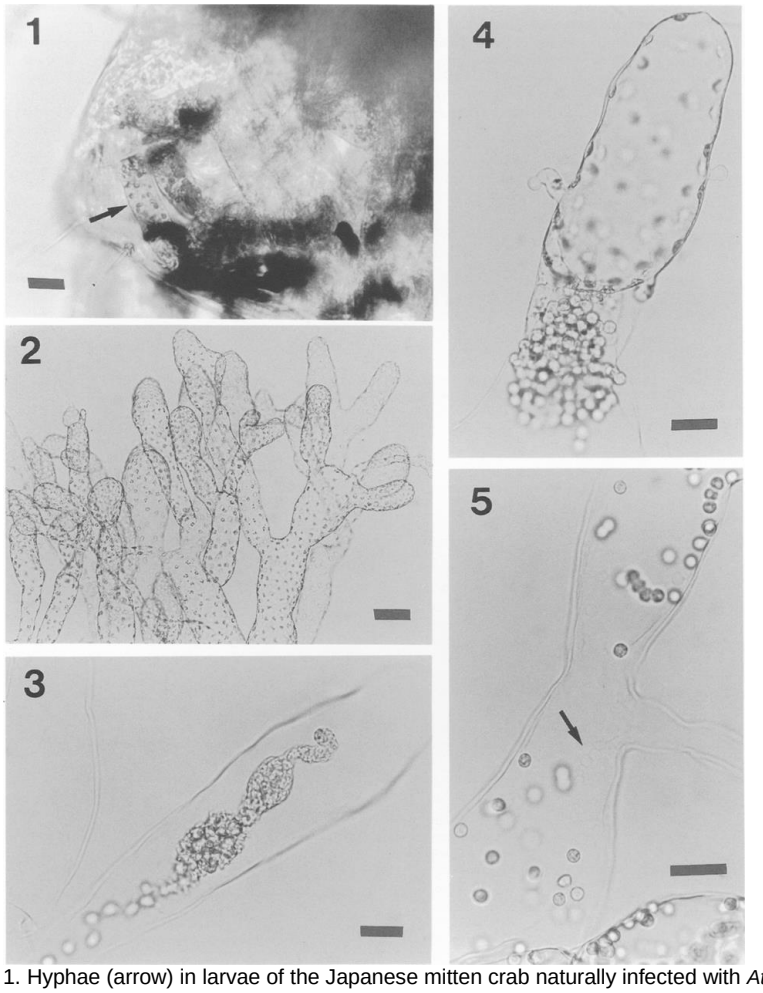
Vishniac (1958) established a new family, Haliphthoraceae (Saprolegniales), for holocarpic biflagellate filamentous fungi, including Haliphthoros milfordensis and Atkins' fungus, which was renamed A. dubia.

Sparrow (1973) reported *Atkinsiella dubia* (Atkins) Vishniac as a parasite in eggs of various crabs in the vicinity of Friday Harbor laboratories. He followed the morphology and development of the marine phycomycete *Atkinsiella dubia* from crab eggs in pure culture on nutrient media. He pointed out the peculiarity of the sequential transformation of the intricate lobed thallus contents into a zoosporangium and of the similar development of the zoospores themselves. He remarked that the thallus itself was sometimes holo-sometimes encarpic and pointed out the resemblances of *Atkinsiella* to *Eurychasma*, a parasite of marine algae.

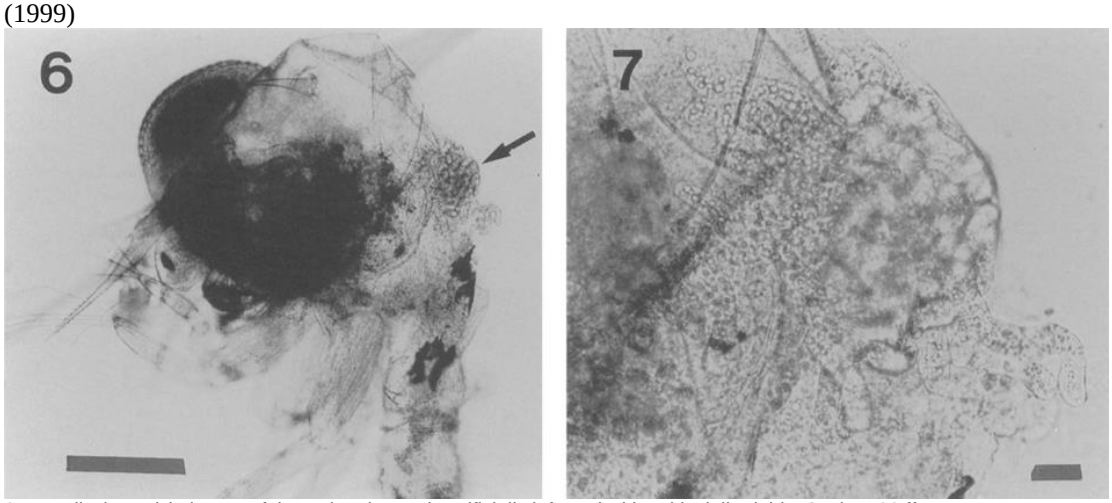
Nakamura and Hatai *(1995)* described and illustrated *Atkinsiella dubia*, isolated from the mantle of abalone (*Haliotis sieboldii*), as a new record from Japan. The fungus was also obtained from the gills of swimming crab (*Portunus*

trituberculatus). Six other species of the genus *Atkinsiella* have hitherto been reported from various aquatic animals. The fungus is distinguished from the other six species by the morphology of its mycelia and the process of zoospore production. The most distinctive feature is that zoospores in the first motile stage of*A. dubia* encyst in zoosporangia, unlike the other species. They therefore proposed *Halocrusticida* gen. nov. (Lagenidiales, Haliphthoraceae) for the other six species of *Atkinsiella*.

Roza and Hatai (1999) reported that heavy mortalities reaching 100% among larvae of the Japanese mitten crab, Eriocheir japonicus, occurred in Yamaguchi Prefecture, Japan. This was the first report of mass mortality in crustaceans due to *A. dubia* infection. Under the microscope, infected zoeal larvae were filled with numerous aseptate hyphae. The infected fungus was inoculated on PYGS agar and incubated at 25°C for 7–10 days. Colonies on PYGS agar were attaining a diameter of about 25 mm in 15 days, crystalline, tuberculate, and moist; moderately heaped at the center. Mycelia in the broth were aseptate, radially branched, stout, swollen up to 150 mm in diameter, with clusters of shiny spherical granules, without oil droplets and vacuoles. Granular clusters were evenly distributed inside mycelia, generally consisting of several granules. Mycelia in seawater developing narrow branches (discharge tubes) were followed by zoospore production. Gemmae were present. Zoospores in the first motile stage were produced after 30 h at 25°C. Protoplasmic masses due to gathering of granular clusters on zoosporogenesis were supported at the center of zoosporangia by several protoplasmic threads; differentiated into loose networks of zoospores, then into free individual zoospores in the first motile stage. Zoosporangia were the same in size and shape as the mycelia, with several discharge tubes extending from each zoosporangium. Zoospores in the first motile stage were swimming dully and encysting within zoosporangia and discharge tubes, and biflagellate, subglobose to globose, 3–6 mm in size. Zoospores in the second motile stage were released one by one from encysted zoospores within zoosporangia and dischargetubes, swimming freely for a long time; laterally biflagellate, pyriform, slipper-shaped, isokont, 2–7 mm. Zoospores were dimorphic and diplanetic. Encysted spores were globose to subglobose, 3–7 mm in the first motile stage and 3.5–6 mm in the second motile stage. Discharge tubes were unbranched or occasionally branched, straight or tapering with flared openings, rarely with a central swelling, 4–9 mm in width, 5–16 mm in length. Germination was observed at 6–8 h after spores with slender germ tube were transferred to broth. This fungus was identified as A. dubia. The optimum growth temperature was at 25°C, and grew only on PYG agar containing 2.5% NaCl and PYGS agar.



1. Hyphae (arrow) in larvae of the Japanese mitten crab naturally infected with *Atkinsiella dubia*..2. Mycelia with granular clusters.3. A protoplasmic mass formed in zoosporangium. 4. Vegetative hyphae and a primary zoospores which encysted in zoosporangium.5. Empty encysted zoospores (arrow) in zoosporangium. Roza and Hatai (1999)



6. Mycelia (arrow) in larvae of the swimming crab artificially infected with *Atkinsiella dubia.* Scale: 100 ffm.
7. High magnification of the lesion shown in Fig. 6. Roza and Hatai (1999)

.

References:

1. Atkins D (1954) A marine fungus Plectospira dubia n. sp. (Saprolegniaceae), infecting crustacean eggs and small Crustacea. J Mar Biol Assoc UK 33:721–732
2. **Des Roza and Kishio Hatai**. ***Atkinsiella dubia*** **infection in the larvae of Japanese mitten crab, *Eriocheir japonicas.*** Mycoscience 40: 235-240, 1999
3. Dick MW (2001) Straminipilous fungi: systematics of the Peronosporomycetes, including accounts of the marine straminipilous protists, the Plasmodiophorids and similar organisms. Kluwer Academic Publishers, Dordrecht, The Netherland
4. Fuller MS, Fowles BE, McLaughlin DJ (1964) Isolation and pure culture study of marine Phycomycetes. Mycologia 56:745–756
5. Hatai. K. Diseases of fish and shellfish caused by marine fungi. Chapter 2 Progress in molecular and subcellular biology 53:15-52 · January 2012
6. Nakamura, K. & Hatai, K. *Atkinsiella dubia* and its related species. Mycoscience (1995) 36: 431. doi:10.1007/BF02268628
7. Roza D, Hatai K (1999) Atkinsiella dubia infection in the larvae of Japanese mitten crab, Eriocheir japonicus. Mycoscience 40:235–240
8. Sparrow FK (1973) The peculiar marine phycomycete Atkinsiella dubia from crab eggs. Arch Mikrobiol 93:137–144
9. Vishniac HS (1958) A new marine Phycomycete. Mycologia 50:66–79

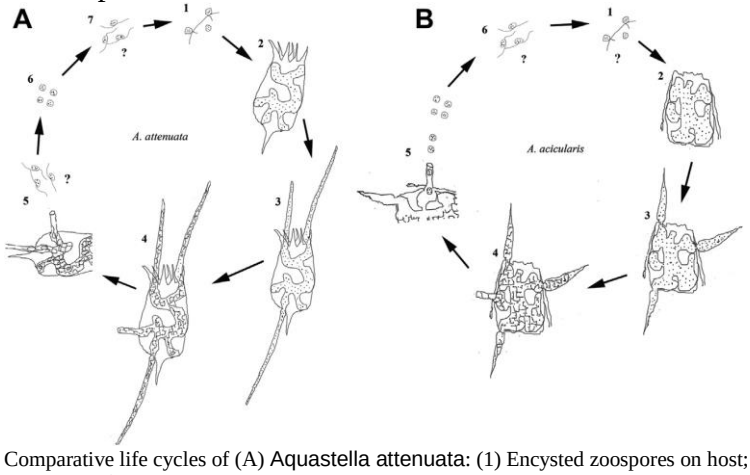
12. Aquastella

- **Molloy *et al.* (2014)** described the oomycete genus Aquastella to accommodate two new species of parasites of rotifers observed in Brooktrout Lake, New York State, USA.
- Three rotifer species--Keratella taurocephala, Polyarthra vulgaris, and Ploesoma truncatum--were infected, and this is the first report of oomycete infection in these species.
- Aquastella attenuata was specific to K. taurocephala and Aquastella acicularis was specific to P. vulgaris and P. truncatum.
- The occurrence of infections correlated with peak host population densities and rotifers were infected in the upper layers of the water column.
- Sequencing of 18S rRNA and phylogenetic analysis of both species placed them within the order Saprolegniales, in a clade closely related to Aphanomyces.
- The Aquastella species were morphologically distinct from other rotifer parasites as the developing sporangia penetrated out through the host body following its death to produce unique tapered outgrowths.
- Aquastella attenuata produced long, narrow, tapering, finger-like outgrowths, whilst A. acicularis produced shorter, spike-like outgrowths. It is hypothesized that the outgrowths serve to deter predation and slow descent in the water column. Spore cleavage was intrasporangial with spore release through exit tubes. Aquastella attenuata produced primary zoospores, whereas A. acicularis released spherical primary aplanospores, more typical of other genera in the Aphanomyces clade.

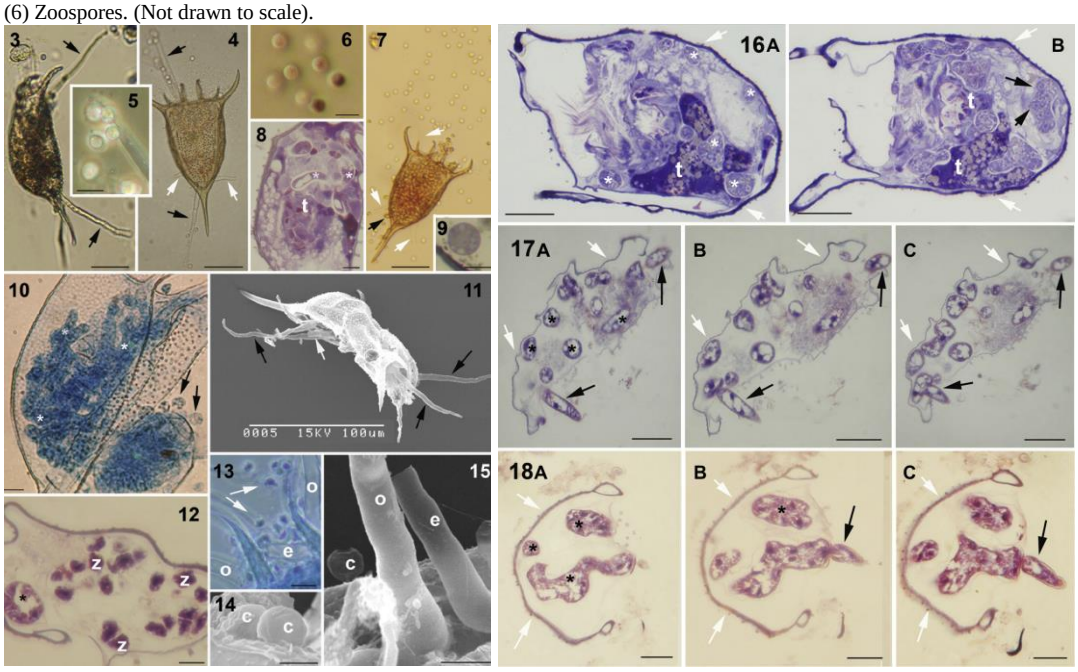
Aquastella attenuata D. P. Molloy & S. L. Glockling, sp. nov

Description: Thallus initially narrow and cylindrical, 5-12 μm diam., coenocytic, aseptate, extensive, and convoluted, becoming broader, lobed, and irregular, 6-20 μm diam., giving rise towards maturity to up to 7 long, gently tapering, rigid, finger-like

outgrowths extending outside the host from the ventral anterior and/or posterior ends. Sporangial outgrowths up to 125 µm long (usually 80-100 µm long) x 4.5-5.5 µm diameter at the base, tapering gradually to 2 µm diameter at the apex. Exit tube(s) up to 30 µm long (usually 15-25 µm long) x 3-6 µm diameter produced vertically from ventral surface or from ventral anterior or posterior end of host. Primary zoospores encysting shortly after release. Cysts spherical, 4.0 - 5.0 µm diam. Infecting Keratella taurocephala rotifers.



Comparative life cycles of (A) Aquastella attenuata: (1) Encysted zoospores on host; (2) Thallus inside host; (3) External outgrowths from maturing thallus; (4) Cleaving sporangium with exit tube; (5) Zoospores released from sporangium via exit tube; (6) Cysts; (7) Secondary zoospores. (B) Aquastella acicularis: (1) Encysted zoospores; (2) Thallus inside host; (3) External outgrowths from maturing thallus; (4) Cleaving thallus with exit tube; (5) Cysts released from sporangium via exit tube; (6) Zoospores. (Not drawn to scale).



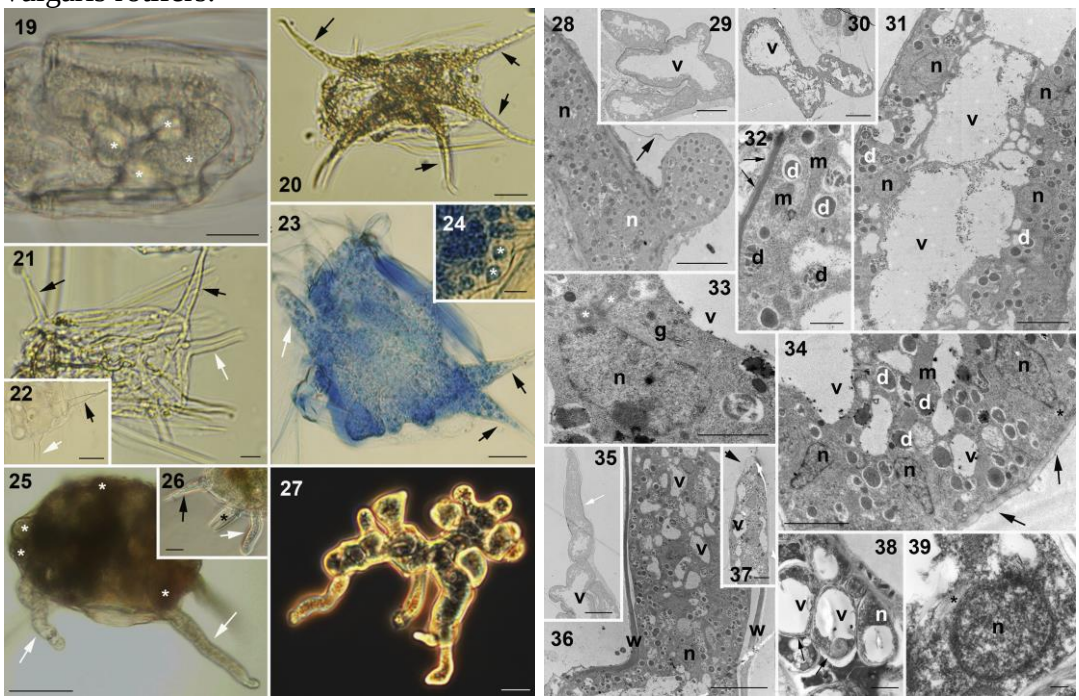
Developmental stages of Aquastella attenuata. (3) Keratella taurocephala with several elongate outgrowths of Aquastella attenuata (arrows). Scale[50mm; (4) Keratellataurocephalawithempty outgrowths (blackarrows)andopenexit tubes (white arrows). Scale[50 mm; (5) Encysting zoospores and cysts. Scale[10 mm; (6) Cysts adhering to the bottomof a glass dish. Scale[10 mm; (7) Keratella taurocephala with several open exit tubes (white arrows) and one intact exit tube (black arrow), having dischargedmany spores which have encysted. Scale[50 mm; (8) Stained histology section of host with profiles of young Aquastella attenuata thalli (*) running through the rotifer tissues (t). Scale[8 mm; (9) Stained histology section througha cyst, revealing nucleus and probablemitochondria. Scale[5 mm;(10) Lactophenol blue-stained Keratella taurocephala containingcylindrical and saccate thalli (*), with an egg into which encysted spores are penetratingwith narrow germtubes (arrows). Scale[10 mm; (11) SEMof Keratella taurocephala with long, narrow outgrowths (black arrows) extending fromunder the lorica at the anterior and posterior ends of the host. An exit tube (white arrow) is extending fromamore

central ventral region. Scale[100 mm; (12) Stained histology section of cleaved and cleaving (*) sporangial profiles, showing fully cleaved spores (z). Scale[10 mm; (13) Lactophenol blue-stained wholemount showing flagellate zoospores (white arrows) near an open exit tube (e) and empty outgrowths (o). Scale[8mm;(14)SEMof two cysts (c)ona Keratella taurocephala. Scale[5 mm;(15)SEMshowingoutgrowth(o)and exit tube (e) extending fromthe host. Note the empty cyst (c) with what appears to be an apical opening. Scale[8 mm.

Thallus profiles of Aquastella attenuata. (16) (A and B). Serial histology section of young infection of Aquastella attenuata in Keratella taurocephala showing thallus profiles (*) amongst the host tissues (t). Note the thick covering of the lorica (white arrows). (B) Some thallus profiles show nuclei with nucleoli (black arrows). Scale [8 mm; (17) (AeC). Serial histology sections of developing, vacuolated (*) thallus, showing outgrowths at the anterior and posterior ends of the host (black arrows), penetrating the body wall. The thick lorica covering the dorsal side is indicated with white arrows. Scale [10 mm; (18) (AeC). Serial histology sections through midcross-section of host showing thick, loricate dorsal covering (white arrows). Thallus is maturing into sporangium and has cleavage furrows visible (*). An exit tube is penetrating out through the midventral body wall (black arrows). Scale [10 mm.

Aquastella acicularis D. P. Molloy & S. L. Glockling, sp. nov.

Description: Thallus irregular, coenocytic, aseptate, and convoluted, with broad saccate, subspherical or spherical lobes, up to 30 µm diam.; giving rise to up to 15 (usually 2-8) rigid, spiked outgrowths projecting out from the host, up to 90 µm long (usually 60-70 µm long) x 7-10 µm wide at the base, tapering to a sharp point at the apex. Exit tube(s) up to 100 µm long (usually 30-50 µm in P. vulgaris, 60-80 µm in Ploesoma truncatum) x 8-10 µm diam. Spore cleavage intrasporangial, forming walled cysts. Cysts 3.5-5.0 µm diam. Infecting Ploesoma truncatum and Polyarthra vulgaris rotifers.



Developmental stages of Aquastella acicularis. (19) Lobed thalli (*) of Aquastella acicularis inside Polyarthra vulgaris. Scale [15 mm; (20) Maturing infection with several outgrowths (arrows). Scale [15 mm; (21) Polyarthra vulgaris containing empty sporangia and outgrowths (black arrows). Exit tube (white arrow). Scale[10 mm; (22) Empty saccate and lobed sporangium with spiked outgrowth (black arrow) and open exit tube (white arrow). Scale [40 mm; (23) Lactophenol blue staine whole mount of mature infection with cleaved content, showing sporangial outgrowths (black arrows) and intact exit tube (white arrow). Scale [30 mm; (24) Lactophenol blue-stained fully cleaved cysts (*) in sporangium

in Polyarthra vulgaris. Scale [5 mm; (25) Infection in Ploesoma truncatum showing dorsal, loricate side with many spherical lobes underneath (*). Note the long exit tubes (white arrows). Scale [30 mm; (26) Lobed thalli inside Ploesoma truncatum with a spiked outgrowth (black arrow) and an exit tube (white arrow). Note the toes (*) under the lorica. Scale [10 mm; (27) Cultivated growth of Aquastella acicularis. Scale [10 mm.

Reference:

1. Daniel P Molloy, Sally L Glockling, Clifford A Siegfried, Gordon W Beakes, Timothy Y James, Sergey E Mastitsky, Elizabeth Wurdak, Laure Giamberini, Michael J Gaylo, Michael J Nemeth AQUASTELLA GEN. NOV.: A NEW GENUS OF SAPROLEGNIACEOUS OOMYCETE ROTIFER PARASITES RELATED TO APHANOMYCES, WITH UNIQUE SPORANGIAL OUTGROWTHS. *Fungal Biol 2014 Jul 12;118(7):544-58. Epub 2014 Feb 12.*

Pythium

Species of *Pytium* are found in various habitats. A large number of these species are recognized as saprophytes and parasites of higher plants. Not only are they parasites of terrestrial plants and aquatic alga-type plants (S p a r r o w, 1931), but are also found on eggs of crabs (A t k i n s, 1955) and fish (S c o t t, O'B i e r, 1962). In fish, only *Pythium ultimum* and a few unidentified species were observed on eggs, which questioned (S c o t t, W a r r e n, 1964; S t u a r t, F u l l e r, 1968) their parasitic nature. These data were confirmed by other authors (W i l s o n, 1976; N e i s h, H u g h e s, 1980; S r i v a s t a v a, 1980; D u d k a et al., 1989). F l o r i n s k a j a (1969), who found *Pythium proliferum* on fish eggs in hatcheries, while C z e c z u g a and W o r o n o w i c z (1993) noted *Pythium artotrogus* on eggs of *Coregonus lavaretus, C. albula* and *Esox lucius*. The above data as well as very scant evidence of the occurrence of species of *Pythium* on fish eggs inclined us to carry out detailed investigations, which would provide more information about the biological properties of some of these fungi species.

Czeczuga (1996)

- Pythium infection was first reported as Lagenidium myophilum infection from marine shrimp (Hatai and Lawhavinit 1988).
- In 1991, a fungal infection occurred in the larvae of coonstripe shrims, Pandalus hypsinotus, artificially produced at Hokkaido in Japan. Mortality was 100%.
- In 1993, the infection also occurred in juvenile coonstripe shrimps, which had been reared in tanks after seed production.
- Mortality was about 70% (Nakamura et al. 1994a, b). The pathogenic fungi isolated from the lesions were same as those caused by Pythium myophilum reported by Hatai and Lawhavinit (1988).
- Later, Muraosa et al. (2009) made clear that the fungus was included into the genus Pythium by phylogenic tree.
- Pythium myophilum (Lagenidium myophilum) infection occurred in the abdominal muscles and swimmerets of adult northern shrimp, Pandalus borealis, cultured at the Japan Seafarming
- P. myophilum is pathogenic toward adult northern shrimp, larval and juvenile coonstripe shrimps and Hokkai shrimp, Pandalus kessleri (Hatai, unpublished). P. myophilum infections have only been in Japan, and these shrimps of the genus Pandalus are known to live only in the deep areas of the sea off the coast of Japan. It was interesting that these hosts seemed to be highly sensitive to P. myophilum.

Pythium species encountered on fish eggs

- Pythium **ultimum** Trow was found on the eggs of
  - Lepomis macrochirus Raf.,1875 (Scott and O´ Bier, 1962)
  - Sturgeon Acipenser nudiventris Lovetzky,1834 (Czeczuga et al., 1995),
  - Tilapia fish (El-Sharouny and Bedran, 1995),
  - Hucho hucho (L.,1758) salmon (Czeczuga et al., 1996).
- **Pythium hydnosporum** (Mont.) J. Schröt. was observed on the eggs of
  - white fish, vendace and pike (Czeczuga and Muszyńska, 1998a; 1999b).
- Pythium middletonii Sparrow was found on the eggs of several fish species in a hatchery in Russia (Florynskaya, 1969).
- **Pythium pulchrum** Minden was found on eggs of Perca fluviatilis (L.,1758) (Czeczuga and Muszyńska, 1999b
- **Pythium rostratum** E. J. Butler on eggs of lamprey Lampetra planeri (Bloch,1784) (Czeczuga, 1997).
- **Pythium monospermum** Pringsh. Was found as a parasite of salmonid eggs often occurred (Kitancharoen and Hatai, 1998; Kitancharoen et al., 1997).

Classification:

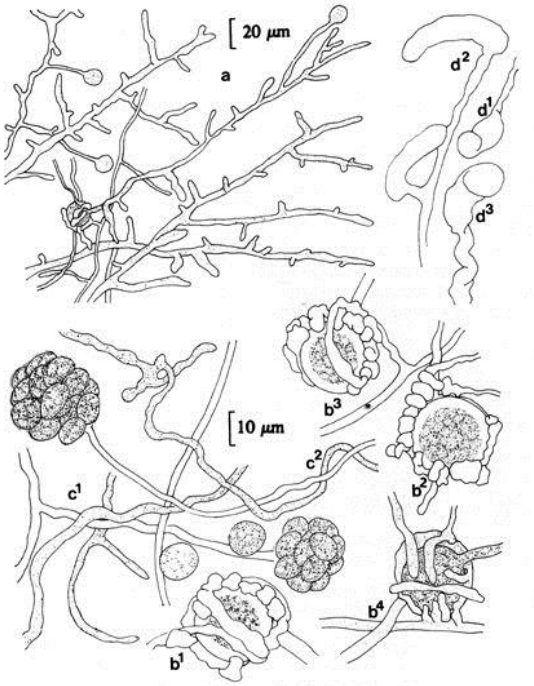
Species 2000 & ITIS Catalogue of Life: April 2013
Chromista +
- Oomycota +
  - Oomycetes +
    - Pythiales +
      - Pythiaceae +
        - Pythium +
          - ***Pythium flevoense*** **Plaäts-Nit. 1972**
          - ***Pythium ultimum***
          - *Pythium ultimum* var. *sporangiiferum* Drechsler 1960
          - ***Pythium undulatum*** H. E. Petersen 1910
          - *Pythium acanthicum* Drechsler 1930
          - *Pythium acanthophoron* Sideris 1932
          - Pythium acrogynum Y.N. Yu 1973
          - *Pythium adhaerens* Sparrow 1931
          - *Pythium afertile* Kanouse & T. Humphrey 1928
          - Pythium amasculinum Y.N. Yu 1973
          - *Pythium anandrum* Drechsler 1930
          - *Pythium angustatum* Sparrow 1931
          - *Pythium aphanidermatum* (Edson) Fitzp. 1923
          - 102 more...

Description:

1. *Pythium flevoense* Plaats-Niterink, Acta Botanica Neerlandica 21: 633 (1972)

Colonies on cornmeal agar submerged, on potato-carrot agar submerged but sometimes with some scanty low aerial mycelium, showing a Chrysanthemum pattern. Main hyphae up to 6 µm wide. Appressoria sickle-shaped. Sporangia filamentous, not differing from the vegetative hyphae. Zoospores produced at 5-20°C. Oogonia only produced in dual cultures of compatible isolates, mostly terminal on short side branches of feather-like hyphae, smooth, 17-20(-30) (av. 19) µm diam. Antheridia diclinous,

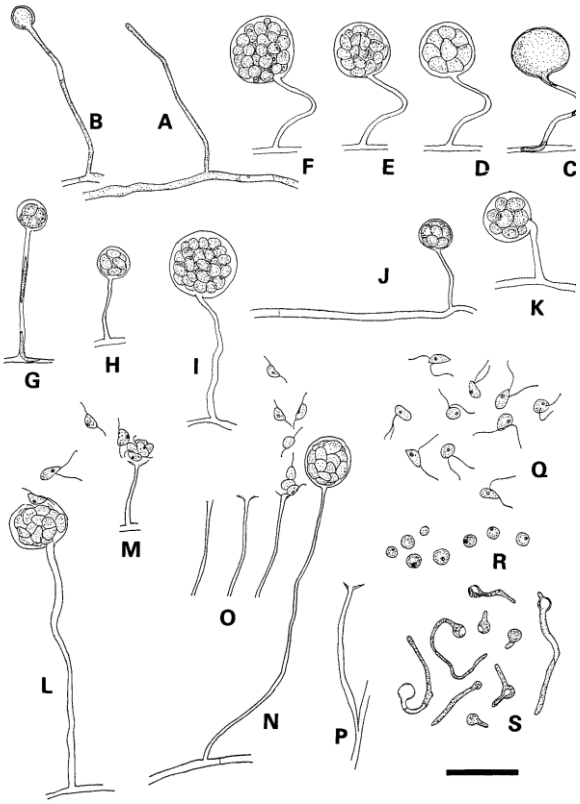
1 to several per oogonium, antheridial stalks mostly bifurcate or differently branched near the oogonium, around which they often intricately entwine. Oospores aplerotic, occasionally nearly plerotic, smooth, (14-)16-18(-24) (av. 17.7) µm diam, wall 24 µm thick. Cardinal temperatures: minimum 5°C, optimum 25°C, maximum over 35°C. Daily growth rate on cornmeal agar at 25°C 7-10 mm.



Mycobank

2.Pythium myophilum

Vegetative hyphae growing in PYGS broth were nonseptate, with numerous oil grobules, branched and *3-8,um* diam. The diameter of the hyphae in the artificial medium was somewhat more uniform than those in the tissue of the shrimps, and did not vary at several incubation temperatures. Zoospore formation occurred within 12-24 h after a thallus was transferred into sterilized artificial sea water. In the process of zoospore formation, protoplasma with numerous oil grobules in the thallus moved into the gelatinous vesicle formed at the orifice of the discharge tube. Mass protoplasma in the vesicle was divided into individual zoospores with two flagella. Vesicles were produced at the top or lateral side of the hyphae. Discharge tubes were *33-242,um* long, 3-4 pm diam, and vesicles were spherical, 25.5-51 ,um diam. Zoospores moved in the vesicle slowly before liberation. Release of zoospores occurred when the vesicle was broken by active zoospore movement. Zoospores were laterally biflagellate, pyriform to subglobose, 5-7.7 (av. 6.7)x7.7-12.8 (av. 10.3) pm, and monoplanetic. Some zoospores were not divided individually, but liberated from the vesicle and encysted as one spore. Zoospores encysted after several minutes' to
several hours' swimming. Encysted zoospores were spherical, 5-9.7 (av. 7.7) ,um diam. Germination was observedwithin 2 h after zoospores were encysted. The fungus was holocarpic and endobiotic. No sexual reproduction was observed.

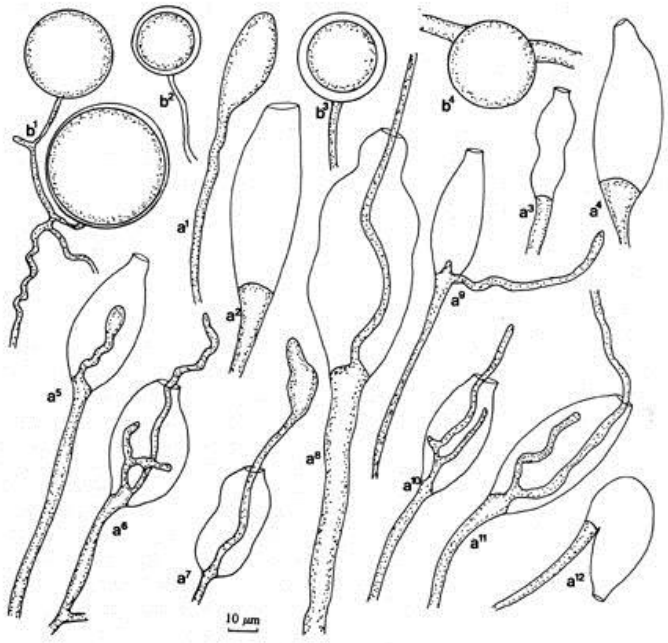


Zoospore formation of P. (*L). myophilum* NJM 9331 isolated from juvenile coonstripe shrimp. A. Discharge tube formation from hypha; B. Vesicle formation; C-F. Zoospore formation in a vesicle; G. Vesicle formation; H-K. Matured vesicles; L-P. Releace of zoospores; Q. Swimming zoospores, laterally biflagellate; R. Encysted zoospores; S. Germination. Scale: 50 pm.

3, Pythium undulatum H.E. Petersen, Botanisk Tidsskrift 29: 394 (1909)

≡Pythiomorpha undulata (H.E. Petersen) Apinis, Acta Horti Botanici Universitatis Latviensis 4: 234 (1930)
≡Phytophthora undulata (H.E. Petersen) M.W. Dick, Mycotaxon 35 (2): 449 (1989)
≡Elongisporangium undulatum (H.E. Petersen) Uzuhasi, Tojo & Kakish., Mycoscience 51 (5): 364 (2010)

Colonies on cornmeal agar submerged, on potato-carrot agar showing a radiate pattern. Main hyphae up to 7 µm wide. Sporangia proliferating internally by 1 or more sporangiophores, sometimes provided with a hyaline papilla, often very long, (27-) 45-118(-156) x (12-)20-44(-50) µm (av. 77.5 x 33 µm), mostly forming short discharge tubes. Zoospores produced at 5-20°C. Chlamydospores present, dark yellow, thick-walled (2-5 µm), (sub)globose, intercalary and terminal, (16-)21-61(-75) (av. 36) µm diam. Oogonia, antheridia and oospores not observed. Cardinal temperatures: minimum 5°C, optimum 20-25°C, maximum 35°C. Daily growth rate on potato-carrot agar at 25°C: 20 mm.

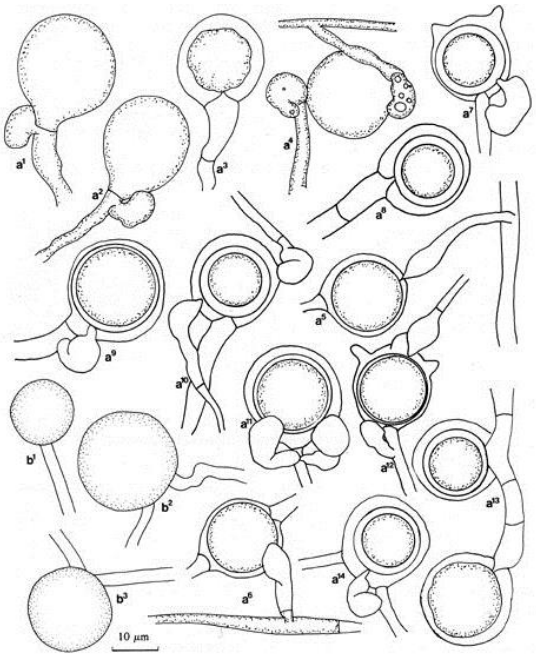


Mycobank

4. Pythium ultimum **Trow, Annals of Botany 15: 300 (1901)**

=Globisporangium ultimum (Trow) Uzuhashi, Tojo & Kakish., Mycosci. 51 (5): 363 (2010)
=Pythium haplomitri Lilienfeld, Bull. Inter. l'Acad. Sciences de Cracovie (1911) =Globisporangium ultimum (Trow) Uzuhashi, Tojo & Kakish., Mycosci.51 (5): 363 (2010)
=Pythium haplomitri Lilienfeld, Bull. Inter. l'Acad. Sciences de Cracovie (1911)

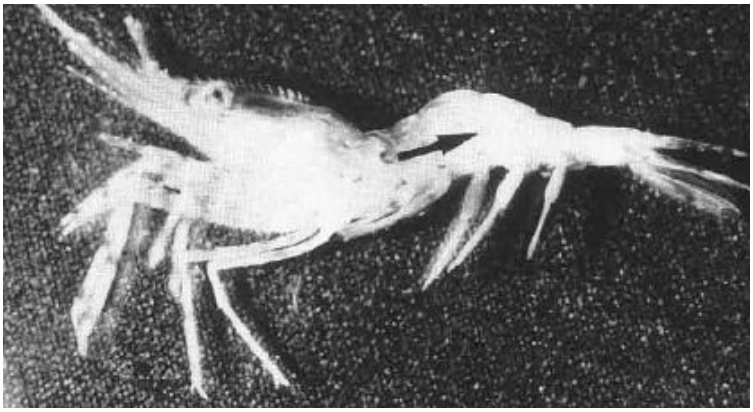
Colonies on cornmeal agar forming cottony aerial mycelium, on potato-carrot agar with a radiate pattern. Main hyphae up to 11 µm wide. Sporangia mostly not formed and zoospores very rarely produced through short discharge tubes at 5°C. Hyphal swellings globose, intercalary, sometimes terminal, 20-25(-29) µm diam. Oogonia terminal, sometimes intercalary, globose, smooth-walled, (14-)20-24(-25) (av. 21.5) µm diam; antheridia either 1(-3) per oogonium, sac-like, mostly monoclinous originating from immediately below the oogonium, sometimes hypogynous, or 2-3 and then either monoclinous or diclinous and frequently straight. Oospores single, aplerotic, globose, (12-)17-20(-21) (av. 18) µm diam, wall often 2 µm or more thick.
Cardinal temperatures: minimum 5°C, optimum 25-30°C, maximum 35°C. Daily growth rate on potato-carrot agar at 25°C: 30 mm.



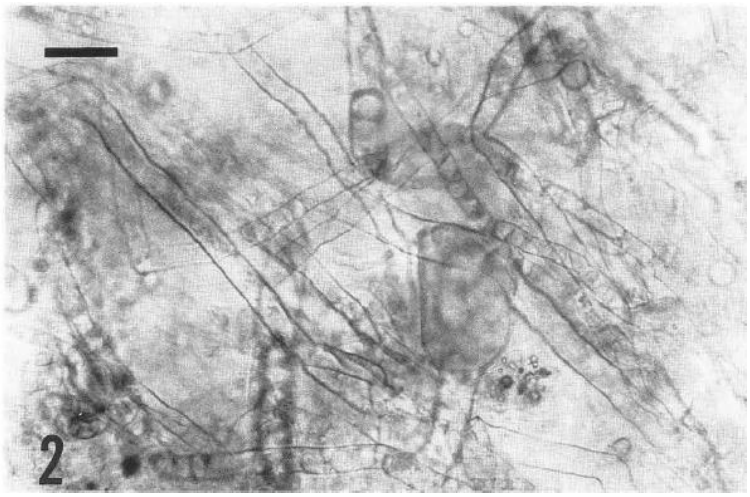
Mycobank

Reports:

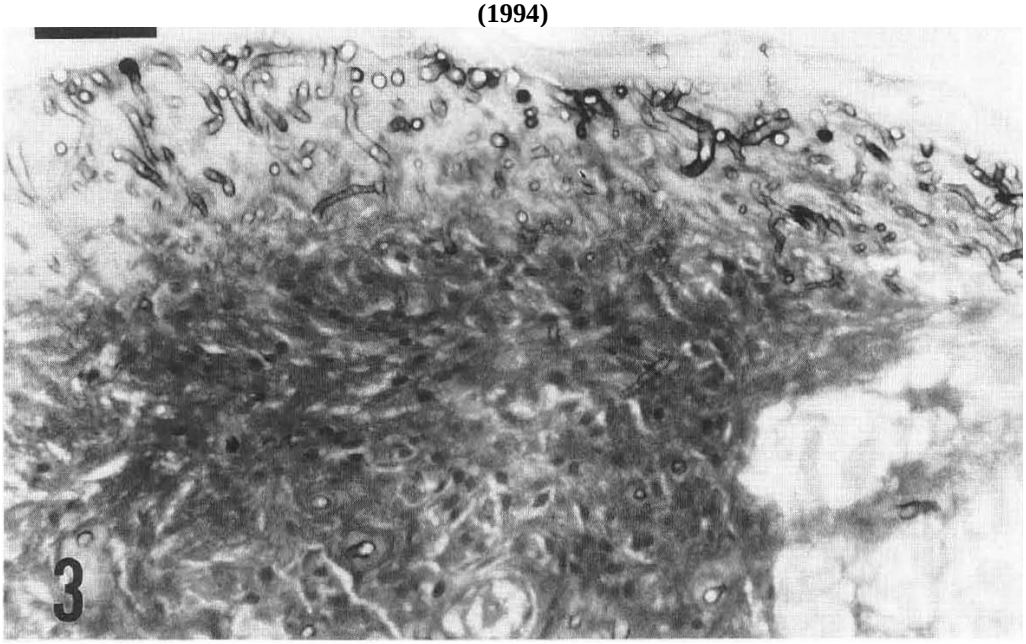
Nakamura *et al.* (1994) identified a fungal infection occurred in juvenile coonstripe shrimps, *Pandalus hypsinotus*, cultured at Hokkaido Institute of Mariculture, Hokkaido, Japan as *Lagenidium myophilum*, the same fungus that had previously been isolated from the abdominal muscle of adult northern shrimps, *Pandalus borealis*, and larvae of the coonstripe shrimp. Histopathologically, numerous nonseptate hyphae were observed in the lesions, and melanized hemocytes were present within the blackened areas. The optimum temperature for growth of the present strain was 25–30°C, and the optimum NaCl concentration for growth was 0.5–1.0%. Its biological characteristics were compared with those of *Lagenidium myophilum* isolated from diseased larval coonstripe shrimp and adult northern shrimp. The fungus was pathogenic toward shrimps of the genus*Pandalus*, which live in deep sea areas. The fungus could infect shrimps at various stages, from larva to adult.



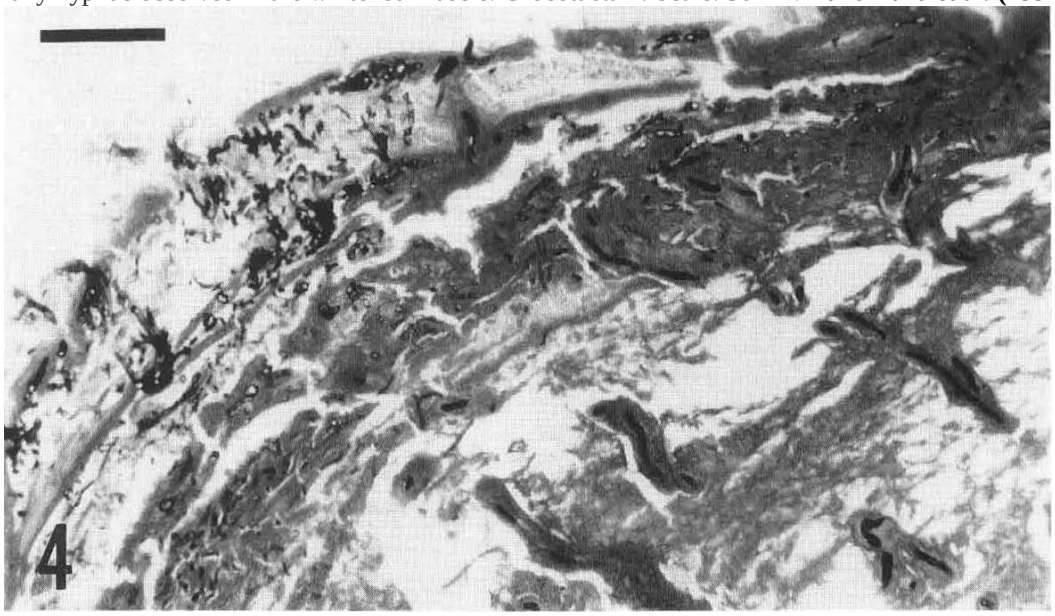
Gross appearance of the diseased coonstripe shrimp, *Panda/us hypsinotus.* Note the muscle with whitish color (arrowl.Scale: 42 mm. **Nakamura *et al.* (1994)**



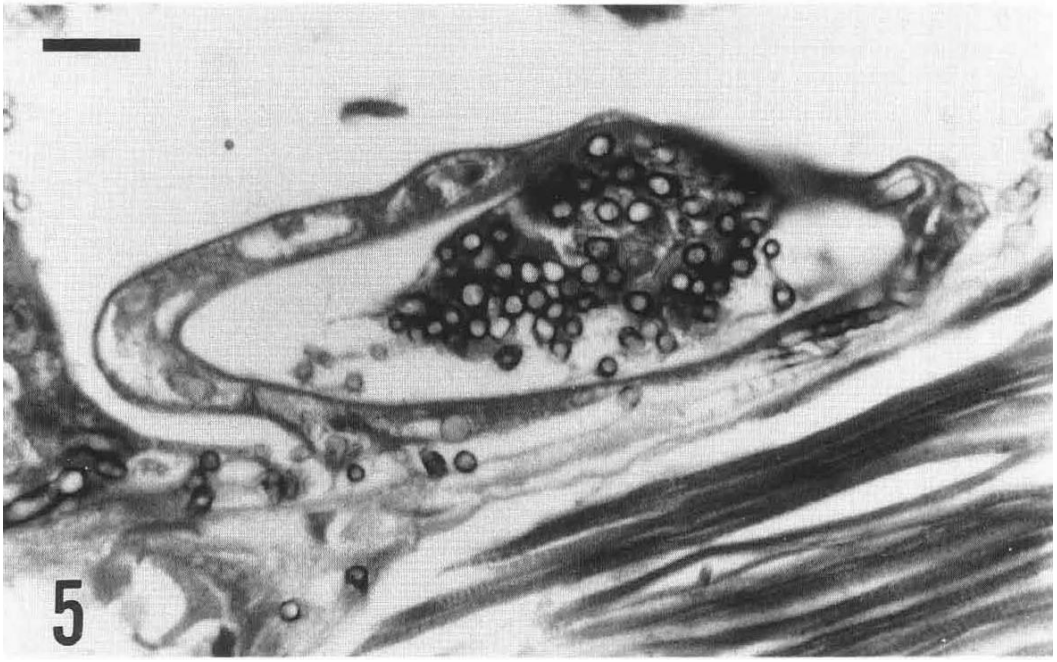
Hyphae observed in the whitened muscle of the infected shrimp . Scale: 20 I'm. **Nakamura *et al.* (1994)**



Many hyphae observed in the whitened muscle. Grocott stain. Scale: 50 I'm. **Nakamura *et al.* (1994)**



Many hyphae observed in the blackened area. Grocott-H & E stains. Scale: 100 I'm. **Nakamura *et al.* (1994)**



Hyphae observed in the blood vessels. Grocott stain . Scale: 20 I'm. **Nakamura *et al.* (1994)**

Nakamura *et al.* (1995) reported a fungal infection in the eggs and larvae of mangrove crab (*Scylla serrata*) in seed production in Bali, Indonesia. The causative fungus was classified as a member of the genus *Lagenidium* (Oomycetes, Lagenidiales). After comparison of its biological and physiological characteristics with those of*L. callinectes* ATCC 24973, a known parasite of various crustaceans, was concluded that the isolate is a new species of*Lagenidium, L. thermophilum*, because of its rapid and thermotolerant growth and unique discharge process. Fungal growth was observed on PYG agar containing 0–5.0% (w/v) NaCl and 0–2.5% (w/v) KCI. Similar pathogenicity toward the zoeae of swimming crab (*Portunus trituberculatus*) was demonstrated.

Czeczuga (1996) observed the growth of 16 fungi species of the genus Pythium on the eggs of fresh-water fish. Some of them were observed sporadically, while others such as P. artotrogus var. macranthum, P. middletonii or P. ultimum occurred commonly. In addition, 13 species of Pythium had never been observed on fish eggs before.

Fungi of the genus *Pythium* were found on the eggs of fish in the different water

Species	Water from		
	pond	lake	river
P. aristosporum Vanterpool		x	
P. artotrogus var. *macranthum* Sideris	x	x	x
P. debaryanum Hesse	x	x	x
P. echinulatum Matthews		x	
P. inflatum Matthews			x
P. intermedium de Bary	x	x	x
P. mamillatum Meurs	x	x	
P. marsipium Drechsler	x	x	
P. middletonii Sparrow	x	x	x
P. oligandrum Drechsler	x		
P. pulchrum Minden in Falck		x	
P. rostratum Butler	x		x
P. spinosa Sawada	x		
P. sylvaticum Campbell et Hendrix		x	x
P. torulosum Coker et Patterson	x	x	x
P. ultimum Trow	x	x	x
Number of species	11	12	9

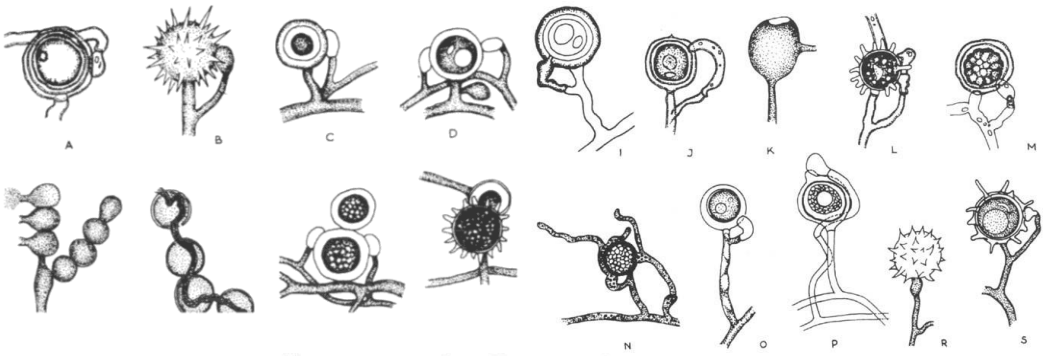


Fig. 1. Species of *Pythium* growing on fish eggs

A — *P. aristosporum* (oospore – 35 µm diamet.), B — *P. artotrogus* var. *macranthum* (oogonium – 25 µm), C — *P. debaryanum* (oogonium – 22 µm), D — *P. inflatum* (oogonium – 22 µm), E — *P. intermedium* (sporangium – 20 µm), F — *P. marsipium* (proliferating sporangia), G — *P. middletonii* (oogonium – 22 µm), H — *P. oligandrum* (oogonium – 27 µm and antheridium), I — *P. pulchrum* (oogonium – 19-23 µm and antheridium), J — *P. rostratum* (oogonium – 20 µm and antheridium), K — *P. rostratum* (sporangium – 30 µm), L — *P. spinosum* (oogonium – 21.8 µm and antheridium), M — *P. torulosum* (mature oogonium – 15 µm and antheridium), N — *P. torulosum* (young oogonium with two antheridia), O — *P. ultimum* (oogonium – 21.5 µm), P — *P. sylvaticum* (oogonium – 20 µm with aplerotic oospores and diclinous antheridium), R — *P. echnulatum* (oogonium – 13.8-24.2 µm), S — *P. mamillatum* (oogonium – 13.5-18.5 µm and antheridium)

Species of the genus Pythium on the eggs of fresh-water fish, Czeczuga (1996)

* *P. aristosporum* Vanterpool 1938; it was reported for the first time from Canada in infected wheat roots. In Japan it caused root decay of various plants, and was noted in rice fields (I w a i z a k o et al., 1976; I c h i t a n i, K a n g, 1988; I c h i t a n i, K i n o s h i t a, 1990). We observed the growth of this species on the eggs of *Leuciscus cephalus* only in the lake.

P. artotrogus var. *macranthum* Sideris 1932; it was described as a saprophyte. It is mainly recognized as a saprophyte living on vegetative parts as a water phytosaprophyte of plants and on dead insects in water (S k i r g i e ł ł o, 1954), and as water phytosaprophyte (C z e c z u g a, 1995 c). We observed it on eggs of *Coregonus lavaretus, Coregonus albula* and *Esox lucius* in hatcheries in north-eastern Poland (C z e c z u g a, W o r o n o w i c z, 1993), on eggs of *Acipenser nudiventris* (C z e c z u g a et al., 1995) and other fish species.

* *P. debaryanum* Hesse 1874. Since the second half of XIX century it has been recognized as a phytopathogenic fungus as well as a soil (J o h n s o n, 1971) and aquatic (B a t k o, 1975) saprophyte. In the aquatic environment it occurrs in springs (C z e c z u g a et al., 1989), running waters (M i l k o, 1965; C z e c z u g a, 1991 a, 1995 b) and stagnant lake-type waters of various trophicity (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970; C z e c z u g a, W o r o n o w i c z, 1993) in spring, autumn and winter (C z e c z u g a, 1991 b, 1995 a). In fish, it was found to grow on the eggs of *Leuciscus cephalus.*

* *P. echinulatum* Matthews 1931 was described as a saprophyte from soil in the United States. It was found to be a saprophyte of waters in Ukraine (M e s h c h e r y a k o v a, 1970) and Iceland (J o h n s o n, 1971). We observed it on eggs of *Leucaspius delineatus* in the lake.

* *P. inflatum* Matthews 1931 was described as a soil saprophyte. It has been hitherto encountered as an aquatic saprophyte (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970). We observed it on eggs of *Cobitis taenia* and *Ictalurus nebulosus.*

* *P. intermedium* de Bary 1881. The species was noted for the first time in soil. It is recognized as a soil saprophyte (A l i - S h t a y e h et al., 1986; H a e d n a n, D i c k, 1987), parasitizing on prothalia of horsetail and fern, and on seedlings of cross plants (S k i r g i e ł ł o, 1954; B a t k o , 1975). It also occurs on in water (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970). We observed it on eggs of *Ctenopharyngodon idella.*

* *P. mamillatum* Meurs 1928. It was first described from the Netherlands. M e s h c h e r y a k o v a and L o g v i n e n k o (1970) noted this species in a body of water in Ukraine; J o h n s o n (1971) isolated it from soil in Iceland. We found it on eggs of *Alburnoides bipunctatus* in the pond and lake.

* *P. marsipium* Drechsler 1941, was first described as *Pythium carolianum* from Kyoto in Japan (I t o, 1936). The above author (I t o, 1942, 1944) recognized it as the species formerly described by Drechsler. This relatively rare species was noted in Japan in the 1940's in water purification deviced in the former Soveit Union (M e s h c h e r y a k o v a, 1970) and in Taiwan (H s i e h, C h a n g, 1976; H s i e h, 1978). It has been recently isolated from a pond in Osaka, Japan (A b d e l z a h e r et al., 1933, 1994 a) and from lakes in Ukraine (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970). We observed this species on eggs of *Rhodeus sericeus amarus.*

P. middletonii Sparrow. It has been recognized as *Pythium proliferum* de Bary since XIX century (de B a r y, 1860). S p a r r o w (1960) initiated a new name, *Pythium middetonii.* The fungus was found to be a saprophyte of ditch water (S z w a n k e, 1938), in lakes (J o h n s on, 1971), decaying algae and dead insects in water (N a u m o v, 1954; K a r l i n g, 1967). In fish, it was observed for the first time by F l o r i n s k a y a (1969) in a hatchery on eggs of several fish species. We observed this species on eggs of many fish species in north-eastern Poland. It has been hitherto recognized as an aquatic saprophyte of rivers and large lakes (C z e c z u g a, 1991 c, 1994 b).

* *P. oligandrum* Drechsler 1930, was first described as a saprphyte and is also recognized as as agressive mycoparasite (D e a c o n, 1976; M a d s e n et al., 1995). It occurrs in the soil of vegetable field (K i n o s h i t a et al., 1994). We observed its growth on eggs of *Pungitius pungitius.*

* *P. pulchrum* Minden 1916. This species was isolated for the first time from soil samples in Germany. It was also found to occur in bodies of waters in Ukraine (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970) and soil samples in Iceland (J o h n s o n, 1971). W e observed the growth of this species on eggs of *Noemacheilus barbatulus* in the pond.

* *P. rostratum* Butler 1907. It is a very common aquatic and soil fungus (L u n d, 1934; S k i r g i e ł ł o, 1954; I c h i t a n i et al., 1992). It was isolated from soil samples in India. This species occurs in various types of water bodies ranging from sunk wells (C z e c z u g a et al., 1987), rivers (S t p i c z y ń s k a - T o b e r, 1965; C z e c z u g a, 1995 b), post-peat pits (S t p i c z y ń s k a, 1962), ponds (M e s h c h e r y a k o v a, 1970) to lakes of various trophicity (C z e c z u g a, 1991 c, 1994 a, 1995 a). We found it on eggs of *Gobio gobio.*

* *P. spinosum* Sawada. It was noted for the first time in Taiwan (S a w a d a, C h e n, 1926) on the damping-off of *Antirrhinum majus.* It is also known to occur in roots systems of other plants (I c h i t a n i et al., 1989 a). We found it only on eggs of *Cottus gobio* in the pond.

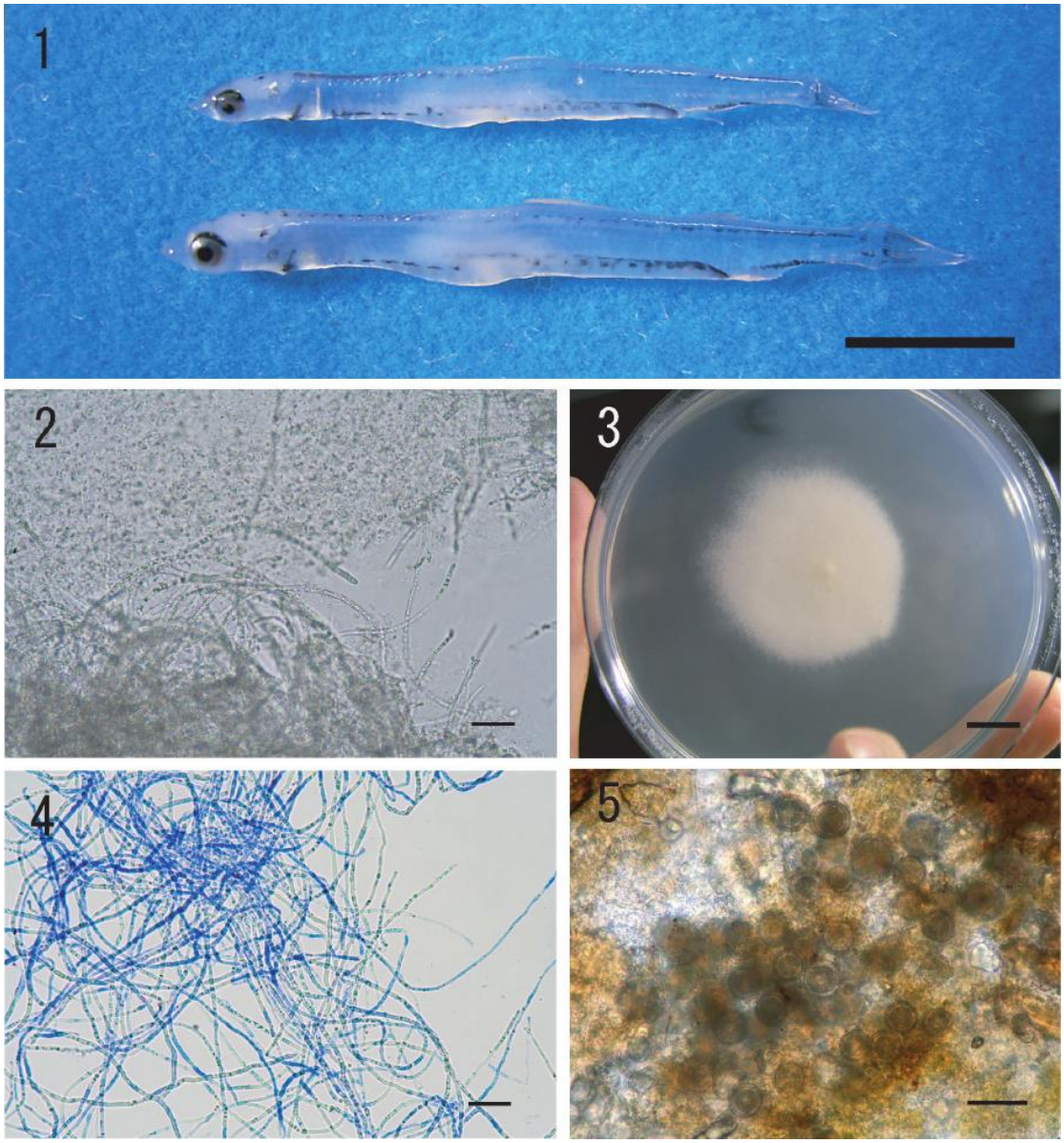
* *P. sylvaticum* Campbell ex Hendrix 1976. The fungus was found to grow on eggs of *Alosa sapidissima.* It was reported for the first time from the south of the United States, and since then it has been recognized as a saprophyte. Recently it has

been recognized in Japan as a pathogen of barley causing browning root rot in these plants (K u s o n o k i, I c h i t a n i, 1994). We observed its growth on eggs of *Pelecus cultratus.*

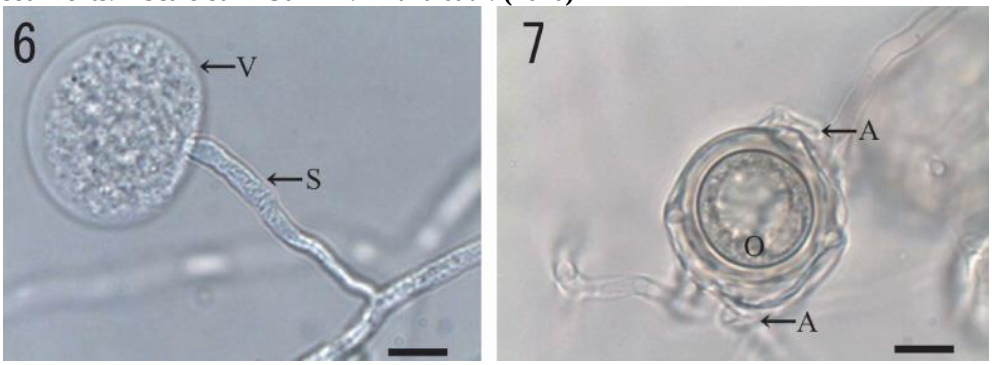
* *P. torulosum* Coker et Patterson 1927. Described for the first time as a saprophyte and recognized as a free-living fungus of different types of grass (I c h i t a n i et al., 1989 b). It also occurs in aquatic habitats (M e s h c h e r y a k o v a, L o g v i n e n k o, 1970; J o h n s o n, 1971; H a l l e t, D i c k, 1981). In fish, it was found to grow on gonads in the 3rd stage of *Anguilla anguilla.*

P. ultimum Trow 1901, described as a soil saprophyte. The species ia a parasite of tulip bulbs (M o o r e, B u d d i n, 1937; M o o r e, 1940, 1979; H u m p h r e y s - - J o n e s, de R o o y, 1975) causing great damage (I c h i t a n i et al., 1991) in crops, and of other plants (M a z e n et al., 1985; T o j o et al., 1992, 1993; K u s u n o k i, I c h i t a n i, 1994). It tolerates high levels of salinity (H a s s a n, F a d l - A l l a h, 1993). In the aquatic environment this saprophyte occurs in a variety of bodies of water ranging from rivers (C z e c z u g a, P r ó b a, 1987) to lakes of various trophicity (C z e c z u g a, 1991 c, 1994 b, 1995 a; C z e c z u g a, W o r o n o w i c z, 1992). In fish, it was observed on eggs of *Lepomis macrochirus* (S c o t t, O'B i e r, 1962) and *Acipenser nudiventris* (C z e c z u g a et al., 1995). In our studies *Pythium ultimum* was found on eggs of many fish species.

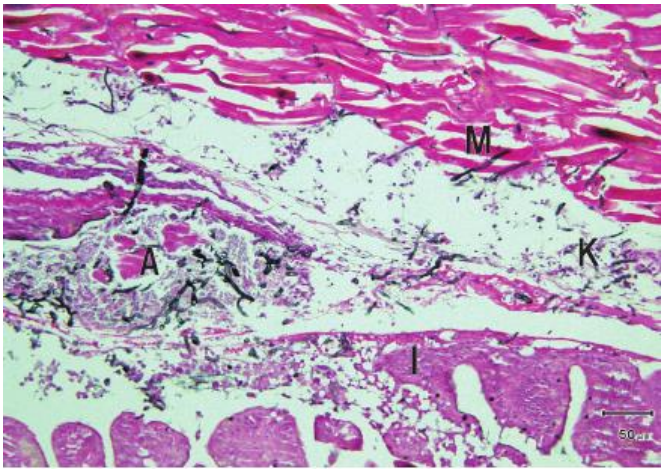
Miura *et al.* (2010) reported visceral mycosis in ayu *Plecoglossus altivelis* larvae at Yamanashi Prefectural Fisheries Technology Center, Japan, in 2007 and 2008. Cumulative mortalities due to the disease were 19–33%. Most diseased fish were characterized by the opaque abdomen. Abundant non-septate hyphae with a width of approximately 5 m m were observed in the opaque areas. Fungi isolated from diseased fish were all identified as *Pythium flevoense* based on the morphological characteristics and sequence analysis of the 5.8S rDNA and adjacent ITS regions. Histopathological examinations showed that non-septate hyphae were present in the airbladder, kidney, intestine, pancreas, spleen, abdominal cavity, musculature and spinal cord. Heavy hyphal propagation in the airbladder and rhexis of the organ suggested that accidental ingestion of *P. flevoense* into the airbladder was the prime cause of this disease



1. Diseased ayu larvae with opaque abdomens. Scale bar = 5 mm. **2.** Microscopic examination of an opaque area of the diseased fish. Abundant non-septate hyphae were observed. Scale bar = 50 m m. **3.** *Pythium flevoense* colony isolated from diseased fish on GY agar after 5 days of incubation at 15°C. Scale bar = 1 cm. **4.** Hyphal appearance of YFTM 0701 on GY agar. Lactophenol cotton blue staining. Scale bar = 50 m m.**5.** Spherical particles, presumably oospores of *Pythium flevoense*, in pond sediments. Scale bar = 50 m m. **Miura *et al.* (2010)**

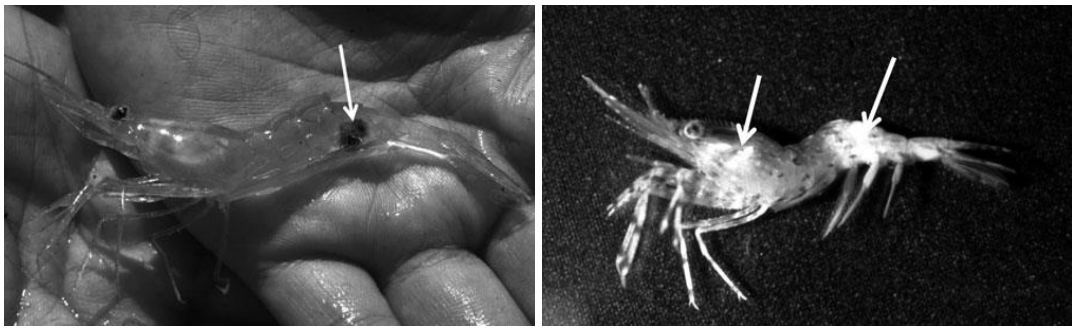


6. Filamentous sporangia (S) of NJM 0702 with a vesicle (V). Scale bar = 10 m m. **7.** NJM 0702 sexual organs. Each oogonium had several antheridia (A), which were intricately entwined around the oogonia. Oospores (O) were aplerotic. Scale bar = 10 m m.



Section of the opaque area on a diseased fish. Fungal hyphae were present in the airbladder (A), kidney (K), intestine (I) and muscle (M). The caudal side of the airbladder was most heavily infected with hyphae and conspicuously degenerative. Grocott & HE staining. Scale bar = 50 m m. **Miura *et al.* (2010)**

Hatai (2012) mentioned that pure cultures of P. myophilum were consistently isolated from the partly blackened abdominal muscle and the inside of the swimmerets of the adult northern shrimps. Growth of the fungus on PYGS agar was observed at 2 days after incubation. Microscopical observation of the blackened areas of the lesions showed them to be filled with hyphae and the pathogenic fungus to grow only in the tissue of shrimp. The optimum temperature for growth of this fungus was 25^{o}C , but it also grew at the low temperature of 5^{o}C. It would thus be able to infect northern shrimps living in cold seawater; the temperature of the Japan Sea was approximately at 5^{o}C. In pure culture, the hyphae were somewhat uniform with a diameter of 7–10 mm and generally vacuolated. Vesicle formed at the end of discharge tube were measuring 86–240 _ 7 10 mm in diameter. Zoospores were 12.9 _ 9.6 mm, globose, reniform, pyriform or elongate, monoplanetic and laterally biflagellate. Encysted zoospores were spherical, 5.5–12.0 mm in diameter. Sexual reproduction was not observed.



Pythium myophilum isolated from the partly blackened abdominal muscle (arrow). A juvenile coonstripe shrimp infected with Pythium myophilum. The lesions look whitish (arrows) **Hatai (2012)**

Mahfujur Rahman and Sarowar (2016) collected 2 types of samples i.e. water, fish mucus and apparently infected muscle samples of fish from a large fish farm consisting of over 100 medium to large ponds in Mymensingh during summer (March to June) in 2015. A total number of 385 samples (284 of water, 79 of mucus and 22 of apparently infected muscle samples) were collected in 15 ml sterile falcon tubes with baits in each. Eleven of the isolates were isolated in Potato Dextrose Agar (PDA) plates and were identified using molecular methods that included DNA extraction, PCR amplification and subsequent sequencing of the ITS region of the genomic DNA of the samples. BLAST analysis to GenBank revealed that two of the isolates were 99% similar to *Pythium* sp. (HQ643814), three of the isolates were 98-99% similar to *Pythium* sp. (KT247392), and each of the remaining four isolates was similar up to 99% to *Pythium* sp. (KF836354), 99% to *Pythium* sp. (EU544193), 99% to *Pythium*

rhizo-oryzae (HQ643757) and 100% to *Pythium catenulatum* (KP862946). Two of the eleven isolates were not assessed due to sequencing error. Phylogenetic analysis revealed that six of the isolates are of clade B1 and three of the isolates are of clade B2 in the *Pythium* phylogeny. The results partially suggest that plant pathogenic oomycetes are more common in summer than animal or fish pathogenic isolates in the sampled farm however; intensive sampling with a broad range of freshwater ecosystems during summer can give a clearer view on oomycete diversity in Bangladesh.

Table 1. Isolated species that are closely related to the species enlisted in GenBank and their source of origin.

Isolates No	Closely related species (From GenBank)	Source of species in the study (fish pond)	GenBank accession No. of the closely related species	Isolation sources (Country of origin)	Similarity to GenBank accession No.
1, 4	Sequencing error	*H. fossilis*	-	-	-
2	*Pythium catenulatum*	*O. niloticus*	KP862946	Certified organic soil (Colambia basin, WA, USA)	100%
3, 5	*Pythium* sp.	*A. testudineus, H. fossilis*	HQ643814	Unknown (Tamberma Land Togo)	99%
6, 7, 11	*Pythium* sp.	*H. fossilis*	KT247392	Greenhouse recycled water irrigation tank (Pennsylvania, USA)	98-99%
8	*Pythium rhizo-oryzae*	*H. fossilis*	HQ643757	Soil of paddy field (Gorakhpur, India)	99%
9	*Pythium* sp.	*A. testudineus*	KF836354	Unknown (France)	99%
10	*Pythium* sp.	*A. testudineus*	EU544193	Sediment (USA)	99%

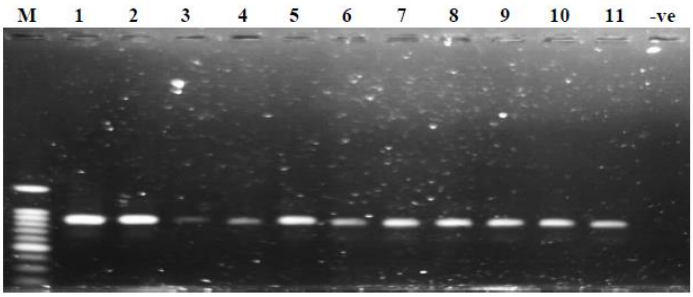


Figure 1.Agarose gel (1%) run of the PCR products of isolates (1 to 11) produce an approximate band size of 800 - 900 bp that is visualize under UV; M = 1 kbp molecular marker, -ve = negative control (DNAse/RNAse free water).

References:

1. Czeczuga, B. (1996): Species of *Pythium* isolated from eggs of fresh-water fish. *Acta Mycol*., 36, 587–588
2. CZECZUGA, B. and MUSZYŃSKA, E., 1999a. Aquatic fungi growing on the eggs fishes representing 33 cyprinid taxa (Cyprinide). Acta Ichthyologica et Piscatoria, vol. 29, p. 53-72
3. CZECZUGA, B. and MUSZYŃSKA, E., 1999b. Aquatic fungi growing on the eggs of various fish families. Acta Hydrobiologica, vol. 41, p. 235-246.
4. DILER, O., 1995. Pythium spp. on infected rainbow trout eggs and fry. Irish Journal of Biology, vol. 19, p. 317-321.
5. EL-SHAROUNY, HM. and BADRAN, RAM., 1995. Experimental transmission and pathogenicity of some zoosporic fungi to Tilapia fish. Mycopatholgy, vol. 132, p. 95-105
6. FLORYŃSKAYA, AA., 1969. Data on the species composition and ecology of moulds-agents of fish saprolegniosis in Leningrad district. Izvvestia Gosuderstwiennogo isoledovatelskogo instituta rybnego choziajtva, Russian, vol. 69, p. 103-123.

7. Hatai, K. (2012). Diseases of Fish and Shellfish Caused by Marine Fungi, in Chandralata Raghukuma, Biology of Marine Fungi, Springer-Verlag Berlin Heidelberg
8. Hatai K, Lawhavinit O-R (1988) Lagenidium myophilum sp. nov., a new parasite on adult northern shrimp (Pandalus borealis Kroyer). Trans Mycol Soc Jpn 29:175–18
9. Mahfujur Rahman, K. M. and Mohammad Nasif Sarowar. Molecular characterisation of oomycetes from fish farm located in Mymensingh sadar during summer. Asian J. Med. Biol. Res. 2016, 2 (2), 236-246;
10. Miura, M., Kishio Hatai, Motoaki Tojo, Shinpei Wada, Sakura Kobayashi and Takumi Okaza. Visceral Mycosis in Ayu *Plecoglossus altivelis* Larvae Caused by *Pythium flevoens*./ Fish Pathology, 45 (1), 24–30, 2010.
11. Nakamura K, Hatai K (1995a) Three species of Lagenidiales isolated from the eggs and zoeae of the marine crab, Portunus pelagicus. Mycoscience 36:87–95
12. Nakamura K, Wada S, Hatai K, Sugimoto T (1994) Lagenidium myophilum infection in the coonstripe shrimp, Pandalus hypsinotus. Mycoscience 35:99–104
13. Nakamura K, Nakamura M, Hatai K, Zafran (1995) Lagenidium infection in eggs and larvae of mangrove crab (Scylla serrata) produced in Indonesia. Mycoscience 36:399–404
14. Sathi SC and RD Khulbe, 1983. *Pythium gracile*, as parasite on fish gills. Indian Phytopathol., 36: 587-588
15. SCOTT, WW. and O'BIER, AH., 1962. Aquatic fungi associated with diseased tropical fish and fish eggs. The Progressive Fish - Culturist, vol. 24, p. 3-15.

15. Aspergillomycosis

- Infection caused by *Aspergillus spp.* has increased in the recent years in fresh water fish.
- Aspergillomycoses was reported in fishes by. Olufemi *et al.*1983,1985 and 1986 ; Salem *et al*.1989m Bhattacharya ,1988
- Bhattacharya *et al*.1988 reported *A. niger* and *A. terreus* as fish pathogen.
- Shrivastava ,1996 reported *A. terreus* from fresh water fishes and tested its pathogenecity on some species of fishes.
- Refai *et al.* (2010) inoculated *Oreochromis* species with *Aspergillus flavus* and maintained at 26°C showed high mortality rates via I.P (70%) rather than I.M route (40%)
- Aspergillus species were isolated from fresh water fishes by Shabazain *et al*. 2010 ; Junaid *et al.* 2010, Fadarfard *et al.*2011 ;Chauhan 2013 and 2014.
- Chauhan *et al*. (2014) proved the pathogenicity of *A. flavus* and *A. terreus* to fish species tested. *A.terreus* was found more virulent causing 100% mortality of experimental fish. Histopathological studies of skin, muscles, gills , liver and kidney showed marked variations showing necrotization and granulomas formations.
- Chauhan *et al*. (2015) studied the haematological and histological alterations in *Channa punctatus* infected with fungi *Aspergillus fumigatus* and *Aspergillus niger.*
- Edoghotu and Hart (2016) detected Aspergillus infection on Chrysichthys nigrodigitatus in a study of the fisheries ecology of the Niger Delta region of Nigeria.

Clinical, postmortem and histopathology

- The reported clinical signs of natural and artificial infection in fish were characterized by abnormal swimming behaviour, high mortality rate, skin darkening, slight abdominal inflation, exophthalmia and /or corneal opacity.
- The postmortem lesions were petechial haemorrhages on the body surface, creamy to haemorrhagic fluid in the abdominal cavity, focal to diffuse peritonitis, congestion and ulceration of gills, congestion and haemorrhages on the surfaces of internal organs along with necrotic foci on the liver and distended gall bladder.
- The picture associated with aspergillomycosis may best be described as a systemic necrotising inflammation characterised by the formation of granulomas.
- The disease may either occur as an acute fulminating or a chronic proliferative form.
- In the acute form, large areas of organs, especially the liver, undergo necrosis, there is usually diffuse distribution of macrophages within a stroma of necrotic tissue and fungal hyphae.
- The chronic form is probably more common under aquacultural conditions and is characterised by the production of granulomas. The granulomas generally have two zones –
    - a central necrotic zone surrounded by a second zone of epithelioid

cells.
    - Giant cells - especially of the Langhan's type-are rare.
    - In some cases, fungal hyphae are easily observed in tissue sections stained by periodic acidSchiff method (PAS) or Grocott's methenamine silver stain.
- The production of a toxin or toxins by the Aspergillus may be involved in the virulence of the fungus, its infectivity and pathological effects, especially in the acute state.

Aspergillus species isolated from fish

1. *Aspergillus candidus*
2. *Aspergillus clavatus*
3. *Aspergillus chevalieri*
4. *Aspergillus flavus*
5. *Aspergillus fumigatus*
6. *Aspergillus niger*
7. *Aspergillus repens*
8. *Aspergillus terreus*

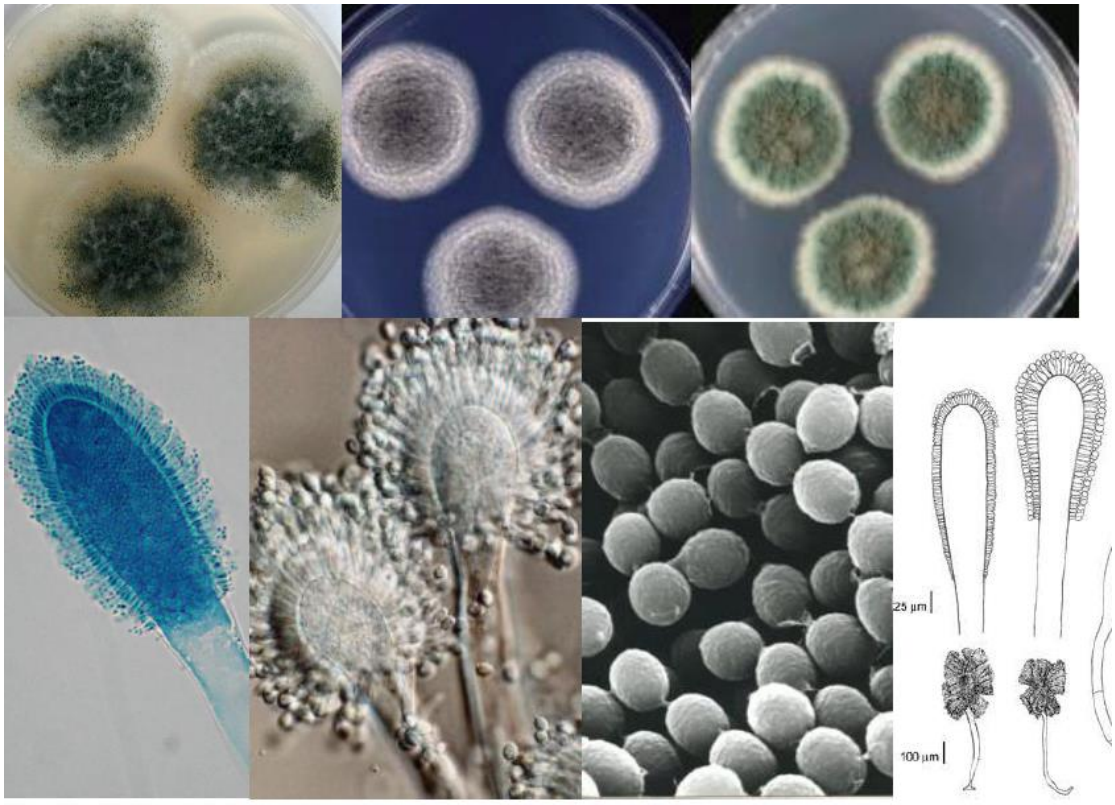
Description of Aspergillus species isolated from fish

i. *Aspergillus candidus* Link, (1809)

Colony diameters on Czapek's Agar 1.5-1.7 cm in 14 days at 25°C, dense, plane; conidial heads radiate, white to ivory yellow; mycelium white; reverse white to cream color or warm buff to light ochraceous-buff, stipes 64-800 × 4.0-8.7 µm, hyaline, smooth; vesicles subglobose, globose, ellipsoidal or obovoid, 5.6-26.0 µm wide. Aspergilla biseriate, occasionally unseriate; metulae 4.4-11.1 × 2.1-3.8 µm, usually swollen, covering the whole surface of the vesicle; phialides 5.8-10.6 × 2.5-3.6 µm. Conidia subglobose or globose to ellipsoidal, smooth, 2.2-3.7 µm wide. Colony diameters on Malt Extract Agar 1.8-2.2 cm in 14 days at 25°C, dense, velutinous; conidial heads radiate, white to pale ivory; mycelium white; reverse ivory yellow to cream color

ii. *Aspergillus clavatus* Desmazières (1834)

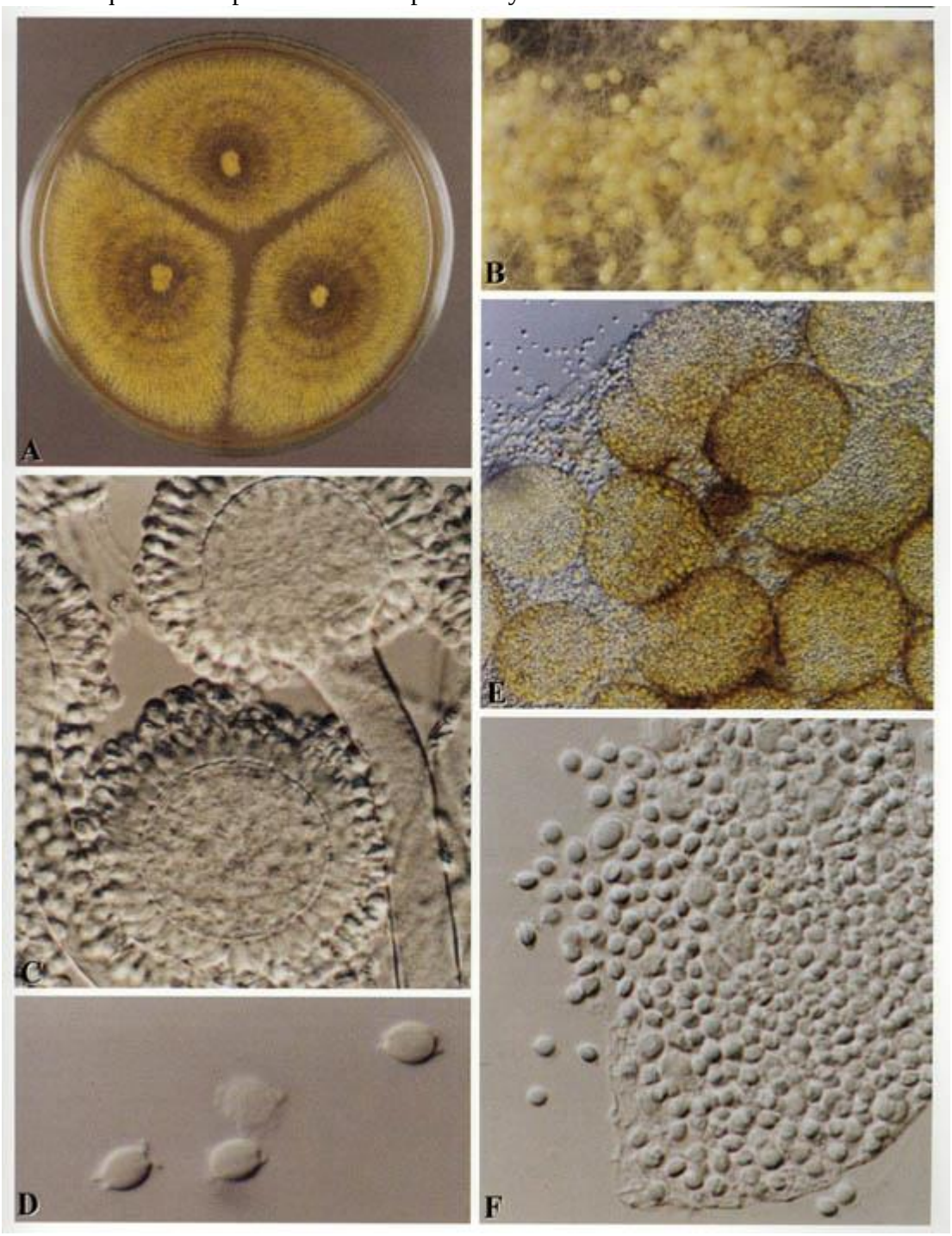
Colony diameters on Czapek's Agar 4.7-5.0 cm in 14 days at 25°C, zonation conspicuous to inconspicuous; conidial heads radiate or splitting into well defined columns in age, niagara green to bice green, or artemisia green to slate-olive; mycelium white; exudate clear; reverse colorless, or ivory yellow to cartridge buff; stipes 250-2300 × 4.8-40.0 µm, uncolored, smooth; vesicles clavate, 8.7-80.0 µm wide. Aspergilla uniseriate, phialides covering the entire surface of the vesicle, 5.3-21.4 × 2.4-5.6 µm. Conidia subspherical, ellipsoidal, occasionally cylindrical, 3.3-7.1 × 2.4-4.4 µm, smooth. Colony diameters on Malt Extract Agar 5.0-5.5 cm in 14 days at 25°C, zonation conspicuous; conidial heads radiate or splitting into well defined columns, bluish gray-green to artemisia green; mycelium white; reverse uncolored.



iii. ***Aspergillus chevalieri*** **(Mangin) Thom. & Church**,1926

Colonies on Czapek's solution agar growing restrictedly, 2.5 to 3.0 cm. in 2 weeks at room temperature (24-26°C), plane, comparatively thin and closely felted, becoming bluish gray in central areas with the development of conidial heads; cleistothecia produced throughout or confined to marginal areas; reverse yellow-orange to maroon. Colonies on Czapek's solution agar with 20 per cent sucrose growing best at 30° C or above, spreading, plane to somewhat wrinkled in central area, with abundant conidial heads in gray-green shades from sage green to andover green or slate-olive distributed evenly over the whole surface or limited to localized areas, usually projecting above a continuous layer of abundant yellow cleistothecia enmeshed in orange-red hyphae at the agar surface; reverse in shades of orange-red to brown, more intense in center. Conidial heads abundant, appearing radiate from divergent conidial chains, mostly

125 to 175 µm in diameter, occasionally larger; conidiophores mostly 700 to 850 µm in length, enlarging to an almost globose vesicular apex 25 to 35 µm in diameter; sterigmata in a single series, closely packed, 5 to 7 µm by 3.0 to 3.5 µm; conidia ovate to elliptical with ends often flattened, spinulose, mostly 4.5 to 5.5 µm in length. Cleistothecia abundant and closely enmeshed in a felt of orange-red encrusted hyphae, mostly 100 to 140 µm, occasionally up to 150 µm, globose to subglobose, yellow to orange; asci 9 to 10 µm; ascospores lenticular, 4.6 to 5.0 µm by 3.4 to 3.8 µm, with walls smooth or very faintly roughened and with equatorial crests prominent, thin and often recurved, and with furrow consisting more of a trough between parallel crests than an equatorial depression in the spore body.



Aspergillus chevalieri A Colonies on MEA +20% sucrose after one week; B ascomata x 40; C conidiophores x 920; D ascospores http://www.aspergillus.org.uk/images/species

iv. *Aspergillus fumigatus* Fresenius, 1863.

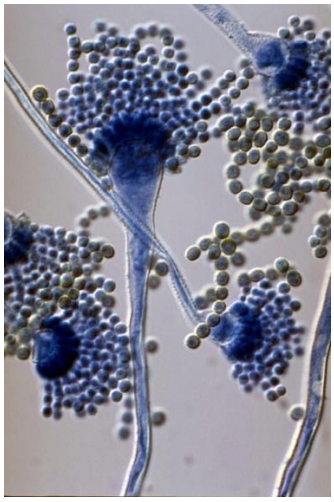
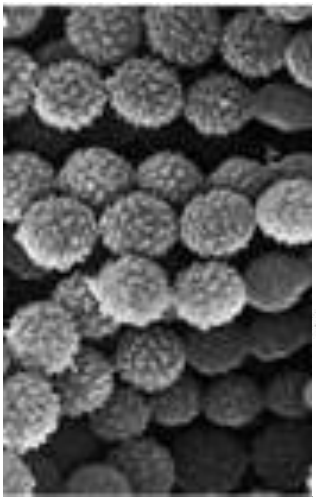
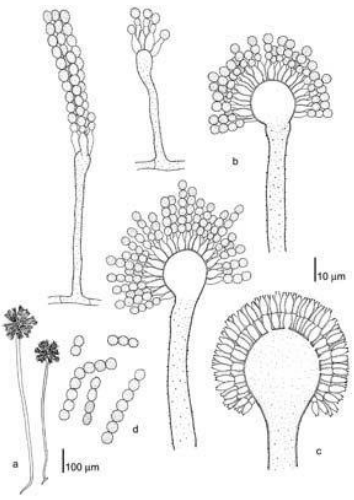
Colony diam (7 d): CYA25: 21-67 mm; MEA25: 25-69 mm; YES25: 48-74 mm; OA25: 34-62 mm, CYA37: 60-75 mm, CREA: poor growth, no or very weak acid production. Colour: greyish turquoise or dark turquoise to dark green to dull green. Reverse colour (CYA): creamy, yellow to orange. Colony texture: velutinous, st. floccose. Conidial head: columnar. Conidiation: abundant, rarely less abundant. Stipe: 50-350 × 3.5-10 µm. Vesicle diam, shape: 10-26 µm, pyriform to subclavate, sometimes subglobose, but rarely globose. Conidia length, shape, surface texture: 2-3.5(-6) µm, globose to ellipsoidal, smooth to finely rough

Aspergillus fumigatus, Mycoba

v. *Aspergillus flavus* Link, 1809

A. flavus is known as a velvety, yellow to green or brown mould with a goldish to red-brown reverse. On Czapek dox agar, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. Conidial heads are typically radiate, mostly 300-400 um in diameter, later splitting to form loose columns .The conidiophores are variable in length, rough, pitted and spiny. They may be either uniseriate or biseriate. They cover the entire vesicle, and phialides point out in all directions. Conidia are globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 mm in diameter. Based on the characteristics of the sclerotia produced, A. flavus isolates can be divided into two phenotypic types. The S strain produces numerous small sclerotia (average diameter ,400 mm).

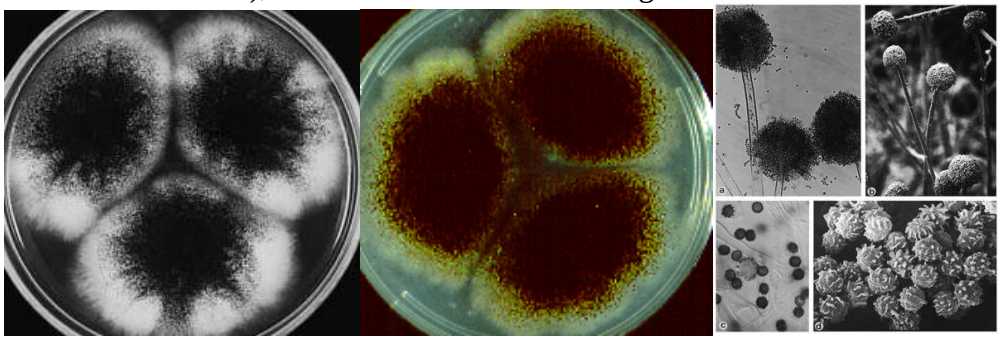
Fungi mycospecies info *w*ww.drjacksonkungu.com *William McDonald*

Rahayu WP **Mycobank**

vi. *Aspergillus niger* van Tieghem 1867

On Czapek dox agar, colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads. Conidial heads are large (up to 3 mm x 15-20 um in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophores are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5.0 um in diameter), dark brown to black and rough-walled.

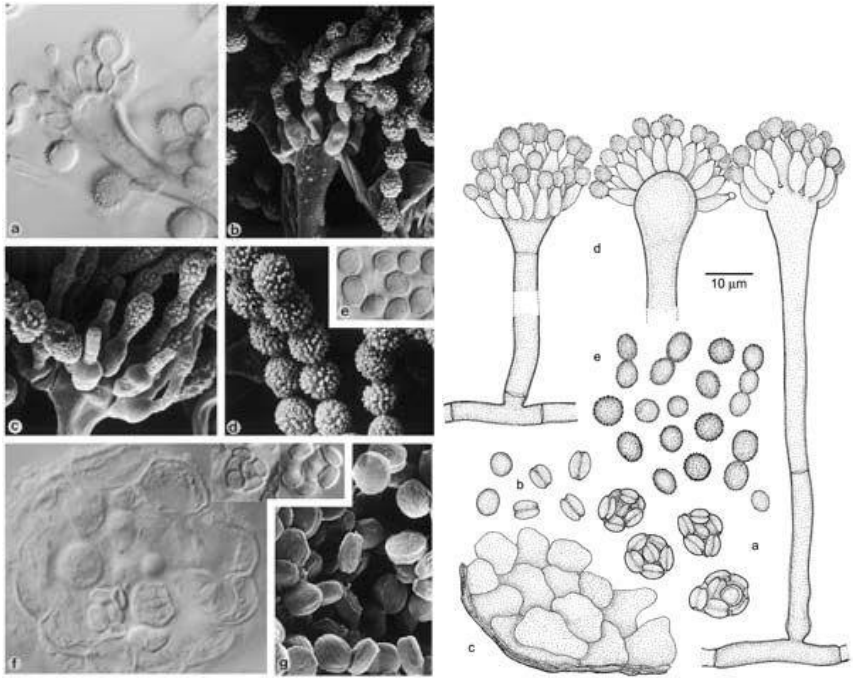


Varga *et al*., 2011 Mycobank

vii. *Aspergillus repens* (Corda) Sacc., Michelia 2 (8): 577 (1882)

Colonies on Czapek's solution agar restricted, plane or somewhat wrinkled forming a rather compact felt, with the marginal area near Scheele's green from developing heads, older areas yellow-green to greenish gray and enmeshing large numbers of aborted cleisto thecia producing few ascospores; normal cleistothecia found only when the substrate dries out or colonies spread over the bare walls of the vessel. Reverse in shades of greenish yellow at colony margin to deep maroon or almost black in older areas. Colonies on Czapek's solution agar with 20 per cent sucrose spreading broadly and often irregularly, attaining a diameter of 5 to 6 cm. in 2 weeks at room temperature (24-26°C), plane or slightly wrinkled, commonly characterized by broad zones or patches of dull green to gray-green conidial heads often alternating with orange-yellow areas more pre-dominantly cleistothecial; surface growth usually

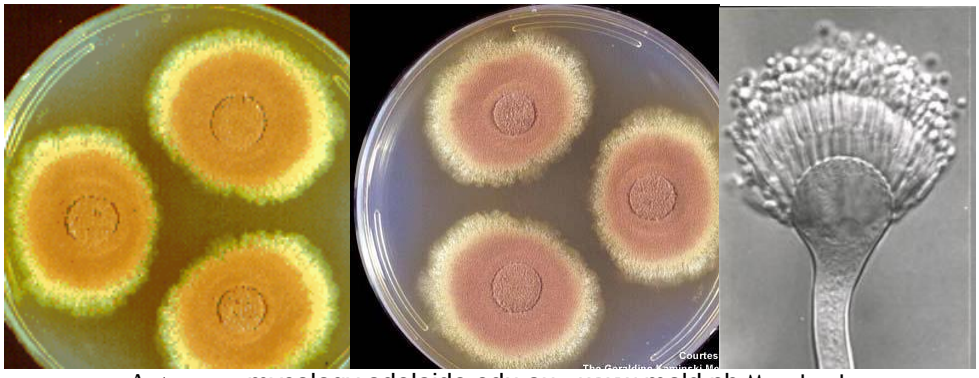
consisting of loosely woven hyphae studded with orange granules and enmeshing the yellow cleistothecia above which project the more or less abundant conidial heads, the whole colony and especially the marginal areas and adjacent wall of the culture dish commonly overgrown by a loose aerial network of hyphae bearing conidial heads and scattered cleistothecia; reverse varying from yellow-orange to deep maroon. Conidial heads abundant, radiate to very loosely columnar, varying in different strains from 125 to 200 µm in diameter, typically consisting of diverging chains of conidia radiating from a hemi spherical vesicular apex of the conidiophore; conidiophores smooth, mostly colorless, 500 to 1000 µm in length, broadening at the apex to a vesicular area about 25 to 40 µm in diameter, occasionally branched; sterigmata in one series 7 to 10 µm by 3.5 to 4.5 µm; conidia ovate to subglobose or globose, spinulose, variable in size from 4.5 to 7 or 8 µm but mostly 5.0 to 6.5 µm. Cleistothecia usually very aubndant, borne in loose networks of yellow to orange-red hyphae, yellow, spherical to subspherical, mostly 75 to 100 µm, occasionally up to 125 µm; asci 10 to 12 µm; ascospores lenticular, mostly 4.8 to 5.6 µm by 3.8 to 4.4 µm, smooth walled, with equatorial area rounded or somewhat flattened and occasionally indented showing a trace of furrow but without crests or ridges.



Aspergillus repens, Mycobank

viii. Aspergillus terreus Thom, (1918)

Colonies on potato dextrose agar at 25°C are beige to buff to cinnamon. Reverse is yellow and yellow soluble pigments are frequently present. Moderate to rapid growth rate. Colonies become finely granular with conidial production. Hyphae are septate and hyaline. Conidial heads are biseriate (containing metula that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle). Conidiophores are smooth-walled and hyaline, 70 to 300µm long, terminating in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae.



A. terreus mycology.adelaide.edu.au www.mold.ph Mycobank

Reports

Easa (1974) isolated *Aspergillus* sp. from skin of apparently healthy cultured common carp. After experimental infection this fungus was only demonstrated in gills and skin.

Olufemi and Roberts (1983) found that after intraperitoneal inoculation of Tilapia with *A. flavus* and/or *A. niger*, *A. flavus* was more pathogenic than *A. niger* at water temperature of 18°C and 26°C. The course of the disease was peracute and/or acute in nature in case of fish maintained at 26°C. The infection with *A. niger* was subclinical at water temperature of 26°C but it was subacute in nature at 18°C. Lesions including skin darkening and slight to moderate abdominal distension were noticed.

Olufemi *et al*. (1983) described an outbreak of aspergillosis among Tilapia *(Sarotherodon* spp.*)* from an intensive fish farm. They recorded sudden increase in the mortality rate following a stress factor of grading. The problem was associated with abdominal distension and dark discolouration of skin. Incision of the abdominal cavity of the distended specimens resulted in a release of copious amount of clear or bloody stained fluid. In extreme cases, the liver showed an extensive liquifactive necrosisand only a small amount of hepatic tissue remained. *A. flavus* and *A. niger* were isolated from heart and liver of affected fish. Histopathological examination of these organs revealed the presence of fungal material in the form of graulomatous reaction. The pellets and meal feed of the fish were suggested to be the source of infection.

Olufemi (1984) showed clearly that fishes are highly susceptible to infection by members of the genus Aspergillus, although there is variability in the pathogenicity of the various species. A. flavus was shown to be more pathogenic to fish than A. niger. The combination of the two species produced a more serious disease than the monospecific infection. This may well explain the serious nature of clinical outbreaks with this species. Most natural disease conditions quite possibly result from infection by more than one Aspergillus species - conditions which may be termed polyspecific infections. The pathogenicity of Aspergillus species may be attributable to their ability to grow under the environmental conditions provided by the host, water temperature appearing to playa significant role in this regard. At 26°C, A. flavus was about twice as pathogenic to Oreochromis niloticus than at 18°C. A. flavus is able to produce mortalities at various temperatures, whereas A. niger is usually only able to initiate the disease when the water temperature is low (18°C).

Olufemi and Roberts (1986) fed *Oreochromis niloticus* at 17°C and 26°C a pelleted diet contaminated with cultures of *Aspergillus flavus* fungus. Affected fish stopped feeding with one week, and became inactive, dark and oedematous. Exophthalmia, often accompanied by corneal or humoral changes, was common and mortalities

commenced after 4 weeks. Surviving fish were sacrificed after 5 weeks and the fungus was isolated from all organs, including--irregularly--the eye. The histopathology was predominantly that of a necrotizing septicaemia, with fungal hyphae present in liver, peritoneum, kidney, intestinal wall and, distinctively, in the orbit and eye of affected fish.

Shaheen (1986) isolated aspergilli from skin, gills, liver kidneys of apparently healthy freshwater fishes, *Aspergillus* and *Mucor* species were the more prevalent fungi recorded from Tilapia sp.

Refai *et al.* (1987) noted 30% deaths in case of cat fish experimentaIly infected with *A. niger* and/or *A. flavus*. Both fungi were re-isolated from heart, liver, gall bladder, kidney, spleen and gills of fish inoculated intraperitoneally (I.P.) or orally, whereas in fish inoculated by scarification of gills the fungus could only be isolated from gills.

Bhattacharya (1988) isolated *Aspergillus niger* from the fish *Heteropneustes fossilis* for the first time. Though the fungus was commonly a soil and air inhabitant, it was found to be virulent pathogen of the fishes.

Eisa (1988) carried out an experimental infection of tilapia fishes by intraperitoneal infection with *A. flavus* and *A. niger* mixture. The fungi caused complete death of Tilapias at 19°C**.** *A. flavus* alone or *A. niger* alone caused 10% mortality. The main post-mortem changes were pale gills, greenish to yellowish brown liver, distended gall bladder and congested spleen and kidney. Clinically fishes had darkened skin, exophthalmia and abdominal distension. Oral infection resulted in mild abnormalities. The main post mortem changes were congested gills, spleen and kidney, pale liver with friable consistency, distended gall bladder and enteritis. The main histopathological changes were granulomatous lesions in liver and gills, haemorrhages in the internal organs and eyes.

Salem *et al.* (1989a) carried out mycological studies on 190 cultured tilapia collected from 4 different freshwater fish farms at Sharkia, Kalubia, Kafr-El-Sheikh and Giza Governorates during different seasons. The isolated fungi were Aspergillus (280), Penicillium (170), Mucor (340) and (40) Rhizopus species from gills, eyes, heart, liver. gall bladder, spleen, kidneys and intestine of both apparently healthy as well as diseased fish.

Salem *et al.* (1989b) studied the pathogenesis of the isolated fungi for Tilapia (*Oreochromis nilotica*) intraperitoneally (I.P.) and by incorporating the fungus spores in rations. I.P. infection revealed 90%, 15% and 80% mortalities within 10 days, when they used doses of $9X10^4$ conidia/mm^3 of *A. flavus*, $2.7X10^5$conidia/mm^3 of *A. niger* and equal mixture of both, respectively. The infected fish showed skin darkening, slight to moderate abdominal distension, exophthalmia and paleness of gills. The liver was congested and friable with multiple small whitish foci. Distended gall bladder and inflammation of the intestine were observed. No mortalities were seen in case of added fungi to the ration but the signs were in the form of deprived food intake after one week and the infected fish became darker, less active with different degrees of exophthalmia and ascites. The liver of sacrificed fish was friable with focal grayish areas whereas the gall bladder was distended with bile tinged with blood.

Kidneys and spleen were contested. Re-isolation of infected *Aspergillus* species were positive in case of gills, heart, liver, spleen, gall bladder, kidneys and intestine of both dead and sacrificed fish.

Abd EI-Monem *et al.* (1995) inoculated 20 Nile tilapia I/P. with 0.2 ml spore suspension of *A. flavus* and 10 tilapia in a control group with 0.2 ml distilled water. Injection of spore suspension caused darkening of the skin, loss of scales, and sloughing of fin rays. The mortality rate was 90%. The liver, spleen, posterior-kidney, and intestine were the main organs affected, 3 weeks after inoculation. Multiple discrete granulomatous reactions were observed in the internal organs. It was suggested that the observed activation of melano-macrophage centers inside the granulomas indicated involvement of these centers in a defensive mechanism against aspergillosis.

Badran *et al*. (1995) diagnosed aspergillomycosis among Nile tilapia *(Oreochromis niloticus)* by recording the clinical signs, post-mortem lesions, histopathological changes and isolation of the causative agent. The ability of the isolated fungi to cause the disease among healthy *O. niloticus* by oral and intraperitoneal (l/P) routes was done. Moreover, the disease control by using variable veterinary fungicide, after determination of their inhibiting effect on the fungal growth, was studied. The causative agent of aspergillomycosis, *Aspergillus flavus,* was isolated in pure culture from liver, kidneys, eyes and orbital cavity of diseased *O- niloticus*. Experimentally, the organism produced the disease in healthy *O. niloticus* by oral and I / P challenges. The typical pictures of natural aspergillomycosis with high mortality rate resulted after challenge. The clinical signs of natural and artificialey infected fish were characterized by abnormal swimming behaviour, high mortality rate, skin darkening, slight abdominal inflation, exophthalmia and /or corneal opacity. While, the postmortem lesions were petechial haemorrhages on the body surface, creamy to haemorrhagic fluid in the abdominal cavity, focal to diffuse peritonitis, congestion and ulceration of gills, congestion and haemorrhages on the surfaces of internal organs along with necrotic foci on the liver and distended gall bladder. Histopathologically, the mycotic elements were observed in most internal organs and induced retrogressive changes, necrosis and circulatory disturbances.

Bhattacharya (1995) isolated *Aspergillus terreus* from the fish *Channa punctatus* collected from the Chakia Sugar Factory, Bihar, where effluents were disposed off. It was one of the causative organism of the disease syndrome "aspergillosis" in fishes. Sensitivity of this pathogen to various antimicrobial agents was investigated in vitro.

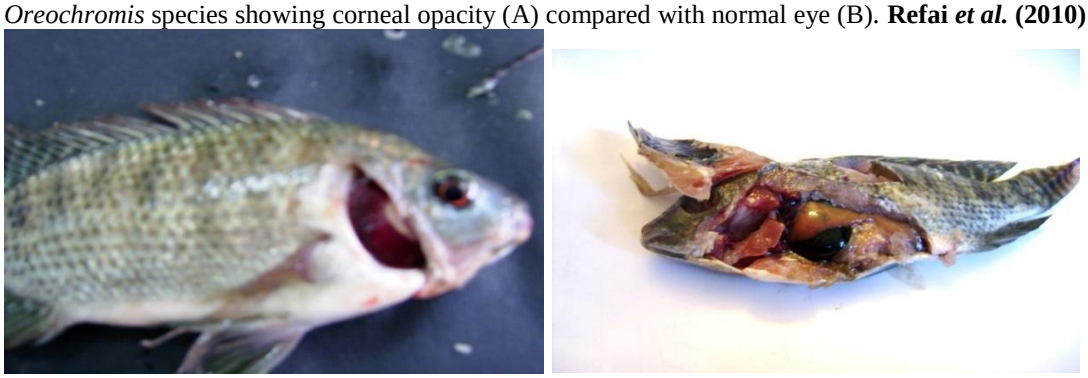
Olufemi and Roberts (2006) fed Oreochromis niloticus maintained at 17°C and 26°C a pelleted diet contaminated with cultures of Aspergitlus flavus. Affected fish stopped feeding within one week, and became inactive, dark and oedematous. Exophthalmia, often accompanied by corneal or humoral changes, was common and mortalities commenced after 4 weeks. Surviving fish were sacrificed after 5 weeks and the fungus was isolated from all organs, including—irregularly—the eye. The histopathology was predominantly that of a necrotizing septicaemia, with fungal hyphae present in liver, peritoneum, kidney, intestinal wall and, distinctively, in the orbit and eye of affected fish.

Refai *et al.* (2010) inoculated *Oreochromis* species with *Aspergillus flavus* and

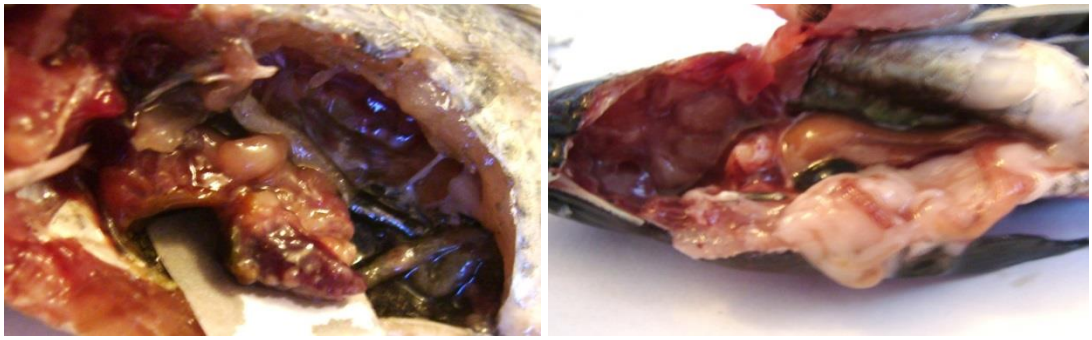
maintained at 26°C showed high mortality rates via I.P (70%) rather than I.M route (40%) and exhibited several clinical signs as skin darkening, exophthalmia, moderat abdominal distention and corneal opacity**.** Postmortem finding revealed congestion and ulceration of gills, haemorrhagic abdominal fluids, necrotic foci within liver and distention of gall bladder, multiple nodules within spleen and severe intestinal congestion were also observed. On the other hand, no clinical or postmortem changes were detected on fish groups maintained at 18ºC. *Aspergillus flavus* was re-isolated from all organs including (skin, fins, eyes, gills, heart, liver, spleen, kidneys and gall bladder.

Oreochromis species showing skin darkening (A), exophthalmia and moderate abdominal distention (B). **Refai *et al.* (2010)**

Oreochromis species showing corneal opacity (A) compared with normal eye (B). **Refai *et al.* (2010)**

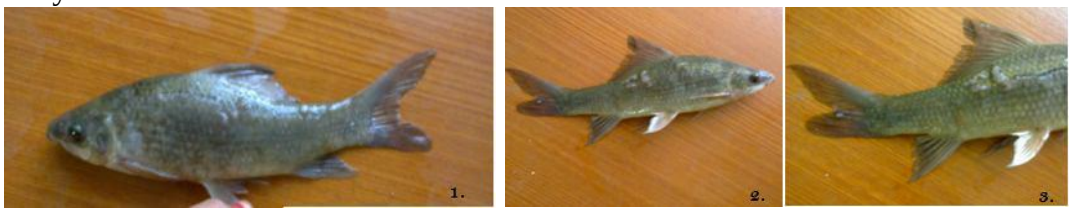


Oreochromis species showing congestion and ulceration of gills.Liver of *Oreochromis* species showing necrotic foci with distention of gall bladder. **Refai *et al.* (2010)**

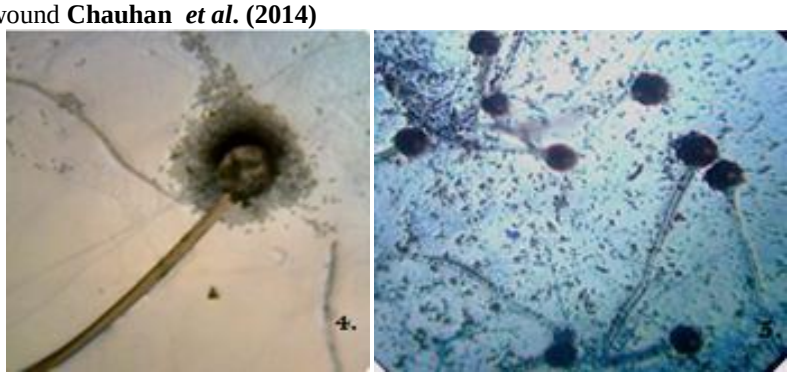


Spleen of *Oreochromis* species showing multiple nodules. *Oreochromis* species showing severe enteritis. Refai *et al.* (2010)

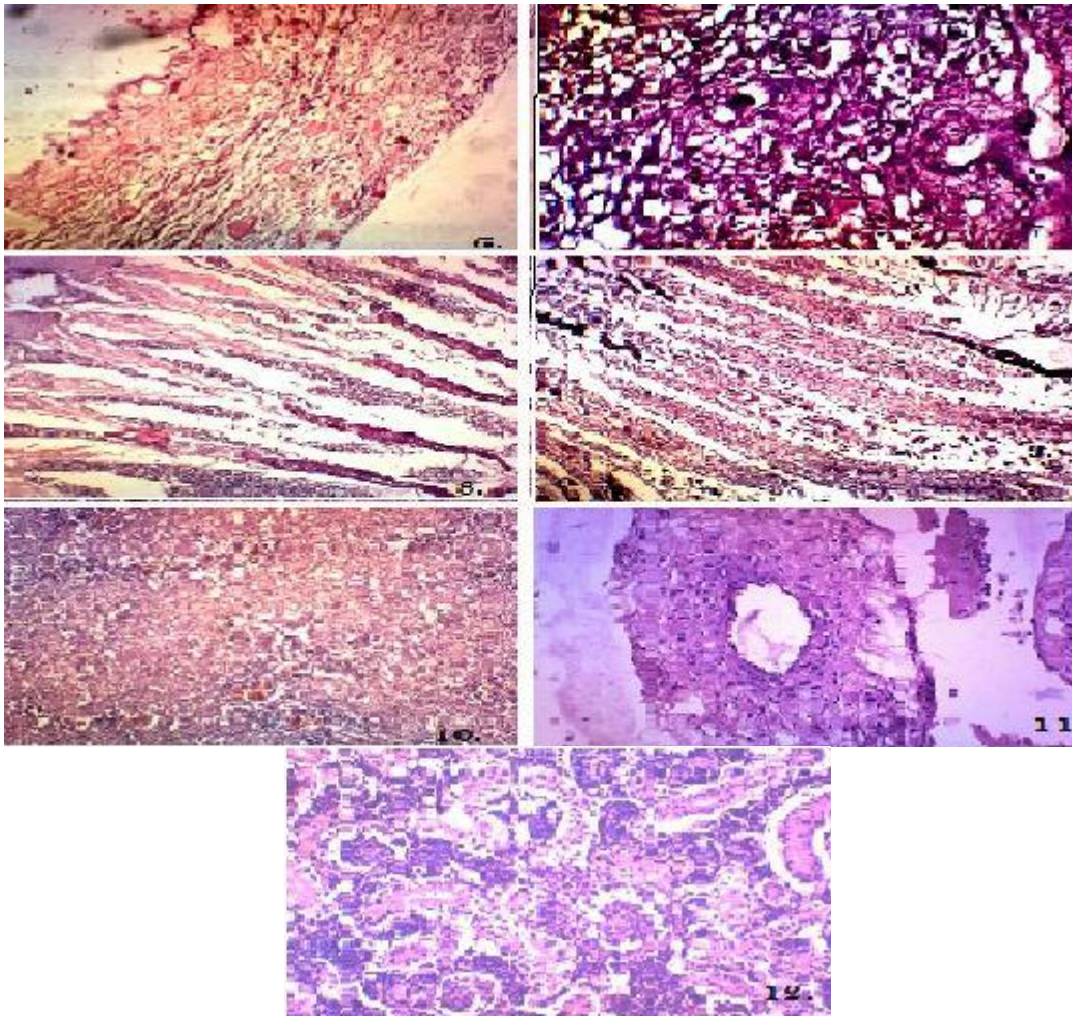
Chauhan *et al*. (2014) collected sixteen specimens of diseased *Labeo calbasu* from Halali reservoir, Bhopal. All the fishes showed external symptoms of fungal growth on body in form of cottony tufts and in some fishes whole body was found covered with fungus. Isolation and identification of fungi showed the presence of two species of *Aspergillus* viz. *A. flavus* and *A. terreus* on all the collected specimens. Both the species of fungi were found in combination. Experimental inoculation studies showed both the isolated fungi were pathogenic to fish species tested. *A.terreus* was found more virulent causing 100% mortality of experimental fish. Histopathological studies of skin, muscles, gills , liver and kidney showed marked variations showing necrotization and granulomas formations. No wounds or lesions were observed on body of infected fish.



Infected *Labeo calbasu* found completely covered with *Aspergillus spp*, cottony outgrowths without any lesion or wound **Chauhan *et al*. (2014)**



Conidia with released conidiospores of *A.terreus* and *A.flavus.* Chauhan *et al*. (2014**)**

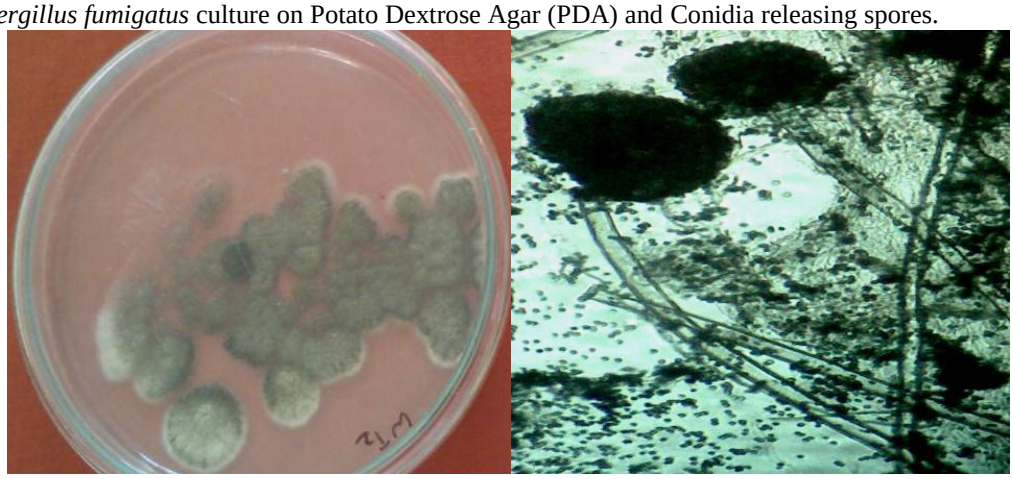


Histological sections of *L calbasu* infected with *A. flavus* and *A.terreus*. 6. Infected skin showing completely lost epidermis and distended dermal and muscular layer. 7. necrotized muscles with conidiospores and formation of fibrillar granulomas. 8 & 9. Gill lamellae with degenerated epithelium and fungal hyphae encapsulated by fusiform hepatocytes in gill tissue. 10&11. Necrotised hepatic tissue and vacuolization of liver cells with hyphal growth. 12. necrotic tissue and haemorrhages in kidney **Chauhan *et al*. (2014)**

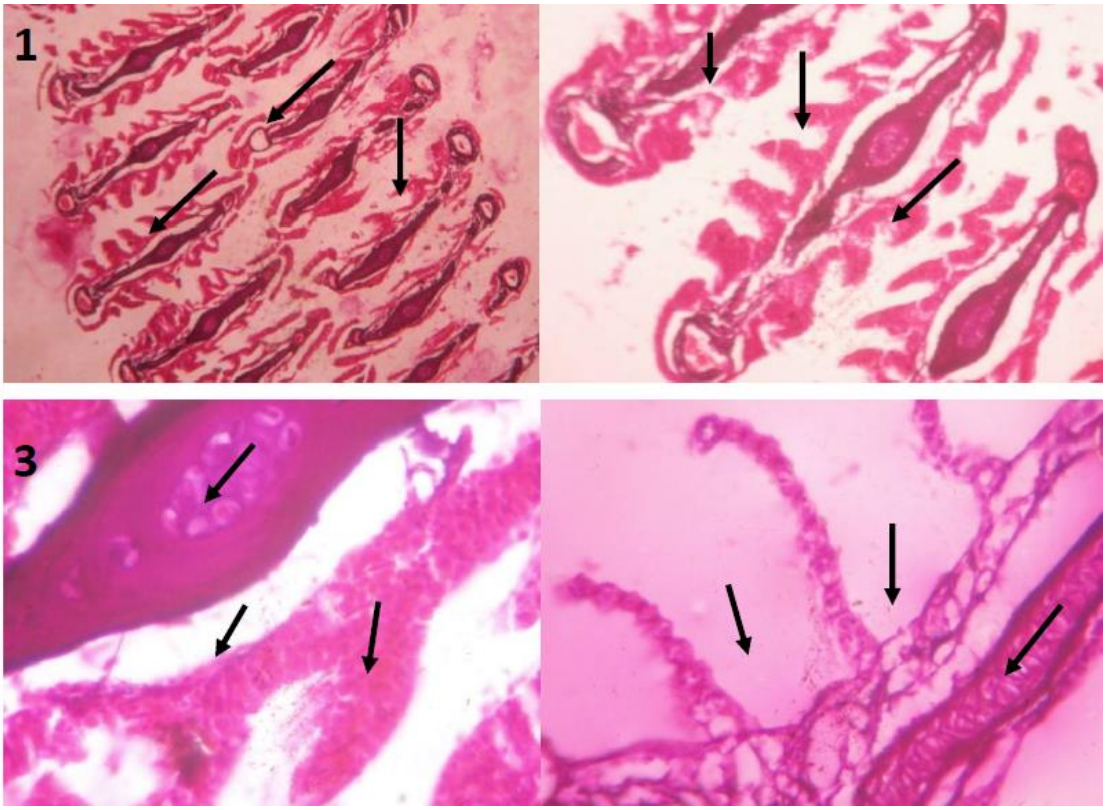
Chauhan *et al*. (2015) studied the haematological and histological altarations in *Channa punctatus* infected with fungi *Aspergillus fumigatus* and *Aspergillus niger*. Fresh water murrels were collected from Hasanparthy and Bhandham lakes and Local fish markets of Warangal district. Isolated fungi from infected fish bodies were identified as *Aspergillus fumigatus* and *Aspergillus niger*. The considerable variations have been observed in the mean values of blood parameters. HB content, RBCs, percentage of Monocytes and Neutrophils were significantly decreased by (9%), (55%), (2.4%) and (18%) respectively. WBCs, Lymphocytes, Esinophils and Basophils were found significantly increased (17%), (5%), (5.8%) and (2.7%) respectively. Histopathologically different kinds of destructions were observed in Skin, Gills and Liver of the infected fish. Penetrating fungal hyphae were observed on skin and complete muscles.

Channa punctatus infected with *Aspergillus fumigatus* and *Aspergillus niger* (External Hemorrhage and tail rot).

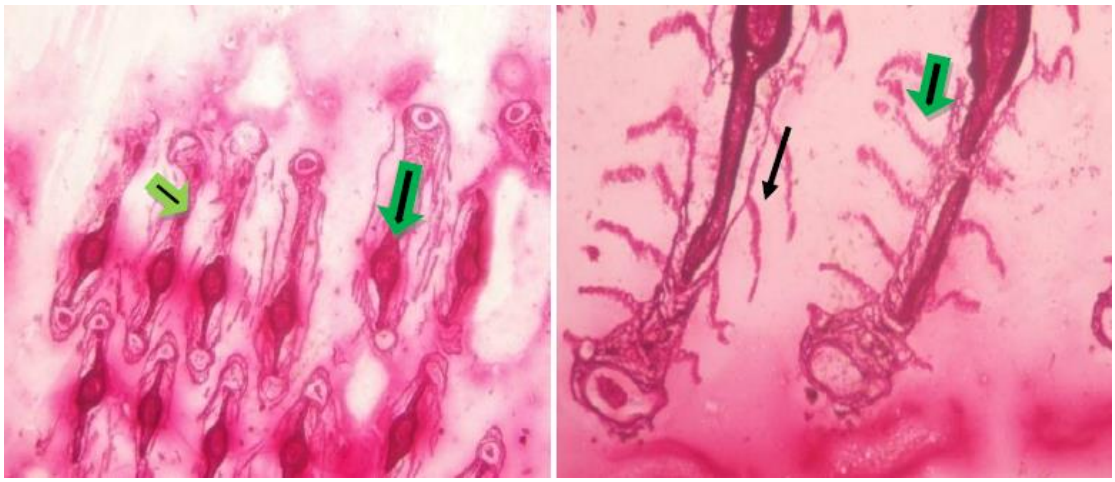
Aspergillus fumigatus culture on Potato Dextrose Agar (PDA) and Conidia releasing spores.



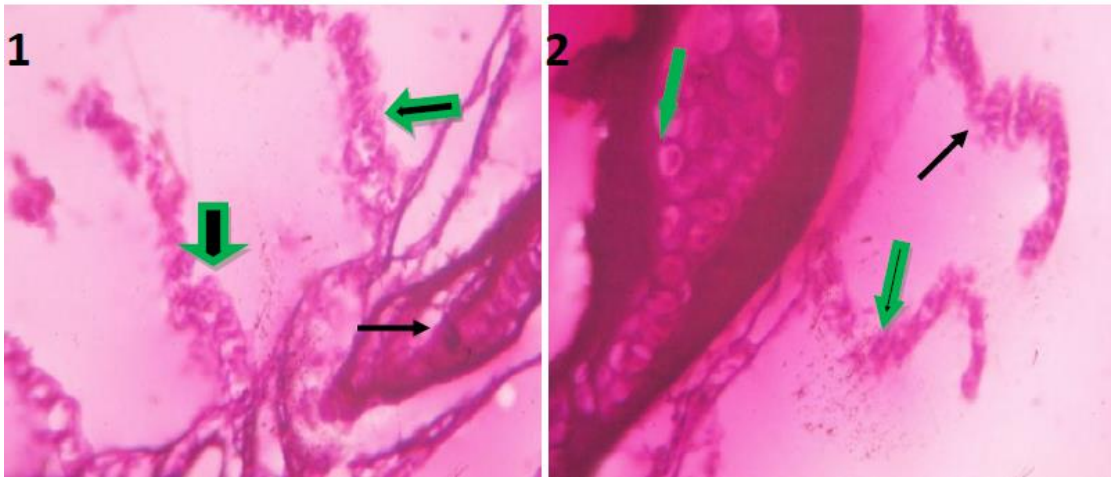
Aspergillus niger culture on Potato Dextrose Agar (PDA) and Conidia releasing spores.



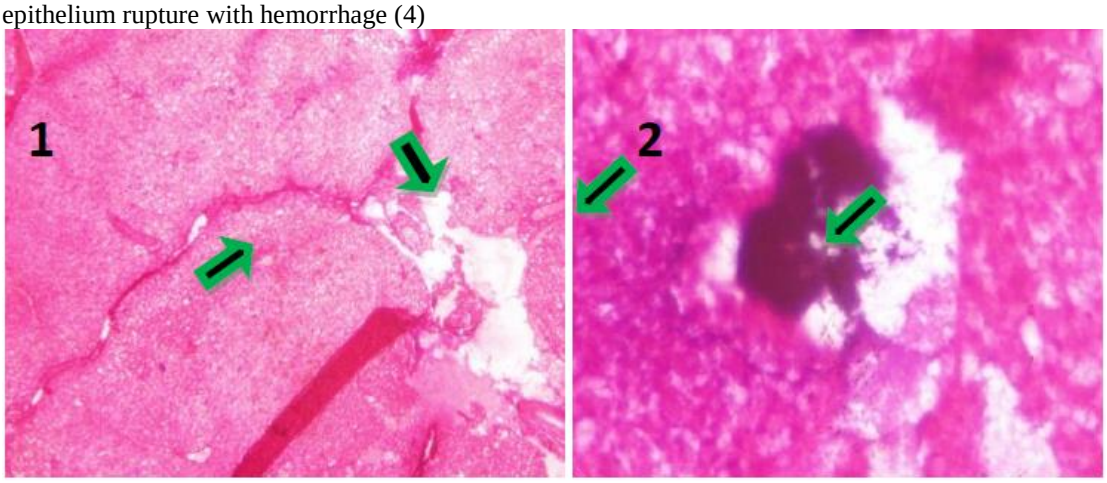
Control gill, showing the filament (Microphotograph. 1 & 2) a pillar cell and an epithelial cell (Microphotograph.3). lamellae with water channel (Microphotograph. 4)



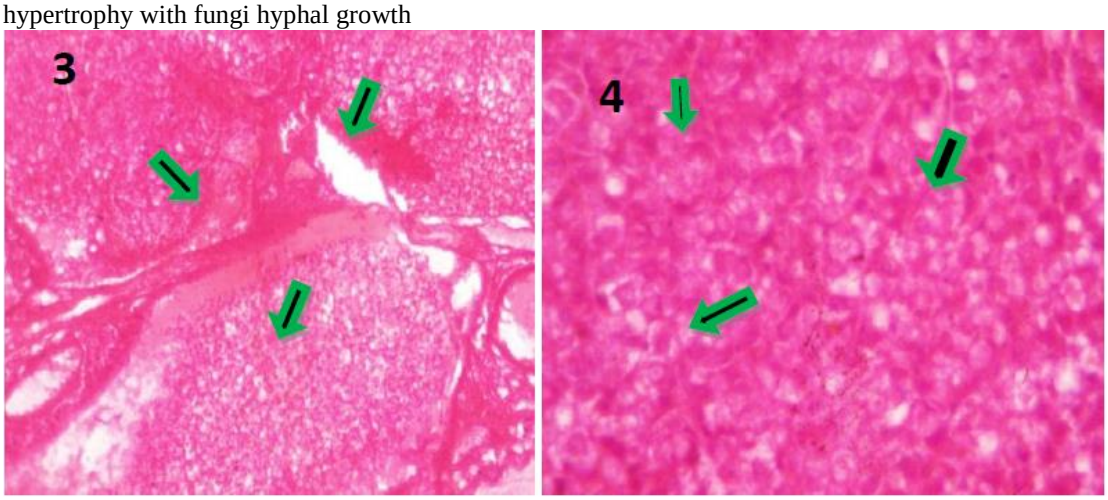
Infected gill Lamellae showed with the marginal channel dilated (1) hyperplasia of the epithelial cells, fusion of 4 lamellae and blood congestion lamellar disorganization. (2)



Infected gill partial fusion of some lamellae and hypertrophy (3). The lamellar epithelium and epithelium rupture with hemorrhage (4)



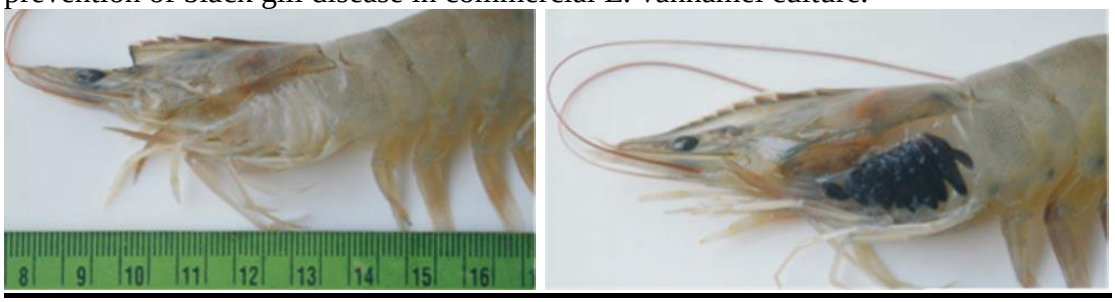
Infected hepatocytes with irregular shaped nucleus, eosinophilic granules in the cytoplasm and nuclear hypertrophy with fungi hyphal growth



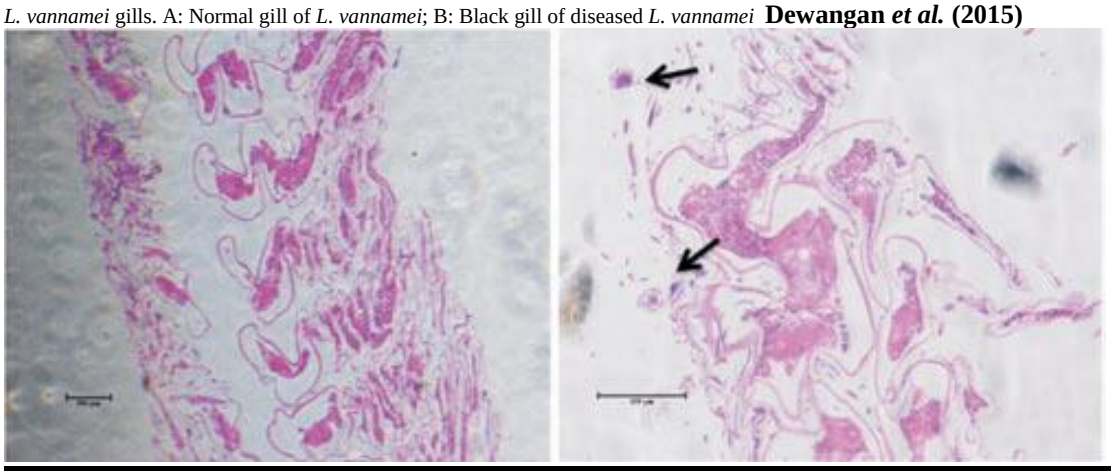
Bile stagnation, nuclear degeneration and cytoplasmic degeneration (3) cytoplasmic vacuolation and hepatic necrosis (4).

Dewangan *et al.* (2015) carried out a study on the epidemiology of black gill disease in white leg shrimp which is a major problem being faced by the commercial shrimp farmers who are culturing Litopenaeus vannamei (L. vannamei) in India. The normal and infected shrimps were collected from shrimp pond and the gill was preserved in appropriate preservative for histopathological examination and scanning electron

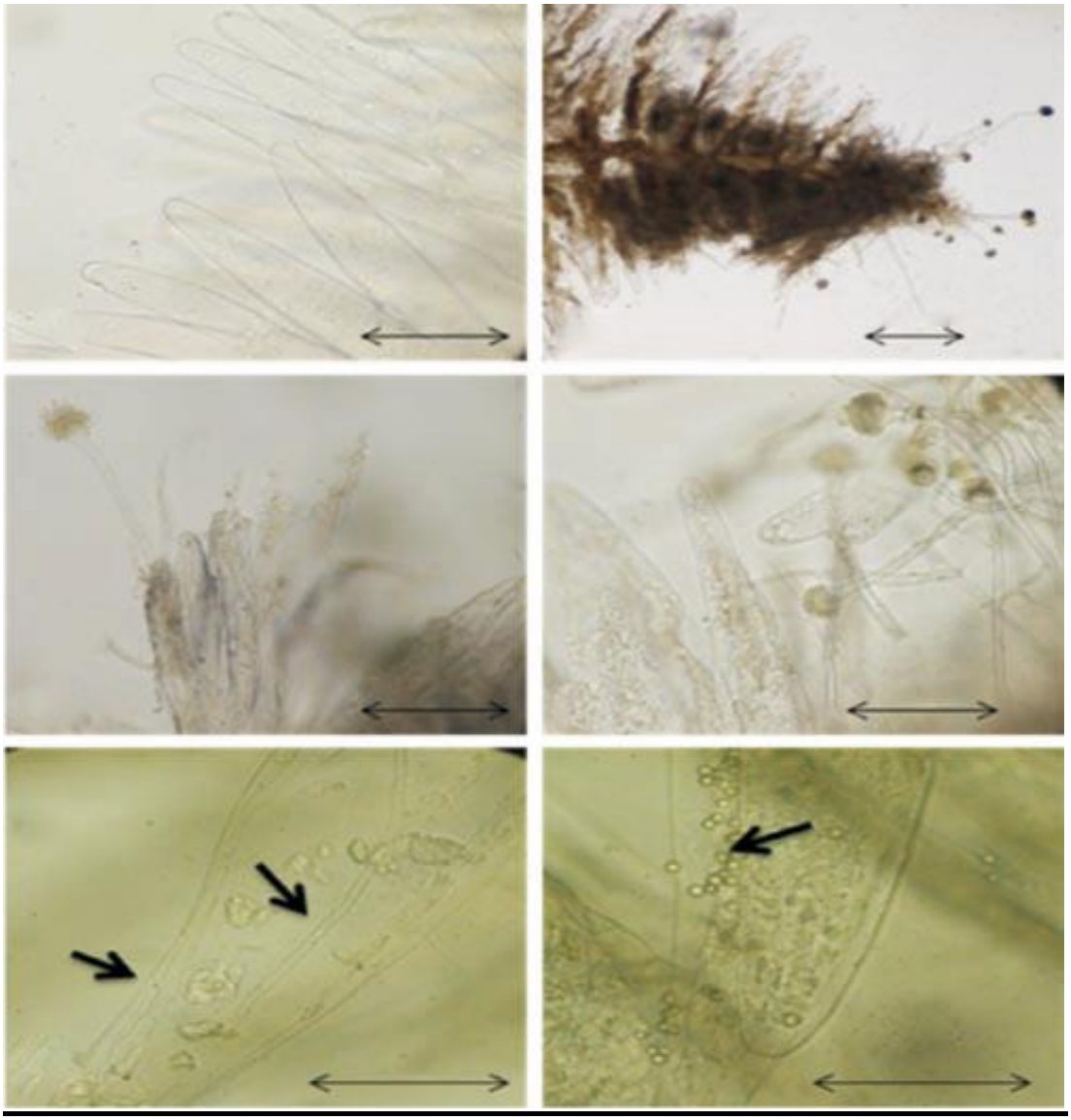
microscope analysis. Pathogenic fungus was isolated from black gill of L. vannamei in potato dextrose agar medium. Morphological study and fungal strain identification were done by using light microscopy and scanning electron microscope. Fungal DNA was amplified by ITS4 and ITS5 primers and gene sequencing was done by Macrogen Inc., Korea. Phylogenetic tree was prepared by using MEGA 6 software. Results: Fungal spores and hyphae were observed both in internal and external gill surface of infected shrimps. Fungal spores were round in shape and mature sporangium was observed. The histopathology study showed clearly that infected gill was damaged by the fungi. Scanning electron microscopic study showed adherence of fungi in infected gill. Internal transcribed spacer gene sequencing revealed that it was caused by Aspergillus flavus. Conclusions: The outcome of the present study would help to know the cause of black gill disease and to understand the effect of pathogenic fungi in shrimp culture. This study will initiate researchers for work in field of treatment or prevention of black gill disease in commercial L. vannamei culture.



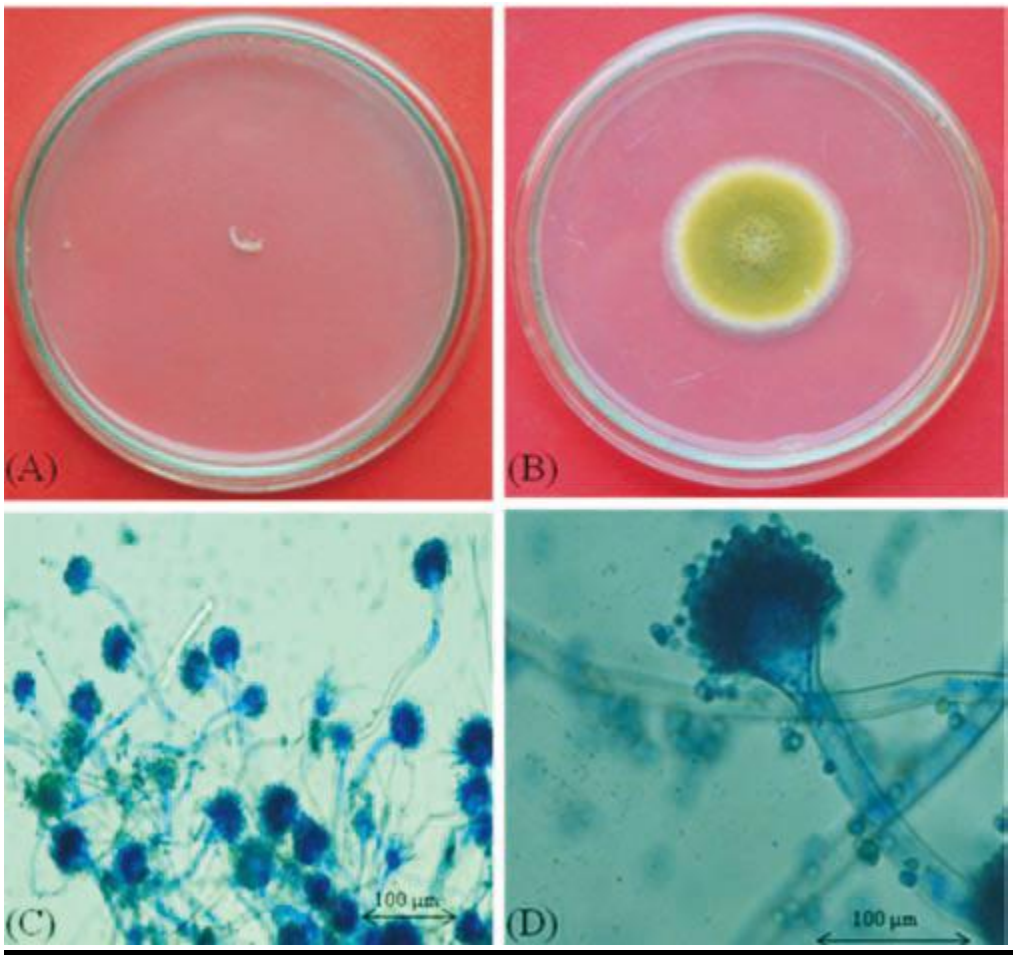
L. vannamei gills. A: Normal gill of *L. vannamei*; B: Black gill of diseased *L. vannamei* **Dewangan *et al.* (2015)**



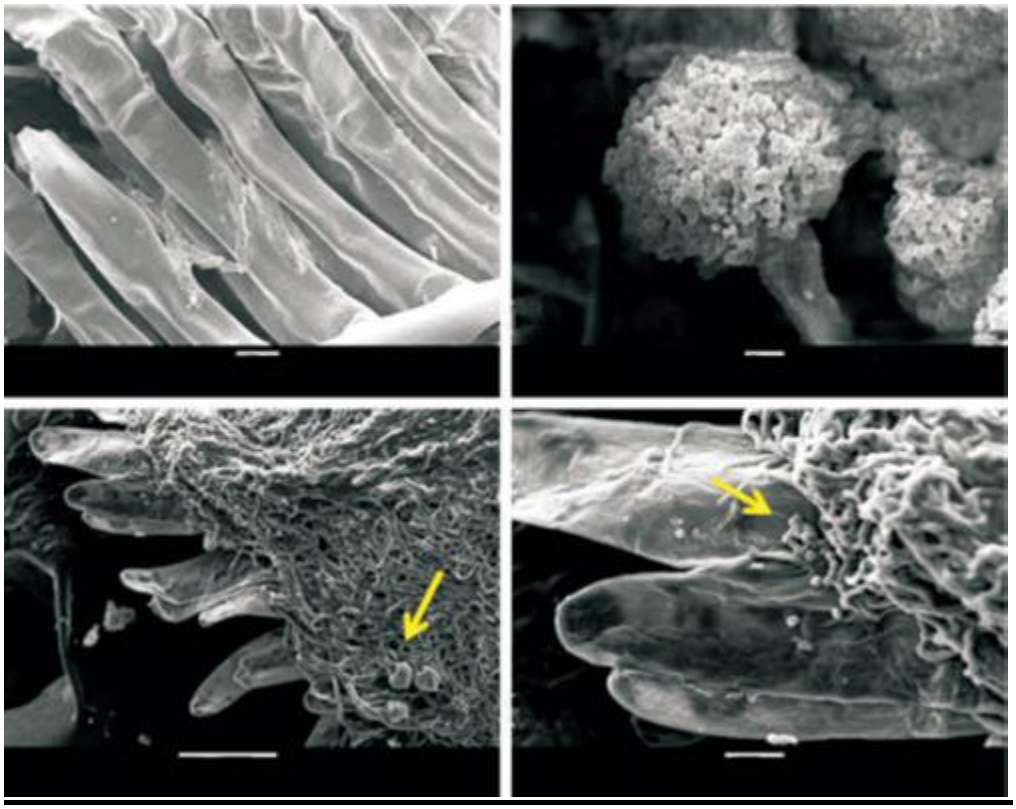
tissue sections of gill lamella. A: Normal gill lamella; B: Black gill lamella. **Dewangan *et al.* (2015)**



Light microscopic observation of gills. A: Image of normal gill lamella; B: Fuzziness of black gill lamella infected with fungi; C: Outward growth of fungi from black gill lamella; D: Presence of fungi around the gill lamella; E: Presence of fungal hyphae inside the affected gill lamella; F: Presence of conidia in the outer surface of black gill lamella. **Dewangan *et al.* (2015)**

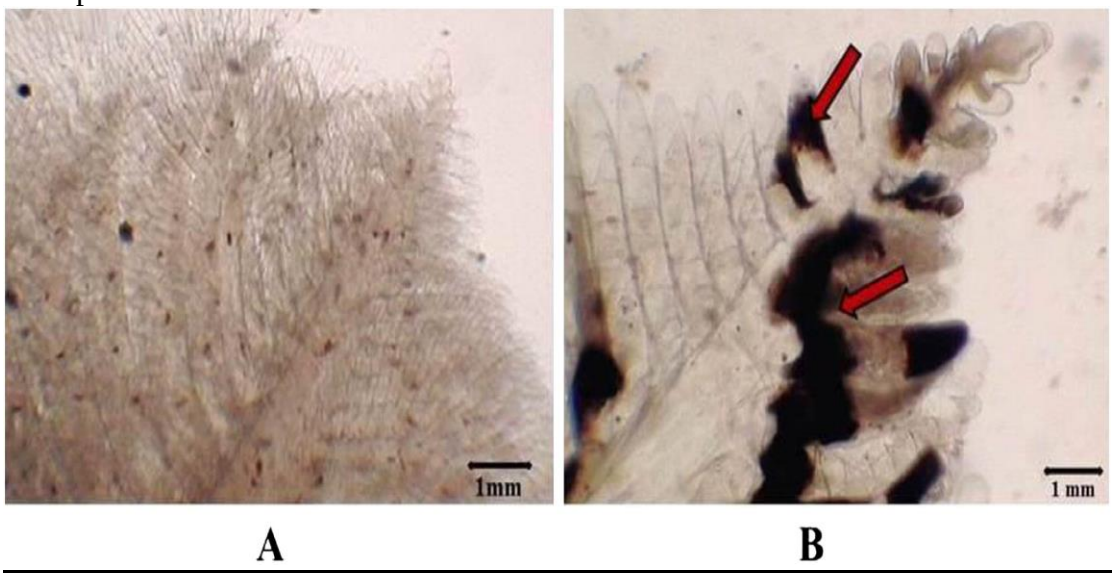


Microscopic observation of fungi. A: Image of control PDA medium plate inoculated with normal gills; B: Colony of A. flavus NKD1 isolated from black gills of L. vannamei in PDA medium; C: Light microscopic view of A. flavus NKD1 stained with lacto phenol cotton blue; D: Close-up view of A. flavus NKD1 in light microscope (100×). **Dewangan *et al.* (2015)**



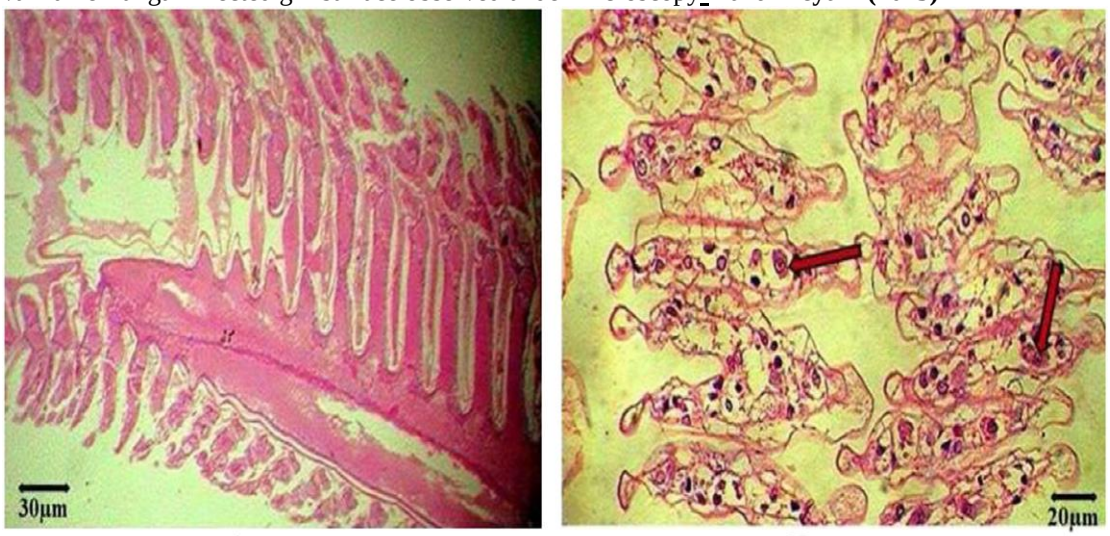
Scanning electron microscopic view of gills. A: Image of normal gill lamella; B: Mature sporangium of A. flavus NKD1 fungi; C: Black gill lamella covered by fungal mycelium; D: Small conidia present in black gill lamella. **Dewangan *et al.* (2015)**

Karthikeyan (2015) reported Aspergillus awamori that caused black gill disease in pacific white shrimp (Litopenaeus vannamei**)** in a pond located at Vellapallam, Nagapattinam District, Tamil Nadu, India. A. awamori was isolated from affected gill of shrimp. Further, its morphological, cultural and phylogenetic characteristics were identified. The histopathological depiction is inflammatory response of L. vannamei against A. awamori were haemocytic infiltration, encapsulation, melanization and collagen-like fibre deposition in the gill. In addition to that, Aspergillus awamori caused dysfunction of gills that leads to chronic mortality in the grow-out pond of shrimps.

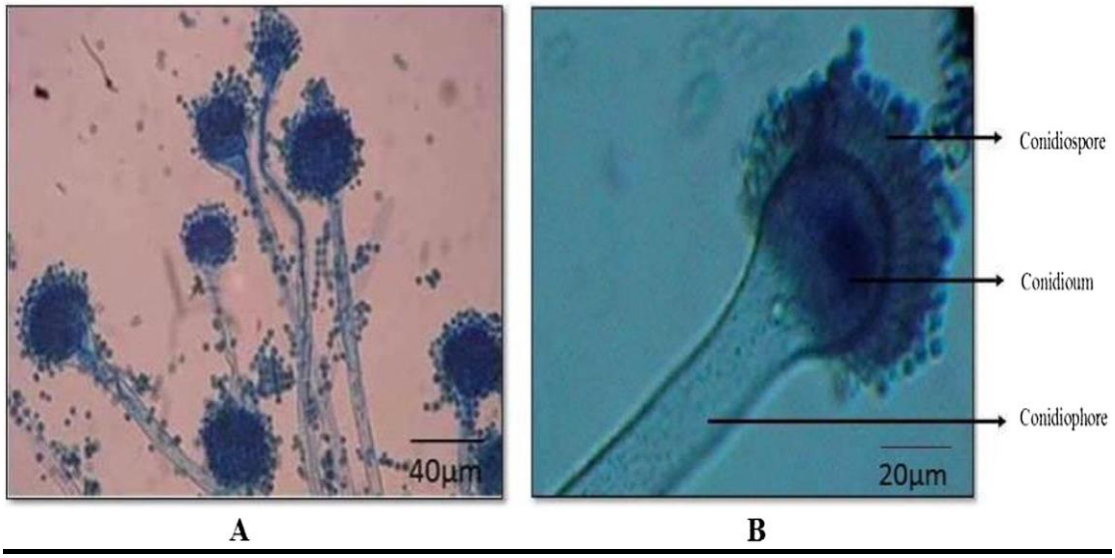


(A) A wet mount preparation of the L. vannamei normal gill. (B) A wet mount preparation of the L.

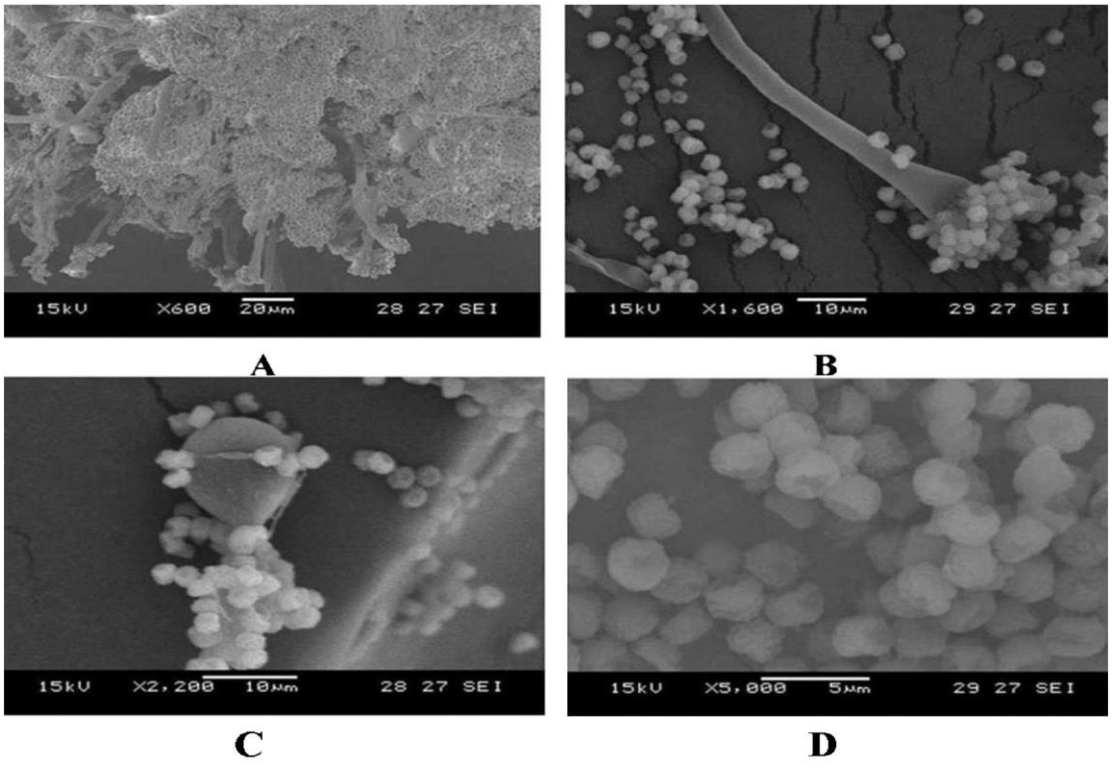
vannamei fungal infected gill surface observed under microscopy **Karthikeyan (2015)**



(A) Normal gill lamella (B) Cross-section of haemocoel in gill tissue, haemocytes are surrounded by large amounts of substances caused by coagulation necrosis. **Karthikeyan (2015)**



(A) Photomicrograph reveals mature conidiophores of A. awamori observed by LPCB mount. (B) LPCB mount showing conidiophores, conidiospore and conidioum of A. awamori **Karthikeyan (2015)**



(A) Photomicrograph reveals many hyphae,mature conidia and conidiophores of A. awamori . (B) A SEMimage of conidia covering thewhole surface of the conidiophore. (C) Photomicrograph view on conidia with spores of A. awamori (D) A SEM close up view on spores of A. awamori. **Karthikeyan (2015)**

Edoghotu and Hart (2016) detected Aspergillus infection on Chrysichthys nigrodigitatus in a study of the fisheries ecology of the Niger Delta region of Nigeria. The discovery of the fungus infection on fish is the first of its kind in the region. Its occurrence was attributed to environmental degradation resulting from incessant oil spillages that pollute the region water. Several scientists in the region had reported involvement of the parasite in the degradation process of spilled crude oil in water bodies of the region. This discovery shall therefore serve as warning signal for precaution against similar potential virulent degrader, yet to be known in the region or elsewhere in the world.

References:

1. Ben Olufemi, R. J. Roberts. Induction of clinical aspergillomycosis by feeding contaminated diet to tilapia, Oreochromis niloticus (L.). Journal of Fish Diseases 9(2):123 - 128 · April 2006
2. Chauhan, R., Zeeshan Nisar and Ashiq Hussian Baig. STUDIES ON ASPERGILLOMYCOSIS IN *LABEO CALBASU* FOUND INFECTED WITH *ASPERGILLUS FLAVUS* AND *A.TERREUS.*, World J. Pharm. Pharmceut. Sci.3, 7, 1842-1848.2014
3. Dewangan, N. K. , Ayyaru Gopalakrishnan, Daniel Kannan, Narayanasamy Shettu, Ramakrishna Rajkumar Singh. Black gill disease of Pacific white leg shrimp (Litopenaeus vannamei) by Aspergillus flavus. Journal of Coastal Life Medicine 2015; 3(10): 761-765
4. Edoghotu A.J , A.I Hart. Aspergillus Infection of Chrysichthys Nigrodigitatus (Silver Catfish) of the Niger Delta, Nigeria. Sci. Agri. 15 (1), 2016: 338-339

5. Karthikeyan, V., Periyasamy Selvakumar, Ayyaru Gopalakrishnan. A novel report of fungal pathogen Aspergillus awamori causing black gill infection on Litopenaeus vannamei (pacific white shrimp). Aquaculture 444 (2015) 36–40
6. OLUFEMI B. E., R. J. ROBERTS. Induction of clinical aspergillomycosis by feeding contaminated diet to tilapia, *Oreochromis niloticus*(L.). J. Fish Dis.9,2,123-128, 1986
7. Refai M. K., Laila A. Mohamed, Amany M. Kenawy and Shimaa El-S M. A., The assessment of Mycotic Settlement of Freshwater Fishes in Egypt, Journal of American scienc, 2010; 6(11): 595-602.
8. Rao, K., Podeti and Benarjee. G. STUDIES ON HAEMATOLOGICAL AND HISTOLOGICAL MYCOSIS VARATIONS OF *CHANNA PUNCTATUS* (BLOCH) FOUND INFECTED WITH *ASPERIGILLUS FUMIGATUS* AND *ASPERGILLUS NIGER SPP* EXHIBITED EUS CHARECTERSTICS. World J. Pharm. Pharmceut. Sci. 4, 7, 1233-1246,2015
9. Refai M, Abdel MM halim, MMH, Afify, H, Youssef and Marzou.K. M. Studies onaspergillomycosis in catfish (Clarias Lasera). All gemeine Pathologic and pathologische Anatomic.Tagung der DeutachenVeterinar–Medizinischen Gesellschaft. DerEuropeischen Gesellschaft fur Vet. Pathol.1987; 63: 1-12.
10. Salem, A., Refai, M., Eissa, I. A., Mmarzouk, M., Bakir, A., Mustafa, M. Mandmanal, A. Some studies on Aspergillo mycosis in Tilapia nilotica. Zagazig Vet. J., 1989; 17(3): 315-328.

16. Fusarium:

Fusarium infections have been identified in a number of marine species:

- In the Pomacanthidae (angelfish), these include:
  - French angelfish (Pomacanthus paru),
  - gray angelfish (Pomacanthus arcuatus),
  - blue angelfish (Holocanthus bermudensis),
  - queen angelfish (Holacanthus ciliaris).
- Susceptible members of the Sphyrnidae (hammerhead sharks) include:
  - bonnethead sharks (Sphyrna tiburo)
  - scalloped hammerhead sharks (Sphyrna lewini)
- Others have been identified as susceptible include:
  - pink-tailed triggerfish (Melichthys vidua) and scrawled
  - filefish (Aluterus scriplus)

Fusarium-associated mortalities have been described in several species of freshwater fish, including:

- Barbus rana,
- Channa punctatus,
- Labeo rohita,
- Mastacembelus armatus,
- Mystus tengra,
- Puntius sophore,
- Wallago attu

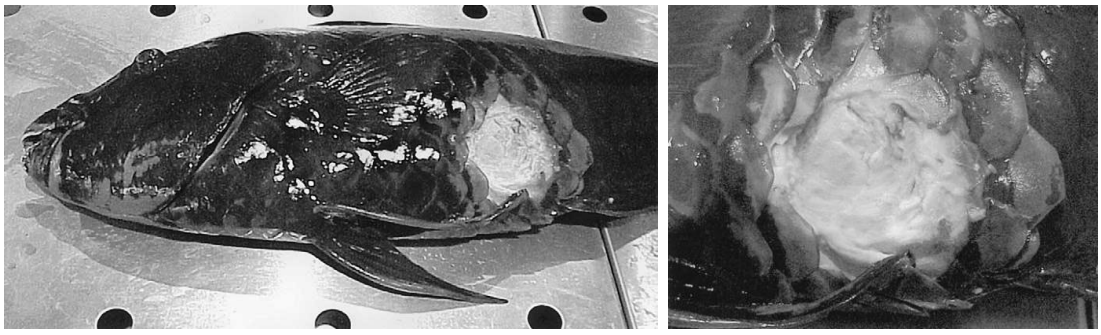
F. solani-induced granulomatous peritonitis was described in an aquarium-kept desert pupfish, Cyprinodon macularis.

Clinical signs:

- Infections may start initially with localized epidermal lesions, usually on the lateral body wall, that included skin defects, ulcers, or lifting of scales
- These progress to necrotizing dermatitis, myositis with perforation of the coelomic cavity, and ultimately, death.
- Progression may be rapid, occurring within several days to 2 weeks, depending upon other factors (eg, water quality or natural sunlight).
- In some cases, underlying liver and spleen infections may be noted
- In several cases, lesions appear to initiate at or around the lateral line system.
- Lesions may begin as raised fluid-filled pustules that rupture on contact.
- Lesions may progresse to severe dermal ulcerations beginning primarily at the head, but rapidly spreading to the operculum and lateral line.
- Infections may be aggressive, often leading to invasion of underlying muscle and bone, and in several cases, becoming systemic and infecting kidney and brain.
- Severe chronic granulomatous dermatitis, cellulitis, and myositis with fungal elements present were seen on histopathology.

Cases described by Yanong, 2003:

- In a pair of newborn bonnet head sharks, clinical signs included lethargy, disorientation, weight loss, appearance of cephalic erosions, and ulceration. Histopathology demonstrated a chronic myositis with myonecrosis present surrounding the cartilaginous skeleton.
- In four of 14 wild female bonnetheads papules developed on the dorsal and ventral surfaces of their heads and along the lateral line. These papules ruptured with application of minimal pressure, releasing a white purulent exudate.
- In two of five scalloped hammerhead sharks presented with behavioral changes and ultimately granulomatous exudative mycotic dermatitis that began in the cephalic canals, but over months, spread into the lateral canal.
- In the triggerfish, ascites and lifting of the scales were noted, and a severe granulomatous peritonitis was diagnosed.
- In the filefish, a perianal swelling and aberrant swimming were noted, but mycotic myositis with necrosis was the primary lesion



Parrotfish with Fusarium infection. Note ulcer on ventrolateral aspect extending into deep musculature. Closer view of deep ulcer in parrotfish. (Courtesy of Scott Terrell, University of Florida,Gainesville, FL.) **Yanong, 2003**

Bonnethead, ventrum of bonnet. Note multifocal to coalescing hemorrhagic areas. (Courtesy of Scott Terrell, University of Florida, Gainesville, FL.) **Yanong, 2003**

Fusarium species reported in fish

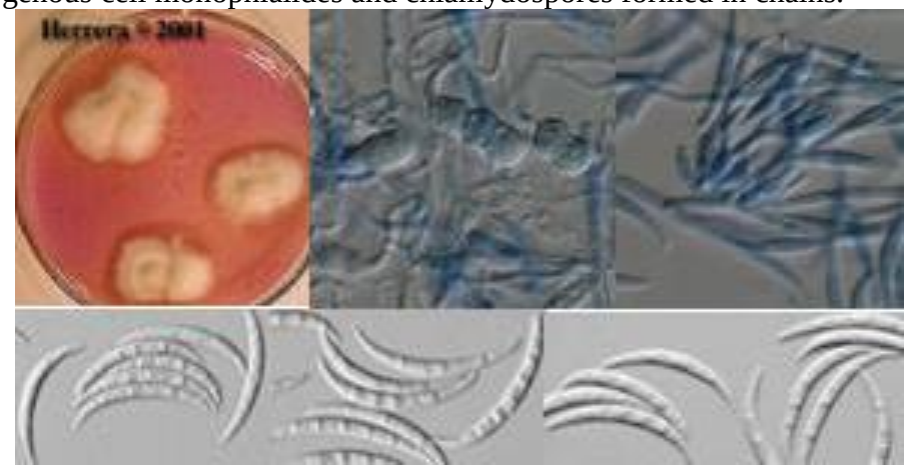
- **Bain and Egusa (1981)** studied the histopathology of black gill disease caused by ***Fusarium solani***
- **Alderman and Polglase (1985)** reported ***Fusarium tabacinum*** Gams, as a gillparasite in the crayfish, Austropotamobius pallipes Lereboullet
- **Hatai *et al.* (1986)** reported a case of infection by ***Fusarium oxysporum*** that occurred among juvenile "ned sea bream" imported from Hong Kong.
- **Rhoobunjongde *et al.* (1991)** isolated ***Fusarium moniliforme*** from gill lesions of *kuruma prawn, Penaeus japonicus* , with black gill disease at a private farm in Okinawa Prefecture (Japan)
- **Zhan *et al.* (1993)** mentioned that 4 species of Fusarium, *F. solani, F.* ***graminearum, F. tricinctum****,* and *F. oxysporum,* were isolated from *Penaeus chinensis*.
- Khoa ***et al.* (2004)** isolated ***Fusarium incarnatum*** from gill lesions of cultured black tiger shrimp, Penaeus monodon
- **Bisht *et al.* (2000)** found that *Fusarium moniliforme* and ***F. udum*** were natural pathogens of freshwater fish in reservoirs, causing mycosis and high mortality in *Barbus rana, Channa punctatus, Labeo rohita, Mastaceamblus armatus, Mystus tengra, Puntius sophore* and *Wallago attu.*
- **Palmero *et al.* (2009)** isolated *Fusarium* ***anthophilum, F. acuminatum, F. chlamydosporum, F. culmorum, F. equiseti, F. verticillioides****, F. oxysporum,* ***F. proliferatum****, F. solani,* and *F. sambucinum* **from** 18 water samples collected from the Andarax River
- **Edsman *et al.* (2015)** isolated ***Fusarium sambucinum*** syndrome from crayfish with eroded swimmeret syndrome (ESS)
- **Cutuli *et al.* (2015)** reported the first case of tilapia infection by ***Fusarium oxysporum*** species complex confirmed by culture, molecular identification and histopathology.

Description of Fusarium species recorded in fish

1. *Fusarium acuminatum* Ellis & Everh., Proc. Acad.Nat. Sci.Philad. 47: 441 (1895)

≡Fusarium scirpi var. acuminatum (Ellis & Everh.) Wollenw., Fusaria Autographice Delineata 3: 930-933 (1930)
≡Fusarium scirpi subsp. Acuminatum (Ellis & Everh.) Raillo, Fungi of the genus Fusarium: 177 (1950)
≡Fusarium gibbosum var. acuminatum (Ellis & Everh.) Bilai, Mykrobiologichnyi Zhurnal Kiev 49 (6): 6 (1987)

Colonies are slow-growing, with white aerial mycelium, developing brownish pigmentation in the center on PDA. The dorsal side of the colony has rose to burgundy pigmentation. Macroconidia are broadly falcate with 3-5 septa, apical cell long and tapered, basal cell foot- shaped. Microconidia are sparse, fusiform, 0-1 septa, conidiogenous cell monophialides and chlamydospores formed in chains.

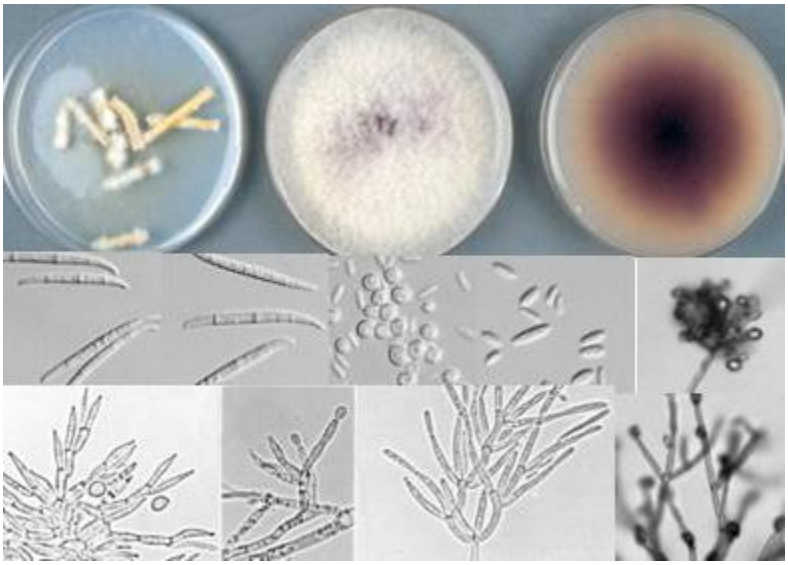


F. acuminatum colony, Paul Cannon Chlamydospores, conidiogenous cells, macroconidia, Leslie and Summerell

2. *Fusarium anthophilum* (A. Braun) Wollenw., Fusaria Autographice Delineata 1: 176 (1916)

≡Fusisporium anthophilum A. Braun, Fung. Europ.: no. 1964 (1875)
≡Fusarium moniliforme var. anthophilum (A. Braun) Wollenw., Fusaria Autograph/ Delin. 3: 975 (1930)
≡Fusarium wollenweberi Raillo, Fungi of the genus Fusarium: 189 (1950)
≡Fusarium tricinctum var. anthophilum (A. Braun) Bilai, Fusarii (Biologija i sistematika): 251 (1955)
≡Fusarium sporotrichiella var. anthophilum (A. Braun) Bilai, Mykrobiol. Zhurnal Kiev 49 (6): 7 (1987)

Colonies on PDA form abundant white floccose mycelium turn to greyish violet in old cultures. Pigmentation in agar violet grey or dark. Sporodochia pale orange. Macroconidia are thin-walled, long, slender, almost straight, 3-5 septa,produced from monophilides on branched conidiophores in the sporodochia or on the hyphae, basal cell notched or foot-shaped, apical cell curved and tapered. Microconidia are abundant, from poly- or monophialides, globose, 1-2 celled, globose, or ovoid, in false heads. Chlamydospores absent.

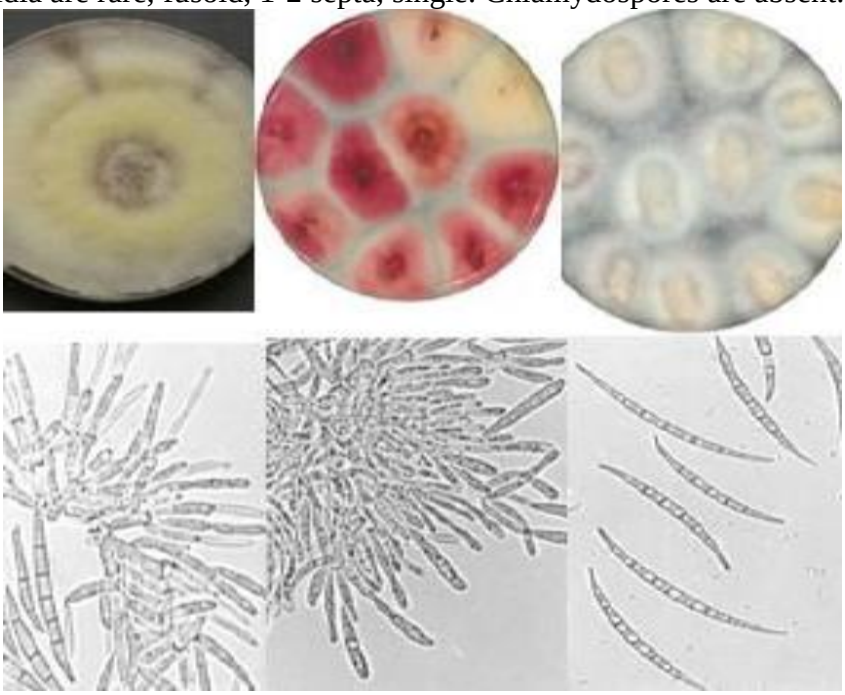


Leslie and Summerell , Hagedorn, Burhenne & Nirenberg

3. Fusarium avenaceum (Fr.) Sacc., Sylloge Fungorum 4: 713 (1886)

≡Fusisporium avenaceum Fries, Systema Mycologicum 3: 444 (1832)
≡Fusarium herbarum var. avenaceum (Fries) Wollenw., Fusaria Autographice Delineata 3: 899 (1930)
=Selenosporium herbarum Corda, Icones fungorum hucusque cognitorum 3: 34, t. 6:88 (1839)

Colonies initially form abundant fluffy white mycelium and produce a golden orange pigment on PDA at 25°C. Sporodochia pale orange, Macroconidia are slightly falcate, thin-walled, usually 3 to 5 septate, with a tapering apical cell , basal cell notched. Microconidia are rare, fusoid, 1-2 septa, single. Chlamydospores are absent.

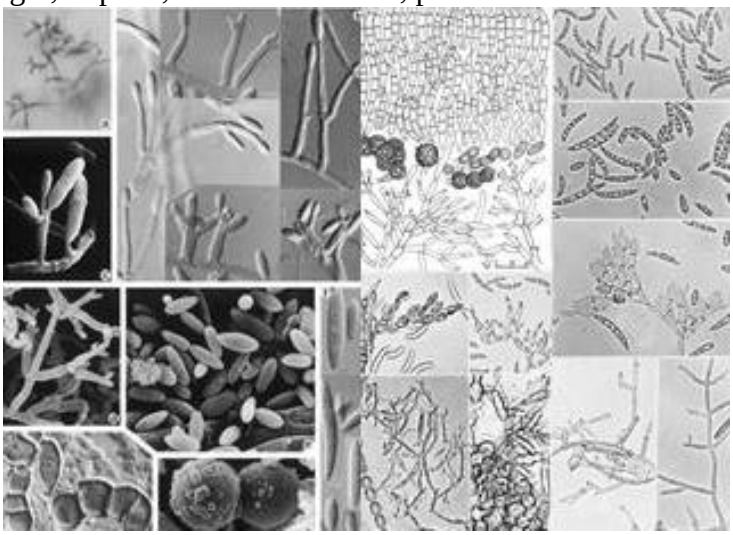


F, avenaceum colonies, www.grainscanada.gc.ca. Mycota, G. Hagedorn, M. Burhenne & H. I. Nirenberg

4. *Fusarium chlamydosporum* Wollenw. & Reinking, Phytopathology 15 (3): 156 (1925)

=Fusarium sporotrichioides var. chlamydosporum (Wollenw. & Reinking) Joffe, Mycopathologia et Mycologia Applicata 52 (1-4): 211 (1974)

Colonies produce white mycelium with grayish rose to burgundy or yellowish to pale brown pigmentation.Macroconidia: abundant, thick-walled, moderately curved, 3-5 septa, apicalcell short, curved and pointed, basal cell notched or foot-shaped. Sporodochia: rare. Microconidia: comma-shaped, 0-2 septe, single or in pairs fro, a phialide, abundant. Chlamydospores : abundant after 2-4 weeks, on aerial hyphae or submerged in agar, in pairs, chains or clusters, pale brown

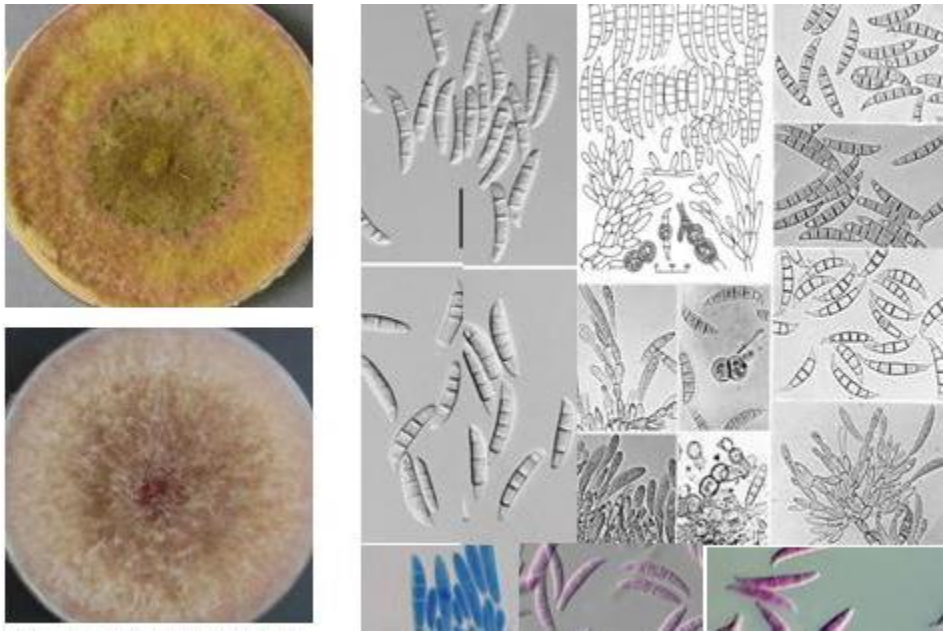


Mycobanc, G. Hagedorn, M. Burhenne & H. I. Nirenberg

5. *Fusarium culmorum* (W.G. Sm.) Sacc., Sylloge Fungorum 11: 651 (1895)

=Fusisporium culmorum Wm.G. Sm., Diseases of field and garden crops: 209 (1884)
≡Fusarium culmorum (W.G. Sm.) McAlpine, Agricul. Gaz. New South Wales 7: 299-306 (1896)

Macroconidia: abundant, relat. Short, thick-walled, dorsal curvature and straight ventrally, 5 septa, apical cell rounded ant blunt , basal cell notched. Sporodochia: orange –brown, abundant. Microconidia: absent. Chlamydospores : abundant in 3-5 weeks, in hyphae and macroconidia, in chains and clusters

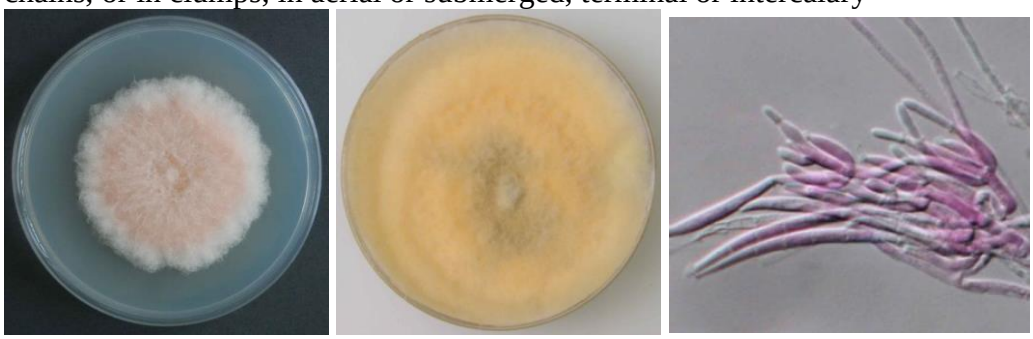


John F. Leslie and Brett A. Summerell , G. Hagedorn, M. Burhenne & H. I. Nirenberg, Wikipedia

6. *Fusarium equiseti* (Corda) Sacc., Sylloge Fungorum 4: 707 (1886)

≡Selenosporium equiseti Corda, Icones fungorum hucusque cognitorum 2: 7, t. 9:32 (1838)
=Fusarium gibbosum Appel & Wollenw., Kaiser. Biologischen Anstalt Land u Forstwirtschaft 8: 190 (1910)
=Fusarium caudatum Wollenw., Journal of Agricultural Research 2: 262 (1914)
=Fusarium bullatum Sherb., Memoirs Cornell Univ. Agri. Exper. Stat. 6: 198-201 (1915)

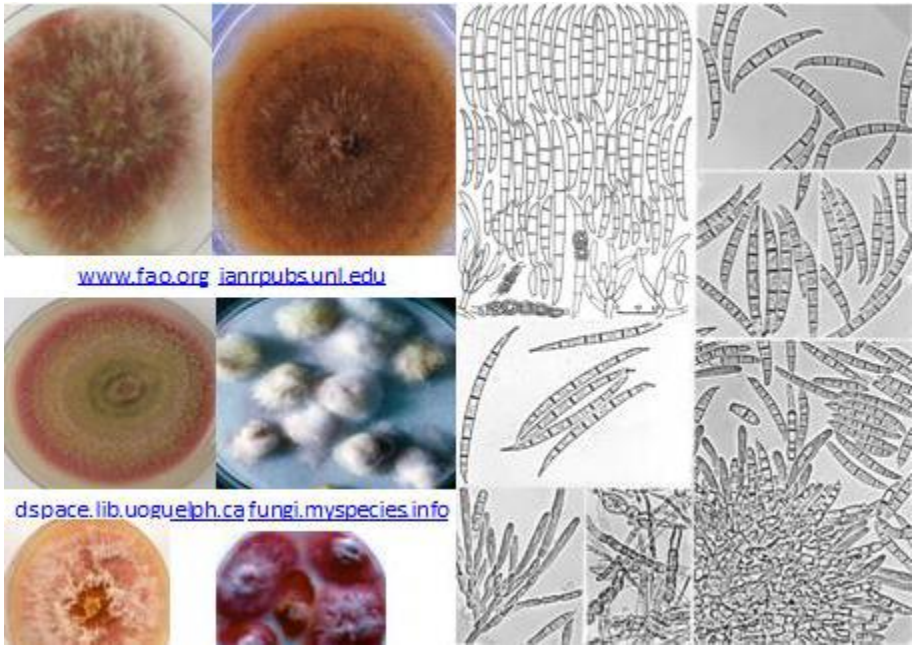
Macroconidia: abundant in sporodochia , long , slender, dorsoventral curvature, 5-7 septa, apical cell elongate and tapering, basal cell foot-shaped. Sporodochia: orange. Microconidia: absent. Chlamydospores abundant in 2 -6 weeks, single, in pairs , in chains, or in clumps, in aerial or submerged, terminal or intercalary



Fusarium equiseti, colony on potato sucrose agar, fungi.myspecies.info *Fusarium equiseti*, macroconidia, conidiogenous cells stained in lactofuchsin. fungi.myspecies.info

7. *Fusarium graminearum* Schwabe, Flora Anhaltina 2: 285 (1839)

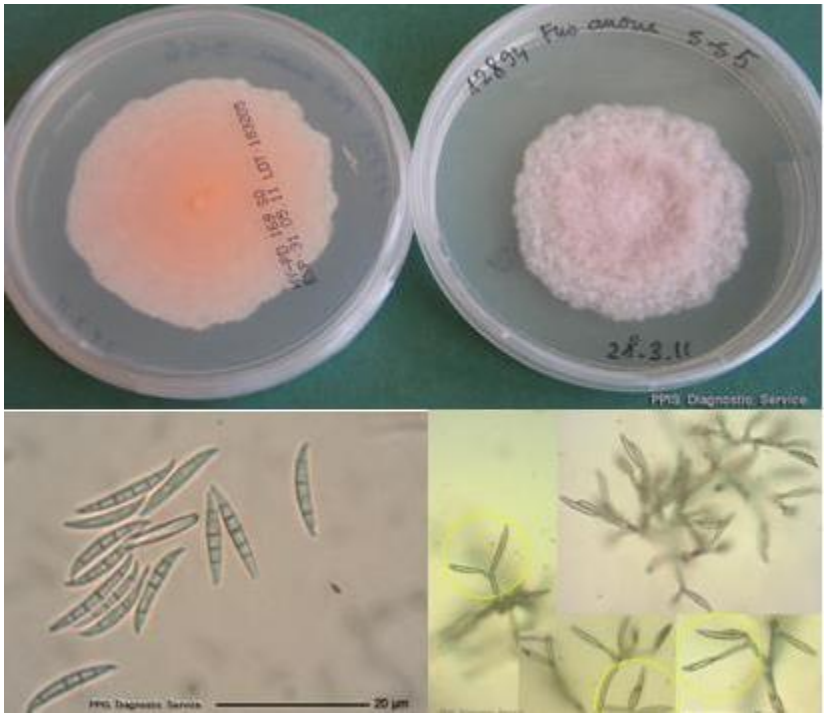
Macroconidia: abundant in sporodochia, slender-slightly curved, thick-walled, 5-6 septa, apical cell tapering, basal cell foot-shaped. Sporodochia: pale orange. Microconidia: absent . Chlamydospores : are formed in the macroconidia, finely roughened, single, in chains or clumps



8. *Fusarium incarnatum* (Roberge) Sacc., Sylloge Fungorum 4: 712 (1886)

≡Fusisporium incarnatum Roberge ex Desm., Ann Sci Natur Bot 11: 274 (1849)
=Fusarium semitectum Berk. & Ravenel, Grevillea 3 (27): 98 (1875)
=Fusarium semitectum var. semitectum (1875)
=Fusisporium pallidoroseum Cooke, Grevillea 6 (40): 139 (1878)
=Fusarium semitectum var. majus Wollenw., Fusaria Autographice Delineata 3: 907-910 (1931)

Colonies produce floccose aerial mycelium, at first whitish, later becoming avellaneous to buff-brown; reverse pale, becoming peach-coloured. Conidiophores scattered in the aerial mycelium, loosely branched; polyblastic conidiogenous cells abundant. Sporodochial macroconidia slightly curved, with foot-cell, 3-7-septate. Conidia on aerial conidiophores (blastoconidia) usually borne singly on scattered denticles, fusiform to falcate, mostly 3-5-septate. Microconidia sparse or absent. Chlamydospores sparse, spherical, intercalary, single or in chains 180

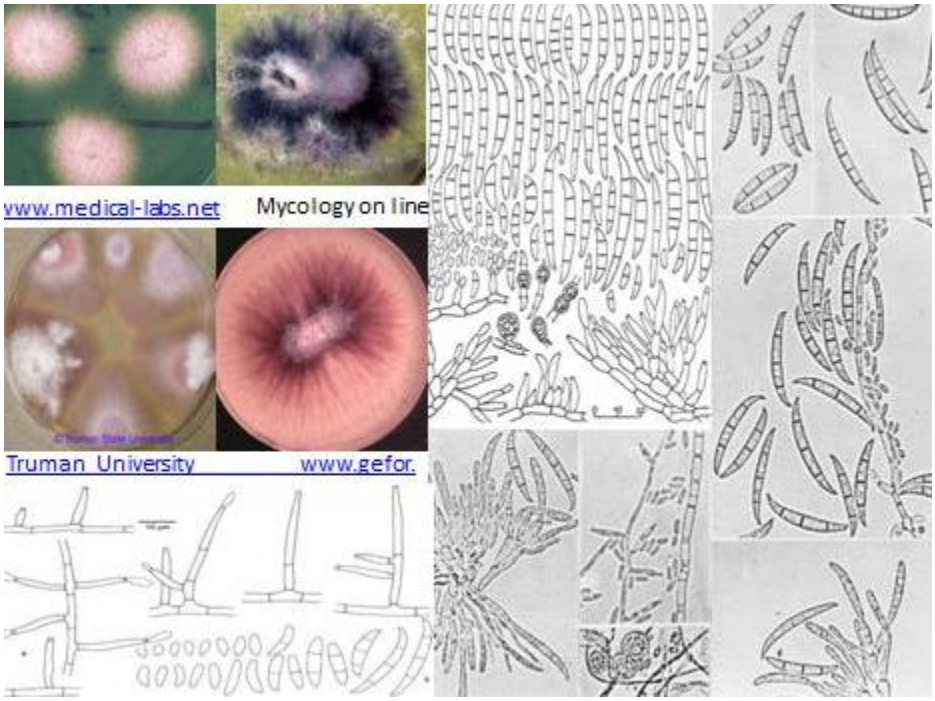


Fusarium incarnatum www.ppis.moag.gov.il

9. *Fusarium oxysporum* Schltdl., Flora Berolinensis, Pars secunda: Cryptogamia: 106 (1824)

=Fusarium bulbigenum Cooke & Massee, Grevillea 16 (78): 49 (1887)
=Fusarium orthoceras Appel & Wollenw., Kaiser. Biol. Anstalt für Land u Forstwirtschaft 8: 152 (1910)
=Fusarium citrinum Wollenw., Bull. Maine Agric. Exp. Sta.: 256 (1913)
=Fusarium angustum Sherb., Memoirs Cornell Univ. Agricult. Experimental Station 6: 203 (1915)
=Fusarium oxysporum var. longius Sherb., Memoirs Cornell Univ. Agricult. Exper.Station 6: 223 (1915)
=Fusarium lutulatum Sherb., Memoirs Cornell Univ. Agricult. Exper.Station 6: 209 (1915)
=Fusarium lutulatum var. zonatum Sherb., Memoirs Cornell Univ. Agricult. Exper.Station 6: 214 (1915)
=Fusarium bostrycoides Wollenw. & Reinking, Phytopathology 15 (3): 166 (1925)
=Diplosporium vaginae Nann., Atti Reale Accad. Fisiocrit. Siena: 491 (1926)

Macroconidia: abundant in sporodochia, 3- septa, thin-walled, short to moderately long, straight , apical cell short and slightly hooked, basal cell notched or foot-shaped. Sporodochia: abundant, pale orange . Microconidia: small, oval , elliptical or kidney-shaped, 0- septa. Chlamydospores: abundant

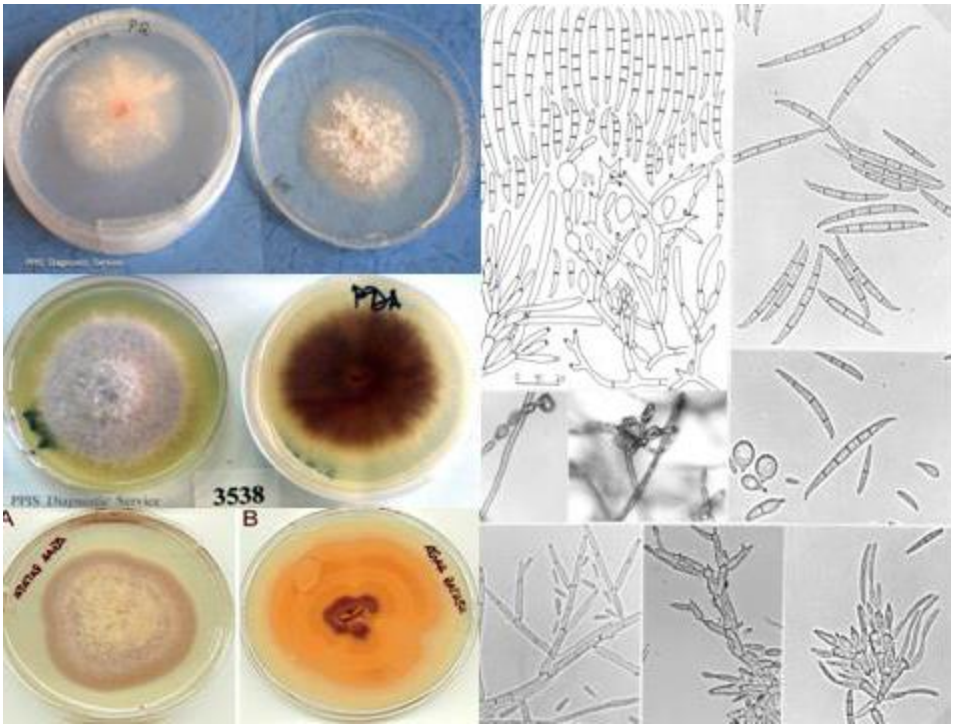


Mycobank G. Hagedorn, M. Burhenne & H. I. Nirenberg

10. *Fusarium proliferatum* (Matsush.) Nirenberg, Biologischen Bundesanstalt für Land- und Forstwirtschaft 169: 38 (1976)

≡Cephalosporium proliferatum Matsush., Microfungi of the Solomon Islands and Papua-New Guinea: 11 (1971)
≡Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach & Nirenberg, Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 209: 309 (1982)

Macroconidia: in chains of moderate length, thin-walled, straight, 3-5 septa, apical cell curved, basal cell poorly developed,. Sporodochia: pale orange. Microconidia, club-shaped to pyriform, 0-septa, may be in chains. Chlamydospores: absent

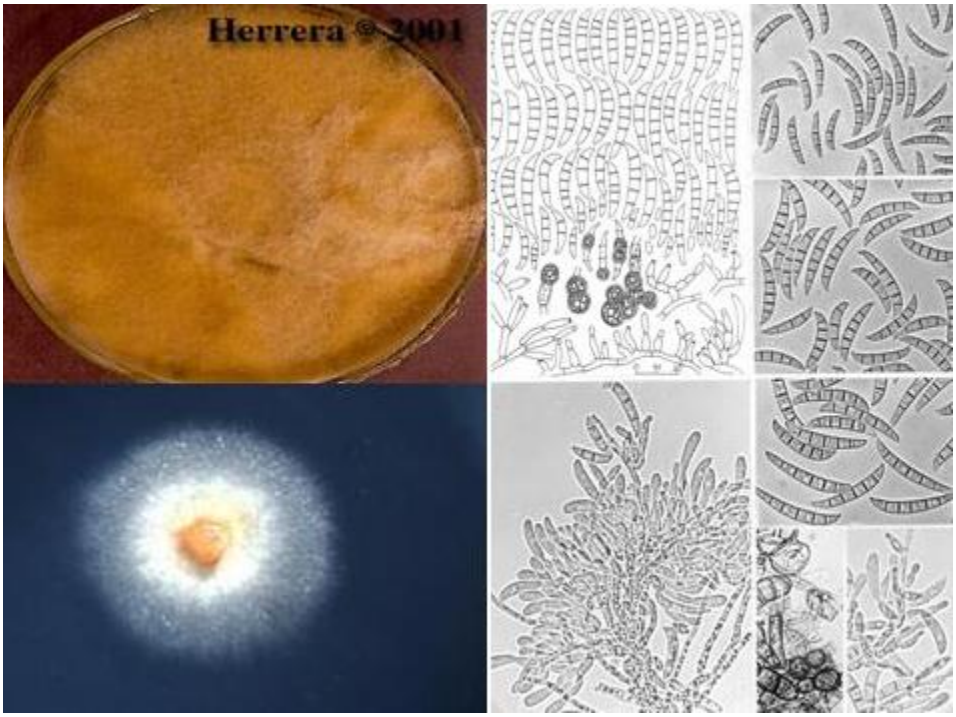


www.ppis.moag.gov.il www.ppis.moag.gov. jcm.asm.org G. Hagedorn, M. Burhenne & H. I. Nirenberg, Ferrer et al., 2005

11.*Fusarium sambucinum* **Fuckel, Hedwigia 2 (15): 135, Fung. Rhen. no 211 (1863)**

=Fusarium roseum Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3: 10, t. 1:10 (1809)
=Fusarium sulphureum Schltdl., Flora Berolinensis, Pars secunda: Cryptogamia: 139 (1824)
=Fusarium sambucinum var. sambucinum , Jahrbücher Nassaui. Vereins Naturkunde 23-24: 167 (1870) [
=Fusarium trichothecioides Wollenw., Journal of the Washington Academy of Sciences 2: 147 (1912)
=Fusarium sambucinum var. minus Wollenw., Fusaria Autographice Delineata 3: 941 (1930)
=Fusarium sambucinum f. 2 Wollenw., Fusaria Autographice Delineata 3: 942 (1930)
=Fusarium sambucinum var. medium Wollenw., Zeitschrift für Parasitenkunde 3: 358 (1931)
=Fusarium sambucinum f. 6 Wollenw., Zeitschrift für Parasitenkunde 3: 358 (1931)

Macroconidia: abundant in sporodochia, 3-5 septa, falcate, slender, short, apical cell pointed, basal cell foot-shaped. Sporodochia: orange, common. Microconidia: oval, 0-1 septa. Chlamydospores: in chains or clusters

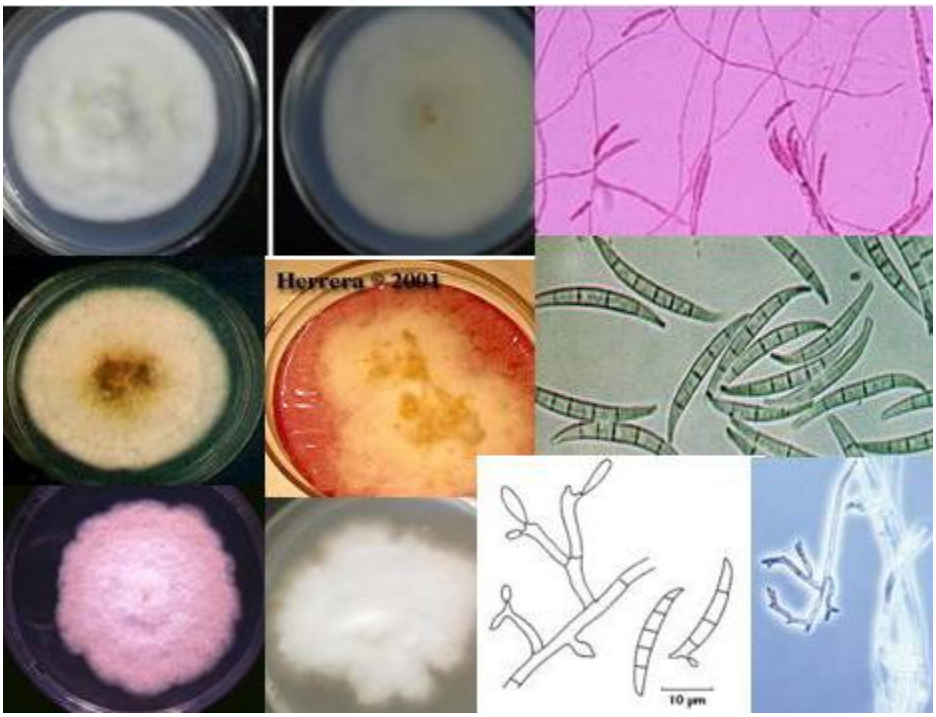


ddis.ifas.ufl.edu G. Hagedorn, M. Burhenne & H. I. Nirenberg

12.*Fusarium semitectum* **Berk. & Ravenel, Grevillea 3 (27): 98 (1875)**

≡Pseudofusarium semitectum (Berk. & Ravenel) Matsush., Icon. Microfung. Matsushima lect.: 119 (1975)
=Fusisporium incarnatum Roberge ex Desm., Annales des Sciences Naturelles Botanique 11: 274 (1849)
=Fusarium semitectum var. semitectum (1875)
=Fusisporium pallidoroseum Cooke, Grevillea 6 (40): 139 (1878)
=Fusarium semitectum var. majus Wollenw., Fusaria Autographice Delineata 3: 907-910 (193]

Macroconidia: abundant, slender, curved dorsal surface, 3-5 septa, apical cell curved and tapering , basal cell foot-shaped. Sporodochia: orange. Microconidia: pyriform, 1-septa, mesoconidia spindle-shaped, 3-5 septa. Chlamydospores: globose

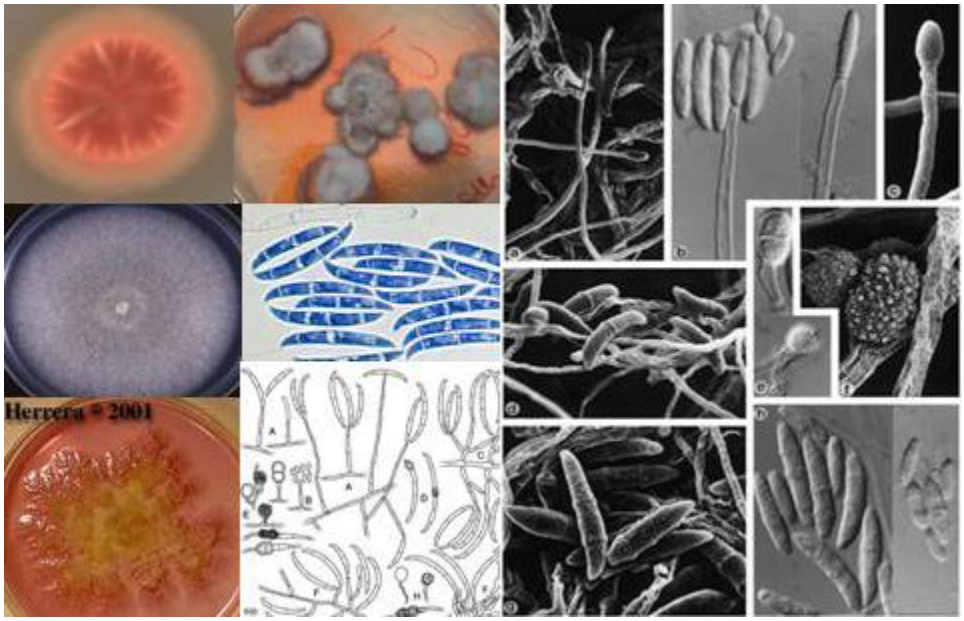


file.scirp.org, ecoport.org, Galería de imágenes**, EcoPort Picture, Databank**

13.*Fusarium solani* (Mart.) Sacc., Michelia 2 (7): 296 (1881)

≡Fusisporium solani Mart., die Stockfäule und Räude der Kartoffeln: 20 (1842)
≡Fusarium solani (Mart.) Appel & Wollenw., Kaiser. Biol. Anstalt Land u Forstwirtschaft 8: 64-78 (1910)
≡Neocosmospora solani (Martius) L. Lombard & Crous, Studies in Mycology 80: 228 (2015)
=Fusarium martii Appel & Wollenw., Kaiser. Biologischen Anstalt Land u Forstwirtschaft 8: 83 (1910)
=Nectria cancri Rutgers, Ann. Jard. Bot. Buitenzorg, II: 59 (1913)
=Fusarium striatum Sherb., Memoirs Cornell Univ. Agricultural Experimental Station 6: 255 (1915)
=Fusarium solani var. minus Wollenw., Fusaria Autographice Delineata 1: 403 (1916)
=Fusarium solani f. 2 W.C. Snyder, Z Iblatt Bakteriol Parasitenkunde Abteilung 2 91: 174 (1934)
=Cephalosporium keratoplasticum T. Morik., Mycopath. Mycol. appl.: 66 (1939)
=Fusarium solani f. keratitis Y.N. Ming & T.F. Yu, Acta Microbiologica Sinica 12: 184 (1966)
=Cylindrocarpon vaginae C. Booth, Y.M. Clayton & Usherw., Proc. Indian Acad. Sci. 94 (2-3): 436 (1985)

Macroconidia: abundant, wide, straight or slightly curved , 3-7 septa, apical cell blunt and round, basal cell foot-shaped or cylindrical with notched end. Sporodochia: abundant, cream, blue or green. Microconidia: oval to fusiform, 0-2 sept. Chlamydospores: abundant, in 2-4 weeks, single, in pairs, in clumps or chains, terminal or intercalary

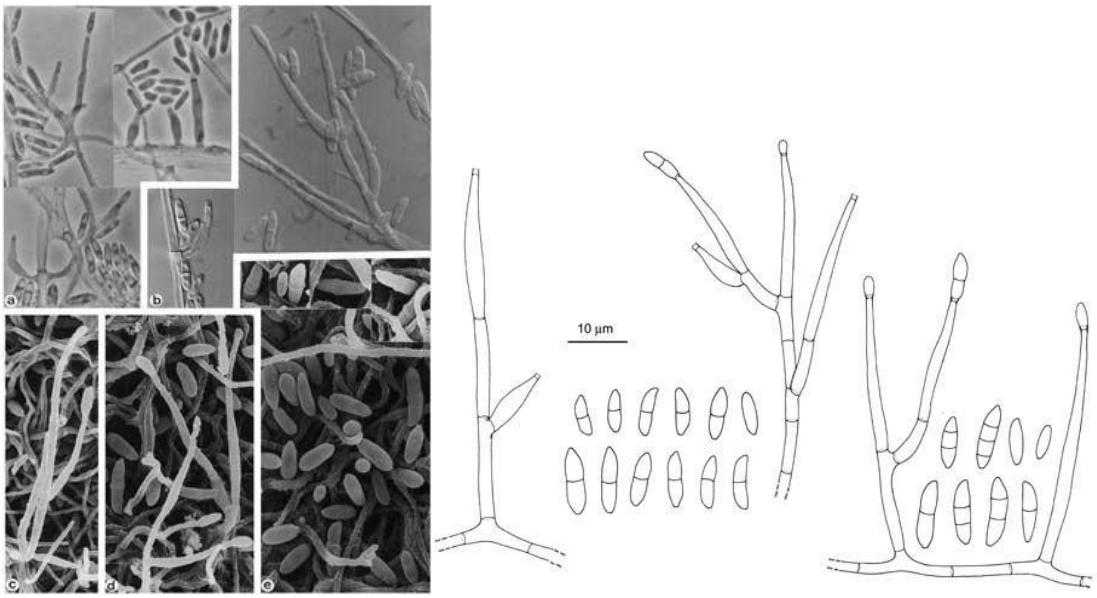


www.mycology.adelaide.edu.au, www.pf.chiba-u.ac.jp, Mycoya, Mycobank, Br J Ophthalmol. 2002, Mycobank

14.*Fusarium tabacinum* (J.F.H. Beyma) W. Gams, Persoonia 5 (2): 179 (1968)

≡Cephalosporium tabacinum J.F.H. Beyma, Zblatt Bakt Parasit/ Abt. 2 89: 240 (1933)
≡Microdochium tabacinum (J.F.H. Beyma) Arx, Trans. Brit. Mycol. Soc. 83 (2): 374 (1984)
≡Plectosporium tabacinum (J.F.H. Beyma) M.E. Palm, W. Gams & Nirenberg, Mycologia 87 (3): 399 (1995)
=Septomyxa affinis Wollenw., Fusaria Autographice Delineata 2: nos 643-644 (1924)
=Cephalosporium ciferrii Verona, Studio sulle microbiche danneggiano la carta ed i libri: 30 (1939)
=Cephalosporiopsis imperfecta Moreau & V. Moreau, Revue de Mycologie 6 (3-4): 67 (1941)

Colonies (OA) growing rather slowly, whitish to beige, somewhat floccose; aerial mycelium generally sparse. Microscopy. Conidiophores at first arising in the aerial mycelium as lateral phialides, later with sparse branching. Conidiogenous cells monophialidic. Macroconidia cylindrical, slightly curved with more or less pointed apex and wedge-shaped base, (0-) 1 (-3)-septate 12-16 x 3-4 µm. Microconidia absent.

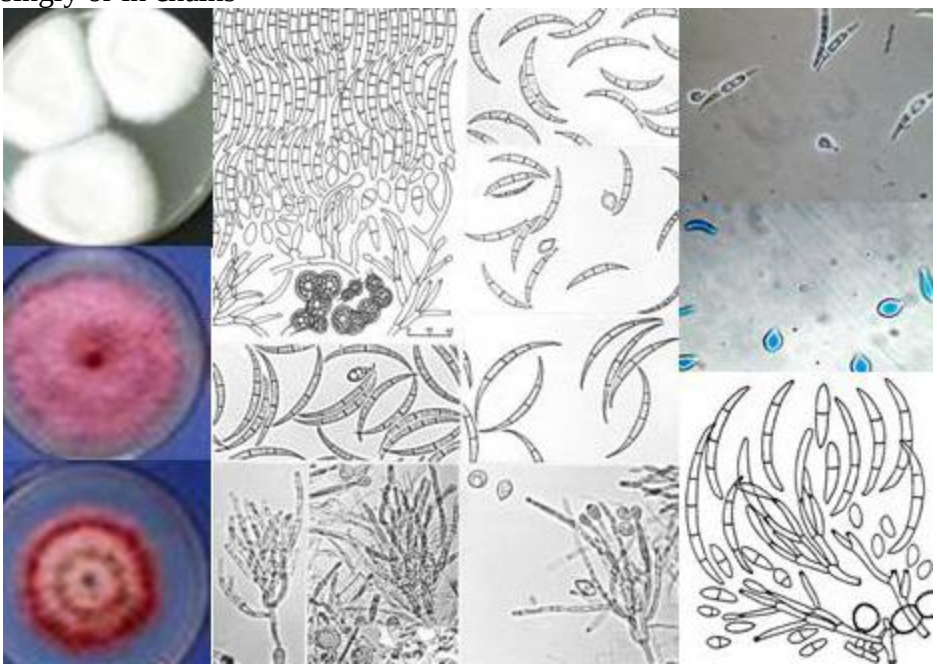


Mycobank

15.*Fusarium tricinctum* (Corda) Sacc., Sylloge Fungorum 4: 700 (1886)

≡Selenosporium tricinctum Corda, Icones fungorum hucusque cogn 2: 7, t. 9:33 (1838)
≡Fusarium sporotrichioides var. tricinctum (Corda) Raillo, Fungi of the genus Fusarium: 197 (1950)
≡Fusarium sporotrichiella var. tricinctum (Corda) Bilai, [Poisonous fungi on cereal seed]: 87 (1953)
≡Fusarium sporotrichiella var. tricinctum (Corda) Bilai, Mykrobiologichnyi Zhurnal Kiev 49 (6): 7 (1987)
=Fusarium citriforme Jamal., Valt. Maatalousk. Julk.: 11 (1943)

Colonies form dense white mycelium, become pink, red or purple. Sporodochia pale orange, abundant. Macroconidia abundant, slender to falcate, 3-5 –septate, apical cell curved and tapering, basa; cell foot-shaped. Microconidia abundant, napiform, oval, pyriform and citriform, 0-1-septate, may be clustered in false heads. Chlamydospores found singly or in chains

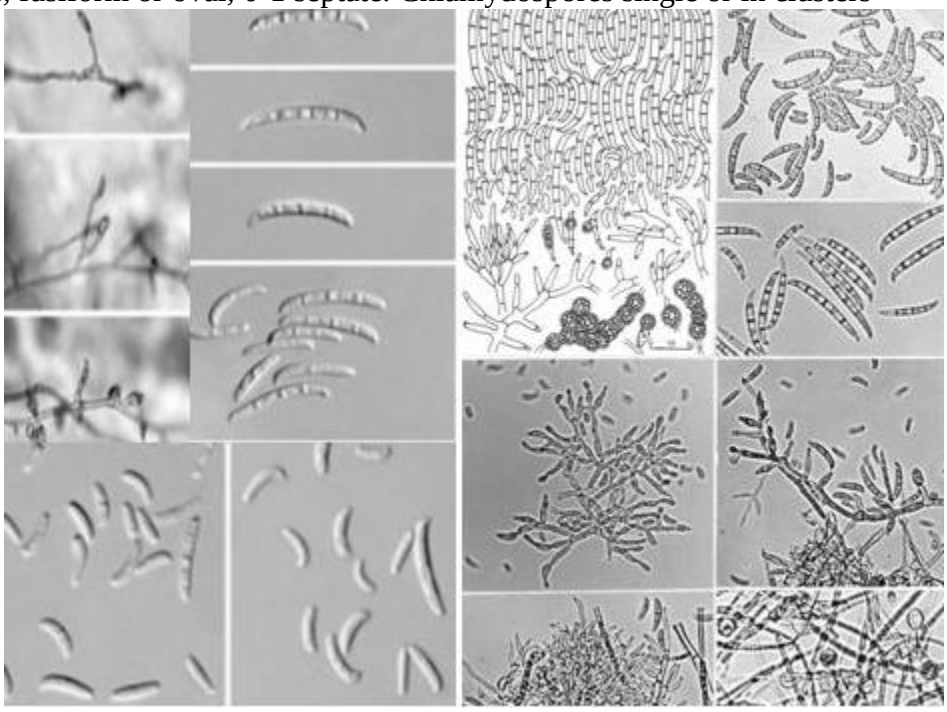


www.invasive.orgwww.andrewmccullagh.com draaf.lorraine.agriculture.gouv.fr, en.engormix.com

16.*Fusarium udum* **E.J. Butler, Mem. Dept. Agric. India: 54 (1910)**

≡Fusarium oxysporum f.sp. udum (E.J. Butler) W.C. Snyder & H.N. Hansen, American Journal of Botany 27: 66 (1940) [MB#509372]
=Fusarium uncinatum Wollenw., Annales Mycologici 15 (1-2): 54 (1917)

Colonies form white mycelium with pink to purple pigments in the agar, pink to salmon sporodochia. Macroconidia abundant in sporodochia, straight to fulcate, thin-walled, 1-5-septate, apical cell curved-hooked, basal cell foot-shaped. Microconidia sparse, fusiform or oval, 0-1 septate. Chlamydospores single or in clusters

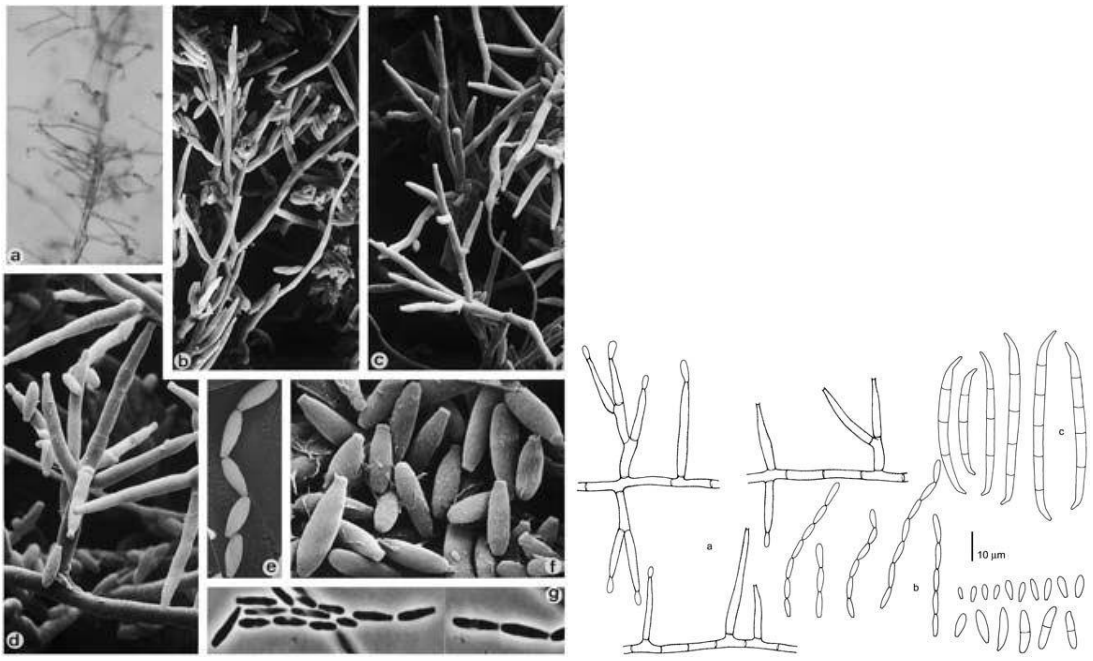


John F. Leslie and Brett A. Summerell , G. Hagedorn, M. Burhenne & H. I. Nirenberg

17.*Fusarium verticillioides* **(Sacc.) Nirenberg, Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 169: 26 (1976)**

≡Oospora verticillioides Sacc., Fung. Ital.: fig. 789 (1881)
≡Alysidium verticillioides (Sacc.) Kuntze, Revisio generum plantarum 3: 442 (1898)
≡Alysidium verticilliodes (Sacc.) Kuntze (1898) =Fusarium moniliforme J. Sheld., Annual Report of the Nebraska Agricultural Experimental Station 17: 23 (1904)
=Fusarium celosiae Abe, Mem. Coll. Agric. Kyoto Univ.: 51-64 (1928)
=Oospora cephalosporioides Luchetti & Favilli, Ann. Fac. Agrar. R. Univ. Pisa N.S.: 399 (1938)

Colonies produce white mycelium, violete pigmenta with age. Macroconidia rare, in pale orange sporodochia, long. Slender,thin-walled, 3-5-septate, apical cell curved and pointed, basal cell notched to foot-shaped. Microconidia, monophilides abundant on the aerial mycelium, club-shaped, 0-septate. Chlamydospores absent



Mycobank

Reports:

Easa (1974) isolated *Fusarium* sp. from skin of mirror carp showing no pathological alterations.

Burns *et al.* (1979) isolated *Fusarium* sp. from cuticular lesions on Malaysian fresh water *prawn Macrobrachium rosen bergii*

Bain and Egusa (1981) studied the histopathology of black gill disease caused by *Fusarium solani* infection in the Kuruma prawn and *Penaeus iaponicus*. They found that the inflammatory responses of *P. japonicus* against *Fusarium solani* were haemocytic infiltration. haemocytic encapsulation, melanization and collagen-like fiber deposition. These responses were more pronounced in lesions in the exoskeleton than in the gill lamellae.

Johnson (1983) reported that the most common moulds affecting adult shrimps was Fusarium. Fusarium may be identified by the presence of conal-shaped macroconidia**.**

Pillai and Freitas (1983) recorded that in year 1977, a case of mass mortality of *Tilapia mossambica* was diagnosed as caused by pathogenic fungus of the Fusarium genus.

Bohm and Fuhrmann (1984) isolated different types of moulds and yeasts from skin and gill lesions in different fresh water fish (rainbow trout, tench, carp, eel and others). Mucor and Fusarium were isolated from carp which had clinically swollen gills.

Hose *et al.* (1984) studied the pathogenesis of *Fusarium solani* in the California brown shrimp, *Penaeus californiensis. F. solani* infections were established in

artificially wounded and infected juveniles and adults of *P. californiensis* as compared with similar control groups, which were wounded but not artificially infected. The progress of *F. solani* infection in 15 g cultured juveniles of *P. californiensis* was followed by gross inspection, where lesions were visible at wound areas on the gills, cuticle, abdominal pleural, and lesions extended into body musculature in the form of burn spots. *F. solani* infections were produced with a success rate of 100% within 14 days post-infection.

Alderman and Polglase (1985) reported *Fusarium tabacinum* (Beyma) Gams, as a gillparasite in the crayfish, Austropotamobius pallipes Lereboullet

Journal of Fish Diseases 1985, **8**, 249–252

SHORT COMMUNICATION

Fusarium tabacinum (Beyma) Gams. as a gill parasite in the crayfish, *Austropotamobius pallipes* Lereboullet

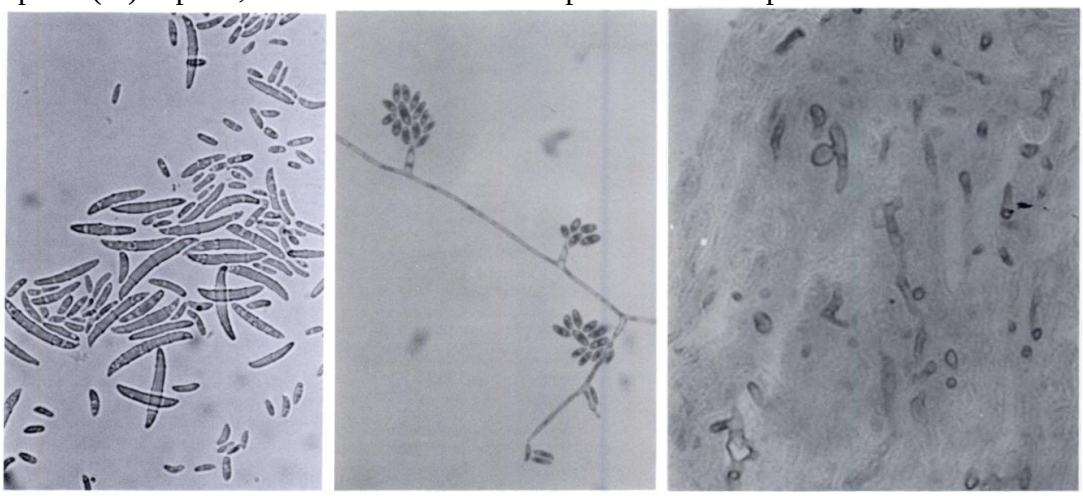
D. J. ALDERMAN *Ministry of Agriculture, Fisheries & Food, Directorate of Fisheries Research, Fish Diseases Laboratory, Weymouth, Dorset, England*

JANE L. POLGLASE *Institute of Aquaculture, University of Stirling, Stirling, Scotland*

Interest in the intensive culture of crustacea in recent years has led to the recognition of an increasing number of cases of fungal disease in these animals. Among these are diseases which have become known as 'burn spot disease' and 'black gill disease'. The large majority of such cases reported from marine crustacea such as the lobsters, *Homarus gammarus* and *H. americanus*, and pink shrimp, *Penaeus duorarum*, (Fischer, Nilsen, Steenbergen & Lightner 1978; Lightner & Fontaine 1975; Alderman 1981) and the Kuruma prawn, *Penaeus japonicus*, (Egusa & Ueda 1972) have been attributed to an infection by *Fusarium* sp. Where specific identification has been made, the species involved has, in the marine environment, been shown to be *F. solani* (Mart.) Sacc. However, the earliest cases of this type were reported from freshwater crustacea by Happich (1900, in Mann & Pieplow 1938) and by Mann & Pieplow (1938). The latter, working with two crayfish species and the mitten crab, described three different fungi as being responsible for exoskeletal infections. These were *Didymaria cambari* from *Cambarus affinis*, *Ramularia astaci* from *Astacus astacus* and *Septocylindrium eriocheir* from *Eriocheir sinensis*. More recent cases of infected crayfish gills have been reported by Vey (1978) and attributed to unidentified *Fusarium* species. This note describes the gross and microscopic pathology of a case of 'black gill disease' in *Austropotamobius pallipes* Lereboullet, the white-footed crayfish, native to British fresh waters, which was caused by *Fusarium tabacinum* (Beyma) Gams.

The animal concerned was being held in aerated running fresh water in a laboratory tank at 18°C and was first noted in an immediate post-moult state. The specimen had been caught in the River Ebble (Wiltshire, England) 12 weeks earlier and was a 6–7 year old adult of 85 mm body length. Recovery from moult was excessively prolonged so that full hardening of the exoskeleton took 20 days. The posterior gills on both sides were displaced to the outside of the carapace, presumably as a result of a faulty moult. On the surface and throughout the body of the displaced gill filaments were numerous small (2–4 mm diameter) dark-brown encapsulations (Fig. 1). The animal was transferred to a static isolation tank at 18°C and kept under observation. Over the following few weeks, the general condition of the animal slowly deteriorated and it became increasingly overgrown by epibionts, until it died at 27 days. During this period additional areas of

Hatai et al. 1986) reported a case of infection by Fusarium oxysporum that occurred on a farm in Mie Prefecture in May 1984 among juvenile “ned sea bream” imported from Hong Kong. Fish dying from the epizootic measured 5.2-6.4 cm in length and 2.7-7.7 g in weight. In almost all cases, no external signs were observed. Detailed examinations were made on five moribund speci- mens. Microbial examination showed that one of the five was infected with a fungus, whereas four were infected with the bacterium Edwardsiella tarda. Kidneys of the fish infected with the fungus were remarkably swollen and discolored. The fungus was isolated by incubating a piece of kidney on Sabouraud dextrose agar (SA agar) at 25 C, and a pure culture was obtained. Fungal colonies were subcultured onto SA agar and identified as Fusarium oxysporum Schlecht. The following are the outstanding characters of the fungus. Colonies were fast growing, floccose, and white on cream with a purple or violet tinge. Microconidia were abundant and borne on short, simple, lateral monophialides or from sparsely branched conidiophores, ellipsoidal or slightly curved, generally one-celled, and produced only in false heads. Macroconidia were slightly sickle-shaped, up to 3(-4)-septate, and with an attenuated apical cell and a pedicellate basal cell.



Microconidia and macroconidia of *Fusarium oxysporurn* NVZC 8401 isolated from the kidney of a red sea bream. x 400. Microconidia of Fusarium oxysporum NVZC 8401 (from a red sea bream) produced on the tip of short monophialides. Cotton blue stain, x400. Section of kidney of red sea bream with mycotic infection. Note the hyphae.**Hatai *et al.* (1986)**

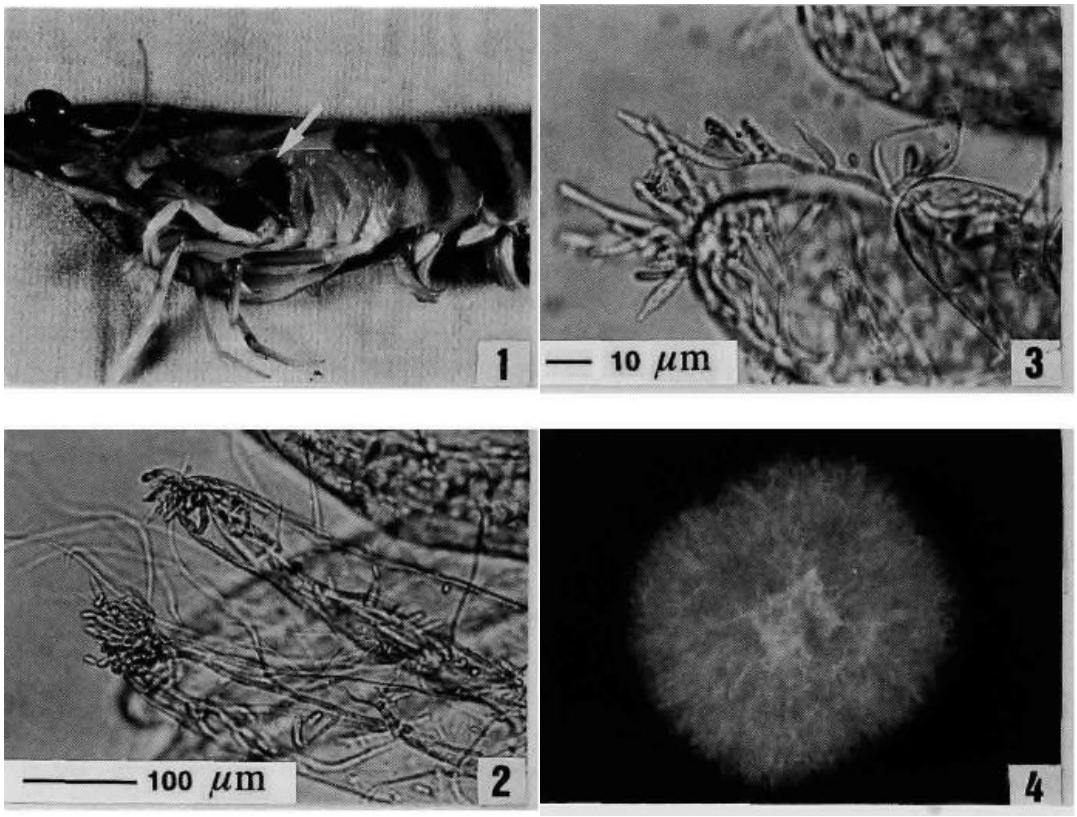
Muhvich *et al.* (1989) reported the occurrence of fatal fusariosis in baby bonnethead sharks (Sphyrna tiburo) born at the National Aquarium, Baltimore, Maryland, . An atypical strain of Fusarium solani was cultured from the tissues of two of the infected sharks following postmortem examination. Histopathology revealed an apparent predilection of the fungus for hyaline cartilage. Invasion of the cartilage resulted in hyphae with a distorted morphology. In slide culture the fungus displayed the unusual characteristic of terminal chlamydoconidium generation on macroconidia; this may be of some taxonomic significance.

Khalil *et al.* (1990) isolated *Fusarium* sp. from *Oreochromis nilotica* and *Bagrus bayad* as well as from water from which the two species were cought. *Fusarium* sp. were isolated from gills, skin, fins, livers, kidneys and intestines.

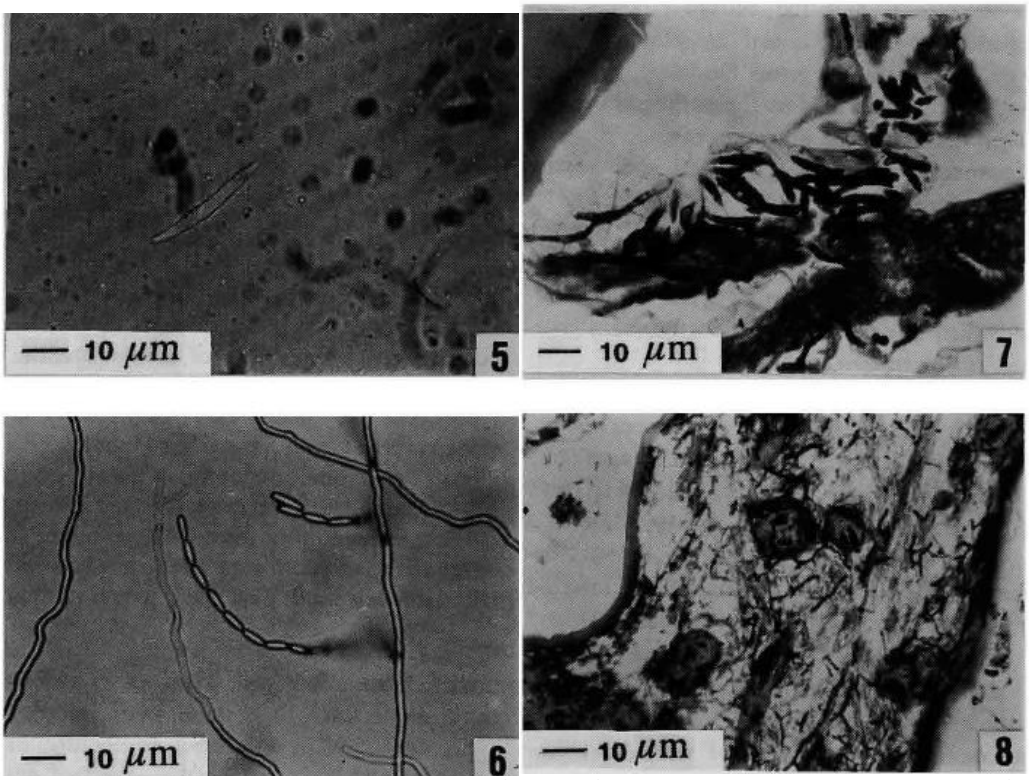
Marzouk *et al.* (1990) isolated different species of moulds from lesions on fins and skin of Tilapia and cat fish. One of them was *Fusarium* sp. , 2 isolates from Tilapia and one isolate from cat fish were recorded.

Rezika (1991) isolated *Fusarium* sp. from integumentry lesions in both *tilapia nilotica* (6 isolates). Fusarium was believed to cause integumentry lesions and was identified as *F. solani*.

Rhoobunjongde *et al.* (1991) isolated *Fusarium moniliforme* from gill lesions of *kuruma prawn, Penaeus japonicus* , with black gill disease at a private farm in Okinawa Prefecture (Japan) in 1989. The colonies of the fungus cultured on upper surface of potato dextrose agar were floccose, creamy white, undersurface a lavender to violet, but did not grow on mycobiotic agar containing cycloheximide. The present report describes the first case of *F. moniliforme* infection in crustacea. An experimental infection using kuruma prawn was made by intramuscular injection with the conidia of F. moniliforme NJM 8995. For comparison, Fusarium solani NJM 8996 isolated also from a kuruma prawn with black gill disease in Okinawa Prefecture in 1989, was used as a reference. The clinical signs and path-ological findings of the disease caused by the two species of the fungi were similar. Identification of the fungi isolated from the lesions was based principally upon the cultural characteristics. The use of a media lacking cycloheximide is recommended for the isolation of F. moniliforme



1. Gross appearance of the gills of a naturally infected kuruma prawn Penaeus japonicus. Note: necrotic tissue fused to a stone-like mass (arrow). 2. Fusarium moniliforme in the gills of a naturally infected kuruma prawn showing hyphae growing out from the tip of gill filament, producing conidiophore and conidia. Fresh mount. 3. The same lesion as Fig. 4. at high magnification. 4. Colony on Potato Dextrose Agar (PDA) 4 days after inoculation. **Rhoobunjongde *et al.* (1991)**



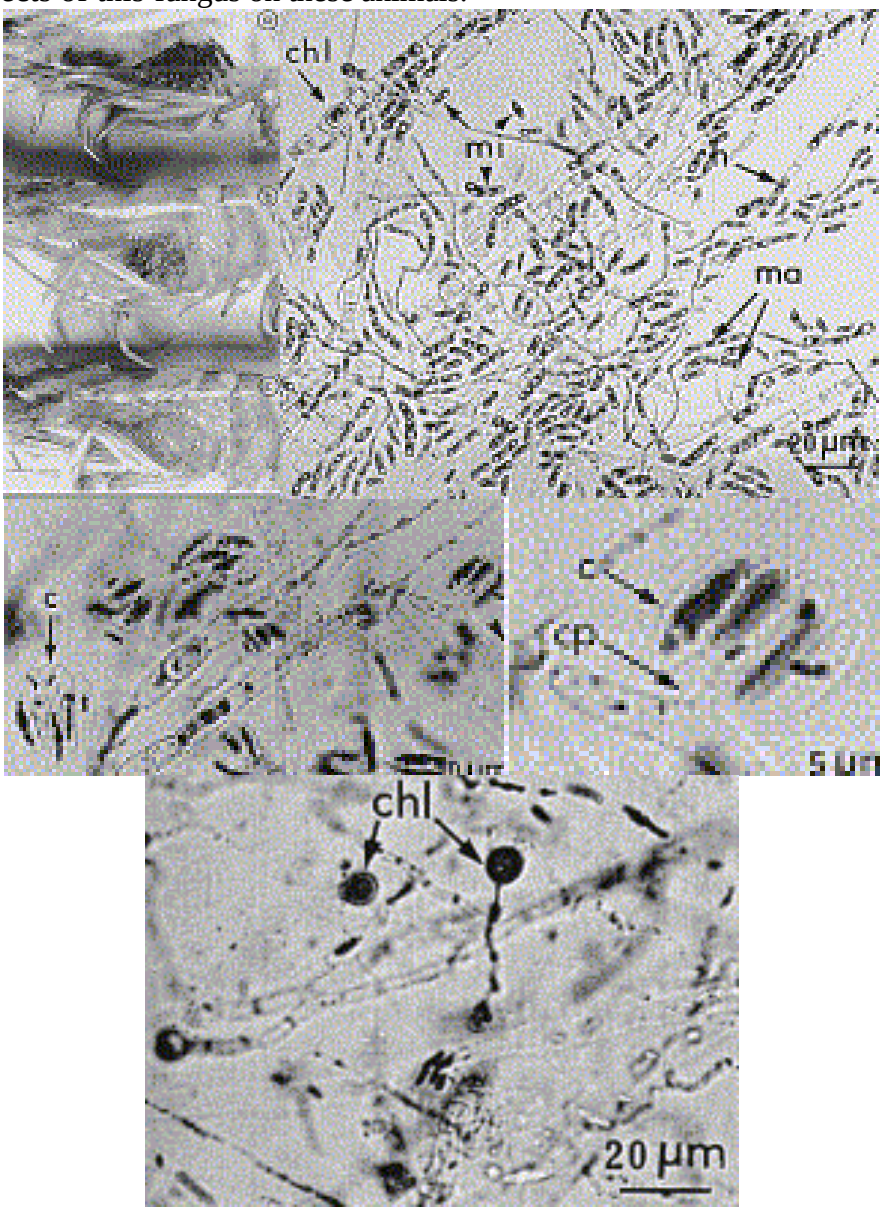
5. Six-celled macroconidia and one-celled microconidia of F. moniliforme producing by aerial hyphae growing out from the tip of gill filament. Fresh mount. 6. Microconidial chains produced from a conidiophore on KCl medium 7 days after inoculation, Fresh mount. 7. Histology of the macroconidia and hyphae within a gill lamella. Grocott.8. Histological section through a blackened gill branch showing the blockage of brachial blood vessels due to encapsulated fungal conidia and hyphae. Grocott. **Rhoobunjongde *et al.* (1991)**

Zhan *et al.* (1993) mentioned that 4 species of Fusarium, *F. solani, F. graminearum, F. tricinctum,* and *F. oxysporum,* were isolated from *Penaeus chinensis*. All species except *F. solani* isolated from prawn were reported for the first time. The infected population was found only in overwinter prawn used for spawning. The artificial infection was caused by three methods, i.e. injection, wounding and dipping. The result showed that *P. chinensis* was highly susceptible to Fusarium. The growth rates of the four species of Fusarium were determined and the sprouting of conidia was also observed.

Crow *et al.* (1995) found that two of five scalloped hammer head sharks (*Sphyrna lewini*) captured May 1987 in Hawaii (USA) developed granulomatous exudative mycotic dermatitis localized in the lateral line canal system. The lesion intitially was noted in the cephalic canals, but over a period of months extended into the lateral canal. *Fusarium solani* and *Vibrio spp*. were isolated from the canal exudate of both shark

Souheil *et al.* (1999) mentioned that a gill-blackening disease in *Penaeus japonicus* was caused by *Fusarium oxysporum*, now considered for the first time to be a parasite of this shrimp. Two different isolates of a strain of *F. oxysporum*, I sub(1) and I sub(2), have been used in experiments. In I sub(2) treated for 3 days with antibiotics, sporulation and growth were inhibited compared to I sub(1) treated for only 3 h. The pathogenic effect of *F. oxysporum* was dose and isolate dependent. With isolate I sub(1), all inoculated animals died within 14 days and their gills were covered in black patches, although they showed no signs of reduced behavioural

activity. In contrast, with isolate I sub(2), all animals died later within 22 days and gill lesions produced were limited but, nevertheless, the behaviour activity of the animals was significantly reduced. Moulting or exposure to low salinities increased animal mortality. In juvenile animals, infection *by F. oxysporum* resulted in a significant decrease in their hypo-osmoregulatory capacity (hypo-OC) in seawater and in their hyper-osmoregulatory in diluted medium. Injections of crude filtrates from shake cultures of the fungus showed that molecules greater than 6-8 kDa caused a significant decrease in the hypo-OC and were likely to be responsible for the toxic effects of this fungus on these animals.

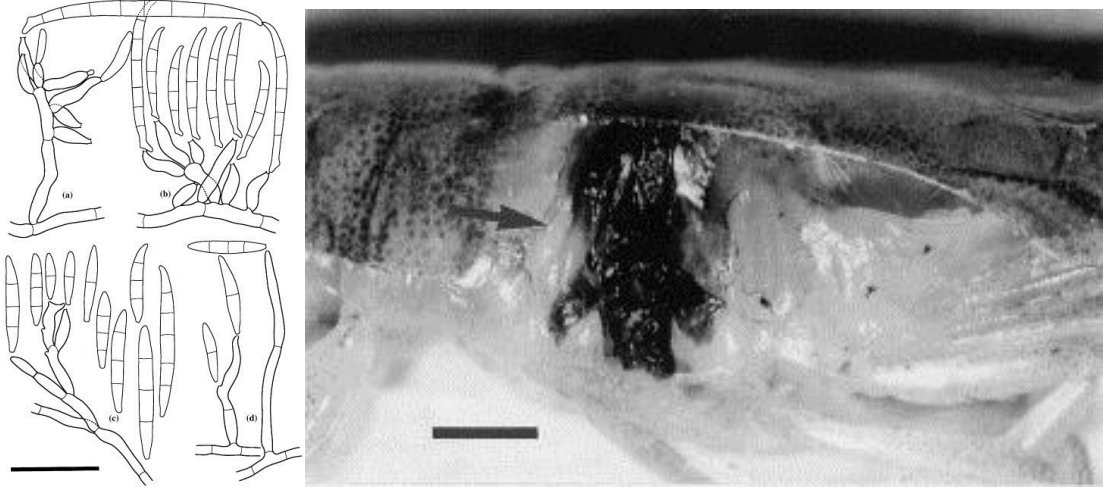


Souheil *et al*. (1999)

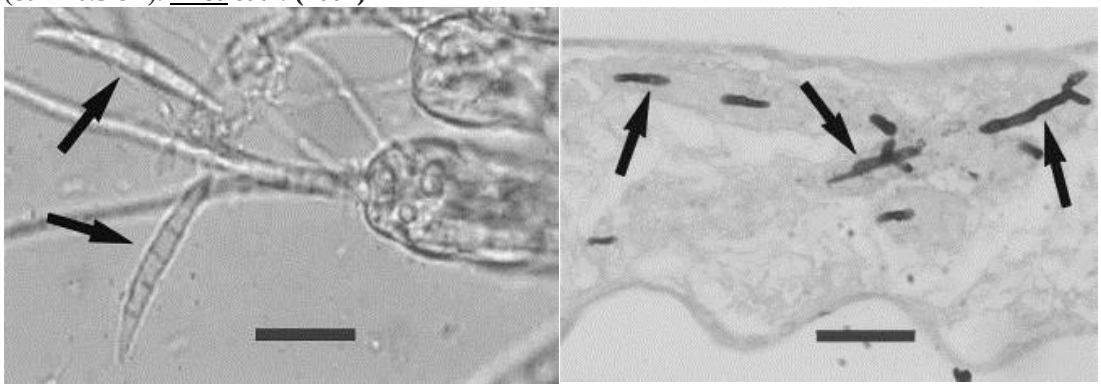
Bisht *et al*. (2000) found that *Fusarium moniliforme* and *F. udum* were natural pathogens of freshwater fish in reservoirs, causing mycosis and high mortality in *Barbus rana, Channa punctatus, Labeo rohita, Mastaceamblus armatus, Mystus tengra, Puntius sophore* and *Wallago attu.* Both the species produced clinical symptoms similar to natural infection in *C. punctatus* and *P. sophore* and caused 40-80% mortality under artificial inoculation. Though 9 other species of extra aquatic fungi belonging to 8 genera of Hyphomycetes were also associated with the diseased

fish in the reservoir, they were unable to infect the test fish. *Fusarium* species parasitize fish more commonly during Summer through rainy season. A temperature above 25°C, coupled with relatively low pH (7.1-7.7) and DO (8.3-9.5 mg l super(-1) encouraged association and infection of these fungi, whereas low temperature during winter (< 20°C) adversely affected their colonization on the fish. Notably mycosis due to water moulds was prevalent during Winter-Spring, while extra-aquatic fungi dominate during Summer through the rainy season, thus posing a continual threat to fish in the reservoirs.

Khoa ***et al.*** **(2004)** isolated **Fusarium incarnatum** from gill lesions of cultured black tiger shrimp, Penaeus monodon, in every crop during 2000-2002 in Nghe An province, Vietnam. Infected shrimps showed typical signs of black gill disease and mortalities about a month prior to harvest. Detailed morphological examinations, as well as molecular phylogenic analyses based on partial nucleotide sequences of ribosomal DNA, were made on the isolates. An artificial infection of kuruma prawn, Penaeus japonicus, using two selected isolates was also conducted and their pathogenicity determined



Microscopic morphology of Fusarium incarnatum isolated from Penaeus monodon (bar = 25 μm). (a) Sporodochial conidiophore forming monophialides verticillately. (b) Sporodochial conidiophores forming slender, cylindrical and slightly curved conidia, with an acuate apical cell and a foot-shaped basal cell from monophialides. (c) Branched aerial conidiophore with polyblastic conidiogenous cells, forming 1-5-septate blastic conidia with a pointed apex and a truncate basal cell. (d) Two unbranched aerial conidiophores bearing mono-polyblastic conidiogenous cells. A P. japonicus artificially infected with F. incarnatum. Note gross appearance of the gills showing a melanized-like mass (arrow) (bar = 0.5 cm). Khoa ***et al.*** **(2004)**



Gill of a P. japonicus artificially infected with Fusarium incarnatum showing fungal hyphae growing out from the tip of the gill filament and producing conidia (arrows) (bar = 30 μm). Fungal hyphae penetrating (arrows) in the gill lamella of a P. japonicus artificially infected with F. incarnatum (bar = 30 μm). Khoa ***et al.*** **(2004)**

Khoa & Hatai (2005) isolated 8 Fusarium strains from five prawns showing black gills at a farm in Kagoshima Prefec ture, Japan in December 2001. On the other hand, no fungi were isolated from five prawns without black gills. Two out of the eight strains were morphologically identified as F. oxysporum and the other six strains as F. solani. Morphological characteristics of F. oxysporum were described and illustrated. The fungus showed pathogenic ity when injected to juvenile kuruma prawns. This is the first case of F. oxysporum infection of kuruma prawn in Japan

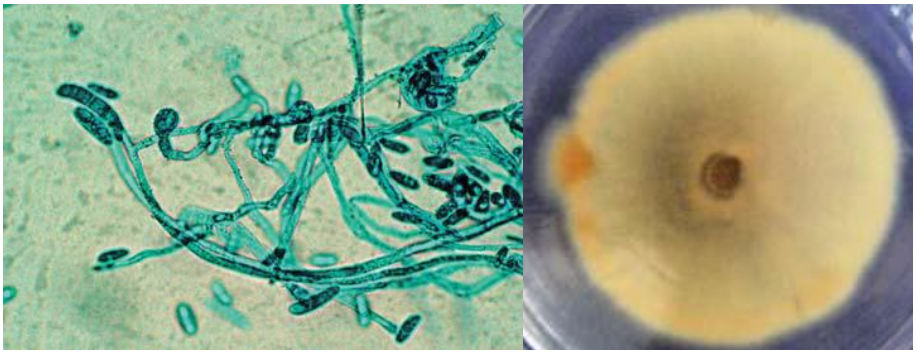
BIAN and EGUSA **(2006)** gave a histopathological description of the black gill disease in Kuruma prawn Penaeus japonicus. The inflammatory responses of P. japonicus against Fusarium solani are haemocytic infiltration, haemocytic encapsulation, melanization and collagen-like fibre deposition. These responses are more pronounced in lesions in the exoskeleton than in the gill lamellae.

Nha *et al.* (2009) mentioned that, based on field observation, lobsters with black gills became weak, lethargic, pale, had difficulty in respiration and were usually observed swimming near the water surface. In some cases, fouling by Balanus sp. and juvenile Pteria sp. were also observed on the shell. Gills became red brown to black. The lesions appeared to eventually destroy the gill filaments in the advanced stage of infection and spread out off the gills. Black spots due to formation of melanotic pigment were always observed in the gills of the infected lobsters. Wet mounts of gill lesions showed the presence of invasive fungal mycelia and conidia in all diseased animals. Septate mycelia, filaments and their conidia were clearly observed under a microscope. Ninety seven fungal isolates were recovered from total 97 infected lobsters (100%) with black gills. All the fungal strains recovered from the 97 diseased lobsters had similar character of conidial shapes and colony. Therefore, a strain NTH 01 was selected for further morphological observation in order to identify into species. The microscopic characteristics of the strain NHT 01 was described as follows: Colonies on PDA at 30o C were white to olive yellow or pale yellow to brownish yellow in aged cultures, 73.1 ± 0.8 mm after 7 days of inoculation. Hyphae were septate and hyaline, 2.42 ± 0.41μm in diameter. Conidiophores were elongated and monophialides forming microconidia in the aerial surface. Conidiophores were simple (non-branched) or branched monophialides. Microconidia were abundant, oval or ellipsoid, usually with one-cell, (11.6 ± 2.07 μm) x (3.8 ± 0.8 μm). Macroconidia were produced after 7 days of inoculation, usually abundant, subcylindric or slightly curved, 2 – 4 septates, predominantly 3-septate (24.7±1.9)μm x (5.0±0.6) μm. Chlamydospores were formed on terminally lateral branches or intercalary and occasionally in chains or in pair. The fungus was identifi ed as Fusarium solani. Pathogenicity challenge Ornate rock lobsters artifi cially infected with NTH 01 showed similar clinical signs to naturally infected animals. Cumulative mortality after 14 days were 57.1%, 72.4% and 77.1% in the 3 groups inoculated with conidial concentrations of 8 x 103 , 8 x 104 and 8 x 105 conidia/mL, respectively. Control groups remained healthy, showed no mortality and no fungal elements in the gills during the course of experiment. Re-isolated fungus was morphologically similar to NTH 01.

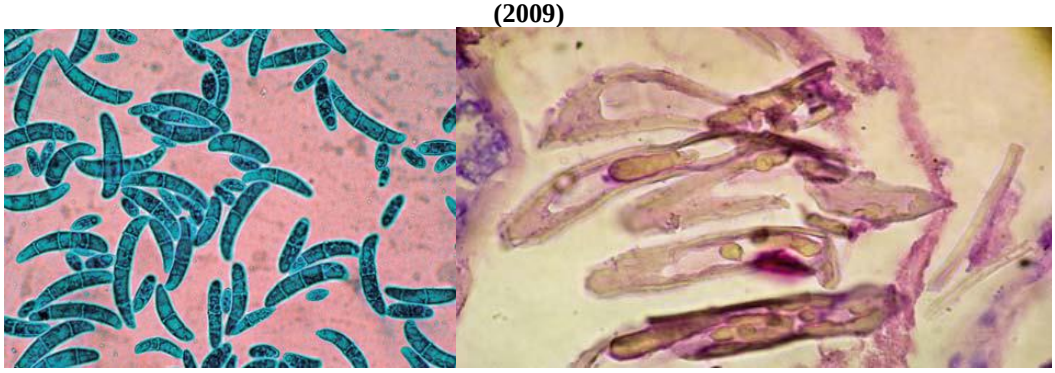
Palmero *et al.* (2009) reported Ornate rock lobster P. ornatus (30 to 220 g in body weight) showing gill discoloration from pale brown to black and/or wounded were collected from cages for examination. Small pieces of the gills were removed from these animals for observation under a light microscope. Species of *Fusarium* were isolated from water samples collected from the Andarax River and coastal sea water

of the Mediterranean in Granada and Almería provinces of southeastern Spain. In total, 18 water samples were analyzed from the Andarax River, and 10 species of *Fusarium* were isolated: *Fusarium anthophilum, F. acuminatum, F. chlamydosporum, F. culmorum, F. equiseti, F. verticillioides, F. oxysporum, F. proliferatum, F. solani*, and *F. sambucinum*. In addition, five species were isolated from 33 sea water samples from the Mediterranean Sea: *F. equiseti, F. verticillioides, F. oxysporum, F. proliferatum*, and *F. solani*. When considering the samples by their origins, 77.8% of the river water samples yielded at least one species of *Fusarium*, with *F. oxysporum* comprising 72.2% of the total isolates. In the case of marine water, 45.5% of the samples yielded at least one species of *Fusarium*, with *F. solani* comprising 36.3% of the total isolates. The pathogenicity of 41 isolates representing nine of the species collected from river and sea water during the study was evaluated on barley, kohlrabi, melon, and tomato. Inoculation with *F. acuminatum, F. chlamydosporum, F. culmorum, F. equiseti, F. verticillioides, F. oxysporum, F. proliferatum F. solani*, and *F. sambucinum*resulted in pre- and post-emergence damping off. Pathogenicity of *Fusarium* isolates did not seem to be related to the origin of the isolates (sea water or fresh water). However, the presence of pathogenic species of *Fusarium* in river water flowing to the sea could indicate long-distance dispersal in natural water environments.

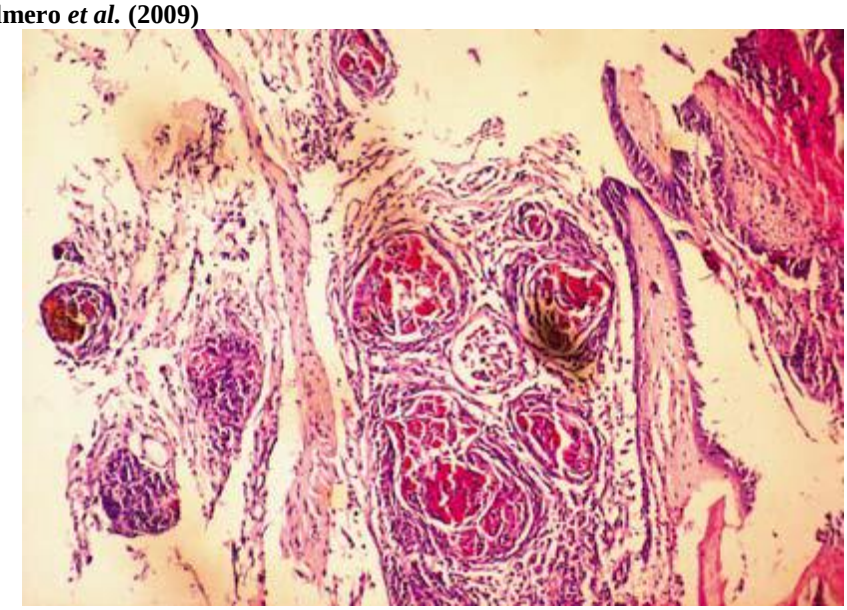
Ornate rock lobster Panulirus ornatus with black gill disease collected from a farm of Khanh Hoa province, Vietnam in 2004. **Palmero *et al.* (2009)**



Fungal hyphae and conidia of an ornate rock lobster naturally infected Fusarium solani (cotton blue stain). Surface of a 7 d-colony of Fusarium solani NHT 01 on PDA at 30C in the dark. **Palmero *et al.* (2009)**



Conidia of Fusarium solani NHT 01 on PDA at 30C in the dark showing 1-4 septates (cotton blue stain). Fungal elements in the degenerative gills (H&E stain) of an artifi cially infected ornate rock lobster. **Palmero *et al.* (2009)**



Fungal hyphae encapsulated by multiple layers of fusiform haematocytes (H&E stain) in the gill tissue of an artifi cially infected lobster **Palmero *et al.* (2009)**

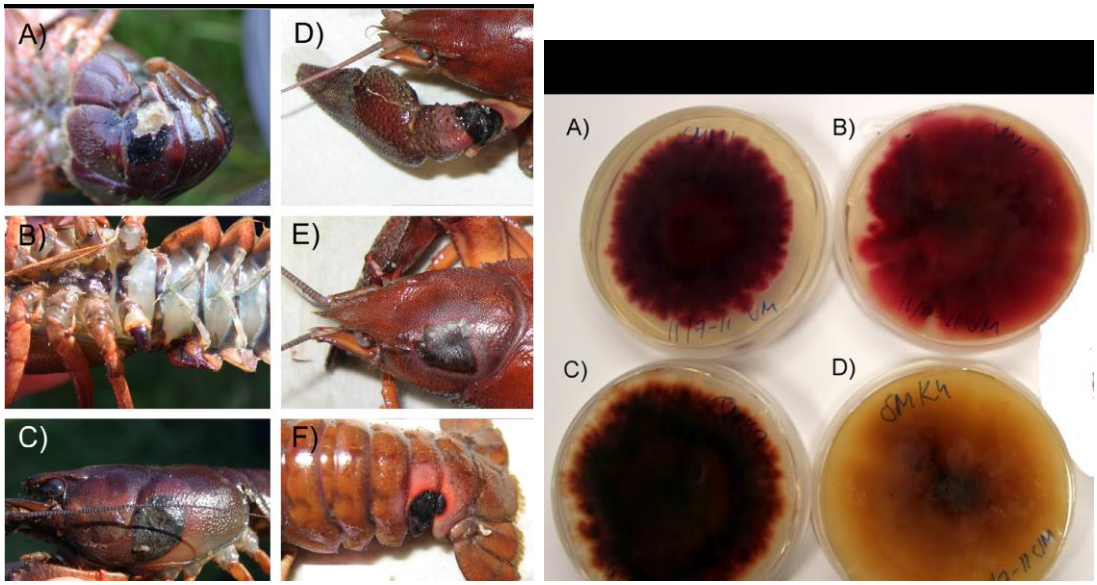
Refai *et al.* (2010) inoculated fish with *Fusarium* species, which caused relatively low mortality rates (40%) via I.P route and (20%) through I.M route. The infected fish exhibited only sluggish movement with detachment of scales. Post mortem examination revealed severe congestion of gills, pale yellow liver and spleenomegally. *Fusarium* species was re-isolated from gills, liver, spleen, and kidneys.

Oreochromis species showing severe congestion of gills. **Refai *et al.*, 2010**

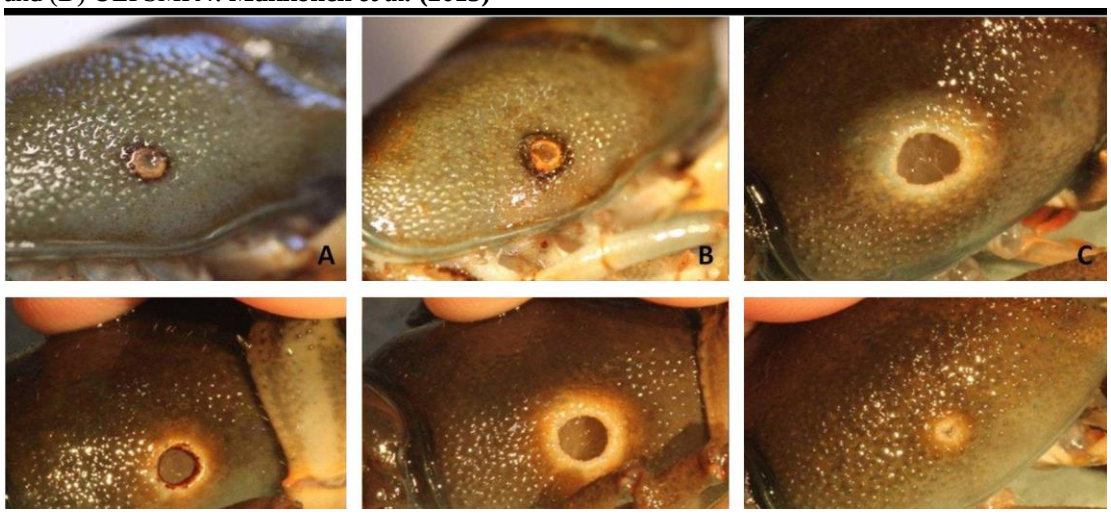
Oreochromis species showing severe enlargement of spleen. **Refai *et al.*, 2010**

Makkonen *et al.* (2013) isolated several Fusarium spp. from Estonian noble crayfish (A. astacus) populations suffering from burn spot disease syndrom The fungi were identified fungi directly from melanised cuticle by their ITS sequences. Then Fusarium spp. was isolated from melanised spots of crayfish showing burn spot disease symptoms, such as melanisation and shell erosion, from two different crayfish populations and watercourses in Estonia. The isolates were then identified based on ITS and EF1 a-gene sequences. Isolates of Fusarium spp. taken from two separate Estonian noble crayfish populations were used in infection studies. Koch postulates confirmed that the studied agent was causing burn spot disease symptoms including shell erosion in the noble crayfish, which were significantly more severe after molts. After the infection period, an identical Fusarium spp. was re-isolated from carapace

lesion s and was thus shown to be the disease agent causing burn spot disease syndrome and shell erosion in noble crayfish. Based on GenBank database searches, the isolates causing burn spot disease symptoms were identified as Fusarium avenaceum in mainland Estonia



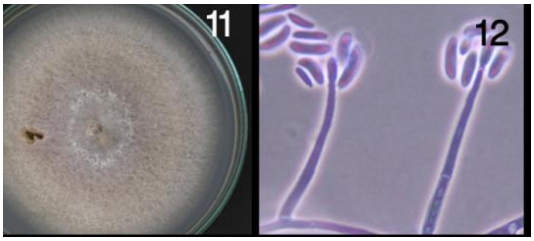
Burn spot disease symptoms in live (A–C) and ethanol fixed (D–F) noble crayfish from Saaremaa, Estonia. Note red coloration around the melanised spot in fixed samples, but not in live crayfish. Isolated and identified Fusarium avenaceum (A–C) and Epicoccum nigrum (D) strains growing on PDA agar. Letters refer to different strains as follows (A) UEFSMM1, (B) UEFSMM2, (C) UEFSMK3 and (D) UEFSMK4. **Makkonen *et al.* (2013)**



Progress of trauma on right lateral side of carapace. Typical symptoms observed during the experiment are used as an example. Figures A to C show trauma site progress in one individual infected using SMM1, after 10, 31 and 52 days, respectively. Figures D to F show final appearance of trauma site in crayfish infected using SMM2, SMK3 and SMK4, respectively, after 52 days. Timing indicated in figures as weeks from initial infection. **Makkonen *et al.* (2013)**

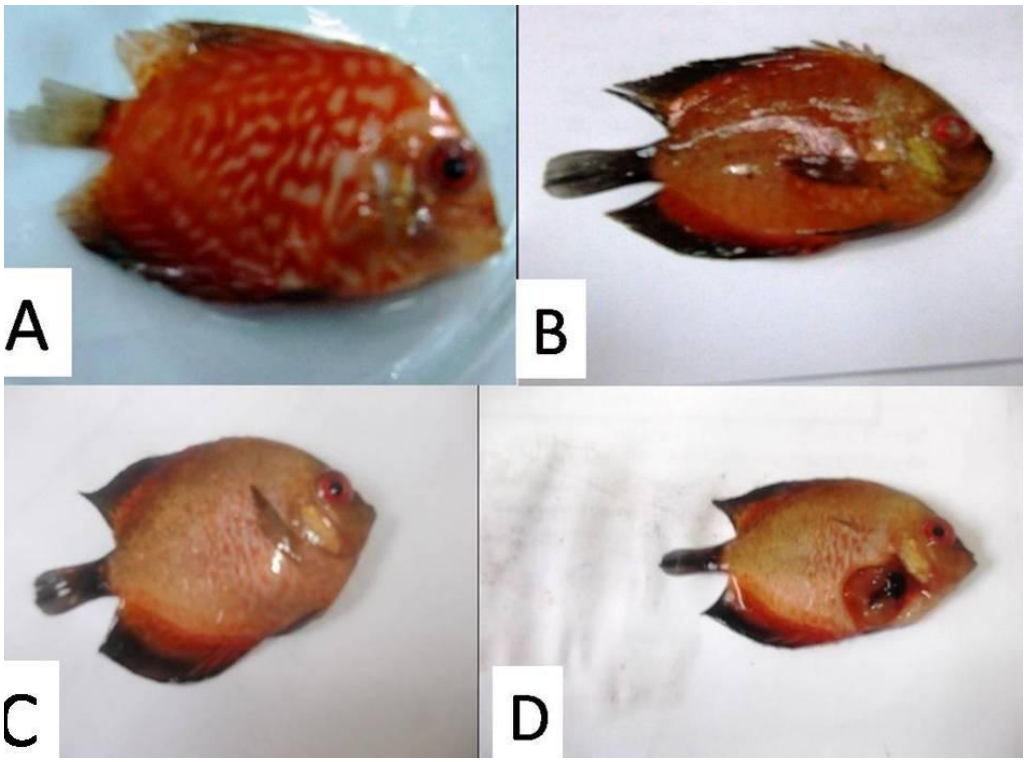
Mohamed et al. (2013) carried out a study on 240 Clarias gariepinus fish collected from The River Nile and El- Ibrahemia canal, Assuit city and the around cities (20 fish /month). The period of study was carried out during October 2011 till the end of September 2012. The clinical finding of naturally infected fish included erosions, ulceration of skin, skin darkening, fin rot, petechial hemorrhage at different parts of

the body, necrotic foci and growth of the fungl hyphe in different sites on the skin and fins. It's colour was from white to brown.Mycological examination of collected samples resulted in isolation of 1200 isolates from 240 fish in presence of 960 isolates as mixed cases. The incidence of moulds isolated from fish were Fusarium solani (210)17.5%

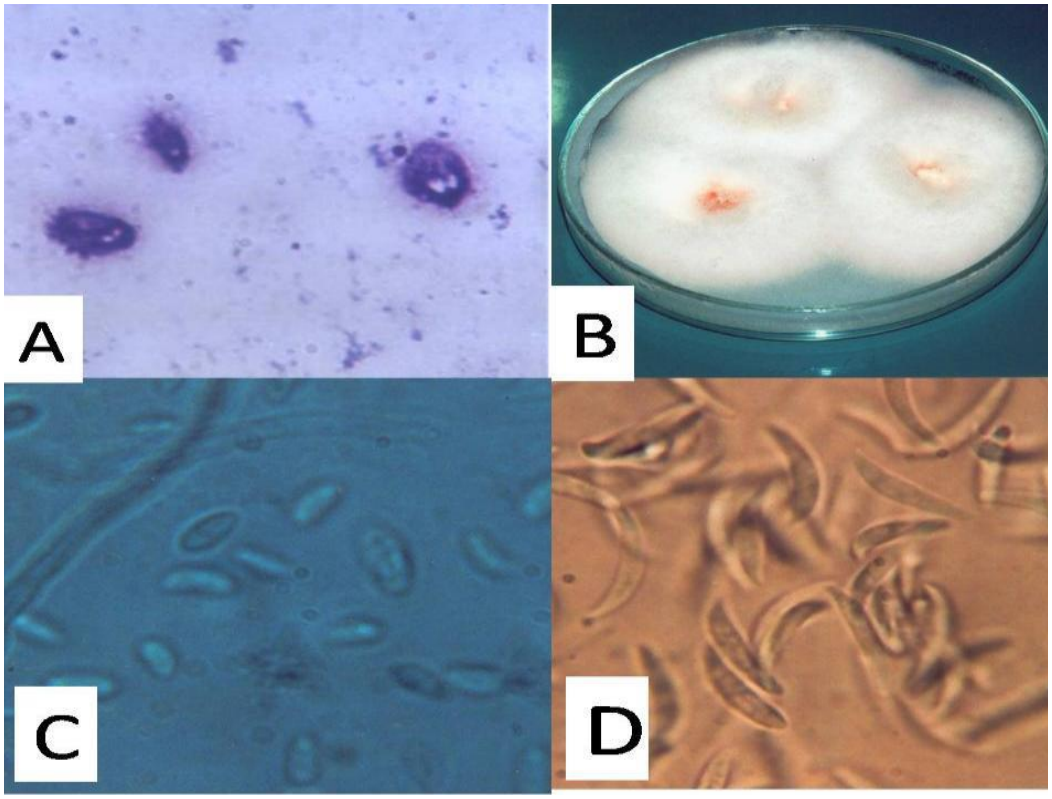


Fusaruim solani on (SDA) with the reverse, Photo. (12): Fusaruim solani with characteristic slender, multicelled conidia **Mohamed et al. (2013)**

Abd El-Ghany *et al.* (2014) collected 30 Symphysodon spp randomly from private freshwater ornamental fish farm in Kalubia Governorate at December 2013. Discus fish suffered from mortality after the onset of anorexia, eye cloudiness, ascites, excessive body mucus, frayed dorsal fin and tail rot. They were subjected to clinical, postmortem, parasitic, bacterial and mycotic examinations to clarify the causative agents of mortality. The recovered fungi were Fusarium solani, F. oxysporum and F. moniliform with the prevalence of 50, 33.34 and 16.66% respectively. The infected *Symphysodon spp* showed ulceration on the skin especially on the head, dorsal fins, tail rot and ascites and severe congestion of internal organs.



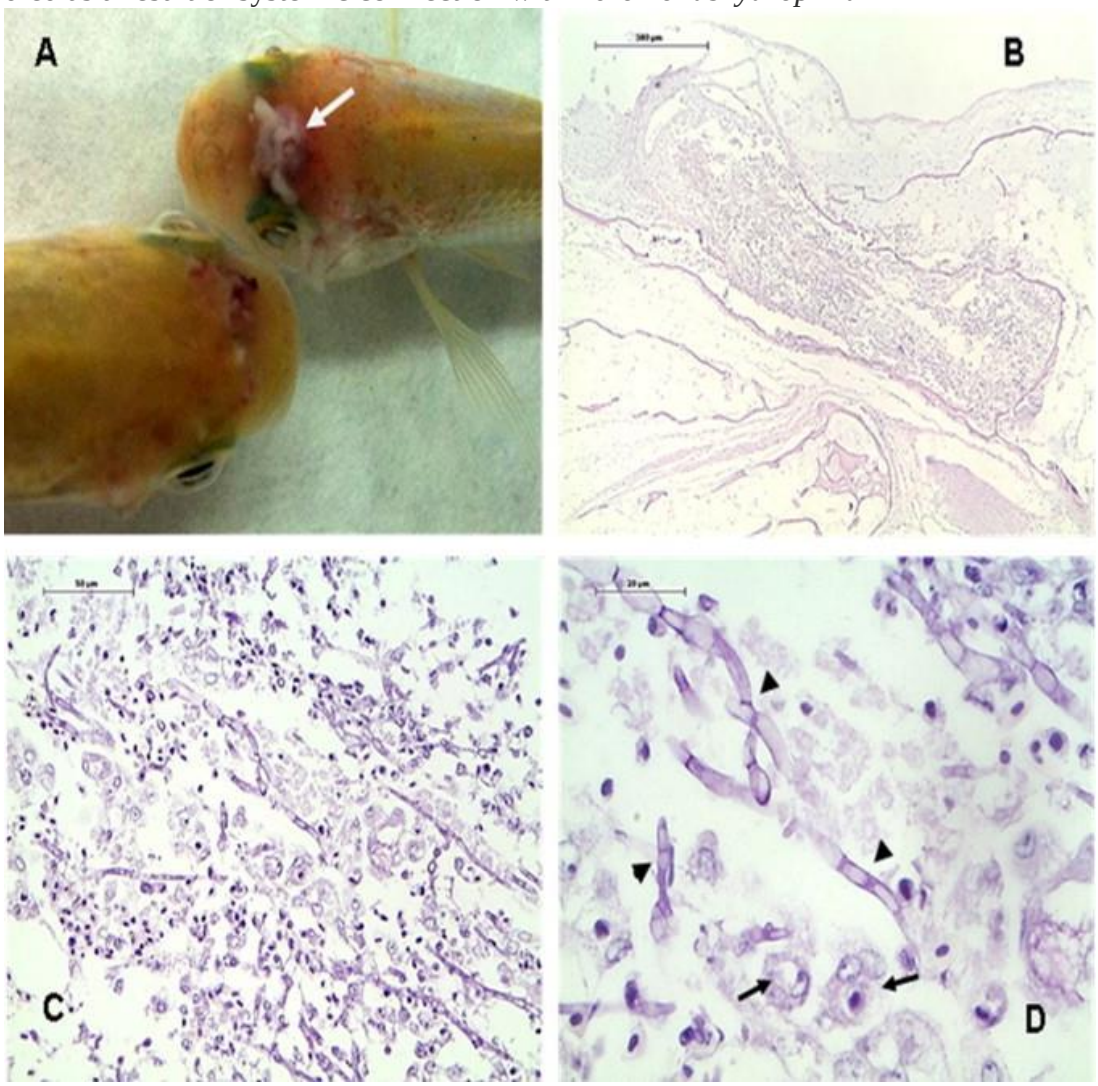
Naturally infected *Symphysodon spp* suffering from ulceration on the skin especially on the head, dorsal fins, tail rot and ascites. Arrow (A, B & C) show severe congestion of internal organs. Arrow (D).



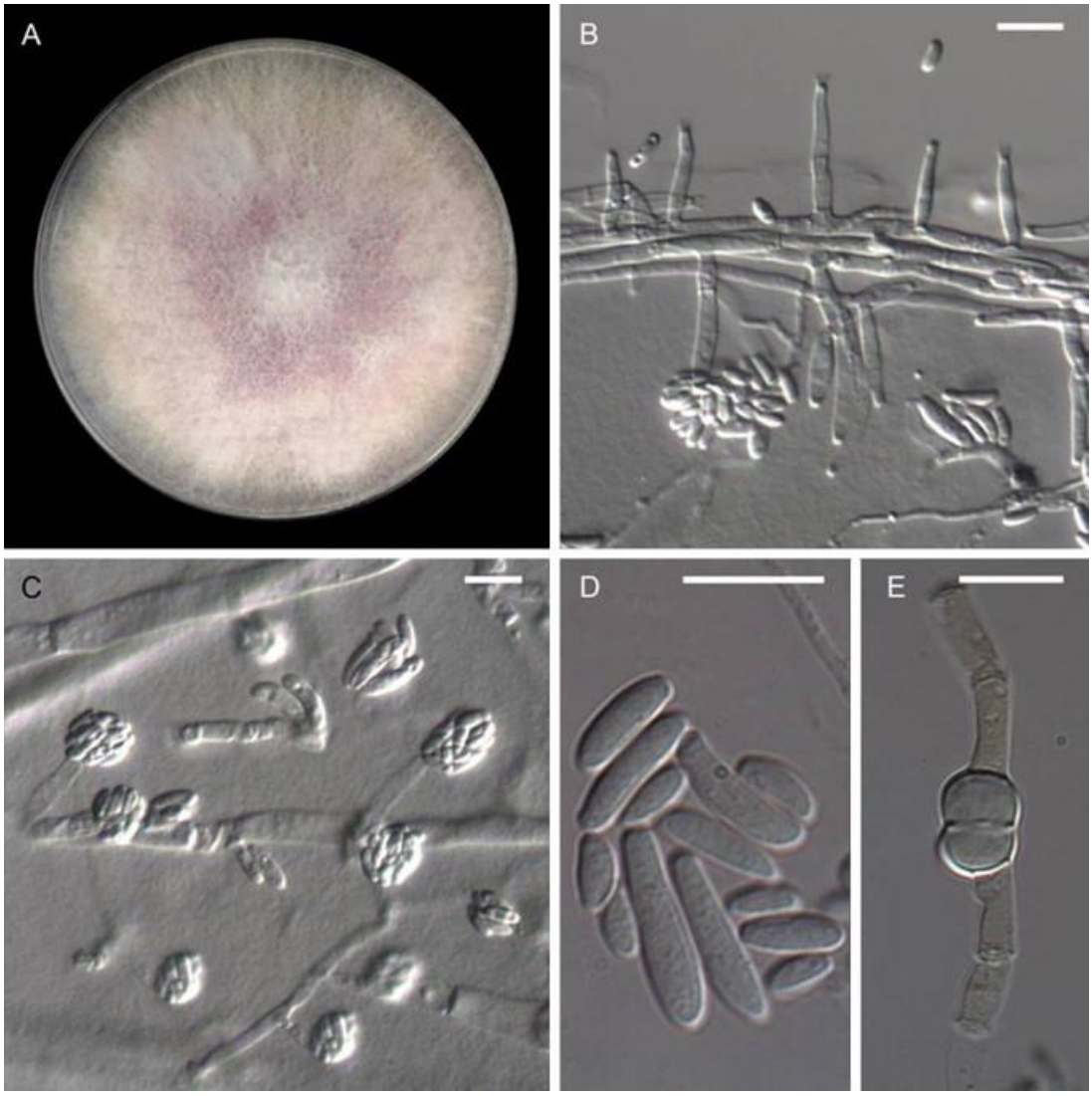
Giemsa stained *Spironucleus* isolated from *Symphysodon spp*. X100, arrow(A). Colonies of *Fusarium solani* on PDA showing aerial, white to cream mycelium in concentric rings (B). Microconidia of *Fusarium solani* after 2-3 days stained with lactophenol cotton blue showing fusiform shape. (X40) arrow(C). Macroconidia of *Fusarium solani* showing slightly curved, more and thin walled (X 40) arrow (D).

Tuxbury *et al.* (2014) housed Captive American horseshoe crabs *Limulus polyphemus* at the National Aquarium presented with a variety of shell and gill lesions over a 3 yr period. Carapace lesions were located on both the dorsal and ventral prosoma and opisthosoma and included multifocal circular areas of tan discoloration, ulcerations, and/or pitting lesions, extending from superficial to full thickness. Gill lesions involved both the book gill cover (operculum) and individual book gill leaflets and included multifocal circular areas of tan discoloration, tan to off-white opaque proliferative lesions, and/or areas of black discoloration. Histopathology revealed fungal hyphae, with variable morphology throughout the thickened and irregular cuticle of the carapace and occasionally penetrating into subcuticular tissues, with associated amebocytic inflammation. Book gill leaflets were infiltrated by fungal hyphae and contained necrotic debris and amebocytes. Thirty-eight of 39 animals (97%) evaluated via histopathological examination had intralesional fungal hyphae. Fungal cultures of carapace and gill lesions were attempted in 26 tissue samples from 15 individuals and were positive in 13 samples (50%), with 10 cultures (77%) yielding identification to genus. *Fusarium* sp. was identified in 8 of the 10 cultures (80%) via culture morphology. The *Fusarium solani* species complex was confirmed in 6 of these 8 (75%) via polymerase chain reaction amplification of 2 different ribosomal-specific sequences of isolated fungal DNA. Ante-mortem systemic and topical treatments were performed on some affected individuals, but no appreciable change in lesions was observed. Mycotic dermatitis and branchitis are serious health issues for captive American horseshoe crabs.

Cutuli *et al.* (2015) reported the presence of *Fusarium oxysporum* in subcutaneous lesions of Nile tilapia (*Oreochromis niloticus*). Histopathologic evaluation revealed granuloma formation with fungal structures, and the identity of the etiological agent was demonstrated by morphological and molecular analyses. Some of the animals died as a result of systemic coinfection with *Aeromonashydrophila*



(A) Gross appearance of the head and skin lesions of fish: soft creamy and yellowish nodules with hyphae and hemorrhagic subcutaneous spot; (B) histological appearance of nodules with low magnification. H&E. 2,5× –Bar – 500 µm; (C) skin granuloma formation composed of numerous foamy macrophages, numerous neutrophils and fungal formations compatible with septate hyphae and conidia. PAS. 10× – Bar – 50 µm. (D) Dermal fungal structures with high magnification: septate hyphae (head arrows) and intracytoplasmatic conidia (arrows) into the macrophages. PAS. 40× – Bar – 20 µm **Cutuli *et al.* (2015)**



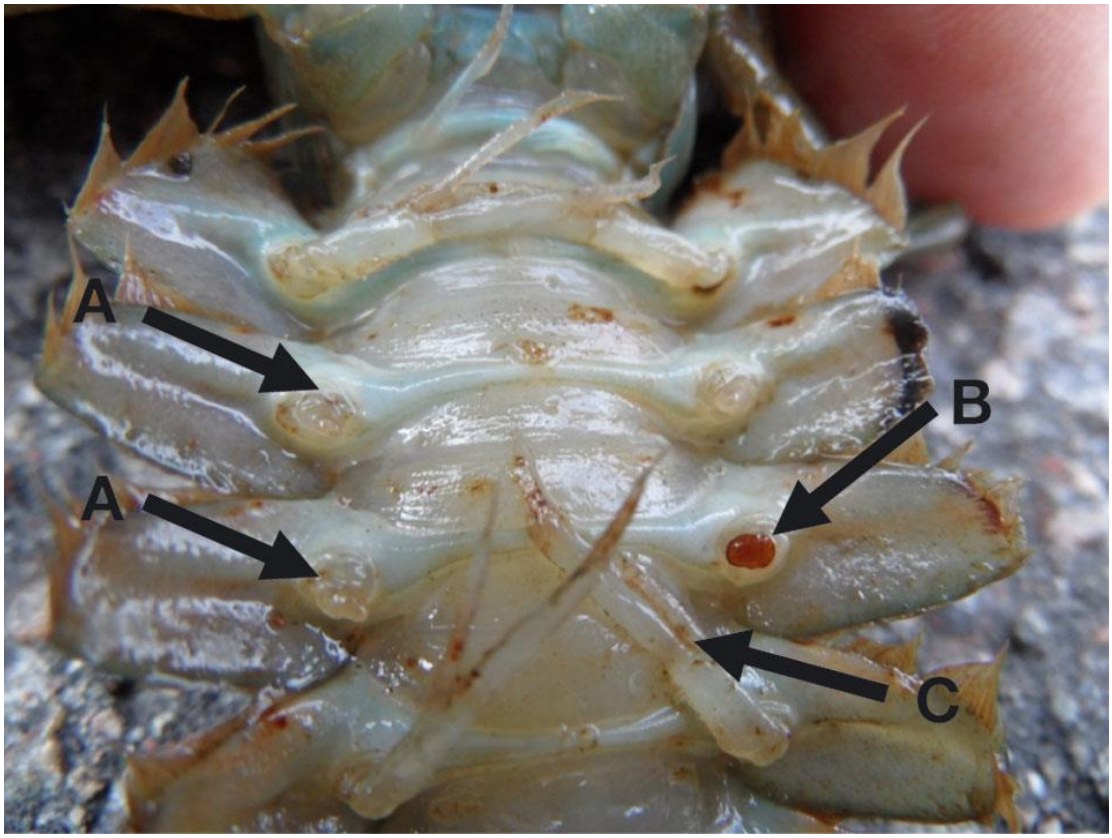
Macroscopic and microscopic features of *Fusarium oxysporum* species complex isolate FMR 13411. (A) Colony on PDA after 14 days at 25 °C. (B) Monophialides. (C) Microconidia arranged in false heads. (D) Mesoconidia and microconidia (E) Intercalary chlamydospores. Scale bar=10 µm. **Cutuli *et al.* (2015)**

Edsman *et al.* (2015) described a novel syndrome in crayfish, eroded swimmeret syndrome (ESS), affecting wild female signal crayfish Pacifastacus leniusculus. ESS causes partial or total swimmeret erosion. We observed ESS only in female signal crayfish larger than 40 mm carapace length, i.e. sexually mature and probably having carried eggs at least once. The eroded swimmerets were melanised, indicating a crayfish immune system response. We isolated Fusarium tricinctum species complex (SC), F. sambucinum SC, Saprolegnia parasitica and S. australisfrom the melanised tissue of the eroded swimmerets. ESS includes chronic Aphanomyces astaci infection and a secondary infection by Fusariumsp. In Sweden, we found female signal crayfish with ESS in 6 out of 11 populations with a prevalence below 1% in lakes with commercially productive signal crayfish populations and higher than 29% in lakes with documented signal crayfish population crashes. In Finland, the ESS prevalence was from 3.4 to 6.2% in a commercially productive population. None of the sampled male signal crayfish showed signs of ESS. A caging experiment indicated that females with at least 1 lost swimmeret carried on average 25% fewer fertilized eggs compared to females with intact swimmerets. ESS could significantly reduce

individual female fecundity and thus could also affect fecundity at the population level. The decline in reproductive success due to ESS could be among the factors contributing to fluctuations in wild signal crayfish populations.

Eroded swimmeret syndrome (ESS) signs (arrows) as observed in Lake Saimaa female signal crayfish *Pacifastacus leniusculus*. (A) swimmerets intact but show melanised spots (stage 1); (B) swimmerets partially or totally eroded with fewintact swimmerets remaining (stage 2) **Edsman *et al.* (2015)**

(C) swimmerets completely eroded with melanised spots remaining (stage 3). ESS stage 0 shows intact swimmerets with no visible signs **Edsman *et al.* (2015)**



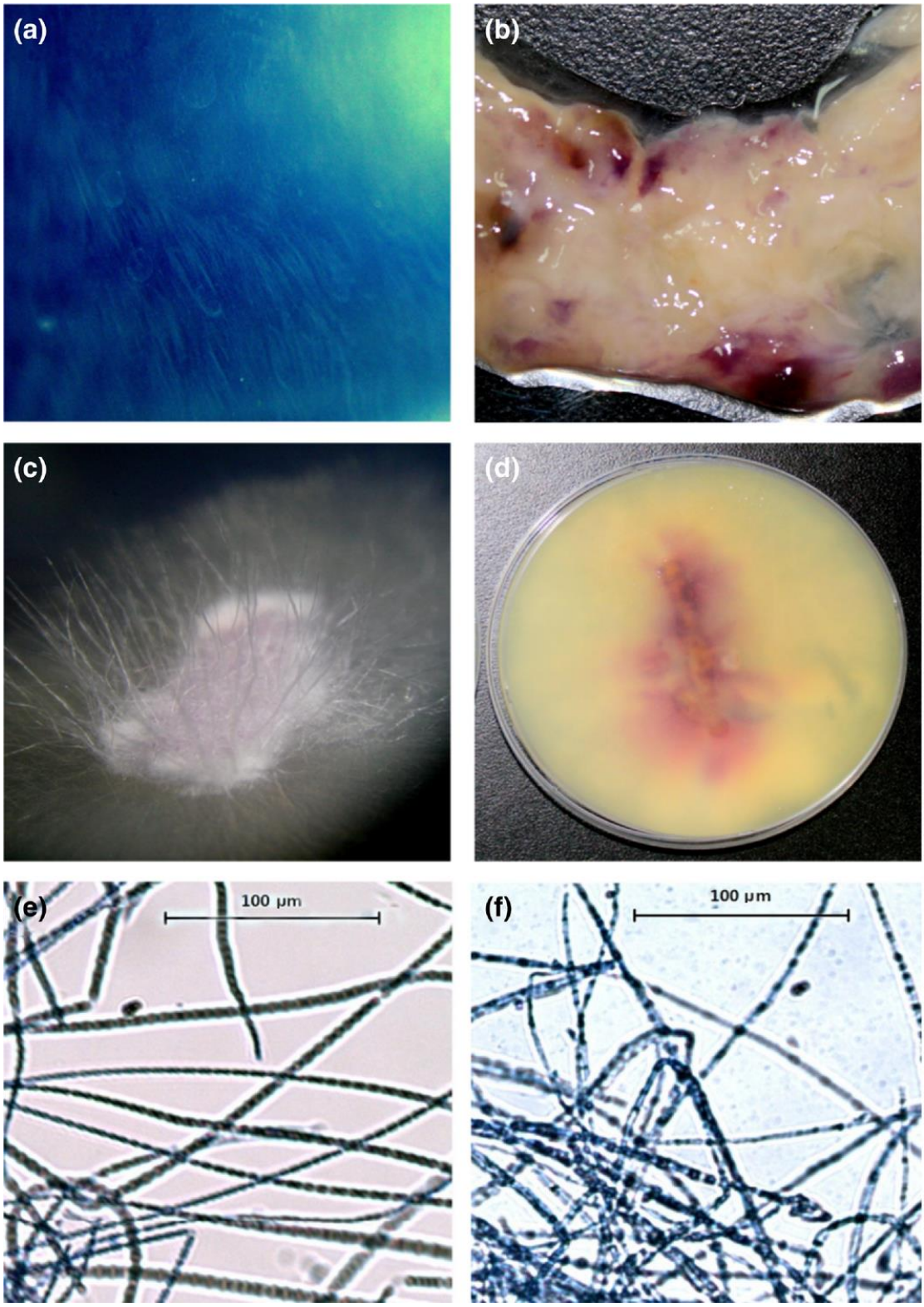
Example of a moulted female signal crayfish *Pacifastacus leniusculus* with eroded swimmeret syndrome (ESS) and eroded swimmeret remains showing scar tissue (A) with the base of one swimmeret still melanised (B). Melanised spots are also visible in the intact swimmerets (C) **Edsman *et al.* (2015)**

Table 1. Results of the isolation and identification of possible disease agents from the melanised and eroded swimmerets of Lake Saimaa female signal crayfish *Pacifastacus leniusculus*. The species identification is based on internal transcribed spacer (ITS) sequence and elongation factor 1 alpha (EF1α) gene blasts in NCBI GenBank (*Saprolegnia* species) and in FUSARIUM-ID (*Fusarium* species) with the GenBank accession numbers provided; na: not analysed

Isolate	Closest species	Match similarity (%)	Accession no.	Species complex	GenBank accession no. EF1α	GenBank accession no. ITS regions
Saimaa1 sampling site						
UEF-LIM5a	*Saprolegnia parasitica*	99.8	AM947031	–	na	KJ920734
UEF-LIM6	*S. australis*	99.9	JN662488	–	na	KJ920736
UEF-LIM5b	*Fusarium negundis*	97.05	NRRL 20682	*Tricinctum*	KJ920740	KJ920735
Saimaa5 sampling site						
UEF-ES6a	*F. tricinctum*	97.30	NRRL 36147	*Tricinctum*	KJ920737	KJ920731
UEF-ES6b	*F. graminearum*	99.84	NRRL 28336	*Sambucinum*	KJ920738	KJ920732
UEF-ES7a	*F. negundis*	97.64	NRRL 20682	*Tricinctum*	KJ920739	KJ920733

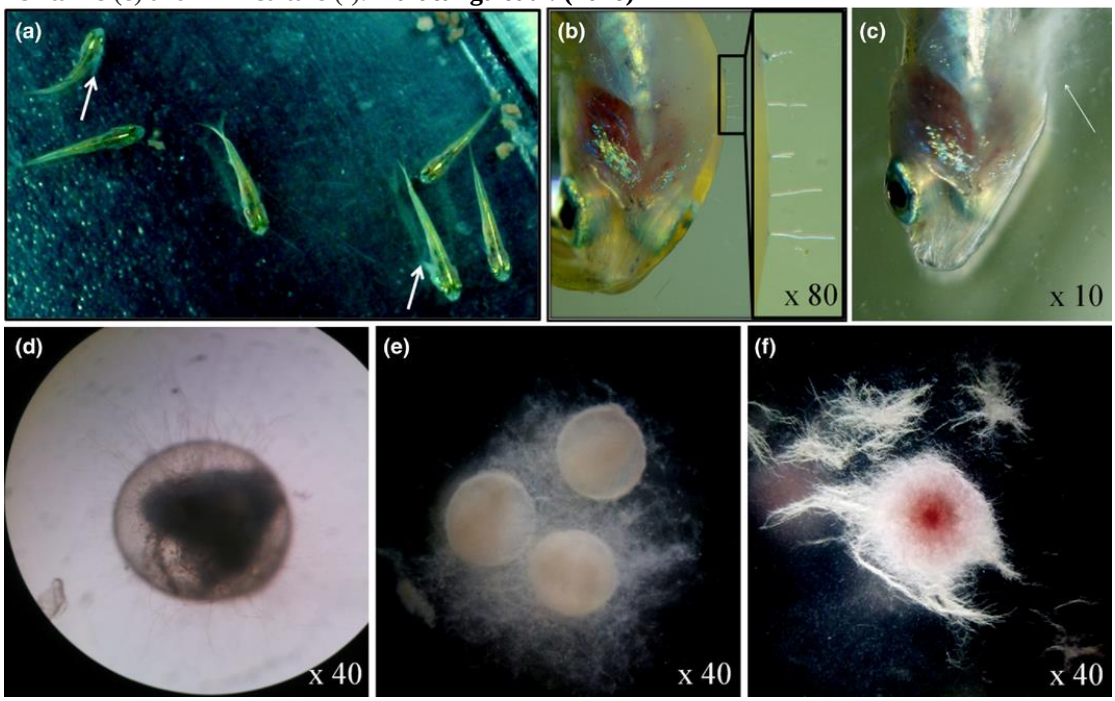
Kulatunga *et al.* (2016) identified F. oxysporum infection from zebrafish (Danio rerio) culturing system in Korea. Initially, a rapid whitish smudge was appeared in the water with the fungal blooming on walls of fish tanks. Microscopic studies were conducted on fungal hyphae, colony pigmentation and chlamydospore formation and the presence of macro- and microspores confirmed that the isolated fungus as F. oxysporum. Furthermore, isolated F. oxysporum was confirmed by internal transcribed spacer sequencing which matched (100%) to nine F. oxysporum sequences available in GenBank. Experimental hypodermic injection of F. oxysporum into adult zebrafish showed the development of fungal mycelium and pathogenicity

similar to signs observed. Histopathologic results revealed a presence of F. oxysporum hyphae in zebrafish muscle. Fusarium oxysporum growth was increased with sea salt in a concentration-dependent manner. This is the first report of FOSC from zebrafish culture system, suggesting it appears as an emerging pathogen, thus posing a significant risk on zebrafish facilities in the world.

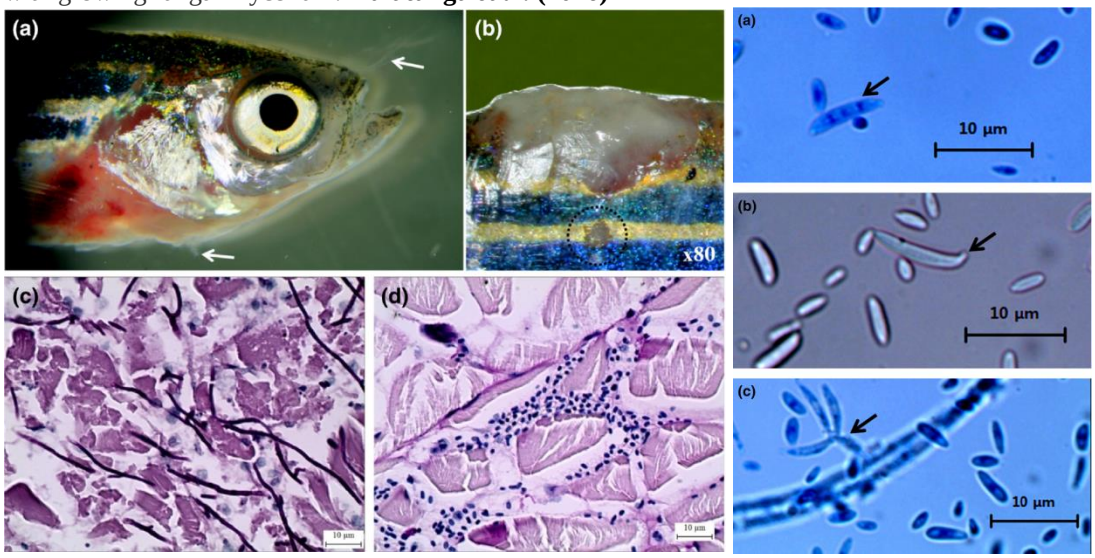


Prominent features of identified *Fusarium oxysporum* in the zebrafish culturing system. (a) Fungal vegetation on tank walls. (b) Detached fungal mass collected from the pipelines bearing characteristic

pigmentation. (c) Isolated fungal mycelium and vegetative hyphae on PDB. (d) Fungi grown on potato dextrose agar showing distinct pigmentation. Microscopic image of fungal filaments collected from fish tanks (e) and PDB culture (f). **Kulatunga *et al.* (2016)**



Gross appearances in adult zebrafish and larvae infected by *Fusarium oxysporum*. (a) Live fish in the aquarium. (b) Dorsal light microscopic view of external fungal vegetation (×80). (c) Ventral light microscopic view of external fungal vegetation (×10). (d–f) Different stages of infected embryos (dead) with growing fungal mycelium. **Kulatunga *et al.* (2016)**



Fusarium oxysporum pathogenicity by experimental infection in zebrafish. Development of infection at 72 hpi on experimentally injected isolated*F. oxysporum* into zebrafish isthmus (a) and dorsal muscle (b). The infection progressing further downward is indicated by the black dotted circle (b) and superficial fungal vegetation (around the mouth and head) indicated by white arrows. Histopathologic indications of *F. oxysporum*infection in zebrafish dorsal muscle tissue at the site of infection showing the invading fungal filaments (c) and inflamed tissues with cell infiltration (d). (×400) Microscopic characteristics of *Fusarium oxysporum*. (a) *F. oxysporum* grown on potato dextrose agar showing the macro- and microspores. Three septate macrospores are indicated by an arrow, while microspores are spread randomly. (b) Macroconidia (spores) of a *F. oxysporum* showing the characteristic foot cell (indicated by arrow). (c) Macrospore bearing conidiophore (indicated by arrow). **Kulatunga *et al.* (**2016**)**

References:

1. Abd El-Ghany, N., A., Nahla R. El-khatib, and Soad S. A. Salama. Causes of mortality in Discus fish (Symphysodon) and trials for treatment. Egy. J. aquac., Vol. 4, No. (2):1-12 (2014)
2. Alderman, D.J., Polglase, J.L., 1985. Fusarium tabacinum (Beyma) Gams, as a gillparasite in the crayfish, Austropotamobius pallipes Lereboullet. J. Fish Dis. 8 (2),249–252.
3. Bain, B. Z. and Egusa, S. (1981): Histopathology of black gill disease caused by *Fusarium solani* (Martins) infection in the Kuruma Prawns, *Penaeus japonicus* Bate. Fish Dis., 4, 195-501.
4. Bisht, D.; Bisht, G.S.; Khulbe, R.D(2000) Fusarium A new threat to fish population in reservoirs of Kumaun India Curr-Sci. 78, (10), 1241-1245
5. Bohm, K.H. and Fuhrmann, H. (1984): Mycological survey of diseased freshwater fish. Bull. Eur. Assoc. Fish. Pathol. 4(2), 26-27.
6. Burns, C.D.; Derrigan, M.E. and Henderson, G.E. (1979): *Fusarium* sp. infection in the freshwater prawn *Macrobrachium henderosonii.* Aquaculture, 16(3), 193-198.
7. Crow, G.L.; Brock, J.A. and Kaiser, S.(1995):*Fusarium solani* fungal infection of the lateral line canal system in captive scalloped hammerhead sharks (*Sphyrna lewini)* in Hawaii J. Wildl. Dis., 31, (4), 562-565.
8. Cutuli MT, Gibello A, Rodriguez-Bertos A, et al. Skin and subcutaneous mycoses in tilapia (*Oreochromis niloticus*) caused by *Fusarium oxysporum in coinfection with Aeromonas hydrophila*. *Medical Mycology Case Reports*. 2015;9:7-11. doi:10.1016/j.mmcr.2015.06.002Easa, M. EI-S (1979):Role of fingi as a cause of gill diseaes in carp of Egyptian fish farm. Ph. D. Thesis, Vet. Akademy (Moscow) USSR.
9. Edsman , L., Per Nyström , Alfred Sandström , Marika Stenberg , Harri Kokko , Vesa Tiitinen , Jenny Makkonen , Japo Jussila. Eroded swimmeret syndrome in female crayfish Pacifastacus leniusculus associated with Aphanomyces astaci and Fusarium spp. Infections. Dis Aquat Org 112: 219–228, 2015
10. Hatai, K., Sabrouh S.; Norihiro Kida, and Shun-ichi Udagawa, *Fusarium oxysporum* in Red Sea Bream *(Pagrus* sp.). *Journal of Wildlife Diseases*, 22(4), 1986, pp. 570-571
11. A visceral mycosis in ayu fry, Pleoglossus altivelis Temminck & Schlegel, caused by a species of Phoma. J. Fish. Dis. 9: 111-116.
12. Hose, J.E. ; Lightner, D.V. ; Redman, R.M. ; and Donald, D.A. (1984): Observations on the pathogenesis of the imperfect fungus, *Fusarium solani*, in the California brown shrimp, *Penaeus californiensis.* J. Invert. Pathol. 44: 292-303.
13. Johnson, P. T. (1983):Viral, bacterial and fungal diseases in the biology of crustacea. chapter 6, pathology. D.E. Bliss (Editor) Academic Press, New York, pp. 1-78.
14. Khalil, A.M.; Ahmed, S.M. and Ahmad, L.S. (1990):Some microbiological studies on two species of Nile fishes.Assiut Vet. Med. J. 23, (46), 88-96.
15. Khoa, L V, Kishio Hatai, T Aoki. Fusarium incarnatum isolated from black tiger shrimp, Penaeus monodon Fabricius, with black gill disease cultured in Vietnam Journal of Fish Diseases 27(9):507-15 · October 2004
16. Khoa L.V. & Hatai K. (2005) First Case of *Fusarium oxysporum* infection in cultured Kuruma prawn *Penaeus japonicus* in Japan. *Fish Pathology* 40,195–196.
17. Kulatunga. D C M , S H S Dananjaya1, B K Park1, C-H Kim2, J Lee3,4 and M De Zoysa. First report of Fusarium oxysporum species complex infection in zebrafish culturing system. Journal of Fish Diseases 2016 doi:10.1111/jfd.12529
18. Makkonen, J., J. Jussila, Margo Hurt, Harri Ilkka Kokko Fusarium avenaceum causes burn spot disease syndrome in noble crayfish (Astacus astacus). Journal of

Invertebrate Pathology · April 2013 DOI: 10.1016/j.jip.2013.03.008 · Source: PubMed
19. Marzouk, M.S.M.; El-Far, F. and Nawal, M.A. (1990): Some investigations on moulds and yeasts associated with tail and fin rot in freshwater fish in Egypt. Alex. J. Vet. Sci., 6.(l), 193-203.
20. MOHAMAD, I. S. MOHAMAD; MOHAMED W. ABD AL-AZEEM; MAHMOUD M. MAHMOUD and AML MOKHTAR M. ABD-EL AAL. MICROBIOLOGY STUDIES ON THE AFFECTIONS OF SKIN IN SHARP TOOTH CATFISH (CLARIAS GARIEPINUS). Assiut Vet. Med. J. Vol. 59 No. 137 April 2013 157
21. Muhvich, A. G.; Reimschuessel, R.; Lipsky, M.M. and Bennett, R. O. (1989):*Fusarium Solani* isolated from newborn bone-thead Sharks, Sphyrnatiburo (L).J. Fish Dis., 12; 57-62.
22. Nha, V.V. , Hoa, D.T. and Khoa, L.V. Black gill disease of cage-cultured ornate rock lobster Panulirus ornatus in central Vietnam caused by Fusarium species. Aquaculture Asia Magazine Volume XIV No. 4, October - December 2009
23. Palmero *et al.* (2009) Palmero D., Iglesias C., de Cara M., Lomas T., Santos M. & Tello J.C. (2009)Species of Fusarium isolated from river and sea water of southeastern Spain and pathogenicity on four plant species.*Plant Diseases* 93, 377–385, C.T.; Freitas, Y.M.(1983): Fungal infection causing mass mortality of freshwater fish *Tilapia mossambica* .Sea food Export. J. 15, (1), 15-17.
24. Refai *et al.* (2010)
25. Rezika, S. (1991):Integumentry mycosis in cultured freshwater fish and shrimps.M.V.Sc. Thesis. Faculity of Vet. Med., Alexandria University.
26. Rhoobunjongde, W.; Hatai, K.; Wada, S. and Kubota, S.S.(1991): *Fusarium moniliforme* (Sheldon) isolated from gills of kuruma prawn *Penaeus japonicus* (Bate) with black gill disease.Bull. Jap. Soc. Sci. Fish. 57, (4), 629-635.
27. Zhan, W.; Yu, K. and Meng, Q. (1993):Studies on pathogen of fungus (Fusarium) disease of prawn (*Penaeus chinensis*) J. Ocean Univ. Qingdao qingdao haiyang daxue xuebao 23, (2), 91-100
28. Souheil, H.; Vey, A.; Thuet, P.; Trilles, J.P.(1999):Pathogenic and toxic effects of *Fusarium oxysporum* (Schlecht.) on survival and osmoregulatory capacity of *Penaeus japonicus* (Bate) Aquaculture , 178, (3-4), 209-224.uxbury *et al.* (2014)
29. Tuxbury, K. A, Gillian C. Shaw, Richard J. Montali, Leigh Ann Clayton, Nicole P. Kwiatkowski, Michael J. Dykstra, Joseph L. Mankowski Fusarium solani species complex associated with carapace lesions and branchitis in captive American horseshoe crabs Limulus polyphemus. DAO 109:223-230 (2014)

.

17. Exophiala

Systemic black yeast infections in fish have been described on many occasions (9). The infections are generally considered to be secondary to metabolic factors or stress of captivity or a consequence of water quality problems, trauma (rough handling or aggression), bacterial disease, or parasites (36, 37). Bacterial or parasitic diseases and toxic or environmental conditions may mimic fungal disease to various extents. Furthermore, mycoses may be masked by overwhelming secondary bacterial infection and therefore remain undiagnosed (9). Captivity may be a contributory factor to reduced immune function.

- **Disseminated infections** by ***Exophiala angulospora*** were identified repeatedly in aquarium-maintained weedy seadragons (*Phyllopteryx taeniolatus*)

- ***Exophiala. aquamarina***, caused infections in leafy seadragons (*Phycodurus eques*). *S*ystemic necrotizing lesions and invasion of blood vessels were consistent features
- **Exophiala salmonis** causes an internal systemic mycosis of marine-reared salmonids of a low prevalence e.g. Atlantic salmon, Salmo salar.
- **E. psychrophila** has been described from rainbow trout, Oncorhynchus mykiss and also in Atlantic salmon in salt water from Norway (Pederson and Langvad, 1989).
- **E. pisciphila** has occurred in Atlantic salmon from Australia (Langdon and McDonald, 1987).

Gross clinical signs

- Infected fish may continue to feed normally, but display erratic swimming movements, which can be followed by whirling behaviour.
- Distension of the abdomen is reported.
- Exophthalmia and cranial cutaneous ulcers are common, although these clinical signs are not considered pathognomonic.
- Internally, an opaque capsule and enlargement of the kidney is characteristic with large, raised, off-white nodules containing variable quantities of hyphae

Light microscopy

- Infected fish attempt to limit vascular invasion, with the
- development of a marked systemic granulomatous response, involving macrophages and multinucleate giant cells, despite the limited number of hyphae in any one location
- Fibrosis and atrophy develop as the hyphae penetrate the kidney tubules and blood vessels, as well as other organs, such as the heart, liver, and spleen, where an acute multi-focal response can be observed.
- An eosinophilic gastritis and enteritis occur within the gut.
- In severe infections, the musculature may be discoloured.
- A cranial location for **E. psycrophila** has been reported for Atlantic salmon following movement of hyphae through the lateral line system. Healing lesions are fibrous in nature,
- the pathology associated with E. psycrophila is similar to that described for E. salmonis.

Control measures and legislation

- Exophiala infection in farmed fish is not generally treated or subject to national legislation.
- Recent work has shown that the secondary metabolite Latrunculin B produced by a marine sponge Negombata magnifica displays potential as an antifungal

agent and could be theoretically developed for use in aquaculture (Devi et al., 2013).

Diagnostic methods –

- A presumptive diagnosis of can be made from gross lesions and the presence of pigmented septate hyphae readily observed in H&E sections.
- Staining sections with periodic acid-Schiff's or Grocott's methenamine silver techniques is also useful for diagnosis (Alderman and Feist, 1985).
- Cultures of Exophiala on Sabouraud's agar are grey, with a darker reverse; abundant spores and colony growth of 5–8 mm occur at 25 ° C, after approximately 14 days.
- Direct ITS1 sequencing and RFLP of PCR-amplified ribosomal genes are published
- Temperature-growth relationships, measured with a continuous temperature gradient incubator, have proven useful for the identification of the four taxa of Exophiala pathogenic on fish (Pederson and Langvad, 1989).

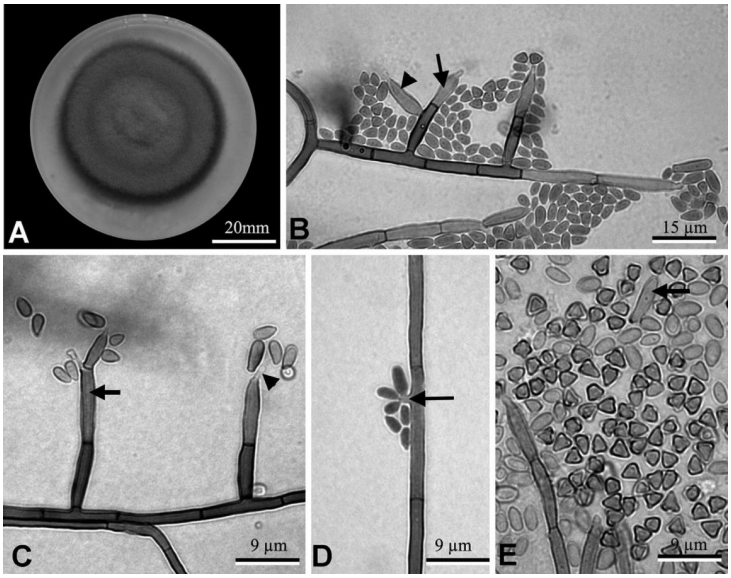
Description of Exophiala species isolated from fish

1.Exophiala angulospora Iwatsu, Udagawa & Takase, Mycotaxon 4: 322. 1991. — MycoBank MB355245

Teleomorph. Capronia coronata Samuels, Trans. Brit. Mycol. Soc. 88: 65. 1967.

Description of CBS 482.92 after 2 wk incubation on MEA, 24 °C.

Colonies restricted, centrally mucous, velvety towards the outside, greyish green to olivaceous black. Germinating cells present, 6–10 × 2.4–4.0 μm. *Hyphae* pale olivaceous, smooth-walled, 1.5–3.0 μm wide. Budding cells present. *Conidiogenous cells* intercalary, lateral and terminal and then 1-celled, flask-shaped, 6–16 × 2.5–3.0 μm; conidia produced from a single short annellated zone per cell. *Conidia* aggregating in slimy heads, 1-celled, smooth- and thin-walled, subhyaline or pale olivaceous, mostly more or less triangular with rounded ends, 2.5–4.0 × 2–3 μm.

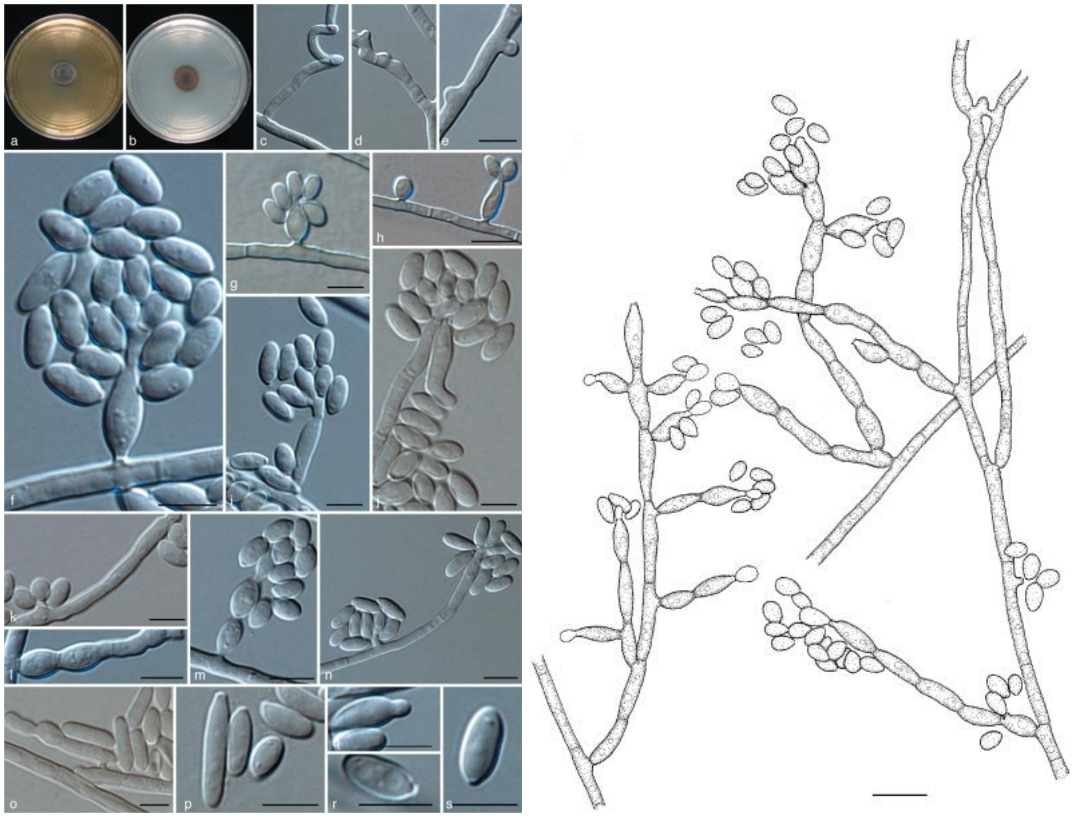


Morphology of Exophiala angulospora isolated from diseased Atlantic cod. (A) Gross colony morphology. (B) Branched, septate conidiophore bearing 4 paler, beaked conidiogenous cells occurring in terminal (arrow) and lateral (arrowhead) positions. (C) Unbranched conidiophores with terminal conidiogenous cells. Arrow indicates an intercalary conidiogenous cell. Arrowhead indicates beak of conidiogenous cell. (D) Vegetative hyphae with conidiogenous peg (arrow). (E) Conidia. Note the diversity of conidium shape and size, ranging from short ellipsoid to more-or-less triangular to long ellipsoid (arrow), **de Hoog GS, 2011**

2.Exophiala aquamarina de Hoog, Vicente, Najafzadeh, Harrak, Badali, Seyedmousavi & Nyaoke, *sp. nov.*— MycoBank

Description of CBS 119918 after 2 wk incubation on MEA, 27 °C.

Colonies restricted, olivaceous black, velvety with aerial mycelium at the centre. Reverse olivaceous black. No diffusible pigment produced. *Conidiogenous cells* flask-shaped, with short annellated zones, sometimes with sympodial conidiogenesis. Spirally twisted hyphae present. *Conidia* ellipsoidal to cylindrical, 6.7–19.2 × 4.0–4.8 µm. Yeast cells rarely present.



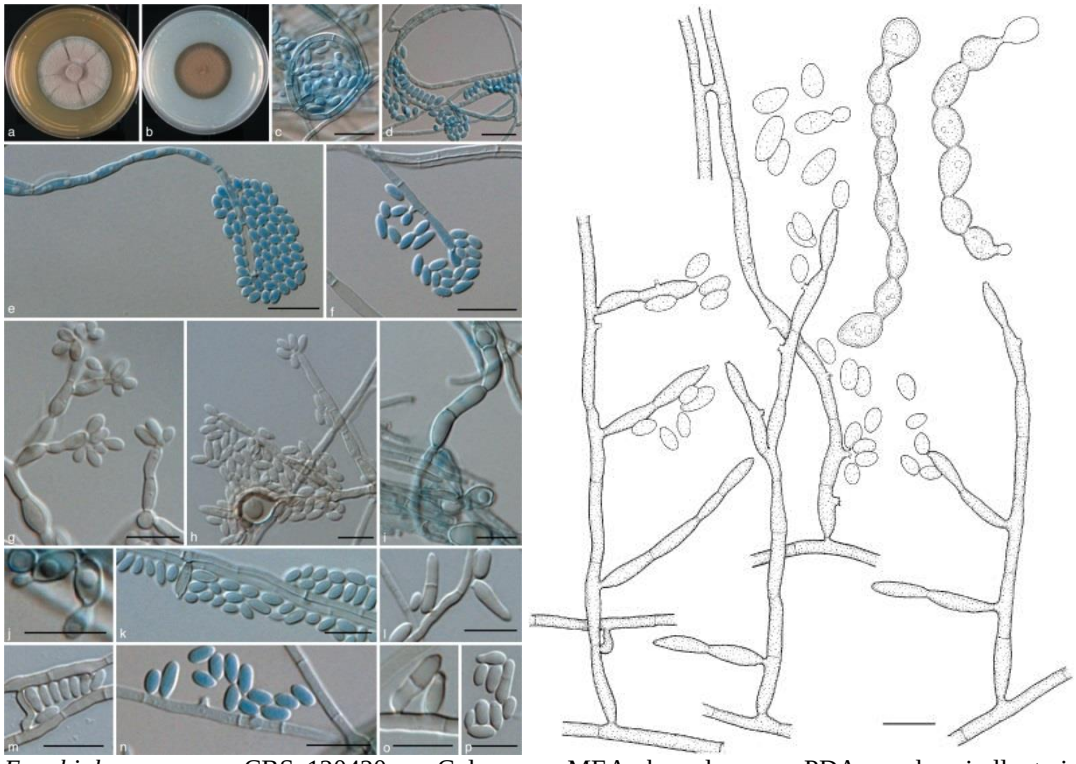
Exophiala aquamarina, CBS 119918. a. Colony on MEA; b. colony on PDA; c, d. spirally twisted hyphae; e–n. conidial apparatus with conidia; f–j. annellidic conidiogeneses with sympodial conidiophores; o. anastomosis between discrete cells; p–s. conidia; q. budding cells. — Scale bars = 10 μm. **de Hoog GS, 2011**

3. Exophiala cancerae de Hoog, Vicente, Najafzadeh, Harrak, Badali, Seyedmousavi & Boeger, *sp. nov.* — MycoBank

Description of CBS 120420 after 2 wk incubation on MEA, 24 °C.

Colonies moderately expanding, circular, initially (on day 3) flat, olivaceous black, slimy with velvety, olivaceous grey centre and flat margin, later (on day 14) becoming velvety, dark olivaceous grey. Reverse olivaceous black, without diffusible pigment. Yeast cells nearly absent. *Conidiophores* short, erect, brown, cylindrical, multi-celled, poorly differentiated. *Conidia* 0–1-septate, subhyaline to pale brown, obovoidal to cylindrical, 4.9–8.0 × 2.7–4.8 μm.

Cardinal temperatures — Minimum ≤ 4 °C, optimum 24–27 °C, maximum 30–33 °C. No growth at 37 °C.



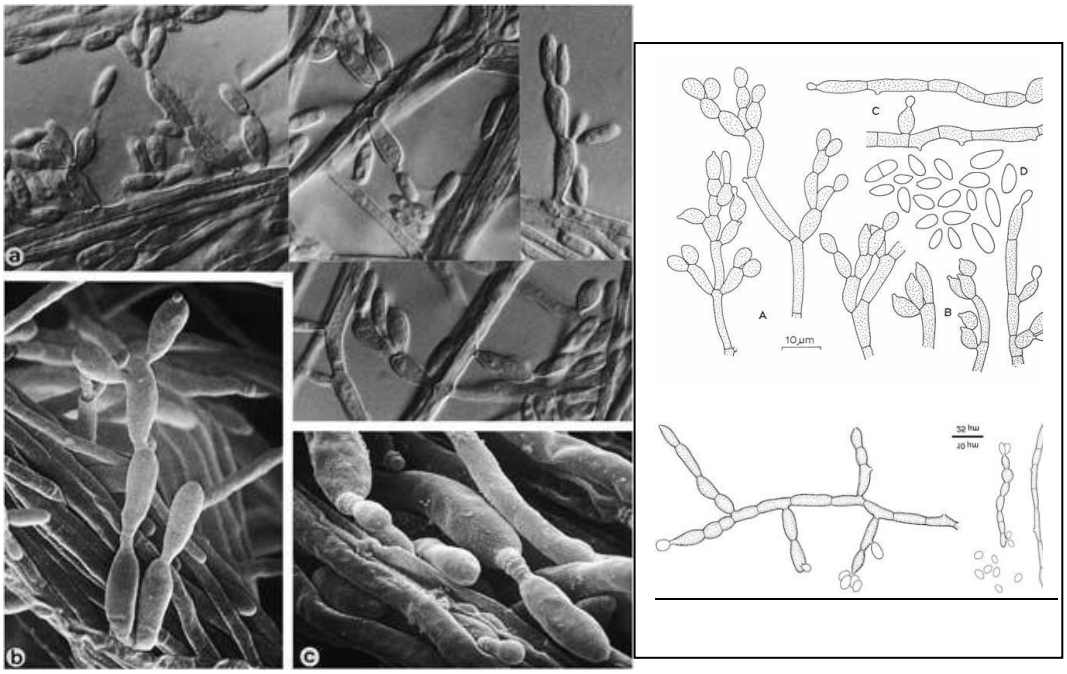
Exophiala cancerae, CBS 120420. a. Colony on MEA; b. colony on PDA; c, d. spirally twisted hyphae; e, f. short, erect, cylindrical, multi-celled conidiophores; h, i. apical and intercalary chlamydospores; j. budding cells; k, l. intercalary conidiogenous cells; m. hyphae and conidia with anastomoses; n–p. conidia. — Scale bars = 10 μm. **de Hoog GS, 2011**

4. Exophiala pisciphila McGinnis & Ajello (as '*pisciphilus*'), Mycologia 66: 518. 1974. — MycoBank

Teleomorph. Unknown.

Description of CBS 537.73 after 2 wk incubation on MEA, 24 °C.

Colonies moderately expanding, dry, floccose, olivaceous black. Yeast cells absent. *Conidiogenous cells*flask-shaped, mostly in loose clusters or branched systems, with inconspicuous annellated zones. *Conidia* 0(–1)-septate, (sub)hyaline, ellipsoidal, 6–8 × 2.5–4.0 μm.



Mycobank

5. Exophiala psychrophila O.A. Pedersen & Langvad, Mycol. Res. 92: 153. 1989. — MycoBank

Description of CBS 191.87 after 2 wk incubation on MEA, 24 °C.

Colonies initially yeast-like and black, gradually becoming effuse and dome-shaped. After 14 d at 18 °C colonies have a dark centre containing the bulk of the conidial mass surrounded by a felt of mouse grey mycelium. Hyphae pale brown, septate, sparingly branched, hyphae 1–3 µm wide. Moniliform cells very common, 3–6 × 5–15 µm, chains consisting of two to several hundred cells, 4–12 being the most common.*Conidiogenous cells* with several enteroblastic proliferations at the apices with 2.0–3.5 µm diam. *Conidia*holoblastic, aseptate, varying in shape from spherical to oblong, sometimes tapered, 1.5–2.5 × 3–6 µm, tending to accumulate in slimy balls at apex of conidiogenous cells. Lipid globules may sometimes give the false impression that the conidia are septate. Conidia may be produced from discrete conidiogenous cells, directly from hyphae, from moniliform cells and from conidia. Yeast-like cells also produce conidia.

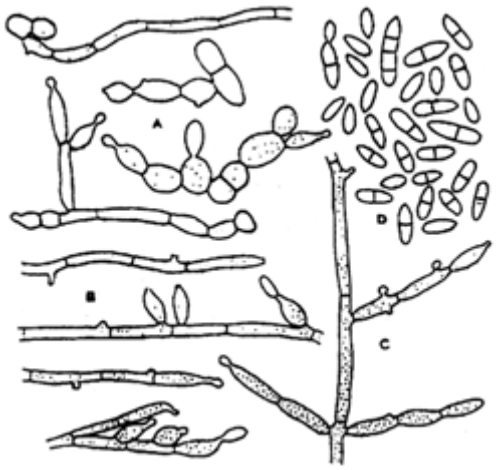
Cardinal temperatures — Minimum 0 °C (growth present after 6 mo at 0 °C); optimum 17–21 °C; maximum 23 °C.

6. Exophiala salmonis J.W. Carmich., Sabouraudia 5: 120. 1966. — MycoBank MB119468

Description of CBS 157.67 after 2 wk incubation on MEA, 24 °C.

Colonies moderately expanding, dry, depressed, hairy, olivaceous black. Yeast cells nearly absent.*Conidiogenous cells* poorly differentiated, intercalary or flask-shaped; annellated zones short, inconspicuous.*Conidia* 0–3-septate, subhyaline to pale brown, ellipsoidal to short cylindrical, 5.5–8.5 × 2.0–3.5 µm.

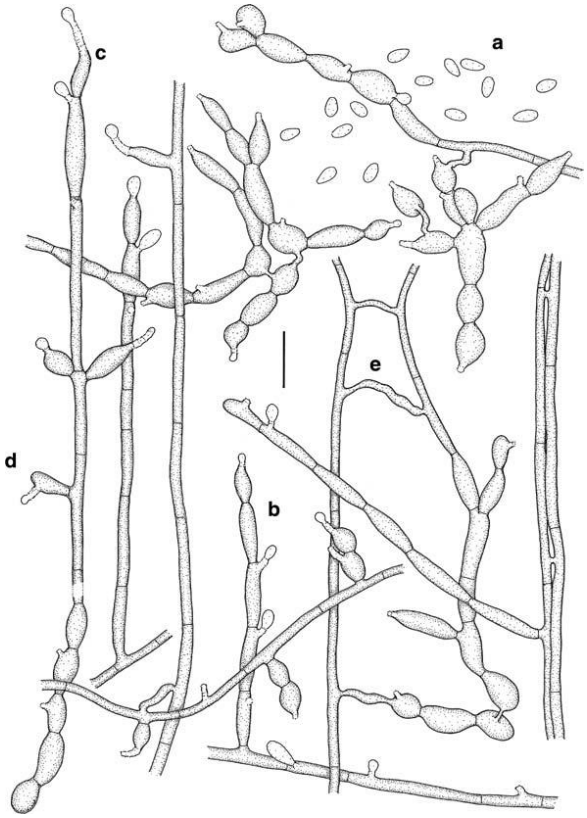
Cardinal temperatures — Minimum ≤ 4 °C, optimum 18–24 °C, maximum 30–33 °C. No growth at 37 °C.



AcuaNatura - Cursos on-line 4: Exophiala salmonis: A-C - conidial apparatus, D – conidia

7. Exophiala xenobiotica De Hoog, Zeng, Harrak and D. A. Sutton, sp. nov. Mycobank

Colonies on PDA and MEA restricted, circular, initially (on day 3) flat, olivaceous black, slimy with velvety, olivaceous grey center and flat margin, later (on day 14) becoming umbonate, felty, olivaceous grey, with velvety, brownish grey center. Reverse olivaceous black on PDA, olivaceous black with brownish black center on MEA. No diffusible pigment produced on any medium. Budding cells initially abundant, pale olivaceous, ellipsoidal, 5–6·2.5–3.0 lm, without capsule in India ink, often inflating and developing into broadly ellipsoidal, brown germinating cells of about 7–10·3–5 lm that often bear a short, irregular annellated zone. Hyphae pale olivaceous to brown, 1.3–2.0 lm wide, irregularly septate every 7–28 lm. Anastomoses abundant. Conidiophores 1–7-celled, arising at acute or right angles from creeping hyphae, with the same color as the hyphae, seldom branched. Conidiogenous cells lemon-shaped or fusiform with a flaring irregular annellated zones. Conidia adhering in small groups, subhyaline, obovoidal, 3.3–4.0·1.6– 2.0 lm. Spherical, subhyaline chlamydospores up to 13 lm diameter may be present. Teleomorph not observed in any of the strains tested after 2 months incubation. Cardinal temperatures: optimum 30C, maximum growth temperature 33–36C.



E. xenobioticaCBS 118157. (a. conidia; b. conidiophores; c,d. conidiogenous cells; e. anastomoses. Bar =10 lm. **G. S. De Hoog, 2006**

Reports:

Carmichael (1966) described a systemic infection of cutthroat trout, Salmo clarki Richardson, and lake trout, Salvelinus namaycush (Walbaum). The diseased fish exhibited cranial ulcers and erratic swimming. The causative agent was initially named a Phialophora-like fungus but later classified as Exophiala salmonis.

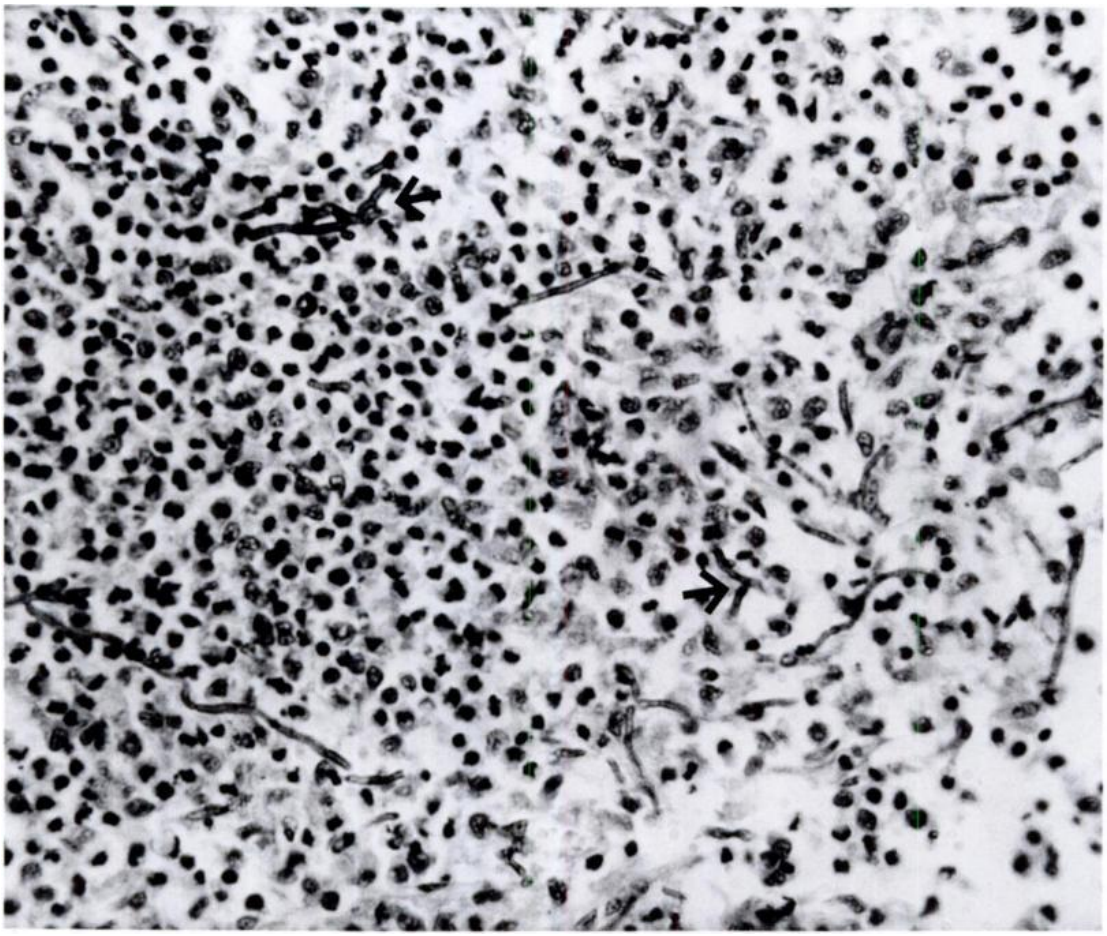
Fijan (1969) reported a systemic mycosis in channel catfish, Ictalurus punctatus (Rafinesque), with skin ulceration and numerous nodules in all internal organs. The aetiologic agent was identified as a Phialophora-like fungus but later reidentified as E. pisciphilus

Richards *et al.* (1978) described infection of Atlantic salmon, *Salmo salar* L., in seawater with ***Exophiala salmonis*** . Histological effects consisted principally of granuloma formation, especially in the posterior kidney and spread appeared to occur both by extension and by the liaematogenous route. The nature of the outbreak suggested that the original infection occurred via contaminated food. A comparison is made between this condition and systemic mycoses in other species

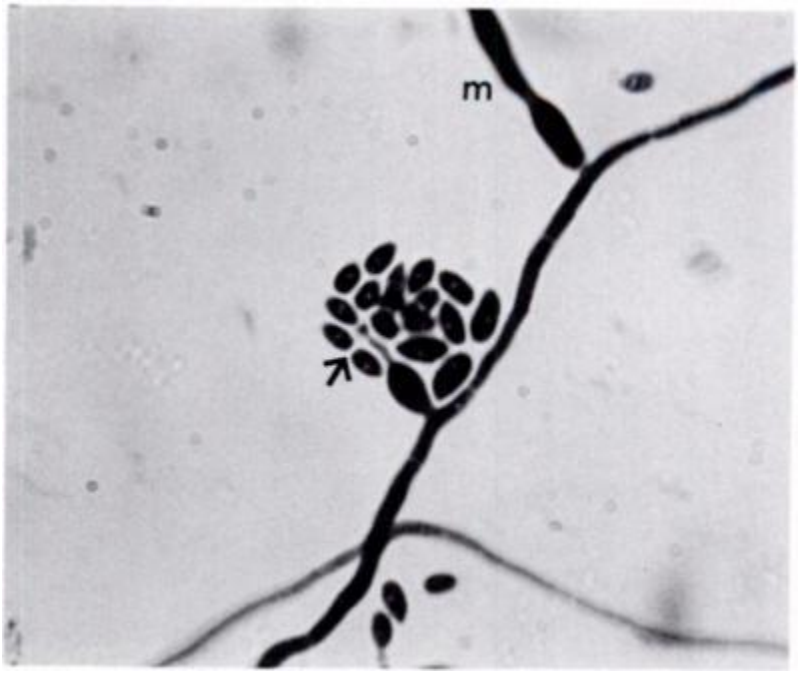
Blazer and Wolke (1979) mentioned that A systemic *Exophiala*-like mycosis occurred naturally in five genera of captive fishes and it was experimentally produced in three additional genera: *Tautogolabrus adspersus* (Walbaum),*Pseudopleuronectes americanus* (Walbaum) and *Fundulus heteroclitus* (L.) by intraperitoneal injection of spores. Histo-pathologically both acute, necrotic and focal granulomatous reactions were present in naturally infected animals. The lesions were reproduced following spore inoculations while a diffuse, proliferative, granulomatous reaction followed

inoculation of hyphae alone. Reports of systemic mycoses in fish and other animals due to pigmented or dematiaceous fungi are discussed and lesions compared.

Otis *et al.* (1985) described the first report of infection by **E. salmonis** in the United States. Over a 4-mo-period (April-June 1982), three adult Atlantic salmon with similar lesions were necropsied. Originally obtained from hatcheries in East Orland, Maine and New Brunswick, Canada, these fish had been held at the University Aquaculture Center in a partial reuse system for up to 20 mo prior to death. City water was maintained at 12 to 18 ppt salinity by the addition of rock salt. Fish were fed raw calf's liver supplemented with vitamins. Tissues from fish were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned at 6 µm and stained with hematoxylin and eosin. Special stains included periodic-acid Schiff (PAS) and Grocott's silver stain. Fish had a reddish discoloration 3.0 mm in diameter on the surface of the liver which extended into the parenchyma. Postmortem examination revealed petechial hemorrhages throughout the viscera. The pyloric cecae contained approximately 50 adult cestodes (Eubothnium sp.). The posterior kidney capsule was thickened and the parenchyma mottled gray. Another fish was cachexic. An ulceration (1.0 cm diameter) was present just posterior to the right pectoral fin. Petechial hemorrhages were present on the ventral surface between the pectorals. The hindgut contained four adult cestodes (Eubothriuni sp.). The posterior kidney was swollen and its capsule opaque. The kidney had three raised gray areas 1.0 cm in diameter. When cut a white opaque fluid appeared. The surrounding parenchyma was red to black and of a watery consistency. A third fish had microscopic changes in the intestine, kidney and liver. The intestinal mucosa was sloughed and there was focal hemorrhage, necrosis and eosinophilic granulocytic inflammation in the submucosa. Kidney lesions consisted of focal tubular necrosis, nephrocalcinosis and a diffuse granulomatous interstitial nephritis reminiscent of bacterial kidney disease. There was a focal acute hepatitis of fungal etiology. Necrotic tissue was infiltrated with polymorphonuclear leucocytes (PMN's) (70%), macrophages (30%) and numerous light brown, branching septate hyphae. Hyphae were frequently up to 30.0 m wide. Septae were 8.0 to 10.0 apart. The fungus was PAS and Grocott positive. A fourth fish had an eosinophilic granulocytic gastritis and enteritis, possibly in response to the cestodiasis. The posterior kidney contained masses of fungal hyphae, most often within multifocal microabscesses. The center of the abscesses contained a high percentage of polymorphonuclear leucocytes (90%), while the periphery was characterized by fibroblastic proliferation and mononuclear cell infiltration. Focal congestion occurred in the liver, spleen, and kidney. Nephrocalcinosis, abscesses, focal interstitial hemorrhage and large accumulations of melanin were present in the kidney. Blood vessels frequently contained histiocytes and PMN's. The abscesses had a central zone of liquefactive necrosis, fungal elements and leucocytes. Bordering this zone were masses of PMN's (70%), histiocytes (20%) and lymphocytes (10%), as well as necrotic interstitial and tubular epithelial cells. Mononuclear cells predominated at the periphery. Fibroblastic proliferation was scant. The kidney of was cultured on Corn Meal Agar (CMA). Raised, mouse to dark gray colonies 7-10 mm in diameter appeared after 10 days incubation at 25 C. Microscopic morphology was examined by the slide culture method. Annellides characteristic of Exophiala sp. produced conidia which accumulated in balls at the tips. The fungus was identified as Exophiala salmonis Carmichael.



Central portion of two microabscesses in the posterior kidney of an Atlantic salmon. Septate, branching fungal hyphae (arrows), masses of polymorphonuclear cells, and macrophages are present. PAS stain. x400, **Otis *et al.* (1985)**

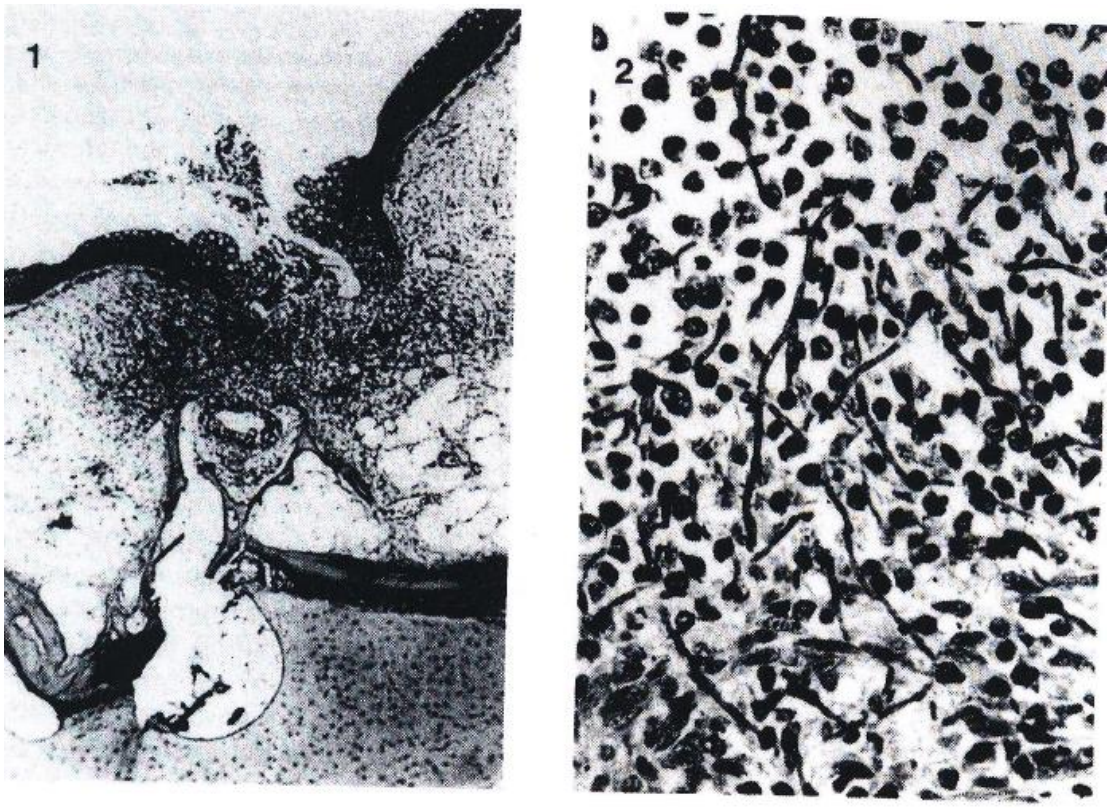


Eight-day-old slide culture of *Exophiala salmonis* showing cylindrical to clavate conidia accumulating at the tip of an annellide. Note cytoplasmic protrusion (arrow) at the apex of the annellide and a chain of moniliform cells (m). 1.0% aqueous phloxine, 10.0% KOH stain. x 1,000. **Otis *et al.* (1985)**

Gaskins and Cheung (1986) reported that Exophiala pisciphila is a dematiaceous

fungus that belongs to a group of fungi known as the 'black yeasts'. It was isolated from the skin lesions of a smooth dogfish, Mustelus canis Mitchill, that had been born in the shark exhibit tank of the New York Aquarium. The different stages of development of this fungus were studied by light microscopy and scanning electron microscopy to illustrate the morphology and surface structures of conidia and mycelium. The list of marine and fresh water fish, which have been infected by Exophiala spp. and Exophiala-like fungi has been up-dated. Potato Dextrose Agar and Malt Agar proved to be the best growth media, while Corn Meal Agar proved to be the best medium for studying the morphological features of the conidia and mycelial development of E. pisciphila, which exhibited polymorphic conidiogenesis.

Langdon and McDonald (1987) detected 15 cases of cranial mycosis over five months in an experimental population of 85 Atlantic salmon parr. Five specimens were found dead and the remainder were killed when moribund. Clinical signs included depression, darkening, and, in some cases, erratic swimming in loose spirals or "whirling". Gross examination usually revealed one or more fistula, about I mm diameter, on the caudo-lateral surfaces of the head, above, below or behind the eye, and on the opercula. Circular depressions of a similar size also occurred adjacent to the fistulae. Erosion of the caudal opercular margin had occurred in some cases. Unilateral or bilateral exophthalmus was seen in five affected fish. The fistulae were found to extend to the deep connective tissues lateral and caudal to the brain, at necropsy. The cranial cartilage was eroded and fragmented, and surrounded by dark red-brown gelatinous rnaterial. Similar material was found in the retrobulbar region of specimens with exophthalmus. Extension to the brain had occurred in three specirnens. Histopattrological examination of the cranial lesions revealecl a diffuse and infiltrating granulomatous inflammatory reaction extending along the fistulae to the cartilaginous erosions. In most cases the lesions involved one or more of the cranial canals of the lateral line system with erosion and necrosis of the cartilage, sometimes extending to the brain and the semicircular canals. Macrophages were the predominant cell type, with lymphocytes, plasma cells, giant cells, and erythrocytes also present. Septate, branching fungal hyphae extended throughout the lesions. The hyphae stained light pink with haematoxylin and eosin and gave a strong positive reaction to the periodic acid Schiff reagents. Material from the lesions was cultured on sheep blood agar and Sabouraud's agar at 28^{o}C. Several opportunist bacterial species including Klebsietta oxytoca and Serrstia fonticola grew on blood agar from the fish with open lesions. Fungal colonies appeared after about five days on Sabouraud's agar. No pathogen was isolated from the kidnev. liver or spleen. The fungal isolate was identified by the Australian National Reference Laboratory in Medical Mycology (Royal North Shore Hospital, St. Leonards. NSW) as **Exophiala pisciphita** McGinnis & Ajello 1974.

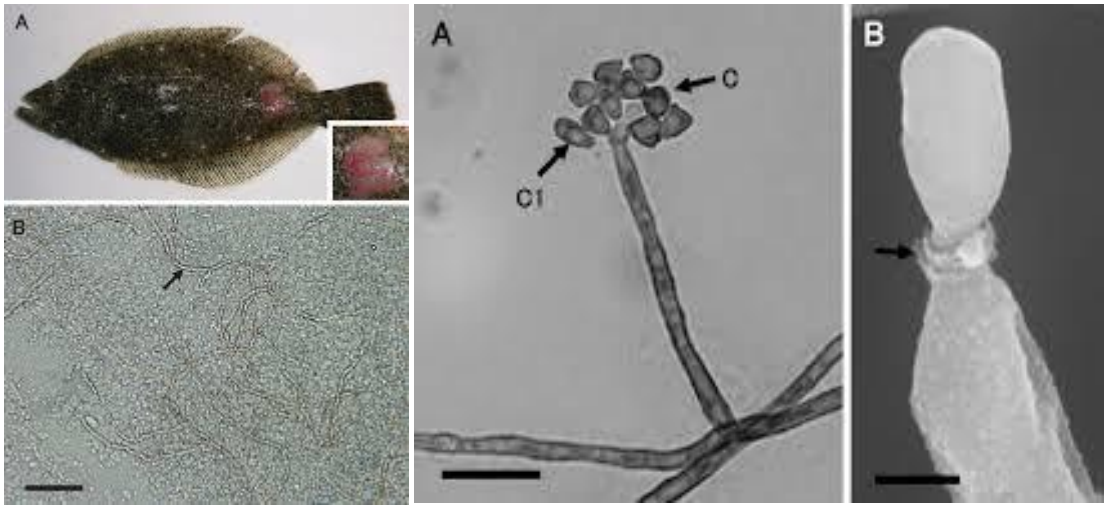


Fistula surrounded by granulomatous inflammatory reaction, extending from exterior. to a supra-orbital canal of the lateral line (H&E, x45, PAS-positive hyphae surrounded by granulomatous inflammatory reaction. **Langdon and McDonald (1987)**

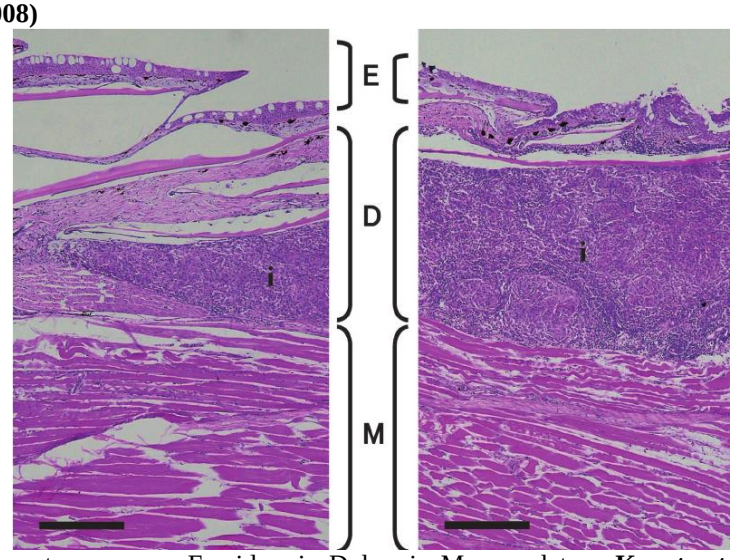
Pedersen and Langvad (1989) described ***Exophiala psychrophila*** sp. nov., isolated from infected Atlantic salmon smolt . It is distinguished from other *Exophiala* species by the combined use of morphological and physiological criteria. The disadvantages of using morphological criteria alone for the classification of *Exophiala* species, which has been common practice up to now, is discussed. Temperature-growth relationships, measured with a continuous temperature gradient incubator, is a valuable tool for the identification of these species. A key to the four taxa of *Exophiala* pathogenic on fish is presented

Richards *et al.* (2006) described infection of Atlantic salmon, *Salmo salar* L., in seawater with *Exophiala salmonis* . Histological effects consisted principally of granuloma formation, especially in the posterior kidney and spread appeared to occur both by extension and by the liaematogenous route. The nature of the outbreak suggested that the original infection occurred via contaminated food.

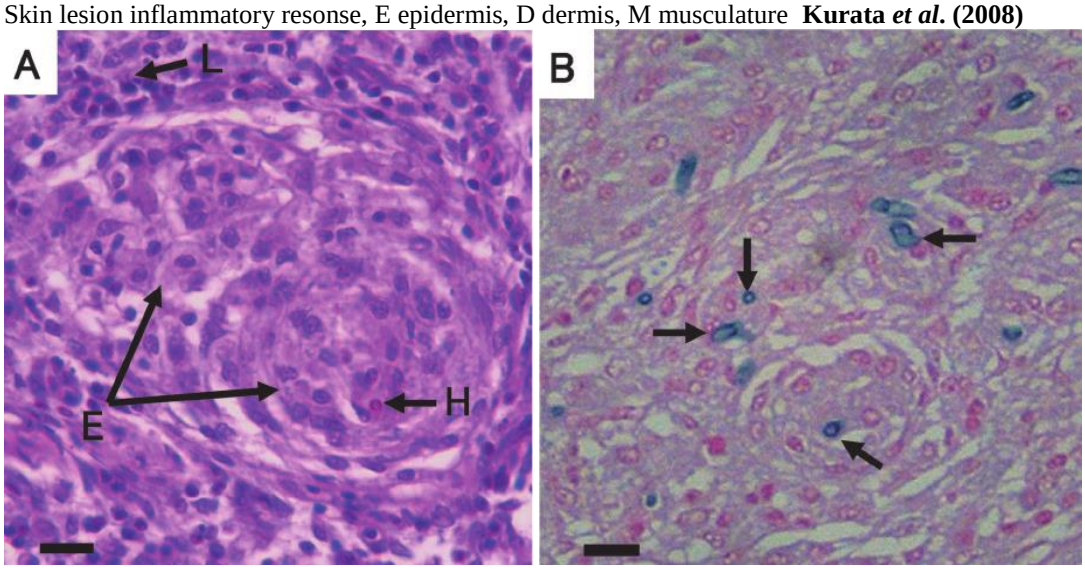
Kurata *et al*. (2008) isolated a novel Exophiala species from ulcerative skin lesions in Japanese flounder *Paralichthys olivaceus*. Fungal hyphae extended laterally in the dermis and were absent in the epidermis and musculature of the skin and kidneys of the diseased fish. An inflammatory response with a granuloma occurred in the dermis. The disease could be repruced by experimental infection.



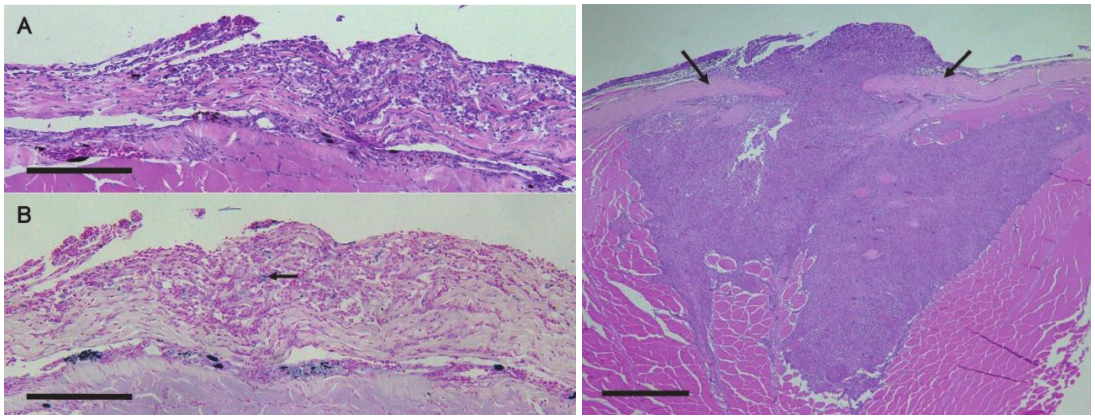
Clinical signs in diseased fish, A: ulcerative skin lesion, B: Numerous fungal hyphae in the lesion. Morphology of the fungus, A: cluster of conidia on the top of a conidiophore, B: SEM of the conidium, **Kurata *et al*. (2008)**



Skin lesion inflammatory resonse, E epidermis, D dermis, M musculature **Kurata *et al*. (2008)**



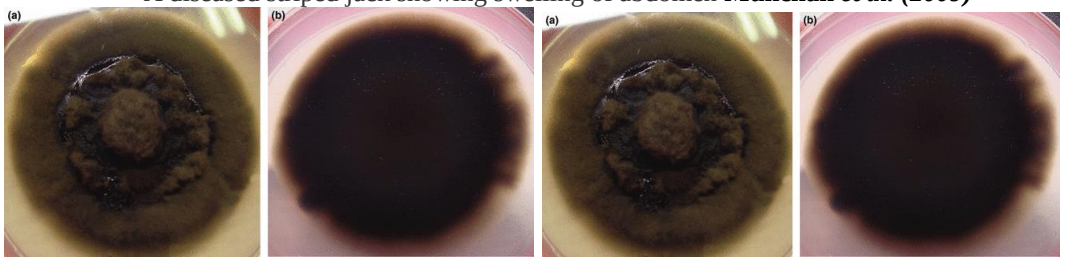
A: epithelial cell granuloma, epithelial cell (E) accumulation around hyphae (H), lymphocyte cell (L), B: arrows show positive hyphae for melanin **Kurata *et al*. (2008)**



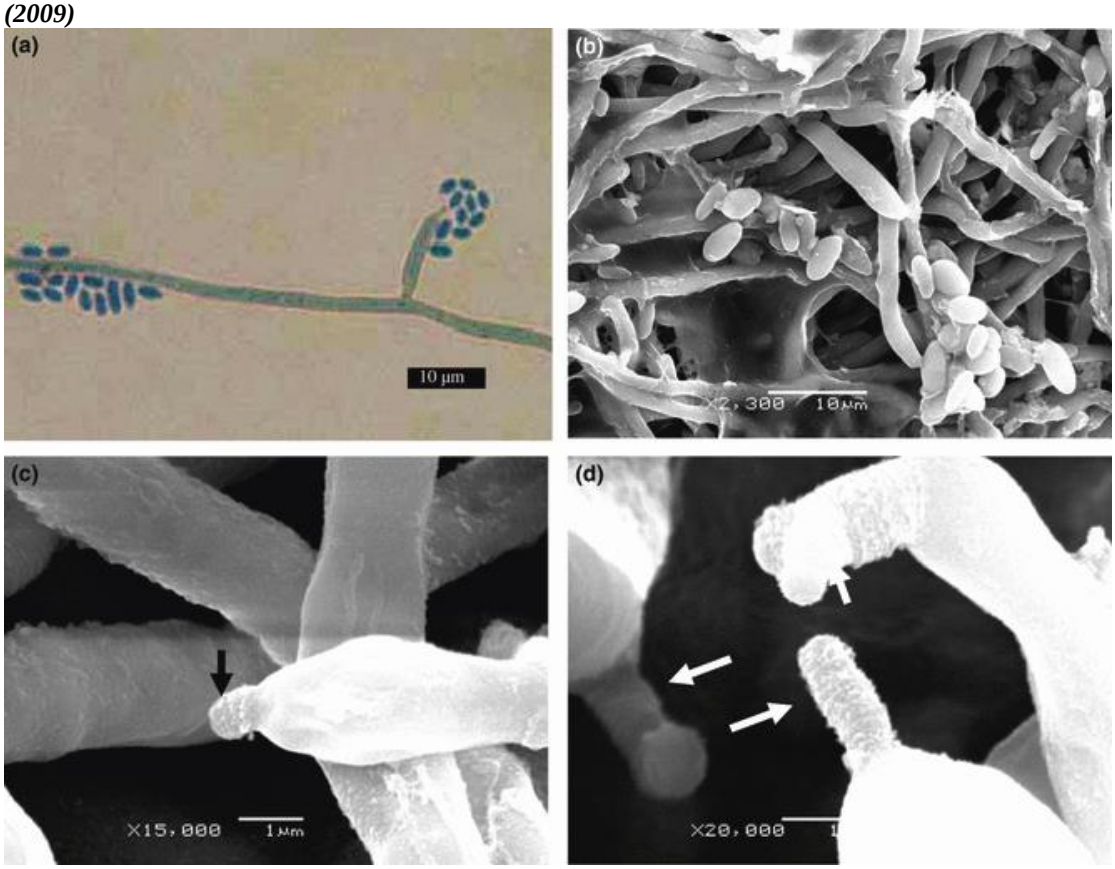
Pathological changes in the skin of experimentally infected fish **Kurata *et al.* (2008)**

Munchan *et al.* *(2009)* *described* *Exophiala* infection in cultured striped jack, *Pseudocaranx dentex*, in Japan in 2005. One hundred out of 35 000 fish died per day and mortalities continued for 1 month. Diseased fish showed swelling of the abdomen and kidney distension. Numerous septate hyphae, pale brown in colour, were seen in kidney in squash preparations. Histology revealed abundant fungal hyphae and conidia in gill, heart and kidney. Fungal hyphae were accompanied by cell necrosis and influx of inflammatory, mainly mononuclear cells. The fungus isolated from the diseased fish had septate hyphae, pale brown in colour and 1.8–3.0 µm in diameter. Conidiogenous cells were conspicuous annellides, short or cylindrical or fusiform in shape. Conidia were one-celled, ellipsoidal with smooth walls, accumulated in balls at the apices of annellides that tended to slide down, 1.5–2.0 µm in width and 3.0–5.0 µm in length. The fungus was classified into the genus *Exophiala* based on its morphology and as ***Exophiala xenobiotica*** based on the sequences of the ITS 1–5.8S–ITS 2 regions of rDNA. This is the first record of this fungus in a marine fish.

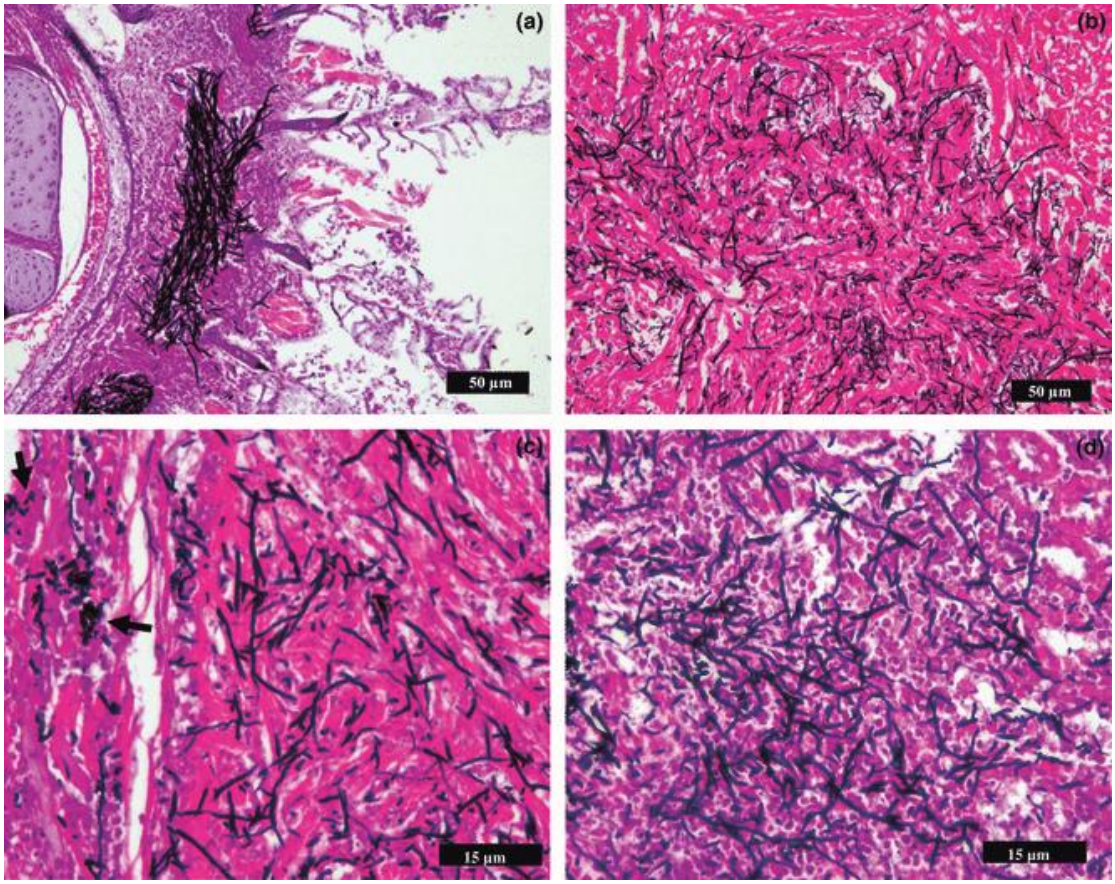
A diseased striped jack showing swelling of abdomen **Munchan *et al.* *(2009)***



Colony of *Exophiala xenobiotica* cultured on PDA at 25°C for 4 weeks. (a) Surface is initially moist, becomes woolly and velvety with age, olive brown in colour. (b) Reverse side is black. **Munchan *et al.* *(2009)***

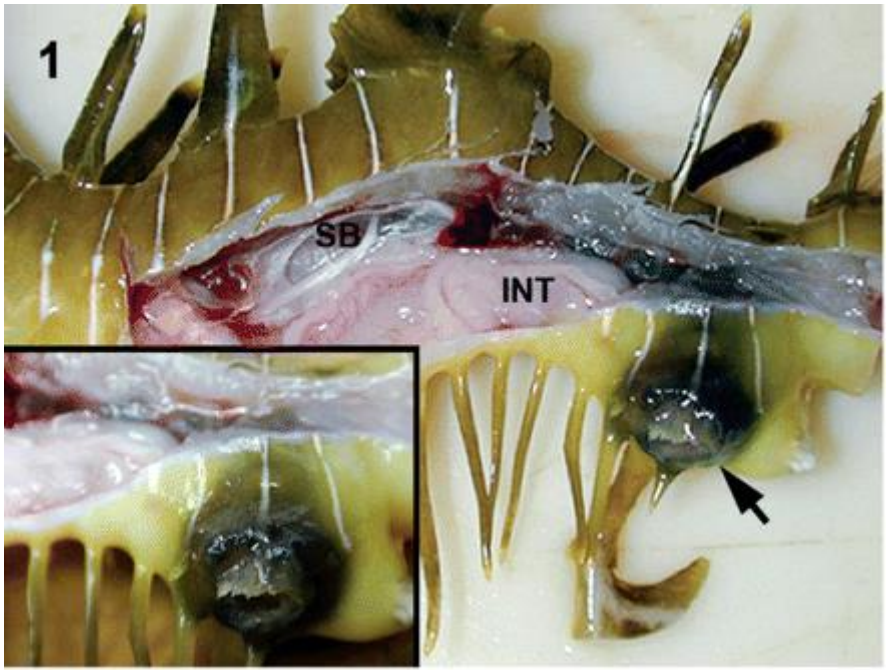


Exophiala xenobiotica. (a) Slide culture on PDA after 4-week incubation at 25°C (lactophenol cotton blue). Conidiogenous cell with conidia accumulated in balls at apices and tending to slide down. (b–d) Scanning electron photomicrographs. (b) One-celled conidia, ellipsoidal with smooth walls. (c–d) Conspicuous annellides conidiophores (arrows). **Munchan *et al.* *(2009)***

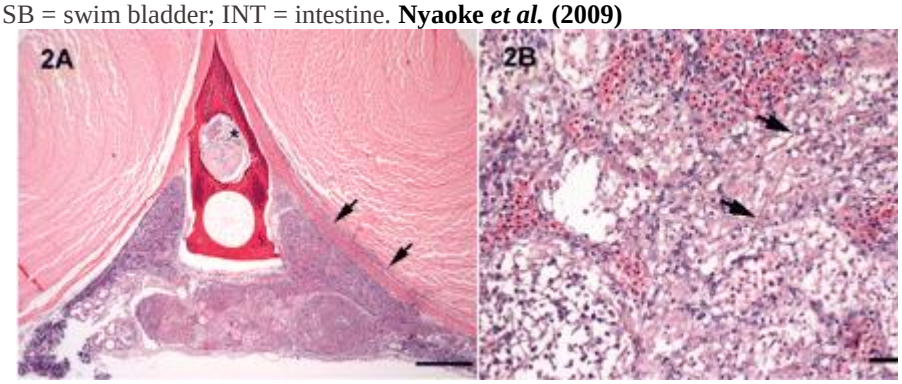


Histopathological features of *Exophiala xenobiotica* infection in striped jack. (a) Gill showing mats of fungal hyphae embedded in gill arch and penetrating into gill lamellae. (b) Cross-section of the heart showing heavy infiltration of fungal hyphae. (c) Higher magnification of heart tissue showing conidial apparatus (arrows). (d) Fungal hyphae invading kidney associated with necrosis and influx of inflammatory cells (a–d = Grocott's-H&E). **Munchan *et al. (2009)***

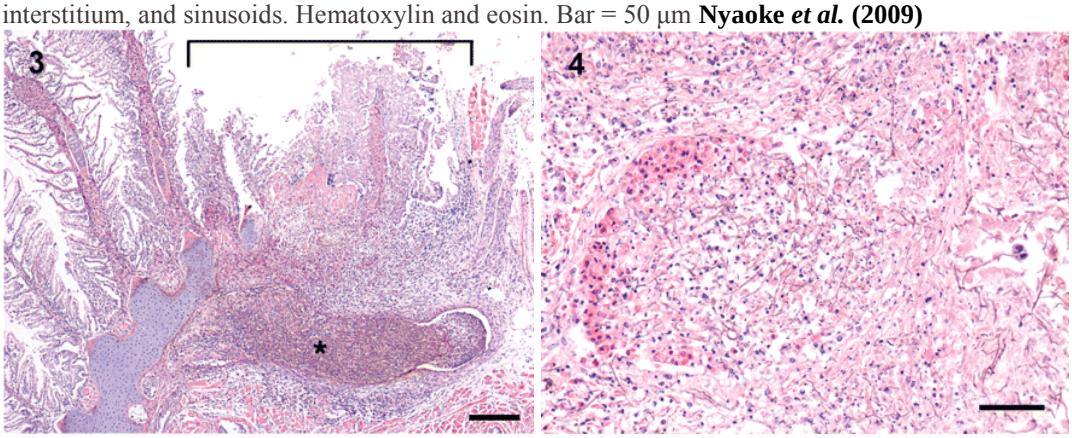
Nyaoke *et al.* (2009) identified infections by melanized fungi with greater frequency in aquarium-maintained leafy seadragons (Phycodurus eques) and weedy seadragons (Phyllopteryx taeniolatus), pivotal species to the educational and environmental concerns of the aquarium industry and conservation groups. Samples from 14 weedy and 6 leafy seadragons were received from 2 institutions and included fresh, frozen, and formalin-fixed tissues from necropsy and biopsy specimens. Fresh and frozen tissues were cultured for fungi on Sabouraud dextrose agar only or both Sabouraud dextrose agar and inhibitory mold agar with gentamicin and chloramphenicol at 30 degrees C. Isolates were processed for morphologic identification and molecular sequence analysis of the internal transcribed spacer region and D1/D2 domains of the large subunit ribosomal RNA gene. Lesions were extensive and consisted of parenchymal and vascular necrosis with fungal invasion of gill (11/20), kidney (14/20), and other coelomic viscera with or without cutaneous ulceration (13/20). Exophiala sp. isolates were obtained from 4 weedy and 3 leafy seadragons and were identified to species level in 6 of 7 instances, namely **Exophiala angulospora** (1) and a novel species of Exophiala (5), based on nucleotide sequence comparisons and phylogenetic analyses. Disseminated phaeohyphomycosis represents an important pathologic condition of both weedy and leafy seadragons for which 2 species of Exophiala, 1 a novel species, have been isolated.



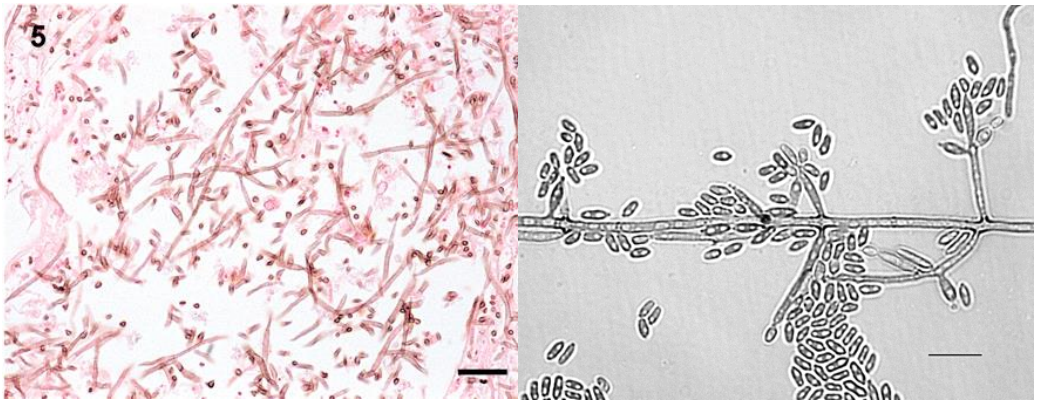
Skin ulcer; leafy seadragon with lateral body wall removed to expose coelomic viscera. An ulcer (arrow) is located in the skin adjacent to the cloaca. Inset: Closer view of the ulcer with raised black margins. SB = swim bladder; INT = intestine. **Nyaoke *et al.* (2009)**



Transverse section of dorsal trunk; weedy seadragon. **A**, there is extensive necrosis involving approximately two-thirds of the renal parenchyma. Note the presence of fibrin and cells in the extradural sinus (asterisk) and an infiltrate along the fascia and margin of adjacent epaxial muscle (arrows). Hematoxylin and eosin. Bar = 500 µm. **B**, higher magnification of renal parenchyma reveals innumerable, filamentous brown fungal hyphae (arrows) coursing through necrotic tubules, interstitium, and sinusoids. Hematoxylin and eosin. Bar = 50 µm **Nyaoke *et al.* (2009)**

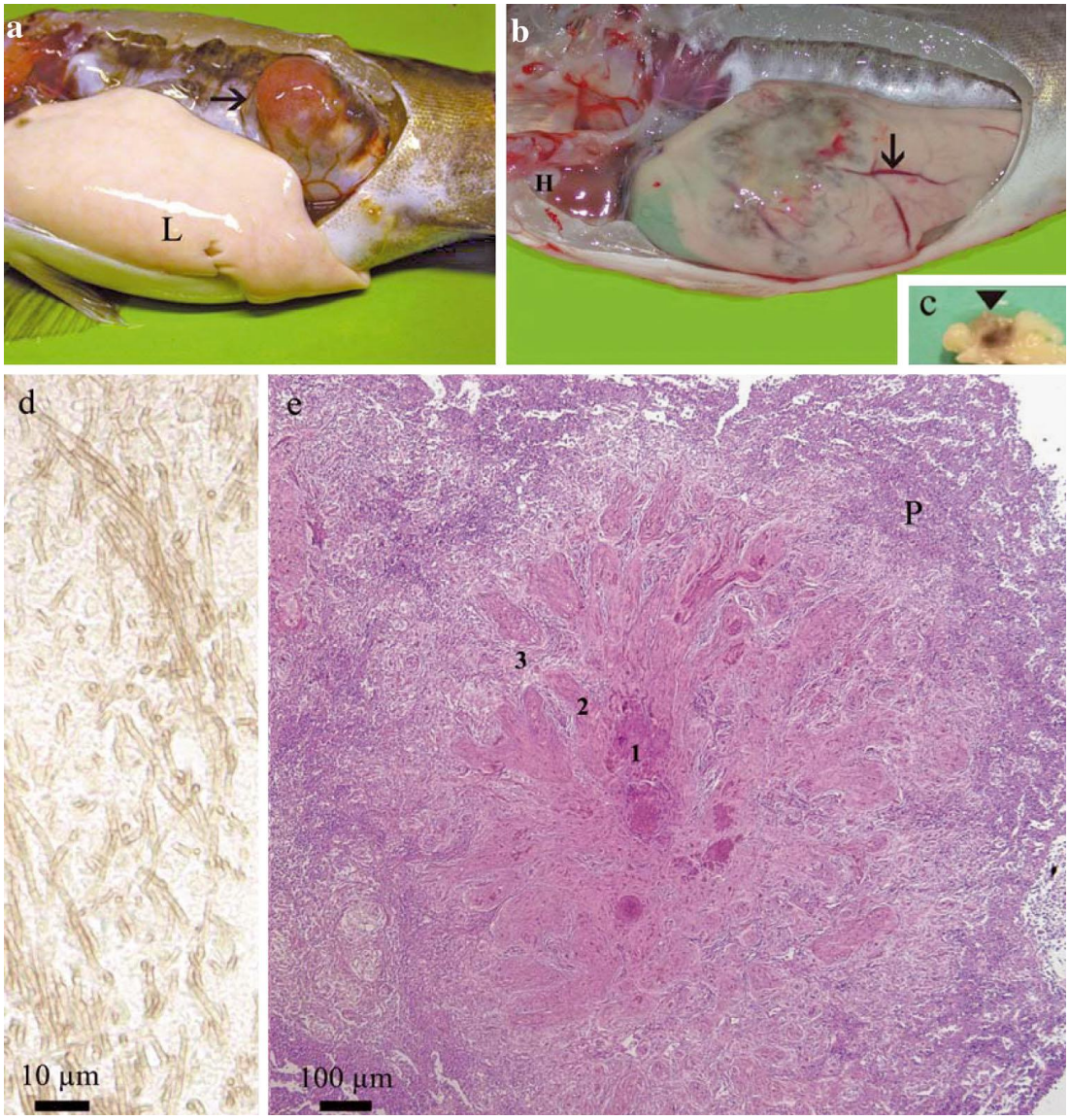


Gill; leafy seadragon. There is focally extensive necrosis of several consecutive filaments and their lamellae (bracket) overlying a region of the arch wherein a mat of densely intertwined brown fungal hyphae (asterisk) resides within the venous sinus. Hematoxylin and eosin. Bar = 200 µm Blood vessel, kidney; weedy seadragon. Intertwined hyphae are present in the blood vessel lumen, and there is necrosis of a segment of the wall. Hematoxylin and eosin. Bar = 200 µm **Nyaoke *et al.* (2009)**

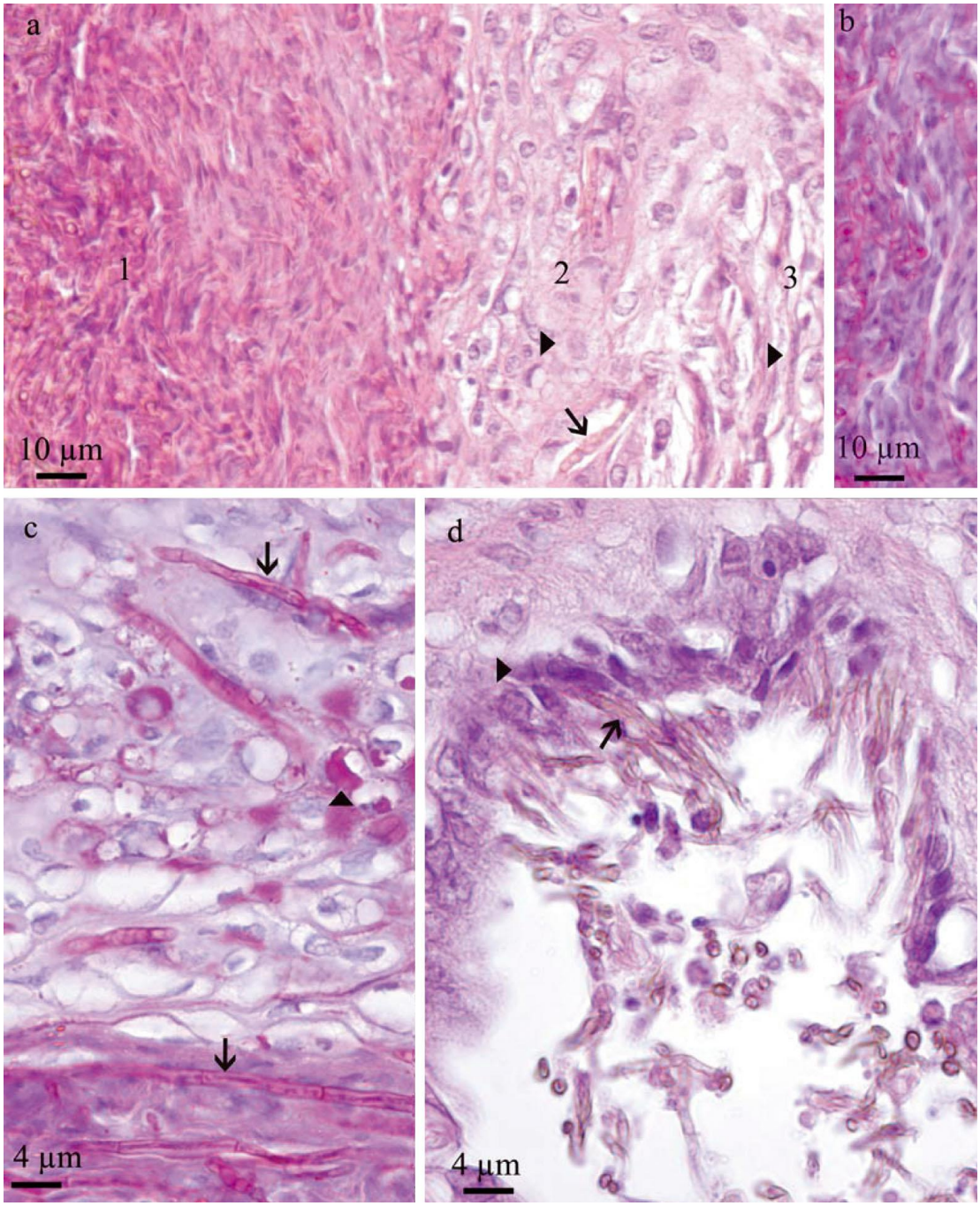


Fungal hyphae, kidney; weedy seadragon. Hyphae are slender, filamentous, and septate with occasional right-angle branches. Walls of hyphae stain brown, indicative of melanin. Fontana-Masson. Bar = 25 μm Microscopic colonial morphology of *Exophiala* sp. nov. showing septate hyphae with multiple annellides and conidiogenous loci bearing single-celled, approximately 2–3 μm × 4–5 μm conidia. Lactophenol cotton blue. Bar = 10 μm **Nyaoke *et al.* (2009)**

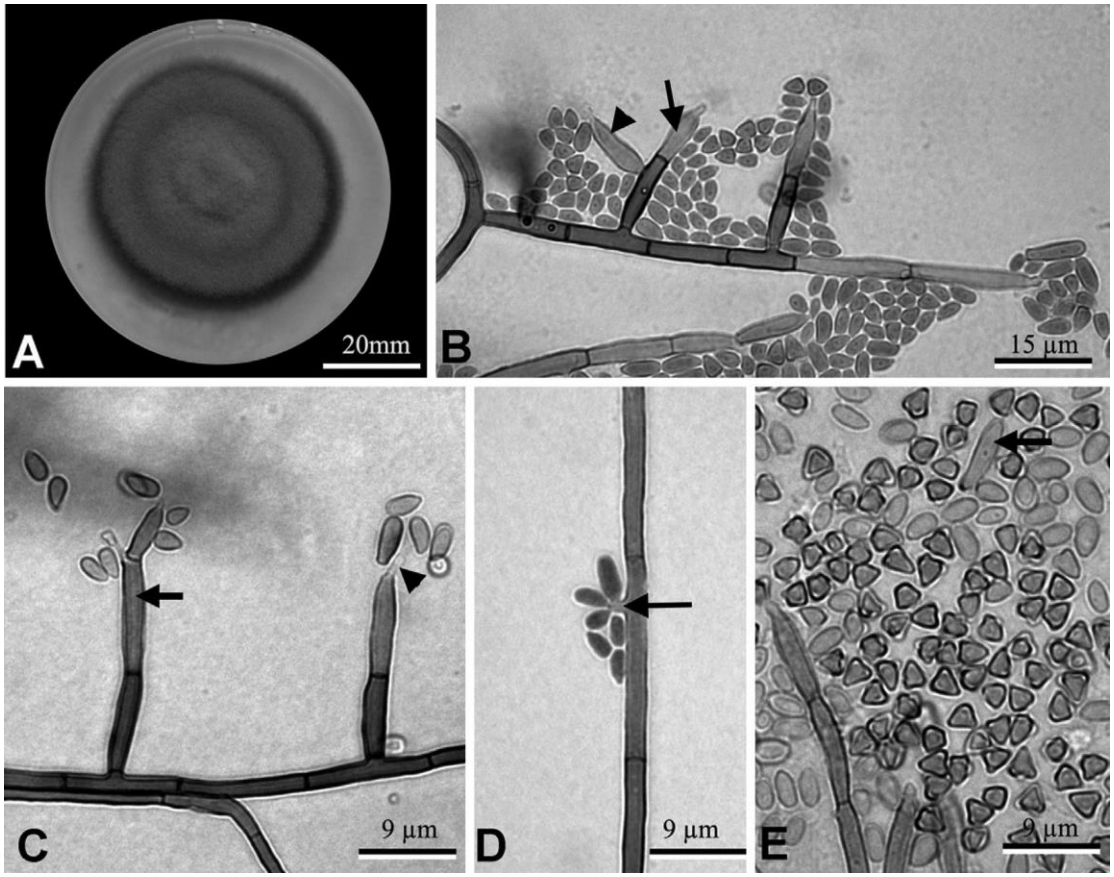
Gjessing *et al.* (2011) observed abnormal swimming behaviour and skin pigmentation and increased mortality in cod kept in an indoor tank. Necropsy revealed foci of different sizes with a greyish to brownish colour in internal organs of diseased fish. The foci consisted of ramifying darkly pigmented fungal hyphae surrounded by distinct layers of inflammatory cells, including macrophage-like cells. In the inner layer with many hyphae, the macrophage-like cells were dead. No apparent restriction of fungal growth by the inflammatory response were observed. A darkly pigmented fungus was repeatedly isolated in pure culture from foci of diseased fish and identified as **Exophiala angulospora** using morphological and molecular characters. This species has not been previously reported to cause disease in cod, but has been reported as an opportunistic pathogen of both marine and freshwater fish. Based on the morphology and sequence analysis presented here, it was conclude that E. angulospora caused the observed chronic multifocal inflammation in internal organs of cod, leading to severe disease and mortality.



Gadus morhua. Atlantic cod infected with Exophiala angulospora. (a–c) Gross pathology; (d,e) histological sections of kidney. (a) Large protruding focus in caudal part of kidney (arrow). L: liver. (b) Large greyish to brownish and partly greenish focus and blood-filled vessels in the liver (arrow). Note also the greenish color outside the focus in the most cranial part of the liver, as compared to normal colour in (a). H: heart. (c) Greyish to brownish focus affecting the whole tectum opticum of the midbrain (arrowhead). (d) Brownish hyphae in a focus. No stain. (e) Large, irregular and poorly-confined foci consisting of centrally-located hyphae and surrounded by inflammatory cells organized in distinct layers denoted layer 1, 2 and 3 as indicated. The invasiveness of hyphae in different directions has resulted in a ramified pattern of eosinophila due to dead cells around hyphae within the focus. Haematoxylin and eosin. P: parenchyma **Gjessing *et al.* (2011)**



Gadus morhua. Atlantic cod infected with Exophiala angulospora. Histological sections of (a–b) kidney interstitium, (c) liver, and (d) a kidney tubule or duct, stained with (a,d) H&E and (b,c) periodic acid-Schiff (PAS). (a) Layer 1; note the high number of hyphae and dead cells (1). Many macrophage-like cells with blastic nuclei (left arrowhead) and and a single hyphae (arrow) are seen in layer 2. Flattened fibrocyte-like cells are seen in layer 3 (right arrowhead). (b) PAS-positive hyphae in layer 1. (c) Some hyphae are seen in layer 1 (lower arrow) and layer 2 (upper arrow). Note branched and septate structure, and some material of unknown nature in putative inflammatory cells staining homogenously for PAS (arrowhead). (d) Many hyphae (arrow) apparently invading from the lumen into the epithelium of a kidney tubule or duct (arrowhead) **Gjessing *et al.* (2011)**



Morphology of Exophiala angulospora isolated from diseased Atlantic cod. (A) Gross colony morphology. (B) Branched, septate conidiophore bearing 4 paler, beaked conidiogenous cells occurring in terminal (arrow) and lateral (arrowhead) positions. (C) Unbranched conidiophores with terminal conidiogenous cells. Arrow indicates an intercalary conidiogenous cell. Arrowhead indicates beak of conidiogenous cell. (D) Vegetative hyphae with conidiogenous peg (arrow). (E) Conidia. Note the diversity of conidium shape and size, ranging from short ellipsoid to more-or-less triangular to long ellipsoid (arrow) **Gjessing *et al.* (2011)**

References:

1. Alderman, D. J., and Feist, S. W. 1985. Exophiala infection of kidney of rainbow trout recovering from proliferative kidney disease. Transactions of the British Mycological Society, 84(1): 157–159.
2. Blazer VS, Wolke RE (1979) An *Exophiala*-like fungus as the cause of a systemic mycosis of marine fish. J Fish Dis 2: 145–152
3. Carmichael, J. W. 1967. Cerebral mycetoma of trout due to a Phialophora-like fungus. Sabouraudia: Journal of Medical and Veterinary Mycology, 5: 120–123.
4. de Hoog, G. S., Vicente, V. A., Najafzadeh, M. J., Harrak, M .J., Badali, H., and Seyedmousavi, S. 2011. Waterborne Exophiala species causing disease in cold-blooded animals. Persoonia Molecular Phylogeny and Evolution of Fungi, 27:46–72.
5. Gaskins JE, Cheung PJ. **Exophiala pisciphila. A study of its development.** Mycopathologia. 1986 Mar;93(3):173-84.
6. Gjessing, M. C., Marie Davey , Agnar Kvellestad , Trude Vrålstad. Exophiala angulospora causes systemic inflammation in Atlantic cod Gadus morhua. Dis. Aquat. Org. Vol. 96: 209–219, 2011

7. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. Leaflet No. 42. 5 pp. Original by F. Langvad and Engjom. Revised and updated by D. W. Bruno. 2016. Infection with Exophiala salmonis.

8. Kurata O, Munchan C, Wada S, Hatai K, Miyoshi Y, Fukuda Y (2008) Novel *Exophiala* infection involving ulcerative skin lesions in Japanese flounder *Paralichthys olivaceus*. Fish Pathol 43: 35–44

9. Langdon, J. S., and McDonald, W. L. 1987. Cranial Exophiala pisciphila infection in Salmo salar in Australia. Bulletin of the European Association of Fish Pathologists, 7: 35–36.

10. Munchan C, Kurata O, Wada S, Hatai K, Sano A, Kamei K, Nakaoka N (2009) *Exophiala xenobiotica* infection in cultured striped jack, *Pseudocaranx dentex* (Bloch & Schneider), in Japan. J Fish Dis 32: 893–900

11. Nyaoke A, Weber ES, Innis C, Stremme D, Dowd C, Hinckley L, Gorton T, Wickes B, Sutton D, de Hoog S, Frasca S Jr. **Disseminated phaeohyphomycosis in weedy seadragons (Phyllopteryx taeniolatus) and leafy seadragons (Phycodurus eques) caused by species of Exophiala, including a novel species.** J Vet Diagn Invest. 2009 Jan;21(1):69-79.

12. Otis, E. J., Wolke, R. E., and Blazer, V. S. 1985. Infection of Exophiala salmonis in Atlantic salmon (Salmo salar L.). Journal of Wildlife Diseases, 21: 61–64.

13. Pedersen OA, Langvad F (1989) *Exophiala psychrophila* sp. nov, a pathogenic species of the black yeasts isolated from farmed Atlantic salmon. Mycol Res 92: 153–156

14. Richards RH, Holliman A, Helgason S (1978) *Exophiala* ***salmonis*** **infection in Atlantic salmon *Salmo salar* L. J FishDis 1: 357–368**

15. Richards, R. H., Holliman, A., and Helgason S. 2006. Exophiala salmonis infection in Atlantic salmon Salmo salar L. Journal of Fish Diseases, 1: 357–368.

18.Lethargic crab disease (LCD)

- The disease was termed lethargic crab disease (LCD) because of these reported set of specific clinical signs (Boeger et al. 2005, 2007).

- The economic impacts of LCD are extensive, with reductions in the fishing yields of 84% and 97.6% in mangroves of Paraíba and Bahia, respectively (Alves and Nishida 2003; Schmidt, 2006).

- Several potential etiological agents have been linked in unpublished accounts with LCD, including protists, fungi, bacteria, introduction of exotic metazoans, and chemical poisoning.

- In some regions, LCD has been associated with:
    - sugar-cane cultures
    - shrimp farming,
    - oil prospection and extraction,
    - wood industry.

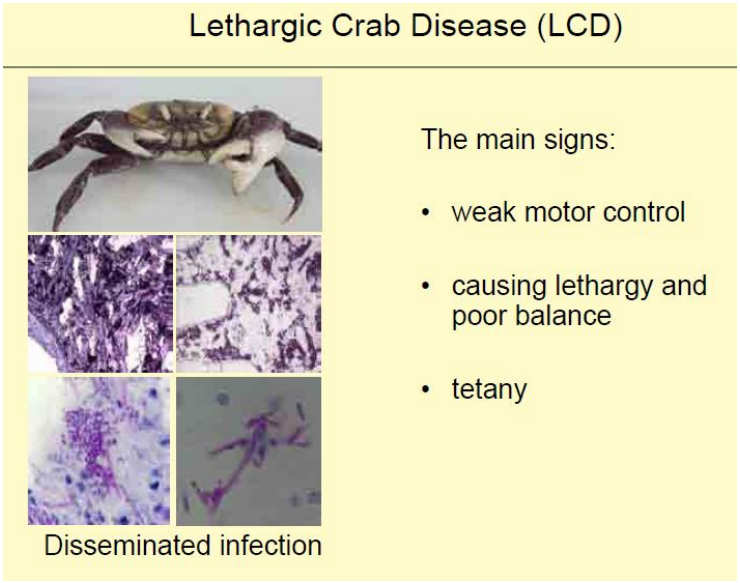
- **BOEGER *et al.* (2005)** provided robust evidence suggesting that LCD is caused by an anamorph Ascomycota of the subphylum Pezizomycotina.
- This conclusion is supported by both morphological (TEM and optical microscopy) and molecular methods.
- Evidence from a variety of sources indicates that there is an association between LCD and a new species of black yeast, *Exophiala cancerae*

Clinical signs included

- increasingly weak motor control, particularly of pereiopods and chelae, causing lethargy and poor balance, followed by the death of the affected crab.
- Tetany of the claws was also observed in many crabs with other signs of the disease.

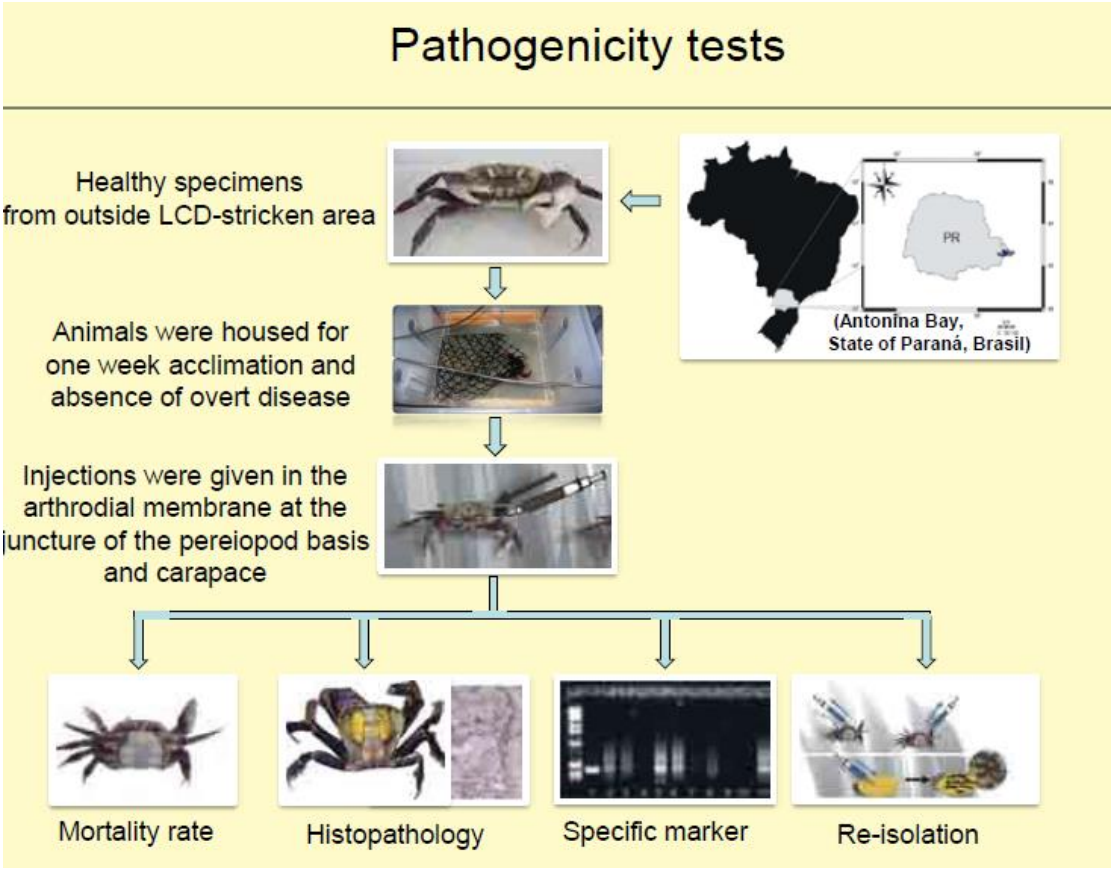
The histopathology of crabs with variable signs of LCD indicated that

- the most affected tissues were the epidermis, connective tissue, heart, hepatopancreas, nervous system, and gills.
- Necrosis, tissue degeneration, and congestion of hemal sinuses (the two principal empty areas along the digestive tube) and vessels were present in heavily infected organs.
- Nerve fibers were compressed by accumulations of yeast-like cells.
- In heavy infections, the tissue of gill lamellae was destroyed with subsequent dilatation or compression.
- Cellular immune responses included hemocytic infiltration, agglutination and encapsulation, and phagocytosis.
- Phagocytosis of yeast-like cells was abundant in the connective tissue associated with the exoskeleto**n**



- **Vicente et al.,2010**

Pathogenecity:



Vicente et al.,2010

Epidemiology:

- LCD spreads towards northern and southern mangroves.
- LCD appears to spread by a pattern of waves, with large mortalities events followed by subsequently smaller events separated from each other by a period of time (usually a year).
- The limit of distribution of LCD to the north appears associated to the high salinity and temperatures of coastal water, as indicated by experimental data.
- The spread of LCD is apparently limited to significant changes in the coastal line of Brazil, which changes from a north-south direction to an almost east-west direction. The change in the pattern of currents, their proximity to the coast and the scarce presence of mangrove areas in the coast immediately south of the limit of distribution likely explain the reduction in the spreading of the LCD.

Causative agents

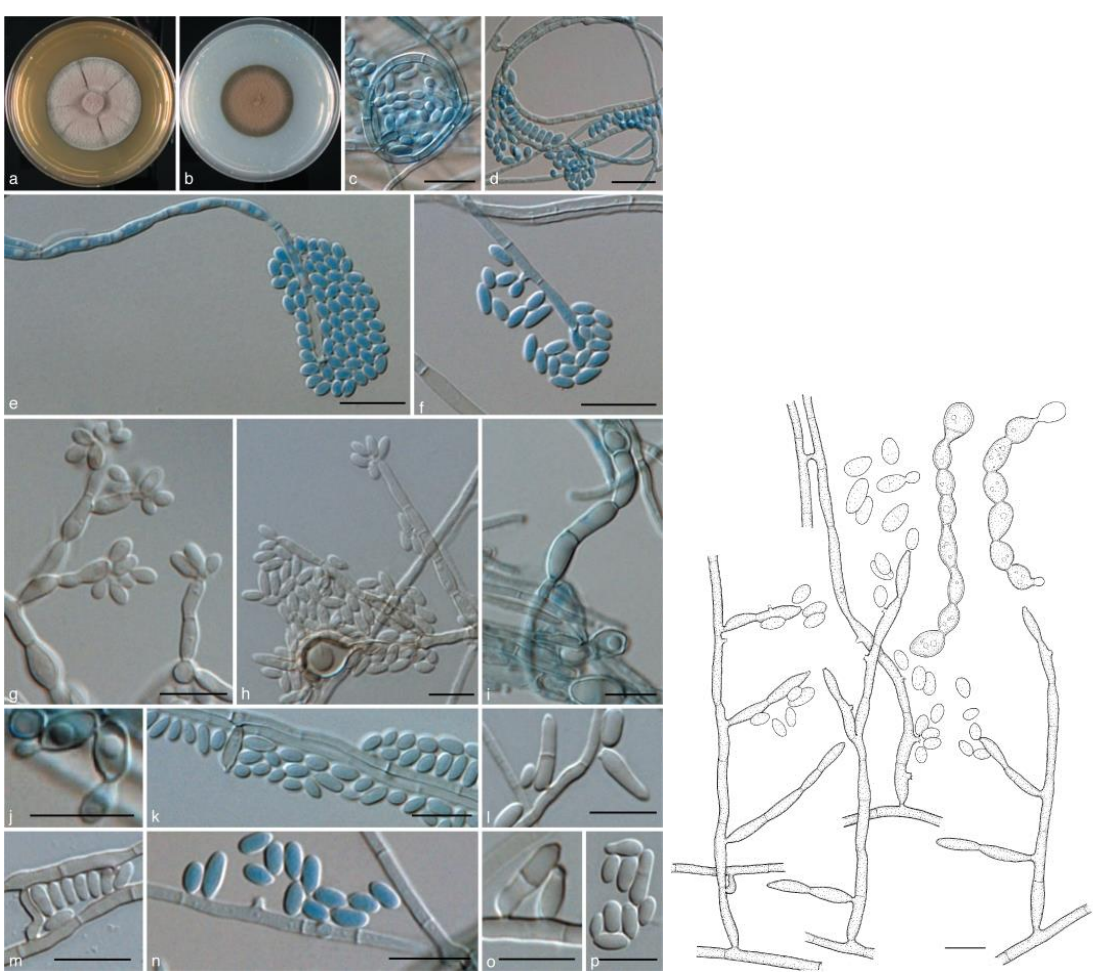
Two species of black yeast-like fungi of the family Herpotrichiellaceae (ascomycete order Chaetothyriales) as confirmed in in vivo and in vitro experiments:

- The *E. cancerae* is considered as the main agent since it was present in all events of Lethargic Crab Disease
- The *C. brasiliensis* takes an advantage of weak crabs affected by *E. cancerae*, suggesting an existence of an opportunistic infection

Description

Exophiala cancerae de Hoog, Vicente, Najafzadeh, Harrak, Badali, Seyedmousavi & Boeger, *sp. nov*

Colonies moderately expanding, circular, initially (on day 3) flat, olivaceous black, slimy with velvety, olivaceous grey centre and flat margin, later (on day 14) becoming velvety, dark olivaceous grey. Reverse olivaceous black, without diffusible pigment. Yeast cells nearly absent. *Conidiophores* short, erect, brown, cylindrical, multi-celled, poorly differentiated. *Conidia* 0–1-septate, subhyaline to pale brown, obovoidal to cylindrical, 4.9–8.0 × 2.7–4.8 µm.

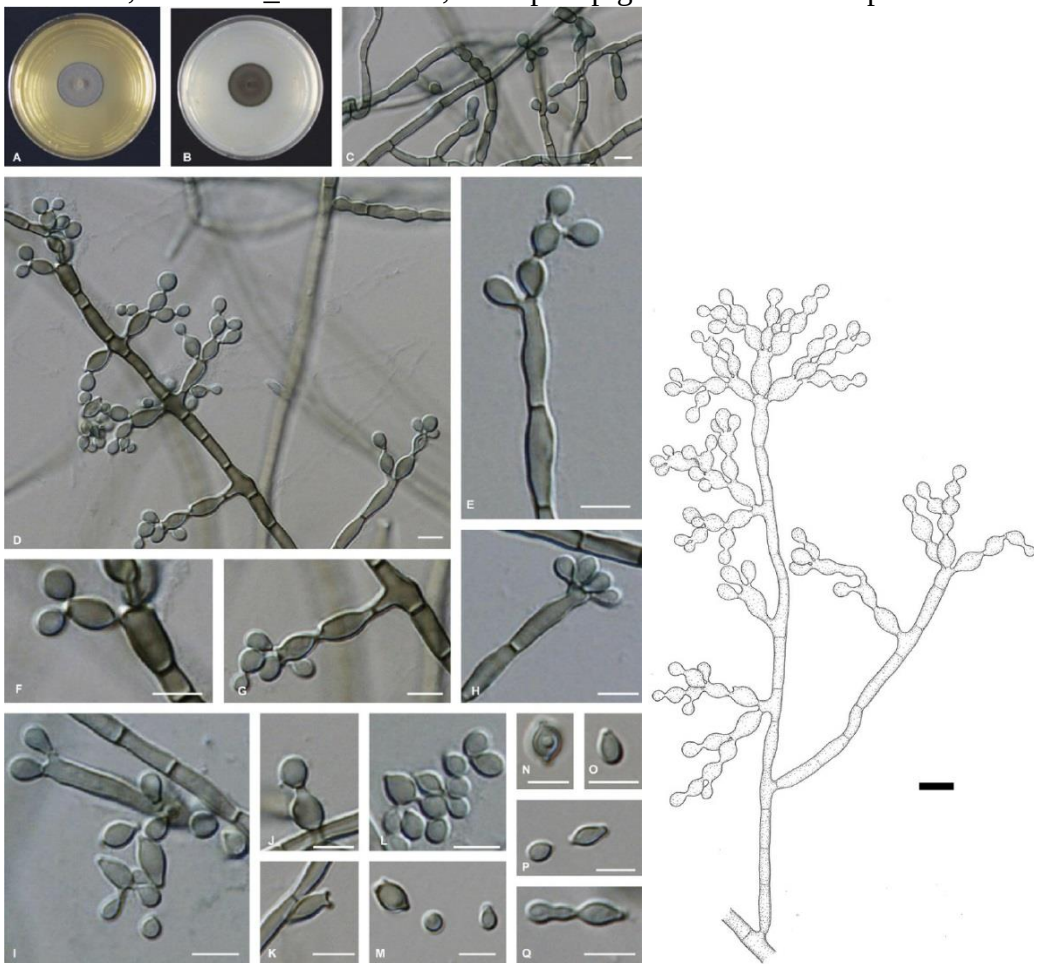


Exophiala cancerae, CBS 120420. a. Colony on MEA; b. colony on PDA; c, d. spirally twisted hyphae; e, f. short, erect, cylindrical, multi-celled conidiophores; h, i. apical and intercalary

chlamydospores; j. budding cells; k, l. intercalary conidiogenous cells; m. hyphae and conidia with anastomoses; n–p. conidia. — Scale bars = 10 μm.

Fonsecaea brasiliensis V.A. Vicente, Najafzadeh, Klaassen and de Hoog, sp. nov. (Figs. 7 and 8). MycoBank MB 561621.

Colonies moderately expanding, 30 mm diam, initially (on day 3) olivaceous black, with olivaceous grey centre and flat margin, later (on day 14) becoming velvety, greyishblack, with grey center. Margin straight, entire. Reverse greyish to olivaceous black. No diffusible pigment produced on any medium. Conida formed in densely branched, acropetal chains of max. 5 conidia, often located on distinct denticles, olivaceous brown, smooth-walled, ellipsoidal to lemon-shaped, narrowed towards both ends, 6.5–10.0 _ 2.0–3.5 mm; scars pale pigmented. Teleomorph unknown.

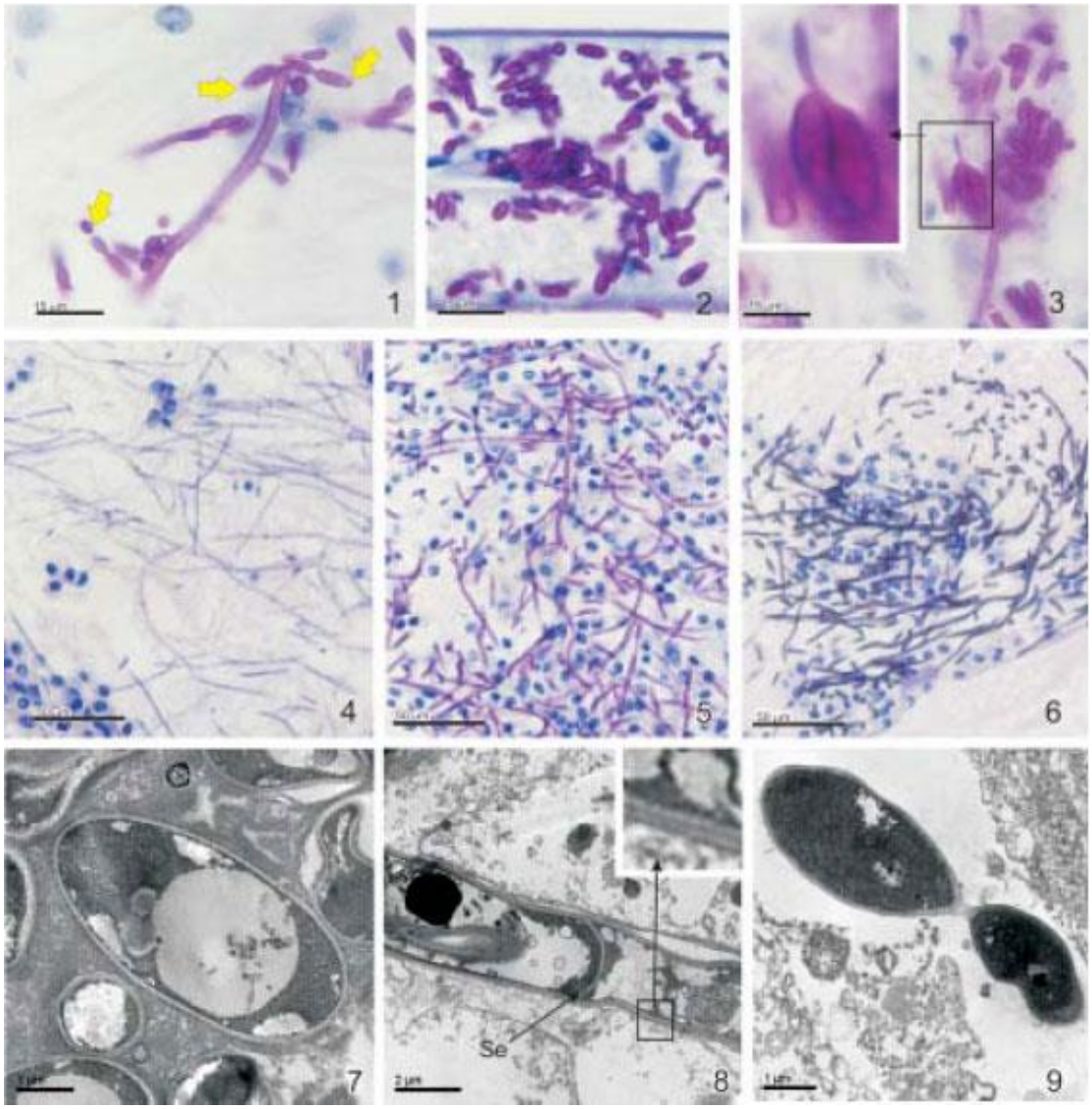


Fonsecaea brasiliensis , CBS 119710. (A and B) Colonies on MEA and PDA, respectively. (C) Olivaceous brown hyphae with conidia; (D–G) Conidia formed in short acropetal chains; (H) Sympodial cluster of conidia; (I–Q) Conidia.

Reports:

BOEGER *et al.* (2005) provided robust evidence suggesting that LCD is caused by

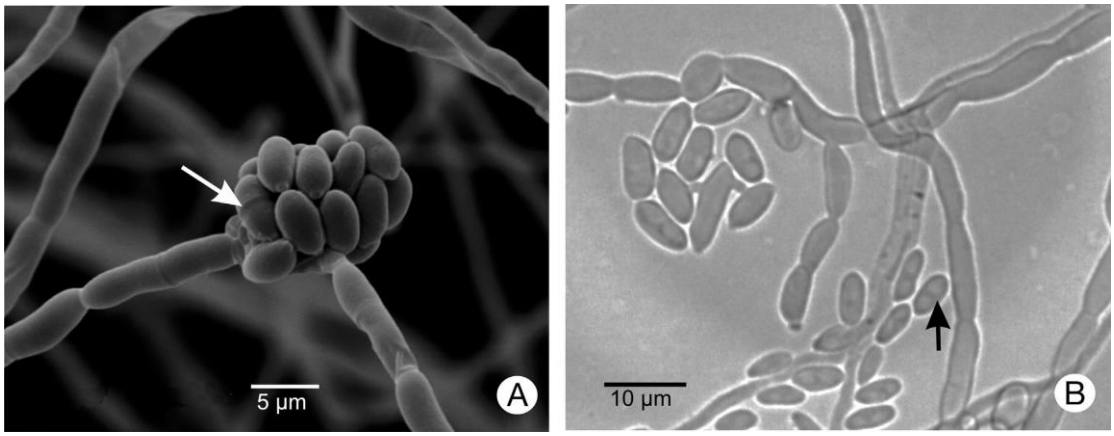
an anamorph Ascomycota (Fungi). Specimens of *U. cordatus* collected from stocks affected by LCD were examined. Histological and TEM methods detected the presence of hyphae, conidia, and condiophores in several host tissues. Moreover, the abundance of fungal stages was negatively associated with crab health. Finally, DNA was isolated from the fungus and a region of its 18S ribosomal gene was sequenced. Phylogenetic analyses not only confirm the diagnosis of the LCD fungus in crab tissues as an ascomycete, but also suggest a close relationship with members of the subphylum Pezizomycotina.



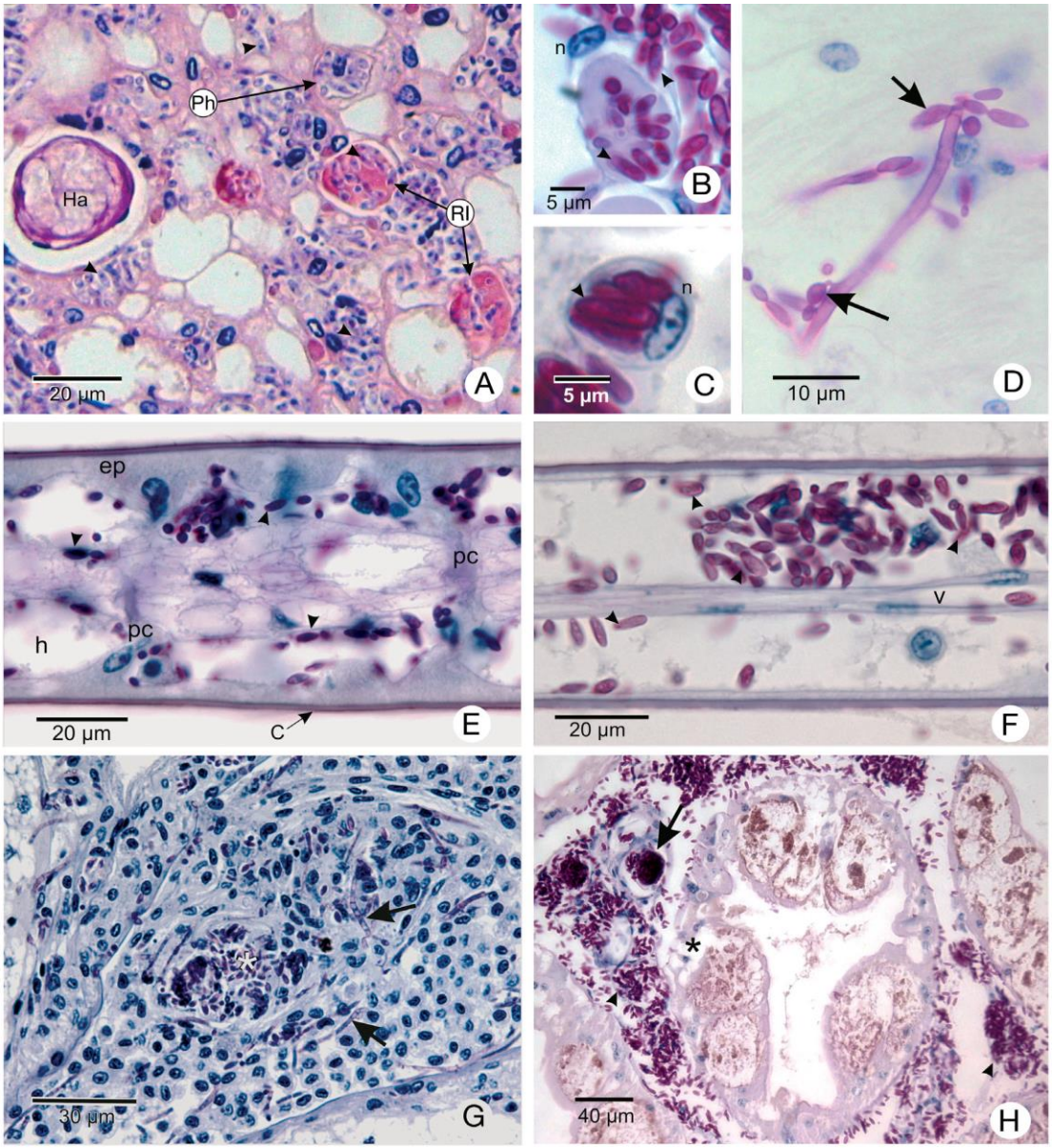
Ascomycota from *Ucides cordatus.* 1: light micrograph of a conidiophore in a histological section of the cardiac tissue (PAS). 2: light micrograph of transverse section of a gill lamella with numerous conidia in lacunae (PAS). 3: light micrograph of a conidium germinating in the cardiac tissue (PAS). 4: light micrograph of cardiac tissue parasitized by hyphae stained with H&E. 5: light micrograph of cardiac tissue parasitized by hyphae stained with PAS (counter stained with H&E). 6: Light micrograph of cardiac tissue parasitized by hyphae stained with GMS (counterstained with H&E). 7: TEM micrograph of a conidium. 8: TEM micrograph of a hypha depicting septum (Se) and cell wall composed of two layers (detail). 9: TEM micrograph of two attached conidia attached (catenate). **BOEGER *et al.* (2005)**

Orélis-Ribeiro ***et al.*** **(2012)** evaluated the efficiency of cooking procedures on the inactivation of the etiologic agent. The variation of the internal temperature of crabs and tests of the activity of *Exophiala cancerae*to temperature under simulated cooking condition were determined and the results were analyzed combined. The results indicate that crab's core body attains the boiling water temperature about an average of 14 min after exposition. Furthermore, short intervals of exposure (30 s) to such boiling temperatures were sufficient to warrant inactivation of *E.cancerae*. Thus, the traditional mode of preparation of the mangrove-land crab is sufficient to inactivate the pathologic agent and the consumption of sick or carrier animals should not represent a potential public health risk.

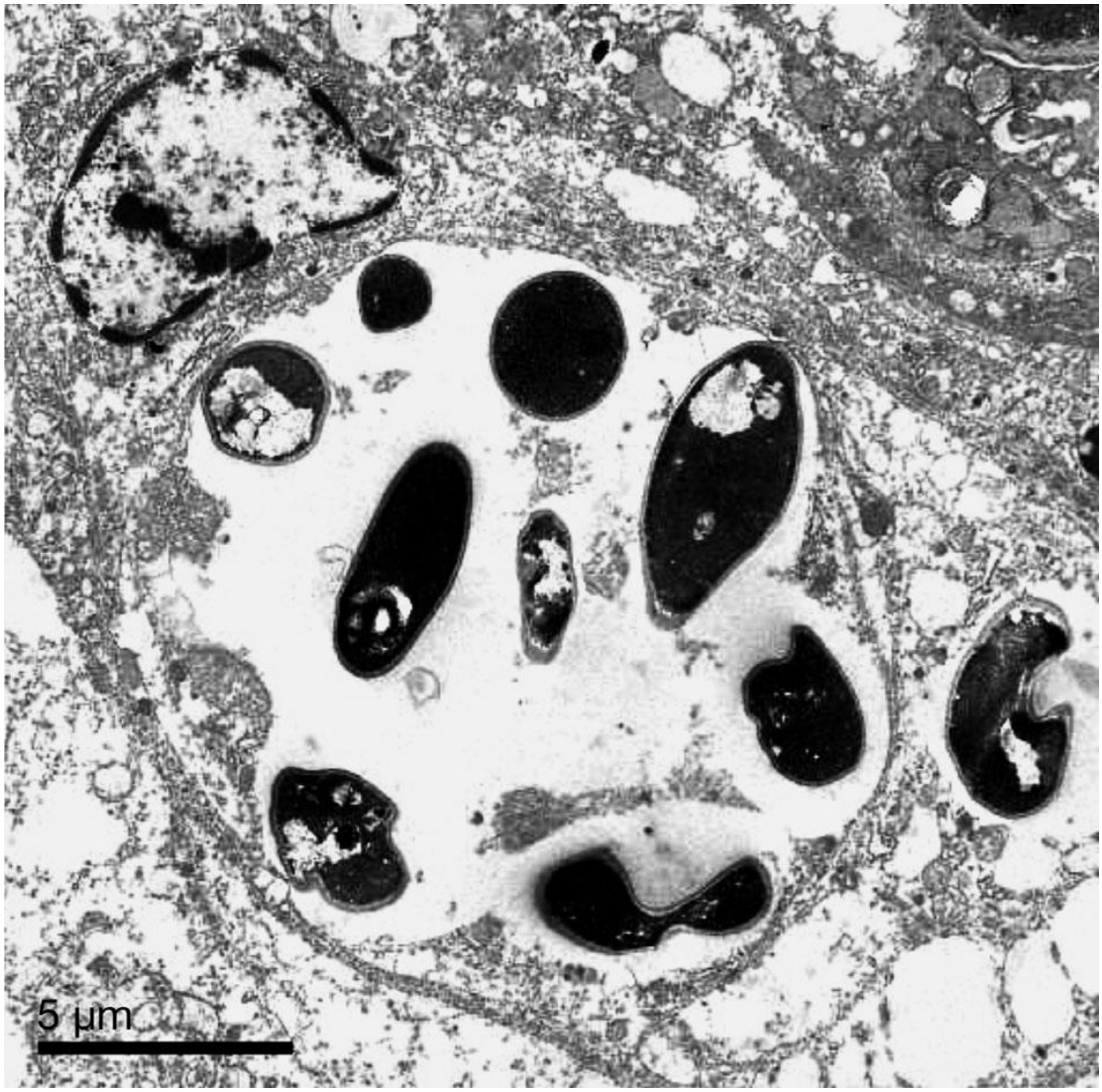
Boeger ***et al.*** **(2007)** mentioned that Lethargic crab disease (LCD) has caused extensive epizootic mortality of the mangrove land crab Ucides cordatus (Linnaeus 1763) (Brachyura: Ocypodidae) along the Brazilian coast. Direct culture of tissue samples from sick crabs and subsequent isolation and purification identified the causative agent as an Exophiala species of fungus. The histopathology of crabs with variable signs of LCD indicates that the most affected tissues are the epidermis, connective tissue, heart, hepatopancreas, nervous system, and gills. Gonads, somatic muscles, and digestive system are less affected by the fungus. The observed pathology is compatible with the clinical signs of LCD. Necrosis, tissue degeneration, and congestion of hemal sinuses and vessels are present in heavily infected organs. Nerve fibers may be compressed by accumulations of yeast-like cells. In heavy infections the tissue of gill lamellae is destroyed with subsequent dilation or compression. Cellular immune responses include hemocytic infiltration, agglutination and encapsulation, and phagocytosis. Phagocytosis of yeast-like cells is abundant in the connective tissue associated with the exoskeleton. These results indicate that LCD is the result of a systemic phaeohyphomycosis caused by a species of Exophiala. The present study also suggests that dispersal of the fungus within the crab occurs through the hemal system.



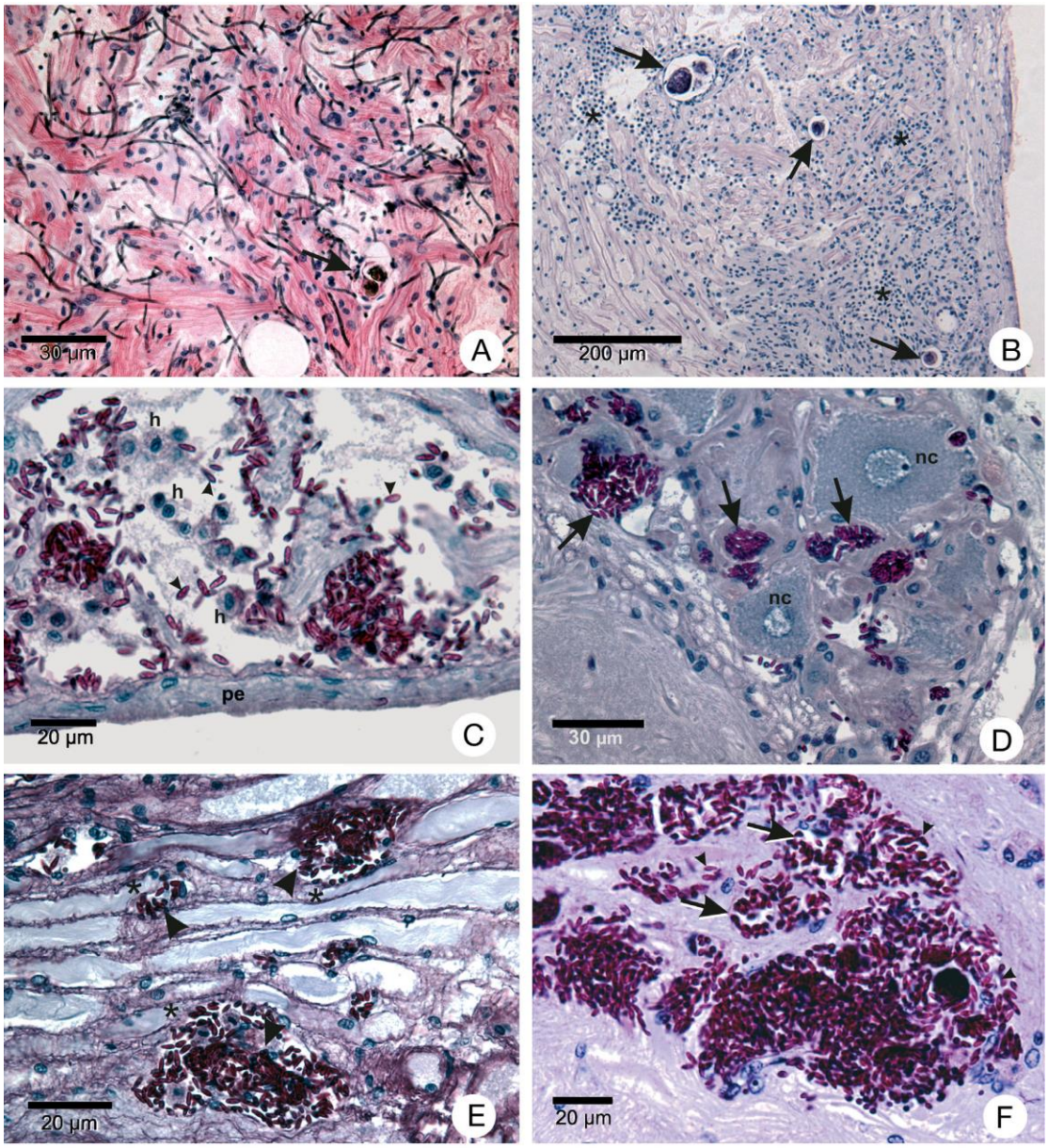
Exophiala sp. isolated from *Ucides cordatus* showing signs of the lethargic crab disease. (A) Scanning electron micrograph of a conidial apparatus and hyphae of *Exophiala* sp. Arrow points to a conidium with external evidence of septum. (B) Light microscopy micrograph of conidia, many evidencing internal septum (arrow) **BOEGER *et al.* (2007)**



Ucides cordatus infected with an Exophiala sp. and showing signs of lethargic crab disease (LCD). (A) Yeast-like cells (arrowheads) concentrated in the intersticial spaces of the connective tissue or within reserve-inclusion cells (RI), phagocytic cells (Ph), and melanized hemocytic encapsulation (Ha). Haematoxylin and eosin (H&E) stain. (B) RI cell with numerous yeast-like cells (arrowheads) in the connective tissue associated with the exoskeleton. n = nucleus of the cell. Periodic acid Schiff (PAS) and H & E stain. (C) Phagocytic cell (probably a hyalinocyte) of the connective tissue associated with the exoskeleton with numerous yeast-like cells (arrowhead). n = nucleus of the cell. PAS and H&E stains. (D) Conidiogenous hypha producing numerous conidia (arrows) in the myocardium. PAS and H&E stains. (E) Gills of mangrove land crab with LCD in intermediate stage of infection. Although many yeast-like cells (arrowheads) are visible, most of its components are relatively intact. c = cuticle. pc = pillar cell. ep = epithelium. h = hemal lacuna. PAS and H&E stains. (F) Gills of moribund mangrove land crab with LCD. Numerous yeast-like cells (arrowheads) are associated with the absence of most cellular components of the gills, except for the central vessel (v). PAS and H&E stains. (G) Large haemocytic agglutination congesting the hemal sinus between tubules of the hepatopancreas, with both yeast-cells (asterisk) and hyphae (arrows). PAS and H&E stains. (H) Yeast-like cells (arrowhead) and hemocytic encapsulations (arrow) occlude hemal sinuses of the hepatopancreas. The hepatopancreas caecum shows signs of necrosis (asterisk). PAS and H&E stains **BOEGER *et al.* (2007)**



Ucides cordatus. Phagocytic cell within the connective tissue associated with the exoskeleton. A large vacuole contains numerous yeast-like cells of a species of Exophiala **BOEGER *et al.* (2007)**

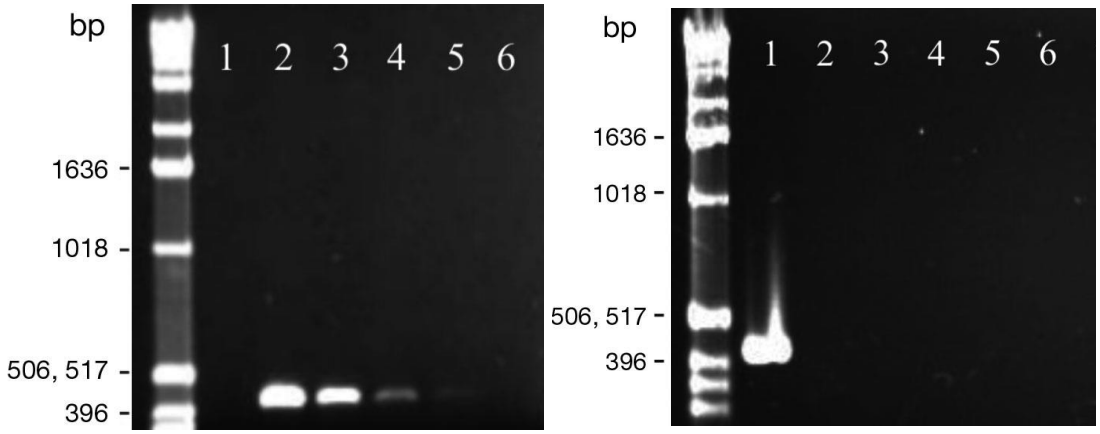


Ucides cordatus infected with Exophiala sp. and showing signs of lethargic crab disease (LCD). (A) Cardiac tissue infected mostly with hyphae showing extensive tissue disorganization and hemocytic encapsulations (arrow). Grocott's methanamine silver (GMS) and haematoxylin and eosin (H&E) stains. (B) Heart of a healthy mangrove land crab showing no gross signs of LCD. Yeast-like cells are present only within hemocytic encapsulations (arrows) and extensive infiltration of hemocytes is evident (asterisk). Periodic acid Schiff (PAS) and H&E stain. (C) Heart of a moribund mangrove land crab with LCD. Disruption and disorganization of cardiac-muscle fibers associated with the yeast-like cells (arrowheads) with extensive hemocytic infiltration (h). Note that pericardia (pe) are free of yeast-like cells. PAS and H&E stains. (D) Neurosecretory area of the thoracic ganglion of a moribund mangrove land crab with LCD with hemal sinuses congested by yeast-like cells (arrows). nc = neurosecretory cells. PAS and H&E stains. (E) Nerve fibers of a moribund mangrove land crab with LCD showing clusters (arrowheads) of yeast-like cells and resulting compression of some individual fibers (asterisk). PAS and H&E stains. (F) Nerve ganglion of a moribund mangrove land crab with LCD with a large cluster of yeast-like cells (arrowheads) and associated necrosis of the tissue (arrows). PAS and H&E **BOEGER *et al.* (2007)**

Boeger (2011) mentioned that Since 1997, the Lethargic Crab Disease (LCD) has caused caused extensive epizootic mortality of the mangrove land crab Ucides cordatus (Brachyura: Ocypodidae) along the Brazilian coast, mainly in the

Northeastern region. Causative agents are two species of black yeast-like fungi of the family Herpotrichiellaceae (ascomycete order Chaetothyriales) as confirmed in in vivo and in vitro experiments. The disease is systemic, causing extensive damages to the heart, hepatopancreas , circulatory system and nervous system of the crab. The animals are usually found death or moribund outside of their burrows, lethargic and often tetanic. Since the first year it was detected, it spread towards northern and southern mangroves. The disease appear to spread by a pattern of waves, with large mortalities events followed by subsequently smaller events separated from each other by a period of time (usually a year). The limit of distribution of LCD to the north appears associated to the high salinity and temperatures of coastal water, as indicated by experimental data. The southernmost limit, the state of Espirito Santo, has been submitted to recurrent mortalities events since 2006. The spread of the disease towards mangroves located to south of this state is apparently limited to significant changes in the coastal line of Brazil in this region, which changes from a north-south direction to an almost east-west direction. The change in the pattern of currents, their proximity to the coast and the scarce presence of mangrove areas in the coast immediately south of the limit of distribution likely explain the reduction in the spreading of the LCD. Presently, mangroves subjected to LCD mortalities present significant recuperation of mangrove-land crab populations but continuation of studies to determine the origin of the outbreaks are necessary to allow prediction of future impacts.

Marcio *et al.* (2011) sequenced the internal transcribed spacer (ITS) of the rDNA region of Exophiala cancerae and developed species-specific PCR primers. Sensitivity tests indicated that the developed protocol is capable of detecting very small amounts of target DNA. Also, the application of the protocol to a variety of other dematiaceous fungi did not generate any false positives. The specific primers provided in the present study represent an important tool for rapidly surveying a large number of crab individuals, as well as environmental samples. Such knowledge will be instrumental in understanding the epidemiological dynamics of LCD.

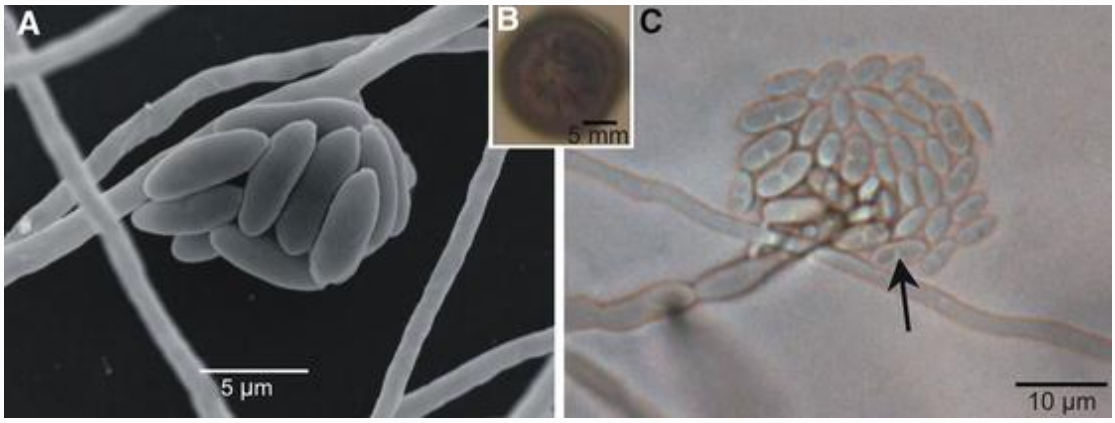


Sensitivity tests of the specific primers for the detection of the Exophiala-like black yeast associated with lethargic crab disease. Template DNA for the PCR in each lane: Lane 1, genomic DNA of Ucides cordatus; Lanes 2–5, respectively, 5, 0.5, 0.05, and 0.005 ng of fungal DNA; Lane 6, negative control

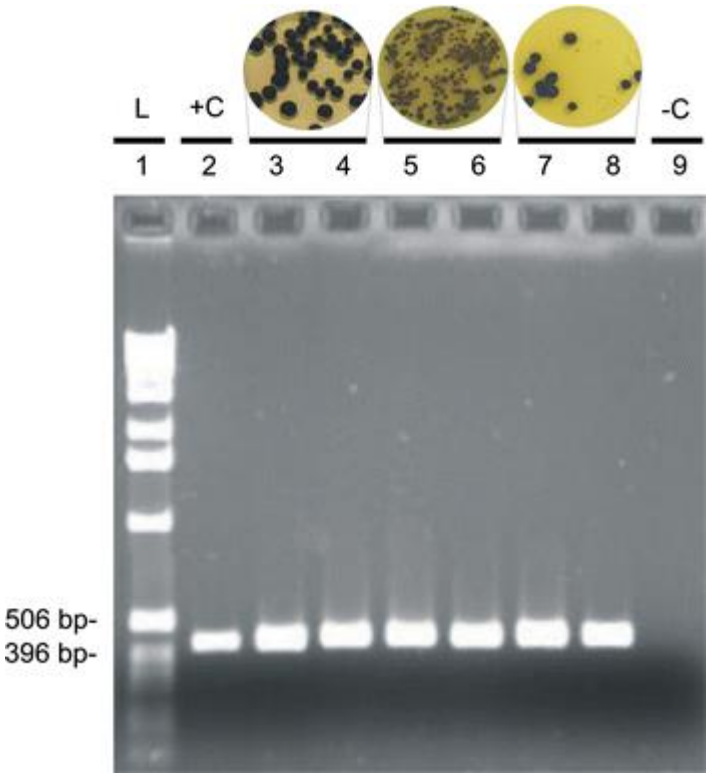
Specificity tests of the specific primers for the detection of the Exophiala-like black yeast associated with lethargic crab disease. Template DNA for the PCR in each lane: Lane 1, E. cancerae (CBS120420); Lane 2, Ramichloridium atrovirens (CBS685.76); Lane 3, R. atrovirens (CBS677.76); Lane 4, Ramichloridium sp. (CBS102238); Lane 5, Fonsecaea pedrosoi (CBS253.49); Lane 6, Cladophialophora immunda (CBS102237); Lane 7, C. saturnica (CBS118724); Lane 8, E. spinifera

(HC-EML); Lane 9, E. jeanselmei (HC-EJ4); Lane 10, negative control **Marcio *et al.* (2011)**

Orélis-Ribeiro *et al.* (2011) experimentally infected LCD-free specimens of *U. cordatus* with *Exophiala cancerae* (strain CBS 120420) isolate. During the 30-day experimental period, only a single death was observed within the control crabs. However, at the end of this period, crabs that were inoculated once or three-times with mycelial elements and hyphae of *E. cancerae* had a 60% and 50% mortality rates, respectively ($n = 6$ and $n = 5$). These results support that the fungal agent is pathogenic and is the causative agent of LCD. Species-specific molecular markers confirm the presence of *E. cancerae* (strain CBS 120420) in recovered colonies and tissue samples from the infected animals. The experimentally infected crabs manifested signs (lethargy, ataxia and tetany) that were consistent to LCD-affected animals in the environment. These results fulfil Koch's postulates and the hypothesis that the tested strain of *Exophiala cancerae* is a causative agent of LCD is accepted.



Macro- and micromorphological characteristics of *Exophiala cancerae* (strain CBS 120420) a Scanning electron micrograph of a conidiophore. b Colony surface growth on Mycosel, incubated for 14 days at 25°C. c Light microscopy image of conidia; the *black arrow* indicates the internal septum **Orélis-Ribeiro *et al.* (2011)**



Amplification of an *Exophiala* sp. (*strain CBS 120420*) specific fragment, which was used to screen colonies that were recovered from the tissue of artificially infected crabs. *Lane 1*: molecular size marker (1 kb ladder) (L); *2*: *E. cancerae* (*strain CBS 120420*) (positive control) (+C); *3–8*: DNA extract of colonies that were recovered from the heart (*3* and *4*), hepatopancreas (*5* and *6*) and thoracic ganglion (*7* and *8*); and *9*: negative control (–C). The pictures above the gel are the respective agar plates with colony growth **Orélis-Ribeiro *et al.* (2011)**

Vicente *et al.* (2012) carried out a study to prove that two species are involved in the disease: the recently described black yeast Exophiala cancerae, but also a less virulent, hitherto undescribed fonsecaea-like species, introduced here as the novel species Fonsecaea brasiliensis. Strains were identified by ITS rDNA sequencing, and species borderlines were established by multilocus sequencing and AFLP analysis. Fonsecaea brasiliensis proved to be closely related to the pathogenic species Cladophialophora devriesii which originally was isolated from a systemic infection in a human patient. The virulence of F. brasiliensis is lower than that of E. cancerae, as established by artificial inoculation of mangrove crabs.

Guerra *et al.* (2013) stated that knowledge of natural ecology is essential for a better understanding of pathogenicity and opportunism in black yeast-like fungi. Although etiological agents of diseases caused by these fungi are supposed to originate from the environment, their isolation from nature is difficult. This is probably due to their oligotrophic nature, low competitive ability, and, overall, insufficient data on their natural habitat. We obtained environmental samples from mangrove areas where mortalities by lethargic crab disease (LCD) are reported and areas without disease recorded. Isolation of chaetothyrialean black yeasts and relatives was performed using a highly selective protocol. Species-specific primers were used to determine if these isolates represented Exophiala cancerae or Fonsecaea brasiliensis, two proven agents of LCD, in order to test hypotheses about the origin of the disease. Isolates, identified by morphology as Fonsecaea- or Exophiala-like, were tested specific diagnostic markers for the fungi associated with LCD. Although several black fungi were isolated, the main causative agent of the LCD, E. cancerae, was not found. Molecular

markers for F. brasiliensis revealed 10 positive bands for isolates from biofilms on mangrove leaves, branches, and aerial roots, of which four were confirmed by ITS sequencing. The absence of E. cancerae in environmental samples suggests that the species is dependent on the crab, as a genuine pathogen, different from F. brasiliensis, which is probably not dependent on the host species, U. cordatus. However, we did not attempt isolation from the marine water, which may represent the pathway of dispersion of the black yeast species between neighbor mangrove.

References:

1. Boeger, Walter A, Pie, Marcio R, Ostrensky, Antonio, & Patella, Luciana. (2005). Lethargic crab disease: multidisciplinary evidence supports a mycotic etiology. *Memórias do Instituto Oswaldo Cruz*, *100*(2), 161-167.
2. Boeger WA, Pie MR, Vicente V, Ostrensky A, Hungria D, Castilho GG (2007) Histopathology of the mangrove land crab *Ucides cordatus*(Ocypodidae) affected by lethargic crab disease. Dis Aquat Org 78:73-81
3. Boeger, W. A. CURRENT STATUS OF LETHARGIC CRAB DISEASE IN BRAZIL Fourth meeting of the ISHAM working groups on Black Yeasts and chromoblastomycosis: "Hidden Danger, Bright Promise.ISHAM, Curitiba, Brazil, 1−4 December, 2011
4. Guerra, R. S., Mariana Machado Fidelis do Nascimento, Stephanie Miesch , Mohammad Javad Najafzadeh, Raphael Ore´lis Ribeiro, Antonio Ostrensky, Gerrit Sybren de Hoog, Vania Aparecida Vicente, Walter A. Boeger. Black Yeast Biota in the Mangrove, in Search of the Origin of the Lethargic Crab Disease (LCD). Mycopathologia (2013) 175:421–430
5. Marcio R. Pie, Walter A. Boeger, Luciana Patella, Vânia A. Vicente, Raphael O. Ribeiro, Antonio Ostrensky. Specific primers for the detection of the black-yeast fungus associated with lethargic crab disease (LCD). DISEASES OF AQUATIC ORGANISMS Dis Aquat Org. Vol. 94: 73–75, 2011 doi: 10.3354/dao02312
6. **Orélis-Ribeiro, R., Boeger, W.A., Vicente, V.A. et al.** Fulfilling Koch's postulates confirms the mycotic origin of Lethargic Crab Disease **Antonie van Leeuwenhoek (2011) 99: 601. doi:10.1007/s10482-010-9531]**
7. Orélis-Ribeiro, R , Marcelo A. Chammas, Antonio Ostrensky, Walter A. Boeger. Viability of the etiologic agent of the Lethargic Crab Disease,*Exophiala cancerae*, during cooking of the mangrove-land crab: Does this traditional dish represent a risk to humans? Food ControlVolume 25, Issue 2, June 2012, Pages 591–593
8. Vania Vicente, M. Javad, Najafzadeh Jiufeng Sun, Hamid Badali Marcio, Pie Stephanie Miesch Walter Boeger, Sybren de Hoog. Lethargic Crab Disease, Causative agents of Lethargic Crab Disease (LCD) in mangrove land crab Ucides cordatus (Ocypodidae) in Brazil. CBS. http://blackyeast2010.bf.uni-lj.si/fileadmin/userfiles/Lectures/Vicente1.pdf
9. Vicente VA, Orélis-Ribeiro R, Najafzadeh MJ, Sun J, Guerra RS, Miesch S, Ostrensky A, Meis JF, Klaassen CH, de Hoog GS, Boeger WA. **Black yeast-like fungi associated with Lethargic Crab Disease (LCD) in the mangrove-land crab, Ucides cordatus (Ocypodidae).** Vet Microbiol. 2012 Jul 6;158(1-2):109-22.

19.Ochroconis

- ***Abbott (1927)** designated the genus Scolecobasidium*
- **de Hoog and von Arx in (1983)** suggested the nomenclature of genus *Ochroconis* to include all morphologically similar fungi,

i.e. *Scolecobasidium constricta, Scolecobasidium tshawytschae and Dactylaria gallopava*.

- **Salkin and Dixon (1986)** compared the morphologic and physiologic properties and proposed that *D. gallopava* be regarded as *D. gallopava var. gallopava* and the remaining isolates be given a separate variety of *D. humicola var. humicola*. The genus *Ochroconis* thus contains *O. gallopava, O.tshawytshae, and O. humicola.*
- *Ochroconis* is identified by the presence of brown hyphae with two-celled, pale brown, smooth-walled, cylindrical conidia, constricted at the septum, inability to liquefy gelatin, inability to grow at 37, 40 and 45 °C, ability to grow at 25 and 30 °C and urease positivity. Nucleotide sequence data of ITS region of rDNA were unable to identify *Ochroconis* to species level as it provided only 98 % identity. However, 28S region identified it to the species level with 99 % similarity.
- *Ochroconis humicola* is a member of dematiaceous fungi, and recognized as an etiological agent of fungal infection in aquatic animals, especially in fishes (Ross and Yusutake 1973; Ajello et al. 1977; Schaumann and Priebe 1994; Wada et al. 1995; Bowater et al. 2003; Wada et al. 2005; Munchan et al. 2006).
- *Ochroconis tshawytschae is* a rare fish pathogen

Classification:

Index fungorum

Fungi +
- Ascomycota +
  - Incertae sedis +
    - Incertae sedis +
      - Incertae sedis +
        - **Ochroconis de Hoog & Arx 1974**
          - *Ochroconis anellii* (Graniti) de Hoog & Arx 1973
          - Ochroconis atlantica A. M. Wellman 1975
          - *Ochroconis constricta* (E. V. Abbott) de Hoog & Arx 1974
          - *Ochroconis crassihumicola* (Matsush.) de Hoog & Arx 1973
          - Ochroconis gallopava (W. B. Cooke) de Hoog 1983
          - *Ochroconis gamsii* de Hoog 1985
          - *Ochroconis humicola* (G. L. Barron & L. V. Busch) de Hoog & Arx 1973
          - *Ochroconis simplex* (Papendorf) de Hoog & Arx 1973
          - Ochroconis tshawytschae (Doty & D. W. Slater) Kiril. & Al-Achmed 1977
          - *Ochroconis variabilis* (G. L. Barron & L. V. Busch) de Hoog & Arx 1973
          - *Ochroconis verruculosa* (R.Y. Roy, R.S. Dwivedi & R.R. Mis

NCBI Taxonomy

Cellular organisms +
- Eukaryota +
  - Opisthokonta +
    - Fungi +
      - Dikarya +
        - Ascomycota +
          - Saccharomyceta +
            - Pezizomycotina +
              - Leotiomyceta +

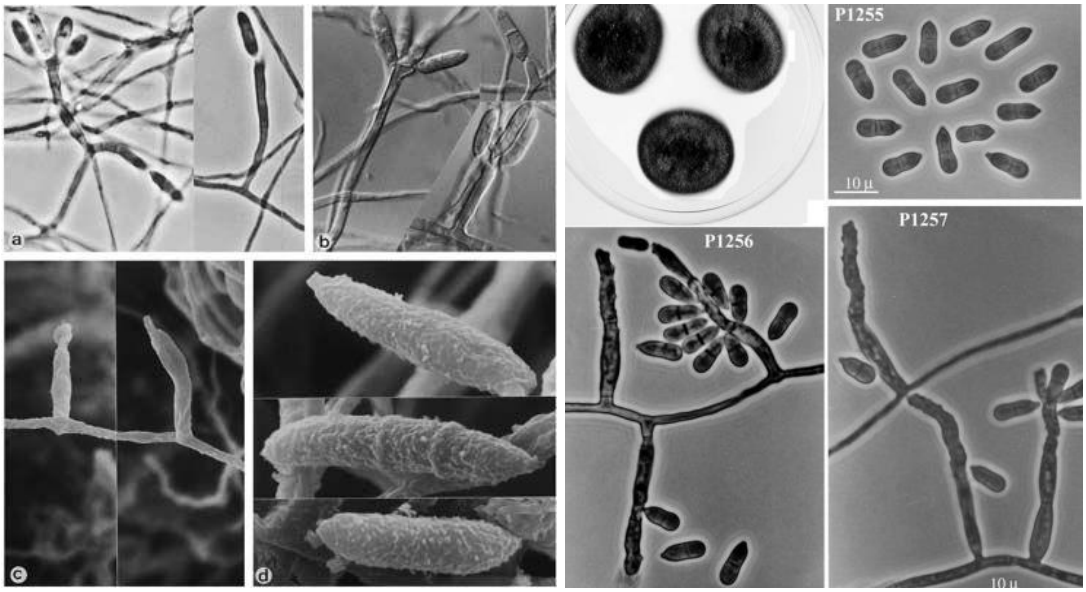
- Dothideomyceta +
  - Dothideomycetes +
    - Dothideomycetes incertae sedis +
      - Venturiales +
        - Sympoventuriaceae +
          - **Ochroconis**
            - Ochroconis aff. gamsii BGE-2014
            - *Ochroconis anellii*
            - *Ochroconis anomala*
            - Ochroconis cf. constricta CBS 124172
            - *Ochroconis constricta* +
            - *Ochroconis cordanae*
            - *Ochroconis gamsii*
            - *Ochroconis humicola*
            - *Ochroconis icarus*
            - *Ochroconis lascauxensis*
            - *Ochroconis longiphorum*
            - *Ochroconis macrozamiae*
            - *Ochroconis minima*
            - *Ochroconis mirabilis*
            - *Ochroconis olivacea*
            - *Ochroconis ramosa*
            - *Ochroconis sexualis*
            - Ochroconis sp. BGE-2014
            - Ochroconis sp. CBS 119644
            - Ochroconis sp. CBS 124172
            - Ochroconis sp. CBS 175.65
            - Ochroconis sp. CBS 206.96
            - Ochroconis sp. CCFEE 5865
            - Ochroconis sp. CCFEE 5991
            - Ochroconis sp. CM13020
            - Ochroconis sp. FF-2011
            - Ochroconis sp. GH-2013
            - Ochroconis sp. L1265
            - Ochroconis sp. LX M3-2
            - Ochroconis sp. LX M6-3
            - Ochroconis sp. MX409
            - Ochroconis sp. SW258
            - *Ochroconis tshawytschae*
            - *Ochroconis verrucosa*

Description:

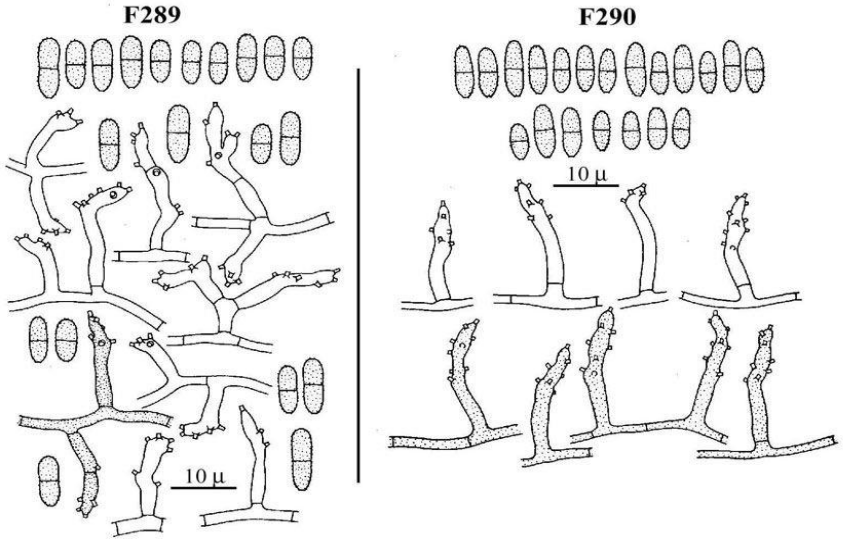
Ochroconis humicola (G.L. Barron & L.V. Busch) de Hoog & Arx, Kavaka 1: 57 (1973)

≡Scolecobasidium humicola G.L. Barron & L.V. Busch, Canadian Journal of Botany 40 (1): 83 (1962)

Colony characteristics. Colonies (OA) growing slowly, flat, velvety, brown, brownish-grey at the centre. Microscopy. Hyphae subhyaline to pale olivaceous, smooth- and thick-walled, aerial hyphae often strongly flexouse; small sclerotial bodies may be present in the submerged mycelium. Conidiophores erect, flexuose, cylindrical, up to about 100 ?m long, 2-3 ?m wide; conidia produced on long denticles. Conidia mostly two-celled, smooth-walled or verruculose, pale olivaceous brown, cylindrical to slightly clavate, with rounded ends, 7-15 x 2.5-4.0 µm.



1-145 *Scolecobasidium humicola*



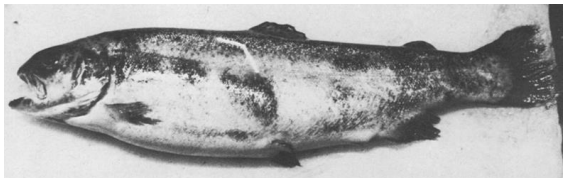
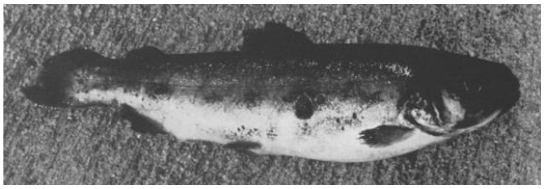
Mycobank

Reports:

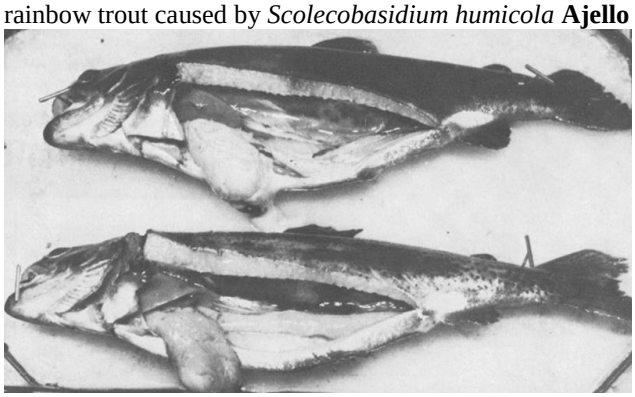
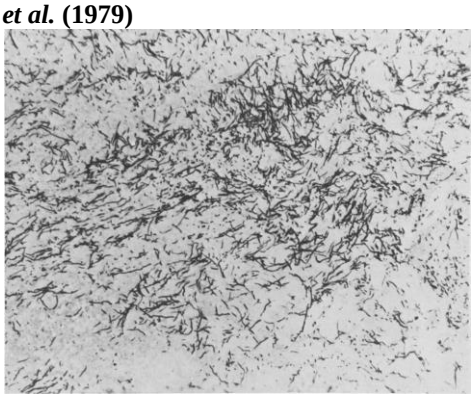
Ross and Yasutake (1973) isolated *Scolecobasidium humicola*, a previously undescribed fungal pathogen of fish from coho salmon (*Oncorhynchus kisutch*). In natural infections the kidney was the organ most affected. The disease was difficult to transmit experimentally and appeared to be only weakly contagious.

Ajello *et al.* (1979) reported a previously undescribed host for the opportunistic dematiaceous hyphomycete, Scolecobasidium humicola, . Several epizootics among rainbow trout, Salmo gairdneri, occurred in a Tennessee fish hatchery from 1969 to 1973. Symptoms included surface lesions, blisters and abscesses. The kidneys and other internal organs were invaded by the mycelium of S. humicola. Tissue morphology of the fungus was typical of that associated with phaeohyphomycosis Experimental infections were reproduced in fingerling rainbow trout after intraperitoneal inoculation of S. humicola. Following a change in the hatchery's water

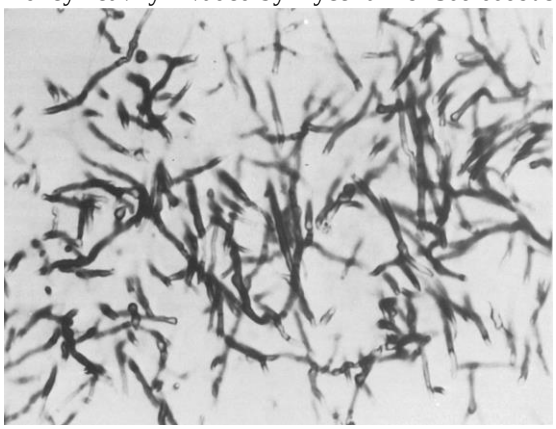
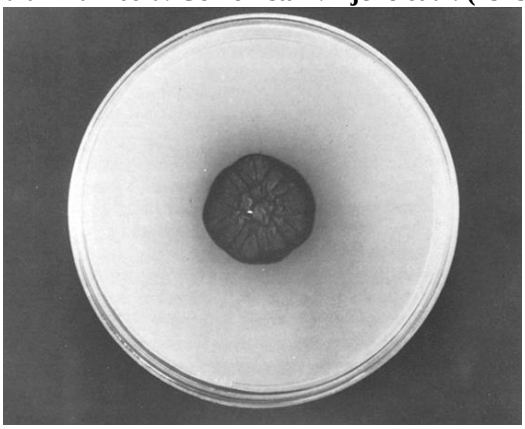
supply, no new epizootics have occurred.

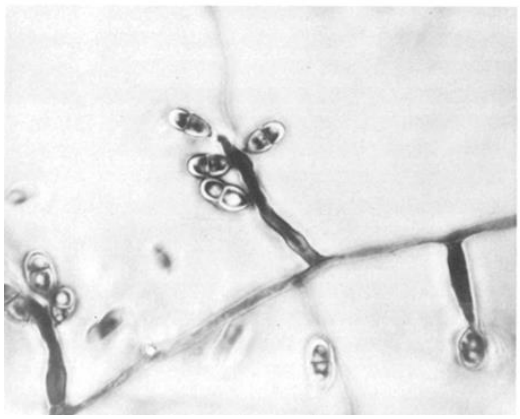
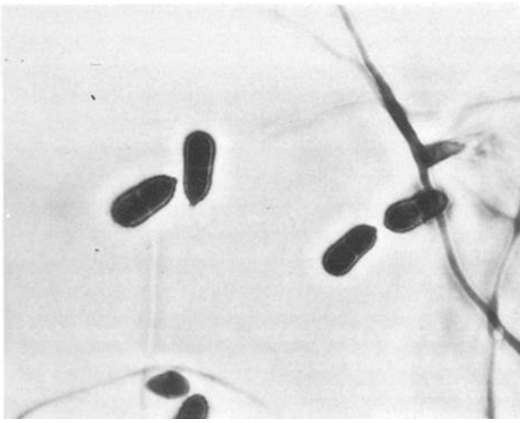
Rainbow trout with furuncle on its side caused by *Scolecobasidium humicola.* Lateral lesions on rainbow trout caused by *Scolecobasidium humicola* **Ajello *et al.* (1979)**

Swollen kidneys of rainbow trout caused by *Scolecobasidium* humicola.Section of rainbow trout kidney heavily invaded by mycelium of *Scolecobasidium humicola.* Gomori stain. **Ajello *et al.* (1979)**

Mycelium of *Scolecobasidium humicola* in kidney tissue of rainbow trout. Gornori stain. Original magnification Three-week-old colony of *Scolecobasidium humicola* on Sabouraud's dextrose agar **Ajello *et al.* (1979)**

Conidiophore of *Sco(ecobasidium humicola.* Eight-dayold slide culture, V-8 juice agar. Finely echinulate, two-celled conidia of *Scolecobasidium humieola.* **Ajello *et al.* (1979)**

Hatai and Kubota (1989) described Ochronconis sp. infection in masu salmon (Oncorhynchus masou) with visceral mycosis in Japan. The external and internal clinical signs were reddening of the anal area, swelling of the abdomen due to accumulation of ascitic fluid in the abdominal cavity and extensive swelling of the posterior kidney. Many pale brown, septate hyphae were found in the kidney by direct

microscopical examination; these were usually not found in the other organs of infected fish. Histopathological examination of the kidney revealed large granulomas with the fungal hyphae and giant cells. The isolated fungus was identified as a species of the genus Ochroconis and was compared with O. tshawytschae, a known fish pathogen. Based on morphological and growth characteristics, we believe that these cases resulted from infection with a different species.

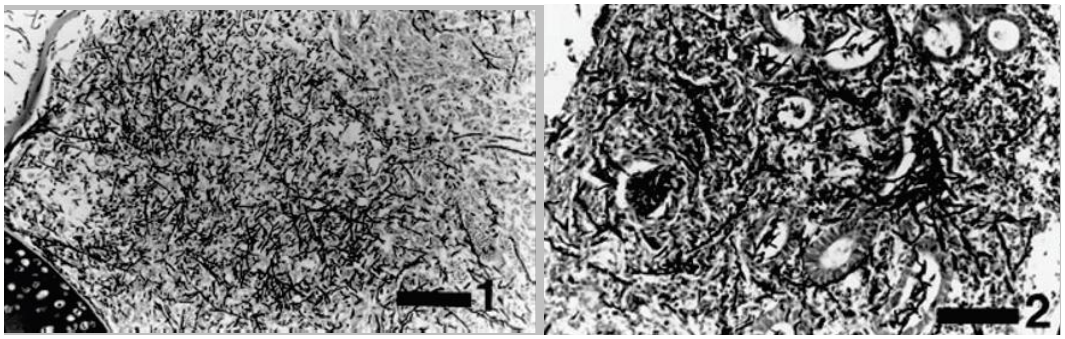
Schaumann and Priebe (1994) isolated a dematiaceous hyphomycete from black discoloured areas of the somatic musculature of a specimen of Atlantic salmon. The fungus caused an endogenous mycosis and obviously must be classified as a facultative or opportunistic pathogen of marine fish. The optimum temperature for the strain was in the range between 20 and 25 °C, and the temperature tolerance for growth ranged from 10 to almost 37 °C. The morphological and physiological investigation revealed that the pathogen belongs to the genus *Ochroconis* de Hoog et von Arx, which is synonymous with *Scolecobasidium* Abbott. However, the definite species identification raised some difficulties, because the characteristics of the pathogenic isolate H 14 670 variably matched with at least four of the known species within the *Ochroconis – Scolecobasidium —Dactylaria* complex, i.e., *O. humicola*, *O. constrictum*, *O. gallopavum*, and *O. simplex*. Because of this multispecies affinity and taking into account the actual confused taxonomic state within this group of hyphomycetes, especially with regard to the pathogenic strains, the identification of the present isolate as *O. humicola* remains with some reservation.

Wada *et al.* (1995) described a new disease characterized by open ulcers on the body surface in cultured devil stinger, Inimicus japonicus (Synanceiidae; Jap anese name: Oni-Okoze) in the south-west region of Japan. They examined a total of five fish averaging 1.4 g in body weight collected from Kagoshima Prefecture at the end of January 1994. They described the isolated potential fungal pathogen, Ochroconis humicola from the lesions and its histopathology. In four out of the five fish examined, open ulcers were formed at dorsal part of the body surface. Although the fish examined showed little appetite, no mortality was recorded. The center of the lesion was necrotic and sloughed, leaving trunk muscles exposed in a crater-shaped cavity surrounded by an erosious periphery. Direct microscopical examination of the exposed trunk muscles revealed numerous fungal hyphae, which were septate and approximately 1 to 2 um in width. Fungi were isolated by inoculating a small piece of trunk muscle of the fish on glucose-yeast extract peptone-seawater (PYGS) agar1) at 25 ^{o}C. To inhibit bacterial growth, 500µg/ml each of ampicillin and streptomycin sulfate were added to the medium. Fungal colonies were subcultured on PYGS agar to obtain pure cultures. The isolate NJM 9471 was used for more detailed examination. The identifica tion was made by the slide culture method at 25°C. Using light microscopy, the slide cultures were ex amined for conidiogenesis. Fungal colonies on PYGS agar were slow growing; colonies were slightly domed, velvety to floccose and pale brown in color. Hyphae were septate, pale brown in color and 1 to 2fÊm in width. Conidia were usually sparse, 1.8-2.2 to 7.0-10.0 um, two-celled, smooth-walled, pale brown in color and cylindrical with rounded ends. The reproductive mode of the conidia was sympodial. It was identified as Ochroconis humicola. After removal of a portion of ventrolateral abdominal body wall, the fish were routinely necropsied. All organs were fixed in 10% phosphate buffered (pH 7.0) formalin solution. The fixed tissues were processed to make paraffin sections and stained with H & E, methenamine silver-nitrate, Grocott's variation and counter-stained with Giemsa (Grocott-Giemsa), perodic acid Schiff (PAS) reaction. Histopathologically, tissue from epidermis to stratum compactum extensively sloughed, leaving wide

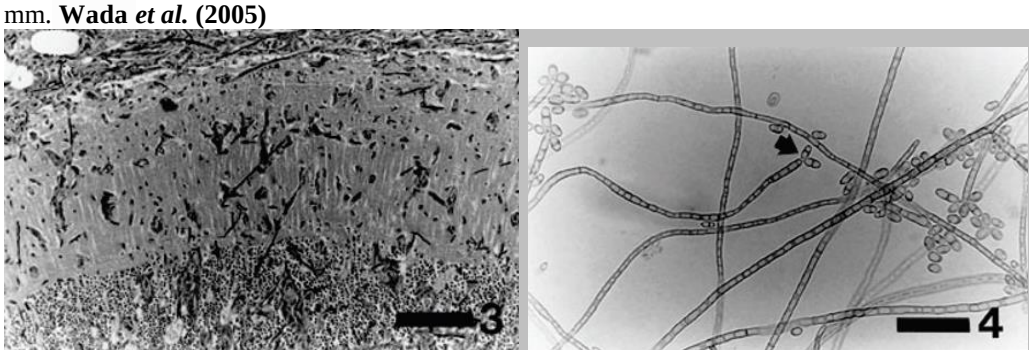
necrotic area in the trunk muscle layer with large numbers of septate fungal hyphae.

Wada *et al.* (2005) described two clinical cases associated with O. humicola found in a new host species in Japan. A total of five red sea bream Pagrus major and seven marbled rockfish Sebastiscus marmoratus were examined. The average body weight of the fish examined was 1.2 g for red sea bream and 1.0 g for marbled rockfish. The red sea bream were collected in Kumamoto Prefecture, Kyushu-island, Japan, in the middle of April 1998, and the marbled rockfish were collected in Yamaguchi Prefecture, Honshu-island, Japan, in the middle of June 1998. Whole bodies of the examined fish were fixed in 10% phosphate-buffered formalin solution after dissection of ventral body walls. After decalcification with 10% EDTA solution, the samples were routinely embedded in paraffin and sectioned at 4–5 mm. The serial sections were stained with hematoxylin and eosin (HE), Gomori's methenamine-silver nitrate, Grocott's variation counterstained with HE (Grocott-HE) and Giemsa (Grocott-Giemsa), perodic acid-Schiff (PAS) reaction, and Schmorl method. The stained sections were examined under a light microscope. In order to isolate the fungi, small pieces of trunk muscles of the lesions were inoculated on glucose yeast extract-peptone-seawater (PYGS) agar and incubated at 25^{o}C. To inhibit bacterial growth, ampicillin and streptomycin sulfate were added to the medium at a concentration of 500 mg/mL each. Fungal colonies were subcultured on PYGS agar to obtain pure cultures. The identification of the fungal isolates was made by the slide culture method at 25^{o}C. Using a light microscope, the slide cultures were examined for conidiogenesis. Both cases showed apparent lesions on the body surfaces. In the red sea bream, severe ulcerations were found around the base of the dorsal fins, while erosive and/or ulcerative lesions mainly appeared at the mouth regions in the marbled rockfish. These erosive and/or ulcerative features on the body surfaces were also reported in devil stinger with O. humicola infection, while other Ochroconis infections in salmonids did not show any external disease signs. Histopathological features of the fungal lesions were quite similar between the two fish species examined in this study, as follows: mats of septate fungal hyphae were widely scattered in the dermal layer with degenerated cellular debris; and many hyphae penetrating into the subcutaneous tissue, trunk muscles and the adjacent internal organs such as the head and trunk kidney. Some hyphae were observed in the renal tubles and glomeruli. The hyphae often infiltrated into the crania, and they sometimes reached to cerebral tissue with a slight to moderate inflammatory response. The hyphae in the dermal and muscular layers were surrounded by epithelioid cell granulomas. In the most part of these lesions, the granulomas were not discrete and formed large mass of granulomatous tissue. Multinucleated giant cells were not observed in the present cases. Although the hyphae were easily recognized in Grocott-HE or Grocott-Giemsa preparation, they were stained pale brown with HE and positively with PAS reaction and Schmor method. The hyphae were approximately 1.5–2.0 mm in width. These characteristics suggested that they belonged to imperfect fungi with melanin pigment and, therefore, they should be classified as dematiaceous fungi. Histopathology of the present cases was characterized by numerous imperfect fungal hyphae surrounded by epithelioid cell granulomas without multinucleated giant cells. These features corresponded to those in many of the imperfect fungal infections in the other fish species. The colonies of the fungi isolated from red sea bream and marbled rockfish grew slowly on the medium and showed same morphological characteristics. The colonies were slightly domed, velvety to floccose, and pale brown in color. Hyphae were septate and pale brown in color. Conidia were usually sparse,

two-celled, smooth-walled, pale brown in color and cylindrical with rounded ends. The reproductive mode of the conidia was sympodial. From these features, the isolates were identified as Ochroconis humicola according to de Hoog and von Arx and de Hoog.10 From the histopathological findings, it was suggested that the main site of infection in these cases were dermis and musculatures, while the hyphae also invaded internal organs such as the head kidney, cranium and brain, indicating that the diseases were invasive fungal infections



Skin lesion of the marbled rockfish showed massive fungal hyphae infiltration in the dermis. Grocott's variation counter-stained with Giemsa. Bar = 100 mm. Cross-section of the kidney of the marbled rockfish. Note the numerous fungal hyphae infiltrated to the adjacent head kidney and some hyphae embedded in renal tubles. Grocott's variation counter-stained with hematoxylin and eosin. Bar = 100 mm. **Wada *et al.* (2005)**



Note the fungal hyphae scattered in the cerebral tissue of the marbled rock fish. Grocott's variation counter-stained with hematoxylin and eosin. Bar = 100 mm. Two-celled conidia (arrow) of the isolated fungi on glucose-yeast extract-peptone-seawater agar. Bar = 20 mm. **Wada *et al.* (2005)**

Munchan *et al.* (2009a) tested the antifungal activities of amphotericin B, fluconazole, 5-fluorocytosine, itraconazole, micafungin, miconazole, terbinafine and voriconazole against four strains of Ochroconis humicola isolated from fish by the broth microdilution method. Three of these drugs (itraconazole, terbinafine and voriconazole) were effective against all isolates. The most active drug was terbinafine (for liniment) with a MIC (MFC) range of 0.06 to 0.13 (0.0625 to 0.125)μg/ml. Itraconazole (for oral administration), with a MIC (MFC) range of 0.5 to 2.0 (0.5 to 1.0)μg/ml, was chosen for in vivo treatment. In vivo treatment with itraconazole of striped jack Pseudocaranx dentex experimentally infected with O. humicola was conducted for 50 days. No fish died, but grey to white nodules were found in the visceral membrane, kidney, liver and spleen in the fish. Granulomatous inflammatory reactions were histopathologically found in all fish injected with conidia of O. humicola NJM 0472. Clinical signs and histopathological findings indicated that itraconazole showed no efficacy for curing the fish infected with O. humicola.

Experimental fish 50 days post-inoculation showed abundant white nodules (white arrows) in internal organs (A) and kidney (B).**Munchan *et al.,* 2009**

Munchan *et al.* (2006) mentioned that, in April 2004, a fungal infection occurred in cultured young striped jack *Pseudocaranx dentex* at a fish farm in Ehime Prefecture, Japan. The cumulative mortality reached about 25% in one month after the disease was first recognized. Moribund fish showed disease signs such as abdominal swelling and distended kidney. A fungus was purely isolated from the kidney of the fish using PYGS agar. The colony was pale brown in color, and the conidia were two-celled, cylindrical to oblong with rounded ends and smooth-walled. From these morphological characteristics, the fungus was identified as *Ochroconis humicola*. This infection of marine fishes has been reported in the skin of juvenile fish, but not known in young fish. This paper describes the first case of *O. humicola* infection in visceral organs of young striped jack.

Munchan *et al.* (2009b) compared the histopathology of young striped jack *Pseudocaranx dentex* experimentally infected with the dematiaceous fungus *Ochroconis humicola* NJM 0472 with that of spontaneously infected fish. Moribund and freshly dead fish from both groups showed similar histopathology, and appeared to have been killed due to hyphae penetrating the visceral organs. Fish that survived the infection appeared to be able to suppress the fungal growth by well-established inflammatory reaction involving mycotic granulomas and granulation tissues. The results suggested that two types of *O. humicola* infection occur in young striped jack: an acute type infection, which is characterized by penetrating hyphae that cause direct tissue destruction and a chronic type infection, which is characterized by severe inflammatory reaction that causes functional disorders of the affected organs.

Machouart *et al. (2014)* stated that *Ochroconis* is a genus of ascomycete fungi that includes oligotrophic saprobes and some opportunistic species causing infections in vertebrates. The most important of these opportunists is the neurotropic species *Ochroconis gallopava*, which occurs in birds and occasionally in immunocompromised humans. Other *Ochroconis* species have been isolated from superficial infections of cats, dogs and fish. In their natural environment, these species are found in litter, soil, and on moist surfaces. Some thermophilic species have been isolated from hot springs, industrial effluents, and self-heated plant material. Although their ecology and epidemiology has been investigated, their classification within the ascomycetes is still unknown

Samerpitak *et al.* (2015) mentioned that the genus *Ochroconis*, typified by *O. constricta*, was morphologically segregated from a genus with lobed conidia, *Scolecobasidium* by de Hoog & von Arx, for melanized fungi with sympodial conidiogenesis and septate, ellipsoidal conidia which were liberated rhexolytically. They studied species diversity by analyzing more variable genes in addition to the partial ribosomal operon, i.e., the partial coding genes, actin (*ACT1*), β-tubulin (*BT2*)

and translation elongation factor 1-α (*TEF1*) and recognized thirteen species in *Ochroconis*. The taxonomic status of *Scolecobasidium* was considered to be doubtful because of ambiguity of the type material, *S. terreum*. The strict morphological parameters to demarcate the genera were abandoned at the expense of a phylogenetic approach. Species with forked conidia similar to *S. terreum* were added to *Ochroconis* on phylogenetic grounds as members of *Sympoventuriaceae*. Some of the species that were morphologically classified in *Scolecobasidium* are currently not available for sequencing, and their classification remains unresolved.

References:

1. Ajello L, McGinnis MR, Camper J. **An outbreak of phaeohyphomycosis in rainbow trout caused by Scolecobasidium humicola.** Mycopathologia. 1977 Nov 30;62(1):15-22.
2. Hatai K, Kubota SS. A visceral mycosis in cultured masu salmon (*Oncorhynchus masou*) caused by a species of *Ochroconis*. *J. Wildlife Dis.* 1989; **25**: 83–88.
3. Machouart M, Samerpitak K, de Hoog GS, Gueidan C. A multigene phylogeny reveals that *Ochroconis*belongs to the family *Sympoventuriaceae* (Venturiales, Dothideomycetes) Fungal Divers. 2014;65:77–88.
4. Munchan, C., O. Kurata, K. Hatai, N. Hashiba, N. Nakaoka and H. Kawakami (2006) Mass mortality of young
5. striped jack *Pseudocaranx dentex* caused by a fungus *Ochroconis humicola. Fish Pathol*., **41**, 179-182.
6. MUNCHAN, C., Kishio HATAI, Shiyuusaku TAKAGI and Azumi YAMASHITA. In Vitro and In Vivo Effectiveness of Itraconazole against Ochroconis humicola Isolated from Fish. Aquaculture Sci. 57（3）, 399－404（2009a）
7. Munchan, C., O. Kurata, S. Wada, K. Hatai, N. Nakaoka and H. Kawakami (2009b) Histopathology of striped
8. jack *Pseudocaranx dentex* experimentally infected with *Ochroconis humicola. Fish Pathol*., **44**
9. Ross AJ, Yasutake WT. *Scolecobasidium humicola*, a fungal pathogen of fish. *J. Fish Res. Board. Can.* 1973; **30**: 994–995.
10. Samerpitak K, Gerrits van den Ende AHG, Menken SBJ, de Hoog GS. Three New Species of the Genus *Ochroconis*. *Mycopathologia*. 2015;180(1-2):7-17.
11. Schaumann, K., K. Priebe. *Ochroconis humicola* causing muscular black spot disease of Atlantic salmon (*Salmo salar*). *Canadian Journal of Botany*, 1994, 72(11): 1629-1634
12. Wada S, Hanjavanit C, Kurata O, Hatai K. *Ochroconis humicola* infection in red sea bream Pagrus major and marbled rockfish Sebastiscus marmoratus cultured in Japan. Fish Sci. 2005;71:682–684.
13. Wada S, Nakamura K, Hatai K. 1995. First case of Ochroconis humicola infection in marine cultured fish in Japan. Fish Pathol. 30:125–126

20.Phoma

- The genus Phoma is ubiquitous and species-rich, with species occurring on a diverse range of substrates, from soil to air, plants to animals, and even humans (Aveskamp et al. 2008, 2010).
- The genus Phoma is notorious because includes many important plant pathogen species, some of which are of quarantine concern (Aveskamp et al. 2008, 2010, Chen et al. 2015).
- Phoma species have been incriminated in infections of fish

- **Ross *et al.* (1975)** isolated Phoma herbarum from diseased hatchery-reared coho salmon (Oncorhynchus kisutch), chinook salmon (O. tshawytscha), and rainbow trout (Salmo gairdneri).
- **Easa (1979)** isolated *Phoma herbarum* from gills of diseased Common carp from El-Abbasa and El-Manzalah fish farms.
- **Sparks and Hibbits (1979)** isolated *Phoma fimeti* from cases of Black mat syndrome in *tanner crabs*.
- **Easa *et al.* (1984)** experimentally infected Armout catfish (*Clarias lazera*) with a strain of *Phoma herbarum* isolated from diseased carp fish (*Cyprinus carpio L.*) obtained from an Egyptian fish farm.
- **Hatai *et al.* (1986)** reported visceral mycosis in ayu fry, *Pleoglossus altivelis*, caused by species of Phoma.
- **Faisal *et al.* (2007)** reported Phoma herbarum in association with two outbreaks of systemic mycosis in hatchery-reared chinook salmon (Oncorhynchus tshawytscha) fingerlings.

Sp. *recognized by Index Fungorum:*

- Fungi +
  - Ascomycota +
    - Dothideomycetes +
      - Pleosporales +
        - Incertae sedis +
          - Phoma Sacc. 1880 +
            - ***Phoma herbarum***
            - *Phoma abdita* Sacc. 1880
            - *Phoma abietella-sibirica* Schwarzman 1952
            - *Phoma abietina* Hartig
            - *Phoma abietinae* Linds.
            - *Phoma abietis* Briard
            - *Phoma abietis-albae* Allesch. 1898
            - Phoma abnormis (Berk. & M. A. Curtis) Sacc. 1884
            - *Phoma abrotani* Oudem. 1902
            - *Phoma abscondita* Pass.
            - 3115 more... show full tree..

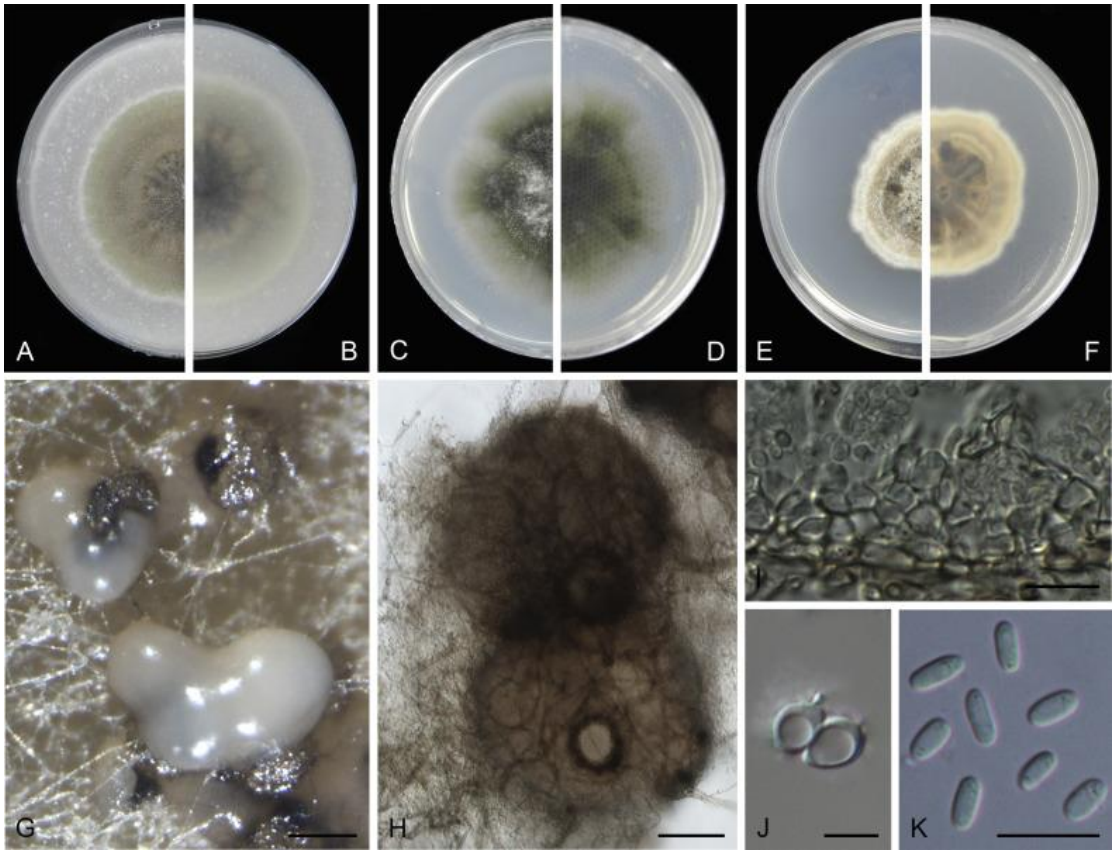
Description:

Phoma herbarum Westend., Bulletin de l'Académie Royale des Sciences de Belgique Classe des Sciences 19: 118 (1852)

=Phoma oleracea Sacc., Michelia 2 (6): 91 (1880) [MB#272816]
=Aposphaeria violacea Bertel, Österreichische Botanische Zeitschrift 54 (6): 205 (1904) [MB#152955]
=Phoma pigmentivora Massee, Bulletin of Miscellaneous Informations of the Royal Botanical Garden

Colony characteristics. Colonies (OA) relatively slow-growing, usually a reddish pigment being exuded into the agar, with sparse grey-green aerial mycelium, turning

purplish-blue instantaneously after application of a drop of 1N NaOH. Microscopy. Pycnidia spherical, 100-200 ?m diam, with distinct, rounded ostioles. Conidia hyaline, in mass hyaline to pinkish, oblong to cylindrical, unicellular, straight, 4-5 x 1.5-2.0 ?m.



Phoma herbarum (CBS 615.75). A–B. Colony on OA (front and reverse). C–D. Colony on MEA (front and reverse). E–F. Colony on PDA (front and reverse). G. Pycnidia forming on OA. H. Pycnidia. I. Section of pycnidial wall. J. Conidiogenous cells. K. Conidia. Scale bars: G = 100 µm; H = 50 µm; I, K = 10 µm; J = 5 µm.

Reports:

Ross *et al.* (1975) isolated Phoma herbarum from diseased hatchery-reared coho salmon (Oncorhynchus kisutch), chinook salmon (O. tshawytscha), and rainbow trout (Salmo gairdneri). The disease was observed at 10 national fish hatcheries in Washington and Oregon, but the low incidence of experimental infections indicate that it is only weakly contagious. Histopathological examination suggests that the air bladder is one of the primary organs infected. The visceral. organs are also affected in both natural and experimental infections

Easa (1979) isolated *Phoma herbarum* from gills of diseased Common carp from El-Abbasa and El-Manzalah fish farms. The histopathological picture as well as the results of re-isolation trials suggested that the gills might be the primary organ affected with *Phoma herbarum* in carp.

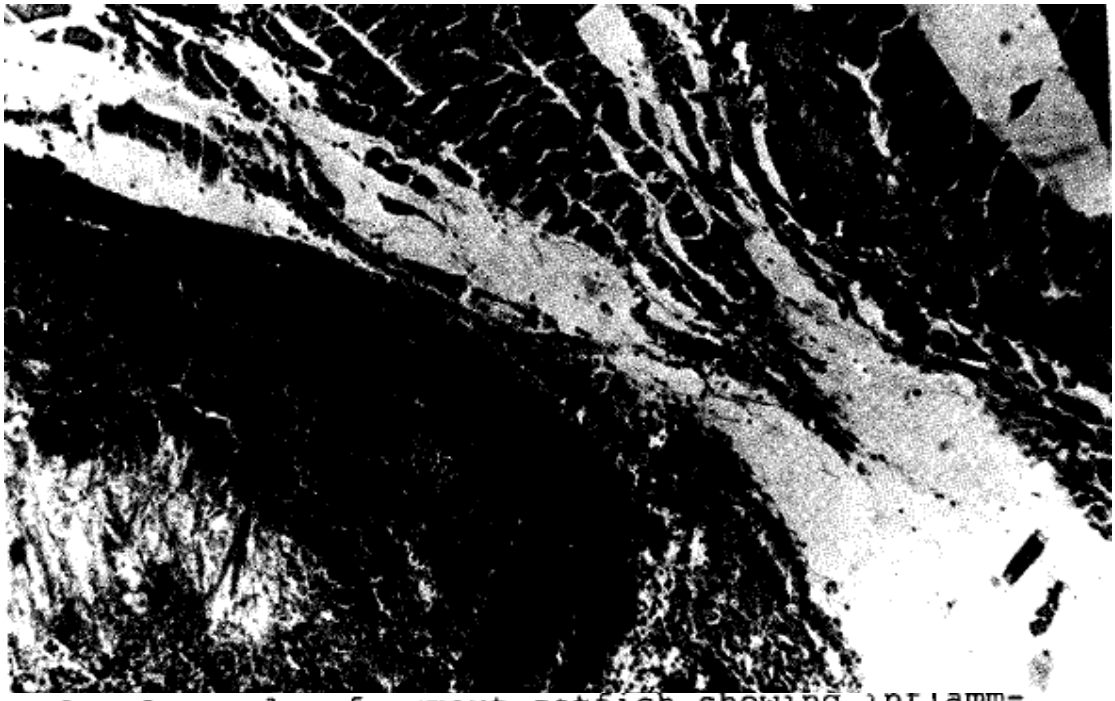
Sparks and Hibbits (1979) mentioned that Black mat syndrome, caused by an

encrusting fungus on the exterior of the carapace of *tanner crabs*, has been known for many years. Eleven *tanner crabs* from the Kodiak area of Alaska with and 9 without grossly recognizable masses of the fungus on the carapace were necropsied and examined histologically. In all individuals with the syndrome, hyphae of the fungus, previously identified as *Phoma fimeti*, penetrated the carapace and virtually replaced the underlying epidermis. In more advanced cases, the eyestalk was invaded and the epidermis destroyed, and hyphae extended into the eyestalk musculature and nervous tissue. Infections of the connective tissue sheaths surrounding the esophagus, stomach, heart, hemopoietic tissue, thoracic ganglion, antennal gland, and ovary have also been observed. None of the crabs without the syndrome contained internal hyphae. Although data on the lethality of the disease were not available, the ease with which the hyphae penetrate the chitinous exoskeleton, their extensive proliferation in the epidermis, and their ability to invade deep tissues causing obvious pathological effects, were highly suggestive that it was a virulent, probably fatal, disease that may have a significant impact on *tanner crab* population dynamics.

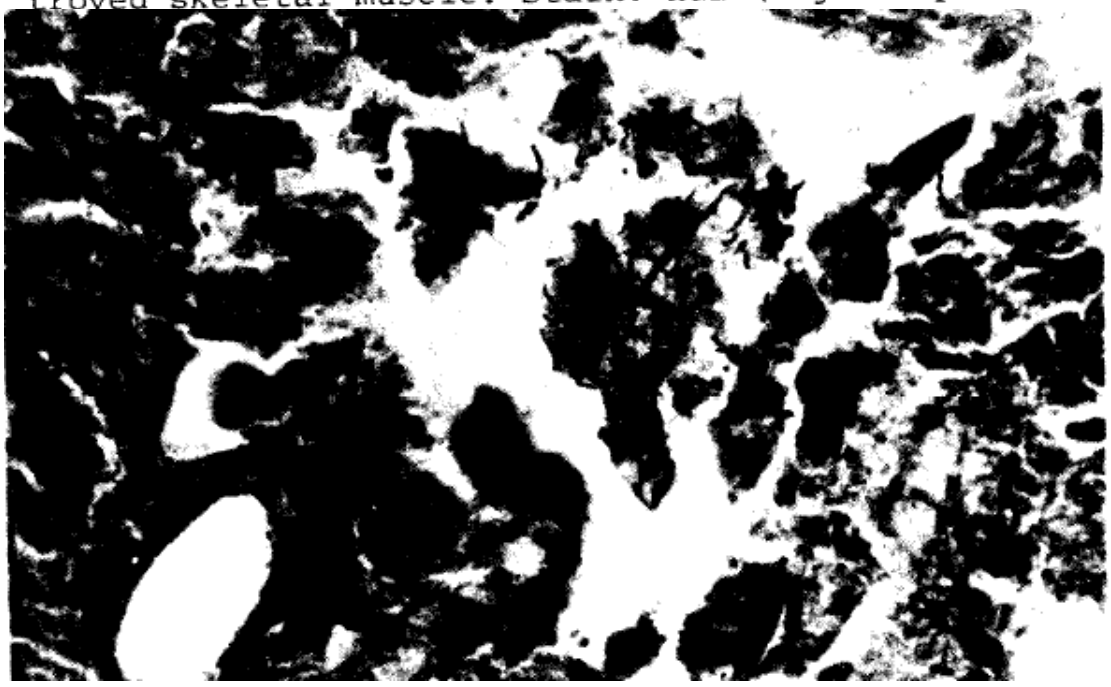
Easa *et al.* (1984) experimentally infected Armout catfish (*Clarias lazera*) with a strain of *Phoma herbarum* isolated from diseased carp fish (*Cyprinus carpio L.*) obtained from an Egyptian fish farm. The strain was administered to fish via different routes (intraperitoneal injection, oral administration, subcutaneous inoculation and swabbing on scarified gills). Re-isolation of the fungus was tried 1, 2, 3 and 4 weeks post-inoculation. *Phoma herbarum* was constantly isolated from gills and skin of fish which received the fungus through gill scarification and subcutaneous inoculation, respectively. However, the fungus was less frequently recovered from the liver of the intraperitoneally inoculated fish.

Table : 1 Experimental infection of Armout catfish with Phoma herbarum

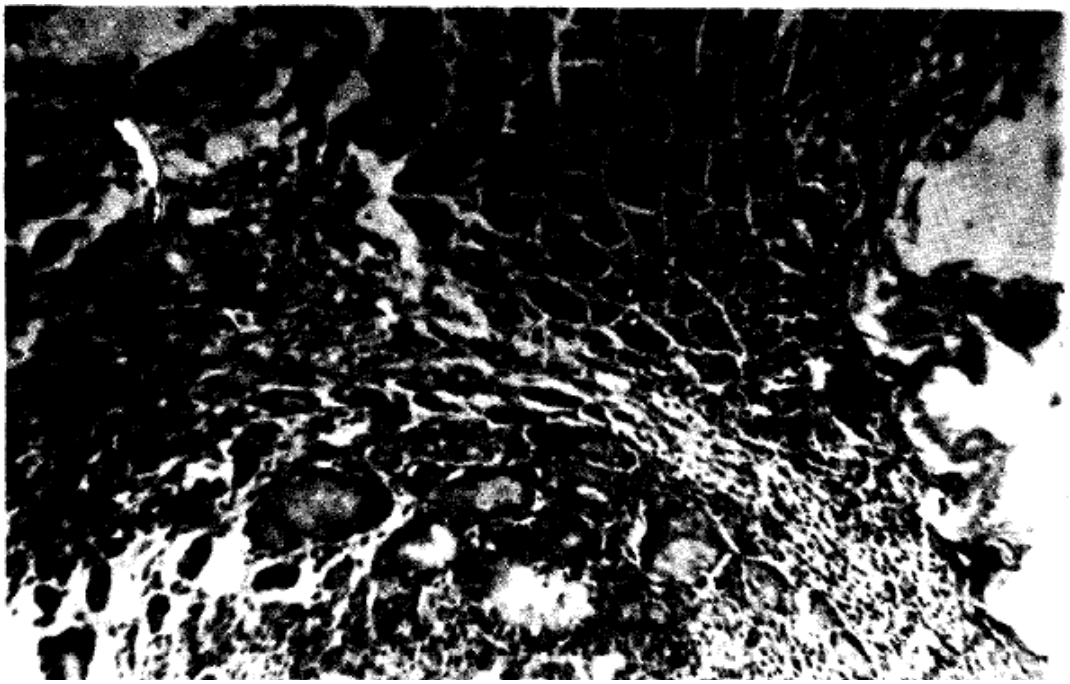
Group	Route of infection	Mortalities		Reisolation of Phoma herbarum							
				Gills		Skin		liver		Fin	
		No.	%	No.	%	No.	%	No.	%	No.	%
I	Control	-	-	-	-	-	-	-	-	-	-
II	Intraperito-neal inoculation	4/10	40	8	80	2	20	10	100	-	-
III	Oral adminis-tration	4/10	40	9	90	5	50	-	-	1	10
IV	Subcutaneous inoculation	3/10	30	7	70	10	100	-	-	5	50
V	Gill scarific-ation	1/10	10	10	100	6	-	-	-	-	-



Skletal muscle of armout catfish showing inflammatory granulomatous reaction formed of Lymphocytes histocytes as well as giant cells (arrow) has destroyed skeletal muscle. Stain: H&E (Obj. 10 p.3.2)



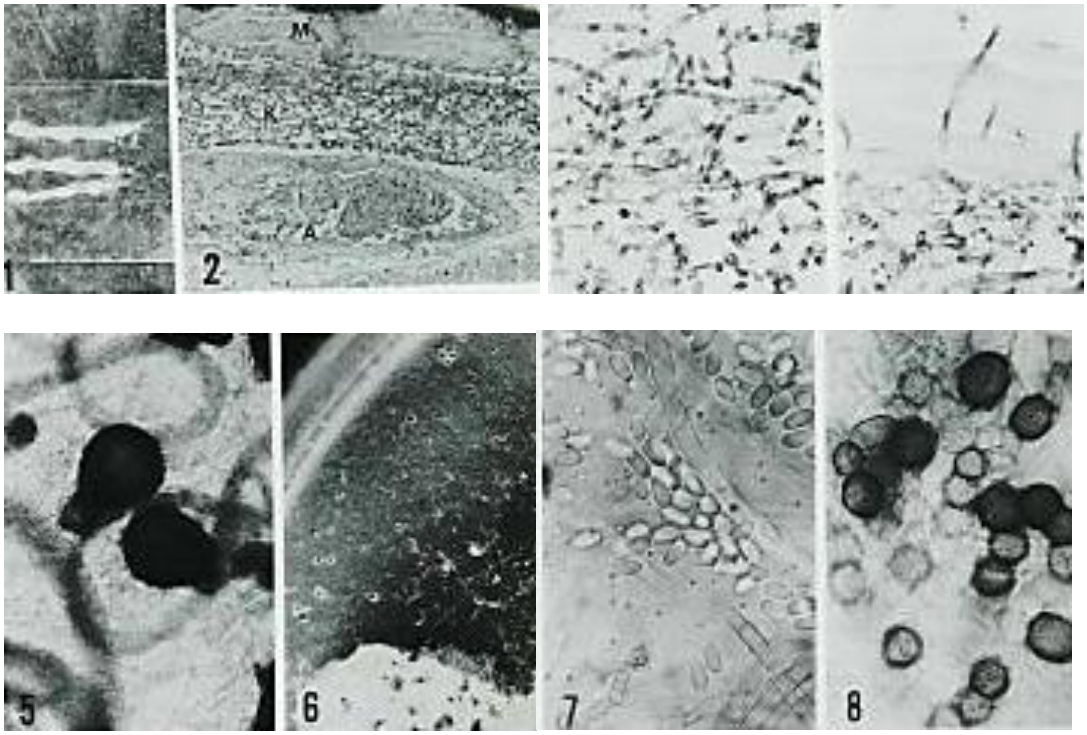
Skeletal muscle showing: Septate hyphae that invaded and destroyed muscle bundles.
Stain: PAS (Obj. 20 p. 3.2)



Regenerated muscle fiber showing reduplication of sarcolemmal nuclei in masses of sarcoplasm (Muscle Giant Cell). Great numbers of inflammatory cells are also seen.
Stain H & E (Obj. 10 p. 3.2)

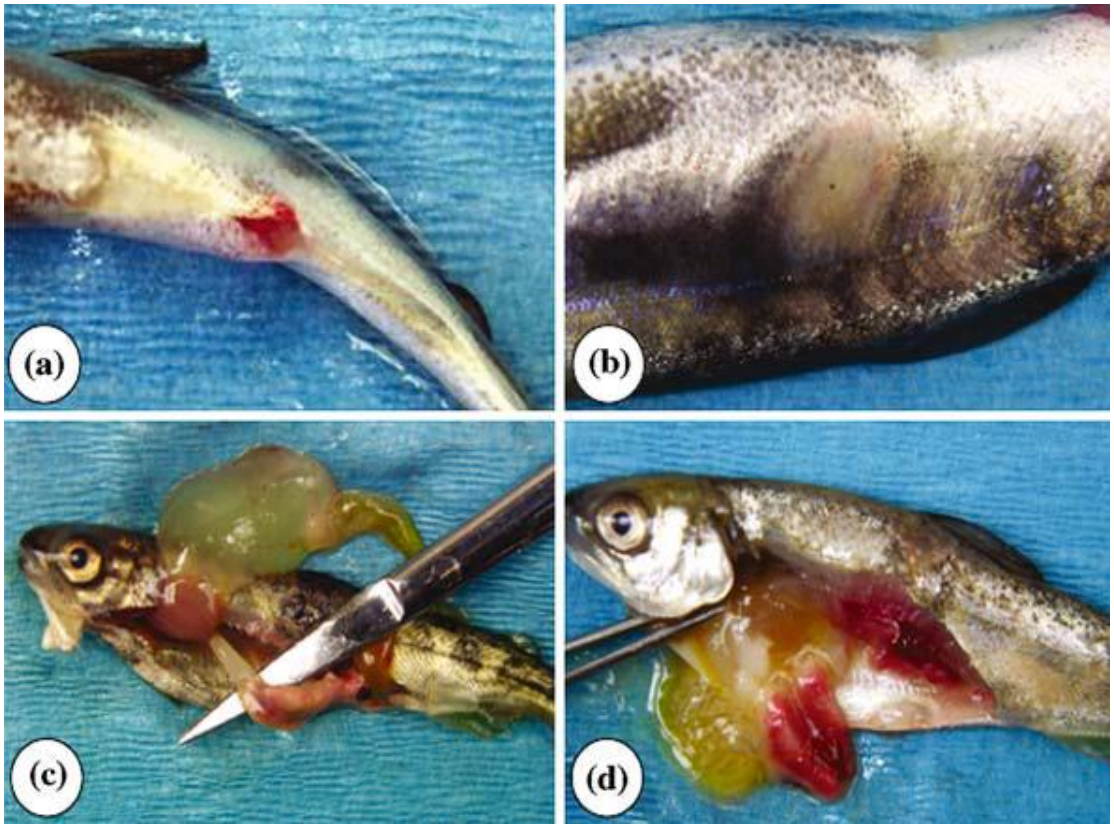
Gill of armout catfish. Notice destruction of primary and secondary gill lamellae, oedema in gill arch together with inflammatory cells surrounding the fungal elements (Arrow).
Stain: PAS (Obj. 10 p. 3.2)

Hatai *et al.* (1986) reported visceral mycosis in ayu fry, *Pleoglossus altivelis*, caused by species of Phoma. The clinical signs on fish were in the form of opaque abdomen. No growth of hyphae was visible on the opaque area of the abdominal wall with the naked eye. Histopathological examination showed that fungal hyphae were present in the air bladder, kidneys, intestine liver, abdominal cavity and surrounding lateral musculature in all examined moribund fish. The air bladder was most heavily infected and the lumen was filled with not only hyphae but also many degenerative necrotized and sloughed cells from the inner wall. Hyphae were abundant in the kidney, but the heart brain and gills did not appear to be infected. In some cases, hyphae had penetrated through the skin to the exterior.

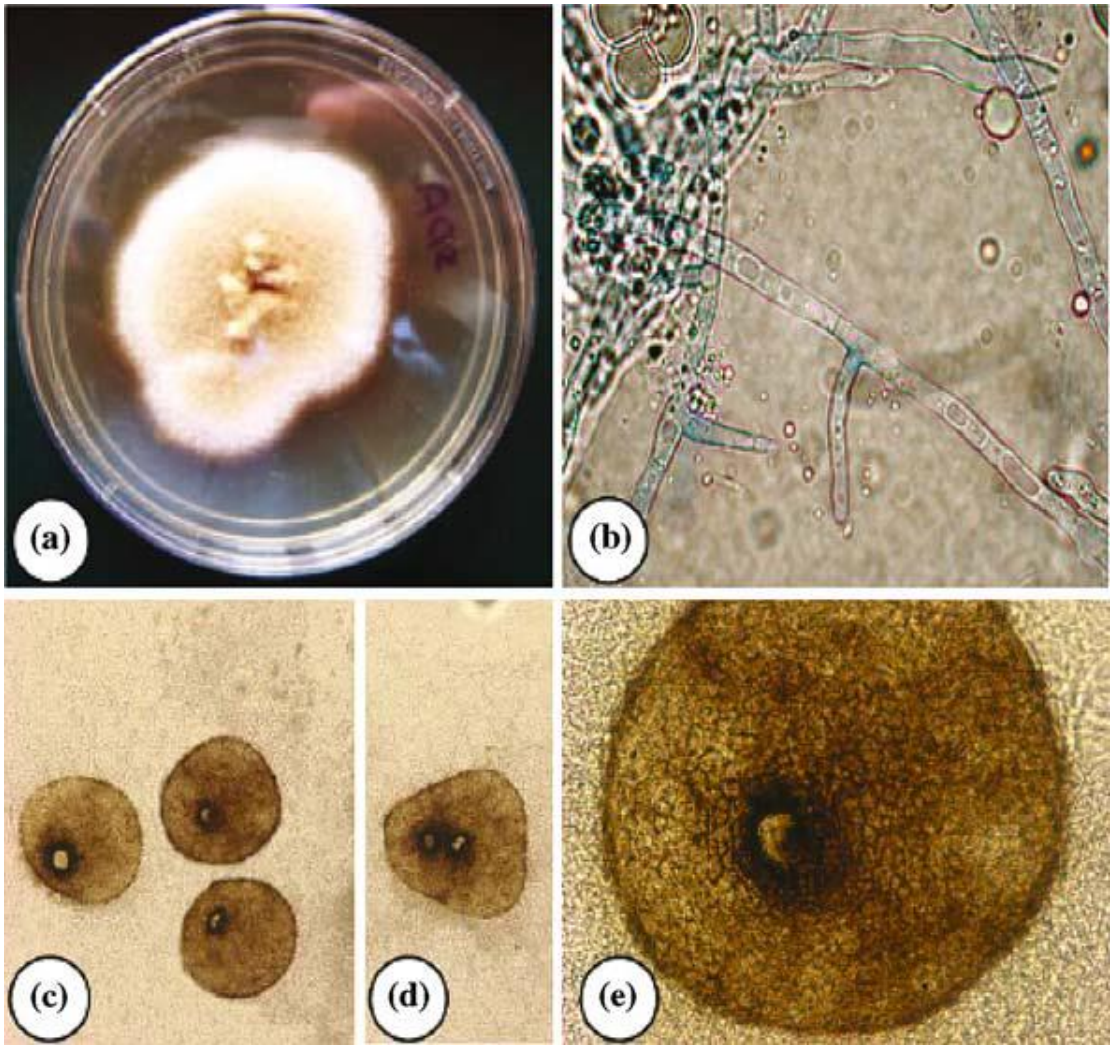


Pathological reactions and morphological features of Phoma herbarum, **Hatai et al., 1986**

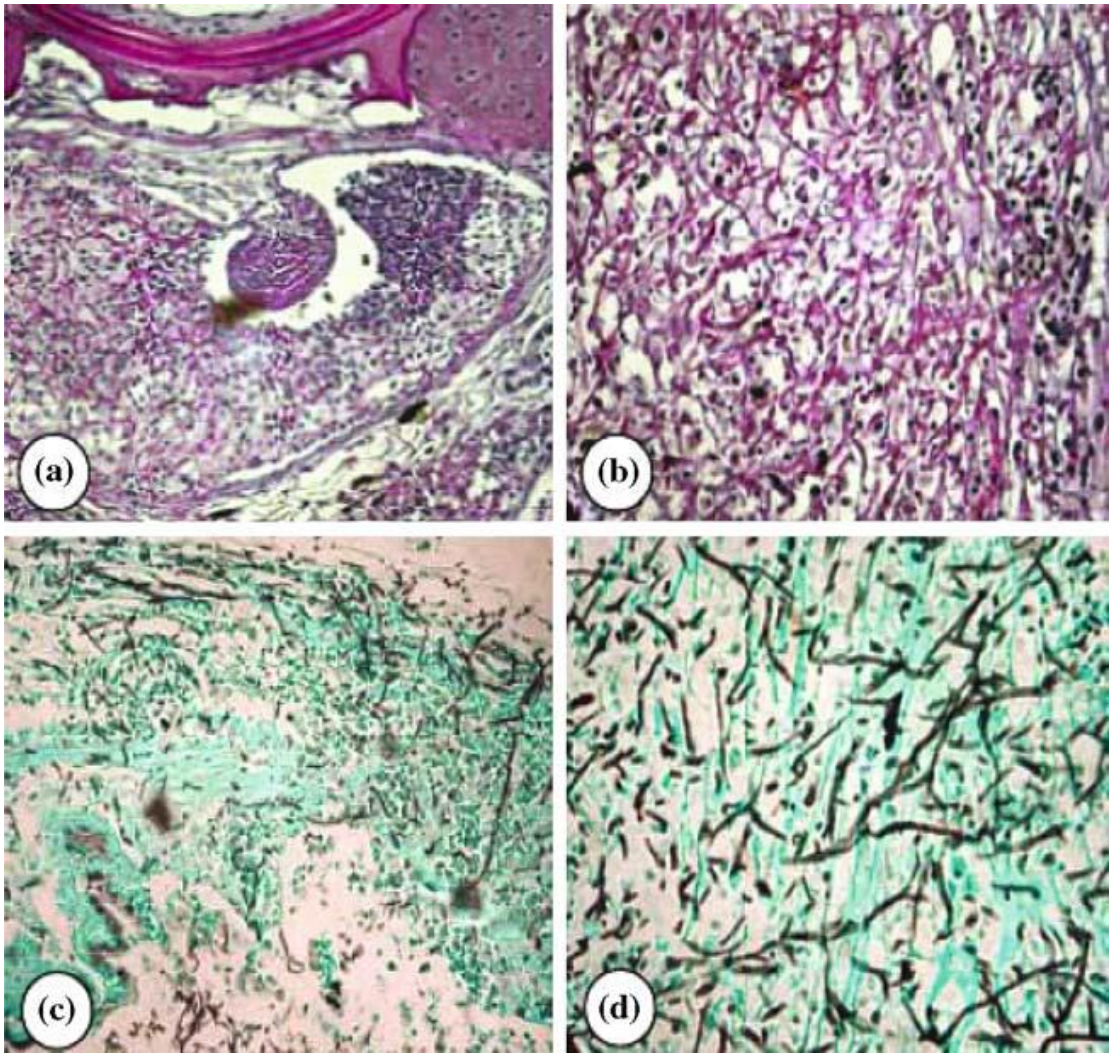
Faisal *et al.* (2007) reported Phoma herbarum in association with two outbreaks of systemic mycosis in hatchery-reared chinook salmon (Oncorhynchus tshawytscha) fingerlings. Affected fish exhibited abnormal swimming behavior, exophthalmia, multiple rounded areas of muscle softening, protruded hemorrhagic vents, and abdominal swelling. In all affected fish, swimbladders were filled with whitish creamy viscous fungal mass, surrounded by dark red areas in swimbladder walls, kidneys, and musculature. Clinical and histopathological examinations suggest that the infection may have started primarily in the swimbladder and then spread to the kidneys, gastrointestinal tract, and surrounding musculature. Consistent microscopical findings included broad septate branched fungal hyaline hyphae, 5-12 microm in diameter within the swimbladder, stomach, and often within and adjacent to blood vessels. Profuse growths of woolly brown fungal colonies were obtained from swimbladders and kidneys on Sabouraud medium. On corn meal agar the formation of pycnidia, characteristic of Phoma spp., was detected within 10 days of incubation. Morphological and molecular analyses identified this fungus as Phoma herbarum. This report underscores systemic fungal infections as a threat to raceway-raised salmon.



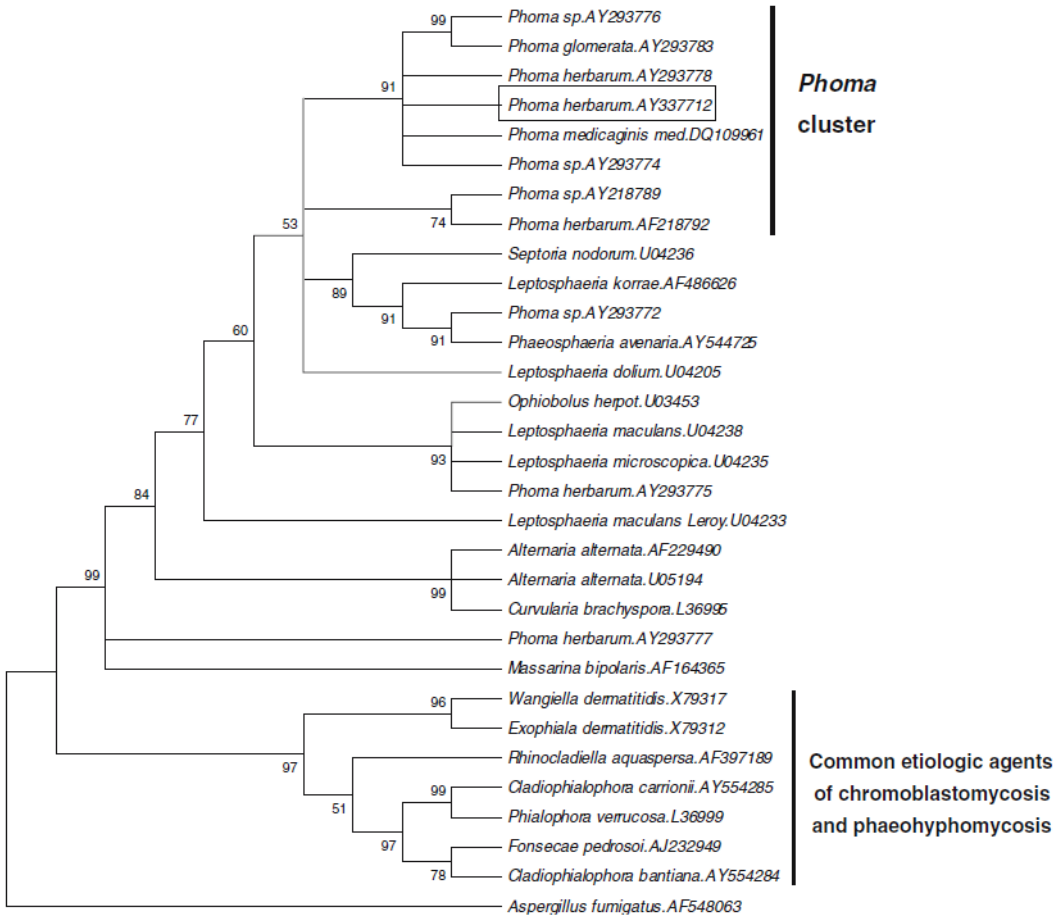
Chinook salmon fingerlings affected by Phoma herbarum showing: (a) hemorrhagic and prolapsed vents, (b) focal areas of skin and musculature softness and discoloration, (c) swimbladder filled with creamy whitish material and distended stomach filled with turbid fluid, and (d) severe inflammation extending from the swimbladder to the surrounding organs and musculature. **Faisal et al., 2007**



Phoma herbarum: (a) colony on 2% dextrose Sabouraud Agar with 0.05 g/L chloramphenicol, (b) Wet mount preparation from necrotic lesion in swimbladder showing fungal hyphae of Phoma herbarum as branched and septated, (c) three pycnidia typical of the genus Phoma, (d) a pycinia exhibiting two osteoles, and (e) a close up of Figure 3c, note the ostiole and the oval pycnidiospores located around the opening. **Faisal et al., 2007**



(a) Photomicrograph of a chinook salmon infected with Phoma herbarum. The wall and lumen of the dorsal aorta is invaded by numerous fungal hyphae. (_200, PAS stain), (b) Photomicrograph of the lumen of the swimbladder of a chinook salmon fingerling infected by Phoma herbarum. Note the 5–8 lm diameter, septate and branching fungal hyphae filling the lumen. (_350, PAS stain), (c) P. herbarum fungal hyphae extending transmurally through the wall of the stomach and are associated with necrosis and mild mixed inflammatory infiltrates. (_200, GMS stain), (d) Higher magnification photomicrograph of the fungal hyphae in Figure 4c. Note the slightly irregular diameter, septate and branching hyphae (_400, GMS stain). **Faisal et al., 2007**



Phylogenetic relationships of several pathogenic phaeoid fungi, including Phoma herbarum causing phaeohyphomycosis in fish (this study), inferred from their 18S SSU rDNA data. Numbers above the branches are percentages of 1000 bootstrap-resample data set support, obtained by neighbor-joining analysis (values below 50% are not shown). The chinook salmon Phoma herbarum strain from this study (rectangular box) was found to be part of a large cluster formed by other Phoma species including two other P. herbarum isolates. The most common agents of chromoblastomycosis and phaeohyphomycosis, grouped in a well supported clade. The accession numbers of the sequences used in this analysis are shown beside the organism names. Aspergillus fumigatus was used as outgroup. **Faisal et al., 2007**

References:

1. Easa, M. EI-S (1979a):Role of fingi as a cause of gill diseaes in carp of Egyptian fish farm. Ph. D. Thesis, Vet. Akademy (Moscow) USSR.
2. Faisal, M., Scott D Fitzgerald, Ehab E Elsayed and Leonel Mendoza . Outbreaks of phaeohyphomycosis in the chinook salmon (Oncorhynchus tshawytscha) caused by Phoma herbarum.Mycopathologia **163(1):41-8** · February 2007
3. Hatai,K.; Fujimaki, Y. and Egusa, S. (1986):A visceral mycosis in ayu fry, Pleoglossus altivelis Temminck & Schlegel, caused by a species of Phoma. J. Fish. Dis. 9: 111-116.
4. Ross, A.J., W.T. Yasutake, and Steve Leek. *Phoma herbarum*, a fungal plant saprophyte, as a fish pathogen. Journal of the Fisheries Research Board of Canada. 32,9, 1648-1652,1975
5. Sparks,A.K. and Hibbits, J.(1979):Black mat syndrome, an invasive mycotic disease of the tanner crab, *Chionoecetes bairdi*. J. Invertebr. Pathol., 34(2), 184-191.

21.Purpureocillium (Paecilomyces)

- ***Paecilomyces lilacinus*** is an ubiquitous, saprobic filamentous fungus commonly isolated from soil, decaying vegetation, insects, nematodes and laboratory air (as contaminant),
- ***Paecilomyces lilacinus*** is a cause of infection in man and other vertebrates.
- ***Paecilomyces lilacinus*** can colonize materials such as catheters and plastic implants and can contaminate antiseptic creams and lotions, causing infections in immunocompetent and immunocompromised patients (**Castro *et al.*, 1990**; **Orth *et al.*, 1996**; **Itin *et al.*, 1998**).
- The prevalence of *P. lilacinus* in patients has increased recently (**Carey *et al.*, 2003**; **Rosmaninho *et al.*, 2010**).

❖ **The phylogenetic analysis** of the 18S rRNA gene region confirms the data of Luangsa-ard *et al.* (2004), showing the polyphyletic nature of *Paecilomyces*.

- ***Paecilomyces variotii***, the type species of *Paecilomyces*, is located in the family of the *Trichocomaceae* (*Eurotiales*) near *Aspergillus*, *Penicillium* and related species, forming a sister clade with the *Onygenales*.
- ***Paecilomyces lilacinus*** belongs to the *Ophiocordycipitaceae*, a family recently introduced by Sung *et al.* (2007).
- ***Paecilomyces marquandii*** is phenotypically similar to *P. lilacinus*, but failed to group with *P. lilacinus* in the phylogenetic analysis using 18S rRNA gene sequences, and this species grouped with green-spored species within the family of *Clavicipitaceae*.

❖ **Detailed phylogenetic analysis** showed that the purple-colored species *Paecilomyces nostocoides, P. lilacinus, Isaria takamizusanensis* and *Nomuraea atypicola* are closely related (Sung *et al.*, 2007.

- None of the 3 species are types of a genus, which warranted the introduction of the new genus *Purpureocillium* for these species.

Infection in fish

- **Lightner *et al.* (1988)** studied experimentally the renal mycosis caused by *Paecilomyces morquandii* on an adult hybrid of red tilapia, *Oreochromis mossambicus* and *Oreochromis hornorum.*
- **Lehmann *et al.* (1999)** reported that swim bladder fungal infection of farmed young Atlantic salmons caused severe clinical symptoms of diseased fishes, but with low mortality relating to the total stock. The fungus, diagnosed in the wall of the swim bladder, possibly belonged to the species *Paecilomyces farinosus*
- **Rand *et al.* (2000)** isolated *Paecilomyces lilacinus* from internal tissue samples of a hatchery-raised blue tilapia *Tilapia aurea* and three of nine feral *Mozambique tilapias T. mossambica* suffering from tilapia wasting disease in Puerto Rico.

- **Marancik *et al*. (2011)** characterized two cases of systemic mycosis in captive sharks. These cases were progressive and ultimately culminated in terminal disease. *Paecilomyces lilacinus*,

Classification

Sp. recognized by Index Fungorum:

- Fungi +
  - Ascomycota +
    - Eurotiomycetes +
      - Eurotiales +
        - Trichocomaceae +
          - Paecilomyces Bainier, 1907 +
            - ***Paecilomyces lilacinus* (Thom) Samson 1974**
            - *Paecilomyces aegyptiacus* S. Ueda & Udagawa 1983
            - *Paecilomyces aerugineus* Samson 1974
            - *Paecilomyces albus* Demelius
            - *Paecilomyces amoene-roseus* (Henn.) Samson 1974
            - *Paecilomyces ampullaris* Matsush. 1971
            - *Paecilomyces ampulliphorus* Matsush. 1975
            - *Paecilomyces andoi* Shimazu & Humber
            - Paecilomyces antarcticus Bridge, M.S. Clark & D.A. Pearce 2005
            - *Paecilomyces aspergilloides* Pidopl. 1950
            - 131 more... show full tree...

NCBI

Cellular organisms +

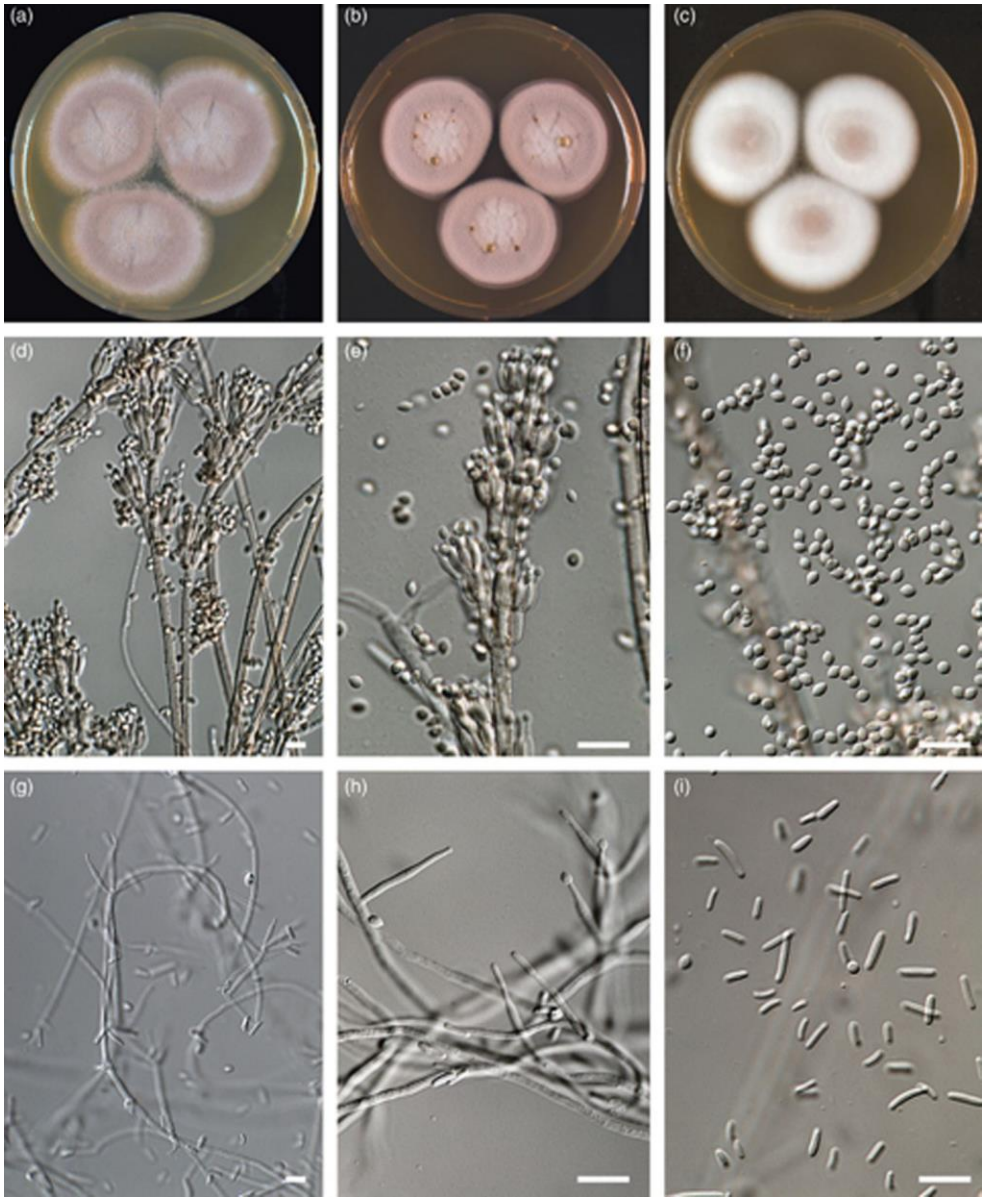
- Eukaryota +
  - Opisthokonta +
    - Fungi +
      - Dikarya +
        - Ascomycota +
          - Saccharomyceta +
            - Pezizomycotina +
              - Leotiomyceta +
                - Sordariomyceta +
                  - Sordariomycetes +
                    - Hypocreomycetidae +
                      - Hypocreales +
                        - Ophiocordycipitaceae +
                          - **Purpureocillium**
                            - Purpureocillium aff. lilacinum ROG-2010
                            - Purpureocillium cf. lilacinum Fun111C
                            - *Purpureocillium lavendulum*
                            - ***Purpureocillium lilacinum***

Description:

Purpureocillium lilacinum (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson, **comb. nov.**Mycobank MB 519530

Basionym: *Penicillium lilacinum* Thom –*Bull Bur Anim Ind US Dep Agric*, **118**: 73 (1910).
=*Paecilomyces lilacinus* (Thom) Samson –*Stud Mycol***6**: 58 (1974).
=*Paecilomyces nostocoides* Dunn –*Mycologia***75**: 179 (1983).

Colonies on MEA (Oxoid) fast growing, attaining a diameter of 25–35 mm after 7 days at 25 °C; no or restricted growth at 37 °C, 0–10 (−20) mm. Colonies consisting of a basal felt with or without floccose aerial overgrowth, some isolates strongly floccose, white at first, becoming vinaceous; reverse mostly in shades of purple or sometimes uncolored. Conidiophores arising from submerged hyphae 4–6 µm in length, occasionally forming loose synnemata up to 2 mm high; stalks with roughened thick walls 3–4 µm wide consisting of verticillate branches with whorls of two to four phialides. Phialides 6–9 × 2.5–3 µm, having a swollen basal portion tapering into a short distinct neck about 1 µm wide. Conidia in divergent chains, ellipsoidal to fusiform, smooth-walled to slightly roughened, hyaline, purple *en masse*, 2–3 × 2–4 µm. Conidial structures formed near the agar atypical: phialides solitary or in verticils, 2–4, variable in length; shaped like typical*Purpureocillium lilacinum* phialides, or very long (up to 30 µm) and *Acremonium*-like. Cylindrical, occasionally slightly curved conidia formed in ‘slimy heads’ on these *Acremonium*-like structures, conidia on these structures variable in size, measuring 2.0–14 × 1.5–2.5 µm This conidiogenesis was also observed by for *P. nostocoides* (=*Purpureocillium lilacinum*). Chlamydospores absent. **Luangsa-Ard *et al.* (2011)**



Purpureocillium lilacinum:(a–c) 14-day-old culture on MEA. (a) DTO 63E5, typical sporulating colonies; (b) DTO 63E1, typical sporulating colonies; (c) floccose colonies, DTO 141C2, (d, e) well-defined conidiophores; (f) typical fusiform conidia; (g, h) Acremonium-like conidiophores; DTO 141C2; (i) cylindrical conidia formed near the agar; DTO 141C2. Scale bar=10 μm. **Luangsa-Ard *et al.* (2011)**

Reports:

Lightner *et al.* (1988) studied experimentally the renal mycosis caused by *Paecilomyces morquandii* on an adult hybrid of red tilapia, *Oreochromis mossambicus* and *Oreochromis hornorum.* Infected fish showed enlarged granulomatous kidney and prominent cottony patches of aerial hyphae on the surface of the peritoneum slightly ventrolateral to the kidney. The affected fish was also slightly darker in pigmentation than the other fish harvested from the same tank at the same time but, otherwise showed no outward signs of the disease.

Journal of Fish Diseases 1988, **11**, 437-440

SHORT COMMUNICATION

A renal mycosis of an adult hybrid red tilapia, *Oreochromis mossambicus* × *O. hornorum*, caused by the imperfect fungus, *Paecilomyces marquandii*

D. LIGHTNER, R. M. REDMAN & L. MOHNEY *Environmental Research Laboratory, University of Arizona, Tucson, Arizona, USA*

J. SINSKI *Department of Microbiology and Immunology, University of Arizona, Tucson, Arizona, USA*

D. PRIEST *Carl Hayden Bee Research Centre, US Department of Agriculture, Tucson, Arizona, USA*

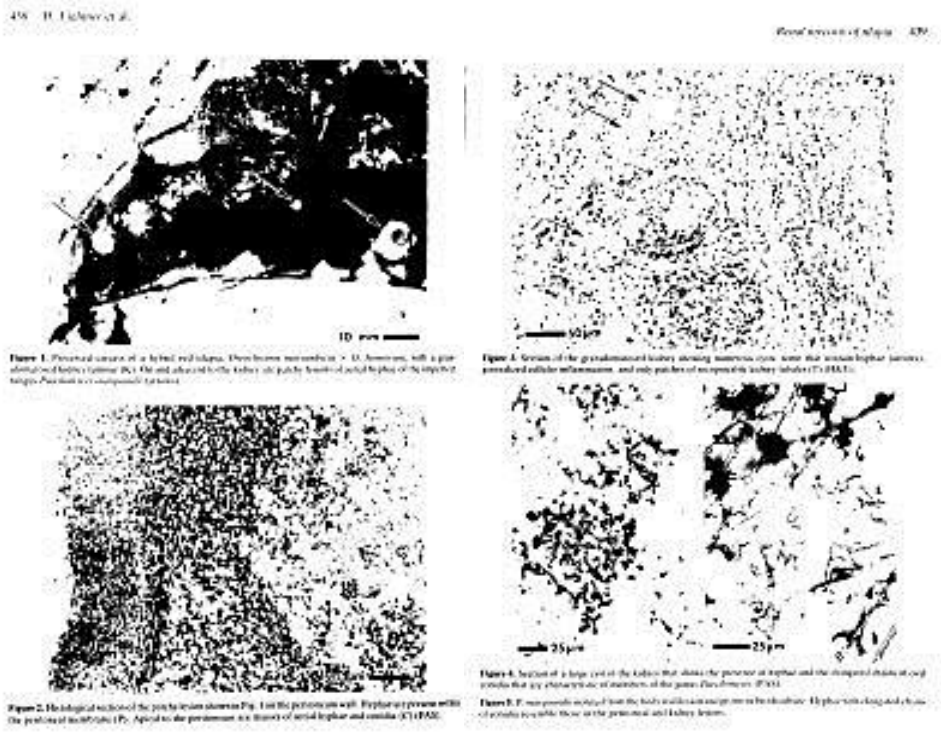
An adult (850 g), tank-reared hybrid red tilapia, *Oreochromis mossambicus* × *O. hornorum*, was found, upon processing, to possess an enlarged granulomatous kidney, and prominent cottony patches of aerial hyphae on the surface of the peritoneum covering the kidney and on the peritoneum slightly ventrolateral to the kidney (Fig. 1). The affected fish was slightly darker in pigmentation than the other fish harvested from the same tank at the same time but, otherwise, showed no outward signs of disease. Its sex was not noted.

The granulomatosed kidney measured 9·0 cm in its anterior to posterior axis, 2·5 cm in maximum width and 3 cm in height relative to the dorsal surface of the peritoneal cavity. Several short projections of the tumour extended dorsally between the ribs and myotomes. The tumour was mostly encapsulated and easily removed, except for the processes that extended between the myotomes, which were not encapsulated. The cottony patch of fungus on the peritoneum near the kidney (Figs 1 & 2) measured 8×9×5 mm in height. It, and a few millimetres of underlying tissue, were excised. A portion of this tissue was easily cultured in a variety of mycological media with and without added antibiotics to inhibit bacterial growth.

The excised portion of the tumour and the remaining portion of the excised cottony fungus lesion from the peritoneum were preserved in 10% neutral buffered formalin and processed for light microscopy using routine histological procedures that included haematoxylin and eosin (H&E), Brown and Brenn tissue Gram stain, and periodic acid-Schiff (PAS) staining.

The histological sections of kidney showed it to be composed of multiple granulomatous cysts, masses of inflammatory cells, and fungal hyphae, with only a few areas of recognizable kidney tissue remaining (Fig. 3). PAS-stained sections showed the kidney (Fig. 4) and cottony lesion (Fig. 2) to contain hyphae. Many of the cysts contained hyphae with long chains of oval conidia that were characteristic of the genus *Paecilomyces*. The morphology of the hyphae and conidia in tissue sections was nearly identical to that displayed by the fungus in culture (Fig. 5), although it was larger in tissue.

Correspondence: Dr D. Lightner, Environmental Research Laboratory, 2061 E. Airport Drive, University of Arizona, Tucson, Arizona 85706, USA.



Lehmann *et al.* (1999) reported that swim bladder fungal infection of farmed young Atlantic salmons caused severe clinical symptoms of diseased fishes, but with low mortality relating to the total stock. The fungus, diagnosed in the wall of the swim bladder, possibly belonged to the species *Paecilomyces farinosus*, already described in 1989 as pathogen for salmon in Scotland and Norway or to the genus Phoma, also known as a swim bladder pathogen in salmonids.

Bull. Eur. Ass. Fish Pathol., 19(2),83, 1999.

SWIM BLADDER INFECTION OF FARMED ATLANTIC SALMON (*SALMO SALAR* L.) BY A FUNGUS: A CASE REPORT.

J. LEHMANN, D. MOCK AND W. SCHÄFER

Summary

This is a report of a seldom observed swim bladder fungal infection of farmed young Atlantic salmons with severe clinical symptoms of diseased fishes, but with low mortality relating to the total stock. The fungus, diagnosed in the wall of the swim bladder, possibly belongs to the species *Paecilomyces farinosus*, already described in 1989 as pathogen for salmon in Scotland and Norway or to the genus *Phoma*, also known as a swim bladder pathogen in salmonids.

Moribund young fish (size 5-6 cm) from a farmed stock in Northrhine-Westfalia, Germany, showed a swollen vent area. No bacterial or viral infections could be prooved. The only observed symptom was a pathological alteration of the swim bladder (Fig.1). The confined lumen of the swim bladder was filled with a whitish mass, and the wall was extremely thickened. The microscopic and histological findings revealed a serious infestation of the swim bladder by septate fungal hyphae. The hyphae had penetrated the whole swim bladder wall destroying the tissue (Fig. 2-3). The fungus was not

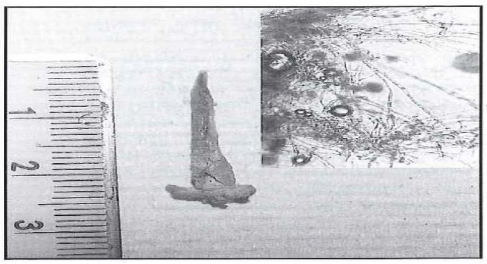
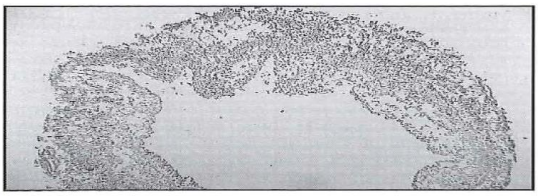


Fig. 1 .A macroscopic demonstration of the swim bladder of a diseased young Atlantic salmon. Detail: Vital crushed preparation of the fungal hyphae.



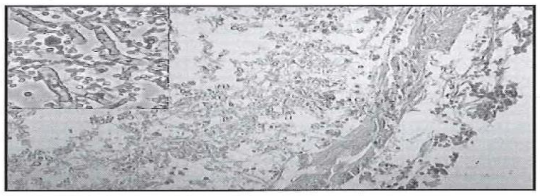
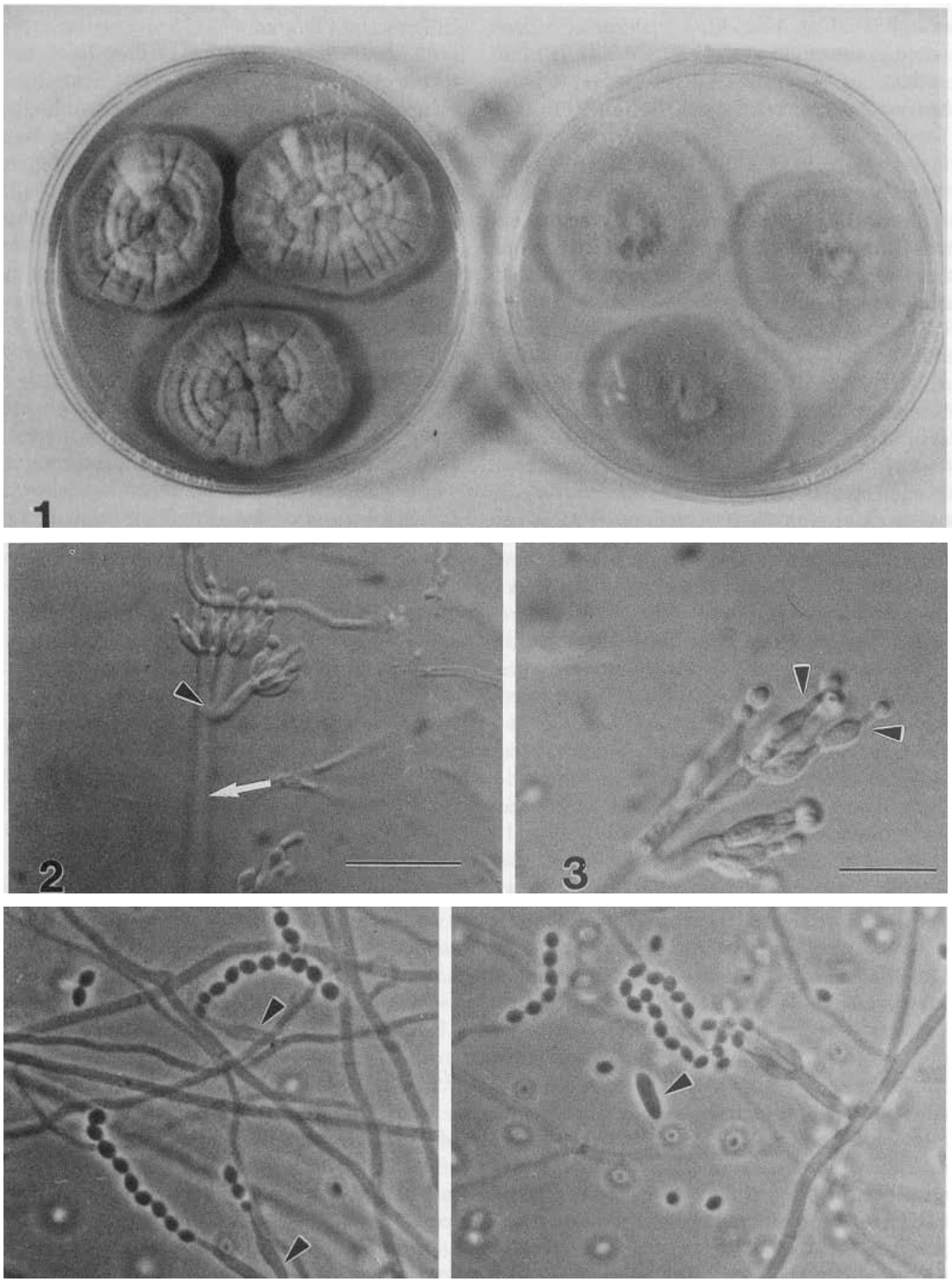


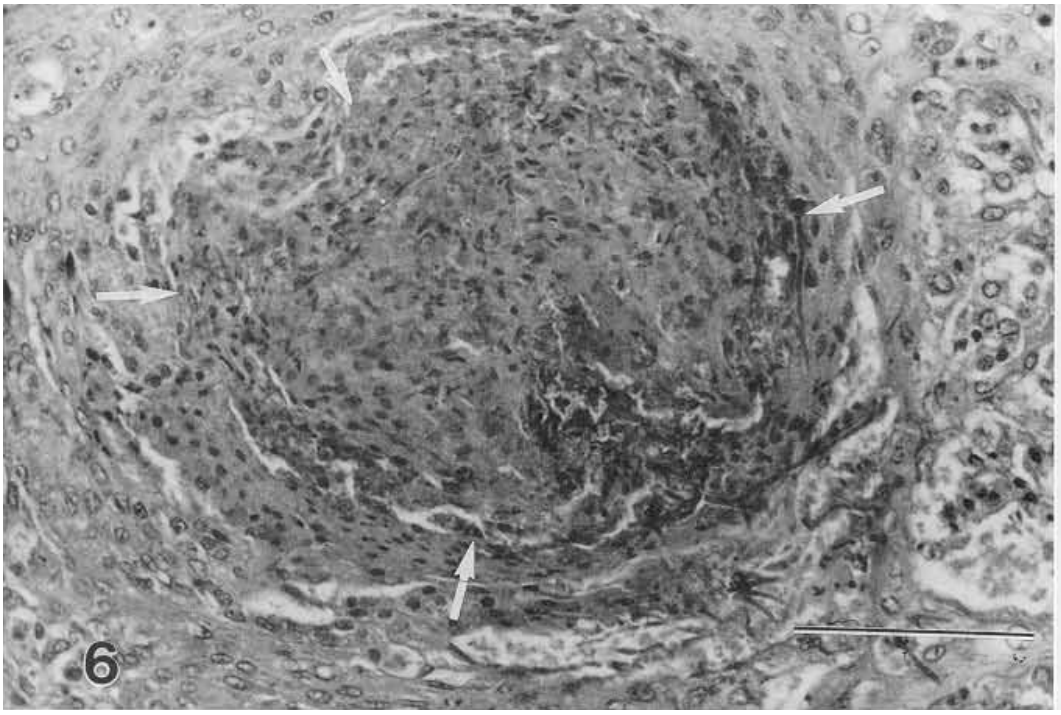
Fig. 2. An histological cross section of a swim bladder, which is infested with the mycosis. The swim bladder wall is extremely thickened. The mycosis has caused a great reduction of the lumen (x 31,2). **Fig. 3**. A high power view of the same swim bladder shown in Fig. 2. The fungal hyphae and conidia are present within the swim bladder wall. Because of the invasion and infiltration of the hyphae, the cellular structure of the swim bladder wall is nearly completely destroyed (x 200). Detail: Hyphae and conidia in phase-contrast image (x 312,5).

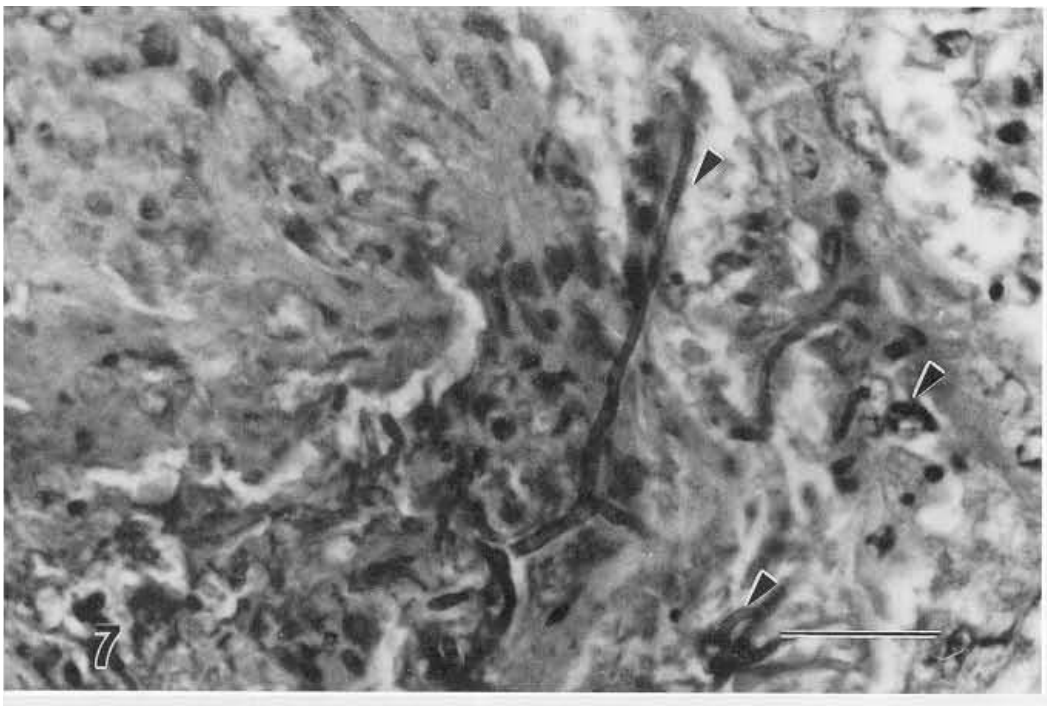
Rand *et al.* (2000) isolated *Paecilomyces lilacinus* from internal tissue samples of a hatchery-raised blue tilapia *Tilapia aurea* and three of nine feral *Mozambique tilapias T. mossambica* suffering from tilapia wasting disease in Puerto Rico. Gross cultural and microscopical features of this fungus closely resembled those of *P. farinosis* and *P. marquandii*, both of which have been previously isolated from fish tissues. They also resembled features of *P. fumoso-roseus*, a species that has been isolated from a captive tortoise. However, the species from tilapia could be distinguished from these other species of Paecilomyces by its production of a brown exudate on Czapek yeast agar (CYA), its deep brown reverse colouration on CYA and Blakeskee malt extract agar (MEA), and its longer, more slender, solitary phialides. It could be further differentiated from these species by growth at 37°C, which was absent in the other three species. Externally, infected fish were emaciated and had sunken eyes and relatively large heads. They also had eroded fins and haemorrhagic, occasionally scaleless lesions up to 5 cm wide on their flanks. Internally, their gastrointestinal tracts and body cavities contained a clear, light amber fluid. Infections were also marked by the presence of numerous golden to reddish-brown granulomas, 0.3-1.3 mm wide, throughout the internal organs. Histopathology revealed that granulomas in spleen, kidney, and liver samples from the blue tilapia and from 12 of 18 Mozambique tilapias collected between 1992 and 1998 were composed of necrotic foci containing invading hyphae, hyphal fragments, conidia, and mixed cellular and caseous material. Bacteria were not observed in the lesion material.



FIGURES 1–5.—(1) Colonies of *Paecilomyces lilacinus* on Czapek yeast agar and malt extract agar after 10 d. (2) Terminal conidiophore of *P. lilacinus* with verticillately arranged branches (short arrow) and smooth-walled stipes (long arrow); differential interference (DIF) microscopy; bar = 25 μm. (3) Terminal conidiophore of *P. lilacinus* revealing phialides with cylindrical basal portion, and short, abruptly tapered tip (arrows); differential interference (DIF) microscopy; bar = 15 μm. (4) Subterminal solitary and dichotomously branched phialides (arrows) of *P. lilacinus* with ellipsoidal conidia in chains; phase-contrast microscopy; bar = 15 μm. (5) Single cylindrical allentoid conidium (arrow) of *P. lilacinus*; phase-contrast microscopy; bar = 15 μm.

Rand *et al.* (2000)





FIGURES 6–7.—(**6**) Granulomatous lesion (arrows) associated with *T. mossambica* spleen; H&E stain; bar = 250 μm. (**7**) Granulomatous lesion with invading hyphae (arrows) extending into surrounding splenic tissue; periodic acid–Schiff stain (PAS) with a hematoxylin counterstain (PASH); bar = 20 μm.

Rand *et al.* (2000)

Luangsa-Ard et al. (2011) *mentioned that Paecilomyces lilacinus* was described more than a century ago and is a commonly occurring fungus in soil. However, in the last decade this fungus has been increasingly found as the causal agent of infections in man and other vertebrates. Most cases of disease are described from patients with compromised immune systems or intraocular lens implants. In this study, we compared clinical isolates with strains isolated from soil, insects and nematodes using

18S rRNA gene, internal transcribed spacer (ITS) and partial translation elongation factor 1-α (TEF) sequences. Our data show that *P. lilacinus* is not related to *Paecilomyces*, represented by the well-known thermophilic and often pathogenic*Paecilomyces variotii*. The new genus name *Purpureocillium* is proposed for *P. lilacinus* and the new combination *Purpureocillium lilacinum* is made here. Furthermore, the examined *Purpureocillium lilacinum* isolated grouped in two clades based on ITS and partial TEF sequences. The ITS and TEF sequences of the *Purpureocillium lilacinum* isolates used for biocontrol of nematode pests are identical to those causing infections in (immunocompromised) humans. The use of high concentrations of *Purpureocillium lilacinum* spores for biocontrol poses a health risk in immunocompromised humans and more research is needed to determine the pathogenicity factors of *Purpureocillium lilacinum*.

Phylogram based on partial 18S rRNA gene sequences showing that Purpureocillium lilacinum belongs Ophiocordycipitaceae and Paecilomyces variotii to the Trichocomaceae. ***Luangsa-Ard et al. (2011)***

Marancik *et al*. (2011) characterized two cases of systemic mycosis in captive sharks. These cases were progressive and ultimately culminated in terminal disease. *Paecilomyces lilacinus*, an uncommon pathogen in human and veterinary

medicine, was associated with areas of necrosis in the liver, heart, and gill in a great hammerhead shark (*Sphyrna mokarran)*. Fungal growth was observed from samples of kidney, spleen, spinal fluid, and coelomic cavity swabs. Dual fungal infection by *Exophiala pisciphila* and *Mucor circinelloides* was diagnosed in a juvenile zebra shark (*Stegostoma fasciatum)*. Both fungi were present in the liver, with more severe tissue destruction associated with *E. pisciphila*. *E. pisciphila* also produced significant necrosis in the spleen and gill, while *M. circinelloides* was associated with only minimal tissue changes in the heart. Fungal cultures from liver, kidney, and spleen were positive for both *E. pisciphila* and *M. circinelloides*. Identification of *P. lilacinus* and *M. circinelloides* was based on colonial and hyphal morphology. *E. pisciphila* was identified by sequence analysis of the 28S rRNA D1/D2 region and the internal transcribed spacer (ITS) region between the 18S and 28S rRNA subunit. These cases, and a lack of information in the literature, highlight the need for further research and diagnostic sampling to further characterize the host–pathogen interaction between elasmobranchs and fungi.

References:

1. Luangsa-Ard J, Houbraken J, van Doorn T, Hong SB, Borman AM, Hywel-Jones NL, Samson RA. Purpureocillium, a new genus for the medically important Paecilomyces lilacinus. FEMS Microbiol Lett. 2011 Aug;321(2):141-9.
2. Lehmann, J.; Mock, D.; Schaefer, W.(1999):Swim bladder infection of farmed Atlantic salmon (*Salmo solar L.*) by a fungus. Bull. Eur. Ass. Fish Pathol.19, (2), 83-84.
3. Lightner, D.; Redman, R.M.; Mohney, L.; Sinski, J. and Priest, D. (1988): A renal mycosis of an adult hybrid red tilapia, *Oreochromis mossambicus* and *O. hornorum*, caused by the imperfect fungus, *Paecilomyces marquandii*.J. Fish. Dis., 1.1: 437-440.
4. Marancik D. P., Berliner A. L., Cavin J. M., Clauss T. M., Dove A. D. M., Sutton D. A., et al. (2011).Disseminated fungal infection in two species of captive sharks. J. Zoo and Wild. Med. 42, 686–693
5. Rand,Th. G., Lucy Bunkley Williams and Ernest H William. A Hyphomycete Fungus, Paecilomyces lilacinus, associated with wasting disease in two species of Tilapia from Puerto Rico **.** Journal of Aquatic Animal Health 12(2):149-156 · June 2000

22. Reports on multiple fungal infections of fish

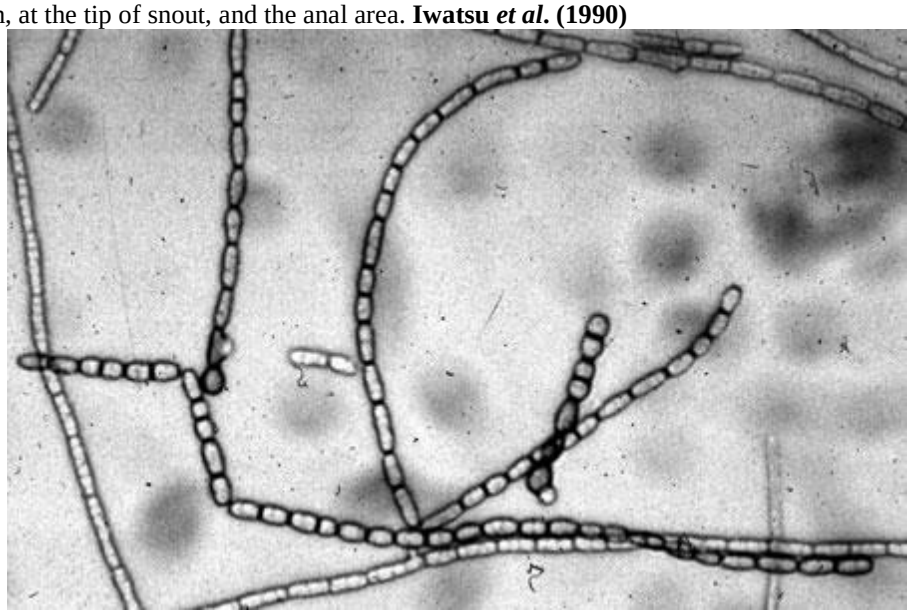
Ellis *et al.* (1983) reported a fungal infection of Atlantic salmon (*S. salar*) occurring at low water temperatures, principally in January, from fish farm hatcheries in western Scotland. Clinical signs and histopathology of the disease were described, illustrated, discussed and compared with those of certain other fungal diseases of salmonids. The fungus, provisionally placed in the hyphomycete genus Phialophora was described and illustrated from pure culture, with a discussion of its taxonomic position.

Bohm and Fuhrmann (1984) isolated members from the family of Saprolegniacaea from 64 samples of freshwater fish. Saprolegnia was found mostly in skin lesions but fins were also affected. In l8 cases the fungus was isolated from pathologically changed gills. On cytophaga agar Saprolegnia grew well. Pure cultures were easily

obtained by applying small agar sections infiltrated with mycelium onto appropriate nutritient media e.g. cannabis seeds. Hyphomycetes of the genus Cephalosporium, Mucor and Fussrium were isolatecl fronr carp which hacl clinically swollen gills. The results indicated that yeasts are of minor importance compared with Saprolegnia. Nine of l2 yeast strains isolated were differentiated as members of the genera Canclida, Rhodotorula, Trichosporon and Torulopsis. Three strains could not be differentiated. Saprolegnia was isolated from skin ancl fins of the examined fish. "I'he frequency of infection differed within the various species, ie. tench (l9%) , rainbow trout (100%), eels (10%) and carp (50%). Saprolegnia could not be isolated from intenral orgaus.
Olufemi (1984) showed clearly that fishes are highly susceptible to infection by members of the genus Aspergillus, although there is variability in the pathogenicity of the various species. A. flavus was shown to be more pathogenic to fish than A. niger. The combination of the two species produced a more serious disease than the monospecific infection. This may well explain the serious nature of clinical outbreaks with this species. Most natural disease conditions quite possibly result from infection by more than one Aspergillus species - conditions which may be termed polyspecific infections. The pathogenicity of Aspergillus species may be attributable to their ability to grow under the environmental conditions provided by the host, water temperature appearing to playa significant role in this regard. At 26°C, A. flavus was about twice as pathogenic to Oreochromis niloticus than at 18°C. A. flavus is able to produce mortalities at various temperatures, whereas A. niger is usually only able to initiate the disease when the water temperature is low (18°C).

Aho *et al.* (1988) diagnosed a fungal swim bladder infection caused by *Verticillium lecanii* in one-year-old Baltic salmon. Haemorrhagic swim bladder inflammation with sloughing of the epithelium was seen histologically. Hyphae were evident in the tissue and secondary bacterial infection was also found. The infection occurred in a period of extremely low water temperature (< 1 degree C) when primary bacterial infections are seldom seen.
Bhattacharya (1988) recorded *Helminthosporium nodulosum* a non-aquatic fungus from fish *Clarias batrachus* . It was found to cause great mortality in the fishes.
Iwatsu *et al*. (1990) reported first Scytalidium infection in striped jack, Pseudocaranx dentex with systemic mycosis in Japan. The external clinical signs were blackish patches and ulcers formed on the surface, especially at the basement of dorsal fin, at the tip of snout, and the anal area. No apparent clinical signs were found in the internal organs. Numerous pale brown, septate hyphae, andarthroconidia were found in the lesions of the surface and various internal organs by direct microscopical examination. The fish was reared in sea water with a temperature of about 18^{o}C. The mortality was about 6% of the original population. A fungus was isolated from the lesions of the surface and the internal organs. Experimental infection using striped jack showed that the fungus was a causal agent of the mycosis. The fungus was isolated on PYGS agar. The colonies were dark green and conidia showing dark green were abundantly produced. Mycelium immersed or superficial, composed of straight or sinuous, sometimes curled, smooth, cylindrical, hyaline to mid-brown, branched, rather thick-walled, septate. Stromata were absent. Conidiophores were micronematous, mononematous, straight or flexuous, hyaline to pale brown and branched or unbranched, smoothwalled. Conidiogenous cells were undifferentiated,

Scytalidium infection in striped jack, Pseudocaranx dentex with systemic mycosis. The external clinical signs were blackish patches and ulcers formed on the surface, especially at the basement of dorsal fin, at the tip of snout, and the anal area. **Iwatsu *et al*. (1990)**



Arthroconidia of Scytalidium infestans formed in extended chains **Iwatsu *et al*. (1990)**

EL-HISSY *et al.* (1992) recovered 80 species which belonged to 34 fungal genera yielding 2992 colonies from surface water (zoosporic fungi) and submerged decaying leaves (aquatic hyphomycetes) samples (160 samples each) during this investigation. Of these fungi, 45 species related to 8 genera of zoosporic fungi (862 colonies) and 35 species related to 26 genera of aquatic hyphomycetes (2130 colonies). Three species of zoosporic fungi (Achlya rodriguazina, Isoachlya toruloides and Saprolegnia luxurians) in addition to fourteen species of aquatic hyphomycetes are new records for Egypt. The richest samples of aquatic fungi (both zoosporic and hyphomycetes) were those collected from water areas with low or moderate temperature and comparatively high total organic matter and dissolved oxygen. Achlya (13 species) and Saprolegnia (12 species) were the commonest zoosporic fungal genera whereas Triscelophorus (2 species), Anguillospora (2 species) and Alatospora (one species) were the most prevalent genera of aquatic Hyphomycetes. The samples collected from Assiut governorate were the richest in zoosporic fungi (23 species and 7 genera) whereas those collected from Aswan governorate were the poorest (6 species and 3 genera). The samples collected from Qena governorate were the richest in aquatic

Hyphomycetes (21 species and 16 genera) whereas those collected from El-Giza governorate were the poorest (8 species and 8 genera). Total counts (TC), per 10 plates and the number of cases of isolations (NCI) of zoosporic fungi recovered from twenty water samples collected from each Governorate of Upper Egypt (8 Governorates) using baiting technique at 22°C.

Governorates	Aswan		Qena		Sohag		Assiut		El-Minia		Beni-Suef		Fayoum		Giza		Total	
Genera and species	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI
Achlya	16	7	35	11	72	20	48	11	76	13	42	11	28	10	33	9	350	92
A. americana Humphrey	0	0	0	0	0	0	14	3	14	3	15	5	0	0	12	4	55	15
A. apiculata deBary	7	3	0	0	0	0	1	1	5	2	0	0	0	0	0	0	13	6
A. abortiva Coker and Braxton	0	0	3	2	0	0	3	1	0	0	0	0	0	0	0	0	6	3
A. caroliniana Coker	0	0	0	0	0	0	0	0	2	1	0	0	0	0	12	3	14	4
A. colorata Pringsheim	0	0	0	0	2	1	0	0	0	0	10	2	18	7	0	0	30	10
A. conspicua Coker	9	5	0	0	0	0	12	3	0	0	0	0	0	0	0	0	21	8
A. dubia Coker	0	0	14	5	0	0	16	4	13	4	12	5	0	0	0	0	55	18
A. flagellata Coker	0	0	0	0	0	0	2	1	3	1	0	0	0	0	0	0	5	2
A. imperfecta Minden	0	0	0	0	0	0	0	0	0	0	0	0	10	4	0	0	10	4
A. klebsiana Pieters	0	0	0	0	15	5	0	0	6	2	0	0	0	0	0	0	21	7
A. prolifera C. G. Nees	0	0	0	0	2	1	0	0	0	0	5	2	0	0	0	0	7	3
A. racemosa Hildebrand	0	0	18	7	53	15	0	0	33	10	0	0	0	0	0	0	104	32
A. rodriguazina F. T. Wolf	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	3	9	3
Aphanomyces laevis de Bary	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	2	1
Allomyces	0	0	3	3	1	1	3	2	0	0	0	0	0	0	2	1	9	7
A. Arbuscula Butler	0	0	0	0	1	1	1	1	0	0	0	0	0	0	2	1	4	3
A. macrogynus (Emerson) Emerson and Wilson	0	0	3	3	0	0	2	1	0	0	0	0	0	0	0	0	5	4
Dictyuchus	9	4	4	2	14	5	36	12	17	7	6	3	9	5	15	6	110	44
D. monosporus Leitgeb	0	0	0	0	4	1	2	1	0	0	0	0	0	0	0	0	6	2
D. polysporus Lendstedt	0	0	0	0	0	0	1	1	2	2	0	0	0	0	0	0	3	3
D. Sterile Coker	9	4	4	2	10	4	33	10	15	5	6	3	9	0	15	6	86	39
Isoachlya	0	0	0	0	9	3	12	4	6	2	0	0	0	0	4	1	31	10
I. monilifera (de Bary) Kauffmann	0	0	0	0	3	2	3	2	0	0	0	0	0	0	0	0	6	4
I. toruloides Kauffman and Coker	0	0	0	0	0	0	0	0	2	1	0	0	0	0	4	1	6	2
I. unispora Coker	0	0	0	0	6	2	9	2	4	1	0	0	0	0	0	0	19	5
Phytophthora	0	0	0	0	4	1	0	0	2	1	0	0	0	0	0	0	6	2
P. cinchonae Sawada	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2	1
P. omnivora de Bary	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	4	1
Pythium	0	0	3	2	4	3	6	3	31	11	7	2	3	1	6	3	60	25
P. acanthicum Drechsler	0	0	0	0	0	0	0	0	0	0	0	0	3	1	2	1	5	2
P. butleri Subram	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
P. marisipium Drechsler	0	0	0	0	0	0	0	0	14	5	0	0	0	0	0	0	14	5
P. indicum Meers	0	0	0	0	1	1	2	1	0	0	0	0	0	0	0	0	3	2
P. ostracodes Drechsler	0	0	0	0	3	2	0	0	4	2	0	0	0	0	0	0	7	4
P. salpingophorum Drechsler	0	0	0	0	0	0	0	0	13	4	0	0	0	0	4	2	17	8
P. torulosum Coker and Patterson	0	0	3	2	0	0	3	1	0	0	0	0	0	0	0	0	6	3
P. ultimum Trow	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0	0	5	1
P. oedochilum Drechsler	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	2	1
Saprolegnia	24	9	34	10	49	14	71	14	28	7	25	7	20	7	43	10	294	78
S. aniospora de Barry	0	0	0	0	0	0	9	3	0	0	0	0	0	0	14	5	23	8
S. diclina Humphrey	7	3	0	0	12	4	12	4	0	0	0	0	0	0	0	0	31	11
S. eccentrica (Coker) Seymour	0	0	0	0	0	0	0	0	7	3	0	0	0	0	12	4	19	7
S. ferax (Gruith) Thuret	0	0	0	0	15	5	0	0	0	0	13	4	17	7	0	0	45	16
S. furcata Maurizio	0	0	7	2	0	0	0	0	0	0	0	0	3	1	0	0	10	3
S. hypogyna de Barry	0	0	0	0	15	5	14	4	18	4	0	0	0	0	0	0	47	13
S. megasperma Coker	10	4	13	4	0	0	0	0	0	0	7	2	0	0	0	0	30	10
S. monoica Prigsheim	7	5	0	0	0	0	13	4	0	0	0	0	0	0	0	0	20	9
S. parasitica Cocer	0	0	10	5	7	2	0	0	0	0	5	2	0	0	0	0	22	9
S. terrestris Cookson and Seymour	0	0	4	2	0	0	14	3	0	0	0	0	0	0	0	0	18	5
S. turfosa Minden	0	0	0	0	0	0	9	4	0	0	0	0	0	0	17	4	26	8
S. luxurians (Bhargava et Srivastava) Seymour	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	3	1
Total counts (colonies)	49		79		153		178		160		80		60		103		862	
Total number of species	6		10		16		23		19		9		6		11		45	
Total number of genera	3		5		7		7		6		4		4		6		8	

Governorates	Aswan		Qena		Sohag		Assiut		El-Minia		Beni-Suef		Fayoum		El-Giza		Total	
Genera and species	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI	TC	NCI
Alatospora	21	9	57	16	107	17	16	5	1	1	47	16	6	4	4	1	259	69
A. acuminata Ingold	21	9	57	16	107	17	16	5	1	1	47	16	6	4	4	1	259	69
Anguillospora	9	3	40	7	88	10	68	9	63	11	71	12	79	17	33	8	451	77
A. crassa Ingold	0	0	0	0	15	3	23	6	7	2	0	0	0	0	0	0	45	11
A. longissima (de Wild.) Ingold	9	3	40	7	73	9	45	9	56	11	71	12	79	17	33	8	406	76
Camposporium	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	2	1
C. pellucidum (Grove) Hughes	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	2	1
Clavatospora Tentacula (umphlett) Nilsson	0	0	0	0	0	0	0	0	0	0	6	2	0	0	0	0	6	2
Culicidospora aquatica Petersen	0	0	0	0	0	0	4	1	0	0	0	0	13	3	1	1	18	5
Dactylella submersa (Ingold) Nilsson	4	2	5	2	52	10	9	4	7	2	3	2	3	2	0	0	83	24
Dendrospora junciola Iqbal	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
Exophialo jeanselmei (Langeron) Mc Ginnis	6	2	4	2	2	1	0	0	2	1	1	1	0	0	0	0	15	7
Flabellospora and Padhye	0	0	8	3	11	4	10	2	12	4	2	1	10	4	0	0	53	18
F. crassa Alasoadura	0	0	2	1	10	3	10	2	12	4	0	0	5	2	0	0	39	12
F. tetracladia Nawawi	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1
Flabellospora sp.	0	0	6	2	0	0	0	0	0	0	2	1	5	2	0	0	13	5
Flagellospora	0	0	57	7	21	7	6	2	2	1	15	3	26	6	1	1	128	27
F. curvula Ingold	0	0	13	3	1	1	0	0	0	0	0	0	0	0	0	0	14	4
F. penicillioides Ingold	0	0	44	7	20	7	6	2	2	1	15	3	26	6	1	1	114	27
Heliscus submersus Hudson	0	0	3	1	5	2	0	0	0	0	1	1	1	1	0	0	10	5
Isthmotricladia laeensis Matsushima	0	0	0	0	0	0	10	3	0	0	0	0	0	0	0	0	10	3
Lateriramulosa uni-inflata Matsushima	0	0	0	0	0	0	12	2	5	1	13	3	19	5	1	1	50	12
Lemonniera	0	0	4	2	0	0	1	1	0	0	1	1	0	0	0	0	6	4
L. aquatica de Wild.	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	1
L. terrestris Tubaki	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
L. filiformis Petersen	0	0	3	1	0	0	0	0	0	0	1	1	0	0	0	0	4	2
Lunulospora curvula Ingold	0	0	0	0	0	0	0	0	5	2	0	0	4	3	0	0	9	5
Mycocentrospora acerina (Harfig) Deighton	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	2	1
Pyramidospora	9	3	2	1	5	2	2	1	13	3	17	6	51	9	36	9	135	34
P. casuarinae Nilsson	5	2	2	1	4	2	0	0	12	2	17	6	51	9	36	9	127	31
P. constricta Singh	4	1	0	0	1	1	2	1	1	1	0	0	0	0	0	0	8	4
Polycladium equiseti Ingold	0	0	2	2	0	0	2	1	6	3	3	1	0	0	0	0	13	7
Speiropsis species	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	4	1
Tetracladium marchalianum de Wild.	13	4	38	8	31	2	1	0	0	0	0	0	0	0	3	2	87	23
Torula herbarum Pers.	1	1	0	0	0	0	0	0	0	0	0	0	2	2	0	0	3	3
Tricladium	0	0	2	2	0	0	0	0	0	0	4	1	4	1	0	0	10	4
T. caudatum Kuzuha	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
T. patulum Mavanova and Marvan	0	0	1	1	0	0	0	0	0	0	4	1	4	1	0	0	9	3
Tripospermum myrti (Lind) Hughes	0	0	2	1	0	0	1	1	0	0	0	0	0	0	0	0	3	2
Triscelophorus	14	2	65	7	68	16	36	4	127	13	170	13	182	19	96	10	758	84
T. monosporus Ingold	13	2	58	7	54	15	28	4	89	13	122	13	181	19	96	10	641	83
Triscelophorus sp.	1	1	7	3	14	8	8	2	38	7	48	6	1	1	0	0	117	28
Trisulcoporium acerinum Hudson and Sutton	0	0	2	1	0	0	0	0	4	1	0	0	0	0	0	0	6	2
Varicosporium giganteum Crane	0	0	2	1	0	0	6	3	0	0	0	0	0	0	0	0	8	4
Total counts (colonies)	77		293		390		189		247		354		404		175		2130	
Total number of species	10		21		15		20		15		15		16		8		35	
Total number of genera	8		16		10		18		12		14		14		8		26	

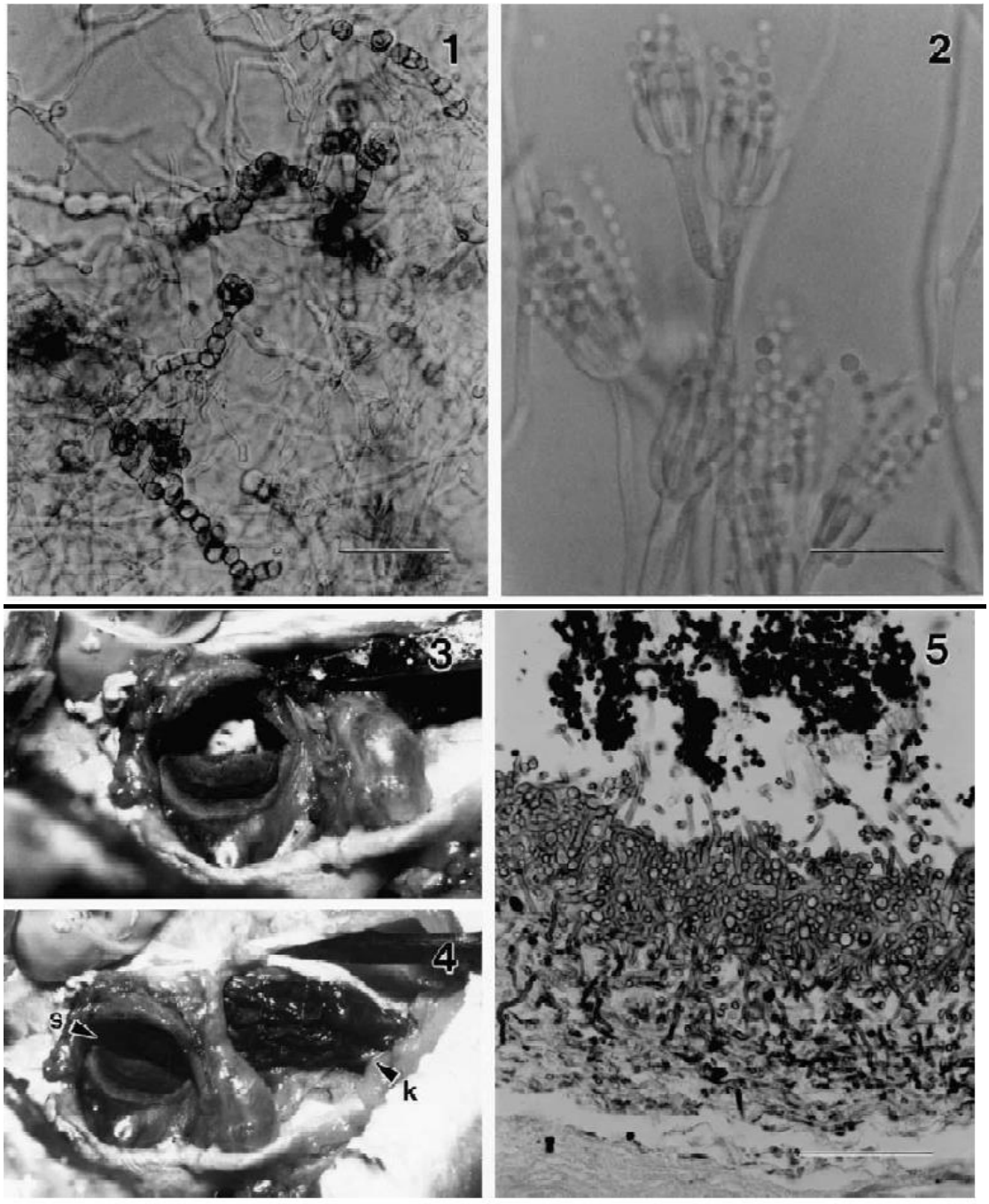
Total counts (TC, per 50 leaf segments in each sample) and the number of cases of isolations (NCI, per 20 samples in each Governorate) of aquatic Hyphomycetes recovered from submerged decaying leaves collected from the Governorates of Upper Egypt.

Khulbe *et al.* (1995) isolated 8 zoosporic fungi viz., Achlya debaryana, A. flagellata, A. klebsiana, Aphanomyces laevis, Saprolegnia diclina, S. ferax, S. parasitica, and Pythium sp. from a large number of adult fishes of the species Mastacembelus armatus, Mystus vitatus, Nandus nandus, Tor putitora and T. tor of Nanak Sagar reservoir in Naini Tal district, India. Species of the parasites and the hosts were different in their pathogenicity and immunity, respectively. However, A. flagellata and S. parasitica appeared to be the most virulent. The severity of mycosis was primarily correlated to moderate water temperatures of 22-25 degrees C. High temperature (> 28 degrees C) retarded the disease process. The experimental inoculation with all the associated fungal species on Puntius conchonius in the laboratory produced clinical signs similar to the ones seen on infected fish in the reservoir. This is the first report on fish mycosis in the large reservoir located in the foot hill of Kumaun Himalaya.

Bocklisch and Otto (2000) examined over 4 years 1.241 fish mycologically. In 182 (14.7%) of them positive results were obtained.Most of the isolates belonged to the following genera: Cladosporium, Saprolegnia, Candida and Penicillium. Fungal infections were correlated with findings of Saprolegnia, Branchiomyces, Pythium, Ichtyophonus and sometimes Cladosporium. Most fungal isolates were etiologically irrelevant for diseases or death.

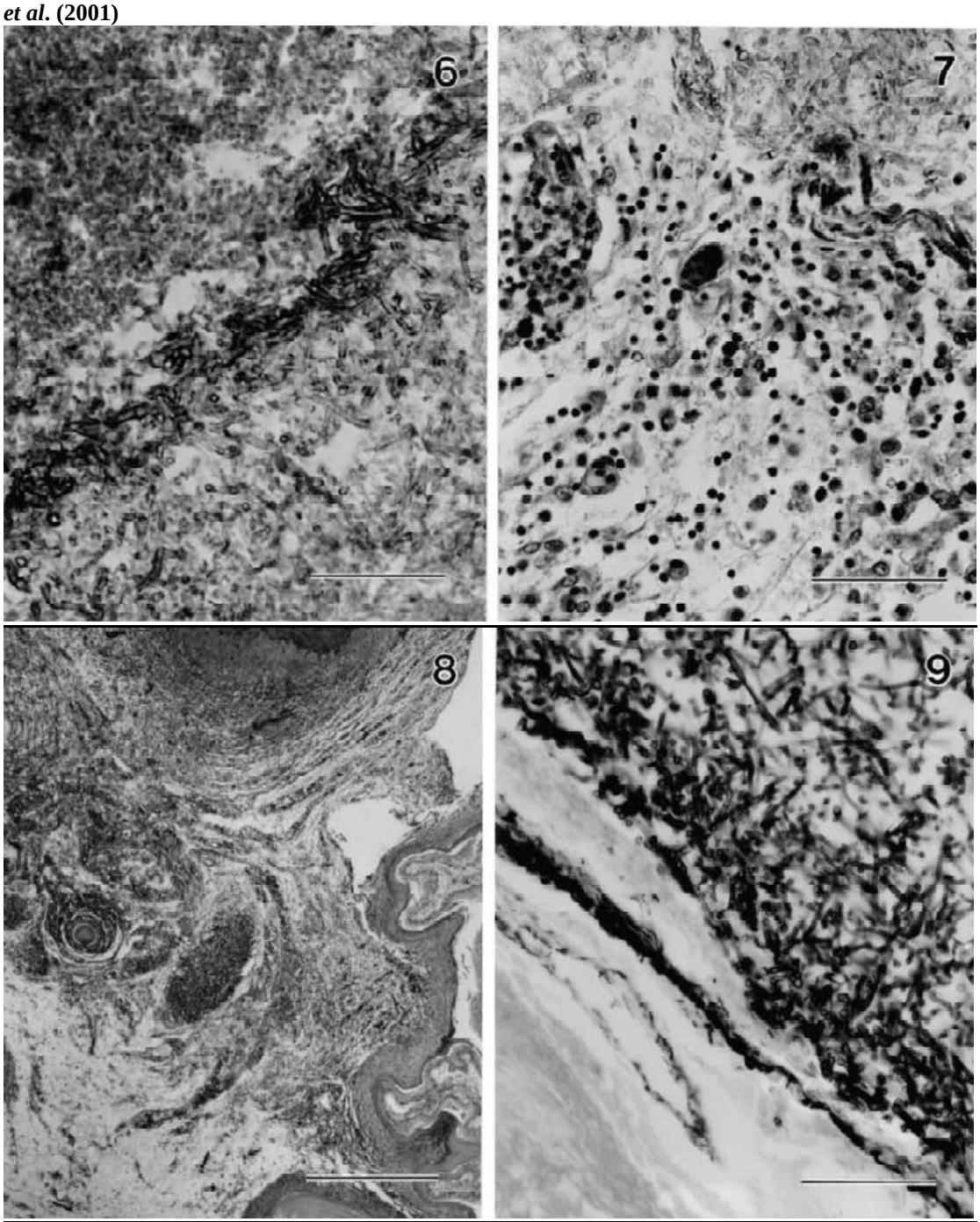
Blaylock *et al.* (2001) reported two species of deuteromycete fungi (*Penicillium corylophilum* and *Cladosporium sphaerospermum*) concurrently infecting the swim bladder and posterior kidney and causing erratic behavior in two specimens of wild-

caught, tank-held red snapper (*Lutjanus campechanus*). Lesions produced by both species infiltrated the immediately surrounding tissue and produced severe pathological changes; however, the infection apparently was not systemic. Only *P. corylophilum* grew in the initial culture from the swim bladder and only *C. sphaerospermum* grew in the initial culture from the kidney. Infection may have occurred upon penetration of a syringe to deflate the swim bladder. There was no horizontal transmission to 13 other specimens of red snapper held in the same tank. This suggests that these fungi are not primary pathogens. Injection of each species into various sites in the Gulf killifish, *Fundulus grandis*, failed to produce infections within 1 month, suggesting differences in susceptibility among species.



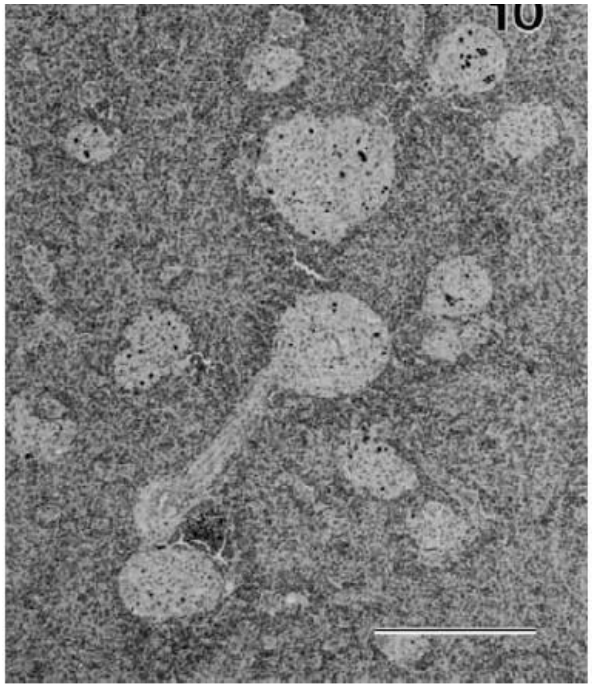
Fungal infections in red snapper (*Lutjanus campechanus*). **Figure 1**. *Cladosporium sphaerospermum* from the kidney, showing hyphae, conidiophores, and conidial chains, wet mount, Nomarski optics.

Scale approximate, bar=30 μm. **Figure 2**. *Penicillium corylophilum* from the swim bladder showing hyphae, conidiophores and conidial chains, wet mount. Scale approximate, bar=20 μm. **Figure 3**. Gross dissection of swim bladder showing cottony appearance of mycelium of *P. corylophilum* overlying extensive lesion. Fungal mat measuring 2–3 cm in diameter. **Figure 4**. Gross aspect showing 'green mold' appearance in dissected swim bladder lesion and distended kidney heavily infiltrated with both *P. corylophilum* and *C. sphaerospermum*. s=swim bladder and k=kidney. **Figure 5**. Matted vegetative hyphae of *P. corylophilum* in swim bladder, with aggregated penicillate conidiophores and conidia protruding into the lumen of bladder, Accustain methenamine silver stain. Scale bar=39 μm. **Blaylock *et al*. (2001)**



Figures 6–9. Histological sections of fungal infections in the kidney of the red snapper (*Lutjanus campechanus*). **Figure 6**. Hyphal mixture of *Penicillium corylophilum* and *Cladosporium sphaerospermum* replacing tissue, Accustain methenamine silver stain. Scale bar=39 μm. **Figure 7**. Variety of inflammatory cells (monocytes, macrophages, granulocytes, and lymphocytes) infiltrating remaining parenchyma adjacent to fungal layer, hematoxylin and eosin. Scale bar=39 μm. **Figure 8**. Fungal lesion exhibiting inflammation, granulomas, hyperplasia, necrotic tissue, and hemorrhage, hematoxylin and eosin. Scale bar=388 μm. **Figure 9**. Hyphae of *Penicillium corylophilum* abutting

fibrotic capsule of kidney wall, Accustain methenamine silver stain. Scale bar=39 μm. **Blaylock *et al.* (2001)**

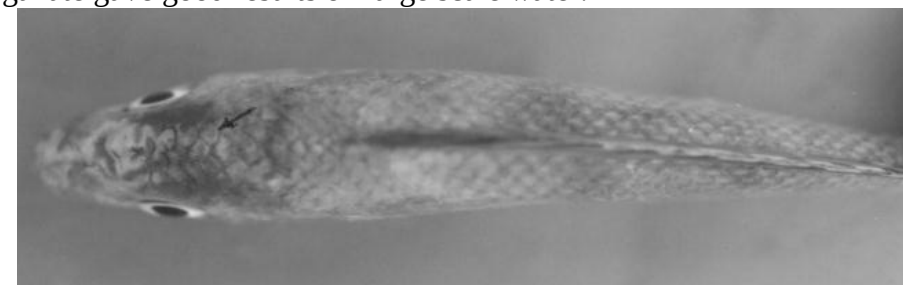


Spleen of red snapper (*Lutjanus campechanus*) infected with *Penicillium corylophilum* and *Cladosporium sphaerospermum* showing an abundance of melanomacrophage aggregates, hematoxylin and eosin. Scale bar=156 μm. **Blaylock *et al.* (2001)**

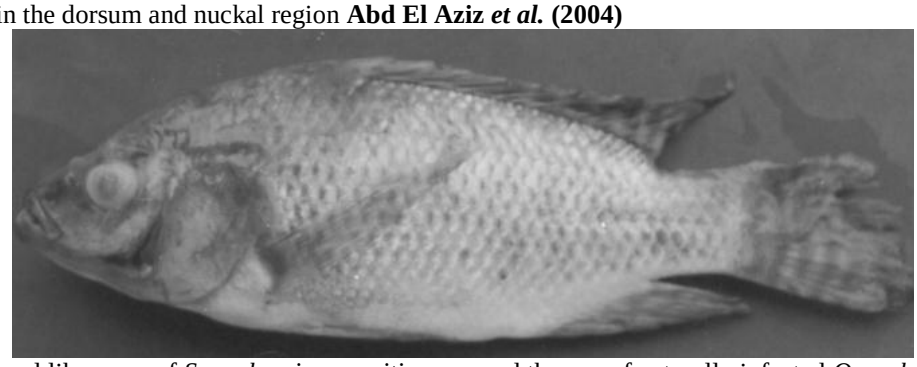
Youssef *et al.* (2003) examined 60 samples of salted fish collected from various moloha markets in Sohag, Qena and Aswan Governorates, Upper Egypt. Moloha contained 52.9% water content, while organic matter content represented 71.79% of dry weight and 33.81% (338.12±8.64 mg g−1) of fresh weight. Total salts and soluble salts represented 13.29% and 10.19% (132.88±7.65 and 101.93±5.76 mg g−1 of fresh weight), respectively. pH values were more or less neutral. Mycological investigation of examined samples revealed that fifty-five fungal species and one variety belonging to 11 genera were identified. The fungal genera of highest occurrence and their respective number of species were Aspergillus (A. flavus, A. niger, A. fumigatus, A. montevidensis, A. ficuum, A. parasiticus and A. mangini) and Penicillium (P. citrinum, P. puberulum, P. aurantiogriseum and P. roquefortii). On the other hand, yeast represented 18.2% and 3.0% of total counts of fungi on Czapeks-dextrose agar and 15%NaCl-Czapeks-dextrose agar media, respectively. Samples were assayed for potential presence of mycotoxins. Ten out of 60 samples (16.7%) were proved to be toxic. It is the first record of mycotoxins contamination of salted fish in Egypt. The ability of 340 isolates of recovered fungi was screened for production of mycotoxins and extracellular enzymes.

Abd El Aziz *et al.* (2004) made a follow up the seasonal occurrence of saprolegniosis in 1600 fish including *Oreochromis niloticus*, catfish *(clarias gariepinus), Muggy cephalus* and *Mugil capito* in 22 fish farms including 3 intensive fish farms in Kafr El-Sheikh and El-Bohaira governorates. The seasonal study extended from May 2003 till April 2004and revealed that saprolegniosis occurred in some *Oreochromis niloticus* as a separate cases following the routine sampling of fish to determine their average weight. In February 2004 an epizootic of saprolegniosis occurred in 9 farms following the passage of a severe cold weather front. The affected species of fish were *Oreochromis niloticus* and *clarias gariepinus* but *Mugil cephalus* and *Mugil capito*

didn't affected although mixed culture *by Oreochromis niloticus* and Mugil species was practiced. The clinical examination revealed the presence of the characteristic clinical picture of saprolegniosis, in *Oreochromis niloticus* the cotton wool like masses found mostly on the head specially at the nuchal region and also covering the eyes either unilateral or bilateral while in *clarias gariepinus* the cotton wool like masses mostly covered the whole body of fish. The wet mount examination of 90 samples of fish from the affected farms revealed the presence of characteristic branched non septated hyphae of saprolegnia with asexual sporangium containing motile zoospores.. The histopathological examination of samples from the skin and muscles showing severe damage of skin and muscles of affected fishes with demonstration of the fungal hyphae by PAS reaction where the hyphae appeared bright red. The treatment trials on affected fishes revealed that the treatment of saprolegniosis were very difficult specially in large scale water and when massive skin affection is found. Malachite green, sodium chloride, hydrogen peroxide and potassium permanganate gave good results in small scale water. Potassium permanganate gave good results on large scale water.

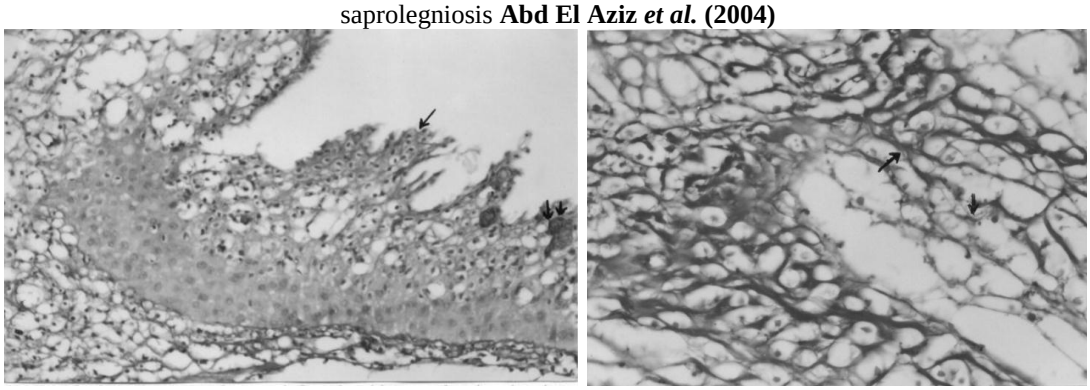


Oreochromis niloticus naturally infected with saprolegnia parasitica in the early stage showing redness in the dorsum and nuckal region **Abd El Aziz *et al.* (2004)**

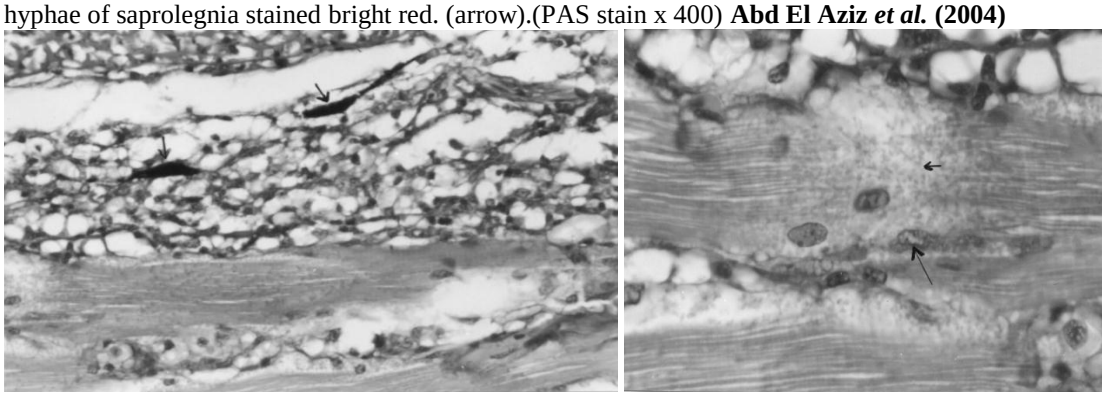


Cotton wool like mass of *Saprolegnia parasitica* covered the eye of naturally infected *Oreochromis niloticus* **Abd El Aziz *et al.* (2004)**

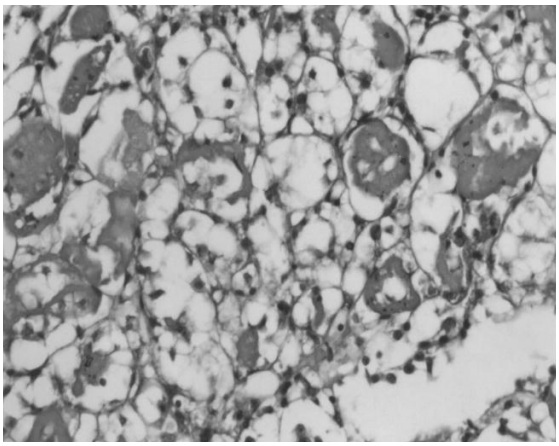
Cotton wool like mass covered the head and body of *Oreochromis niloticus* infected with saprolegniosis **Abd El Aziz *et al.* (2004)**



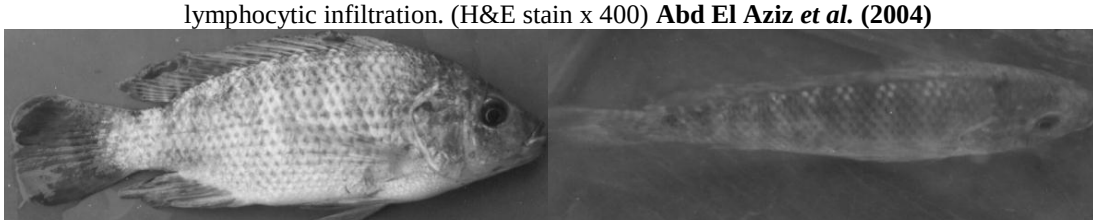
Skin of *Oreochromis niloticus* infected with saprolegnia showing marked spongiosis of the epidermis and epidermal hyperplasia (arrow). Notice: activated mucus cells (2 arrows). (H&E stain x200). Skin of *Oreochromis niloticus* naturally infected with *Saprolegnia parasitica* showing unsegmented fungal hyphae of saprolegnia stained bright red. (arrow).(PAS stain x 400) **Abd El Aziz *et al.* (2004)**



Muscle of *Oreochromis niloticus* naturally infected with *Saprolegnia parasitica* showing severe edema between the muscle fibers, lymphocytic infiltration and melanophores aggregation (arrows). (H&E x 400). Muscle of *Oreochromis niloticus* infected with saprolegnia showing myolysis (small arrow) and macrophage cells engulfing the necrotic muscle fiber (myophagia) (large arrow). (H&E stain x 1000). **Abd El Aziz *et al.* (2004)**



Muscle of *Oreochromis niloticus* infected with saprolegnia showing zunker ś necrosis, edema and lymphocytic infiltration. (H&E stain x 400) **Abd El Aziz *et al.* (2004)**



Oreochromis niloticus treated by potassium permanganate showing the brown discoloration of the cotton wool like masses on the affected lesions inside and outside water **Abd El Aziz *et al.* (2004)**

Ahmed *et al.* (2005) randomly collected 60 samples of salted fish (Molouha) from Ismailia City, Egypt to evaluate the quantitative, qualitative and toxigenicity of xerophilic mold. The results revealed that 50 (83.3%) out of 60 salted fish analyzed were contaminated by xerophilic mold, while 16.7% were negative. The mean value of total xerophilic mold was 2.45±0.95 log10 cfu/g. Aspergillus spp. was the most predominant xerophilic mold (58.2%) in the fish followed by Penicillium spp. (32.7%). Aspergillus niger and P. verrecosum were the most predominant mold strains in investigated samples. Thirty-one (18.8%) toxigenic xerophilic mould strains out of 165 isolated xerophilic mould species found to be Sterigmatocystin and Aflatoxin B 2 , G1 mycotoxin producers. Salted fish processing should be controlled by theauthority in concern to solve the poor mycological quality of retail salted fish in Ismailia city. Measures must be developed to control the growth and activity of xerophilic mold in salted fish

Jakic-Dimic *et al.* (2005) presented the results of hygienic safety of carbohydrate feed (corn, wheat, barley) investigated in the laboratory of Veterinary Research Institute of Serbia in Belgrade within regular control, or with the aim of establishing the causes of disturbance of health status and decreased production results in the pond. During 2004 we performed microbiology and mycotoxicology investigations of the total of 43 samples, namely: 31 corn samples, 8 barley samples and 4 wheat samples. The obtained results point at a high level of mould contamination (Aspergillus, Penicillium, Fusarium, and Rhizopus) and the presence of their secondary mycotoxin metabolites (aflatoxin, ochratoxin, trichothecenes and zearalenone) in feed.

Ali (2009) isolated 16 identified and three unidentified species belonging to six genera of zoosporic fungi from forty water samples which were collected from different fish and fish hatcheries farms at Abbassa city, Sharkiya governorate, Egypt, using sesame seeds baiting technique at 20±2°C. *Saprolegnia* and *Achlya* contributed the broadest spectra of species diversity amongst the other genera of zoosporic fungi. *Saprolegnia diclina* and *Aphanomyces* sp. were the most prevalent species of zoosporic fungi. The abundance of zoosporic fungal species in these aquacultures was correlated with some physicochemical characteristics of the water samples. The two dominant species of zoosporic fungi were tested for their tolerance of NaCl solution and its impact on some morphological and metabolic activities of these fungi. *Saprolegnia diclina* tolerated concentrations of NaCl solution till 12000 µg/ml whereas the maximum resistance of *Aphanomyces* sp. was 8000 µg/ml. The examined morphological aspects of the two studied fungal species, which included the colony diameters, the vegetative hyphae, zoosporogenesis, zoospores discharge, sexual reproductive structures and gemmae formation, were generally affected depending upon the tested fungal species and the applied dose of NaCl solution. The low treatments of NaCl solution were significantly stimulative compared with the control for protease production by *S. diclina* but higher doses were significantly suppressive. A significant decline in protease activity at all applications was found when *Aphanomyces* sp. was treated with NaCl solution. The total free amino acids and total protein content of *S. diclina* and *Aphanomyces* sp. mycelia were almost significantly increased relative to untreated controls at the low dose of NaCl solution and they were significantly dropped at the higher concentrations by the two zoosporic fungi.

Total counts, cases of isolations and occurrence remarks of zoosporic fungi collected from fish and fish hatcheries farms

Genera and species of zoosporic fungi	Number of isolates	Cases of isolations	Occurrence remarks
Achlya	156	23	H
A. diffusa Harvey ex Johnson	19	2	R
A. debaryana Humphrey	37	6	L
A. dubia Coker	72	12	M
A. racemosa Hildebrand	20	5	L
Achlya sp.	8	2	R
Allomyces macrogynus Emerson & Wilson	13	4	R
Aphanomyces	115	26	H
Aphanomyces laevis de Bary	6	2	R
A. scaber de Bary	17	6	L
Aphanomyces sp.	92	21	H
Dictyuchus	103	16	M
D. monosporus Leitgeb	58	11	M
D. sterilis Coker	45	5	L
Saprolegnia	257	32	H
S. diclina Humphrey	162	24	H
S. ferax (Gruith.) Thuret	45	6	L
S. furcata Maurizio	21	4	R
S. glomerata (Tiesenhausen) Lund	10	2	R
Saprolegnia sp.	18	2	R
Pythium	60	15	M
P. debaryanum Hesse	10	3	R
P. rostratum Butler	43	12	M
P. ultimum Trow	7	2	R
Total number of isolates	704		

Abbreviations: H – high occurrence; more than 20 water samples; M – moderate occurrence between 10-20 water samples; L – low occurrence; between 5-9 water samples; R – rare occurrence less than 5 samples.

Makkonen et *al.* (2010) collected samples from melanised spots on the abdominal cuticle and walking legs of Noble crayfish (Astacus astacus), and a variety of fungi and oomycetes were isolated including; **Saprolegnia parasitica, Saprolegnia australis, Mucor hiemalis and Mucor racemosus.** A combination of several of these pathogens may cause the atypical symptoms of burn spot disease seen in Estonia, or the isolated crayfish populations may just express different disease symptoms.

Refai *et al.* (2010) carried out a study on 360 freshwater fishes (240 *Oreochromis* species and 120 *Clarias gariepinus*). They were collected from different governorates and during different seasons. Naturally infected fishes showed clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body. Fishes were then subjected to post mortem examination which revealed many abnormalities. Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples (1658 mould and 423 yeast isolates), of which 1334 were isolated from Oreochromis species and 747 isolates from Clarias gariepinus. Isolated moulds belonged to the following genera: Saprolegnia (4.2%), Aspergillus (43.0%), Fusarium (14.1%), Mucor (14), Penicillium (17.2), Rhizopus (4.8%), Scopulariopsis (1.2%), Paeciliomyces (1%) and Curvularia (0.4%). Yeasts isolated also from both fish species had the following incidence: Candida albicans (35.9 %), other Candida species (19.1%), Rhodotorula species (31.4%) and Torulopsis species (13.5%). Experimental infection with the most predominant fungi (Aspergillus flavus, Fusarium species and Candida albicans) was conducted to evaluate the pathogenicity of these isolates. Clinical pictures of experimentally infected fish were similar to those of natural infection. Inoculated fungi were re-isolated from different organs. Results were confirmed with

histopathological examination, which revealed the presence of fungal hyphae and spores in different organs.

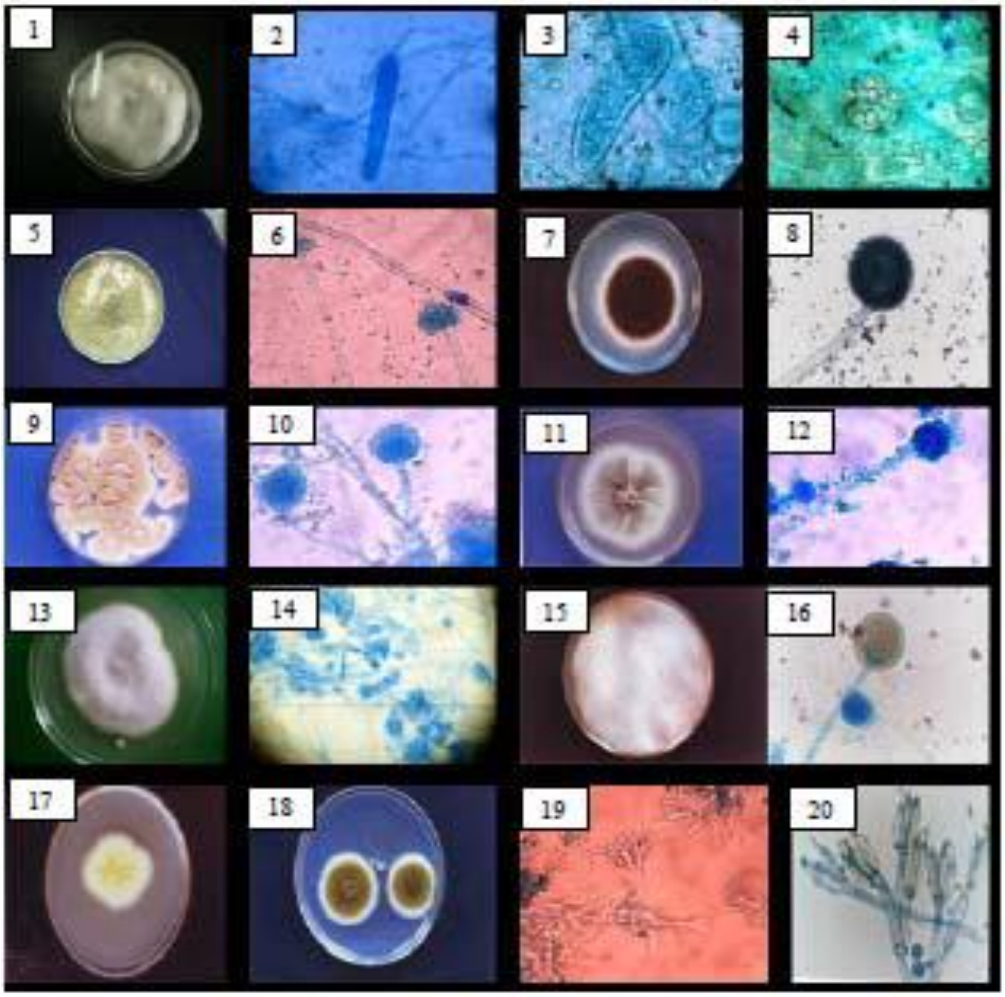


Photo. (1): A colony of *Saprolegnia* species with the characteristic cotton- wool like growth. **Photo. (2):** Non-septated broad hyphae of *Saprolegnia* species (X 200). **Photo. (3&4):** Different stages of reproductive structures of *Saprolegnia* species on hemp seeds (X 400). **Photo. (5):** Colonies of *Aspergillus flavus* on SDA, one weak old. **Photo. (6):** Typical heads *Aspergillus flavus* (X 400). **Photo. (7):** A colony of *Aspergillus niger* on SDA. **Photo. (8):** *Aspergillus niger* showing characteristic round head with black conidia (X 400). **Photo. (9):** Colonies of *Aspergillus terreus* on SDA. **Photo. (10):** *Aspergillus terreus* with small hemispherical vesicle (X 400). **Photo. (11):** A colony of *Aspergillus fumigatus* on SDA. **Photo. (12):** *Aspergillus fumigatus* with columnar head (X400). **Photo. (13):** A colony of *Fusarium* species on SDA with rose pigments on the center. **Photo. (14):** *Fusarium* species with characteristic slender, multicelled conidia (X 200). **Photo. (15):** Colonies of *Mucor* species showing spread over the surface of SDA. **Photo. (16):** Round sporangia of *Mucor* species containing sporangiospores (X 400). **Photo. (17):** *Penicillium* species on SDA with different colour and texture. **Photo. (18):** *Penicillium* species showing brush- like arrangement of fruiting head "A" (X400) and "B" (X 200). **Photo. (19):** *Rhizopus* species colony on SDA showing dens woolly mycelia. Sporangia are seen as small black dots. **Photo. (20):** *Rhizopus* species showing long, branched Sporangiophores and terminate with rhizoids (X200). **Refai et al., 2010**

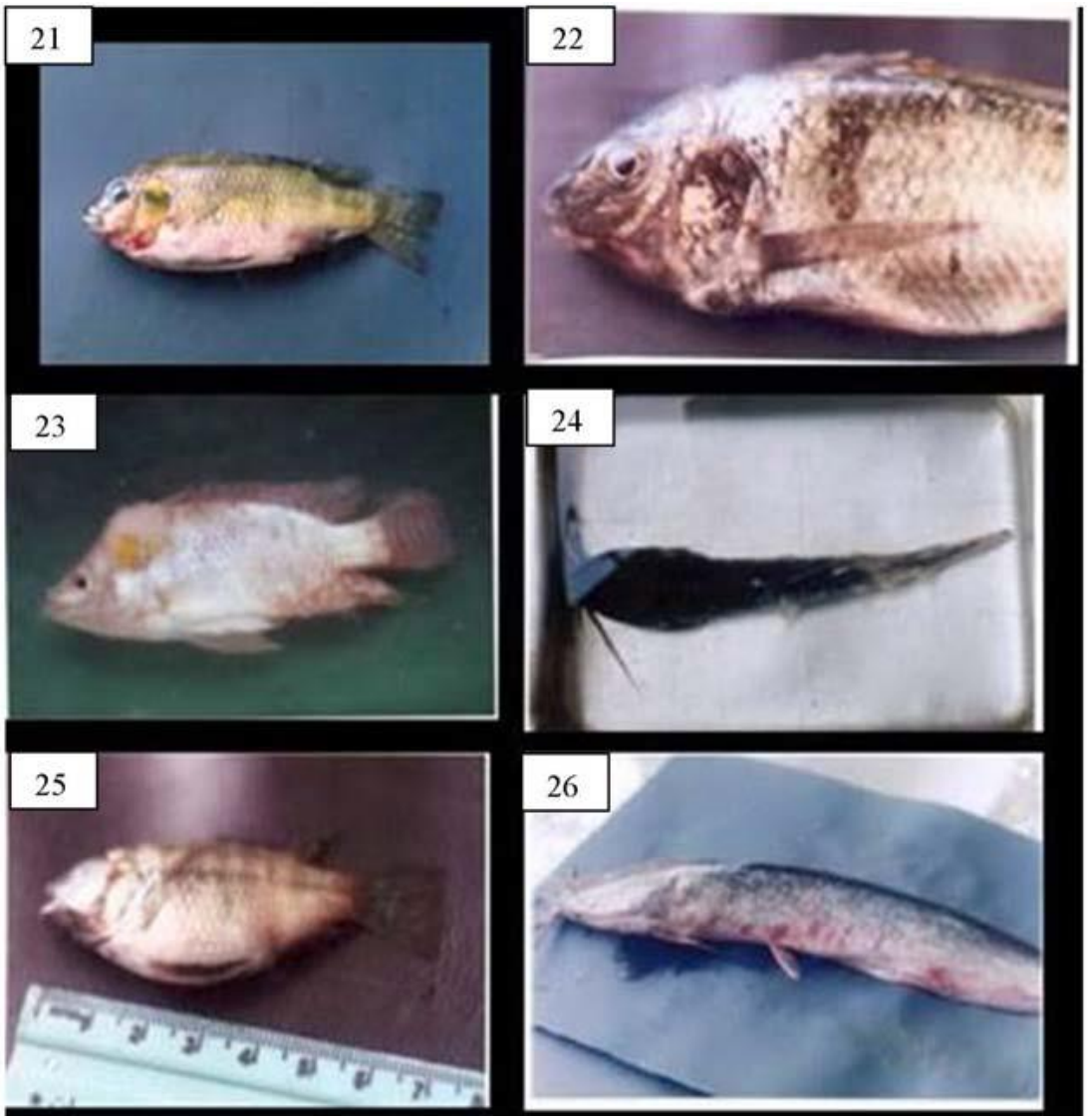


Photo. (21): *Oreochromis* species showing exophthalmia. **Photo. (22):** *Oreochromis* species showing skin darkening. **Photo. (23&24):** *Oreochromis* species and *Clarias gariepinus* showing cotton wool-like growth on various parts of the body. **Photo. (25):** *Oreochromis* species showing ascitis. **Photo. (26):** *Clarias gariepinus* showing haemorrhages allover the body surface. **Refai et al., 2010**

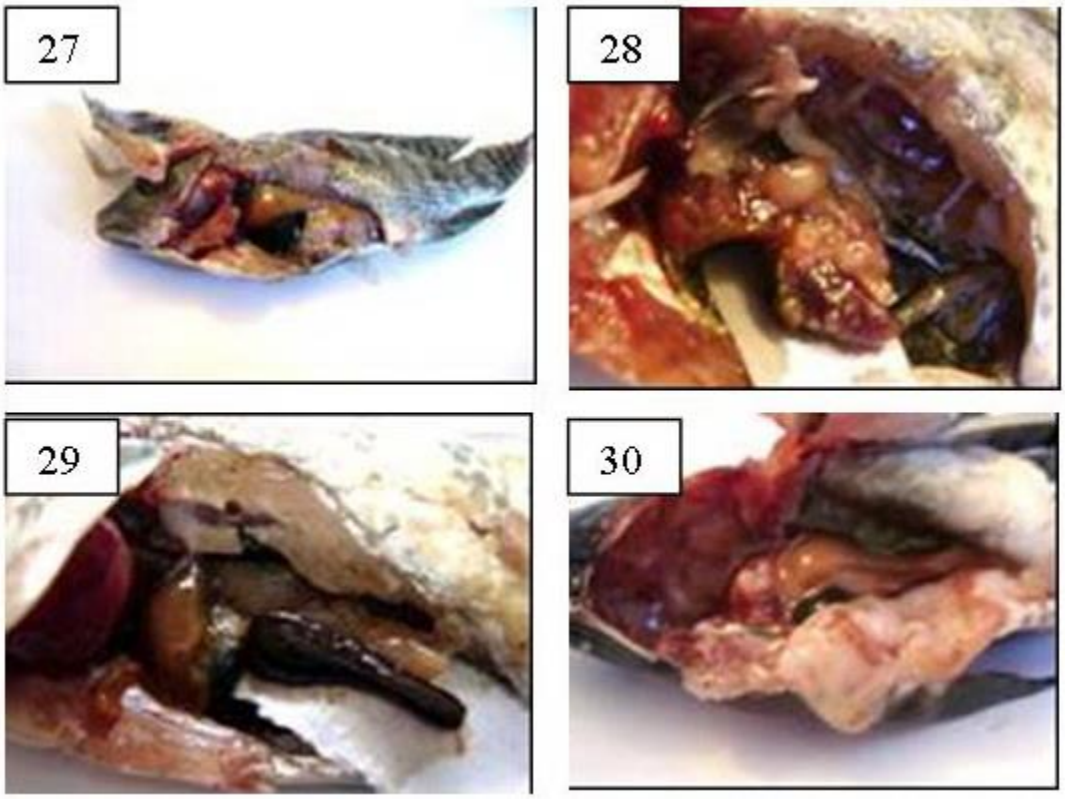


Photo. (27): Liver of *Oreochromis* species showing necrotic foci with distention of gall bladder. **Photo. (28):** Spleen of *Oreochromis* species showing multiple nodules **Photo. (29):** *Oreochromis* species showing severe enteritis. **Photo. (30):** *Oreochromis* species showing severe enlargement of spleen. **Refai et al., 2010**

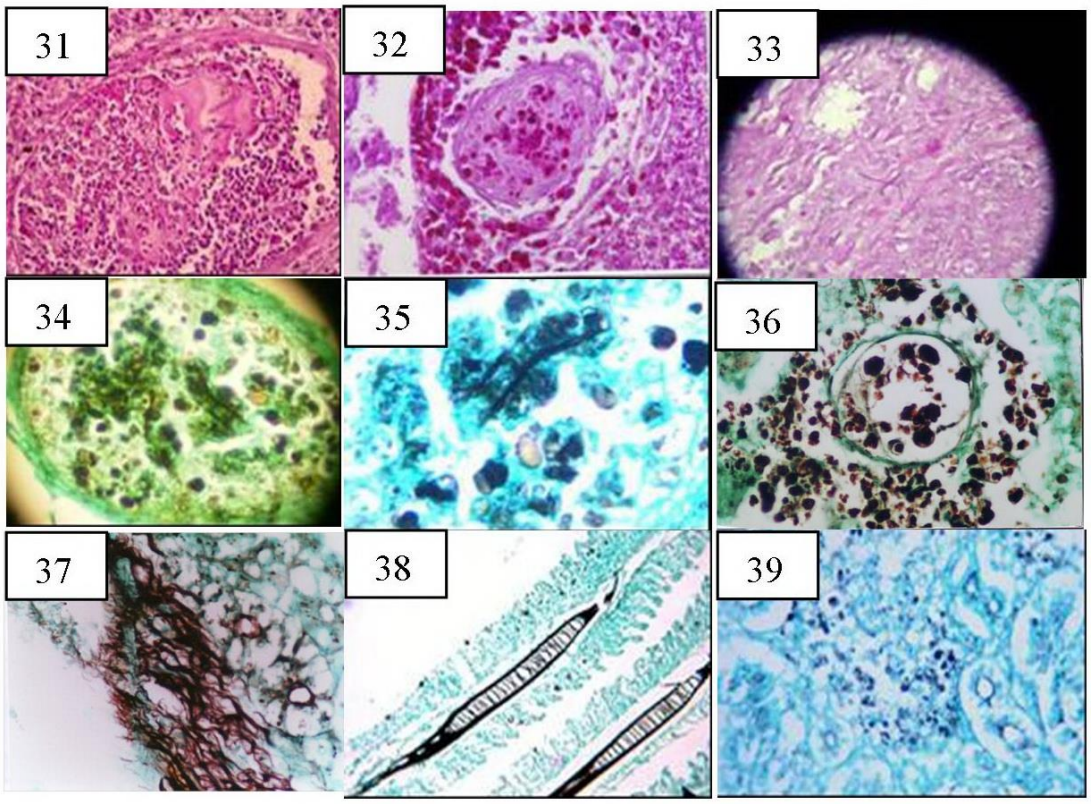


Photo. (31): Spleen section stained with PAS (X400) showing a granuloma formed of epithelioid cells and macrophages surrounded with fibroblasts and fibrous connective tissue capsule. Fungal hyphae appear within the granuloma. **Photo. (32):** Spleen section stained with PAS (X400) showing granuloma

consists of epitheloid cells, macrophages and surrounded with connective tissue capsule. Large number of fungal spores appear within and surrounding granuloma.**Photo. (33):** Liver section showing fungal hyphae between the hepatocytes stained with PAS (X200). **Photo. (34):** Liver section stained by GMS (X400) showing granuloma consists of aggregation of epithelioid cells, macrophages and fibrous connective tissue capsule. Fungal hyphae and spores appear within granuloma. **Photo. (35):** Liver section stained by GMS (X 1000) showing fungal hyphae and spores between the hepatic tissue. **Photo. (36):** Spleen section stained by GMS (X 400) showing focal aggregation of spores surrounded with proliferating fibroblasts and fibrous connective tissue in between. **Photo. (37):** Kidney section stained by GMS (X 400) showing hyphal threads in between the interstitial tissues with marked severe degenerative changes in the tubular epithelium. **Photo. (38):** Gills section stained by GMS (X 400) showing yeast cells investing necrosed areas of epithelial lining the secondary lamellae. **Photo. (39):** Kidney section stained by GMS (X 400) showing yeast cells investing the interstitial tissues. **Refai et al., 2010**

Ke *et al.* (2010) examined infected yellow catfish (*Pelteobagrus fulvidraco*) from Niushan Lake Fishery, Hubei Province, China. Macroscopic daffodil yellow mold was observed on the heads and fins of the fish and one *Mucor* species was isolated. Based on the morphological and molecular analysis, the species was identified as *Mucor circinelloides*. Its optimum growth temperature was 30 °C and it could not grow at 40 °C. The infectivity results showed wound infection could cause 100% cumulative mortalities at all experimental CFU (10^6, 10^7 and 10^8). The cumulative mortalities of the intraperitoneal infection increased along with the sporangiospore concentrations; the highest mortality was 90% with 10^8 CFU. Histopathological studies showed *M. circinelloides* could cause a series of pathological changes in the host tissues and they disseminated in different viscera, perhaps by the blood. This is the first report of *M. circinelloides* infection in yellow catfish.

Ali *et al*. (2011) isolated identified (n = 17) and unidentified (n = 1) fish-pathogenic fungal species from 10 genera of Oomycetes and soil fungi from 40 infected freshwater fish samples of the species *Oreochromis niloticus niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish). Samples were collected from various fish farms in the Nile Delta, Egypt. Nile tilapia were tested in aquaria for their susceptibility to the commonest Oomycetes species, *Aphanomyces laevis* and*Achlya klebsiana,* and also against the 2 most prevalent pathogenic soil fungi, *Paecilomyces lilacinus* and *Phoma herbarum*. Two techniques were used: water bath exposure and intramuscular (subcutaneous) injection. Water bath exposure to the 2 species of Oomycetes caused greater mortalities of *O. niloticus niloticus* than intramuscular injection, but the reverse was true of the soil fungal species. Regardless of the infection method, the 2 Oomycetes species were more potent pathogens than the soil fungal species. In both gills and mytomal muscles of fish infected by *A. laevis* and *P. herbarum*. We measured and compared with controls the oxidative stress parameters total peroxide (TP), lipid peroxidation (LPO) and nitric oxide (NO); and levels of the antioxidants vitamin E and glutathione (GSH), and superoxide dismutase (SOD) and catalase (CAT) activities. Infection by these 2 fungal species through either spore suspension or spore injection significantly increased oxidative damage in gills and induced marked decrease in most studied antioxidants. In addition, both routes showed similar effects and *A. laevis* depressed the antioxidants CAT, vitamin E and GSH more than *P. herbarum*.

Hassan *et al.* (2011) randomly collected 100 fish samples including; 40 of fresh fish (Tilapia nilotica), 30 each of (smoked fish and salted fish) from different shops and retail markets at different sanitation levels at Giza Governorate. Also, one hundred and fifty samples of fish feeds, worker hands and water surrounding the collected fish (50 of each) were collected. All collected samples were subjected for detection of fungal and aflatoxins contamination. The results showed that 7 genera of mould and 2

genera of yeast were recovered from different types of fish. The most commonly isolated mould species in the examined Tilapia nilotica were Alternaria spp. (90%), followed by Penicillium spp., Cladosporium spp. and Candida spp. (70.0% for each). Other moulds were recovered in a variable frequency .However, in salted fish samples, Candida spp., Rhodotorula spp. and Aspergillus spp. were the most common isolates (93.3%, 80% and 83.3 %). Of genus Aspergillus; A.flavus was recovered from (66.6%) of salted fish. On the other hand, in smoked fish samples, members of Aspergillus spp. were also the most common isolates (100%), A.flavus was recovered from (70%), A. niger (36.6%), followed by Candida spp.(`73.3%), Rhodotorula spp.(66.6%), Penicillium spp. (60%), P. citrinum and P. expansum (33.3% and 26.6%) respectively. Six genera of fungal spp. and one genus of yeast were recovered from fish feeds; worker hands and utilized water with a nearly similar to the incidence of contamination in fish particularly genus Aspergillus spp. Where, the A. flavus was predominantly recovered from fish feed. Moulds of A. flavus that isolated from different types of fish and fish feed were able to produce aflatoxins. Regarding fish feed, ten isolates of A. flavus out of 18 (55.5%) were aflatoxins producer strains. On the other hand, smoked fish was highly contaminated with aflatoxins producing strains, followed by the isolated strains from salted fish and Tilapia nilotica (53.3, 45 and 40%) respectively. It is interstice to report here that the aflatoxins were detected in fish feeds and different types of fish in significant higher levels. Forty percent of fish feeds and salted fish were contaminated with aflatoxin at mean levels of (105.2±1.3 and 44.1±0.4 ppb) respectively. Accordingly, the safe alternatives methods to conventional chemical antimicrobial therapy are needed due to the emergence of multi-drug resistance. Therefore, herbal antifungal oils were evaluated as camphor, clove and rosemary oils. Camphor oil had an inhibitory effect on all tested C. albicans isolates, the inhibitory zone in the well or disc-diffusion technique varied between (11±0.71 and 1±0.15 mm) diameter. Whereas, the Inhibitory zones of camphor oil against A. flavus were of (9±0.71 and 7±0.52 mm) diameter that were obtained by the well and disc-diffusion technique, respectively. On the other hand, the crud clove oil gave a stronger antifungal effect than other tested oils; the inhibitory zones against A. flavus were (15±0.63 and 15±0.25 mm) diameter and in case of C.albicans the inhibitory zone (13±0.55 and 9±0.52 mm) in diameter by the well and discdiffusion technique, respectively. In general the well diffusion test gave a wider zone of inhibition for fungal growth by all tested oils or chemicals antifungal. The quality of fish flesh was preserved after treatment with antifungal included normal taste, odor and palatability of flesh. The continuous investigation is necessary to device drug tested to combat fungal infection

Marancik *et al*. (2011) characterized two cases of systemic mycosis in captive sharks. These cases were progressive and ultimately culminated in terminal disease. *Paecilomyces lilacinus*, an uncommon pathogen in human and veterinary medicine, was associated with areas of necrosis in the liver, heart, and gill in a great hammerhead shark (*Sphyrna mokarran)*. Fungal growth was observed from samples of kidney, spleen, spinal fluid, and coelomic cavity swabs. Dual fungal infection by *Exophiala pisciphila* and *Mucor circinelloides* was diagnosed in a juvenile zebra shark (*Stegostoma fasciatum)*. Both fungi were present in the liver, with more severe tissue destruction associated with *E. pisciphila*. *E. pisciphila* also produced significant necrosis in the spleen and gill, while *M. circinelloides* was associated with only minimal tissue changes in the heart. Fungal cultures from liver, kidney, and spleen were positive for both *E. pisciphila* and *M. circinelloides*. Identification of *P.*

lilacinus and *M. circinelloides* was based on colonial and hyphal morphology. *E. pisciphila* was identified by sequence analysis of the 28S rRNA D1/D2 region and the internal transcribed spacer (ITS) region between the 18S and 28S rRNA subunit. These cases, and a lack of information in the literature, highlight the need for further research and diagnostic sampling to further characterize the host–pathogen interaction between elasmobranchs and fungi.

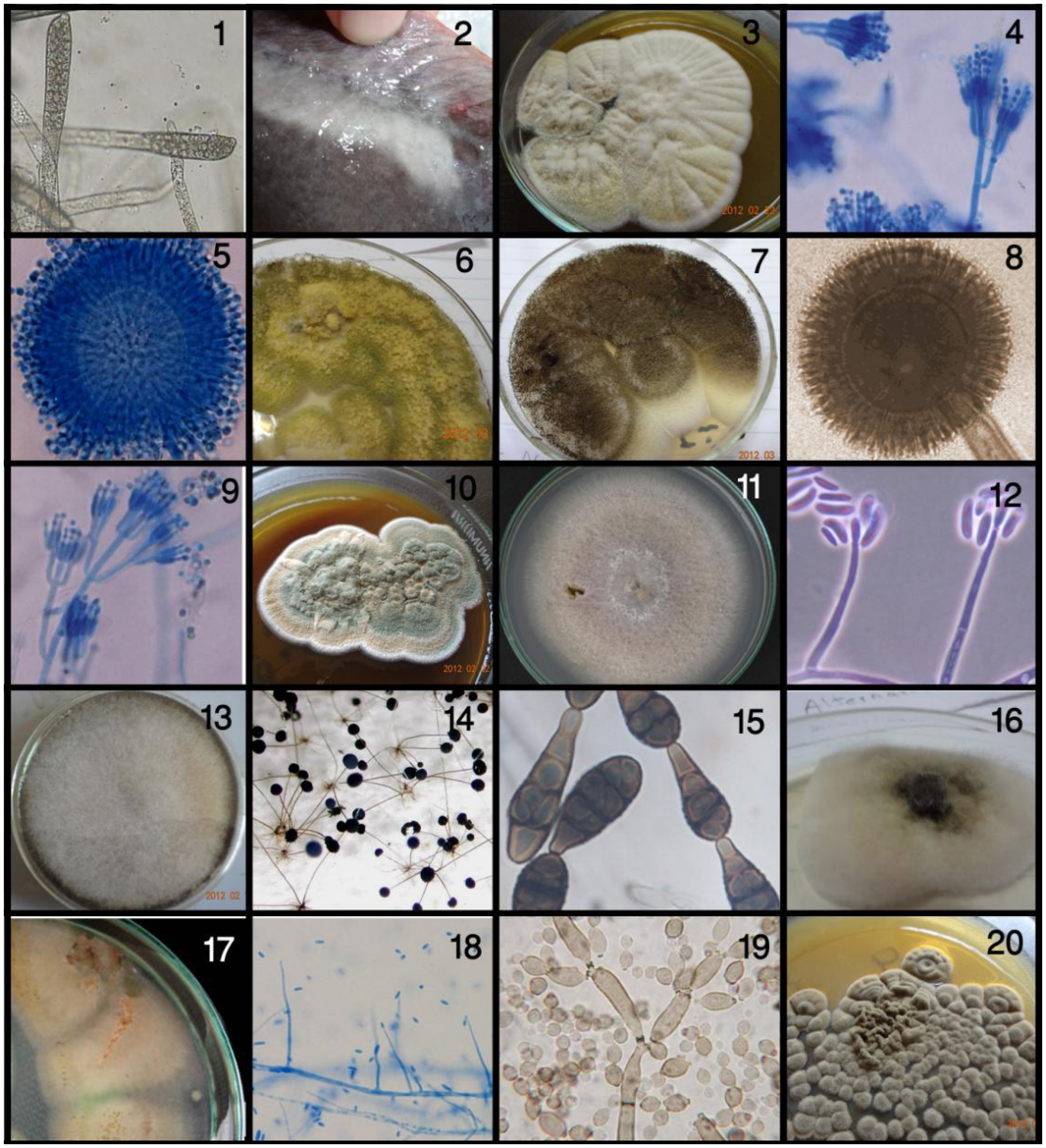
AHMED *et al.* (2012) collected 80 fresh samples of Oreochromis niloticus of family Cichildae from Jable Aulia dam – Al shagara farm, University farm – Wad Al Mamon farm, (40 from the Nile and 40 from the farms), during the period April to July 2009. The spacemen's were examined for the presence of fungal contamination, based on the generic names of the isolated organisms. The density of contamination of the total samples was found to be 54% while the density of contamination in fishes collected from Jable aulia dam 70% (which represent the natural environment) Alshagara farm 38% Sudan University farm 47% and Wad Al Mamon farm 42% (which represent the culture environment the density of contamination was found to be 42%). The fungal organism was identified as Saprolegnia spp, Aphanomyces spp, Achlaya spp, Asperigulus niger, pencilium spp and Rhizopupus species

Edrisa *et al.* (2012) collected 125 random samples of fish products after different periods from production, 25 each of vacuum- packed salted Mugil cephalus (Fesiekh) ; plastic jars containing salted Fesiekh; vacuum-packed cold smoked herring roe; plastic jars containing cold smoked herring fillets and plastic jars containing salted sardine .These products were produced by a single company where they were subjected to bacteriological examinations for aerobic plate count ,total Enterobacteriaceae count, total Staphylococci count, Staphylococcus aureus count and Clostridium perfringens count, as well as mycological examination for count, isolation and identification of moulds and yeasts. The results revealed that the plastic jars containing salted Fesiekh showed relatively higher values of aerobic plate mean count(5.3×105 /g) than the other products. While the vacuum-packed cold smoked herring roe showed relatively the lowest values in Staphylococcus aureus mean count (1.7×102 /g). Moreover, Clostridium perfringens was absent in all products. Candida albicans was the only yeast genera isolated from Vacuumed packed feseikh, Feseikh in jars and Salted sardine fillets, but in vacuum-packed cold smoked herring roe and plastic jars containing cold smoked herring fillets couldn't isolate any yeast genera. While,the mould count was relatively higher in plastic jars containing cold smoked herring fillets. The isolated mould genera form these products were A. niger, A. flavus, Alternaria, Cladosporium , Pencillum , Fusarium and Mucor species.

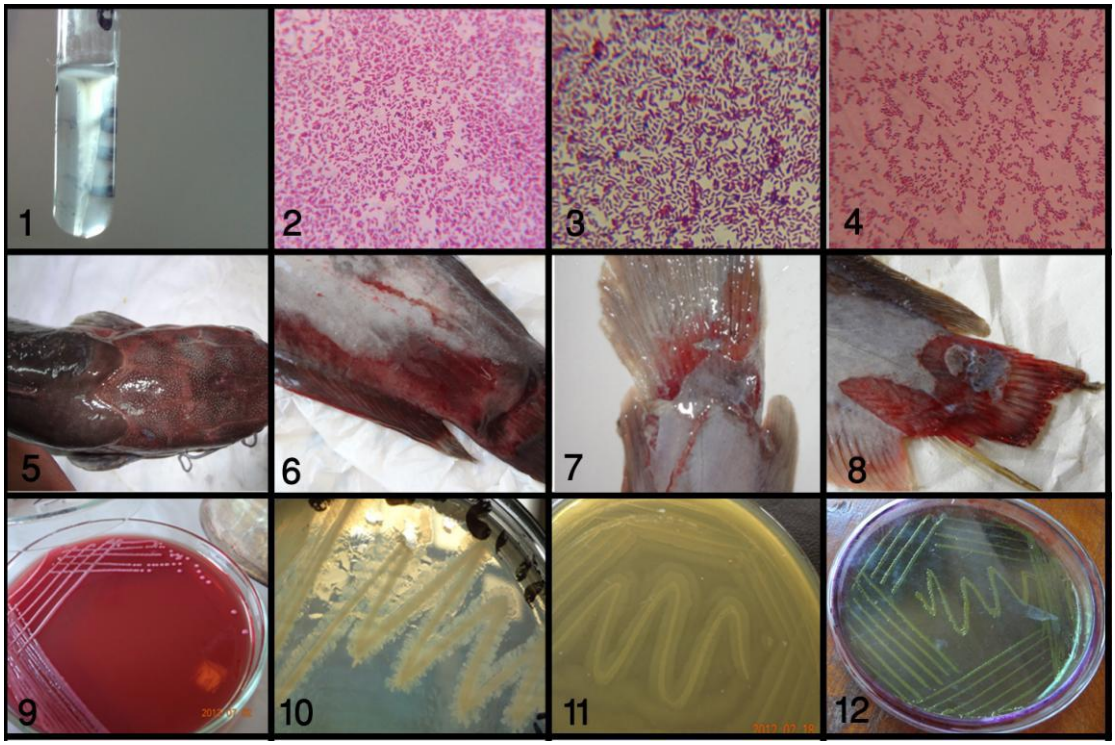
Chauhan (2013) investigated conidial fungi infection in fresh water fishes. Naturally infected fishes showed symptoms like eroded scales, skin darkening, damaged caudal, pectoral, pelvic fins and ulcerations in various parts of the body. Mycological examination revealed the isolation of 68 fungal isolates from 174 diseased fish samples. Seven species of fungi were isolated from infected fishes which belong to four genera viz. Aspergillus (47.4%), Alternaria (38.6%), Fusarium (6.5%) and Penicillum (2.1%). From the isolated seven species of conidial fungi maximum isolates (41.17%) were of Aspergillus fumigatus and minimum isolates were of Aspergillus sydowii and Penicillum sp. (2.94%) each. Twelve different species of fishes were found infected viz. Channa punctatus, C.striatus, Cirrhinus mrigala,

Clarias batrachus, Labeo rohita, Macrognathus aculeatus, Mastacembalus armatus, Mystus cavasius, M.seenghala, Puntius sarana, P.ticto and Trichogaster fasciatus. The most affected fish species was M.seenghala (19.5%). It was observed that among all the four genera of conidial fungi Aspergillus was most prevalent genera causing infection in fishes.

ISMAIL *et al.* (2013) carried out a study on 240 Clarias gariepinus fish collected from The River Nile and El- Ibrahemia canal, Assuit city and the around cities (20 fish /month). The period of study was carried out during October 2011 till the end of September 2012. The clinical finding of naturally infected fish included erosions, ulceration of skin, skin darkening, fin rot, petechial hemorrhage at different parts of the body, necrotic foci and growth of the fungl hyphe in different sites on the skin and fins. It's colour was from white to brown.Mycological examination of collected samples resulted in isolation of 1200 isolates from 240 fish in presence of 960 isolates as mixed cases. The incidence of moulds isolated from fish were Fusarium solani (210)17.5%, Aspergillus flavus (184)15.2%, Aspergillus niger (170)14.3%, Mucor hiemalis (162)13.5%, Penicillium chrysogenum (97) 8.1%, Penicillium aurantiogriseum (95) 7.9%, Chladosporium herbarum (85)7.1%, Saprolegnia Sp. (60) 5% , Rhizopus Sp. (54) 4.5%, Chladosporium sphaerospermum (53) 4.4% Acremonium strictum (18)1.5%, Alternaria alternate (12)1%. Bacteriological examination of collected samples resulted in isolation of 370 isolates from 240 fish in the presence of 130 isolates as mixed cases. The incidence of Gram negative bacilli bacterial isolated from fish were Flavobacterium columnare (115) 31.1%, Aeromonas hydrophila (75) 20.3%, Edwardsiella tarda (57) 15.4%, Pseudomonas sp. (43)11.6%, E. coli (21) 5.7%, Proteus sp. (19) 5.1%, Klebsiella (12) 3.2% . The incidence of Gram postive cocci isolated from fish were Streptococcus sp. (15) 4.1%, Staphylococcus sp. (13) 3.5%. All fish in this study infected by 1-3 types of bacteria with 3-5 types of fungi at the same time.



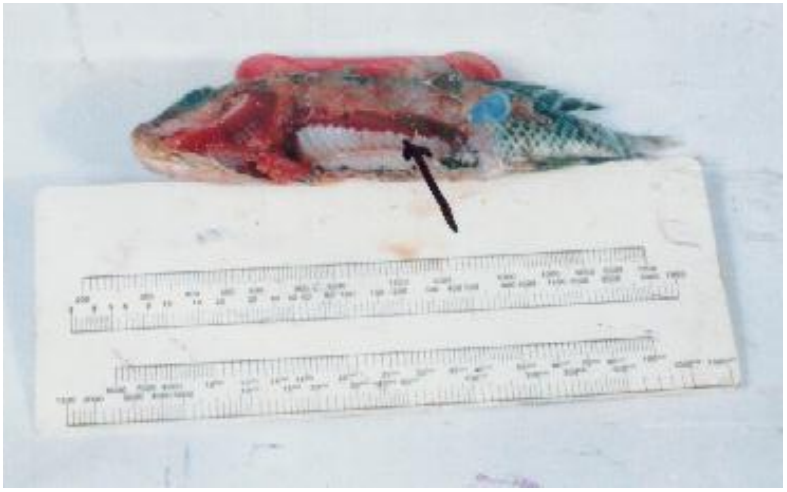
Non septated broad hyphae of Saprolegnia sp., Photo. (2): Characteristic cotton –wool like growth of Saprolegnia, Photo. (3): P.aurantoigriseum on (SDA), Photo. (4): P.aueantiogriseum showing brush-like arrangement of fruiting head, Photo. (5): Uni and biseriate conidophores with conidia of Aspergillus flavus by lactophenol cotton blue stain, Photo. (6): Colonies of Aspergillus flavus on (SDA), Photo. (7): Colonies of Aspergillus niger on (SDA), Photo. (8): Aspergillus niger showing characteristic round head with black conidia, Photo. (9): Conidiophores and smooth-walled, ellipsoidal conidia, Photo. (10): Penicillium chrysogenum with different colour and texture on (SDA), Photo. (11): Fusaruim solani on (SDA) with the reverse, Photo. (12): Fusaruim solani with characteristic slender, multicelled conidia, Photo. (13): Rhizopus sp. colony on SDA showing dens wooly mycelia, Sporangia was seen as small black dots, Photo. (14): Rhizopus sp. showing long branched sporangiophores and terminate with rhizoids, Photo. (15): Conidiophores, part of a conidial chain, and liberated conidia of Alternaria alternate, Photo. (16): Grey, felty and powdery colonies of Alternaria alternate, Photo. (17): Pink colonies of Acremonium strictum on (SDA), Photo. (18): Conidiophores and conidia of Acremonium strictum, Photo. (19): Conidiophores and conidia of Cladosporium herbarum, Photo. (20): Charactarestic velvety, olive-green to olivaceous brown colonies of Cladosporium sphaerospermum on (SDA) **ISMAIL *et al.* (2013)**



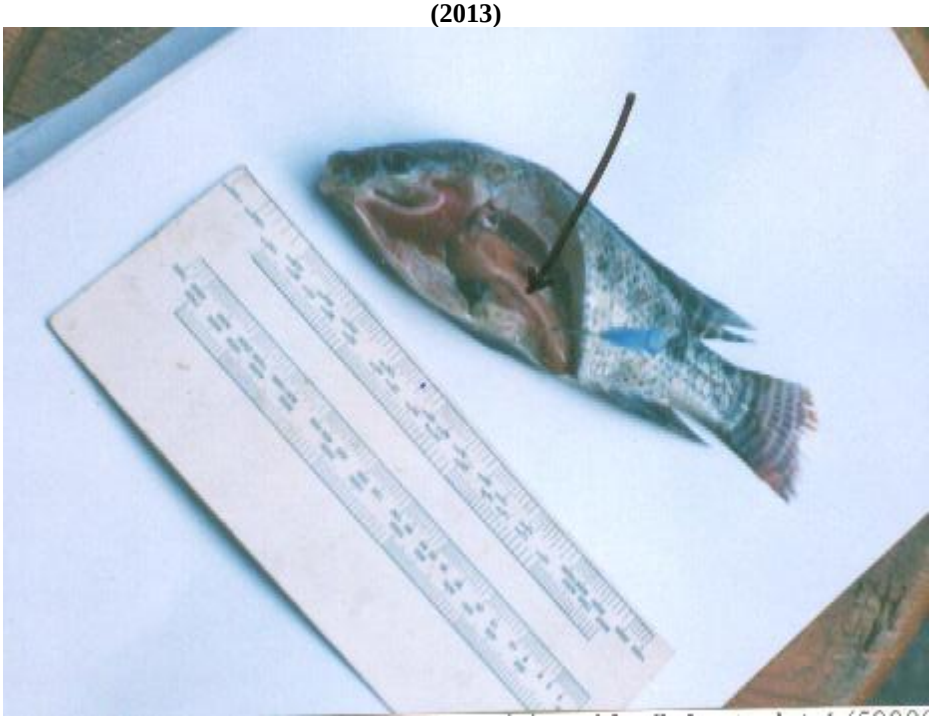
Motility test +ve , Photo. (2): Gram –ve short rod bacilli E. tarda, Photo. (3): Gm –ve short rod of Flavobacterium columnare, Photo. (4): Gram –ve Areomonas hydrophila, Photo. (5): Sever heamorrhage on head Columnaris infection, Photo. (6): f. columnare infection, Photo. (7): haemorrhage and ulceration oftail, Photo. (8): Fin rot columnaris infection, Photo. (9): Pink colonies of Klebsiella on MacConcy agar, Photo. (10, 11): Swarming with irregular edges of F.columnare on (cytophaga agar), Photo. (12): Blue –black colonies with greenish metallic sheen of E.coli on (EMB), **ISMAIL *et al.* (2013)**

Saad *et al.* (2013) concluded that, when the ration or the fish suffered from fungal infection the addition of black seed, garlic and onion will reduce the infection and improve fish health. In Post mortem lesions the fish suffered from mycotic infection showed severe degenerative changes in internal organs especially in the liver, heart and kidneys. The result cleared that, the blackseed is the best herbs that prevented and improve the aflatoxin effect followed by garlic and onion, respectively. The result also showed that level of RBCs and WBCs, differential leucocytic counts, phagocytosis process, serum protein, biochemical analysis of fish body, body weight and body weight gain improved with addition of blackseed, garlic and onion. The residue of aflatoxin in fish flesh decreased in the groups treated with blackseed, garlic and onion than the control or fish fed on the aflatoxin. The results also showed that, frequent supplementation of fish ration with black seed, garlic and onion can reduce the aflatoxin hazards in the fish. The results also concluded that, the higher economic efficiency measures (total return, total costs, net profit, total returns/total costs and net return to total costs) improved in the groups fed with blackseed, garlic, onion and all of them improved economic efficiency measures than the control groups and when all of them added to the fish treated with aflatoxin diet improved economic efficiency results than the group treated with aflatoxin only.

1. *O. niloticus* exposed to (AFTB1), showing fin erosions, eye cataraca and petechial heamorrhages distributed over the body. *O. niloticus* exposed to (AFTB1) showing fin erosion and corenal opacity as well as rusty spots formation on belly and dorsal region. **Saad *et al.* (2013)**



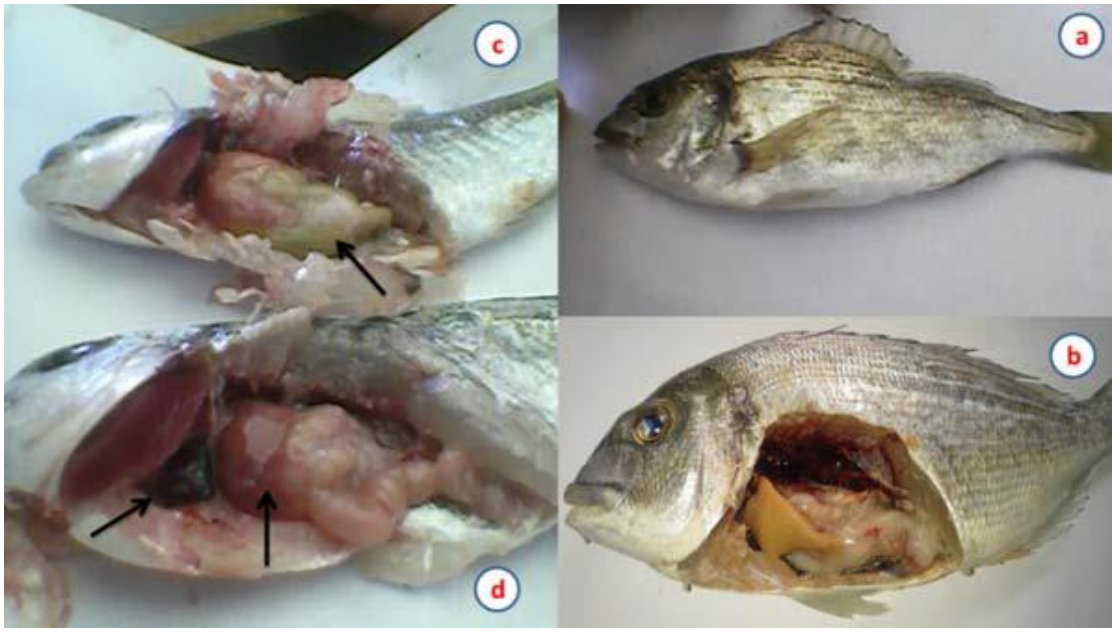
O. niloticus exposed to (AFTB1), showing severe congestion of gills and kidney (Arrow). **Saad *et al.* (2013)**



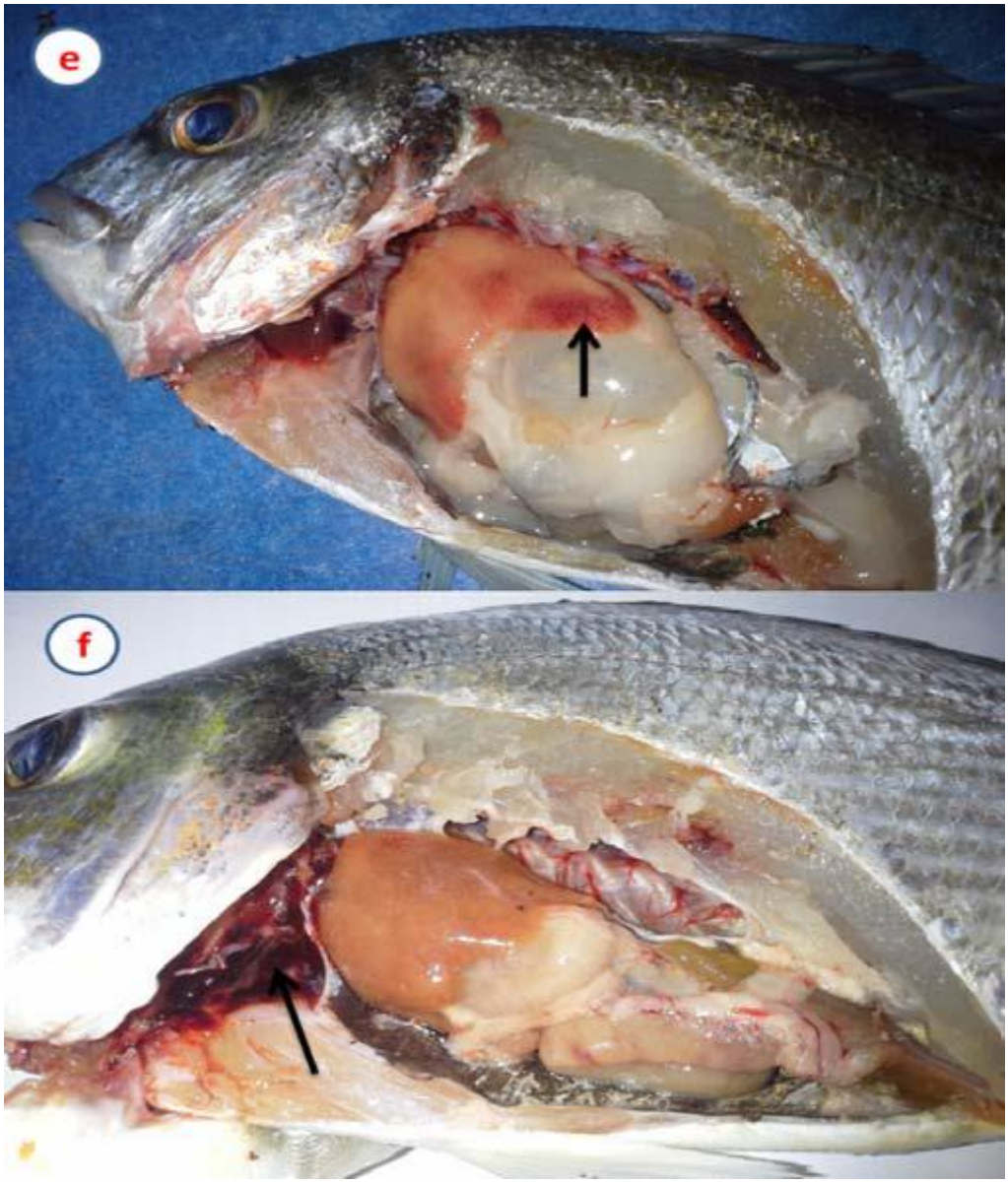
O. niloticus exposed to *(AFTB1),* showing spots of gongested areas in the periphery of the liver. As well as planes of liver (Arrow). **Saad *et al.* (2013)**

Electrophoretic pattern of different groups exposed to immunostimulents during the experiment in the 4th week. **Saad *et al.* (2013)**

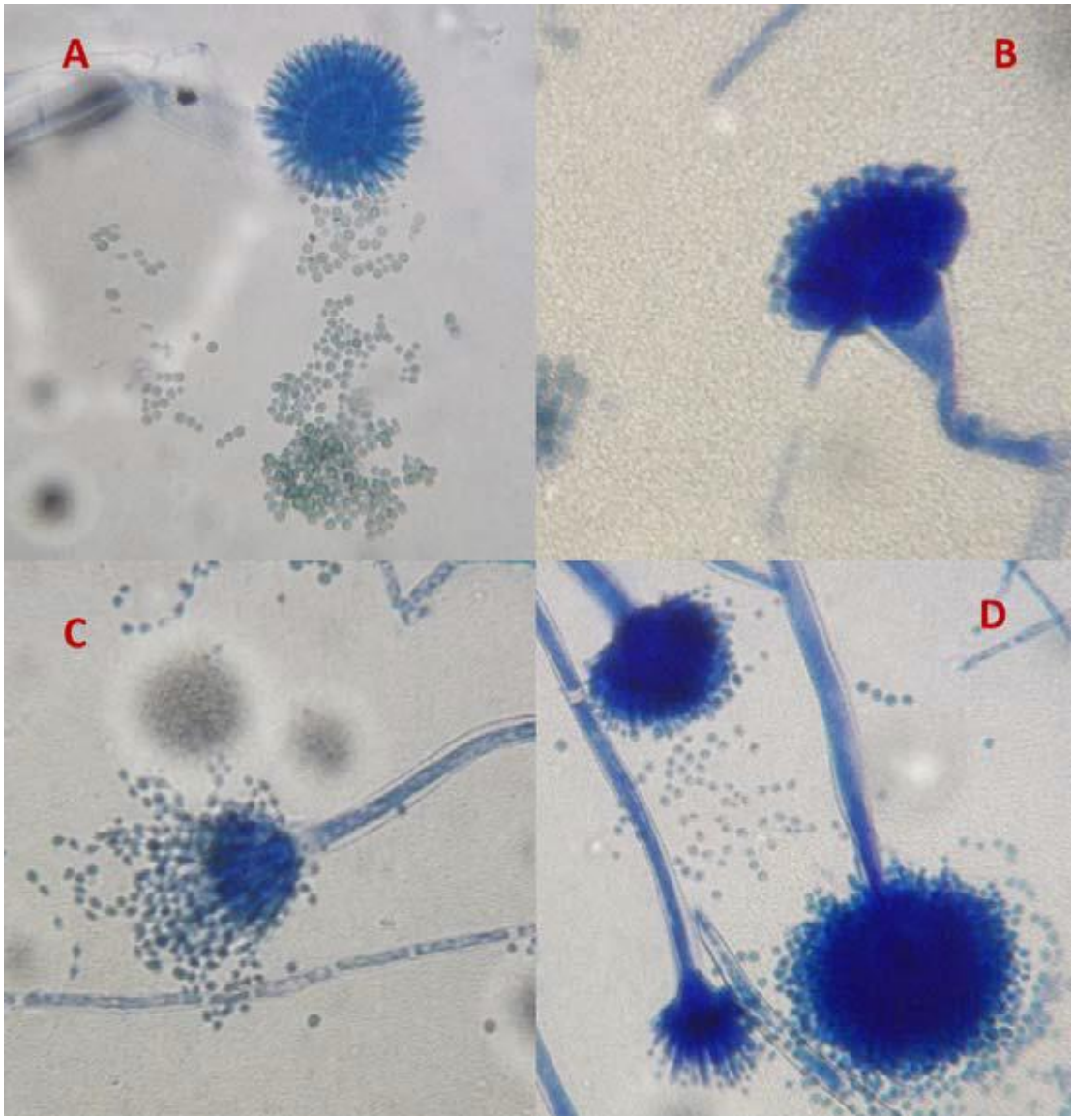
Abdel-Latif *et al.* (2014) performed surveillance and descriptive studies of mycotic infections throughout a period of one year (2013 to 2014). A total number of one hundred of cultured Gilthead seabream at Marriott Lake were surveyed for mycotic infections, whereas clinical and PM lesions were defined. Morphological and cultural characters of the isolated fungi and yeast were identified from fish tissues and organs. Moreover, their prevalence, incidence and relationship with physico-chemical properties and heavy metals content in water and tissues were evaluated. Infected fish have torned vertebral column, congested kidney with pale liver, fungal patches on the GIT and mottled appearance of the liver with severely congested heart. Results were confirmed with histopathological examination, which revealed the presence of fungal hyphae and spores in different organs. It was found that about eighty percentage (80%) of the examined fish were infected and total Aspergillus species were predominant in prevalence of mycotic isolates (32.12%) followed by Cladosporium (20.86%) and Fusarium species (14.45%). Moreover, the incidence of the mycotic isolates was higher in liver and kidney of the infected fish. The results of water quality parameters indicate that levels of nitrite, ammonia, organic matter as well as cadmium (Cd), lead (Pb) and copper (Cu) were higher than the permissible limits. We can conclude that the higher mycotic infections of cultured seabream were parallel together with unsuitable water quality and higher heavy metal levels.



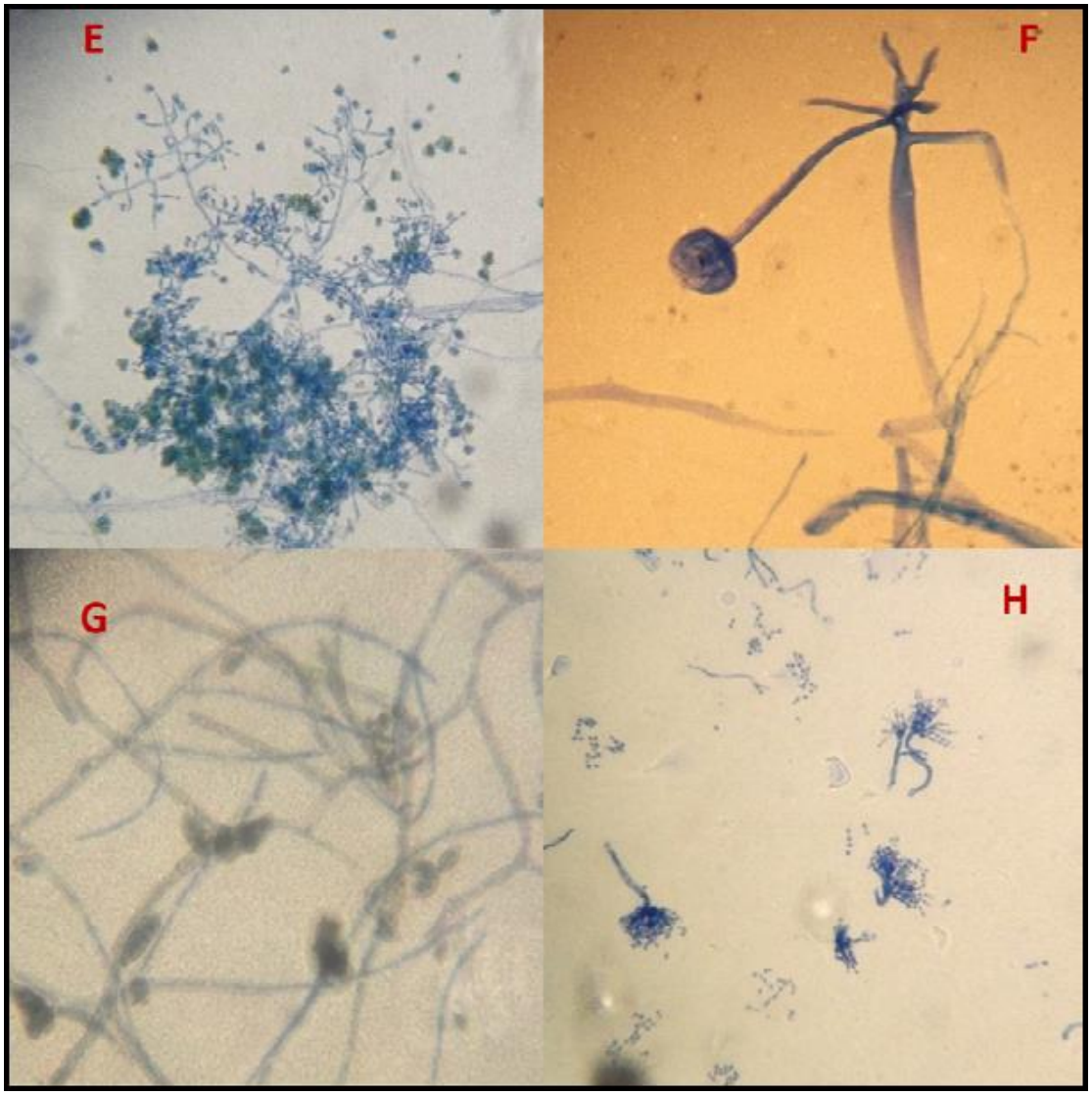
Naturally examined Seabream with torned vertebral column (Photo a), congested kidney with pale liver (Photo b), fungal patches on the GIT (Photo c) and mottled appearance of the liver (arrow) with severely congested heart (arrow) (Photo d). **Abdel-Latif *et al.* (2014)**



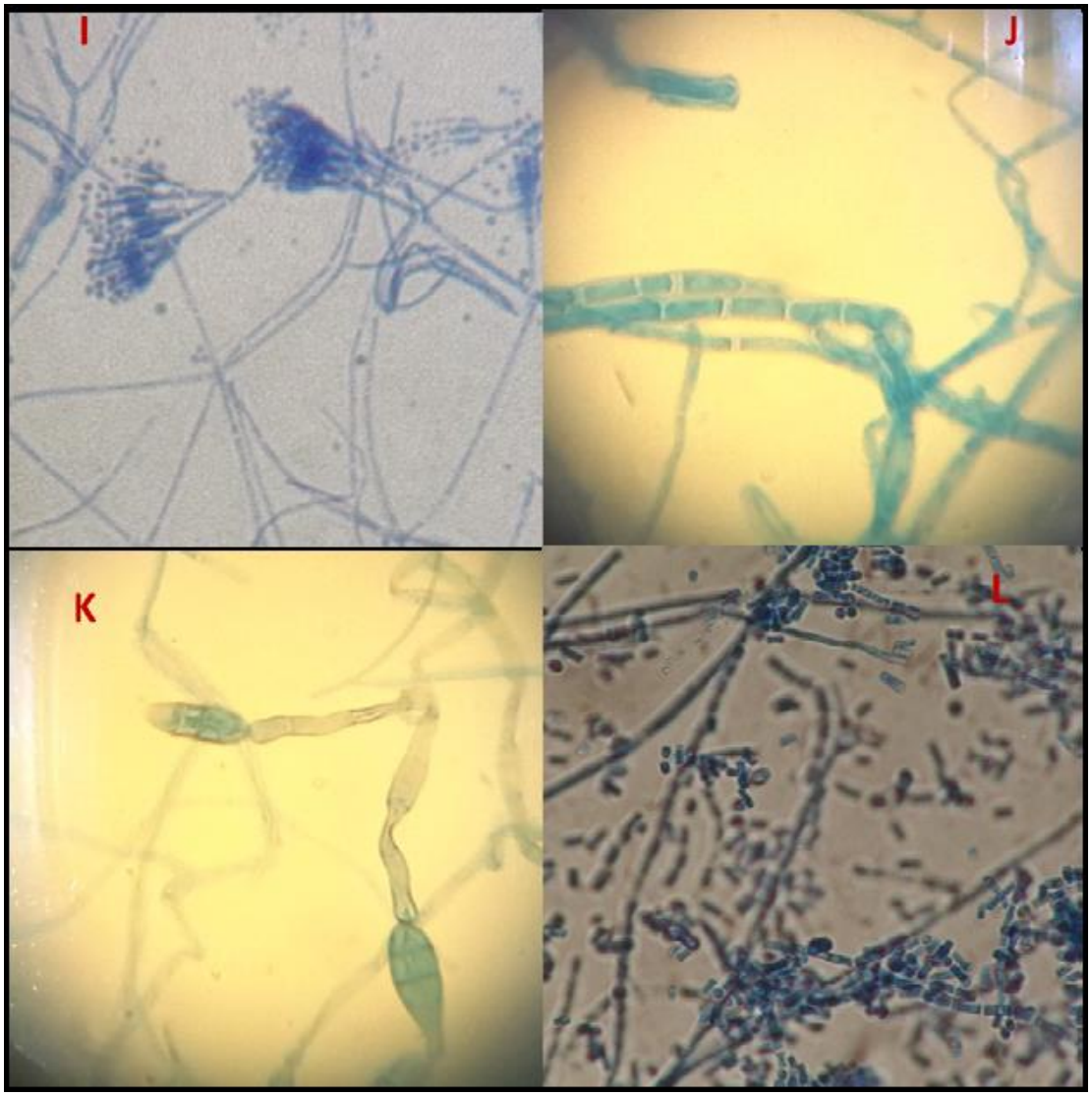
PM lesions of naturally examined Seabream characterized with pale liver with focal hemorrhages on its surface and have mottled appearance (arrow) (Photo d), congested heart and gills (arrow) (Photo e) **Abdel-Latif *et al.* (2014)**



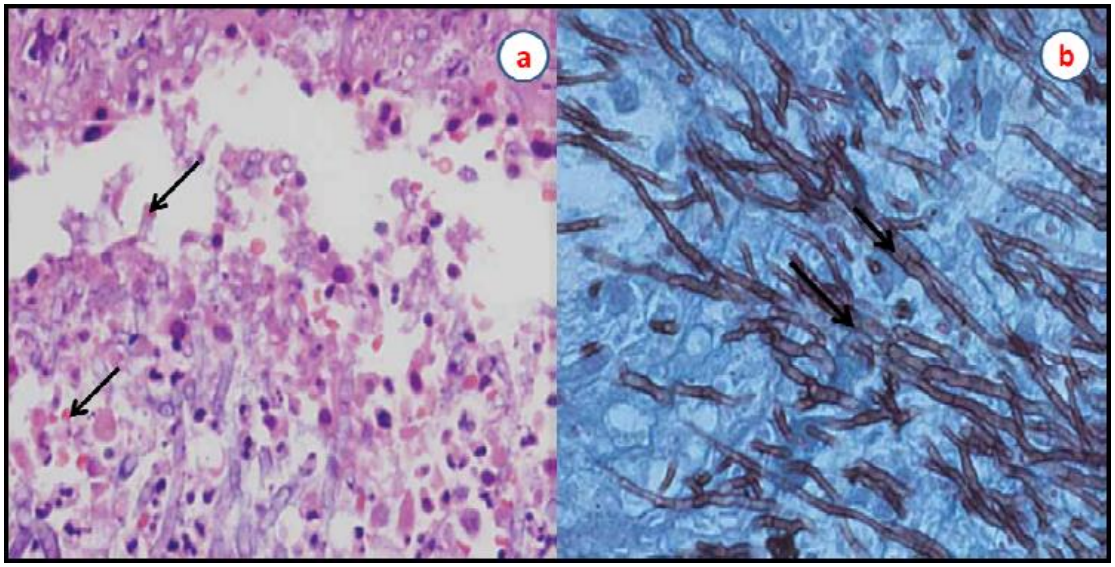
Light photograph of Lacto phenol cotton blue X 400 have A. flavus, 5-7 days old (Photo A), showing the large rounded vesicles were bearing the biseriate, loose and radiate strigmata which gave rise to ovoid rough conidia, A. niger (Photo B), showing conidial heads are short columnar in and biseriate. Conidiophore stipes is usually short, brownish and smooth walled Conidia are globose and rough-walled, A. fumigatus (Photo C), showing conidial heads are typically columnar but often much shorter and smaller) and uniseriate. Conidiophore stipeses are short, smooth-walled and have conical-shaped terminal vesicles which support a single row of phialides on the upper two thirds of the vesicle. Conidia are produced in basipetal succession forming long chains and are globose to subglobose and A. terreus (Photo D) showing conidiophore stipes are hyaline and smooth-walled Conidia are globes to ellipsoidal, hyaline to slightly yellow and smooth-walled. **Abdel-Latif *et al.* (2014)**



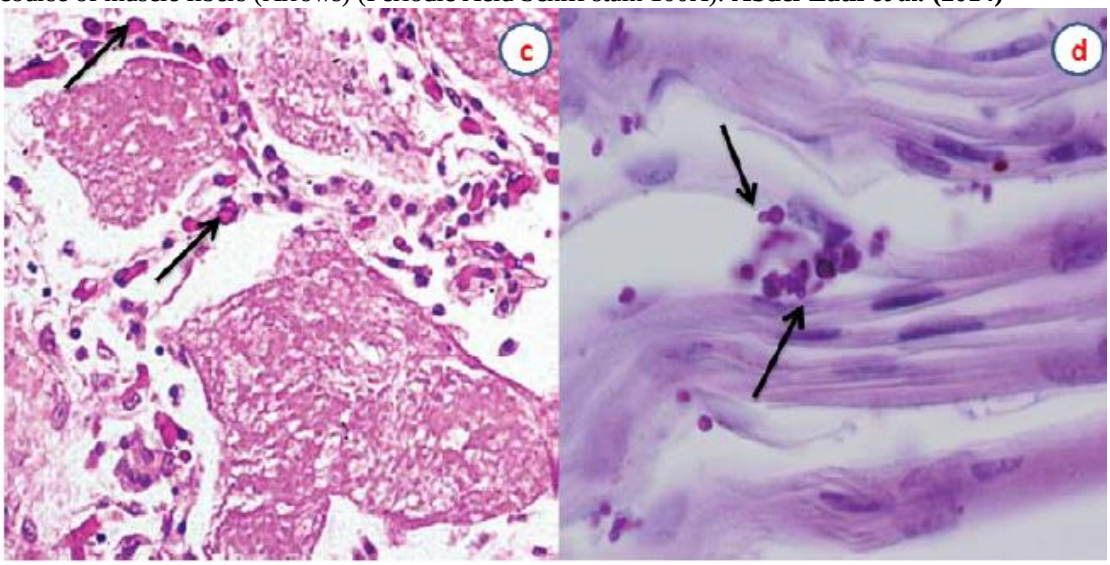
Light photograph of Lacto phenol cotton blue X 400 have Paecilomyces spp. showing phialides are long, slender and graceful and broad, non-septated hyphae (Photo E) and Conidiophores bearing dense, vertically arranged branches bearing phialides. Phialides are cylindrical or ellipsoidal, tapering abruptly into a rather long and cylindrical neck (Photo H), Rhizopus spp. (Photo F) showing rhizoids of the colony formation and Cladosporium carrionii (Photo G) showing ascending to erect, apically branched, elongate conidiophores producing branched acropetal chains of smooth-walled conidia. Conidia are pale olivaceous, smooth-walled or slightly verrucose, limoniform to fusiform. **Abdel-Latif *et al.* (2014)**



Light photograph of Lacto phenol cotton blue X 400 have Penicillium spp. (Photo I) showing conidiophores are hyaline, smooth walled and bear terminal verticals of 3-5 metulae, each bearing 3-7 phialides. Conidia are globose to subglobose, smooth-walled and are produced in basipetal succession from the phialides, Aphanomyces spp. (Photo J) Showing arrangement of zoospores in one row, Exophiala spp. (Photo K) showing aggregations of cylindrical spores at the end of hyphae and Alternaria spp. (Photo L) showing macroconidia divided by alteration of spore **Abdel-Latif *et al.* (2014)**



: Histopathological section of liver of seabream (Photo a) showing hydropic degeneration and distributed fungal elements (arrows) in the most disarrangement hepatic cells, while the musculature (Photo b) showing myelitis of the muscle fibers associated with embedded hyphal elements along the course of muscle fibers (Arrows) (Periodic Acid Schiff stain 100X). **Abdel-Latif *et al.* (2014)**



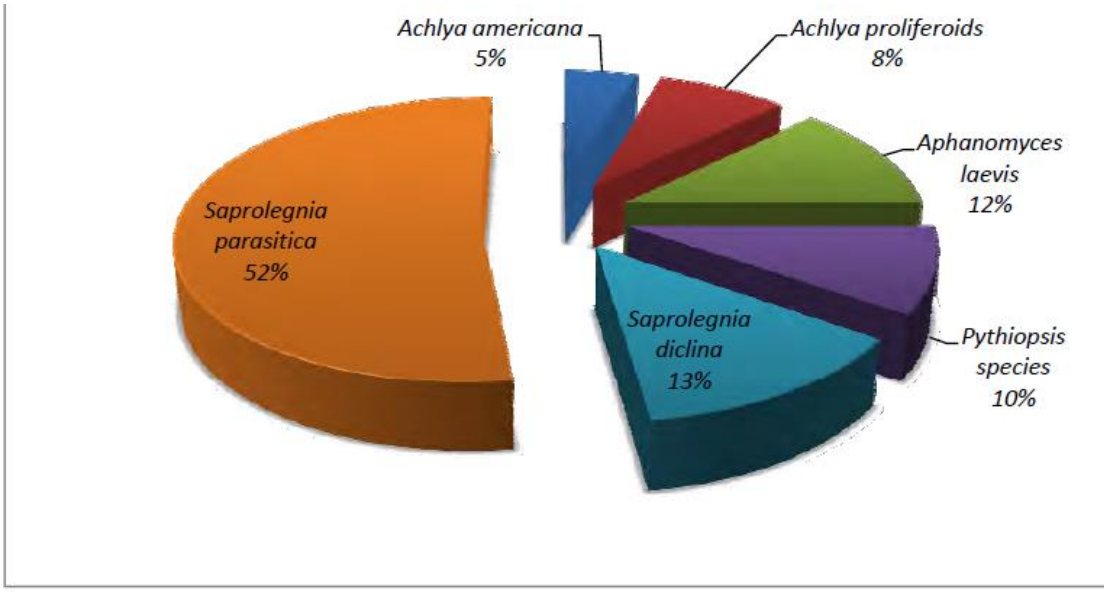
Histopathological section of the liver of Seabream (Photo c) showing severe congestion and hyaline cost masses as well as distributed fungal elements (arrows) in the most disarrangement hepatic cells while the musculature of Seabream (Photo d) showing myelitis of the muscle fibers associated with aggregation of budding spores of fungus in the center of muscle cells (Arrows) (Periodic Acid Schiff stain 100X). **Abdel-Latif *et al.* (2014)**

Chauhan (2014) conducted a study on mass mortality of Tilapia mossambicus in culture pond of University campus , Bhopal. In December, 2012 fungal infection was observed on body of fishes in form of cottony mycelium .Anterior region of body was most affected area and fishes suffered from severe infection followed by death. Fishes were examined regularly for the period of one month. Isolation of fungi revealed the presence of six species of fungi viz. Achlya Americana, Achlya proliferoids, Aphanomyces laevis, Pythiopsis species, Saprolegnia diclina and Saprolegnia parasitica .Total 196 isolates were were cultured by using three different agar media viz. Corn Meal Agar and Potato Dextrose Agar Maximum percentage of isolates were contributed by Saprolegnia parasitica (52%) and minimum were of Achlya Americana(5%) Temperature, pH and DO of water were measured.

fungal hyphae covering whole body surface of Tilapia. **Chauhan (2014)**

moribid fishes infected with fungus collected from culture pond **Chauhan (2014)**

cottony mycelium covering eye and fungal growth inside mouth. **Chauhan (2014)**



Species wise% of isolated species of fungi from infected Tilapia, **Chauhan (2014)**

Gozlan *et al.* (2014), in their review, discussed true fungal pathogens and then focused on commonly reported zoosporic and amoeboid fungal-like pathogens in the Oomycota and Mesomycetozoea. In general, the number of reported fungal and fungal-like pathogens responsible for diseases in animals is on the increase globally. As such, they are truly emerging diseases with increasing incidence, geo-graphic range, virulence, and some of these fungal and fungal-like pathogens have recently been found in new hosts or are newly discovered. The underpinning drivers of this observed increase remain unclear but these pathogens are known to be opportunistic, to have resilient and relatively long-lived environmental and may have benefited from recent increase in global trade and spread of invasive species. Thus increasingly infectious outbreaks are reported in a broad range of species. In aquatic ecosystems fungi and fungal-like pathogen detection in fish hosts is more complicated due to the lack of direct observation of their hosts. This is particularly true in freshwater systems where, despite being responsible for pan-continental population extinctions, some diseases caused by fungal and fungal-like pathogens are chronic with no clear external symptoms. This is very well illustrated, for example, by the rosette agent *Sphareothecum destruens*, which has been rapidly spreading all over Europe via an invasive healthy fish host carrier. This fungal-like pathogen is intra-cellular, causing high mortality (up to 90%) after about 20–30 days but it can only be confidently detected by PCR analysis.

Table 1 | List of Fungi, Mesomycetozoea and Oomycetes species, which have been recorded as fish parasites in the Web of Knowledge since 1997.

Species	Order	Reported hosts	Generalist index	References
FUNGI				
Cladosporium sphaerospermum	Capnodiales	*Lutjanus campechanu*	NA	Blaylock et al., 2001
Exophiala angulospora	Chaetothyriales	*Gadus morhua*	NA	Gjessing et al., 2011
Exophiala pisciphila	Chaetothyriales	*Stegostoma fasciatum*	NA	Marancik et al., 2011
Exophiala xenobiotica	Chaetothyriales	*Pseudocaranx dentex*	NA	Munchan et al., 2009
Paecilomyces lilacinus	Eurotiales	*Clarias gariepinus* *Oreochromis niloticus niloticus* *Tilapia aurea*	2	Rand et al., 2000a,b; Ali et al., 2011
Penicillium corylophilum	Eurotiales	*Lutjanus campechanus*	NA	Blaylock et al., 2001
Ochroconis humicola	Incertae sedis	*Pseudocaranx dentex* *Pagrus major* *Sebastiscus marmoratus*	3.3	Wada et al., 2005; Munchan et al., 2009
Mucor circinelloides	Mucorales	*Pelteobagrus fulvidraco* *Pseudocaranx dentex*	5	Ke et al., 2010; Marancik et al., 2011
Phoma herbarum	Pleosporales	*Clarias gariepinus* *Oncorhynchus tshawytscha* *Oreochromis niloticus niloticus*	4	Faisal et al., 2007; Ali et al., 2011
Phialemonium dimorphosporum	Sordariales	*Mugil cephalus*	NA	Sosa et al., 2007a,b
Ochroconis humicola	Incertae sedis	*Pseudocaranx dentex* *Pagrus major* *Sebastiscus marmoratus*	3.3	Wada et al., 2005; Munchan et al., 2009
MESOMYCETOZOEA				
Dermocystidium cyprini	Dermocystida	*fluviatilis* *Gymnocephalus cernuus* *Cyprinus carpio*	3.3	Lotman et al., 2000; Pekkarinen and Lotman, 2003
Dermocystidium fennicum	Dermocystida	*PercaPerca fluviatilis*	NA	Pekkarinen and Lotman, 2003
Dermocystidium koi	Dermocystida	*Cyprinus carpio*	NA	Gjurcevic et al., 2008
Dermocystidium percae	Dermocystida	*Perca fluviatilis*	NA	Morley et al., 2008
Dermocystidium branchiale	Dermocystida	*Salvelinus alpinus* *Salmo trutta*	2	Kristmundsson and Richter, 2009
Sphaerothecum destruens	Dermocystida	*Abramis brama* *Cyprinus carpio* *Leucaspius delineatus* *Oncorhynchus kisutch* *Oncorhynchus mykiss* *Oncorhynchus tshawytscha* *Pseudorasbora parva* *Rutilus rutilus* *Salmo salar* *Salmo trutta* *Salvelinus fontinalis*	3.6	Arkush et al., 1998; Gozlan et al., 2005; Andreou et al., 2012; Paley et al., 2012
Ichthyophonus hoferi	Ichthyophonida	*Citharichthys stigmaeus* *Clupea harengus* *Clupea pallasi* *Hypomesus pretiosus* *Microgadus proximus* *Oncorhynchus kisutch* *Oncorhynchus mykiss* *Oncorhynchus tshawytscha* *Pleuronectes flesus* *Salmo trutta* *Sebastes alutus* *Sebastes emphaeus* *Sebastes flavidus* *Sprattus sprattus*	3.6	Rahimian, 1998; Criscione et al., 2002; Hershberger et al., 2002; Schmidt-Posthaus and Wahli, 2002; Gavryuseva, 2007; Kocan et al., 2010; Kramer-Schadt et al., 2010; Rasmussen et al., 2010; Gregg et al., 2012; Hamazaki et al., 2013

Species	Order	Reported hosts	Generalist index	References
Ichthyophonus irregularis	Ichthyophonida	*Limanda ferruginea*	NA	Rand et al., 2000a,b
OOMYCETES				
Achlya bisexualis	Saprolegniales	*Mugil cephalus*	NA	Sosa et al., 2007a
Achlya klebsiana	Saprolegniales	*Oreochromis niloticus niloticus*	2.3	Ali et al., 2011; Cao et al., 2013
		Clarias gariepinus		
		Pelteobagrus fuvidraco		
Achlya americana	Saprolegniales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Achlya. oblongata	Saprolegniales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Achlya racemosa	Saprolegniales	*Odonthestes bonariensis*	NA	Pacheco Marino et al., 2009
Achlya ambisexualis	Saprolegniales	*Oncorhynchus mykiss*	NA	Vega-Ramirez et al., 2013
Aphanomyces parasiticus	Saprolegniales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Aphanomyces frigidophilus	Saprolegniales	*Coregonus lavaretus holsatus*	2	Czeczuga et al., 2004, 2005
		Salmo trutta		
Aphanomyces invadans	Saprolegniales	*Alosa sapidissima*		
		Anguilla anguilla		
		Ameiurus melas		
		Archosargus probatocephalus		
		Bairdiella chrysoura		
		Brevoortia tyrannus		
		Brycinus lateralis		
		Barbus poechii		
		Barbus paludinosus		
		Barbus unitaeniatus		
		Catla catla		
		Channa marulius		
		Clarias gariepinus		
		Clarias ngamensis		
		Cyprinus carpio		
		Fundulus heteroclitus		
		Fundulus majalis		
		Hepsetus odoe		
		Hydrocynus vittatus		
		Ictalurus punctatus		
		Leiopotherapon unicolor		
		Labeo lunatus		
		Labeo cylindricus		
		Lepomis macrochirus		
		Macquaria ambigua		
		Macculochella peelii		
		Marcusenius macrolepidotus		
		Micralestes acutidens		
		Micropterus salmoides		
		Mugil cephalus		
		Mugil curema		
		Nematalosa erebi		
		Oncorhynchus mykiss		
		Oreochromis andersonii		
		Oreochromis macrochir		
		Petrocephalus catostoma		
		Pharyngochromis acuticeps		
		Pogonias cromis		
		Sargochromis codringtonii		
		Sargochromis giardi		
		Serranochromis robustus		
		Serranochromis angusticeps		
		Serranochromis macrocephalus		
		Schilbe intermedius		
		Silurus glanis		
		Tilapia sparrmanii		

Species	Order	Reported hosts	Generalist index	References
Tilapia rendalli		*Trinectus maculates*	3.7	Thompson et al., 1999; Hawke et al., 2003; Harikrishnan et al., 2005; Kiryu et al., 2005; Webb et al., 2005; Vandersea et al., 2006; Sosa et al., 2007b; Oidtmann et al., 2008; Saylor et al., 2010; Boys et al., 2012; Go et al., 2012; Huchzermeyer and Van der Waal, 2012; Saikia and Kamilya, 2012
Aphanomyces irregularis	Saprolegniales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Aphanomyces laevis	Saprolegniales	*Aplocheilus panchax* *Clarias gariepinus* *Oreochromis niloticus niloticus*	4	Mondal and De, 2002; Ali et al., 2011
Aphanomyces salsuginosus	Saprolegniales	*Salangichthys microdon*	NA	Takuma et al., 2010
Saprolegnia australis	Saprolegniales	*Oncorhynchus nerka* *Plecoglossus altivelis* *Salmo trutta*	3.3	Hussein et al., 2001; Chang et al., 2002; Fregeneda-Grandes et al., 2007
Saprolegnia brachydanis	Saprolegniales	*Danio rerio*	NA	Ke et al., 2009a,b
Saprolegnia diclina	Saprolegniales	*Acipencer persicus* *Oncorhynchus mykiss Salmo salar* eggs *Salmo trutta* *Sciaenops ocellatus*	3.3	Leano et al., 1999; Fregeneda-Grandes et al., 2007; Ghiasi et al., 2010; Shahbazian et al., 2010; Thoen et al., 2011
Saprolegnia ferax	Saprolegniales	*Carassiuus auratus* *Coregonus lavaretus holsatus* *Odonthestes bonariensis* *Oncorhynchus mykiss* eggs *Salmo trutta*	3.6	Czeczuga et al., 2004; Fregeneda-Grandes et al., 2007; Ke et al., 2009a,b; Pacheco Marino et al., 2009; Shahbazian et al., 2010; Cao et al., 2013
Saprolegnia furcata	Saprolegniales	*Salmo trutta*	NA	Fregeneda-Grandes et al., 2007
Saprolegnia hypogyana		*Oncorhynchus mykiss* eggs *Salmo trutta*	2	Fregeneda-Grandes et al., 2007; Shahbazian et al., 2010
Saprolegnia parasitica	Saprolegniales	*Acipencer persicus* *Astyanax eigenmanniorum* *Astyanax fasciatus* *Bidyanus bidyanus* *Coregonus lavaretus holsatus* *Ictalurus punctatus* *Odontesthes bonariensis* *Oncorhynchus mykiss* *Oncorhynchus masu* eggs *Oncorhynchus nerka* *Salmo salar* eggs *Salmo trutta* *Salvelinus leucomaenis*	3.3	Bangyeekhun et al., 2001; Hussein and Hatai, 2002; Czeczuga et al., 2004; Fregeneda-Grandes et al., 2007; Mancini et al., 2008, 2010; Mifsud and Rowland, 2008; Ghiasi et al., 2010; Shahbazian et al., 2010; Thoen et al., 2011

Species	Order	Reported hosts	Generalist index	References
Saprolegnia polymorpha	Saprolegniales	*Cyprinus carpio*	NA	Willoughby, 1998
Saprolegnia salmonis	Saprolegniales	*Coregonus lavaretus holsatus* *Oncorhynchus masu* *Oncorhynchus mykiss* *Oncorhynchus nerka* *Plecoglossus altivelis* *Salmo trutta* *Salvelinus leucomaenis*	2.4	Hussein et al., 2001; Chang et al., 2002; Hussein and Hatai, 2002; Czeczuga et al., 2004, 2005
Saprolegnia shikotsuensis	Saprolegniales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2005
Pythium aquatile	Pythiales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Pythium pulchrum	Pythiales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Pythium thalassium	Pythiales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004
Pythium torulosum	Pythiales	*Coregonus lavaretus holsatus*	NA	Czeczuga et al., 2004

Saad *et al.* (2014) carried-out a study on a random sample from private and

governmental sector . Three localities were the area of this study regarding to their importance in farmed fish production in Egypt related to El- behera and Kafr El-Sheikh provinces. Four species of fish used in this study which include, Tilapia (Oreochromis niloticus), Common carp, Mugil cephalus and Mugil capito. This study concluded that, the main important economic diseases affecting the Tilapia (Oreochromis niloticus), carp under Egyptian conditions includes Saprolegnia, Aeromonas, parasitic and the mycotoxins from the previous fish species respectively, and the cycles spread in it this diseases achieved the lower net income level which reached to 65.45 , 12.42, 35.51 and 16.20 LE/1000 fish respectively.

Effect of Fish Species and Different Causes of Death on Length of Cycle, Livability Feed Consumption, Total Weight and Feed Conversion for each 1000 Fish. **Saad *et al.* (2014)**

Species	Cause of death	N	Length of cycle (months)	Livability (%)	Amount of feed consumed (kg)	Feed conversion (%)	Total weight (kg)
			Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Tilapia	Aeromonus	25	7.88 ± 0.38^{i}	78.92 ± 1.23^{j}	485.77 ± 23.58^{ef}	2.08 ± 0.11^{ab}	236.00 ± 6.64^{fgh}
	Saprolegnia	16	8.50 ± 0.50^{ghi}	87.60 ± 0.88^{def}	483.41 ± 14.01^{f}	2.02 ± 0.06^{ab}	240.63 ± 5.04^{fgh}
	Parasitic	14	9.29 ± 0.49^{fgh}	90.71 ± 1.09^{abcd}	509.29 ± 15.97^{ef}	1.90 ± 0.10^{bc}	273.81 ± 10.44^{dgh}
	Mycotoxins	10	8.20 ± 0.44^{ghi}	88.48 ± 1.10^{cde}	489.25 ± 22.59^{ef}	2.16 ± 0.13^{a}	230.00 ± 8.16^{gh}
	Control	80	10.00 ± 0.23^{cdef}	93.08 ± 0.44^{ab}	483.19 ± 8.04^{f}	1.66 ± 0.04^{cd}	298.52 ± 6.57^{ef}
	Total	145	9.28 ± 0.17^{C}	89.49 ± 0.56^{A}	486.60 ± 6.53^{B}	1.83 ± 0.03^{A}	274.24 ± 4.66^{C}
Carp	Aeromonus	19	11.05 ± 0.39^{bcd}	72.89 ± 1.54^{k}	754.10 ± 88.83^{ab}	0.61 ± 0.05^{e}	1202.63 ± 44.34^{c}
	Saprolegnia	10	11.40 ± 0.34^{ab}	82.10 ± 2.49^{hij}	640.61 ± 51.69^{cd}	0.49 ± 0.03^{e}	1300.00 ± 44.72^{ab}
	Parasitic	10	10.80 ± 0.44^{bcde}	85.98 ± 2.54^{efg}	694.00 ± 87.09^{bc}	0.57 ± 0.07^{e}	1215.00 ± 40.86^{c}
	Mycotoxins	2	8.00 ± 1.00^{hi}	70.00 ± 5.00^{k}	801.28 ± 32.05^{a}	0.64 ± 0.03^{e}	1250.00 ± 41.01^{bc}
	Control	19	12.68 ± 0.54^{a}	90.20 ± 1.55^{bcd}	521.94 ± 23.78^{ef}	0.40 ± 0.02^{e}	1321.05 ± 44.31^{a}
	Total	60	11.48 ± 0.26^{A}	81.99 ± 1.32^{B}	653.23 ± 35.26^{A}	0.52 ± 0.02^{C}	1260.00 ± 22.82^{A}
Mugil Cephalus	Aeromonus	13	8.92 ± 0.40^{fghi}	83.11 ± 1.22^{ghij}	489.11 ± 29.38^{ef}	1.76 ± 0.12^{cd}	282.05 ± 11.70^{dgh}
	Saprolegnia	14	10.64 ± 0.55^{bcde}	86.79 ± 1.14^{ef}	514.13 ± 16.28^{ef}	1.88 ± 0.11^{bc}	285.71 ± 18.97^{dgh}
	Parasitic	5	11.00 ± 0.55^{bcd}	92.20 ± 0.80^{ab}	478.36 ± 28.96^{f}	1.72 ± 0.15^{cd}	283.33 ± 20.41^{dgh}
	Mycotoxins	2	9.00 ± 1.00^{fghi}	85.00 ± 5.00^{fgh}	575.81 ± 205.44^{de}	1.91 ± 0.43^{abc}	291.67 ± 41.67^{efg}
	Control	51	11.14 ± 0.31^{bcd}	93.43 ± 0.49^{ab}	534.92 ± 13.27^{ef}	1.54 ± 0.06^{d}	365.03 ± 12.30^{d}
	Total	85	10.66 ± 0.23^{B}	90.49 ± 0.60^{A}	522.13 ± 10.40^{B}	1.65 ± 0.05^{B}	332.74 ± 9.30^{B}
Mugil Capito	Aeromonus	8	9.00 ± 0.57^{fghi}	81.50 ± 2.26^{ij}	511.90 ± 36.66^{ef}	2.06 ± 0.16^{ab}	250.45 ± 9.79^{fgh}
	Saprolegnia	3	11.33 ± 0.67^{bc}	88.33 ± 1.67^{cde}	554.50 ± 16.87^{def}	2.04 ± 0.22^{ab}	277.78 ± 27.78^{dgh}
	Parasitic	4	9.50 ± 0.87^{efg}	91.25 ± 0.75^{abc}	519.86 ± 22.83^{ef}	2.07 ± 0.22^{ab}	258.33 ± 27.64^{dgh}
	Mycotoxins	2	8.00 ± 1.00^{hi}	91.00 ± 1.00^{abc}	463.77 ± 19.32^{f}	2.08 ± 0.14^{ab}	225.00 ± 25.00^{h}
	Control	20	9.90 ± 0.48^{def}	93.50 ± 0.77^{a}	518.18 ± 18.93^{ef}	1.72 ± 0.11^{cd}	316.67 ± 18.65^{de}
	Total	37	9.68 ± 0.32^{C}	90.11 ± 1.02^{A}	517.01 ± 13.15^{B}	1.88 ± 0.08^{A}	287.93 ± 12.01^{C}

Cause of Death: Means within the same column carrying different superscripts (small litters) are significant at ($p \leq 0.05$).
Species: Means within the same column carrying different superscripts (capital litters) are significant at ($p \leq 0.05$).

Saad *et al.* (2014)

Effect of Fish Species and Different Causes of Death on Cost Parameter (Fixed, Variable and Total), Price of A Kilogram of Fish, Total Return as well as Net Income for each 1000 Fish.

Kilogram of Fish, Total Return as well as Net Income for each 1000 Fish.

Species	Cause of death	N	Total cost	Return (L.E)	Net income (L.E)
			Mean ± S.E	Mean ± S.E	Mean ± S.E
Tilapia	Aeromonus	25	1284.96 ± 45.86^{h}	1352.00 ± 46.58^{h}	67.04 ± 31.45^{fg}
	Saprolegnia	16	1300.96 ± 46.85^{h}	1366.41 ± 51.05^{gh}	65.45 ± 24.85^{fg}
	Parasitic	14	1462.46 ± 68.10^{h}	1633.93 ± 70.35^{fgh}	171.47 ± 51.61^{defg}
	Mycotoxins	10	1192.97 ± 74.04^{h}	1322.50 ± 81.65^{h}	129.53 ± 75.21^{efg}
	Control	80	1387.31 ± 29.02^{h}	1718.26 ± 48.19^{efg}	330.94 ± 30.98^{bcd}
	Total	145	1353.99 ± 20.91^{C}	1580.85 ± 32.73^{D}	226.86 ± 21.73^{B}
Carp	Aeromonus	19	2924.98 ± 199.87^{b}	3017.11 ± 194.13^{ab}	92.13 ± 69.23^{fg}
	Saprolegnia	10	2789.85 ± 189.97^{b}	2995.00 ± 169.35^{ab}	205.15 ± 67.48^{cdefg}
	Parasitic	10	2827.21 ± 201.27^{b}	2923.75 ± 168.04^{b}	96.54 ± 51.12^{fg}
	Mycotoxins	2	3293.67 ± 271.44^{a}	2950.66 ± 133.28^{ab}	12.42 ± 115.19^{g}
	Control	19	2635.21 ± 120.60^{b}	3281.25 ± 156.25^{a}	315.45 ± 51.06^{bcde}
	Total	60	2806.69 ± 87.31^{A}	2985.63 ± 83.01^{A}	178.93 ± 32.97^{B}
Mugil Cephalus	Aeromonus	13	2286.60 ± 123.21^{cd}	2322.10 ± 117.63^{cd}	35.51 ± 53.17^{g}
	Saprolegnia	14	2206.56 ± 90.82^{cde}	2355.65 ± 156.09^{cd}	149.10 ± 91.48^{defg}
	Parasitic	5	$2058.36 \pm 121.32^{cdefg}$	2312.50 ± 181.98^{cd}	254.14 ± 88.90^{cdef}
	Mycotoxins	2	1998.27 ± 63.50^{defg}	2385.42 ± 447.92^{c}	387.14 ± 384.42^{bc}
	Control	51	2314.10 ± 69.45^{c}	2923.77 ± 99.63^{b}	609.67 ± 59.64^{a}
	Total	85	2269.71 ± 48.80^{B}	2689.56 ± 74.83^{B}	419.85 ± 47.94^{A}
Mugil Capito	Aeromonus	8	1983.60 ± 175.01^{efg}	2018.30 ± 138.09^{de}	34.70 ± 66.49^{g}
	Saprolegnia	3	1897.05 ± 19.05^{fg}	2069.44 ± 256.94^{cde}	172.40 ± 237.9^{defg}
	Parasitic	4	$2107.98 \pm 118.81^{cdef}$	2204.17 ± 210.76^{cd}	96.18 ± 118.79^{fg}
	Mycotoxins	2	1809.95 ± 102.77^{g}	1793.75 ± 143.75^{ef}	16.20 ± 40.98^{g}
	Control	20	2297.99 ± 106.59^{c}	2754.38 ± 147.47^{b}	456.39 ± 84.65^{ab}
	Total	37	2150.58 ± 74.11^{B}	2428.28 ± 106.70^{C}	277.70 ± 60.85^{B}

e of Death: Means within the same column carrying different superscripts (small litters) are significant at ($p \leq 0.05$).
ies: Means within the same column carrying different superscripts (capital litters) are significant at ($p \leq 0.05$).

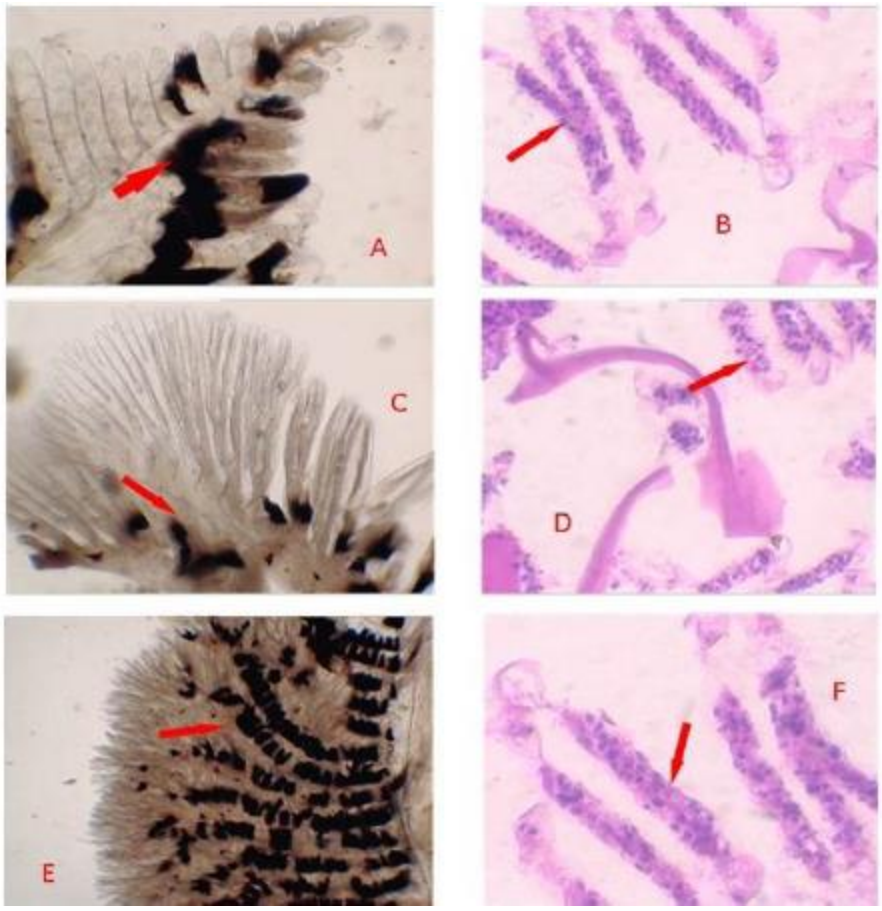
. **Saad *et al.* (2014)**

Velmurugan and Ayyaru (2014) collected Penaied shrimp (21-30g) samples during August 2011 to July 2012 by trawl net from Nagapattinam district, Tamil Nadu, India. For each collection, 8-20 number of fungal brown-gill infected Penaeus sp., were examined mycologically and hjstologically. Totally 427 colonies were isolated from the three browngill diseased shrimps such as P.monodon, P.indicus and P.vannamei collected from grow out pond from Vellapallam. Totally 20 fungal species were isolated from diseased samples and identified six genera viz Mucor hiemalis, M. racemosus, Rhizopus nigricans, R. oryzae, R. stolonifer, Aspergillus fumigatus, A. japonicus, A. niger, A.terreus, A. versicolor, Fusarium aquaeductum, F. oxysporum, F. solani, Pencillum chrysogenum, P. grisofulvum, P. implicatum, P. oxalicum, P. rubrum, Trichoderma harzianum and T. viridae. Out of those 20 fungal species, 16 were isolated from P. indicus and P. vannamei. 15 fungal species were from P. indicus alone. The genus Aspergillus was the predominant species and occurred in all the samples. It is important to accentuate that this is the first report of the isolation of conidial fungi from shrimp 4 , demonstrated a significant diversity of cultivable fungi from adult shrimp Litopenaeus vannamei

Penaeusmonodon infected brown–gill disease*; B-Penaeusindicus* infected brown–gill disease**;** C and D - *Penaeus*vannamei infected brown–gill disease; D-Stacked*Penaeusvannamei* showing Brown-gill infection. **Velmurugan and Ayyaru (2014)**

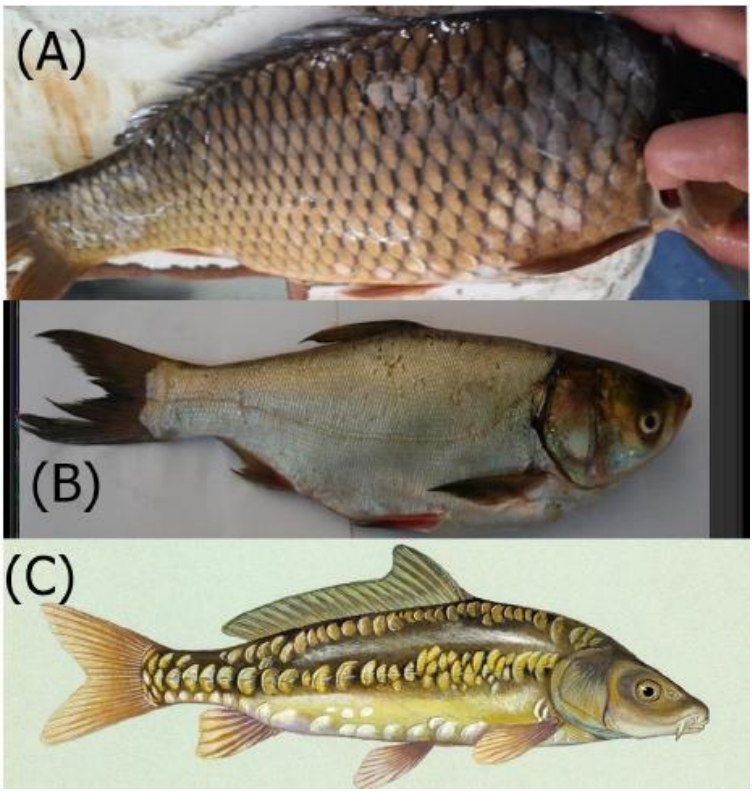
Table 1. Fungal infection on *Penaeus* species collected from the grow out pond

Fungal strains	*Penaeusmonodon*						*Penaeusindicus*						*Penaeusvannamei*					
	Days of Sampling																	
	20	40	60	80	100	120	20	40	60	80	100	120	20	40	60	80	100	120
Mucorhiemalis	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Mucorracemosus	+	+	+	++	+	+	-	-	-	+	+	+	-	-	+	+	-	-
Rhizopusnigricans	+	+	+	++	+	+	+	+	+	++	+	-	-	-	+	+	+	-
Rhizopusoryzae	-	-	-	+	+	-	+	+	+	++	+	-	-	-	-	++	+	-
Rhizopusstolonifer	+	+	+	++	+	-	-	-	-	++	+	-	+	+	+	-	-	-
Aspergillusflavus	+	+	+	+	-	-	+	+	+	++	+	-	+	+	+	++	+	-
Aspergillusfumigatus	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	++	+	+
AspergillusJaponicus	-	-	-	+	+	-	-	-	+	+	-	-	-	-	+	+	-	-
Aspergillusniger	+	+	+	++	+	+	-	-	+	++	+	-	-	-	+	++	+	-
Aspergillusterreus	+	+	-	+	-	-	-	-	+	-	-	-	+	+	+	++	-	-
Aspergillusversicolor	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusariumaquaeductum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Fusariumoxysporum	-	-	+	+	+	+	-	+	+	++	+	+	-	-	+	+	-	-
Fusariumsolani	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	++	+	-
Penicilliumchrysogenum	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
Penicilliumgrisofulvum	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Penicilliumimplicatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Penicilliumoxalicum	-	-	-	+	+	-	-	-	+	++	+	-	-	-	-	+	+	-
Penicilliumrubrum	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichodermaharzianum	-	-	-	-	+	-	+	+	+	-	-	-	-	-	-	+	+	+
Trichodermaviridae	-	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-

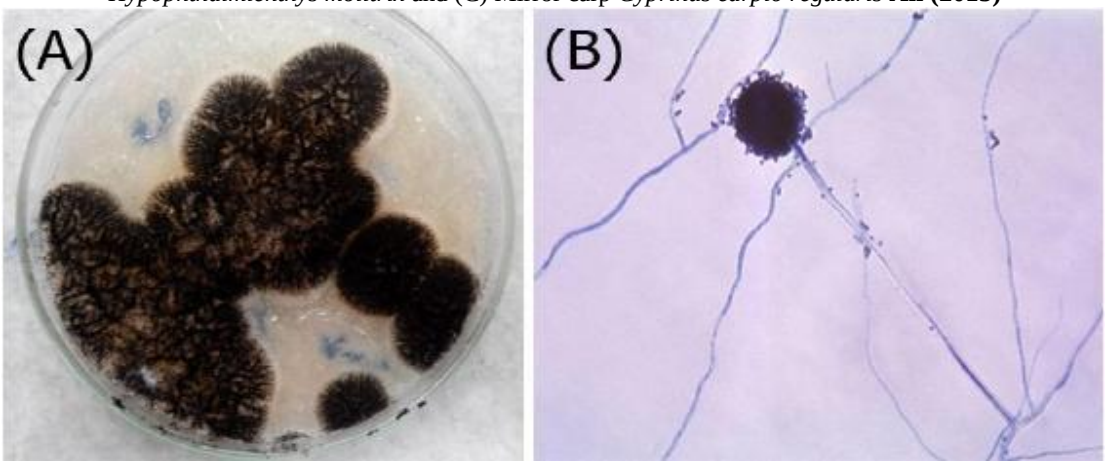


A, C & E- Microcopicalview of adhered fungi on infected gill ofP.monodon, P. indicusand P. vannameirespectively (×40 view); B, D & F- Pathological view of fungal adherence on infected gill of P.monodon, P. indicusand P. vannameirespectively (hematoxylin and eosin,×40) **Velmurugan and Ayyaru (2014)**

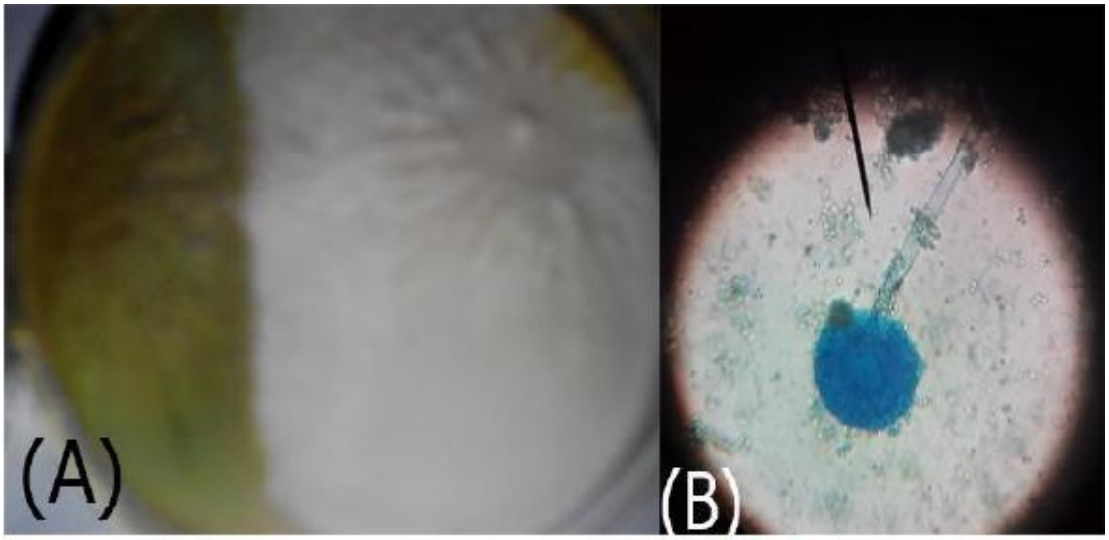
Ali (2015) investigated fungal infections in different species of carps including common carp (*Cyprinus carpio*); silver carp, Hypophthalmichthys (H.) molitrix; *Carpinus carpio* regularis (Mirror carp). Thirty specimens were collected randomly and studied for the presence of fungal infections. Infected fishes showed clinical signs such as fungal growth on skin, fins, eyes, Oral cavity , eroded fins and scales, hemorrhages on body surface and abdominal distension .The specimens from infected organs of fish were inoculated on each, malt extract, Sabouraud dextrose and potato dextrose agars.The fungal colonies of white, black, green, grey and brown colors were observed in the forty agar plates. Slides were prepared and stained with 0.05% Trypan blue in lactophenol. The incidence of fungal infection according to different types of carps recorded that Cyprinus carpio showed the highest infection rate (55 %) followed by *H. molitrix* and *L. rohita* (25.5 % each respectively). The five fungal genera of Aspergillus spp. (32.5%), Blastomyces sp. (7.5%), Penicillium sp. (20%) Rhizopus sp. (25%) and Candida sp. (15%) were isolated from the fish. Eyes (25 %) and gills (20 %) were most affected areas followed by skin (17.5%), buccal cavity (15 %) and operculum (12.5 1%) and Head (10 %) respectively..



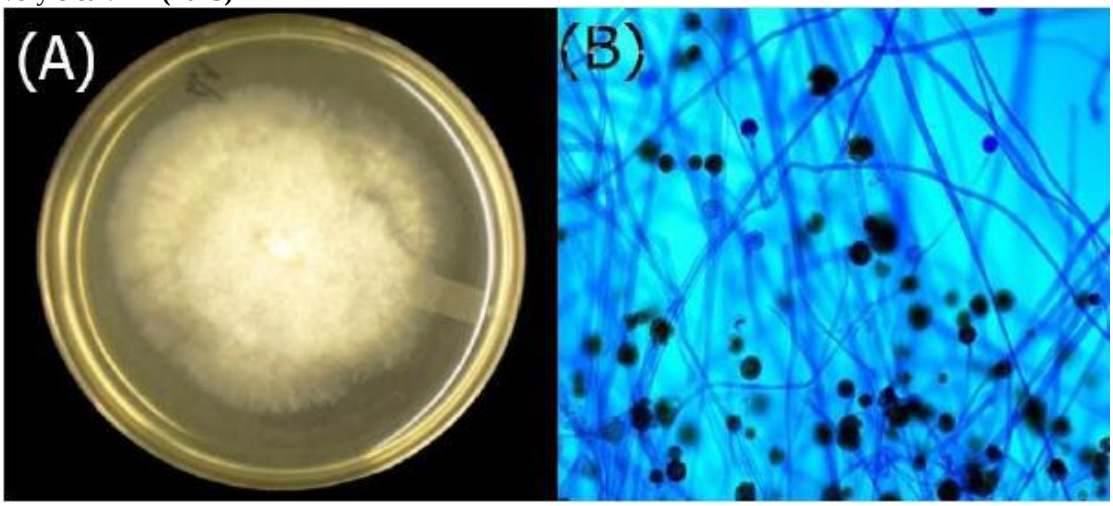
Several types of carps fish (A) common carp *Cyprinus carpio* (B) Silver carp *Hypophthalmichthys molitrix* and (C) Mirror carp *Cyprinus carpio regularis* **Ali (2015)**



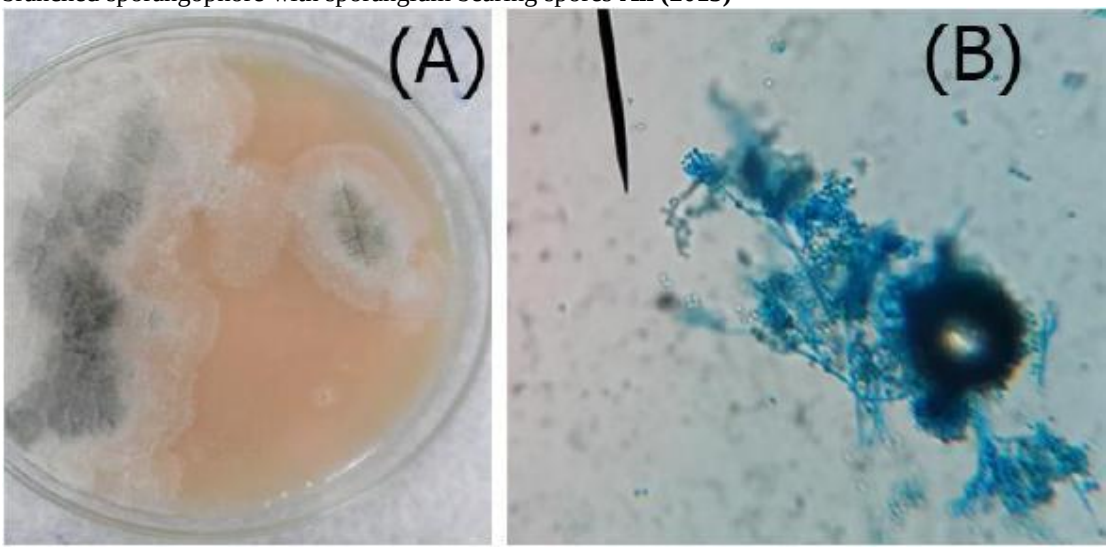
A. Colony of *Aspergillus niger* (Black colony) on SDA and PDA from Site of infection,B. *Aspergillus niger* isolated from A, reproductive head on hyphae very clear **Ali (2015)**



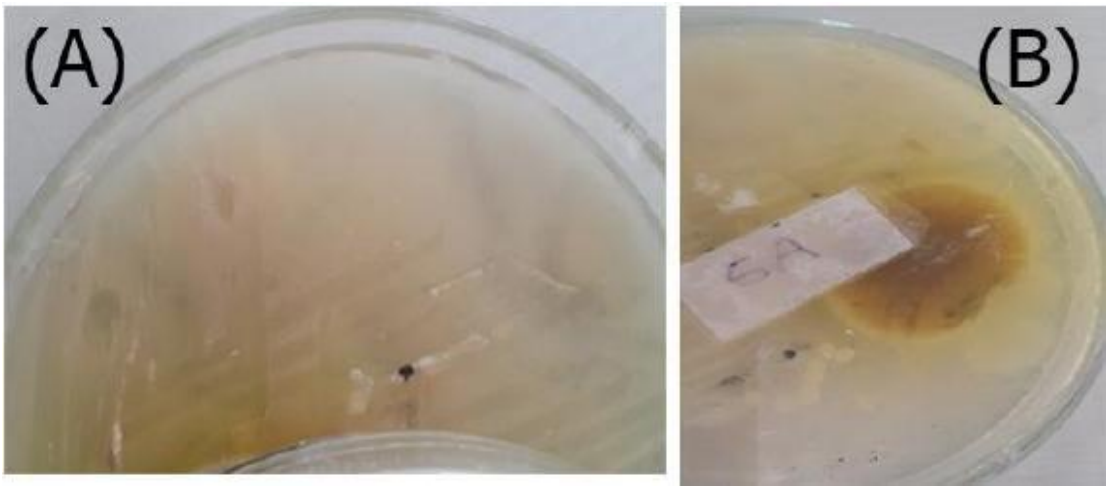
A. Aspergillus flavus colonies greenish - yellow color B , reproductive head on Conidiophores very clear. **Ali (2015)**



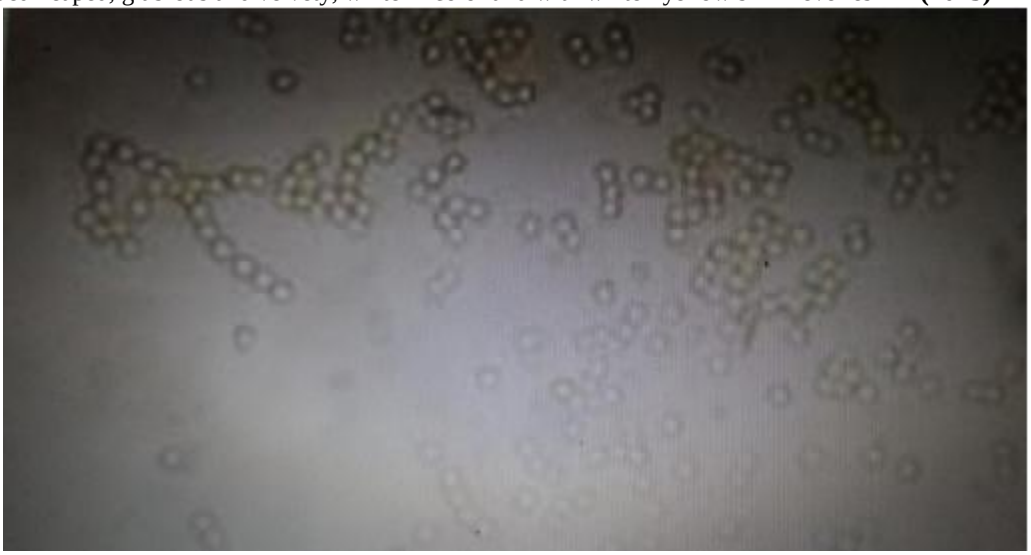
A, Colonies of Rhizopus on MEA, (fish 4,C.auratus). B, Rhizopus (from plate–A) showing long branched sporangophore with sporangium bearing spores **Ali (2015)**



A. Colonies of Penicillium on SDA, (fish 1, C. Carpio). B, Penicillium sp. showing brush like arrangement of fruiting head Microscopically . **Ali (2015)**



ABranchiomyces spp. culture after 3 days of cultivation, (B) After 7 dayes , the colonies shows as folded heaped, glabrous and velvety, white in color and with white –yellowish in reveries **Ali (2015)**



Spores of Branchiomyces spp Lactophenol cotton Blue **Ali (2015)**

A Candida colonies creamy muciod appear on SDA and Malt extract A, B, Unicellular cell of Candida stained by Lacto phenol cotton blue under X 400 **Ali (2015)**

Ismail *et al.* (2015) performed a study to evaluate the mycological quality of 25 samples of commercially available salted fish (Hydrocynus forskalii) sold in retails outlets in Assiut Governorate, Egypt. Three isolation media [Dicloran Rose Bengal

Chloramphenicol (DRBC); 10% NaCl malt extract agar and 20% NaCl malt extract agar] were used for counting and identification of fungi. Also, sensory quality, pH values and sodium chloride percentage were assessed. Sensory evaluation revealed that 12% of the samples were unacceptable while the remaining 88% samples were acceptable. Mean pH values were 7.04 ± 0.27 and 6.81 ± 0.35 for skin and muscular parts, respectively. Sodium chloride percentage ranged from 10.23 to 17.55% with a mean value of 15.03 ± 1.77. A total of 75 species in addition to some unidentified species of yeasts, dematiaceous hyphomycetes and pure mycelia were isolated from all samples on DRBC (61 species), 10% NaCl malt extract agar (46) and 20% NaCl malt extract agar (19). Aspergillus, Petromyces, Penicillium, Eurotium, Cladosporium and yeasts were the most common fungi recovered on the three media. Some of the isolated fungi are toxigenic and have the ability to produce mycotoxins which have potential hazards on human health.

Samaha *et al*. (2015) collected 100 samples of four types of frozen fish (25 from each of Barbone, Sardine, Baca and Mackerel) from different localities of Alexandria markets. The samples were subjected to mycological examination to evaluate both of yeasts and moulds load of these frozen fish. The result recorded that the predominant genera of the isolated mould from the 4 types of fish were Asperigellus spp. and Penicillium spp. moulds could be isolated as Cladosporium spp., Fusarium spp., Alternaria spp., Nigrosporium spp., Paecilomyces spp., Mucor spp. and Rhizopus spp. In addition to other the predominant genus of isolated yeasts, was Candida spp.as well as Torulopsis spp., Rhodotorulla spp. and Geotrichium spp. This study showed how these types of frozen fish were being contaminated from different sources by yeasts and moulds. Also, the hazardous and public health importance of such contaminants were fully discussed and suggested recommendations to improve its quality and safety were explained

Job *et al*. (2016) conducted a research to determine the occurrence of aflatoxigenic fungi in smoke-dried fish at marketing centers in the Jos metropolis. Total fungal load per sample was derived from plate counts and expressed as colony-forming units per gram of sample (cfu/g). *In-vitro* aflatoxigenicity of mould isolates was evaluated on coconut extract agar by exposing reverse side of plates to 365 nm ultra violet light. The results showed that mean fungal load of smoke-dried fish ranged between 2.00x103±8.49x102 to 3.09x104±8.85x103 cfu/g. Generally, the processed fish was contaminated with combinations of eight fungal genera: *Fusarium, Aspergillus, Saccharomyces, Penicillium, Mucor, Rhodotorula, Schizosaccharomyce, Acremonium* and *Rhizopus*. Strains of *Penicillium digitatum, Fusarium equiseti* and *Fusarium semitectum* were the most predominant at 61.67%, 30.00% and 26.67% respectively. Comparatively, the assessment shows that smoke-dried fish from Terminus were the most contaminated ($P < 0.05$) followed by samples from Chobe and Katako markets. Out of 164 fungal isolates, only strains of *Aspergillus flavus* 5(8.33%) from Terminus market exhibited aflatoxin producing potential. In view of sea food safety and quality, thepresence of toxigenic fungi on smoke-dried fish is of health significance and increase the risk of mycotoxin poison. The findings of this study call for stiff regulation and monitoring of smoke-dried fish in our open markets.

References:

1. **Abd El Aziz M. A, Gehan M. Kamel, and Mahmoud A. Mahmoud**. **SEASONAL STUDY, HISTOPATHOLOGICAL AND TREATMENT TRIAL ON**

SAPROLEGNIOSIS IN SOME FISH FARMS. *1rst Ann. Confr. , FVM., Moshtohor, Sept, 2004*

2. Abdel-Latif , H. M. R., Riad H. Khalil, Hanaa R. El-hofi, Talaat T. Saad and Shaimaa M. A. Zaied. Epidemiological investigations of Mycotic infections of cultured Gilthead seabream, Sparus aurata at Marriott Lake, Egypt. International Journal of Fisheries and Aquatic Studies 2014; 2(3): 05-13
3. AHMED SH, MOHAMED FA, YOUSIF RA, ABDELWAHAB SK AND SALIH RRM. ISOLATION OF FUNGAL SPECIES FROM OREOCHROMIS NILOTICUS FROM TWO ENVIRONMENTS, IJBPAS, August, 2012, 1(7): 927-932
4. Ali E. H.: *Antifungal activity of sodium chloride on Saprolegnia diclina and Aphanomyces sp.* Acta Mycol. 44 (1): 125–138, 2009.
5. Ali. H. H., ISOLATION AND IDENTIFICATION OF PATHOGENIC FUNGI FROM CARP FISH IN SULIAMANIA PROVINCE**.** G.J.B.B., VOL.4 (4) 2015: 356-363
6. Ali, E. H, Mohamed Hashem, M. Bassam Al-Salahy. Pathogenicity and oxidative stress in Nile tilapia caused by *Aphanomyces laevis* and *Phoma herbarum* isolated from farmed fish Dis Aquat Organ. 2011 Mar 16;94(1):17-28. doi:
7. Bader, J.A.; Nusbaum, K.E. and Shoemaker, C.A. (2003): Comparative challenge model of Flavobacterium columnare using abraded and unabraded channel catfish, Ictalurus punctatus (Rafinesque). J. Fish Dis. 26: 461-467.
8. Badran, R.A.M. (1989): Studies on fungi associated with Tilapia fish in River Nile water. Ph.D. Thesis, Botany Dept., Faculty of Science, Assiut University, Egypt.
9. Bagy, M.M.K.; Hemida, S.K. and Mahmoud, U.M. (1993): Terrestrial fungi inhabiting certain species of Nile fishes in Egypt. Zentralbl. Mikrobiol., 148: 289-297.
10. Bakeer, A.M.; Marzouk, M.S.M.; Abozid, A.; Moustafa, M. and Husean, M. (1991): Experimental morphopathological studies on tilapia infected by F. columnrae. Beni-Suef. Vet. Med. Vol.1: 53-65.
11. Blaylock, R. B. R. M. Overstreet, M. A. Klich. Mycoses in red snapper (Lutjanus campechanus) caused by two deuteromycete fungi (Penicillium corylophilum and Cladosporium sphaerospermum). Hydrobiologia. Hydrobiologia (2001) 460: 221.
12. Bocklisch, H. and Otto, B. (2000): Mycosis in fish. Mycosis. 43 (suppl 1) 76-78.
13. Bohm, K.H. and Fuhrmann, H. (1984):Mycological survey of diseased freshwater fish. Bull. Eur. Assoc. Fish. Pathol. 4(2), 26-27.
14. Chauhan, R. Studies on conidial fungi isolated from some fresh water fishes. IJALS, Volume (6) Issue (4) August - 2013.
15. Chauhan. R. Fungal attack on Tilapia mossambicus in culture pond, leading to mass mortality of fishes. International Journal of Pharma Sciences and Research (IJPSR). Vol 5 No 07 Jul 2014, 425-428
16. **El-Bouhy, Mahboub, H. Heba, 2014**.Branchiomycosis in Nile tilapia (Oreochromis niloticus) in Behiera Governorate with Trials for Treatment.Zag. Vet. J. (ISSN. 1110-1458) Vol. 42 No. 3 pp. Vol. 42 (No. 3): pp. 29-42.
17. El-Hissy, F.T.; Khallil, A.M. and El-Nagdy, M.A. (1989): Aquatic fungi associated with seven species of Nile fishes (Egypt). Zentralbl. Mikrobiol., 144: 305-314.
18. El-Hissy, F. T., A.M. Khallila.A. Abdel-Raheem. Occurrence and Distribution of zoosporic fungi and aquatic hyphomycetes In Upper Egypt. Journal of Islamic Academy Of Sciences 5:3, 173-179 1992
19. El-Zayat, S.A.M. (1988): Studies on freshwater fungi of Aswan high Dam Lake Ph.D. Thesis, Botany dept. Faculty of Science Aswan University, Egypt
20. Faisal,M., Carolyn A. Schulz, Thomas P. Loch, Robert K. Kim, John Hnath, and Gary Whelan, Current Status of Fish Health and Disease Issues in the Laurentian Great Lakes: 2005–2010
21. **Gozlan, R.E., Wyth L. Marshall, Osu Lilje , Casey N. Jessop4, Frank H. Gleason and Demetra Andreou.** Current ecological understanding of fungal-like pathogens of fish: what lies beneath? **REVIEW ARTICLE** fmicb.,5, 1-15, 2014.00062
22. Ibrahim, K. S., 2011. Isolation and pathological study of Branchiomycosis from the commercial pond of common carp (Cyprinus carpio) fish, in Governorate of Duhok / Iraq. The Iraqi Journal of Veterinary Medicine; 35 (1), 1 – 9.
23. ISMAIL S. MOHAMAD; MOHAMED W. ABD AL-AZEEM; MAHMOUD M. MAHMOUD and AML MOKHTAR M. ABD-EL AA/ MICROBIOLOGY STUDIES ON

THE AFFECTIONS OF SKIN IN SHARP TOOTH CATFISH (CLARIAS GARIEPINUS).Assiut Vet. Med. J. Vol. 59 No. 137 April 2013, 157

24. Khalil, R.H. (1993): Some studies on mycotic infection in some freshwater fish with special reference to its control. Thesis; M.Sc.; Fish Diseases & Hygiene Alex. Univ. Fac. of Vet. Med.
25. Khalil RH, Safinaz G, Mohmad N, Mahfouz El-Banna S, Soliman MK. A New Fungal Disease in Cultured Monosex Oreochromis Niloticus Caused By Paecilomyces Marquandii. Issn 110-2047. Alex J Vet Science 2004; 21(1).
26. Khulbe RD, Joshi C, Bisht GS. **Fungal diseases of fish in Nanak Sagar, Naini Tal, India.** Mycopathologia. 1995 May;130(2):71-4.
27. **Makkonen, J., Harri Ilkka Kokko. Paula Henttonen, J. Jussila/** Fungal Isolations from Saaremaa, Estonia: Noble Crayfish (Astacus astacus) with Melanised Spots. **Freshwater Crayfish 17:155–158, 2010**
28. Manal, A. (1988): Studies on mycotic infections in freshwater fish. M.V.Sc. Thesis, Zagazig University.
29. Marancik D. P., Berliner A. L., Cavin J. M., Clauss T. M., Dove A. D. M., Sutton D. A., et al. (2011).Disseminated fungal infection in two species of captive sharks. J. Zoo and Wild. Med. 42, 686–693
30. Marzouk, M.S.; Samira, S.R. and El-Gamal, M.H. (2003): Mycological investigations on cultured Tilapia in Kafcr El- Sheikh Governorate. Kafer El-Sheikh Vet. Med. J., 1 (2): 97-114.
31. Mohamed Nagla, A. (1994): Some studies on mycoflora of freshwater fish with special reference to Aspergiliosis. Ph.D. Thesis, Fac. Vet. Med. Assiut Univ.
32. Refai, M.K., Laila, A. Mohamed, Amany, M. Kenawy, Shimaa, El-S.M.A. The Assessment Of Mycotic Settlement Of Freshwater Fishes In Egypt. Journal of American Science 2010;6(11):823-831.
33. Saad, T.T., Ahmed, H.A., El-Gohary, M., Ali, M.A. 2013. Economic studies on immunostimulents in relation to mycotoxin infection in cultured fish. *Online J. Anim. Feed Res.*, 3(1): 47-57.
34. Saad, T. T.; Atallah, S. T* and El-Bana, S.A**Fish Diseases and Its Economic Effect on Egyptian Fish Farms./ J. Agric. Food. Tech., 4(5)1-6, 2014
35. Salem, A.A.; Refai, M.K.; Eissa, I.A.M.; Marzouk M.S.; Moustafa, M. and Manal, A. (1989): Mycological investigations on cultured Tilapia in Egypt. Alex. J. Vet. Sci, 5(2): 625-636.
36. Shagar, Gehan E and Ahmed M.E. El-Refaee. **Studies on Cultured Silver Carp** (*Hypophthalmichthys Molitrix*) **Diseases Induced by Some Bacterial, Fungal and Parasitic Pathogens in Sharkia Governorate. JOURNAL OF THE ARABIAN Aquaculture Society Vol. 7 No 2 December 2012**
37. Velmurugan, K. and Gopalakrishnan Ayyaru. Culturable fungal diversity of brown-gill disease in three Penaeus species. International Journal of Research in Marine Sciences 2014; 3(1): 1-4

23.Yeasts

- Yeasts are ubiquitous microorganisms that can grow in various environments where organic substrates are available (Gatesoupe, 2007). Being rich in nutrients, the micro-environment of the GI tract of fish presents a favourable culture environment for the microorganisms (Mondal et al., 2008).
- Ability of the yeasts to colonize within the fish GI tract has been documented previously with rainbow trout and turbot (Andlid et al., 1995, 1998; Vazquez-Juarez et al., 1997).
- Mandal and Ghosh (2013a) later detected tannaseproducing yeasts (Pichia spp. and Candida spp.) in the GI tract of some freshwater fishes. In another study,

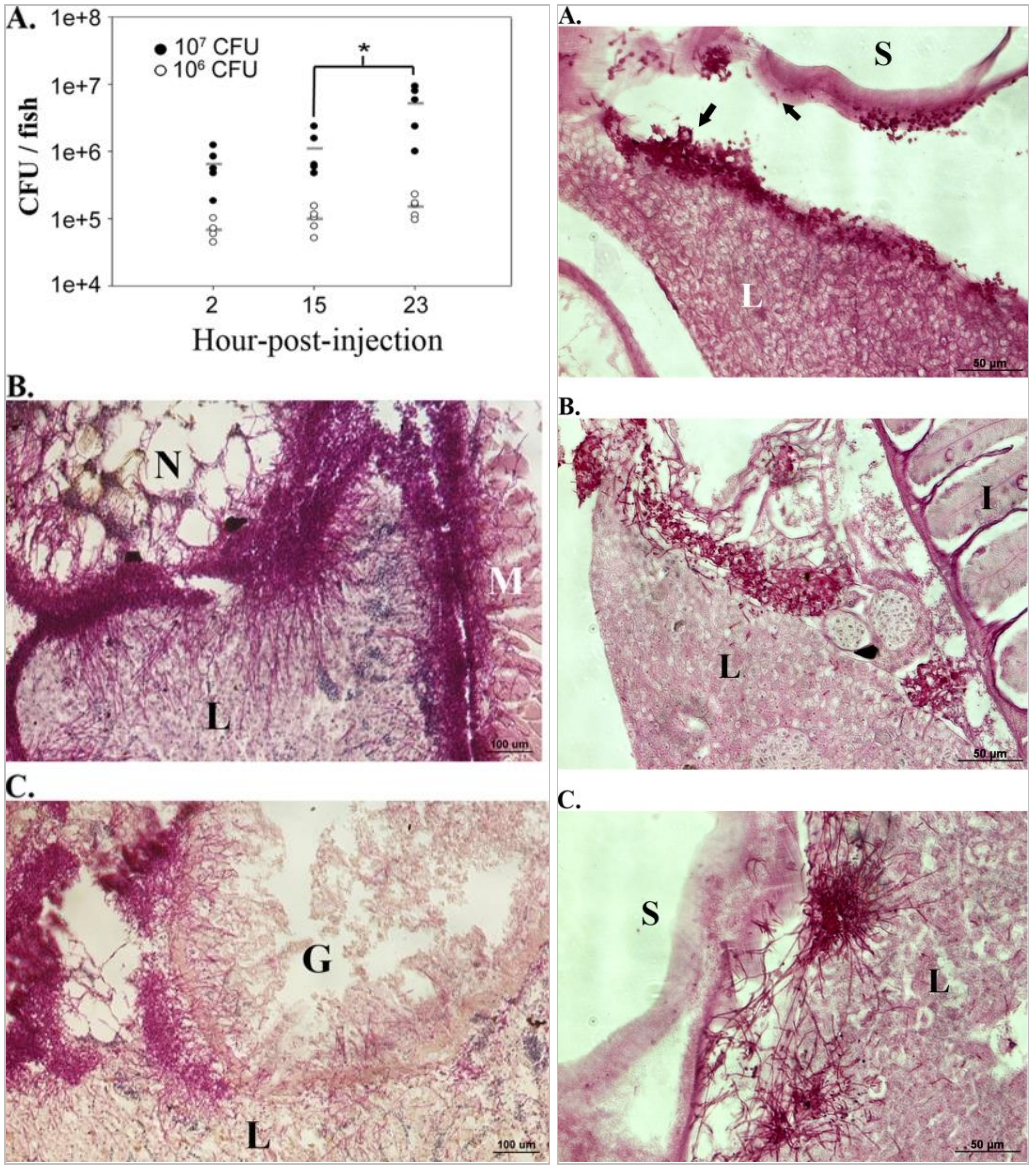
- Das and Ghosh (2014) documented phytase-producing yeasts, Candida tropicalis, within the GI tracts of silver carp, Hypophthalmichthys molitrix and climbing perch, Anabas testudineus.

Candida

Easa (1979) isolated ***Candida albicans*** from infected gills of a bottom feeder fish *common carp* (*Cyprinus, carpio L.)* bred at high temperatures of 20 to 28ºC and high level of organic matters. Experimentally, he infected common carp of different ages kept at various temperatures of 12-24ºC via gill scarification, subcutaneous inoculation and per os. He reported that all ages had gill necrosis although those kept at higher water temperatures (20-24ºC) had more rapid progressing lesions than those kept at (12-15ºC) or at 7-10ºC. Lesions other than in the gill area and caudal peduncle were in the form of button-like ulcers and/or small multiple vesicles that coalesced to form large vesicles, ruptured and ended with necrosis of the caudal fins. Internally, spleen and kidneys appeared enlarged and dark in colour. The liver appeared pale, while intestines were congested and filled with excessive mucous. Histologically, the gills showed hyperplastic proliferation of its epithelial lining, then degenerative changes followed by necrosis. Necrosis extended to the gill arch and hyphal elements were seen in stained preparations between the necrotized tissues. Degenerative changes were observed in internal organs and blastospores also noticed in the spleen and blood vessels.

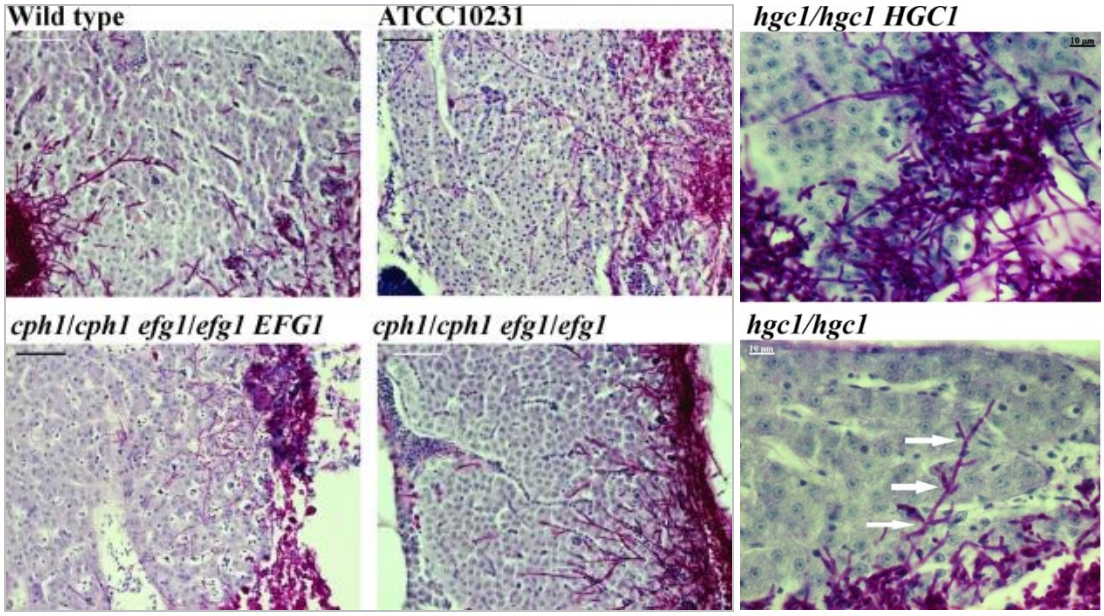
Tikhonova *et al.* (1988) fed yearling carp with **Candida species** at different doses for a month. They observed degeneration of renal tubules and fatty change of the liver in all inoculated fishes.

Chao *et al.* (2010) developed the zebrafish model organism to obtain a minivertebrate host system for a Candida albicans infection study. They demonstrated that C. albicans can colonize and invade zebrafish at multiple anatomical sites and kill the fish in a dose-dependent manner. Inside zebrafish, we monitored the progression of the C. albicans yeast-to-hypha transition by tracking morphogenesis, and they monitored the corresponding gene expression of the pathogen and the early host immune response. We performed a zebrafish survival assay with different C. albicans strains (SC5314, ATCC 10231, an hgc1 mutant, and a cph1/efg1 double mutant) to determine each strain's virulence, and the results were similar to findings reported in previous mouse model studies. Finally, using zebrafish embryos, they monitored C. albicans infection and visualized the interaction between pathogen and host myelomonocytic cells in vivo. Taken together, the results of this work demonstrated that zebrafish can be a useful host model to study C. albicans pathogenesis, and they highlight the advantages of using the zebrafish model in future invasive fungal research.

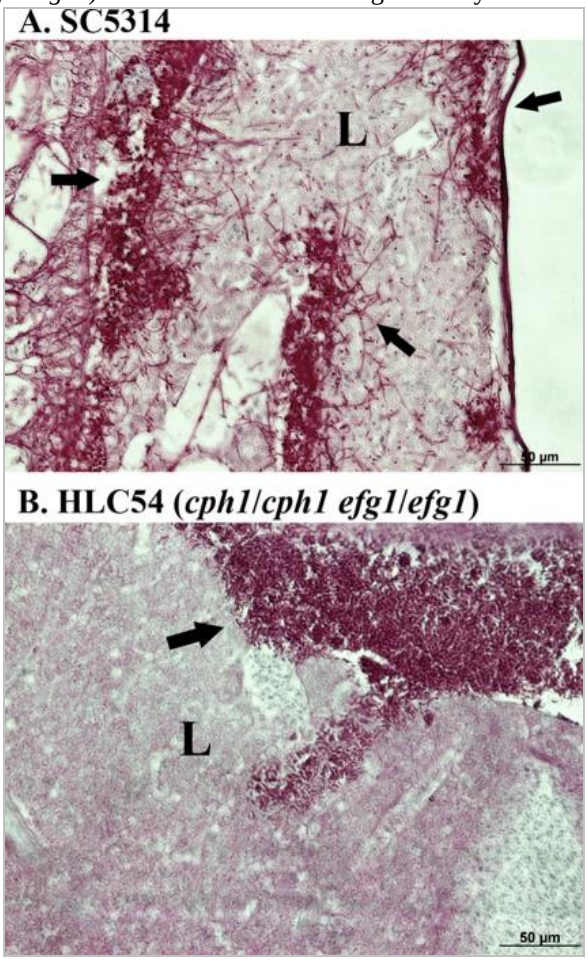


Colonization and invasion of *C. albicans* in zebrafish. (A) *C. albicans* burden in infected adult zebrafish. Five *C. albicans*-injected fish for each dose group were collected at 2, 15, and 23 hpi. Each dot indicates the number of *C. albicans* CFU in one infected fish. The horizontal lines indicate the mean values of the groups. *, $P < 0.05$. (B and C) Histological analysis of *C. albicans*-infected zebrafish. Transverse sections were prepared from zebrafish injected with and killed by 1×10^8 CFU of *C. albicans*. Abbreviations: L, liver; G, gastrointestinal tract; N, connective tissue; M, muscle

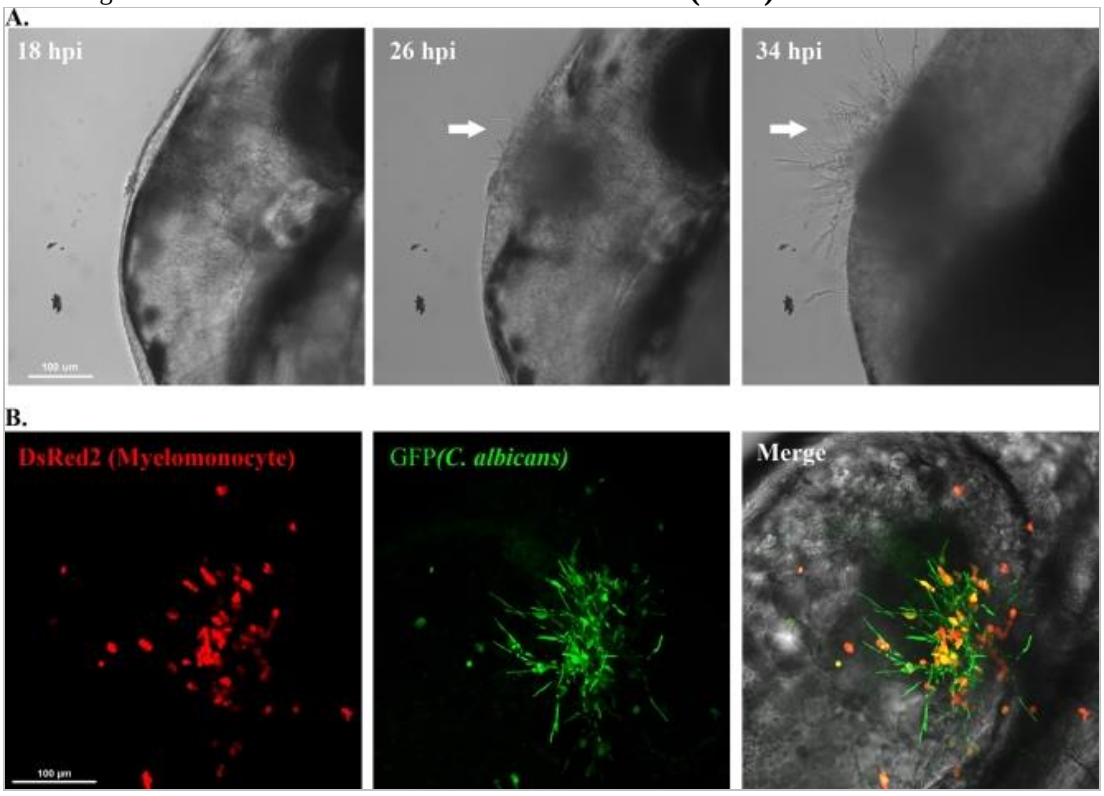
Progression of *C. albicans* hyphal formation in zebrafish. Time-lapse tissue sections were obtained from fish injected with 1×10^8 CFU of *C. albicans* cells at (A) 2, (B) 8, and (C) 15 hpi. The arrows indicate *C. albicans* cells. L, liver; S, swim bladder; I, intestine. **Chao *et al.* (2010)**



The hyphal form of *C. albicans* was found in dead zebrafish. SC5314, ATCC 10231, HLC84 (*cph1/cph1 efg1/efg1 EFG1*), and HLC54 (*cph1/cph1 efg1/efg1*) utilized the dimorphic transition to invade the liver of infected zebrafish. The infected zebrafish were obtained for histological analysis after they died. Scale bar = 50 μm.
C. albicans Hgc1 knockout strain formed only pseudohyphae. The *Hgc1* mutant of *C. albicans* was defective in hyphal formation in the liver of infected zebrafish. It only formed pseudohyphae. The arrows indicate the pseudohyphal branches. Zebrafish killed with 10^8 CFU of WYZ12.1 (*hgc1/hgc1 HGC1*) or WYZ12.2 (*hgc1/hgc1*) were obtained for histological analysis. **Chao *et al.* (2010)**



Delayed morphological transition of HLC54. *C. albicans* SC5314 cells attached to the zebrafish liver became filamentous at 15 hpi, but HLC54 cells were in the yeast form at 15 hpi. The fish were injected with 1×10^8 CFU of *C. albicans* strain SC5314 (A) or HLC54 (B) and sacrificed at 15 hpi for tissue sectioning. The arrows indicate *C. albicans* cells. **Chao *et al.* (2010)**

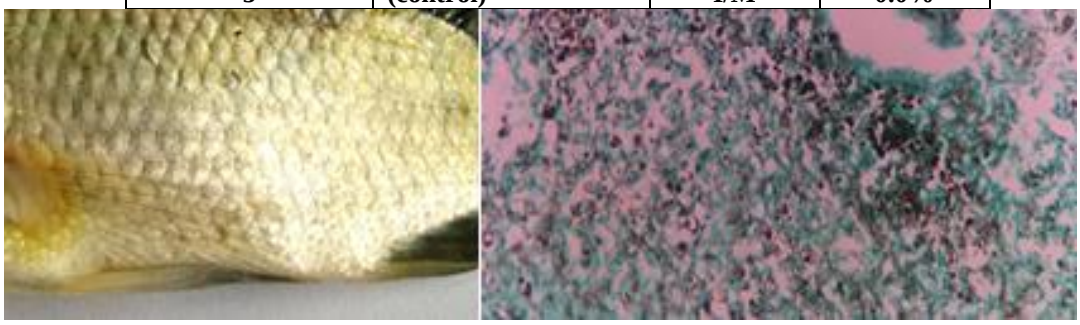


In vivo visualization of *C. albicans* in an infected zebrafish embryo. (A) Hyphae of *C. albicans* form in the hindbrain cavity of the zebrafish embryo. A zebrafish embryo was injected with *C. albicans* strain OG1 in its hindbrain and observed at 18, 26, and 34 hpi using a microscope equipped with a live-imaging apparatus. The arrows indicate the extruding fungal hyphae. (B) Interactions between *C. albican*s and zebrafish myelomonocytic cells. The yolk of the *lyz:DsRed2* zebrafish embryo was injected with GFP-labeled *C. albicans* strain OG1 and examined at 24 hpi using a Nikon A1R confocal microscope **Chao *et al.* (2010)**

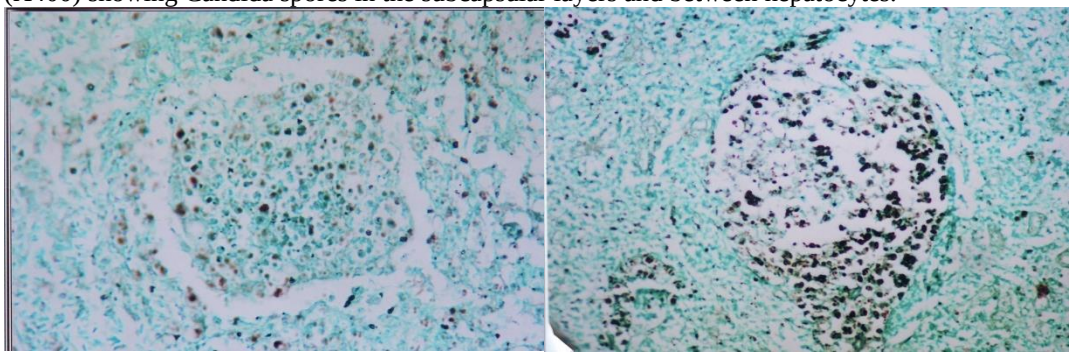
Refai *et al.* (2010) carried out experimental infection of *Oreochromis* species with ***Candida albicans***. A full loop of one day old pure yeast culture of *Candida albicans* was added to test tube containing 5 ml of sterile phosphate buffer saline and mixed gently to reach equal distribution. Spores were counted by using haemocytometer then suspension was adjusted to reach $2x10^3$ Candida spores per ml. Twenty animals were injected by Candida albicans spores either i.p or i.m. and 10 animals were injected by saline as controls. Several clinical abnormalities appeared among *Oreochromis* species inoculated with *Candida albicans* as ascitis, scales detachment and button like ulcer on muscles Postmortem finding include pale gills, distended gall bladder and granulomma of liver and spleen. The mortality rates were recorded among fish inoculated with *Candida albicans* (70%) by I.P route and (60%) through I.M. *Candida albicans* was re-isolated from skin, fins, gills, liver, spleen, kidneys and gall bladder.

Number of fish in each one	**Injected material**	**Inoculated material**	mortality rates
10	***Candida albicans***	**I/P**	**70%**
10	***Candida albicans***	**I/M**	**60%**
5	**Normal saline**	**I/P**	**0.0%**

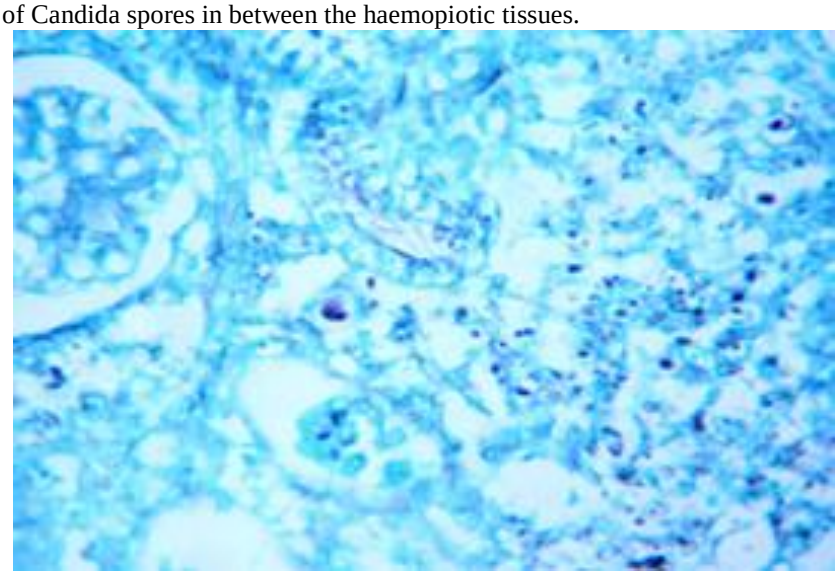
5	**(control)**	**I/M**	**0.0%**



***Oreochromis* species showing button like ulcer near the dorsal fin.** Liver section stained with GMS (X400) showing Candida spores in the subcapsular layers and between hepatocytes.



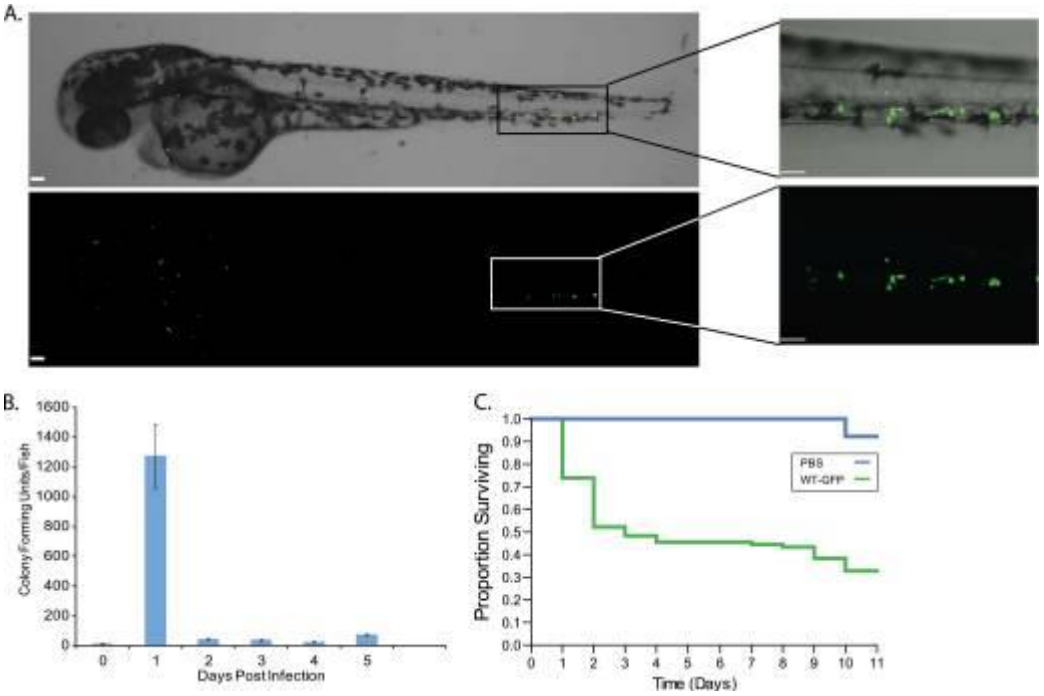
Liver section stained with GMS (X400) showing candida spores within the granuloma and in between the hepatic tissue. Spleen section stained with GMS (X400) showing circumscribed areas of aggregation of Candida spores in between the haemopiotic tissues.



Kidney section stained with GMS (X 400) showing Candida spores in between interstitial haemopiotic tissue and in between the necrosed tubular epithelium

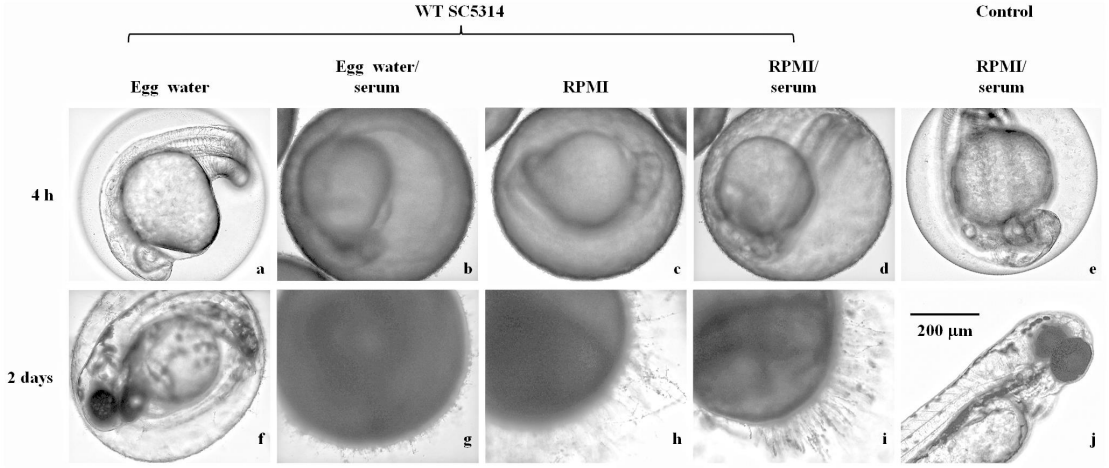
Brothers *et al.* (2011) attempted several routes of infection (immersion, caudal vein, Duct of Cuvier, and hindbrain ventricle) to establish a disseminated infection in transparent zebrafish larvae. They initially attempted bath infection with up to 10^8 *C. albicans* yeast cells/ml of water. These bath infections of fish aged to 3 to 6 days postfertilization resulted in no mortality and no fungal invasion past the gastrointestinal tract. The attempts at infection of the yolk have resulted in universal lethality within 24 h, while common cardinal vein infections yielded yolk-focused infections. When these infected fish were examined, however, there was little to no apparent dissemination. Thus, these methods did not immediately offer a good model

for disseminated candidiasis, which is the lethal form of human candidiasis. It was also attempted to inject fish at 2 to 3 days post-fertilization via the caudal vein, but these attempts resulted in too much lethality, even using buffered saline as a control. This was probably due to the relatively large bore needle required to inject *C. albicans* yeast (3 to 5 μm in diameter). In contrast, the initial infections of the hindbrain of prim25-stage fish embryos yielded a disseminated *Candida albicans* infection in which the fungus replicated rapidly and killed over half of the fish within the first 2 days postinfection. Utilizing the hindbrain infection route, it was found that *C. albicans* disseminated throughout the fish, and both yeast and filamentous fungi were found as far away as the tail (Fig. 1A and insets). Fish were microinjected in the hindbrain ventricle with 10 to 20 yeast cells/fish, as determined retrospectively by counting viable CFU from homogenates immediately following infection (Fig. 1B). After infection, the fungi proliferated rapidly, and by 24 h postinfection (hpi), they were at 100-fold greater numbers per surviving fish (Fig. 1B). By 48 hpi, the fungal burden in surviving fish dropped precipitously, to only five times that of the initial injection amount. The remaining live *C. albicans* and fish continued to survive for several days. In parallel with fungal burden measurements, the authors also monitored fish for mortality. In over 20 experiments performed, it was found that 49% ± 16% of infected fish succumbed to infection within 5 days postinfection, with the majority of mortality in the first 48 hpi (Fig. 1C). To determine if the mortality was pathogen specific, fish were also infected with heat-killed *C. albicans* and prototrophic *Saccharomyces cerevisiae*. Neither dead *C. albicans* nor live *S. cerevisiae* caused mortality.

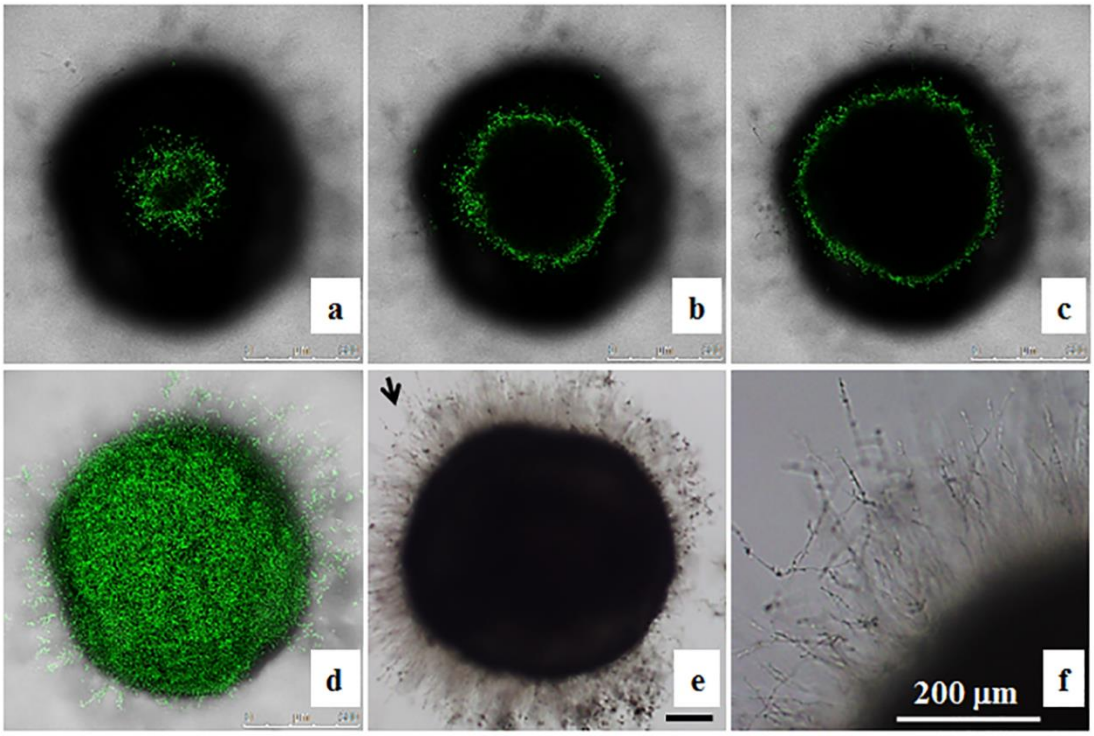


Injection of *C. albicans* into zebrafish larvae caused disseminated infection and significant mortality. Wild-type GFP-expressing (WT-GFP) *C. albicans* cells (11 ± 4 CFU, as measured in homogenates at 0 hpi) were injected into the hindbrain ventricle of wild-type AB larvae at the prim25 stage. The results in each panel are representative of at least three independent experiments. (A) Confocal imaging of disseminated infection at 24 hpi. Bars, 100 μm in large images and 50 μm in insets. (B) Fungal burdens determined by serial dilution and growth on YPD plates. Error bars represent standard deviations. (C) Kaplan-Meier survival curve from a representative experiment. WT-GFP strain-infected fish had significantly more mortality than PBS-injected controls ($P < 0.0001$ by log rank test). **Brothers *et al.* (2011)**

Chen *et al.* (2015) established a simple, noninvasive zebrafish egg bath infection model, defined its optimal conditions, and evaluated the model with various *C. albicans* mutant strains. The deletion of *SAP6* did not have significant effect on the virulence. By contrast, the deletion of *BCR1*, *CPH1*, *EFG1*, or *TEC1* significantly reduced the virulence under current conditions. Furthermore, all embryos survived when co-incubated with *bcr1/bcr1*,*cph1/cph1* *efg1/efg1*, *efg1/efg1*, or *tec1/tec1* mutant cells. The results indicated that our novel zebrafish model is time-saving and cost effective.

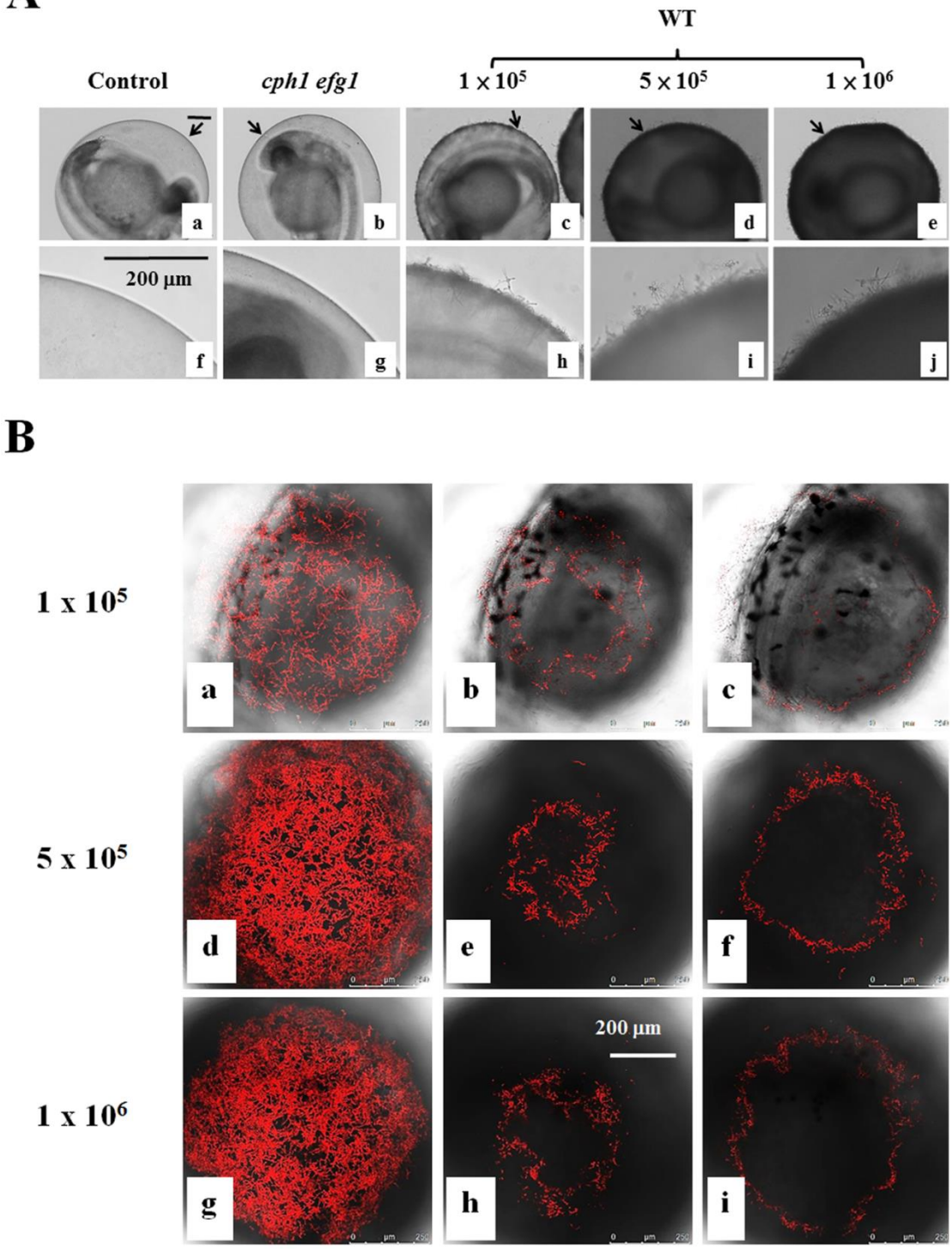


Zebrafish egg bath infection model in various media. Representative embryos were co-incubated with 1 × 10^6 cells/mL of SC5314 (a-d, f-i) or without *C. albicans* (control, e, j) in egg water (a, f), egg water/serum (b, g), RPMI medium (c, h), RPMI/serum (d, i) with shaking at 80 rpm and 30°C for 4 h. The embryos were photographed immediately after non-adhered *C. albicans* cells were removed through washing (a-e) or after an additional 2 days of incubation (f-j). Scale bar = 200 μm. This data are from 3 repeat experiments. Approximately 30 embryos were tested for each treatment. **Chen *et al.* (2015)**

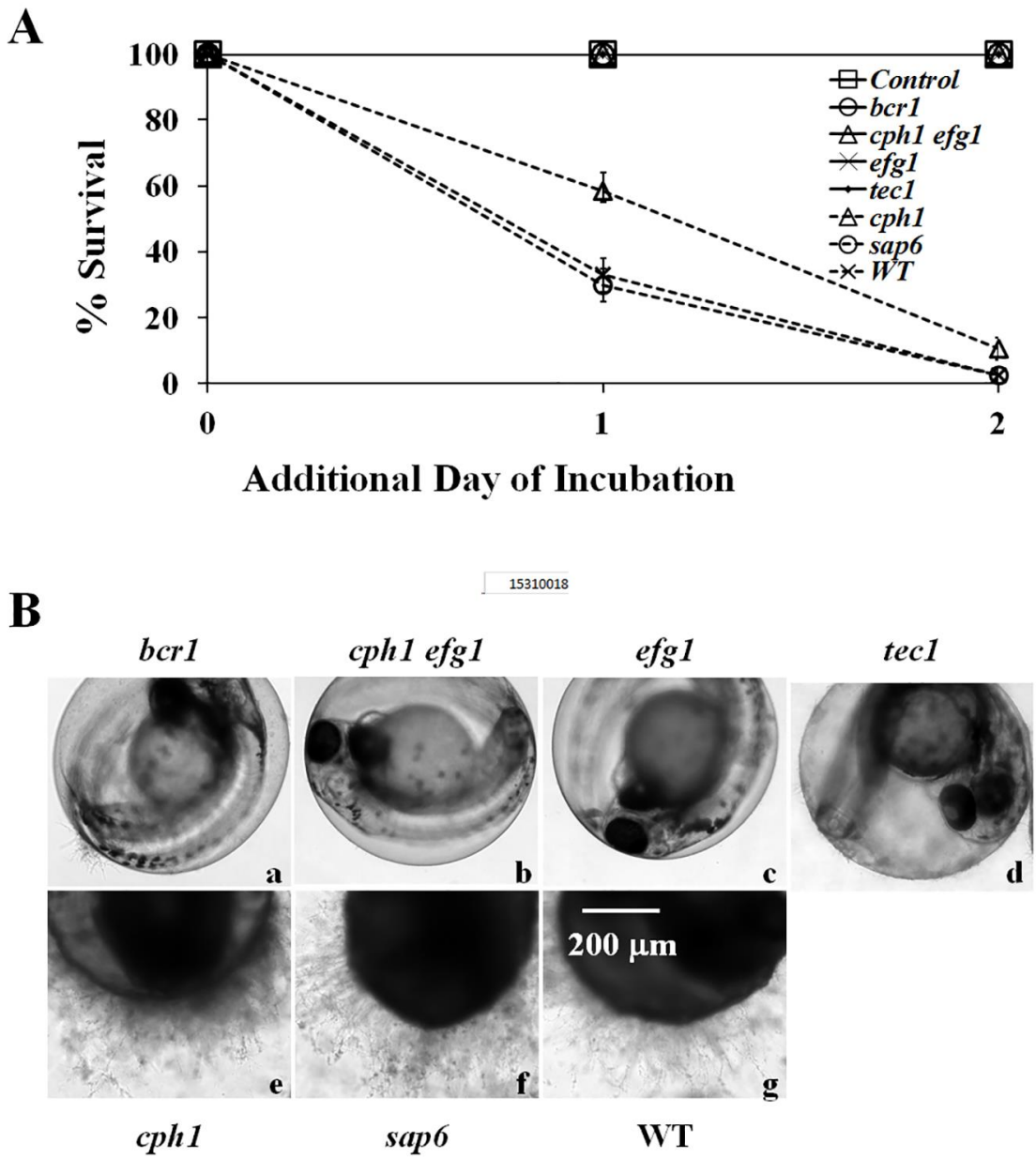


Localization of OG1 *Candida albicans* cells in zebrafish egg bath infection model. Embryos were co-incubated with 1 × 10^6 cells/mL of OG1 *C. albicans*. The representative slices of confocal images (a-c) are shown. The distance between two slices was approximately 55 μm. The whole merged images

are presented (d). The phase contrast photos showing *C. albicans* hyphae were taken by an inverted microscope (e, f). f is the enlargement of the arrow area in e. Scale bars = 200 μm **Chen *et al.* (2015)**



Zebrafish egg bath infection model with different inocula. (A) Embryos were co-incubated in the absence of *C. albicans* (a, f) or in the presence of 1×10^5 (c, h), 5×10^5 (d-i), or 1×10^6 (e-j) cells/mL of wild-type SC531cells, 1×10^6 (e-j) cells/mL of *cph1/cph1 efg1/efg1* mutant cells (b, g) for 4 h. f-j are the enlargement of the arrow areas in a-e. (B) Embryos were co-incubated with 1×10^5 (a-c), 5×10^5 (d-f), or 1×10^6 (g-i) cells/mL of CAF2-dTomato *C. albicans*. The representative slices (b-c, e-f, h-i) are shown. The distance between two slices was approximately 16 μm. The whole merged images for 1×10^5 (a), 5×10^5 (d) or 1×10^6 (g) cells/mL are presented. Scale bars = 200 μm **Chen *et al.* (2015)**

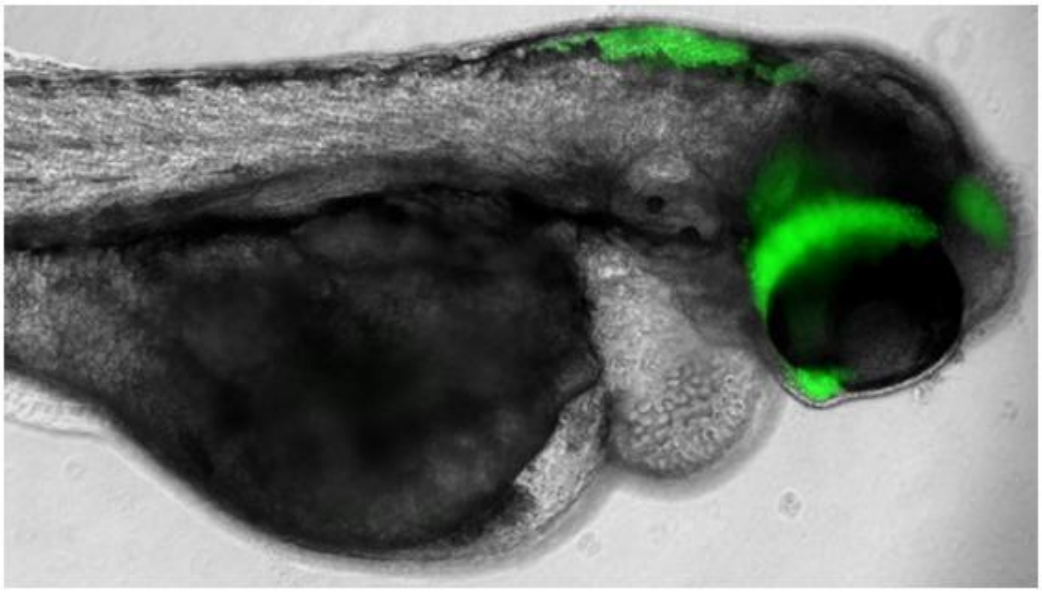


Virulence of *C. albicans* mutant strains in the infection model.(A) Survival rates of embryos. Embryos alone (Control) or embryos with 5 × 10^5 cells/mL of *bcr1/bcr1*, *cph1/cph1 efg1/efg1*, *efg1/efg1*, *tec1/tec1*, *cph1/cph1*, *sap6/sap6*, or WT (SC5314) cells in RPMI/serum were incubated at 30°C for 4 h. Survival rates were determined after an additional 1 day and 2 days of incubation. (B) Representative embryos were co-incubated with (a) *bcr1/bcr1*, (b) *cph1/cph1 efg1/efg1*, (c) *efg1/efg1*, (d) *tec1/tec1*, (e) *cph1/cph1*, (f)*sap6/sap6*, or (g) WT (SC5314) cells, and photographed after an additional 2 days of incubation. Scale bar = 200 µm. The data are from 4 repeat experiments. Approximately 70 embryos were tested for each strain. **Chen *et al.* (2015)**

Cryptococcus

Faisal *et al.* (1986) reported that ***Cryptococcus neoformans*** was isolated from eyes showing exophthalmia and internal organs of *Latus niloticus.* The isolated organism proved to be highly pathogenic to *L. niloticus*, with moderate virulence to *Oreochromis niloticus* and *common carp* and less virulent to *Muqil cephalus* and *Liza ramada* after intramuscular inoculation ($2X10^3$ yeast cells/fish). The infected fish showed severe protrusion of both eye balls as well as haemorrhages at the base of fins and around the vent.

Sabiiti *et al.* (2012) reviewed the range of experimental models that are available for cryptococcosis research and compare the relative advantages and limitations of the different systems. They mentioned that the zebrafish has not yet been used to investigate infection and disease in relation to Cryptococcus—however this application is under development.



Zebrafish embryo 48 hours after infection with Cryptococcus neoformans strain H99 expressing GFP. Image Courtesy of S. A. Johnston, University of Birmingham, UK. GFP-expressing yeast was developed by Voelz et al.

Bojarczuk *et al.* (2016) described a high-content imaging method in a zebrafish model of cryptococcosis that permits the detailed analysis of macrophage interactions with ***C. neoformans*** during infection. Using this approach we demonstrate that, while macrophages are critical for control of *C. neoformans*, a failure of macrophage response is not the limiting defect in fatal infections. We find phagocytosis is restrained very early in infection and that increases in cryptococcal number are driven by intracellular proliferation. We show that macrophages preferentially phagocytose cryptococci with smaller polysaccharide capsules and that capsule size is greatly increased over twenty-four hours of infection, a change that is sufficient to severely limit further phagocytosis. Thus, high-content imaging of cryptococcal infection *in vivo* demonstrates how very early interactions between macrophages and cryptococci are critical in the outcome of cryptococcosis.

Rhodotorula

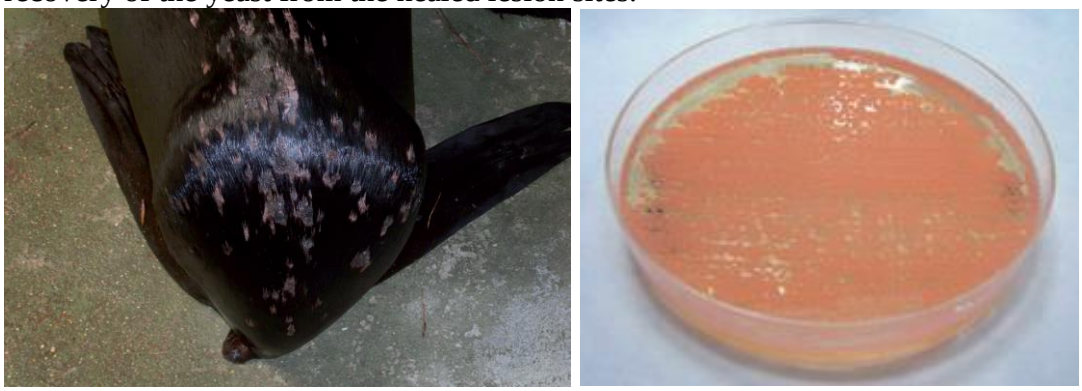
Faisal and Refai (1986) Rhodotorula marina as a cause of red eye syndrome in fish. In Winter 1986/1986, 400 mature fish (Sparus aurata) were observed in a pond in El-Max Fish bream, 500±25 g in weight, 31±4 cm long in 4 water cages. The inspection of the fish revealed that 99 of the 400 fish showed eye cataract just before or shortly after the barking of the eggs. In 26 fish one eye was affected and in73 both eyes were affected. Within few days, the eyes became swollen and bulging and stained deep red. In some cases the eyes ruptured. The wet preparation of the eye content revealed the presence of massive yeast cells. The isolated yeast was identified as Rhodotorula marina

Rhodotorula eye infection in Sparus aurata. **Faisal and Refai. 1986**

Table: Rhodotorula eye infection in Sparus aurata

Cage no.	Number of fish	Number of diseased fish		
		unilateral	bilateral	Total
1	63	4 (6.3$)	10 (15.9%)	14 (22.2%)
2	104	6 (5.8%)	22 (21.2%)	28 (27.0%)
3	133	12 (9.0$)	15 (11.3%)	27 (20.3%)
4	100	4 (4.0%)	26 (26.0%)	30 (30.0%)
Total	400	26	73	99 (24.8%)

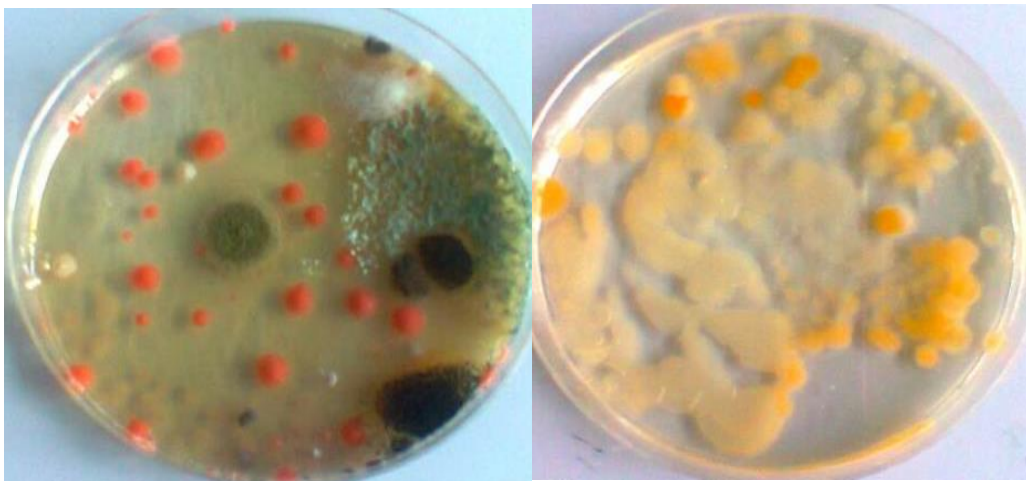
Alvarez-Perez *et al*. (2010) reported the isolation of Rhodotorula mucilaginosa from skin lesions in a Southern sea lion (Otaria flavescens). The microorganism was isolated from cutaneous lesions, identified by the commercial API 20 C AUX system, and confirmed by sequencing. Topical treatment with sertaconazol resulted in complete clinical recovery of the animal and repeat testing did not result in the recovery of the yeast from the healed lesion sites.



Skin lesions in the Southern sea lion at the moment of sampling for microbiological Analysis Growth in pure culture of *Rhodotorula mucilaginosa* on Sabouraud Agar with chloramphenicol **Alvarez-Perez *et al*. (2010)**

Sanusi *et al.* (2016) monitored the trend of colonization of e-waste soil polluted fish

aquaria by *Rhodotorula sp*. The aquaria containing the specie *Oreochromis niloticus* were polluted separately with different quantities of soil from e-waste dumpsite and the soil without e-waste. The soil sample from e-waste dumpsite differed from soil without e-waste in all of the parameters determined. Higher organic contents (17.60%), moisture content (3.86%), organic carbon (10.17%) and higher value of organic nitrogen (0.35%) were recorded. Four species of fungi were isolated from soil of e-waste dumpsite while two species of fungi were isolated from soil without e-waste. *Rhodotorula* presence in the aquaria was only observed in the first and second week of the research. The highest isolation was from the aquarium polluted with 75 g of soil without e-waste (34 isolates) at week one while the lowest was from the control aquarium (15 isolates) also at week one. It was also observed that plates and week where *Rhodotorula* sp population was high, the populations of other fungi were lower. Most of the other fungi isolated within the two weeks period of *Rhodotorula* colonization were inversely proportional to the population of *Rhodotorula* sp. The pH values and the biochemical oxygen demand were significantly affected by the pollutant. The momentary colonization of the aquaria by *Rhodotorula* sp, posed health risk to both the living organisms in the aquaria and human having contact with the aquaria while the antagonistic effect on other fungi could lead to imbalance in the fungi community in the aquaria.



Mixed culture of fungi - *Rhodotorula* spp growing with other fungi *Rhodotorula* spp growing on Potato dextrose agar plate **Sanusi *et al.* (2016)**

Different types of yeasts reported in fish:

Cantoni *et al.* (1976) examined trout (*Salmo irideus)*, eel (*Anguilla angwilla*), *Barbus barbus* and *Coregonus lavaretus.* The yeasts isolated from the skin, gills and viscera were: *Rhodotorula aurantica*, *R. graminis* and *R. lactosa* in 70%, , *C. hungaricus* in 2%, *Torulopsis* sp.in 14% and *Saccharomyces* sp.

Bohm and Fuhrman (1984) isolated different types of yeasts from different freshwater fish. Isolated yeasts were differentiated as members of the genera Candida, Rhodotorula, Trichosporon and Torulopsis. They concluded that these organisms could be regarded as secondary invaders.

El-Bassiouny *et al*. (1989) stated that the Nile fish were affected by different types of yeasts identified as *C. albicans, C. tropicalis, C. parapsillosis, Rhodotorulla* spp. and *Saccharomyces* spp.

Salem *et al.* (1989a) isolated different types of yeasts from gills, eyes, heart, liver, gall bladder, spleen, kidney and intestine from apparently healthy as well as diseased

Tilapia nilotica obtained from different localities during different seasons.

Marzouk *et al.* (1990) isolated different types of yeasts from both tilapia and catfish, which were numerated respectively in both types of fish; (10 & 8) isolates of *Rhodotorula glutinis*, (7 & 5) *Torulopsis sp.*, (1 & 0) *Torulopsis anomala*, (2 & 2) *Cryptococcus diffluens*, (1 & 1) *Cryptococcus uniquttlatus*, (1 & 0) *Cryptococcus albidus*, (1 & 0) *Cryptococcus pnurenti*, (1 & 0) *Cryptococcus* sp., and finally (5 & 6) *Candida albicans*. They concluded that yeasts might have cooperated with other pathogens in causing tail and fin rot in cultured freshwater fish namely tilapia and Nile catfish (*Clarias lazera*).

Shaheen (1991) studied yeast infection in case of naturally infected Oreochromis Tilapia, Common carp and Catfish collected from different localities of fresh water sources using conventional methods. He was able to identify 49 isolates of Candida; 15 Rhodotorulla isolates; 5 Cryptococcus isolates; one Trichosporon; 5 *Saccharomyces* spp.*;* 3 *Debaromyces* spp. and 2 *Trichosporon* spp. The methods for identification included microscopical examination on rice agar, sugar fermentation, sugar assimilation and nitrate assimilation tests and by using the Auxanographic (API20C) system.

Abdel-Alim (1992) isolated different types of yeasts from skin, gills, intestine and musculature of *Oreochromis niloticus* and *Mugil cephalus*. The isolated yeasts were *Rhodotorula mucilagenose,* Torulopsis*, Candida albicans, C. Tropicalis species, C. krusei* and *C. parapsillosis.*

Hsu and Liu (1994) cultured a total of 115 yeast isolates strains from giant freshwater prawn, *Macrobrachium rosenbergii*, during January 1991 to March 1993 in Kaoshiung-Pingtung areas of Taiwan. The disease outbreaks occurred mostly in cool season from October to May, especially, from December to February. No isolates were obtained in high temperature months from June to September. The morbidity rates in different age groups were found as 75%, 23%, 2% and 0% in adult, middle size, juvenile, and larvae, respectively. The major gross lesions of infected prawns showed fulvous-colored in general appearance, swollen hepatopancreas, milky muscle and hemolymph. Microscopically, the hepatopancreas showed vacuolization of tubular epithelial cells and accumulations of yeast clumps in sinusoid which were encapsulated with a thin membrane. Zenker's degeneration and necrosis were found in the affected muscles. Budding of yeasts presented here and there within the haemolymph vessels throughout the whole body. The data of yeast cell concentrations in tissues were shown as 3×10^{10} , 2×10^{9}, 1×10^{8} and 2×10^{5} CFU/gm, in hemolymph, hepatopancreas, muscle and gill, respectively. The giant freshwater prawns experimentally inoculated (I.M) with the isolated yeast, *Debaryomyces hansenii*, in different temperature groups resulted 100% mortalities in 15°C and 20°C groups, whereas no mortalities found in 30°C group.

Shaheen and El Bouhy (1995) infected 144 fingerlings of Tilapia nilotica *(Oreochromis niloticus)* with 2×10^{3} yeast suspension of *Candida albicans* and *Rhodotorula glutinis.* Trials of their treatment with flagyl (2.50 mg/kg ration), acriflavine and ketoconzole (50 mg/kg ration) were done with determination of their growth rate. Gross examination, postmortem examination and reisolation of the infected yeasts from examined cases on Sabouraud's dextrose agar were studied and discussed. The mortality rate reached 66.7% and 41.7% among experimentally infected fish with C. *albicans* and *Rhodotorula glutinis,* respectively. Trials of treatment with flagyl, acriflavine and ketoconazole revealed decreased mortality rate of *C. albicans* infected fish and 16.6%. 8.3% and 16.7% of *R. glutinis* infected fish. With respect decreased mortality and increased growth rate, acriflavine and ketoconazole, proved to be more effective than flagyl in fish infected with C. *albicans.* While flagyl and ketoconazole were more suitable in treatment of fish infected with *R. glutinis.*

Slavikova and Vadkertiova (1995) found a total of 15 genera and 29 yeast species in the water of 3 fish ponds located in the area of low land Zahorie, Slovakia. The fish

ponds were sampled in Summer and Autumn. Aureobasidium, Sporoholomyces, Candida and Cryptococcus *spp*. occurred most frequently. The composition of yeast species was more heterogeneous in Summer than in Autumn, but the number of yeasts observed in Autumn was 5,5-fold higher than that isolated in Summer. Some species of Candida and *Cryptococcus laurentii* were frequently isolated in Summer with high counts.

Andlid ***et al.*** **(1995)** isolated yeasts from the intestine of farmed rainbow trout (*Salmo gairdneri*), turbot (*Scophtalmus maximus*), and free-living flat-fish (*Pleuronectes platessa* and *P. flesus*). The average number of viable yeasts recovered from farmed rainbow trout was 3.0×10^3 and 0.5×10^2 cells per gram homogenized intestine for white and red-pigmented yeasts, respectively. The dominant species were *Debaryomyces hansenii, Saccharomyces cerevisiae, Rhodotorula rubra,* and *R. glutinis*. In 5 of 10 free-lving marine fish, > 100 viable yeast cells per gram intestinal mucus were recovered. Red-pigmented yeasts dominated and composed >90% of the isolates. Colonization experiments were performed by inoculating rainbow trout and turbot with fish-specific, isolated yeast strains and by examining the microbial intestinal colonization at intervals. Inoculation of experimental fish with pure cultures of *R. glutinis* and *D. hansenii*HF1 yielded colonization at a level several orders of magnitude higher than before the inoculation. Up to 3.8×10^4, 3.1×10^6, and 2.3×10^9 viable yeast cells per gram intestine or feces were recovered in three separate colonization experiments. The high level of colonizing yeasts persisted for several weeks. The concentrations of yeasts in the tank water never exceeded 10^3 viable cells per milliliter. No traces of fish sickness as a result of high yeast colonization were recorded during any of the colonization experiments. For periods of the experiments, the concentration of aerobic bacteria in the fish intestine was lower than the intestinal yeast concentration. Scanning electron microscopy studies demonstrated a close association of the yeasts with the intestinal mucosa. The mucosal colonization was further demonstrated by separating intestinal content, mucus, and tissue. All compartments were colonized by $>10^3$ viable yeast cells per gram. No bacteria were detected on the micrographs, indicating that their affinity for the intestinal mucosa was less than that of the yeasts.

Nagornaia *et al*. (1996) studied quantitatity and species compositions of yeasts contaminating eggs, fry and fingerlings of Salmo gairdneri Rich under artificial breeding. Prevalence of species of genera Candida, Rhodotorula, Cryptococcus and Debaryomyces was noted. Yeast isolated from perished eggs and sick fry did not possess pathogenic properties. Certain strains of yeast made stimulating effect on the studied microorganisms.

Paškevičius **and** ***Varnaitė*** **(2010)** carried out a study to ascertain yeast contamination of raw herring, the processed products and manufacturing environment, and to evaluate biochemical peculiarities of dominant species of the isolated yeasts, which could have influenced the product quality. A total of 36 yeast strains were isolated from herring products, raw material and their production environment during the manufacturing process. These yeasts were identified as 8 species belonging to *Saccharomyces, Candida, Pichia, Rhodotorula, Debaryomyces, Yarrowia* yeast genera. *Saccharomyces cerevisiae* and *Candida glabrata* yeasts dominated in herring products. *Candida blankii, Debaryomyces hansenii* var. *hansenii, Yarrowia lipolytica,*

and *C. glaebosa* yeasts were isolated from raw herring. The studies on contamination of the production room air, equipment, dishes, packing and other surfaces showed that the majority of samples were infected by *Saccharomyces cerevisiae* and

Refai *et al.* (2010) carried out a study on 360 freshwater fishes (240 *Oreochromis* species and 120 *Clarias gariepinus*). They were collected from different governorates and during different seasons. Naturally infected fishes showed clinical abnormalities such as skin darkening, exophthalmia, corneal opacity, abdominal distention, ulceration of the skin and cotton wool like growths on various parts of the body. Fishes were then subjected to post mortem examination which revealed many abnormalities. Mycological examination revealed the isolation of 2081 fungal isolates from 150 diseased and 210 apparently healthy fish samples (1658 mould and 423 yeast isolates), of which 1334 were isolated from Oreochromis species and 747 isolates from Clarias gariepinus. Yeasts isolated also from both fish species had the following incidence: Candida albicans (35.9 %), other Candida species (19.1%), Rhodotorula species (31.4%) and Torulopsis species (13.5%).

Banerjee and Ghosh (2014) detected yeasts in the intestine of three Indian major carps (Labeo rohita, Catla catla, Cirrhinus mrigala), three exotic carps (Hypophthalmichthys molitrix, Ctenopharyngodon idella, Cyprinus carpio), as well as Nile tilapia (Oreochromis niloticus), and identified the most promising extracellular enzyme-producing (e.g. amylase, protease, lipase, cellulase, xylanase and phytase) yeast strains by 18S rDNA sequence analysis. Selected for qualitative enzyme assay were 121 yeast strains, from which 28 were further studied for quantitative enzyme assay. The strain CMH6A isolated from C. mrigala exhibited the best extracellular enzyme activities except for amylase and cellulase. The strain ONF19B isolated from O. niloticus was noted as the best extracellular enzyme producer among the strains that produced all of the extracellular enzymes studied. Sequencing of the 18S rDNA fragment followed by nucleotide blast in the National Centre for Biotechnology Information (NCBI) GenBank revealed that strains CMH6A and ONF19B were similar to Pichia kudriavzevii (Accession no. KF479403) and Candida rugosa (Accession no. KF479404), respectively. The test of antagonism (in vitro) revealed that the isolated yeasts could not affect the growth of the autochthonous gut bacteria. This might indicate likely co-existence of autochthonous yeasts and bacteria in the fish gut. Further research is necessary to explore the possibilities of utilizing the extracellular enzyme-producing yeasts detected in the present study for commercial aquaculture. Introduction

References:

1. Abdel-Alim, K. (1992): The role of fish in transmitting some bacterial and fungal diseases to man. M.V.Sc. Thesis, Faculty of Vet. Med., Alexandria, University.
2. Alvarez-Perez, S. , A. Mateos , L. Dominguez , E. Martinez-Nevado , J.L. Blanco, M.E. Garcia. Veterinarni Medicina, 55, 2010 (6): 297–301 Case Report 297 Isolation of Rhodotorula mucilaginosa
3. Andlid, T.; Juárez, R. V.;Gustafsson, L., 1995: Yeast colonizing the intestine of rainbow trout (*Salmo gairdneri*) and turbot (*Scophtalmus maximus*).*Microb. Ecol.* **30**, 321–334
4. Banerjee, S., and K. Ghosh. Enumeration of gut associated extracellular enzyme-producing yeasts in some freshwater fishes. J. Appl. Ichthyol. 30 (2014), 986–993 © 2014 Blackwell Verlag GmbH ISSN 0175–8659 Received: September 7, 2013 Accepted: March 13, 2014 doi: 10.1111/jai.12457

5. Bojarczuk, A., Katie A. Miller, Richard Hotham, Amy Lewis, Nikolay V. Ogryzko, Alfred A. Kamuyango, Helen Frost, Rory H. Gibson, Eleanor Stillman, Robin C. May, Stephen A. Renshaw &Simon A. Johnston. *Cryptococcus neoformans* Intracellular Proliferation and Capsule Size Determines Early Macrophage Control of Infection *Scientific Reports* 6, Article number: 21489 (2016)
6. Brothers KM, Newman ZR, Wheeler RT. Live Imaging of Disseminated Candidiasis in Zebrafish Reveals Role of Phagocyte Oxidase in Limiting Filamentous Growth . *Eukaryotic Cell*. 2011;10(7):932-944.
7. Cantoni, C.; Siano, S. and Colcinordi, C. (1976): Yeasts in freshwater fish. Arch.Vet. Ital. 27: 3-4, 64-65
8. Chen Y-Z, Yang Y-L, Chu W-L, You M-S, Lo H-J (2015) Zebrafish Egg Infection Model for Studying *Candida albicans* Adhesion Factors. PLoS ONE 10(11): e0143048. doi:10.1371/journal.pone.0143048
9. Chao CC, Hsu PC, Jen CF, Chen IH, Wang CH, Chan HC, Tsai PW, Tung KC, Wang CH, Lan CY, Chuang YJ. **Zebrafish as a model host for Candida albicans infection.** Infect Immun. 2010 Jun;78(6):2512-21.
10. El-Bussiouny, A.; Soad, S.M.; Edris, A.M. and Mousa, M.M. (1989): Nile fish as a carrier of some fungi and food poisoning bacteria in connection with river Nile pollution by abattoir sewage. Alex. J. Vet. Sci., 5(1), 335-343.
11. Hata, N. K, K, Iwata E, Takeo K (2000): *Malassezia pachydermatis* isolated from a South American sea lion (*Otaria byronia*) with dermatitis. Journal of Veterinary Medical Science 62, 901–903.
12. Hsu, J.P. and Liu, C.I.(1994): Studies on yeast infection in cultured giant freshwater prawn (*Macrobrachium rosenbergii*) Coa-fish.-ser. (47), 55-68
13. Marzouk, M.S.M.; El-Far, F. and Nawal, M.A. (1990): Some investigations on moulds and yeasts associated with tail and fin rot in freshwater fish in Egypt. Alex. J. Vet. Sci., 6.(l), 193-203.
14. Nagornaia, S.S.; Ignatova, E.A.; Isaeva, N.M.; Davydov, O.N. and Podgorskii, V.S.(1996): Yeasts contaminating salmon roe. Mikrobiol Z. 58(2):8-12.
15. *Paškevičius, A., Regina Varnaitė*. YEAST OCCURRENCE IN HERRING PRODUCTS AND PROCESSING ENVIRONMENT AND THEIR BIOCHEMICAL PECULIARITIES. Pol. J. Food Nutr. Sci. 2010, Vol. 60, No. 4, pp. 369-373
16. Salem, A.A.; Refai, M.K.; Eissa, I.A.M.; Marzouk; M.; Moustafa, M. and Manal Adel. (1989a): Mycological investigations on cultured tilapia in Egypt. Alex. J. Vet. Sci., 5, (2), 625-634.
17. Sabiiti, W., Robin C. May, and E. Rhiannon Pursall, "Experimental Models of Cryptococcosis," International Journal of Microbiology, vol. 2012, Article ID 626745, 10 pages, 2012. doi:10.1155/2012/626745
18. Sanusi. A. I., D. V. Adegunloye, A. M. Orimoloye and T. M. Olorunnusi. Colonization Pattern of *Rhodotorula* sp. in Polluted Tilapia Fish Aquaria and the Risk of *Rhodotorula* Caused Infection. *British Microbiology Research Journal*
19. *11(5): 1-9, 2016*
20. Shaheen, A.A. (1991) . Studies on yeast in Freshwater fish. Ph. D. Thesis, Fac. Vet. Med., Zagazig Univ.
21. Shaheen, A.A.and El Bohy,Z.M.(1995): Effect of *Candida albicans* and *Rhodotorula glutinis* on the health and growth of Tilapia nilotica with trials of their treatment. Vet. Med. J. 6, 2.
22. Slavikova, E. and Vadkertiova, R. (1995):Yeasts and yeast-like organisms isolated from fish-pond waters. Acta Microbiol. Pol.;44(2):181-9.
23. Tikhonova, L.S.; Voinova. N.V.; Kachan, S.N. and Litvinenko, S.(1988 Effect on fish of yeast-like fungi in the feed. Veterinariya, Moscow, USSR, No. 10, 31-32.

24.Mycotoxins

Generally, most of the mycotoxins that have the potential to reduce growth and health status of aquaculture-farmed animals are produced by Aspergillus, Penicillium and Fusarium sp. Toxic metabolites produced by these fungi are known to be either carcinogenic (e.g. aflatoxin B1, ochratoxin A, fumonisin B1), oestrogenic (zearalenone), neurotoxic (fumonisin B1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes) or immunosuppressive (aflatoxin B1, ochratoxin A and T-2 toxin).

Effects of mycotoxins

Aflatoxins

- **Fish**
  - Carcinogenic effects
    - Higher incidence of cancer in exposed animals Liver tumors
  - Decreased performance
    - Reduced growth,
    - Lower weight gain,
    - Higher mortality
  - Hepatotoxic effects
    - Severe hepatic necrosis,
    - Liver lesions
  - Hematopoietic effects
    - Impaired blood clotting,
    - Anemia
  - Dermal effects
    - Pale gills
  - Nephrotoxic effects
    - Pale to yellow kidney lesions
- **Shrimp**
  - Decreased performance
    - Reduced growth,
    - Low apparent digestibility,
    - Higher mortality
  - Gastro-intestinal effects
    - Negative effect on digestive enzymes
  - Hepatotoxic effects
    - Hepato-pancreatic damage
  - Hematopoietic effects
    - reduced number and size of red blood cells)

Ochratoxin A

- **Fish**
  - **Decreased performance (reduced growth, poor FCR)**
  - **Hepatotoxic effects (lower weight gain, high mortality)**

- **Nephrotoxic effects** (Pale and swollen kidneys)

Trichothecene

- **Fish**
  - **Decrease performance**
    - Reduced growth
    - Reduced feed consumption
    - Poor FCR
    - Lower weight gain
    - Higher mortality
  - **Hematopoietic effects**
    - Lower hematocrit value (reduced number and size of red blood cells)
    - Lower blood hemoglobin value
- **Shrimp**
  - **Decrease performance**
    - Poor growth
    - Lower weight gain
    - Inhomogeneous growth
  - **Immunosuppression**
    - Decreased resistance to environmental and microbial stressors
    - Increased susceptibility to diseases
  - **Hematopoietic effects**
    - Lower hematocrit value (reduced number and size of red blood cells)

Fumonisins

- **Fish**
  - **Histopathological changes**
    - Lesions in the exocrine and endocrine pancreas
  - **Hematopoietic effects**
    - Lower hematocrit value (reduced number and size of red blood cells)
  - **Nephrotoxic effects**
    - Lesions in the inter-renal tissue
  - **Gastro-intestinal effects**
    - Lesions in the exocrine and endocrine pancreas

1. Aflatoxins:

Historical:

- **Haddow and Blake (1933)** described hepatoma in trout in two fish in an English trout farm.

- **Wales and Sinnhuber (1966)** mentioned that, during the period 1937-42, two epizootics of hepatoma occurred in trout hatcheries in California and caused some local interest.
- **Wolf and Jackson (1963)** showed that the unidentified cause of hepatoma occurred in the cottonseed meal fraction of a particular pelleted trout feed.
- **Ashley *et al.* (1964)** indicated that aflatoxins consistently induced aflatoxicosis in trouts with a high incidence of hepatoma
- **Coates *et al.* (1967)** fed cottonseed meal to trout and found hepatoma to be contaminated with aflatoxins. The suspicion that the causes of such hepatomas derived from aflatoxin was proved in a number of experiments where crude and crystalline aflatoxins obtained from fungi cultured on shredded wheat media were fed at various dosages to trout.
- **Sinnhuber *et al*. (1968)** produced hepatoma in trout by feeding aflatoxin-contaminated cotton seed meal.
- **Wales (1979)** described an effective technique for the induction of hepatomas in rainbow trout has been described by. This involved brief immersion of embryonated eggs in an aqueous solution of aflatoxin B 1 while maintaining the ambient water temperature.
- **Wales (1970)** reported that some individual rainbow trout developed hepatoma after having been fed the control diet plus 20 ppm aflatoxin B1 for a single day or having received a continuous feeding of levels as low as 0.4 ppb aflatoxin Bl for six months. As the dosages increased in level or in the duration of administration, the incidence of hepatoma increased. The extent of these parenchymal cell abnormalities is generally indicative of the level of aflatoxin in the diet.

- The main aflatoxins commonly found in aquaculture feedstuffs are aflatoxin B1, (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) (Ottinger & Kaattari 1998, 2000; Huang et al. 2011).
- These mycotoxins occur especially in subtropical and tropical areas contaminating mainly feedstuffs with high starch and lipid content, such as cottonseed, corn, peanut, wheat and soya bean (Ostrowski-Meissner et al. 1995).

Aflatoxicosis outbreak cases in fish

have been reported in:

- United States (**Ashley and Halver, 1963)**
- Denmark **(Rasmussen *et al*., 1968)**
- Germany (**Wunder and Korn, 1982)**
- Mexico (**Ruiz Pérez *et al.* 1984)**

Aflatoxin carcinogenicity

- Rainbow trout was the first species in which AFB_1 carcinogenicity was recognized and intensively researched (**Ashley *et al.***, 1965; **Sinnhuber *et al.***, 1968).

- The carcinogenic effect of AFB_1 has also been studied in other fish, such as the channel catfish, *Ictalurus punctatus*; Nile tilapia; and the guppy, *Poecilia reticulata* (**Sato *et al.*, 1973; Jantrarotai and Lovell 1990; Chàvez-Sànchez *et al.*, 1994).**
    - **Wolf and Jackson (1967)** fed young coho salmon (0. kisutch) and young chinook salmon (0. tshawytscha) a diet containing aflatoxin for ten months but failed to induce hepatoma.
    - **Ashley (1970)** described chronic and acute aflatoxicosis in aflatoxin-fed rainbow trout, coho salmon and channel catfish (Ictalums punctatus). He found that coho salmon and channel catfish fed a diet containing 320 ppb aflatoxin B1 for two years had histologically normal livers but when they were force fed I 5 mg aflatoxin B1 per kg body weight, acute aflatoxicosis appeared in 21 days.
    - **Wales (1970)** found sockeye salmon (0. nerka) to be susceptible, providing cyclopropene triglyceride was fed with the aflatoxin.

Susceptibility of fish to aflatoxins

- There is significant variation among 'strains' of rainbow trout in their susceptibility to aflatoxins **(Wales, 1970)**
    - The wild stock of rainbow trout has been found to be more sensitive to high level aflatoxicosis than the domesticated rainbow trout
    - The brook trout (Salveliflus Iontinalis) is susceptible to aflatoxin carcinogenesis, but tests indicate that it is less so than the domesticated rainbow trout.
    - The five species of salmon (Oncorhynchus spp.) in North America are relatively insensitive to the carcinogenicity of aflatoxin.
- Some fish species are extremely sensitive to AFB_1 because of differences in the pattern of enzymes involved in AFB_1 metabolism **(Bailey *et al*. 1988).**
- A pronounced difference in the susceptibility of different fish species and fish classes has been observed, with fish fry, for example, being more sensitive and succumbing quicker to aflatoxicosis than adult fish.
- Variation in AF sensitivity in salmonids, with rainbow trout displaying extreme sensitivity, while coho salmon, *Oncorhynchus kisutch*, were more resistant was recorded by **Hendricks (1994).**
- Many other researchers have demonstrated the extreme sensitivity of rainbow trout to AFB_1 (e.g., **Halver and Mitchell 1967; Bailey *et al.*, 1996).**

Aflatoxicosis occurs in three forms:

- **Acute aflatoxicosis**
    - Acute toxicity appears after ingestion of moderate to high doses of AFs. Determination of orally administered lethal AFs doses for rainbow trout is impractical as AFs induce regurgitation of the stomach contents (**Bauer *et al.*, 1969).**

- Acute toxicity causes anemia, pale gills, reduced hematocrit values, edema, frequent hemorrhaging, liver damage, and alterations to nutrient metabolism in rainbow trout.
- Acute toxicity of AFB_1 in rohu, *Labeo rohita*, following intraperitoneal (i.p.) application, with doses of 7.5, 11.25, and 13.75 mg/kg AFB_1 caused a reduction in food intake, sluggish movement, loss of equilibrium, rapid opercular movement, and dose-dependent mortality by the end of the 10-d experiment**.**
- Necropsy and histopathological examination revealed hepatomegaly with subcapsular focal congestion, necrotic and vascular changes to the liver and gill lamellae, meningitis, brain congestion, degeneration and inflammatory injury of the heart, degenerative and necrotic changes to the kidney tubules, and sloughing of the intestinal mucosa **(Sahoo *et al.*, 2001)**.

- **Subacute aflatoxicosis**
  - symptoms in fish include moderate to severe liver damage, yellow eyes, yellowed mucous membranes or skin, blood clotting abnormalities, lowered feed conversion ratio (FCR), anemia, reproductive failure, impaired immune response, renal damage, and premature death (**Hamilton, 1990; Santacroce *et al.*, 2008).**
  - AFB_1 at concentrations of 1.25 and 2.5 mg/kg (i.p.) in rohu caused cachexia, darkened scales, and preneoplastic liver lesions, along with changes to the spleen, intestine, gill, and pancreas over the 90-d trial (Sahoo ***et al.***, 2001).
  - AFB_1 at concentrations of 1.25 and 5.0 mg/kg (i.p.) in rohu caused disruption of the immune system over 90 d, shown as a reduction in total protein, globulin levels, bacterial agglutination titer, and serum bactericidal activities (Sahoo and Mukherjee, 2001).

- **Chronic aflatoxicosis**
  - occurs after long-term intake of low to moderate doses of AFs.
  - This chronic form of the disease is related to carcinogenic and genotoxic effects, followed by teratogenic, hormonal, neurotoxic, and hematological changes (**Pier *et al.*, 1980).**

Common effects of aflatoxicosis reported in fish

- **in finfish**
  - poor growth,
  - pale gills,
  - reduced RBCs,
  - anemia,
  - impaired blood clotting,
  - damage to liver,
  - decreased immune responsiveness and
  - increased mortality.
- **In rainbow trout**
  - Rainbow trout is extremely sensitive to aflatoxin B1 (AFB1), causing

        - liver damage
        - anemia,
        - hemorrhage,
        - weight loss,
        - increased susceptibility to secondary infectious diseases and mortality
        - Acute aflatoxicosis causes liver failure
        - chronic exposure provokes immunosuppression
    - LD_{50} for 50 g rainbow trout is 500 – 1000 ppb.
- **In warm water fish, such as catfish**,
    - are less sensitive to AFB_1
    - IP-LD_{50} of 11.5 mg/Kg body weight.
    - Feeding catfish on at least 10 ppm AFB_1 – contaminated feed for 10 weeks had adverse effects on the fish (**Lovell, 1992**):
        - growth rate, PCV%, Hb concentration and erythrocyte count were lower than those from the other treatments (0, 100, 500, 2000 ppb).
        - at the highest level, AFB_1caused necrosis and basophilia of hepatocytes, enlargement of blood sinusoids in the head kidney, accumulation of iron pigments in the intestinal mucosa epithelium, and necrosis of gastric glands.
        - the sub chronic toxic level of AFB_1 for catfish is approximately 6000 ppb of diet
    - Mean leukocyte count was significantly higher in the fish fed the highest concentration of AFB_1(**Jantrarotai and Lovell, 1990**).
    - AFB_1 administration (12 mg/kg body weight) caused regurgitation of stomach contents by channels catfish (**Jantrarotai *et al.*, 1990**).

- **The major clinical symptoms** include
    - impaired liver function,
    - reduced feed conversion efficiency,
    - weight loss,
    - increased susceptibility to secondary infectious diseases,
    - occurrence of necrosis and tumors, and
    - increased mortality (**Santacroce *et al.* 2008).**

Reported effects of variable aflatoxin concentrations in feeds on fish

- **In sea bass**, *Dicentrarchus labrax*, AFB_1 at concentration of 0.018 mg/kg in feed induced liver damage, manifested as an increase in serum transaminases and alkaline phosphatase activity and a significant decrease in plasma proteins after 42-d exposure (**El-Sayed and Khalil 2009**).
- **In common carp**, *Cyprinus carpio*, AFB_1 at concentrations of 0.2 mg/kg in feed caused circulation disturbances and reactive infiltration around the hepatic bile duct, dystrophy of liver tissue, serious dystrophic changes in nerve cells, and kidney damage, with appearance of polymorphonuclear elements in tubules after 120-d exposure. While feed containing 0.02 mg/kg AFB_1 resulted in circulation failure in carp, shown as venostasis in the hepatopancreas, spleen, kidney, central nervous system, and gills (**Svobodova *et al.*, 1982),** concentrations of 0.002 mg/kg in feed had no effect on Fulton's coefficient or hematological and biochemical indices of pathoanatomical and histological change over a 5-mo trial **(Svobodova and Piskac, 1980).**

- The presence of AFB_1 in the diet at a level of 0.2 mg/kg or higher negatively affected growth performance, bactericidal activity, lysozyme activity, and concentration of total serum proteins **in yellow catfish**, *Pelteobagrus fulvidraco*, after a 12-wk trial (**Wang *et al.*, 2016**).
- AFs in naturally contaminated feed in a concentration of 0.16 mg/kg had no adverse effects on the production variables of weight gain, feed intake, and feed efficiency ratio (FER) in channel catfish, *I. punctatus* (**Manning *et al.*, 2011).**
- Similar results were shown by a 12-wk study on juvenile channel catfish fed diets containing up to 0.22 mg AFs/kg. No reduction in body weight gain, FER, survival, or hematocrit was recorded ***(Manning et al., 2005).***
- Species of the genus *Oreochromis* tend to show low susceptibility to AFB_1 exposure. The effect of diets with 0.25, 2.5, 10, and 100 mg/kg AFB_1 on Nile tilapia for 8 wk was investigated by **Tuan *et al.*** (2002).
- Diets containing 100 mg/kg AFB_1 caused weight loss, severe hepatic necrosis, and mortality, while 10.0 mg/kg evoked hepatic injury characterized by an excess of lipofuscin and irregularly sized hepatocellular nuclei. Diets containing more than 2.5 mg/kg AFB_1 had a negative effect on hematocrit levels and growth performance. No obvious effects were observed from the diet containing 0.25 mg/kg AFB_1. The toxic effects of AFB_1 in tilapia (Nile tilapia × blue tilapia, *Oreochromis aureus*) over 20 wk using food containing 0.019, 0.085, 0.245, 0.638, 0.793, and 1.641 mg/kg AFB_1 were monitored by

Reports:

Halver (1967) reported most rainbow trout force fed crude aflatoxin at 1, 3 or 5mg/kg body weight in single dose or Img/kg body weight daily for 5 days were moribund by day 10 and only six fish survived in the groups fed Img/kg body weight daily for 5 days. All fish had gross multiple haemorrhagic areas in liver and adjacent viscera. Moribund fish had dark skin, nearly white gills, indicative of severe anaemia, and were listless. Death usually occurred in less than 24 hours after symptoms appeared.

Bauer *et al.* (1969) used nine-month-old Mt. Shasta strain rainbow trout averaging 60 g each to determine the median lethal doses (LD50) of aflatoxins B_1 and G_1. The toxins were isolated from cultures of *Aspergillus flavus* (ATCC 15517) grown on rice. Statistical treatment of mortality occurring during the 10-day period after intraperitoneal injection of the mycotoxins gave LD50 values of 0.81 mg/kg and 1.90 mg/kg for aflatoxin $B_1$and G_1, respectively. Trout given oral doses of aflatoxins, or those given an LD50 ip dose, regurgitated their stomach contents. It is suggested that acute toxicity studies of aflatoxins given by the oral route in this manner are not practical in the case of the rainbow trout.

Schoenhard *et al.* (1981) administered aflatoxicol (AFL), a major metabolite of aflatoxin B1 (AFB1), in a casein diet to duplicate groups of 120 fingering trout. In the same manner, additional duplicate groups received one of the following: no toxicant; AFB1; the diastereomer of AFL (AFL'); cyclopropenoid fatty acids (CPFA); and CPFA plus AFB1, AFL, and AFL'. Eight months after the initiation of the study, the following incidences of carcinoma were observed: control (0%); 20 ppb AFB1 (56%); 29 ppb AFL (26%); 61 ppb AFL' (0%); 50 ppm CPFA (3%); 20 ppb AFB1 plus 50

ppm CPFA (96%); 29 ppb AFL plus 50 ppm CPFA (94%); and 61 ppb AFL' plus 50 ppm CPFA (55%), showing both the carcinogenicity of AFL and the synergistic effects of CPFA. Twelve-month incidences were correspondingly higher in all cases. Aflatoxin M1, another metabolite of AFB1 in rainbow trout, was reported previously to be carcinogenic in trout. These results support the hypothesis that metabolism in rainbow trout does not effectively detoxify AFB1, but rather the formation of AFL extends the carcinogenicity of AFB1 and may contribute to the high sensitivity of rainbow trout to AFB.

Loveland *et al.* (1984) fed Rainbow trout *Salmo gairdneri* a diet containing the mixed-function oxidase system inducer, β-naphthoflavone or were fed a control diet. For the two respective diets, as much as 50 and 12% of an i.p.-injected dose of [^{3}H]aflatoxin $B_1$was recovered in the bile.The major product in the bile of β-naphthoflavone-fed trout was an aflatoxicol-M_1glucuronide, whereas the major product in the control bile was an aflatoxicol glucuronide.

Bailey *et al.* (1988) exposed Rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhyn-chus kisutch*) to aflatoxin B1(AFB1) either by passive embryo uptake or by dietary treatment after hatching and feeding onset. Trout exposed as embryos to an aqueous solution of 0.5 p.p.m. AFB1 for 15 min showed a 62% tumor incidence 12 months later, whereas coho salmon exposed to a similar solution for 30 min showed only a 9% incidence. The difference between salmon and trout response was even greater by dietary AFB1 treatment. Trout exposed for 4 weeks to 20 p.p.b. dietary AFB1 had a 62% tumor response 12 months later, whereas salmon exposed to 40 p.p.b. dietary AFB1 for 4 weeks failed to develop tumors. A 5% tumor incidence was observed in salmon 12 months after 3 weeks exposure to 5000 p.p.b. dietary AFB1, a lethal dose for trout. In addition to a lower tumor incidence when compared to trout, the neoplastic response of salmon to AFB1 is to produce benign hepatic adenomas in contrast to the malignant hepatocellular carcinomas seen in trout. AFB1 metabolism, DNA adduct formation, adduct persistence *in vivo* and *in vitro* and cytochrome P-450 isozyme composition were compared in livers of trout and salmon to understand the role of metabolism and initiation in this species difference. AFB1-DNA binding was 7–56 times greater in trout than salmon liver at various times after AFB1 injection, 20 times greater in embryos or in freshly isolated trout hepatocyte preparations after a 1 h incubation with aflatoxin Bl, and 18 times greater in trout liver after a three week dietary (80 p.p.b.) exposure. The major AFB1-DNA adduct was 8, 9-dihydro-8-(N^7-guanyl)-9-hydroxyaflatoxin B1 in both species. Persistence of AFB1-DNA adducts *in vivo* in liver was high compared to mamalian systems, implying that active enzymatic removal of bulky DNA adducts is low in both species and probably not a factor in their differential response to aflatoxin. Species differences in other phase I and phase II metabolism pathways and in AFB1 elimination were, overall, much less striking than those previously observed for trout fed inhibitors of aflatoxin carcinogenesis. Rates of bileelimination of AFB1 detoxication products, and total excretion of aflatoxins into water after AFB1 exposure, were not significantly different between trout and salmon. Since detoxication differences were not observed, the species difference in AFB1-DNA binding appears to reflect less efficient cytochrome P-450 metabolism of aflatoxin to the reactive 8, 9-epoxide in salmon, compared to trout. In support of this hypothesis, trout liver microsomes displayed a K_m (7.5 μM)for AFB1-DNA adduction *in vitro* that was 7-fold lower than salmon (52 μM). Furthermore, immunoquantitation of various P-450 isozymes suggest that salmon liver microsomes have much lower amounts of an isozyme immunochemically related to trout P-450

LM_2 which has previously been shown to be the major isozyme catalyzing AFB1 8, 9-epoxidation. Other, post-initiation differences were not ruled out by these studies and may contribute to the differential response of rainbow trout and coho salmon to AFB1 hepatocarcino-genesis.

Jantrarotai and Lovell (1990) indicated that means for growth rate, hematocrit, hemoglobin concentration, and erythrocyte count of channel catfish *Ictalurus punctatus* fed 10,000 μg aflatoxin B_1 (AFB_1) per kilogram of feed for 10 weeks were significantly lower than those of fish fed 2,154 μg/kg or a lower concentration ($P < 0.05$). Mean leukocyte count was significantly higher in the fish fed the highest concentration of AFB_1 ($P < 0.05$). Gross appearance and behavior of all fish were normal. Histopathological effects were observed only in fish fed the highest concentration of AFB_1. These fish had foci of necrotic hepatocytes mixed with basophilic hepatocytes. Spaces, apparently resulting from hepatocellular necrosis, were present within the basophilic foci. Sinusoids in the head (hematopoietic) kidney were dilated and circular in profile. Increased hematopoietic activity of blood-forming tissues was apparent from the presence of numerous immature blood cells. The intestinal mucosal epithelium accumulated excessive amounts of iron pigments. Gastric glands in the stomach were necrotic and contained infiltrating macrophages.

Nakatsuru *et al*. (1990) noted variation between species, since a high rate of DNA binding was observed in rainbow trout, whereas significantly lower values were evident in Coho salmon, indicating a direct relationship between binding levels and susceptibility to mycotoxin carcinogenicity.

Plakas *et al*. (1991) found that AFB_1-residues in catfish muscles were rapidly depleted. So, it is concluded that catfish has a very low potential for the accumulation of AFB_1 and its metabolites in the edible flesh through the consumption of AFB_1-contaminated feed.

El-Banna *et al.* (1992) fed tilapia fingerlings AFB_1-cntaminated diets. They showed no effect on 50 ppb concerning fish performance and body composition; yet, AFB_1-residue showed a cumulative effect related to the level of AFB_1 and feeding period.

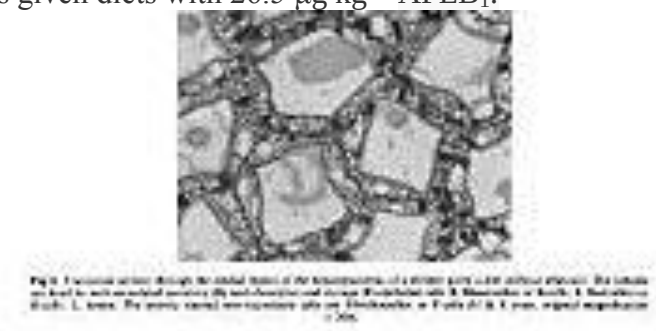
Zhang *et al.* (1992) proved that aflatoxin-induced tumors in fish increased with environmental temperature. Since, acute shift of trout to lower temperature reduced AFB –DNA adduct formation

Hussain *et al*. (1993) fed Walleye fish for 30 days on 50 or 100 ppb aflatoxin reflected 8% mortality rate, pale livers, and degenerative changes. Residues of aflatoxins B_1, G_1 and G_2 were detected in fish muscles at up to 20 ppb. After 2 weeks withdrawal period, no aflatoxin residues were detected but marked histo-pathological lesions were still seen**.**

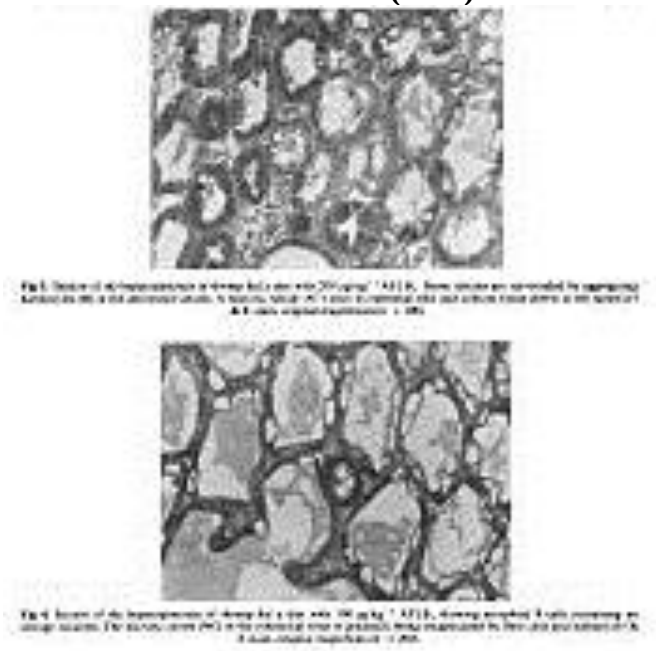
Ngethe *et al.* (1993) conducted a study with the Nile tilapia in order to investigate the disposition of AFBl after oral administration. Tilapia weighing 200 -t 20 g were kept in 500 1 glass tanks supplied with fresh running water (1l/min, 20^{o}C) . They were fed a commercial pelleted salmon diet (Tess-Norway) containing 30% crude protein and 25% crude fat, and were adapted to the experimental conditions for 3 weeks. Tritiated aflatoxin B1 (3H-AFB1), specific activity 20 Ci/mmol (Moravek Biochemicals, Brea, CA, USA), was dissolved in corn oil to a final concentration of 0.17 pCi/@. The test solution was administered through a stomach tube. They reported species differences in hepatic concentration of orally administered 3H-AFB1 between

rainbow trout (Oncorhynchus mykiss) and tilapia (Oreochromis niloticus).

Bautista *et al.* (1994) carried out a survey of aflatoxin B_1 ($AFLB_1$) levels in commonly used commercial shrimp finisher feeds in the Philippines showed a various range of values from not detected to 120 μg kg^{-1} using high-performance thin-layer chromatography. Six experimental diets were prepared to contain various levels of $AFLB_1$ based on survey results to determine the effects of such contamination in pre-adult shrimp *Penaeus monodon* (17.5 ± 0.6 g). Results showed that shrimps fed diets containing $AFLB_1$greater than or equal to 73.8 μg kg^{-1} gave comparatively poor growth rate and higher susceptibility to shell diseases. No $AFLB_1$ residues were detected in sampled whole shrimp tissues after 62 days of exposure to $AFLB_1$ containing diets indicating a low potential for transmission of the toxin from edible shrimp tissues to consumers. Histopathological alterations in the hepatopancreas of shrimp chronically exposed to AFLB, were observed in all samples. The degree of alterations correlated with the level of $AFLB_1$. Based on growth performance, pre-adult shrimps can tolerate $AFLB_1$ levels of up to 52.3 μg kg^{-1} in the feeds although histopathological changes were already evident in the tissues of shrimps given diets with 26.5 μg kg^{-1} $AFLB_1$.



Bautista *et al.* (1994)



Bautista *et al.* (1994)

Chavez – Sanchez *et al*. (1994) offered diets supplemented with 7 different levels of aflatoxin B_1 (0, 0.94, 1.88, 0.375, 0.752, 1.50., 3.0 mg/kg diet) to 0.5 g Nile tilapia for 25 days to study its effects on growth, behavior and potential histopathological

changes. Fish were subsequently maintained for 50 days and fed the basal diet without added aflatoxin. Fish samples from each treatment were taken on days 15, 26, 54 and 75 and preserved for histological examination. From the first days of the experiment, a clear reduction in growth rate and feed consumption in direct relation to the aflatoxin level was observed. Feed consumption resumed when normal feed was offered. However, growth rate remained unchanged until the end of the experiment. Mortality during the experiment was not related to dietary aflatoxin B_1 level. The liver was severely affected by the aflatoxin. The following histological changes were observed: fatty liver and characteristic neoplastic changes such as nuclear and cellular hypertrophy, nuclear atrophy, increase in number of nucleoli, cellular infiltration, hyperemia, cellular basophilia and necrosis. Some changes in the kidney were also observed such as congestion, shrinking of the glomeruli and melanosis. With low doses of aflatoxin, the fish did not show any external signs of toxicity other than growth reduction. In intensive culture systems of tilapia, this could be of economic significance.

Marzouk *et al*. (1994) fed Nile tilapia a diet contaminated with crude aflatoxins for 22 successive weeks showed a significant decrease in growth rate, PCV, Hb conc., erythrocyte count, total leukocyte count and lymphocytes. The mortality rate was 60% and aflatoxin residues were detected in fish at the end of week 16.

Sarcione and Black (1994) suggested that serum alpha foetoprotein measurements may be useful to confirm the appearance of hepatocellular carcinoma in experimental fish carcinogen-assay system and to detect hepatocellular neoplasia in high-risk wild fish populations exposed to carcinogenic pollutants.

Ostrowski-Meissner *et al.* (1995) tested Pacific white shrimp, Penaeus vannamei, in two indoor trials to identify dietary aflatoxin B1 (AF B1) levels that adversely affect performance and to describe histopathological changes. Trial I (0–15 000 ppb nominal AF B1 levels; 21-day duration; 1.61 g per shrimp initial mean weight) was a range-finder test for acute and chronic AF B1 toxicity, including histopathological responses. Trial II (0–900 ppb AF B1; 56-day duration; 1.51 g per shrimp initial mean weight) quantified AF B1 effects on growth, feed conversion and apparent digestibility coefficients for digestible energy, dry matter and crude protein. AF B1 at 15 000 ppb caused 100% mortality within 2 weeks. Abnormal hepatopancreas and antennal gland tissues were caused by 2 weeks of AF B1 at 50 ppb. Feed conversion and growth were significantly affected at ≈400 ppb AF B1. Apparent digestibility coefficients decreased significantly at 900 ppb AF B1.
Omar *et al*. (1996) showed that grey mullet is highly sensitive to AFB_1followed by common carp, red tilapia and Nile tilapia, respectively. Dietary aflatoxin treatment decreased feed consumption, growth performance and feed and nutrient utilization. Additionally,

Abdelhamid *et al*. (1997) revealed that catfish was more resistant than tilapia for aflatoxicosis. Yet, catfish contained more residual aflatoxin than tilapia. This led to a conviction that aflatoxin metabolism is different, depending on fish species. Moreover,

Troxel *et al.*(1997) verified that zebrafish can bioactivate AFB_1 and the resulting DNA adducts suggest sensitivity to this carcinogen.

Bailey *et al.* (1998) found that relative tumorigenic potencies of aflatoxins were AFB_1 1.0, AFL 0.936, aflatoxin M_1 0.086, and AFL M_1 0.041.

Ottinger and Kaattari (1998) reported that *in vitro* exposure of rainbow trout (*Oncorhynchus mykiss*) peripheral blood leucocytes to aflatoxin B_1(AFB_1) caused a dramatic suppression of immunological function as indicated by decreased lymphocyte proliferation and immunoglobulin production in response to the mitogen lipopolysaccharide. These leucocytes were up to 1000-fold more sensitive to this carcinogen than were murine leucocytes. Additionally, leucocytes procured from trout during July–December were significantly (P=0·01) more sensitive to AFB_1than those obtained during January–June. Immunosuppression was observed with AFB_1concentrations that were not toxic as measured by cell viability. This decrease in AFB_1sensitivity appears to occur subsequent to a generalised winter-associated period of decreased immune reactivity that has been observed in a number of ectothermic species and in this study.

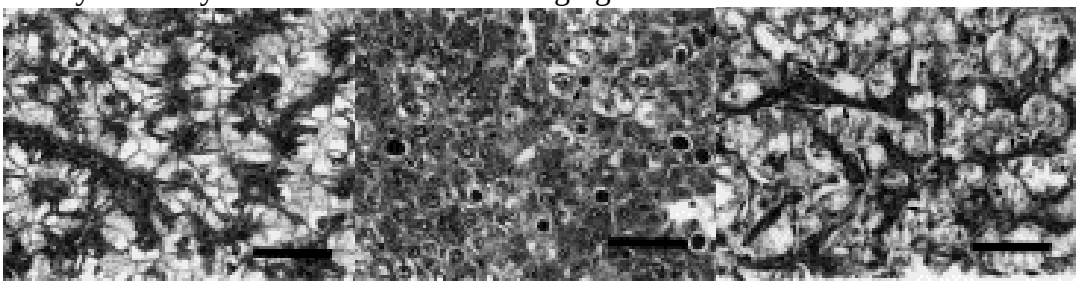
Abd-Allah *et al.* (1999) suggested that the Comet assay is a useful tool for monitoring the genotoxicity of mycotoxins such as AFB_1 and for evaluating organ specific effects of these agents in different species.
Ottinger and Kaattari (2000) reported that AFB_1 is a polycyclic aromatic hydrocarbon that is associated with hepatic carcinogenesis and immunomodulation in a broad spectrum of vertebrates; so, exposure to AFB_1 resulted in the reduction of cytokine, macrophage function and lymphocyte activity, i.e. trout exposed to very low concentrations of AFB_1 in feed or exposed as embryos have a very high incidence of carcinogenesis

Boonyaratpalin *et al.* (2001) mentioned that AFB1 levels between 50–100 ppb showed no effect on growth in juvenile black tiger shrimp (Penaeus monodon). Nevertheless, growth was reduced when AFB1 concentrations were elevated to 500–2500 ppb. Survival dropped to 26.32% when 2500 ppb AFB1 was given, whereas concentrations of 50–1000 ppb had no effect on survival.. There were marked histological changes in the hepatopancreas of shrimp fed diet containing AFB1 at a concentration of 100–2500 ppb for 8 weeks, as noted by atrophic changes, followed by necrosis of the tubular epithelial cells. Severe degeneration of hepatopancreatic tubules was common in shrimp fed high concentrations of AFB1.

Sahoo and Mukherjee (2001) injected graded levels (0, 1.25, 5.00 mg/kg of body weight) of purified AFB1 intraperitoneally (i.p.) into rohu (Labeo rohita) fingerlings weighing 30-50 g, and the fish were observed for a period of 90 days. At the end of the trial, blood samples were collected from the control group as well as the AFB1 injected fish and were screened for nitroblue tetrazolium (NBT) assay, serum total protein, albumin, globulin, albumin-globulin ratio (A:G), serum bactericidal activity and bacterial agglutination titre against Edwardsiella tarda. The aflatoxin-treated fish revealed a reduction of total protein, globulin levels, bacterial agglutination titre, NBT and serum bactericidal activities, as well as an enhanced A:G ratio without change in albumin concentration, irrespective of dose levels of toxin treatment, when compared to the control group. Thus, AFB1 proved to be immunosuppressive in rohu even at the lowest dose (1.25 mg/kg body weight) of toxin treatment. This could be of economic significance in intensive culture systems of rohu.

Abdelhamid *et al.* (2002) reported negative effects of aflatoxicosis on Nile tilapia including reduces in body weight, growth rates, feed conversion and survival rate. Also, protein content of the fish as well as its utilization from the contaminated diets were reduced, whereas fish fat and ash contents as well as muscular RNA increased. Blood profile was negatively also affected, since AFB_1 reduced PCV, Hb, RBCs and protein contents, but increased some enzyme activity and WBCs. The aflatoxic diets led to pathological alterations in all tested tissues of gills, intestine, liver, subcutaneous tissue and muscle, spleen, kidneys, and brain. The AFB_1 – contaminated diets led to gross clinical symptoms and mortality. It reduced fish muscles area, elevated internal organs indices, and caused chromosomal aberrations besides lower mitotic index of gill cells. Severity of its harmful effects correlated positively with its dietary levels. Its effects varied between fish sizes, so its dietary LC_{50} was calculated as 1006 and 1318 ppb for Nile tilapia weighting 2 and 30 g, respectively.

Tuan *et al.* (2002) investigated responses of Nile tilapia to varying concentrations of aflatoxin B_1 (AFB) under controlled laboratory conditions. Nile tilapia (2.7 g) were fed semipurified diets containing 0, 0.25, 2.5, 10, or 100 mg AFB/kg of diet for 8 weeks. Weight gain and hematocrit of fish fed with 0.25 mg AFB/kg were not significantly different from that of the control; however, diets containing higher levels of AFB had significantly (P<0.05) reduced weight gain and hematocrit. Histologically, livers of fish fed with diets containing 10 mg AFB/kg contained excess lipofuscin and irregularly sized hepatocellular nuclei. Diets containing 100 mg AFB/kg caused weight loss and severe hepatic necrosis; 60% of the fish in this treatment died by the end of the 8-week feeding period. No lesions were observed in the spleen, stomach, pyloric intestine, head kidney, or heart of fish in all treatments. These results indicate that acute and subchronic effects of AFB to Nile tilapia are unlikely if dietary concentrations are 0.25 mg/kg or less.

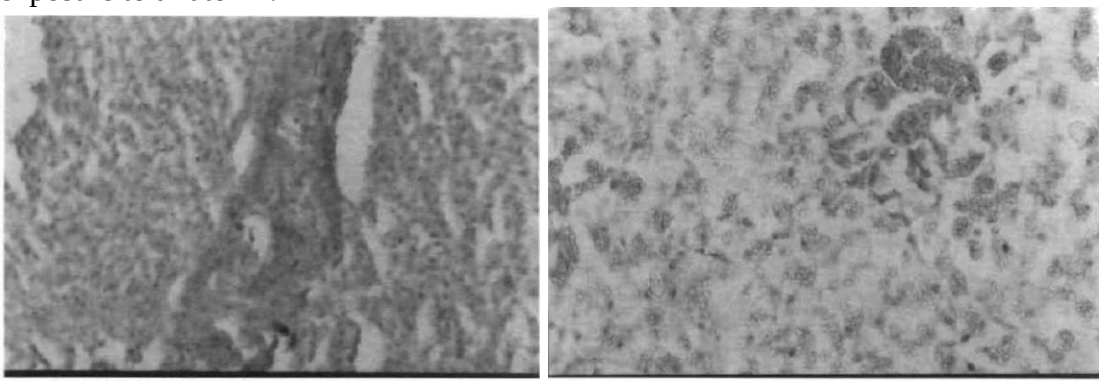


Normal control liver liver of tilapia fed 100 mg/kg AFB1 **Tuan *et al.* (2002)**

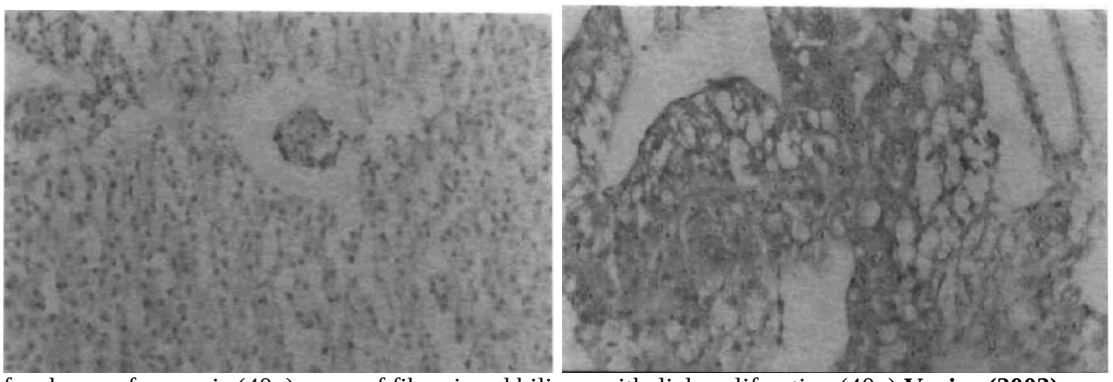
Bintvihok *et al.* (2003) collected 150 samples of shrimp feed from the eastern and southern regions of Thailand, and aflatoxins B1, B2, G1, and G2 (AFB1, AFB2, AFG1, and AFG2) in them were analyzed. AFB1 contamination ranged from a nondetectable level (< 0.003 ppb) to 0.651 ppb. Metabolites of AFB1 were less abundant than AFB1. To study the effects of aflatoxin in feed on shrimp production, black tiger shrimp were divided into four groups of 30 shrimp per group, tested in triplicate, and fed diets containing 0 (control), 5, 10, or 20 ppb of AFB1 for 10 consecutive days. After 7 or 10 days of consumption on each diet, the shrimp were weighed and sacrificed for laboratory examination. AFB1 and its metabolites were not detected in shrimp muscle. The mortality rate was slightly higher in the AFB1-treated groups than in the control group. The body weight of the surviving shrimp was decreased to 46 to 59% of the initial body weight in the AFB1-treated groups but not in the control group. Histopathological findings indicated hepatopancreatic damage by

AFB1 with biochemical changes of the hemolymph. These results show that aflatoxin contamination in shrimp feed may cause economic losses by lowering the production of shrimp. Feed contaminated at the level of 20 ppb or lower (i.e., at the observed natural contamination level) may pose a very low risk, if any, to human health.

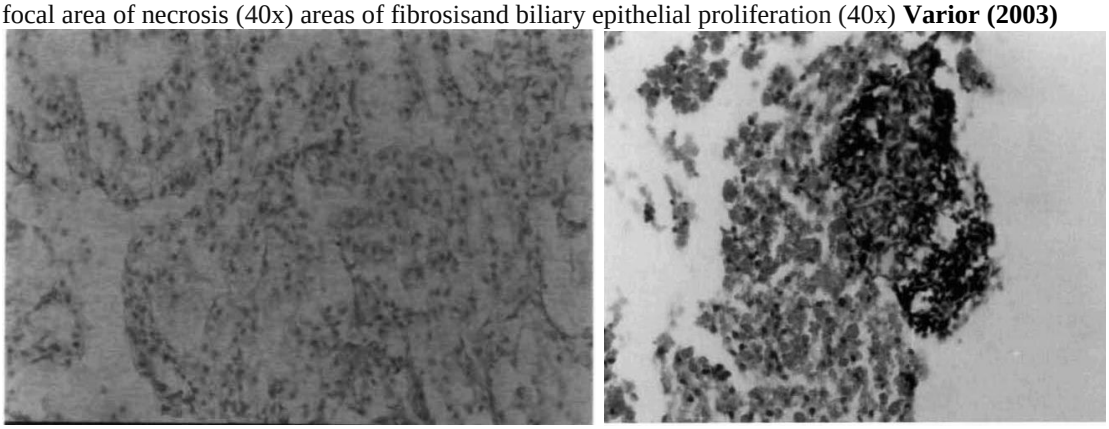
Varior (2003) performed a study to evaluate the changes induced by aflatoxin in the teleost, *Oreochromis mossambicus* through different approaches like biochemistry, histopathology and molecular biology. Diets supplemented with different levels of aflatoxin B1 (0.375ppm, 2.5 ppm and 6 ppm/kg body weight) were fed to the experimental groups for time periods of two weeks and six weeks. Fish fed on the diet containing the highest dose of aflatoxin namely 6 ppm refused to rake the experimental diet for the first few days but later on began to gradually accept the entire feed. The biochemical parameters namely alanine transaminase, aspartate transaminase, alkaline phosphatase and free amino acids were found to increase in the aflatoxin treated groups. The concentration of pyruvate in the liver and muscle were found to decrease after a six-week aflatoxin stress. The lipid peroxidation products namely conjugated dienes, hydroperoxide and malondialdehyde recorded an increase in level up to two weeks after which they registered a decrease in concentration up to six weeks. The antioxidant enzymes namely catalase, superoxide dismutase and glutathione reductase showed an increase in their levels with the duration of exposure, the rate being significantly high after six weeks. The antioxidant, glutathione recorded the same trend as above. The levels of cholesterol, triglycerides, LDL cholesterol and VLDL were found to increase in the aflatoxin exposed groups where as the levels of HDL cholesterol which recorded an initial increase was found to decrease in the highest dose namely 6 ppm. The haemoglobin and erythrocyte count values indicated a decrease in proportion to the aflatoxin exposure. The decrease in the packed cell volume was not significant. Histopathological damages to the liver and kidney tissues intensified with increase in concentration and duration. The histopathological changes observed in the liver were extensive to focal necrosis, biliary proliferation, loss of architecture and preneoplastic stage of the liver tissue after a six week exposure. Important changes observed in the kidney were severe necrosis of tubular epithelial cells, thickening of the bowmans capsule and shrinkage of the glomeruli. Levels of enzymes in serum namely aspartate transaminase, alanine transaminase, lactate dehydrogenase, acid phosphatase and alkaline phosphatase; and other parameters like blood urea, creatinine and glucose also corroborated the damaging effects of aflatoxin to the animal. Significant reduction in the concentration of serum protein, albumin and globulin were noticed with increase in concentration as well as duration of exposure to aflatoxin.



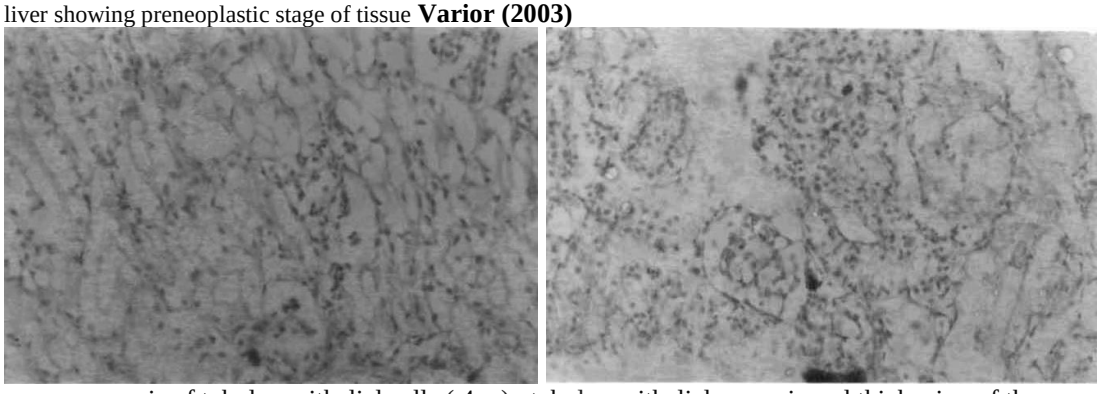
massive proliferation of biliary epitiiellum (4ox) loss of architecture and accumulation of ceroid pigments (4ox) **Varior (2003)**



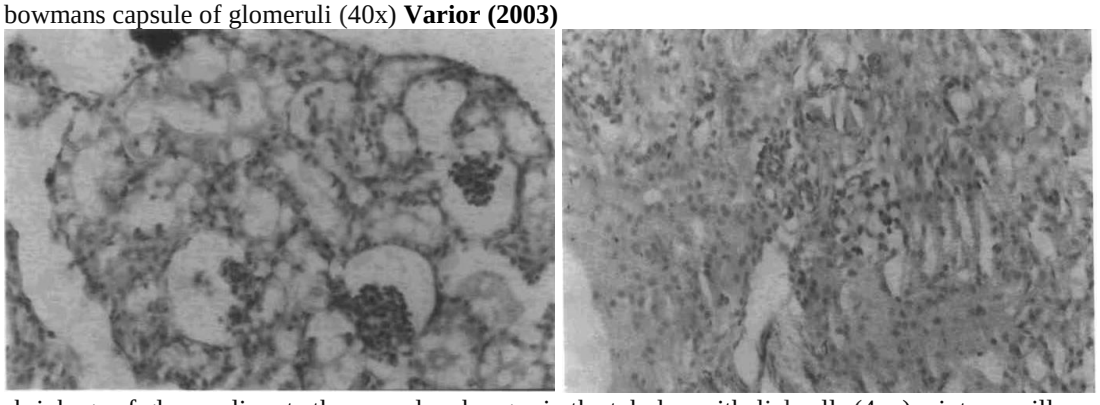
focal area of necrosis (40x) areas of fibrosisand biliary epithelial proliferation (40x) **Varior (2003)**



degeneratlve changes in the hepatocytes, loss of architecture, pyknotic nature of hepatic nuclei (40x) section of liver showing preneoplastic stage of tissue **Varior (2003)**



severe necrosis of tubular epithelial cells (4ox), tubular epithelial necrosis and thickening of the bowmans capsule of glomeruli (40x) **Varior (2003)**



shrinkage of glomeruli. note the vacuolar changes in the tubular epithelial cells (4ox)m inter capillary thickening of glomeruli and sclerotic changes in the glomeruli (4ox)

Cagauan *et al*. (2004) evaluated aflatoxin-contaminated feeds at different levels (good feed, 10% moldy feed, 50% moldy feed and 100% moldy feed) on the growth, survival and histology of liver of Nile tilapia (O. niloticus) reared under aquarium

conditions for 120 days. The aflatoxin content ranged from 0.05). However, percent survival of fingerlings was significantly influenced by aflatoxin.

Burgos-Hernadez *et al.* (2005) mentioned tha**t** the effect of AFB1 toxicity to shrimp resulted in the modification of digestive processes and abnormal development of the hepatopancreas due to exposure to mycotoxins. These effects might be due to alterations of trypsin and collagenase activities, among other factors, such as the possible adverse effect of these mycotoxins on other digestive enzymes (e.g. lipases and amylases).

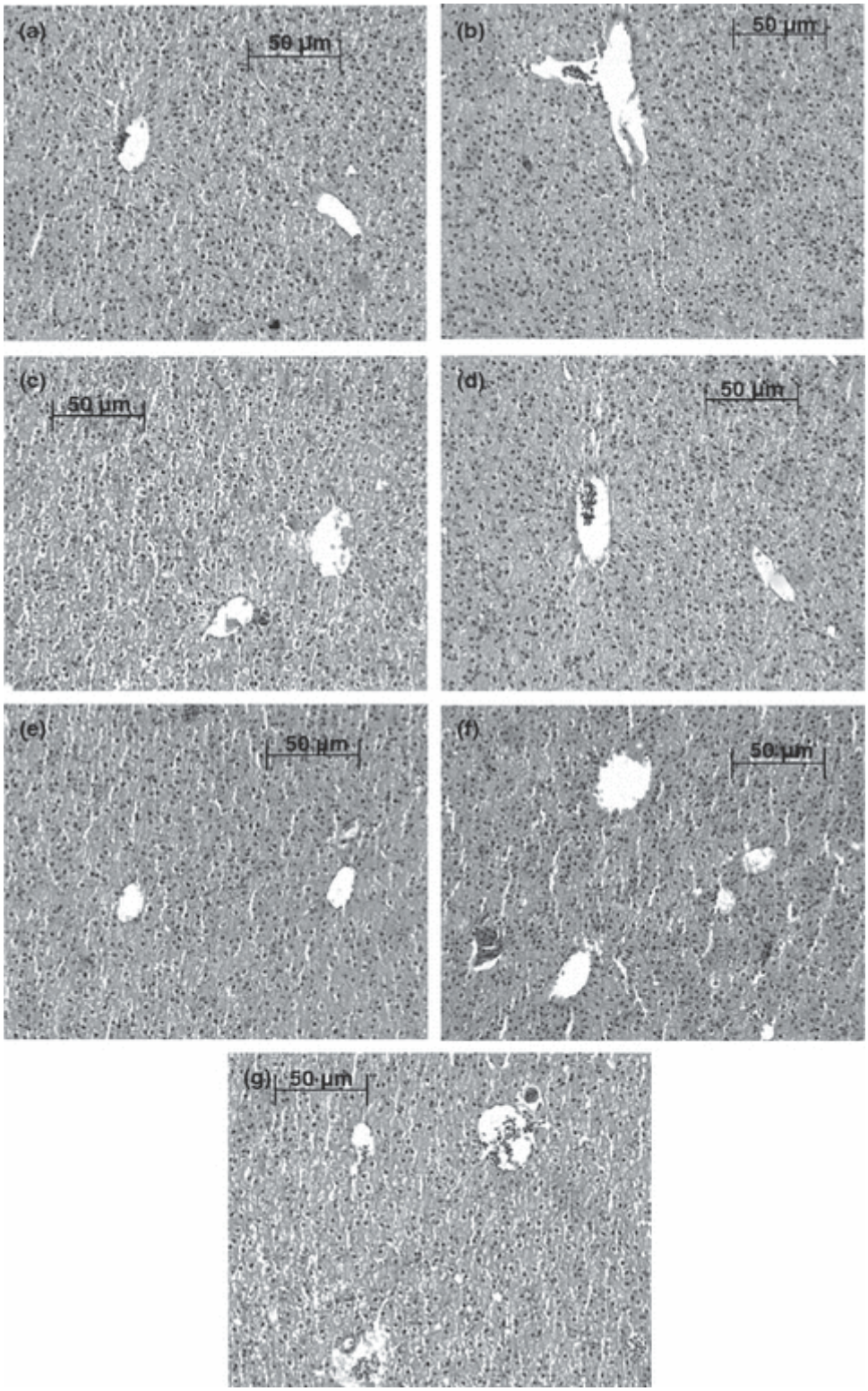
Manning *et al.* (2005) incorporated diets containing aflatoxin from a natural source, moldy corn (MC) naturally contaminated with a high concentration (550 pg/kg) of total aflatoxins into diets fed to Juvenile catfish in two experiments. Experiment 1 consisted of feeding catfish (mean body weight 7.1 g/fish) four diets containing 20% or 40% of two lots of corn; one with no apparent mold contamination, which was designated as clean corn (CC), or the previously described MC. Each diet was fed twice daily to five 100-L aquaria of 20 fish each for 12 wk. Experiment 2 consisted of three diets containing either 50% CC or MC, or a combination of 25% CC and 25% MC prepared by the cooker-extrusion method. Each diet was fed once daily for 130 d to five replicate 0.04-ha ponds of catfish fingerlings. Results of these experiments indicate that feeding diets containing aflatoxin from moldy corn does not affect channel catfish weight gain, feed consumption, feed efficiency, survival, hematocrit, or hepatosomatic ratio. No liver abnormalities were observed upon gross examination. Levels of aflatoxin were reduced approximately 63% in the diets used in experiment 2 after exposure to the high temperature (ca. 120 C) of the cooker-extrusion process used to manufacture commercial catfish diets.

Santacroce *et al.* (2008), in their review, gathered the currently available scientific information, summarised existing data on aflatoxin contamination on feeds and fishmeals, and toxicological effects induced in reared aquatic species; made a comparative analysis of AFB1 metabolism in the most representative species studied with the objective to gain new insights on the risk of DNA damage caused by aflatoxins on fish genomes and their role in cancer development

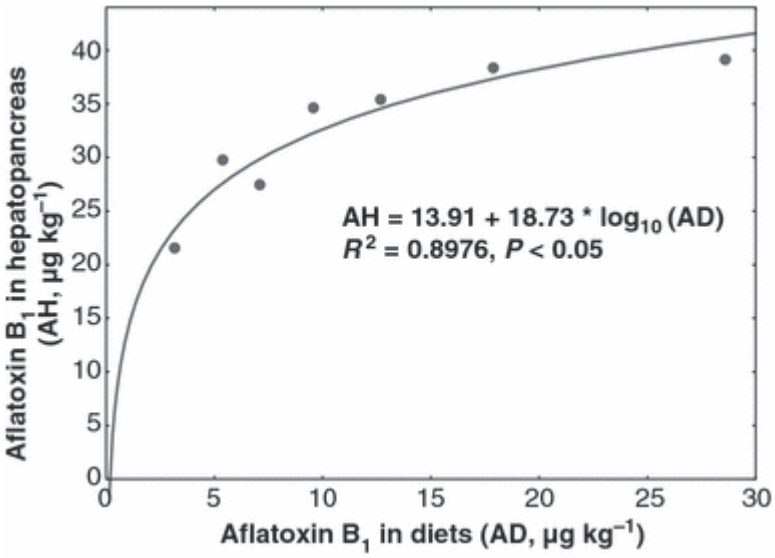
El-Sayed and Khalil (2009) assessed the susceptibility and toxicity of AFB(1) to sea bass (Dicentrarchus labrax L.) by behavioral and biochemical evaluations. The estimated oral acute median lethal concentration (96 h LC(50)) of AFB(1) for sea bass was 0.18 mg/kg bwt. The abnormal behavioral responses and signs of toxicity were described. The prolonged oral administration of 0.018 mg/kg bwt AFB(1) to sea bass for 42 successive days induced a significant increase in serum transaminases and alkaline phosphatase activities, and significant decrease in plasma proteins. Residual AFB(1) was detected at high levels (approximately 5 ppb) in fish musculature at the end of the experimental period. We conclude that marine water sea bass is a species highly sensitive to AFB(1). In addition, consumption of sea bass reared on AFB(1)-contaminated diet could have a negative health impact on human health.

Han *et al.* (2009) investigated the effects of aflatoxin B_1 (AFB_1) on growth, physiological responses and histological changes in juvenile gibel carp (*Carassius auratus gibelio*). Triplicate groups of gibel carp (3.53 ± 0.02 g) were fed seven semipurified diets (Diet 1 to 7) containing 3.20, 5.37, 7.08, 9.55, 12.70, 17.90 and 28.60 μg AFB_1 kg^{-1} diet for 3 months. The results showed fish weight gain fed Diet 6

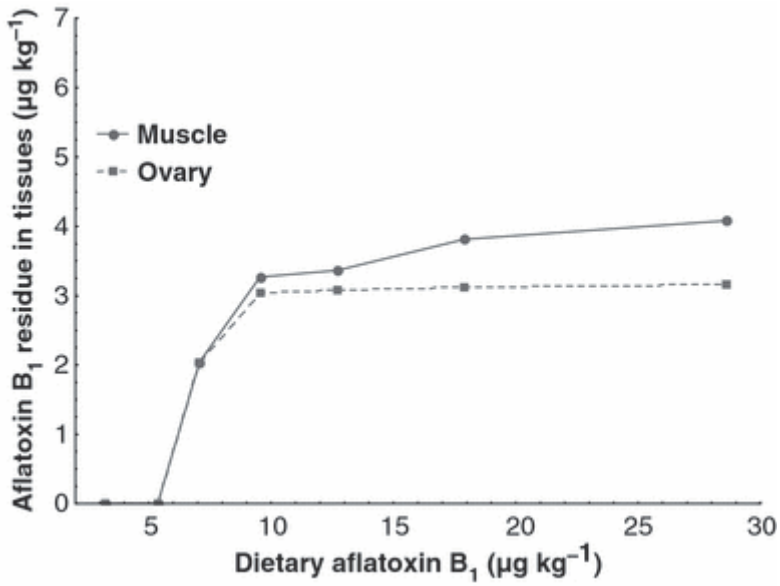
was 112.6% of that of control group (Diet 1) after 3 months, but there was no significant difference of weight gain between fish fed Diet 7 and the control group. Alanine aminotransferase (ALT) of fish hepatopancreas fed Diet 7 was significantly higher than the control group ($P < 0.05$), but no significant difference was observed in ALT activities of the fish fed with more than 10 μg AFB_1 kg^{-1} (Diet 4, 5, 6 and 7). No significant histological lesions were identified between the control and increasing AFB_1treatments. AFB_1 accumulated in hepatopancreas was logarithmically related to the dietary AFB_1levels, and AFB_1 also accumulated in muscles and ovaries of gibel carp fed Diet 3 to Diet 7. The present results indicated that fish fed with more than 10 μg AFB_1 kg^{-1} diet showed impaired physiological responses and more AFB_1 residue of muscles and ovaries above the safety limitation of European Union.



Hepatopancreas of gibel carp fed with (a) the control diet, (b) Diet 2, (c) Diet 3, (d) Diet 4, (e) Diet 5, (f) Diet 6 and (g) Diet 7 for 3 months. H&E, Bar = 50 µm. **Han *et al.* (2009)**



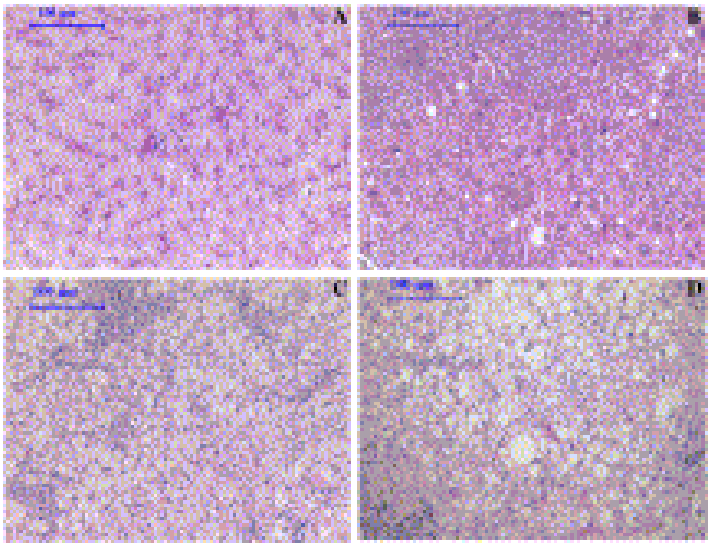
The relationship between AFB1 in hepatopancreas (AH, μg kg^{-1}) and in diets (AD, μg kg^{-1}). **Han *et al.* (2009)**



AFB1 residue in muscle and ovary of gibel carp fed with different dietary AFB1 for 3 months, and AFB1 residue in tissues of fish was not detected in the control group and Diet 2. **Han *et al.* (2009)**

Deng *et al.* (2010) investigated the toxic effects and residue of AFB1 in tilapia during a long-term trial of 20 weeks, during which the tilapia grew to a commercial size (around 500 g). Tilapia were fed six diets containing different levels of AFB1 (19, 85, 245, 638, 793 and 1641 μg/kg), which were prepared with AFB1-contaminated peanut meal. AFB1-related physiological and toxicological properties in fish were determined during the 20-week period. The results indicated that dietary AFB1 led to aflatoxicosis effects in tilapia in a dose- and duration-dependent manner. No toxic effects of AFB1 were found during the first 10 weeks, but by 20 weeks, the diet with 245 μg AFB1/kg or higher doses reduced the growth and induced hepatic disorder, resulting in decreased lipid content, hepatosomatic index, cytochrome P450 A1 activity, elevated plasma alanine aminotransferase activity and abnormal hepatic morphology, but such dietary AFB1 doses did not affect the survival rate of

experimental fish. The AFB1 residue was only detected in liver, in a dose-dependent manner, but not in edible flesh. Taken together, under good culture conditions, tilapia is a rather tolerant species for dietary AFB1 exposure up to 1641 μg/kg diet during 20 weeks. Long-term exposure for more than 15 weeks is necessary to evaluate aflatoxicosis in tilapia. Consuming only tilapia flesh would not increase the risk of exposure to AFB1 for human consumers.



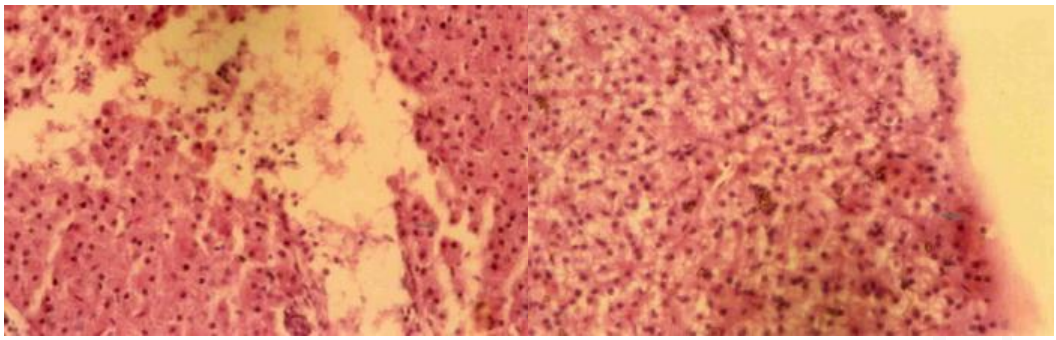
Hepatic histology of tilapia exposed to aflatoxin, **Deng *et al.* (2010)**

Almeida *et al.* (2011) peformed a preliminary study to evaluate fungi contamination and the presence of aflatoxins in 87 samples of feed for sea bass, collected in Portugal. Molds were found in 35 samples (40.2%) in levels ranging from 1 to 3.3 log10 $CFU \cdot g^{-1}$. Six genera of molds were found. *Aspergillus flavus* was the most frequent, found in all positive samples, with a range from 2 to 3.2 $\log_{10} CFU \cdot g^{-1}$. *Aspergillus niger* was found in 34 samples (39.1%), ranging from 1 to 2.7 $\log_{10}$ $CFU \cdot g^{-1}$. *Aspergillus glaucus* was found in 26 samples (29.9%) with levels between 1 and 2.4 $\log_{10}$ $CFU \cdot g^{-1}$. *Penicillium* spp. and *Cladosporium* spp. were both found in 25 samples (28.7%). *Fusarium* spp. was found in 22 samples (25.3%), ranging from 1 to 2.3 $\log_{10}$ $CFU \cdot g^{-1}$. All feed samples were screened for aflatoxins using a HPLC technique, with a detection limit of 1.0 $\mu g \cdot kg^{-1}$. All samples were aflatoxin negative

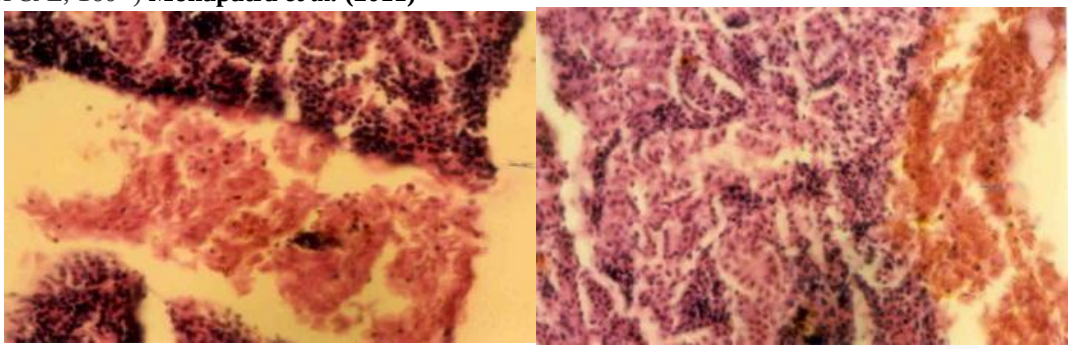
Manning *et al.* (2011) used Channel catfish, Ictalurus punctatus, fingerlings with an average body weight of 8.19±0.32 g to conduct an experiment to determine the effect of feeding diets containing aflatoxin on disease resistance. Twenty fish were randomly sorted into each of 48 80-L capacity flowing water aquaria. Eight replicate aquaria were assigned to each of six experimental diets that contained graded levels of aflatoxin. Graded levels of aflatoxin were obtained by blending calculated, weighed amounts of moldy corn with an aflatoxin concentration of 850 μg/kg with weighed amounts of clean corn with 0 μg/kg aflatoxin. Dietary levels of aflatoxin were 0, 10, 20, 40, 80, and 160 μg/kg. The catfish were fed weighed amounts of the experimental diets twice daily for 7 weeks, after which, fish in each aquarium were group weighed and counted. Fish were continued on assigned diets through week-10 when the immersion challenge with Edwardsiella ictaluri was implemented. Results show that mean body weight gains of catfish fed any of the aflatoxin diets were not significantly ($P > 0.05$) different than the mean weight gain of catfish fed the control diet. Also, cumulative 21-day post-challenge mortality of catfish fed the aflatoxin diets was not

significantly (P >0.05) different than that of the control diet catfish

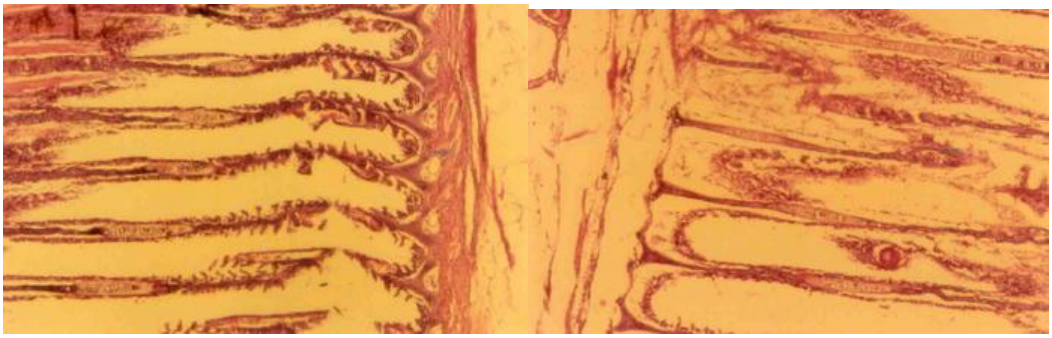
Mohapatra *et al.* (2011) conducted a feeding trial of 60 days for delineating the effect of dietary aflatoxin (AFB1) with or without supplementation of a mixture of mould inhibitor (0.25% clove oil + 0.32% sodium propionate) on haematology, respiratory burst activity and histology of Labeo rohita fingerlings. Three hundred and sixty fishes (avg. wt. 1.48-1.54 g) were randomly distributed into eight treatment groups. Eight experimental diets with four different levels of aflatoxin (0, 10, 20 and 40 ppb) with or without mould inhibitor were prepared. Haematological parameters like total serum protein, albumin, globulin and A:G ratio were significantly (P < 0.05) reduced with increasing levels of aflatoxin in the diet. However, supplementation of mould inhibitor showed enhanced values when compared to non-supplemented counter parts suggesting ameliorating effects of mould inhibitor on aflatoxin. Total leucocyte count was higher in aflatoxin-treated groups. Histological observations were complementary to haematological parameters. Respiratory burst activity was significantly (P < 0.05) decreased in higher aflatoxin-treated groups but not affected significantly (P > 0.05) due to inclusion of inhibitor alone and/or interaction of aflatoxin level and inhibitor in the diet. From this study, it was concluded that up to 20 ppb aflatoxin level in the diet the haemato-immunological parameters are protected.



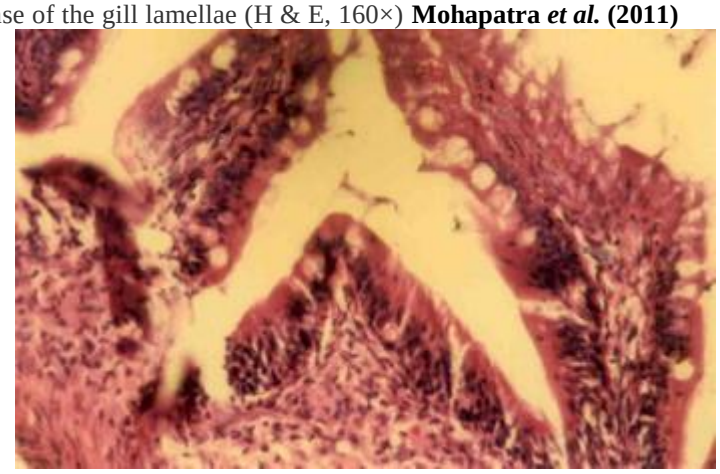
Liver of *Labeo rohita* fingerlings fed with T4 diet showing mild oedema (H & E, 160×) Liver of *Labeo rohita* fingerlings fed with T8 diet showing swollen hepatocytes and constricted sinusoids (H & E, 160×) **Mohapatra *et al.* (2011)**



Kidney of *Labeo rohita* fingerlings fed with T3 diet showing mild haemorrhages (H & E, 160×) Kidney of *Labeo rohita* fingerlings fed with T4 diet showing wide spread haemorrhages. Accumulation of homogenous mass was also evident inside the tubular lumen (H & E, 160×) **Mohapatra *et al.* (2011)**



Gill of *Labeo rohita* fingerlings fed with T3 diet showing degeneration of secondary filaments (H & E, 160×) Gill of *Labeo rohita* fingerlings fed with T4 diet showing massive loss of secondary filament at the base of the gill lamellae (H & E, 160×) **Mohapatra *et al.* (2011)**

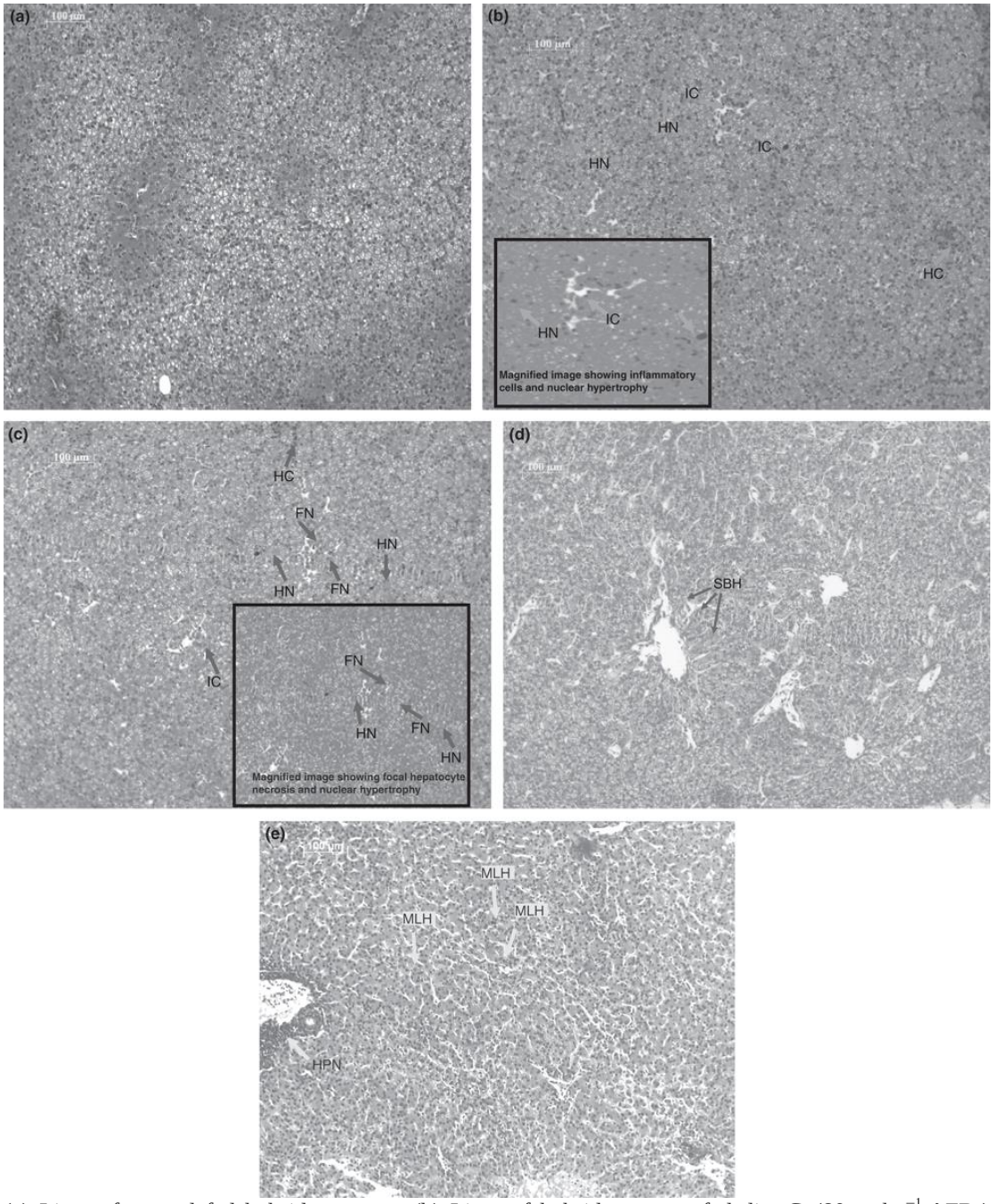


Intestine of *Labeo rohita* fingerlings fed with T7 diet showing activated goblet cells (H & E, 160×) **Mohapatra *et al.* (2011)**

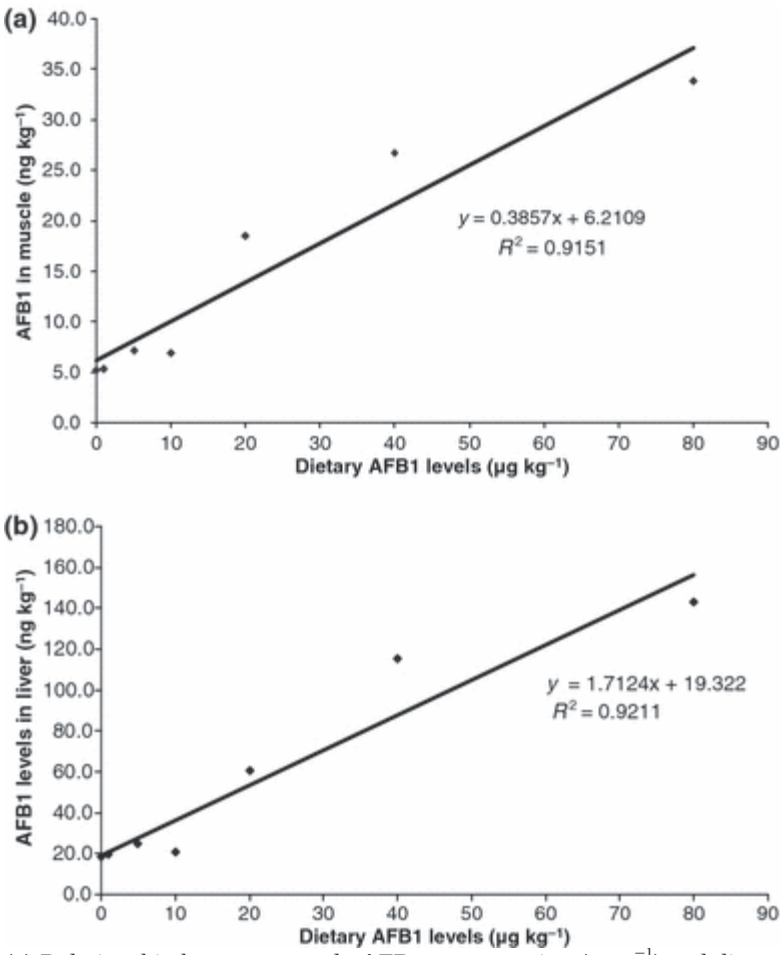
Nomura *et al.* (2011) studied the uptake and elimination of aflatoxins (AFs) by rainbow trout (*Oncorhynchus mykiss*) during a long-term (21 days) dietary exposure to assess contamination by AFs in aquaculture fish fed AF-containing feed. The uptake factor (UF) of aflatoxin B_1 (AFB_1) in muscle ranged from 0.40×10^{-3} to 1.30×10^{-3}. AFB_1 concentrations in liver were 165–342 times higher than in muscle. AFs from feed were more highly accumulated in liver than in muscle. Aflatoxicol (AFL) and aflatoxin M_1 (AFM_1) were detected in muscle and liver and also in the rearing water. AFL concentrations were higher than AFM_1 by 2 orders of magnitude in muscle, and AFL was a major metabolite of AFB_1. The elimination rate constants (α) of AFB_1 and AFL in muscle (1.83 and 2.02 day^{-1}, respectively) and liver (1.38 and 2.41 day^{-1}, respectively) were very large. The elimination half-life ($t_{1/2}$) of AFB_1 was 0.38 days (9.12 h) in muscle and 0.50 days (12.00 h) in liver. The elimination half-life of AFL in muscle and liver was 0.34 day (8.16 h) and 0.29 day (6.96 h), respectively. These data show that AFs are eliminated rapidly and are not biomagnified in fish. Thus, AFB_1 concentration in muscle of fish fed AFB_1-containing feed (ca. 500 μg/kg) decreased to below the detection limit (20 ng/kg) of the most sensitive analytical method at 1.54 days (36.96 h) after the change to uncontaminated feed.

Rajeev-Raghavan *et al.* (2011) investigated toxicity of aflatoxin B_1 (AFB_1) in juvenile hybrid sturgeon *Acipenser ruthenus* ♂ × *A. baeri*♀, an important coldwater finfish farmed in China and other countries. Seven experimental diets (Diet A–G) containing different levels of AFB_1 (0, 1, 5, 10, 20, 40 and 80 μg kg^{-1} diet) were fed to juvenile sturgeon weighing 10.53 ± 0.17 g kg^{-1} to determine its effect on survival, growth, feed consumption, hematocrit, liver histology as well as muscular and hepatic

toxin accumulation. The experiment lasted for 35 days and was conducted in two periods of 25 and 10 days each. No external changes or unusual behaviour was observed in the fish fed diets with AFB_1. Mortality was observed in fish fed with highest levels of AFB_1 (80 μg kg^{-1}– Diet G) from day 12 onwards. After 25 days, fish fed the diet of 80 μg AFB_1 kg^{-1} showed significant lower survival (50 ± 5.77%) followed by those fed 40 μg AFB_1 kg^{-1} diet (80 ± 5.77%) and 20 μg AFB_1 kg^{-1} diet (86.66 ± 3.33%). No significant difference was observed in specific growth rate (SGR) or hepatosomatic index (HSI) between groups. Hematocrit was significantly higher in the fish fed the diet of highest AFB_1. The fish were weighed at day 25 in some treatments (Diets F and G) because of high mortality. However, feeding was continued for another 10 days to observe mortality or behavioural changes if any in the other groups. After 35 days, survival in the fish fed Diet F (40 μg AFB_1 kg^{-1}) was 40% and those fed Diet E (20 μg AFB_1 kg^{-1}) was 36.2%. Significant histopathological changes including nuclear hypertrophy, hyperchromasia, extensive biliary hyperplasia, focal hepatocyte necrosis and presence of inflammatory cells were observed in the liver of fish fed high levels of aflatoxin (40 and 80 μg kg^{-1}). AFB_1 accumulation in fish muscle and liver increased with increased dietary AFB_1 levels. It could be confirmed that 10 μg AFB_1 kg^{-1} diet was the maximum allowable level in hybrid sturgeon diet.



(a) Liver of control fed hybrid sturgeon. (b) Liver of hybrid sturgeon fed diet G (80 µg kg^{-1} AFB_1) showing hypertrophy (HN) and hyperchromasia (HC) of nuclei and cytoplasmic vacuoles and also the presence of inflammatory cells (IC). (c) Liver of hybrid sturgeon fed diet F (40 µg $kg^{-1}$$AFB_1$) showing hypertrophy (HN) and hyperchromasia (HC) of nuclei and cytoplasmic vacuoles presence of inflammatory cells (IC) and focal hepatocyte necrosis (FN). (d) Liver of hybrid sturgeon fed diet G (80 µg kg^{-1} AFB_1) showing severe biliary hyperplasia (SBH). (e) Liver of hybrid sturgeon fed diet G (80 µg kg^{-1} AFB_1) showing basophilic hepatocytes with hyperchromatic and pleiomorphic nuclei (HPN) and occasional multinucleated large hepatocytes (MLH) indicating a progress towards hepatoma. **Rajeev-Raghavan *et al.* (2011)**

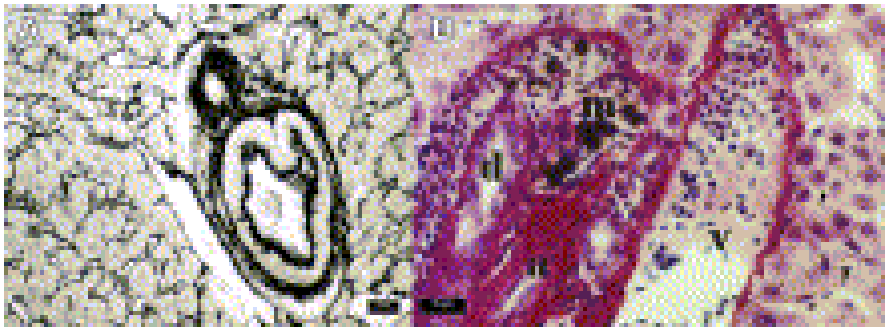


(a) Relationship between muscle AFB_1 concentration (ng g^{-1}) and dietary AFB_1 concentration (µg kg^{-1}) of hybrid sturgeon after 25 days of exposure. (b) Relationship between liver AFB_1 concentration (ng g^{-1}) and dietary AFB_1concentration (µg kg^{-1}) of hybrid sturgeon after 25 days of exposure. **Rajeev-Raghavan *et al.* (2011)**

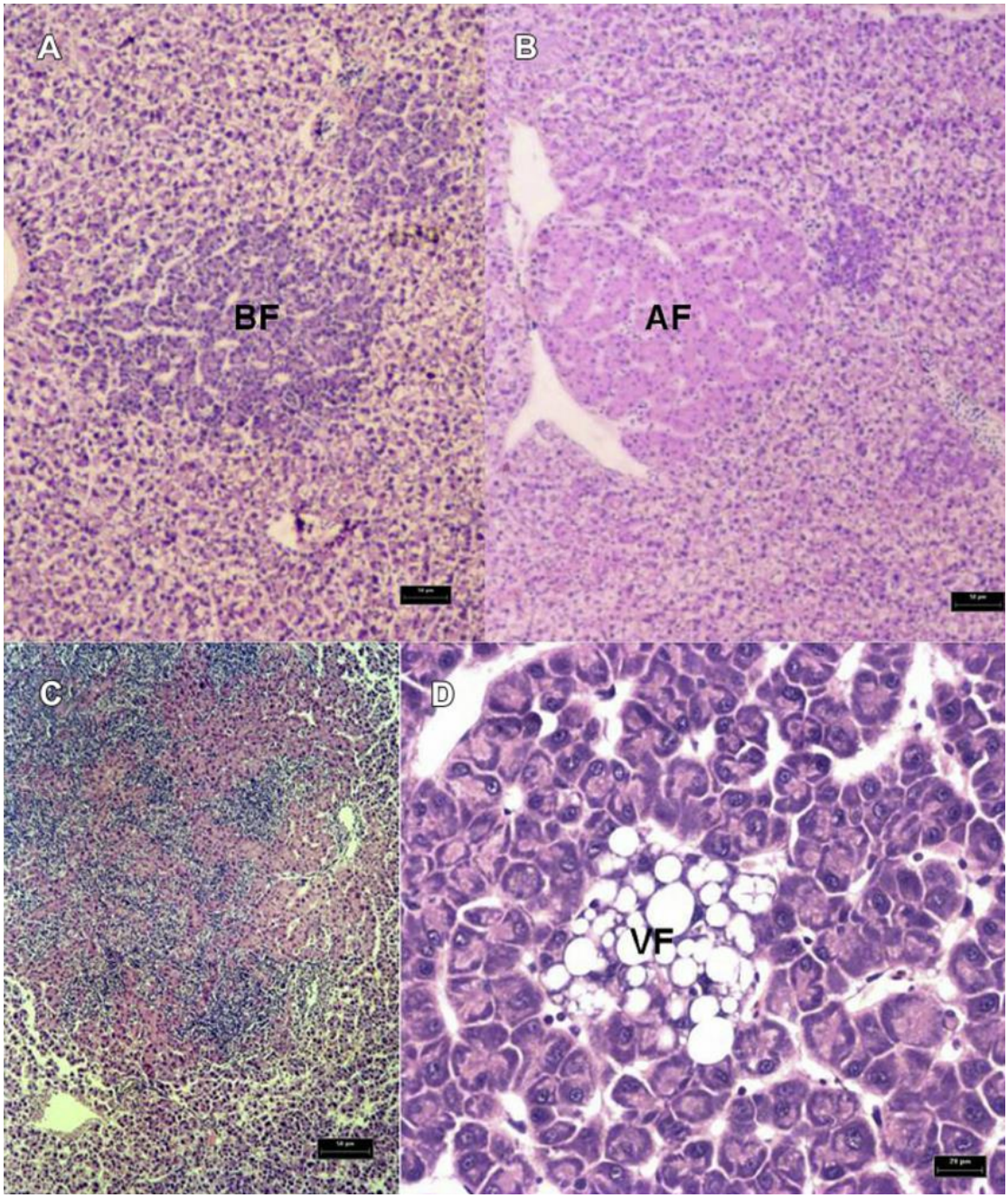
Zychowski *et al.* (2013) designed a study to: (1) evaluate AFB_1 impact on cultured red drum, *Sciaenops ocellatus*, over the course of seven weeks; and (2) assess NS supplementation as a strategy to prevent aflatoxicosis. Fish were fed diets containing 0, 0.1, 0.25, 0.5, 1, 2, 3, or 5 ppm AFB_1. Two additional treatment groups were fed either 5 ppm AFB_1 + 1% NS or 5 ppm AFB_1 + 2% NS. Aflatoxin B_1 negatively impacted red drum weight gain, survival, feed efficiency, serum lysozyme concentration, hepatosomatic index (HSI), whole-body lipid levels, liver histopathological scoring, as well as trypsin inhibition. NovaSil inclusion in AFB_1-contaminated diets improved weight gain, feed efficiency, serum lysozyme concentration, muscle somatic index, and intraperitoneal fat ratios compared to AFB_1-treated fish. Although not significant, NS reduced AFB_1-induced histopathological changes in the liver and decreased Proliferating Cell Nuclear Antigen (PCNA) staining. Importantly, NS supplementation improved overall health of AFB_1-exposed red drum

Arana *et al.* (2014) investigated the involvement of myofibroblast-like cells in rainbow trout (*Oncorhynchus mykiss*) with hepatic damage induced by aflatoxin B1 (AFB1). Histopathological and immunohistochemical analyses characterized alterations in the liver stroma during the carcinogenic process. Anti-human α-smooth muscle actin (SMA) and anti-human desmin primary antibodies were used in immunohistochemistry. Only the anti-SMA reagent labelled cells in trout liver. In the

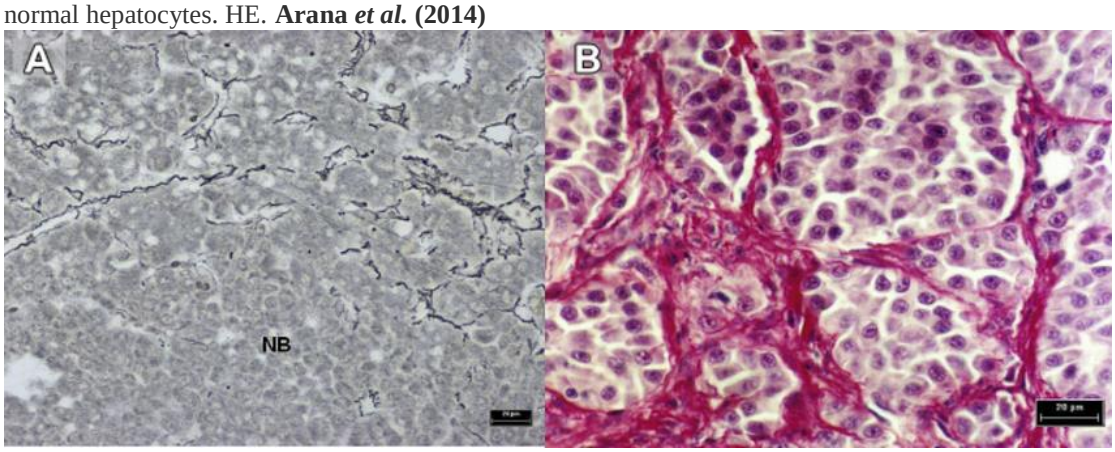
livers of control fish, only smooth muscle in blood vessels and around bile ducts was labelled. In the livers from AFB1-treated fish, SMA-positive cells were present in the stroma surrounding neoplastic lesions and in areas of desmoplastic reaction. These observations indicate that in teleosts, as in mammals, the myofibroblast-like cell is involved in fibrosis associated with liver injury. Chronic liver injury induced in trout by aflatoxin may provide a useful model system for study of the evolution of such mechanisms.



Liver from fish in the CG stained by (A) reticulin and (B) Sirius red. Reticulin fibres (arrowhead) surround tubules of hepatocytes and sinusoids. Dense connective tissue is distributed around blood vessels (a, artery; v, vein) and bile ducts (d); melanomacrophages are also observed in this connective tissue (m). **Arana *et al.* (2014)**



Altered foci in liver of trout treated with AFB1. (A) BF in the liver parenchyma of a trout treated for 4 months. (B) AF near a vein; a small BF can also be seen. (C) An AF infiltrated by leucocytes. There is an area of cell degeneration around the lesion at the bottom of the image. (D) A VF surrounded by normal hepatocytes. HE. **Arana *et al.* (2014)**



Connective tissue distribution in the liver of treated fish in sections stained by (A) reticulin and (B) Sirius red. (A) Reticulin fibres are scarce inside a nodule of basophilic cells (NB) in comparison with the adjacent parenchyma. (B) An HCC arranged in micronodules surrounded by fibrous septa where more prominent connective tissue can be seen. **Arana *et al.* (2014)**

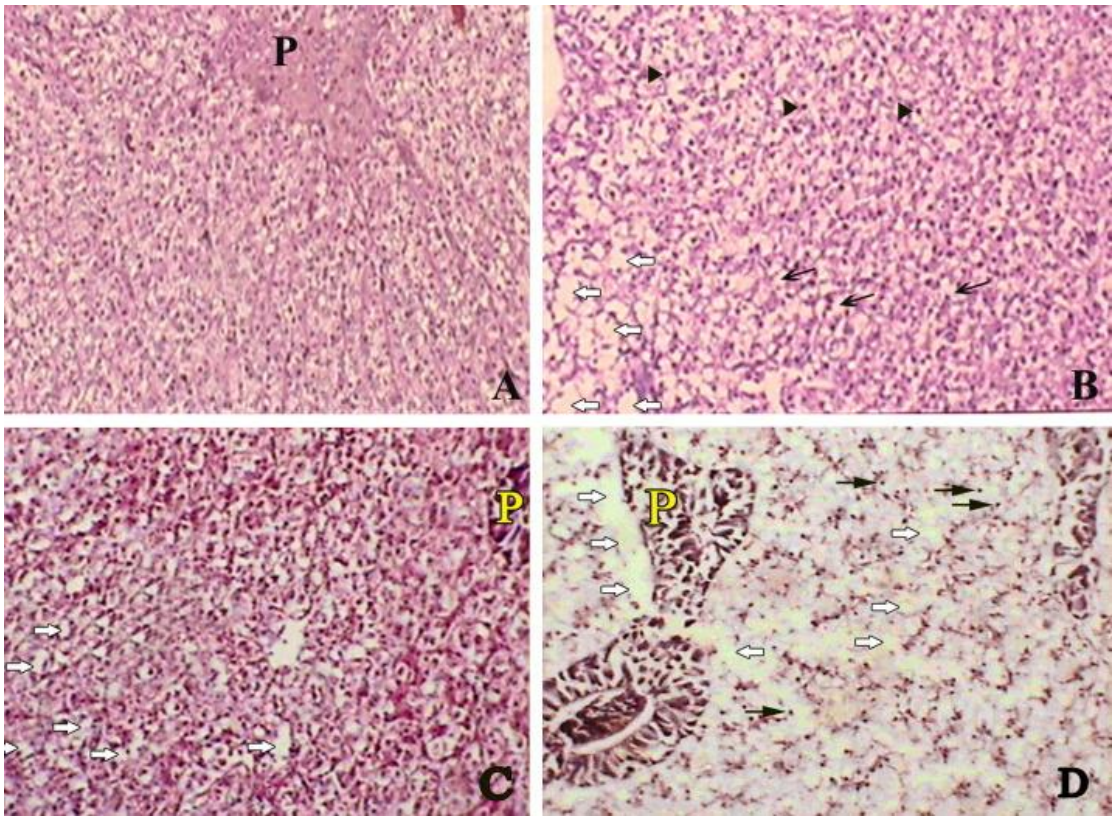
Mohebbi *et al.* (2014) determined aflatoxins content in tissues and diets of white shrimp collected from farms of Helleh, Delvar, Mond, Bandar Rig sites, located in Bushehr province by isocratic reverse-phase liquid chromatography (HPLC). Results showed that the highest content of aflatoxin (4.12±0.14 μg kg−1) was obtained from the food that had been used in Mond shrimp farm among all the examined farms. Although there were negligible differences between all the groups, significant difference were found between AFG1 group and the other groups (P< LOD), 0.01 and 0.03, respectively, while they were 0.057, 0.112, 0.278, and 0.745 ppb for their corresponding diets. In conclusion, continuous aflatoxin measurement of foods is suggested to prevent the contamination of shrimp farms in Bushehr province.

Selim *et al.* (2014) conducted a study to assess the efficacies of three adsorbents, a hydrated sodium calcium aluminosilicates (HSCAS), *Saccharomyces cerevisiae* (*S.C.*) and an esterified glucomannan (EGM), against feed contaminated with contained 200 μg/kg (ppb) aflatoxin B_1 (AFB_1). A total of 240 Nile tilapia fingerlings, *Oreochromis niloticus* (15 ± 2 g), were randomly divided into eight experimental groups (30 fish per group) with three replicates. Group T_1 represented the negative control fed on a basal diet, and T_2 was the positive control group fed on a basal diet supplemented with 200 ppb AFB_1. Groups T_3, T_4 and T_5 were fed the AFB_1-contaminated diet (200 ppb) supplemented with 0.5 % HSCAS, 0.25 % *S.C* or 0.25 % EGM, respectively. Groups T_6, T_7 and T_8 were fed a basal diet supplemented with 0.5 % HSCAS, 0.25 % *S.C* or 0.25 % EGM, respectively. The reduction in AFB_1-bioavailability was judged by toxin residues in fish musculature throughout the study beginning at the second week of exposure. AFB_1 reduced the survivability, total weight gain, average daily gain and specific growth rate, evident as early as the second week of exposure. The total erythrocyte count, hemoglobin content and total leukocyte count were significantly decreased after AFB_1 exposure for 6, 8 and 10 weeks, respectively. Prolonged administration of AFB_1 led to significant increases in serum alanine transaminase, aspartate transaminase and creatinine activity, and produced significant decreases in plasma proteins, including serum

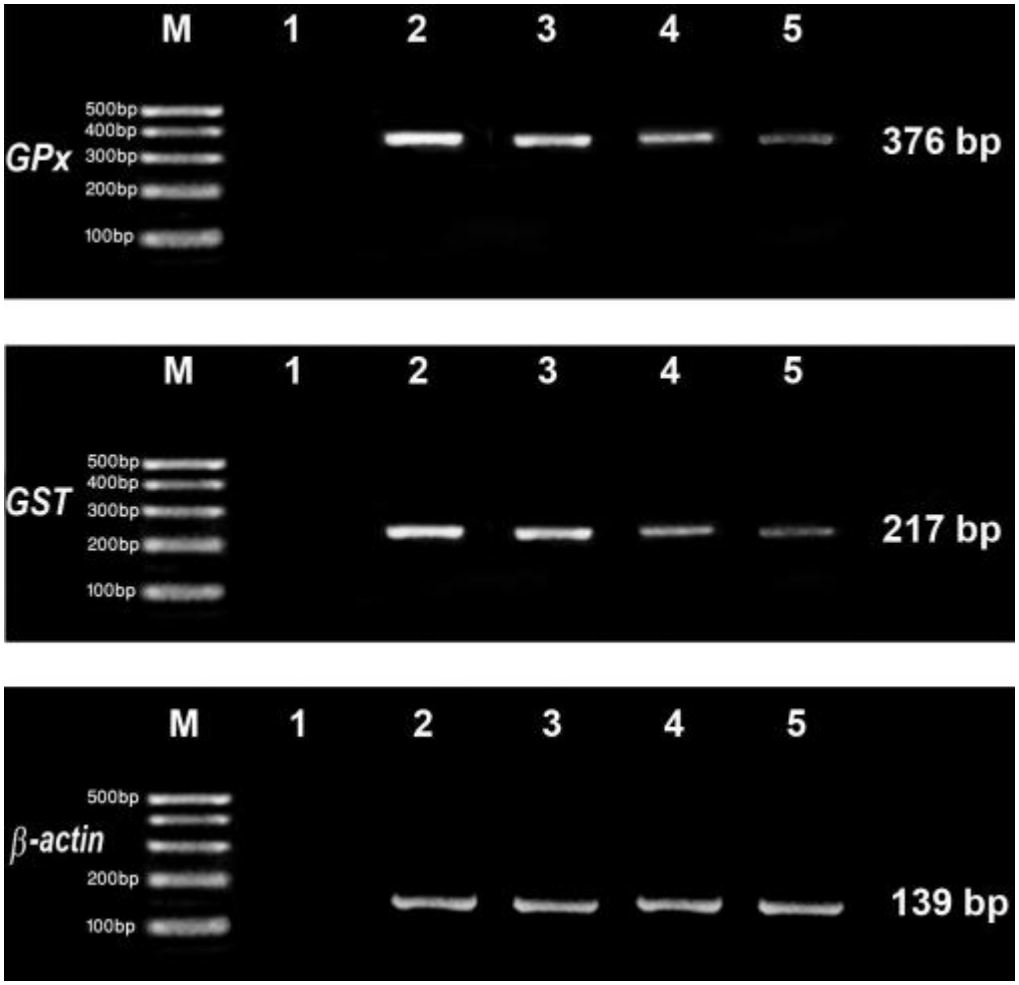
globulin. The specific immune response was assessed by an agglutinating antibody titer after immunization of the fish with an *Aeromonas hydrophila* vaccine. The antibody titer and relative level of protection of fish challenged with *Aeromonas hydrophila* were reduced throughout the period of examination in AFB_1-exposed fish. Supplementation with HSCAS, *S.C.* or EGM significantly improved growth performance, blood parameters and immune status; in addition, these groups showed decreased AFB_1residues in fish musculature when compared with AFB_1-treated fish. HSCAS effectively reduced AFB_1 toxicity, whereas *S.C.* and EGM were less efficacious.

Gonçalves-Nunes *et al.* (2015) carried out a study to determine aflatoxin B1 contamination from raw materials and finished feed intended for fish farm localized in Piaui, Brazil. *Aspergillus flavus* and *P. citrinum* were isolated with a high relative density from all samples. In general, a high percent of samples exceeded the levels proposed as feed hygienic quality limits (CFU g-1) according to Good Manufacture Practice. Aflatoxin B1 was analyzed by enzyme-linked immunosorbent assay. All raw materials and finished feed showed aflatoxin B1 levels. Although in this study AFB1 levels below recommended limits (20 μg kg-1) were found, it is important to emphasize the feed intake with toxin in low concentrations along time, since it produces chronic deleterious effects in animal production. This fact requires periodic monitoring to prevent the occurrence of chronic aflatoxicosis in aquaculture, to reduce the economic losses and to minimize hazards to animal health.

Mahfouz and Sherif (2015) evaluated adverse effects of aflatoxin B1 (AFB1) toxicity on health status in the Nile tilapia *Oreochromis niloticus*. Fsh were fed diet contaminated with either 20 or 100 ppb AFB1 for 6 or 12 weeks. Growth indices, survival rate and hepatosomatic index (HSI) were assessed. Blood samples were collected for hematological profiles (e.g. RBCs and WBC count, Hb content). Liver enzyme activity; aspartate aminotransferase (AST), alanine aminotransferase (ALT) as well as alkaline phosphatase (ALP), were evaluated and toxin residues in the liver and musculature were detected. Liver histopathological investigations were carried out, whereas antioxidant glutathione peroxidase (GPx) and glutathione S-transferase (GST) gene expression were determined in this tissue by semi-quantitative RT-PCR. Furthermore, to test the fish immune status, challenge against *Aeromonas hydrophila* was conducted. Results indicated that 100 ppb AFB1 negatively impacted *O. niloticus* weight gain, feed efficiency, hematological profiles, HSI as well as liver histopathology, while increase in AST, ALT, ALP liver enzymes activity was evidenced. Further, the expression of liver *GPx* and *GST*down-regulated and AFB1 residues were always detected in the liver and only in the musculature in fish fed 100 ppb AFB1 for 12 weeks. The ability of fish to withstand *A. hydrophila* infection was remarkably lowered. Overall, the results herein demonstrate the toxic effects of AFB1 in *O. niloticus*.



Photomicrographs of transverse sections of *O. niloticus* liver for fish fed AFB1, stained with hematoxylin and eosin, P indicates pancreatic tissue scattered throughout the liver (×100). (A) Apparently normal liver of fish exposed to 20 ppb AFB1 for 6 weeks. (B) Fish exposed to 20 ppb AFB1 for 12 weeks with mild hepatocytes vacuolation (black arrows), pyknosis (arrows heads) and moderate fatty changes of hepatocytes (white arrows). (C) Fish exposed to 100 ppb AFB1 for 6 weeks showing pronounced fatty changes of hepatocytes (white arrows). (D) Fish exposed to 100 ppb AFB1 for 12 weeks presenting severe vacuolation (white arrows) and pyknosis (black arrows), indicating liver degeneration **Mahfouz and Sherif (2015)**



***GPx*, *GST* and *β-actin* mRNA expression in *O. niloticus* fed AFB1 contaminated diet. Electrophoresis of RT-PCR products of gene mRNA was performed in ethidium bromide-stained agarose gel (1.5%). Shown are amplicons: M, 100-bp marker; 1, −ve control; 2, 6 w-20 ppb; 3, 12 w-20 ppb; 4, 6 w-100 ppb; 5, 12 w-100 ppb. Mahfouz and Sherif (2015)**

Samuel and Odunigba (2015) investigated storage fungi and aflatoxin in fish feed stored under three different storage conditions. Storage fungi were isolated and identified using direct isolation technique; detection and identification of aflatoxins using the High Performance Liquid Chromatography and Proximate analysis of the stored feed were also carried out. Four types of Aflatoxins (G1, G2, B1 & B2) were identified in the stored feed.

Table 2: Aflatoxins Concentration in the Stored Fish Feed

Parameters	Container label B (ug/ml)				Container label C (ug/ml)			
	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2
y	1.774	5.505	25.571	3.212	7.171	7.171	25.743	27.745
m	31.21	38.63	10.82	20.03	31.21	38.63	10.82	20.03
c	12.46	4.368	12.77	2.97	12.46	4.368	12.77	2.97
X =y-c/m	0.342	0.029	1.183	0.012	0.170	0.072	1.199	1.237

*Where **y** = mean peak area, **m** = slope, **c** = intercept and **x** = aflatoxin concentration.

Wang *et al.* (2016) evaluated the response of yellow catfish *(Pelteobagrus*

fulvidraco) to increasing concentrations of AFB1 and test the protective effect of dietary supplementation with a bentonite (dioctahedral montmorillonite) based AFB1 binder. Triplicate groups of yellow catfish with an average weight of 2.0 ± 0.1 g were fed diets containing 0, 200, 500, and 1,000 μg/kg of AFB1 alone, or diets containing 0, 200, 500, and 1,000 μg/kg of AFB1 along with 2 g/kg AFB1 binder, for 12 weeks. Results showed that diets containing increasing amounts of AFB1 had a significantly lower (P = 0.002) survival rate. There was a statistical significant reduction in weight gain, final body weight, and specific growth rate, and an increase in feed conversion ratio (FCR) influenced by the levels of AFB1 in the diet (P < 0.001 for all parameters), as well as increasing protection due to the presence of the binder (P = 0.046, *P* = 0.014, *P* = 0.038, and *P* = 0.485, respectively). The immunosuppressive nature of AFB1 in yellow catfish diets was confirmed through observation of lower bactericidal activity *(P* = 0.001), lower lysozyme activity (P = 0.006), reduced total protein (P = 0.002), and enhanced albumin/globulin ratio (P = 0.004). Fish fed diets contaminated with AFB1 and supplemented with the AFB1 binder showed better improvement in FCR (P = 0.019). These results indicated that AFB1 has a negative impact on yellow catfish growth and survival rate. The AFB1 binder protected fish from the toxic effects of AFB1.

2. Sterigmatocystin

- Sterigmatocystin (STC) is closely related to aflatoxin as a precursor in aflatoxin biosynthesis and classified as an IARC Group-2B carcinogen.
- STC naturally contaminates grains and feeds.
- STC is a hepato- carcinogenic mycotoxin produced by aspergilli and penicillia species.
- STC-contaminated diets caused gradual decrease in growth rate as well as in muscular protein content and gradual increase in mortality, serum transaminases activity and muscular dry matter and ether extract contents in addition to some pathological findings in carp in proportion to the dietary levels of STC.
- The LD_{50} of STC was estimated to be as 211 ppb STC in carp diet.
- Three months feeding of catfish on STC (250 ppb) led to loss of body weight, increased mortality rate and muscular contents of ether extract, decrease of muscular content of protein as well as to some pathological findings in addition to the presence of residual STC in the fish muscles.

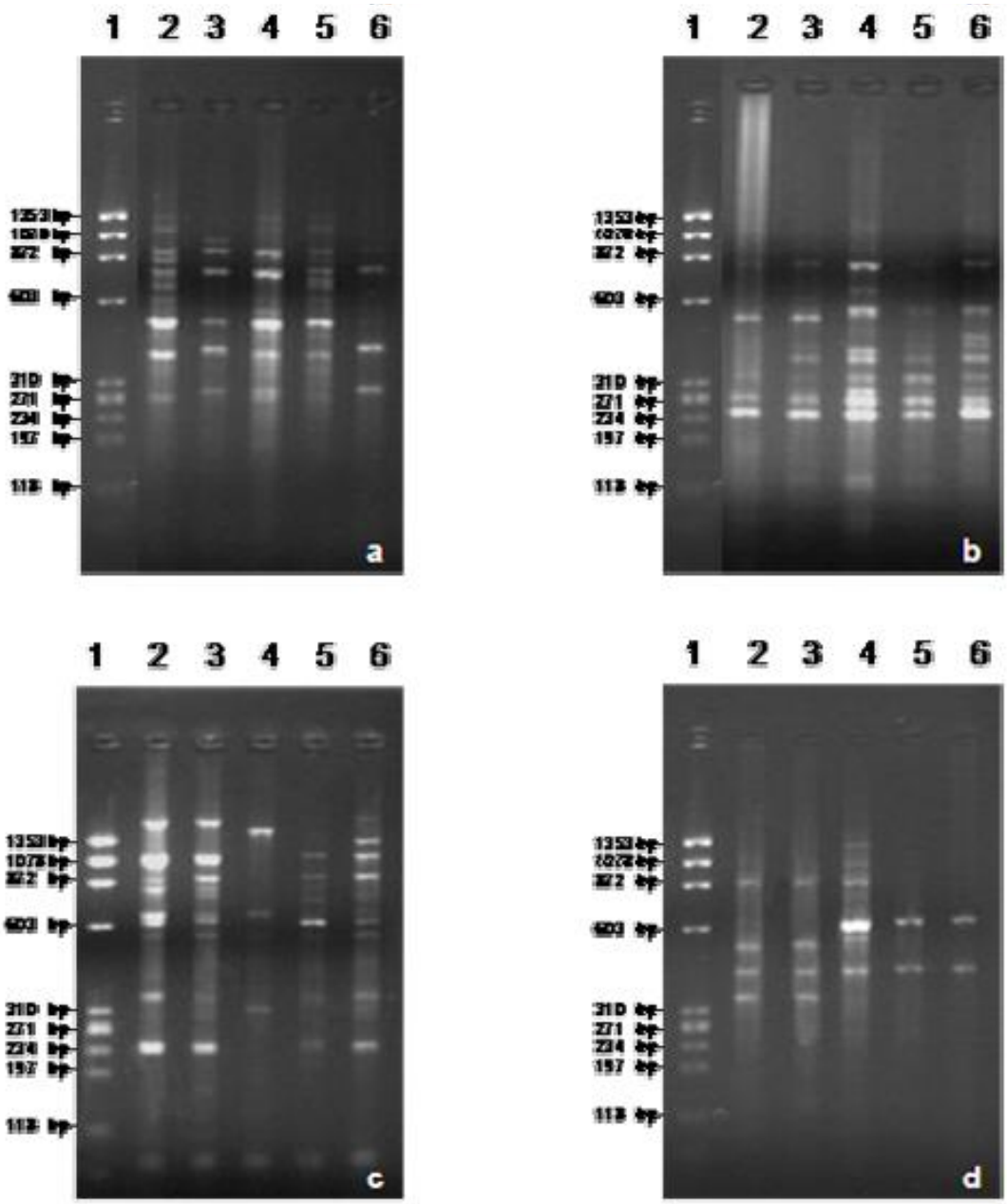
Reports:

Abdelhamid (1988) added sterigmatocystin (Stg) in 2 experiments in the fish feed. On 1st attempt carp seedlings received (Cyprinus carpio) for 3 weeks graded doses of the mycotoxin (0, 10, 50, 250 and 1 250 myg Stg / kg dry feed). The contaminated diets caused - besides some pathologies - a gradual decrease in the growth rate and the muscle protein content and a gradual increase in mortality, the Activity of serum transaminases and muscular Tr Subst.- and fat content. In the 2nd experiment with catfish (catfish, Clarias lezera), both in the control groups (without SN) as well as 250 micrograms SN / kg mixed feed. The contaminated diets led to a loss of body weight, an increase the muscular fat content and decrease in crude protein and asch content,

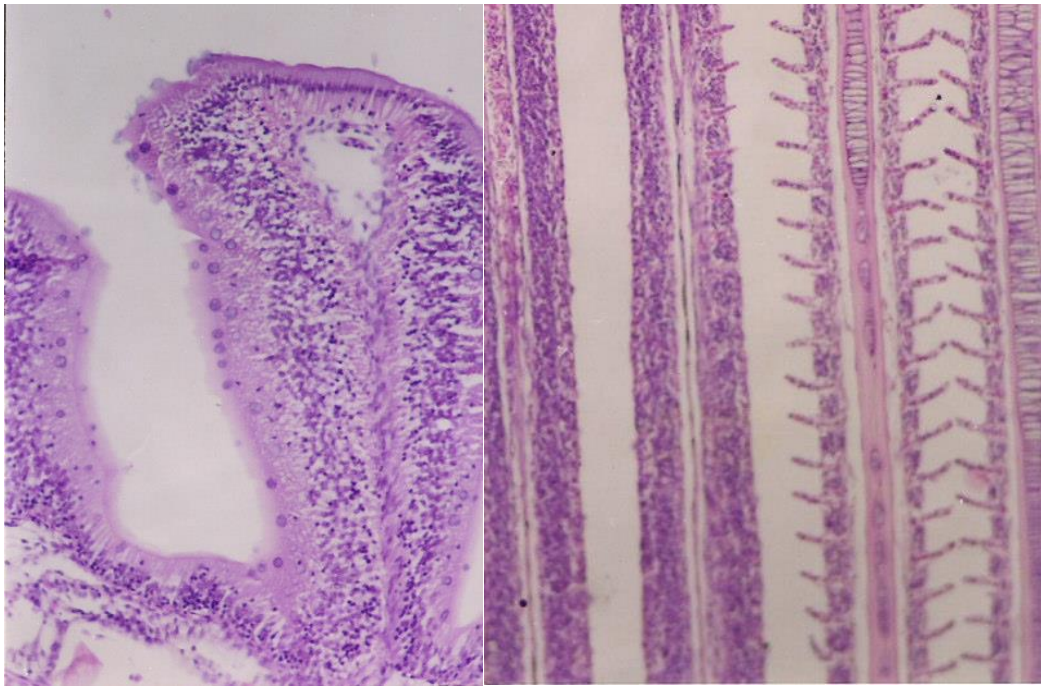
alongside some pathologies with increased mortality rate and Sterigmatocystin residues were detected in muscle meat.

Abdel-Wahhab *et al.* (2006) investigated the efficacy of Egyptian montmorillonite (EM), a clay mineral, to adsorb Stg, to test the stability of the resulting complex under different conditions in vitro, and to utilize the Nile tilapia fish as an in vivo model to evaluate the protective effect of EM against Stg-induced toxicity and clastogenicity. In the in vitro study, four concentrations of EM (0.5, 1, 2 and 4 mg/L aqueous solution) and three concentrations of Stg (5, 10 and 50 microg/ml) were tested. The results show that EM had a high capacity of adsorbing Stg at different concentrations tested. The adsorption ranged from 93.1 to 97.8% of the available Stg in aqueous solutions. The complex was stable at different pHs at 37 degrees C in different organic solvents. An in vivo experiment was conducted to evaluate the ability of EM to prevent the toxicity and chromosomal aberrations induced by Stg in the Nile tilapia fish. Fish received an intragastric dose of EM in corn oil (0.5 mg/kg bw) with or without Stg (1.6 microg/kg bw) twice a week for 4 weeks. Body weight was recorded during dosing, and blood and tissue samples were collected at the end of treatment. Stg residues were determined in fish tissue. The results show that Stg was toxic and clastogenic to fish as indicated by the significant decrease of body weight and the increase in frequencies of micronucleated red blood cells (MN RBC) and chromosomal aberrations in the kidney. The intragastric administration of EM combined with Stg to fish resulted in a reduction of the number of MN RBC and the frequency of chromosomal aberrations in the kidney compared with the group treated with Stg alone. It could be concluded that EM itself was safe and successful in the prevention of Stg toxicity and clastogenicity.

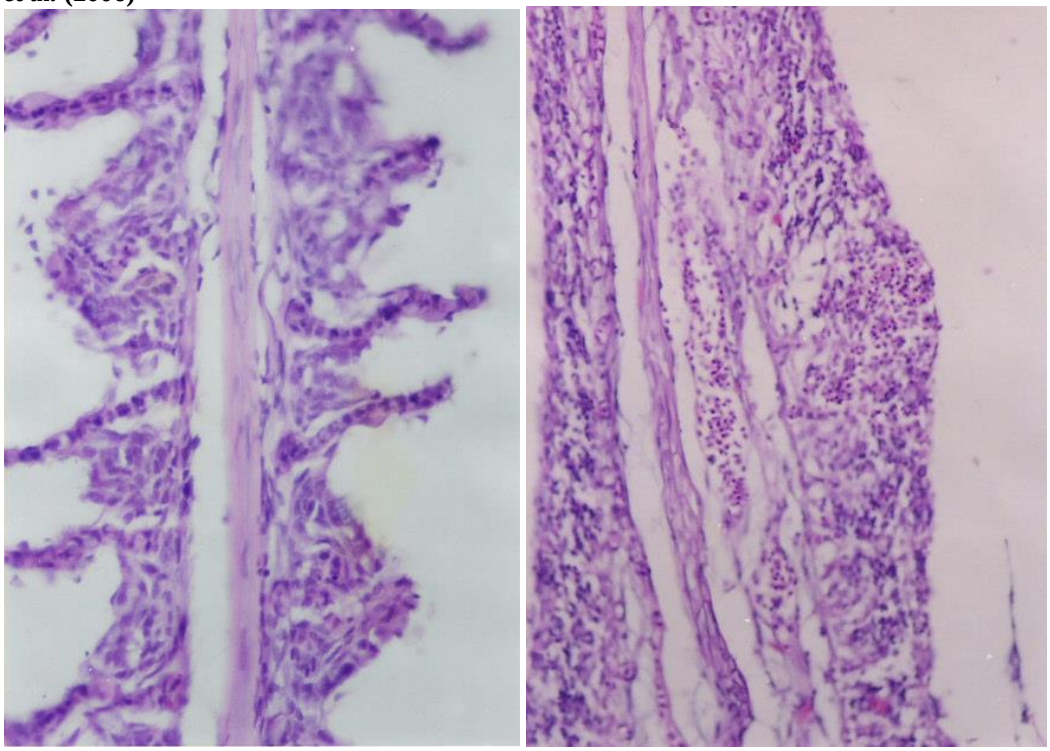
Mahrous *et al.* (2006) used the random amplified polymorphism DNA (RAPD) method to evaluate the genotoxic effects of Stg and to determine if the Egyptian montmorillonite (EM) has a protective effect against Stg. The experiment was conducted *in vivo* to evaluate the ability of EM at a level 0.5 mg/kg body weight (bw) to prevent the toxicity and genotoxicity induced by Stg in the Nile tilapia fish. Fishes were orally administrated with EM in corn oil with or without Stg (1.6 µg/kg bw) twice a week for 4 weeks. Blood and tissue samples were collected at the end of the treatment. The results revealed that Stg had genotoxic and toxicopathological effects in *Oreochromis niloticus* fish. The genotoxic effects were indicated by appearance of some changes in polymorphism band patterns including lost of stable bands or occurrence of new bands. There also exists a distinct distance between the band patterns of exposed fish and protected or control fish samples. The effects on the tissues were manifested by different histopathological lesions in different organs including hyperplastic proliferation of branchial epithelium, necrobiotic changes in hepatic tissue and destruction of components of the spleen. These responses were virtually abolished or markedly decreased when fishes were exposed to EM combined with Stg. It could be conclude that addition of EM resulted in the inhibition of the toxicity and clastogenicity of Stg.



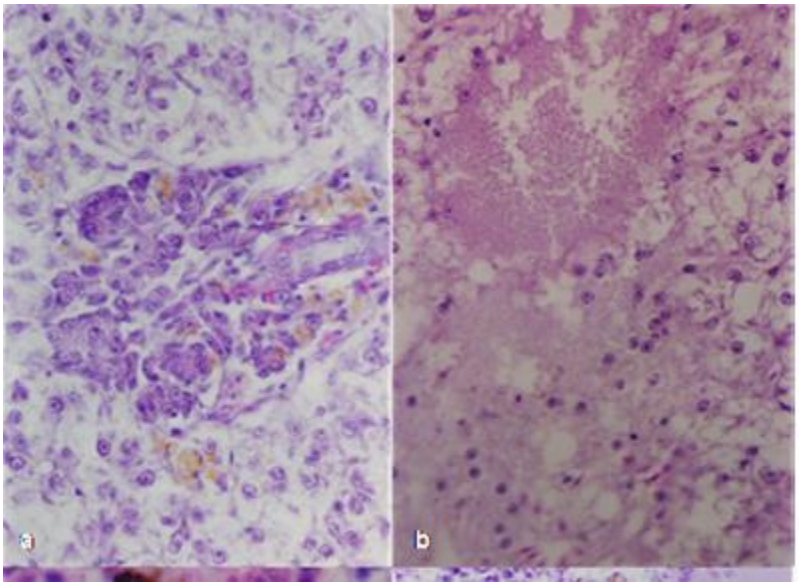
Comparison of RAPD fingerprinting profiles of different tilapia genomic DNA. (a) Represents PCR products with primer A06, (b) represents PCR products with primer A09, (c) represents PCR products with primer C07 and (d) represents PCR products with primer C20. The DNA marker is in lane 1. Lane 2 represents fish treated with corn oil, lane 3 represents untreated fish, lane 4 represents fish exposed to Stg (1.6 µg/kg body weight dissolved in corn oil), lane 5 represents fish treated with EM plus Stg and lane 6 represents fish exposed to EM alone. **Mahrous *et al.* (2006)**



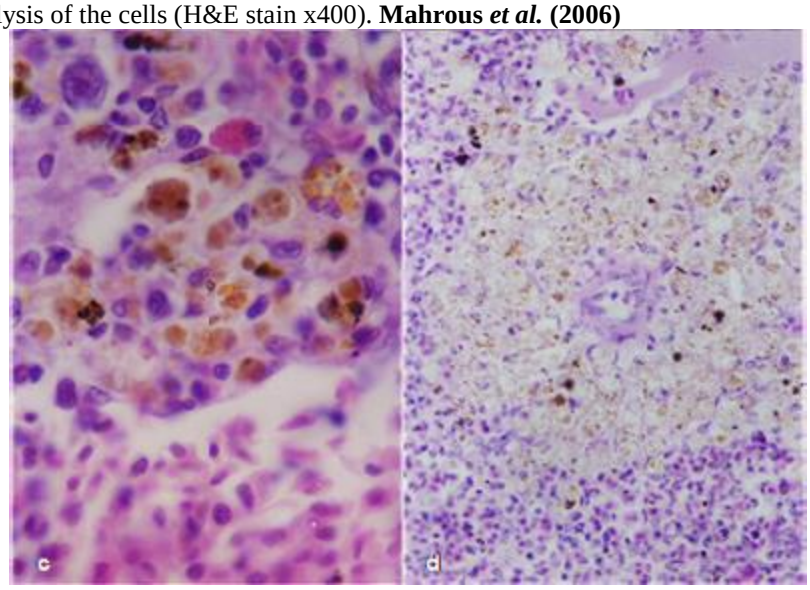
Photomicrographs of several organs of *o. niloticus* fish treated with Stg in combination with EM or with Stg alone: (a) Intestine of *o. niloticus* fish treated with both Stg and EM showing hyperplasia of the epithelial lining with marked activation of mucous secreting cells (H&E stain x200). (b) Gills of *o. niloticus* fish treated with Stg only showing diffuse lamellar hyperplasia (H&E stain x100). **Mahrous *et al.* (2006)**



(c) Gills of *o. niloticus* fish treated with Stg only showing focal hyperplasia in the form of three dimensional lamellar hyperplasia (H&E stain x400). (d) Gills of *o. niloticus* fish treated with Stg only showing hemorrhages between the branchial tissue epithelium (H&E stain x200). **Mahrous *et al.* (2006)**



Oreochromis niloticus fish treated with Stg alone: (a) Liver of *O. niloticus* fish showing melanophores infiltrating the area of hepatopancrease (H&E stain x400). (b) Liver of *O. niloticus* fish showing necrosis and lysis of the cells (H&E stain x400). **Mahrous *et al.* (2006)**



Oreochromis niloticus fish treated with Stg alone: (c) Spleen of *O. niloticus* fish showing marked hemorrhages and eosinophilic granular cells aggregation in the area of melano-macrophage centers (H&E stain x1000). (d) Spleen of *O. niloticus* fish showing melanophores aggregated around the blood capillary of the splenic ellipsoids (H&E stain x400). **Mahrous *et al.* (2006)**

3. Ochratoxins

- Ochratoxins are toxic compounds produced mainly by fungi of the *Aspergillus* and *Penicillium* genera. The most abundant and most toxic mycotoxin within the ochratoxins is OTA (**Marquardt and Frohlich 1992),** which occurs in maize, cereal grains such as wheat and barley, and oil seeds such as soybean and peanuts (**Manning *et al.*, 2003**).
- In fish, the main target organs of OTA toxic impact are the liver and kidney.
- Acute toxicity and metabolization of OTA in rainbow trout, with 10-d mortalities were recorded after single i.p. doses of OTA at 4.0, 6.0, and 8.0 mg/kg body weight (acute i.p. lethal dose 50 was 5.53 mg/kg body weight) **Doster (1973).**

- The livers of trout dosed with OTA (4.0 mg/kg or more) had normal architecture but many necrotic parenchymal cells. The most obvious effect of OTA on the liver was an increase in the number of cytoplasmic and nuclear vacuoles. (**Fuchs *et al.*, 1986).**
- A doses of OTA (8.0 mg/kg body weight) evoked necrosis in all parts of the kidney (i.e., tubules, glomeruli, and hematopoietic tissue). (**Fuchs *et al.*, 1986).**
- Highest concentrations of OTA in tissue 24 h after exposure were in the pyloric ceca, intestine, and liver (**Doster, 1973**). The elimination half-life of OTA in fish is 0.68 h (**Hagelberg *et al.* 1989**).
- **Acute toxicity of OTA** and associated behavioral changes in marine-reared sea bass (average body weight 40 g) were determined by **El-Sayed et al. (2009).**
- The immunosuppressive effect of OTA using juvenile channel catfish fed 2.0 or 4.0 mg/kg diet of OTA was confirmed by **Manning *et al.* (2005**).

Reports:

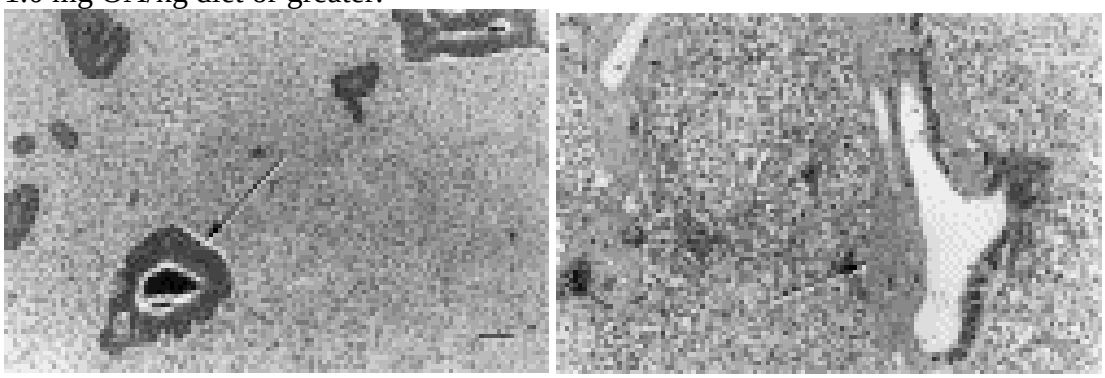
Doster *et al.* (1972) studied the acute intraperitoneal toxicities of two metabolites of *Aspergillus ochraceus*, ochratoxins A and B, and their dihydroisocoumarin derivatives, ochratoxins *a* and *b*, in 6-month-old Mt. Shasta strain rainbow trout (*Salmo gairdneri*). Ochratoxin A was the only compound found to be lethal to trout at the levels administered, its acute intraperitoneal LD_{50} being 4·67 mg/kg. Pathological changes in the liver and kidneys were produced by ochratoxins A and B but not by ochratoxin *a* or *b*. Ochratoxin A produced degenerative changes in the hepatic parenchymal cells, including nuclear swelling and cytoplasmic and nuclear lipid vacuolation, necrosis in the proximal tubules, haematopoietic tissue and glomeruli of the kidneys and pycnotic nuclei, cast formation and lipid vacuolation in the renal tubules. Ochratoxin B administered at levels up to 66·7 mg/kg caused no deaths but the highest dose induced pathological changes in the liver and kidneys similar to those produced by relatively low levels of ochratoxin A. Ochratoxins *a* and *b* administered at levels up to 28·0 and 26·7 mg/kg, respectively, failed to cause any deaths or induce any microscopic lesions that were not seen in control trout dosed with 0·1 N-sodium bicarbonate. It is suggested that ochratoxins A and B are metabolized to their non-toxic water-soluble dihydroisocoumarin moieties, which are readily excreted.

Fuchs *et al.* (1986) studied the nephrotoxic mycotoxin ochratoxin A in rainbow trout by whole-body autoradiography and scintillation counting using 14C-labelled toxin. After one single intravenous injection of 10 muCi/fish, corresponding to 160 ng toxin/g body weight, the tissue affinity was studied during an eight day period. As soon as 5 min. after injection the concentration of the radioactivity in the blood had dropped to one tenth of that in the kidney and the urinary bladder. The autoradiograms showed two patterns of blackening in the kidney, one diffuse in the pronephros and one very strong spotty blackening in the opistonephros. In addition to the kidney very high concentrations of radioactivity were also noticed in the bile and the pseudobranch. The muscular tissue of treated trouts contained almost no radioactivity during the whole experiment. Chemical analysis revealed that the radioactivity that could be extracted from the organs was mainly ochratoxin A.

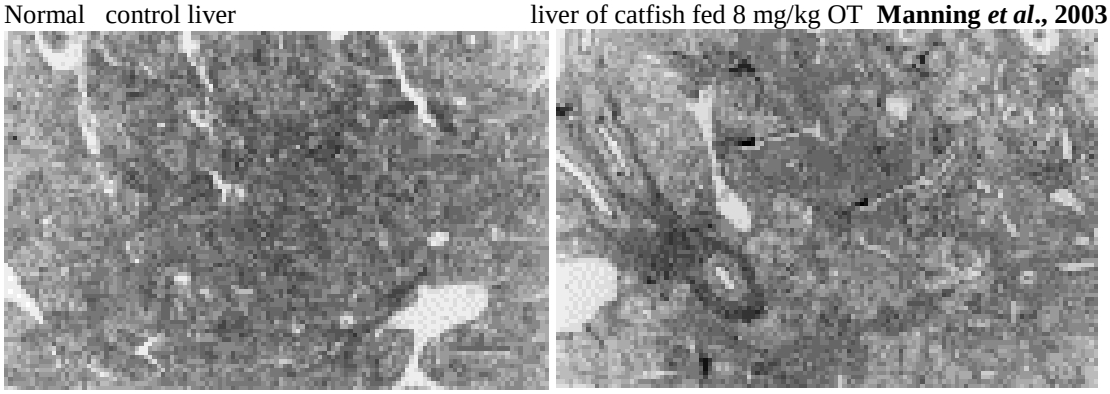
Lovell (1992) reported that the oral LD_{50} for ochratoxin-A in six-month-old rainbow

trout was 4.7 mg/Kg. Pathological signs were severe necrosis of liver and kidney tissues, pale kidney, light swollen livers and death.

Manning *et al.* (2003) conducted an experiment in aquaria with juvenile channel catfish to evaluate the effect of feeding graded levels of OA in a semipurified diet for 8 weeks on growth, feed conversion ratio (FCR), hematology, survival, and histopathology of liver and kidney. Channel catfish, initial body weight 6.1 g/fish, were fed diets containing 0, 0.5, 1.0, 2.0, 4.0, or 8.0 mg OA/kg diet supplied from culture material containing 80 mg OA/kg. Significant ($P \leq 0.05$) reductions in body weight gain were observed after only 2 weeks and at each successive 2-week weighing interval for catfish fed diets containing 2.0 mg OA/kg diet or above. At week 8, weight gain was significantly reduced in catfish fed diets containing 1.0 mg OA/kg or above. Feed conversion ratio was significantly poorer for catfish fed diets containing 4.0 or 8.0 mg OA/kg of diet. Hematocrit was significantly lower for catfish fed 8.0 mg OA/kg, but no significant ($P > 0.05$) differences in white blood cell (WBC) count were observed for catfish at any dietary levels of OA. Survival was high for catfish fed diets containing 0–4 mg OA/kg, but fish fed the diet containing 8.0 mg OA/kg had significantly lower survival compared with those of the other treatments. Histopathological examination of liver and posterior kidney at 8 weeks revealed that there was increased incidence and severity of melanomacrophage centers in hepatopancreatic tissue and posterior kidney for catfish fed dietary concentrations of 2.0 mg OA/kg or above. Exocrine pancreatic cells that normally surround the hepatic portal veins of channel catfish were reduced in number or absent in livers of fish fed 1.0 mg OA/kg diet or greater.



Normal control liver liver of catfish fed 8 mg/kg OT **Manning *et al*., 2003**



Normal control kidney kidney of catfish fed 8 mg/kg OT, **Manning *et al*., 2003**

Srour (2004) showed that increasing OCTA levels in the diet ofNile tilapia resulted in decreasing growth performance and feed utilization parameters. Carcass dry matter, protein and ash contents were negatively correlated with OCTA levels but

carcass lipids had positively correlated with OCTA levels.

Manning *et al.* (2005) confirmed the immunosuppressive effect of OTA using juvenile channel catfish fed 2.0 or 4.0 mg/kg diet of OTA**.** Feeding took place over 6 wk, whereupon the catfish were challenged by *in situ* immersion with a virulent isolate of *Edwardsiella ictaluri*, resulting in mortality at both concentrations. After 21 d, catfish fed a 4.0 mg/kg OTA diet displayed significantly higher mortality (80.49%) than control-fed catfish (68.28%).

El-Sayed *et al.* (2009) conducted a flow-through bioassay test system in two series and a total 180 of adult marine-reared sea bass was used to estimate the acute oral 96 h median lethal concentration (LC_{50}) value and behavioral changes of OTA. The data obtained were statistically evaluated using Finney's Probit Analysis Method developed by EPA. The 96 h LC_{50} value for adult *D. labrax* was found to be 277 μg kg^{-1} bwt with 95% confidence limits of 244–311 μg kg^{-1} bwt. This value was calculated to be 285 μg kg^{-1} bwt with Behrens–Karber's method. The two methods were relatively comparable. The acute dietary 96 h LC_{50} of OTA is 9.23 mg kg^{-1} diet. Additionally, the behavioral changes of sea bass were primarily observed as nervous and respiratory manifestations. We concluded that sea bass is a species highly sensitive to OTA making them a useful experimental model for aquatic mycotoxigenic problems.

Náscher-Mestre *et al.* (2015) surveyed commercially available plant ingredients (19) and PAP (19) for a wide range of mycotoxins (18) according to the EU regulations. PAP showed only minor levels of ochratoxin A and fumonisin B1 and the mycotoxin carry-over from feeds to fillets of farmed Atlantic salmon and gilthead sea bream (two main species of European aquaculture) was performed with plant ingredient based diets. Deoxynivalenol was the most prevalent mycotoxin in wheat, wheat gluten and corn gluten cereals with levels ranging from 17 to 814 and μg kg(-1), followed by fumonisins in corn products (range 11.1-4901 μg kg(-1) for fumonisin B1+B2+B3). Overall mycotoxin levels in fish feeds reflected the feed ingredient composition and the level of contaminant in each feed ingredient. In all cases the studied ingredients and feeds showed levels of mycotoxins below maximum residue limits established by the Commission Recommendation 2006/576/EC. Following these guidelines no mycotoxin carry-over was found from feeds to edible fillets of salmonids and a typically marine fish, such as gilthead sea bream. As far we know, this is the first report of mycotoxin surveillance in farmed fish species.

4. Fumonisins

- Fumonisins are mycotoxins produced mainly by *Fusarium verticillioides* (synonym: *Fusarium moniliforme*) and *Fusarium proliferatum*, the most frequent fungal contaminants of maize (**Scott, 2012**).
- The most abundant and the most toxic FB is fumonisin B_1 (FB_1) (**Escrivá et al. 2015**).
- The toxic dose for FB_1 in fish has a broad range (**Voss *et al.*, 2007**).

- FB_1 causes mortality in fish either by direct tissue damage (**Li *et al*.**, **1994**) or by immunosuppression that results in higher sensitivity to infection (**Lumlertdacha and Lovell, 1995; Pepeljnjak *et al*., 2003**).
- **Chronic effects** of FB_1 include a decrease in body weight gain and changes to hematological blood parameters. In fishes and mammals alike, FBs have a disruptive effect on neural and liver tissues. Sensitivity to FB_1 in fish is dependent on both species and individual body weight.
- Nile tilapia fed a diet containing 40.0 mg/kg FB_1 or more had a significantly lower mean weight gain than control fish, while levels of 150 mg/kg significantly decreased hematocrit and significantly increased the SA/SO ratio in the liver (**Tuan *et al.*, 2003**).
- Dose of 100 mg/kg fed for 42 d led to degenerative changes in the brain of 1-yr-old common carp (**Kovacić *et al*.** (2009).

Reports:

Brown *et al.* (1994) performed a study to determine the toxicity of fumonisin B1 from Fusarium cultures to adult channel catfish, Ictalurus punctatus. Fusarium moniliforme M1325a cultures were grown on whole kernel corn, extracted with acetone : chloroform, dried, and ground. The finely ground F. moniliforme culture material was analyzed for fumonisin B, by high-performance liquid chromotography, blended with crumbled commercial catfish fingerling feed to obtain 5 different levels of FB1 and repelletized. Diets were analyzed for FB1 and found to contain 0, 35, 62, 170, and 313 mg FB1 / kg. The diets were free of the following mycotoxins: aflatoxin, citrinin, sterigmatocystin, zearalenone, ochratoxin A, T-2 toxin, diacetoxyscirpenol, and vomitoxin. Sixty adult channel catfish from various catfish ponds in the Delta region of Mississippi were randomly assigned to 378-liter black circular plastic tanks with flow-through water systems. Water was maintained at 22 ± 2 C, and constant aeration was provided by air stones. Twelve catfish were assigned randomly to each of 5 dietary treatments, and following acclimation for 3 days, experimental diets were fed as the only nutrient source for 5 weeks. At the end of weeks 2, 3, and 5, 4 fish from each group were anesthetized with tricaine methanesulphonatec (100 mg/liter), blood samples were taken for hematocrit determination, and the fish were euthanized by anesthetic overdose and necropsied. Tissues of all major organs were fixed by immersion in 10% neutral buffered formalin. Complete histologic examinations were performed on half the fish from each group (30 fish total). Samples were embedded in paraffin, and stained with hematoxylin and eosin, and cut into 6-µm-thick sections, which were prepared by routine methods. The catfish were generally in good health during the study. Feed was noted in the gastrointestinal tract of all fish at necropsy. Two fish in the 313 mg/kg group (1 at week 2 and 1 at week 3) had mild focal enteritis, characterized microscopically by lymphocytes and lesser numbers of macrophages in the lamina propria of the small intestine. Hematocrits varied from 21.5 to 47 and did not show a dose response. No significant lesions were seen in the brain, heart, liver, spleen, gills, head and trunk kidneys, stomach, intestines, skin, or gonads of the control or treatment groups. At week 2, 1 fish in the 35 mg/kg group had severe multifocal granulomatous hepatitis and granulomatous interstitial nephritis

that was thought to be related to bacterial infection. Some fish from control and treatment groups shared incidental histologic features with mild to moderate hyperplasia of immature hematopoietic cells in the head and trunk kidneys that was thought to be related to low dissolved oxygen levels in the water, cutaneous ulcerations thought to be from tank and fish contact, mild internal parasite infestation, and hepatocellular glycogen accumulation that did not correlate with specific treatment groups. These findings suggest adult channel catfish can tolerate feed contaminated with FB1 at concentrations up to 313 mg/ kg for periods of up to 5 weeks. With the exception of a mild enteritis noted in 2 fish in the 313 mg/kg group, no remarkable macroscopic or microscopic lesions were found.

Li *et al.* (1994) found that FB_1levels below 20mg/kg diet were not a problem in commercial catfish feed. Since, higher levels of FB_1 depressed growth, lowered hematocrit, increased liver glycogen, increased vacuolation in nerve fibers, and perivascular lymphohistiocytic investment in the brain of catfish. Consumption of feed containing 240.0 mg/kg FB_1 for 12 wk led to a reduction in survival in channel catfish (initial weight 6.1 g), while diets containing 40.0 mg FB_1 or more caused increased liver glycogen and histological changes in nerve fibers and the brain

Lumbertdacha *et al.* (1995a) fed year-1 (average initial weight 1.2 g) and year-2 channel catfish (average initial weight 31 g) diets containing various amounts of *Fusarium moniliforme* corn culture to provide 0.3 (control), 20, 80, 320, or 720 mg of fumonisin B_1 (FB_1)/kg of diet for 10 and 14 weeks, respectively. Year-1 fish fed 20 mg or more of FB_1/kg of diet gained significantly less weight than the control and those fed 80 mg or more of FB_1/kg of diet had significantly lower hematocrits and red and white blood cell counts than those fed lower doses. Mortality among year-1 fish fed 80 mg or less of FB_1/kg of diet was not significantly different from controls but over 70% of fish fed 320 or 720 mg of FB_1/kg of diet died during the experiment compared to 0% in controls. Year-2 fish fed 80 mg or more of FB_1/ kg of diet gained significantly less weight than fish fed lower amounts of fumonisin. Dietary concentrations of 320 mg of FB_1/kg caused significantly lower hematocrit and red cell counts, and higher white cell counts. There were no significant differences in mortalities among year-2 fish fed 80 mg or less of FB_1/kg of diet, but over 50% of the fish fed 320 mg or more of FB_1/kg diet died from *Cytophaga columnaris* infection. Fish fed the two highest doses of FB_1 reduced their food consumption after 1 week and lost weight during the feeding trial. Small (2- to 4-mm diameter) white foci of subcapsular adipocyte hyperplasia were observed in the livers of year-1 and year-2 channel catfish fed 20 mg or more of FB_1/kg of diet. Livers of year-1 and year-2 channel catfish fed 20 mg or more of FB_1/kg of diet had swollen hepatocytes with lipid-containing vacuoles, lymphocyte infiltration, and scattered necrotic hepatocytes. These results indicate that diets containing *Fusarium moniliforme* culture material with FB_1 concentrations of 20 mg/kg or above are toxic to year-1 and year-2 channel catfish.

Lumlertdacha *et al.* (1995b) fed year-2 channel catfish *Ictalurus punctatus* (average initial weight, 31 g) nutritionally balanced diets containing various amounts of corn culture material contaminated with the fungus *Fusarium moniliforme*. Quantities of culture material used provided 0.3 (control), 20, 80, 320, or 720 mg of the mycotoxin fumonisin B_1 (FB_1) per kilogram of diet. Fish fed the two highest concentrations of FB_1 lost weight during the 14-week feeding period and experienced high mortality caused by *Flexibacter columnaris*. Fish fed the three lower concentrations for 14

weeks experienced no mortality, but those fed 80 mg FB_1/kg showed significantly less weight gain. When challenged by immersion in an aqueous cell suspension of a virulent strain of *Edwardsiella ictaluri*, fish that had been fed 80 mg FB_1/kg diet for 14 weeks had a significantly lower percentage survival than the fish fed 0.3 or 20 mg FB_1/kg. Antibody production by fish fed 20 or 80 mg FB_1/kg diet and inoculated with killed *E. ictaluri* cells was significantly lower after 14 d than antibody production by inoculated control fish. These results indicate that feeding year-2 channel catfish corn material contaminated with *F. moniliforme* and containing fumonisins can reduce the fishes' growth and resistance to *E. ictaluri* infection.

Yildirim *et al.* (2000) investigated growth, histological lesions, and biochemical changes in channel catfish *Ictalurus punctatus* fed various concentrations of moniliformin with or without fumonisin B_1. Channel catfish (average initial weight, 1.5 g) were fed diets formulated to contain 0, 20, 40, 60, and 120 mg moniliformin/kg; 0, 20, and 40 mg fumonisin B_1/kg, or two combinations of moniliformin and fumonisin B_1 for 10 wk. Fish fed diets with the lowest concentration of moniliformin or fumonisin B_1(20 mg/kg diet) had significantly ($P < 0.05$) less weight gain than the control fish. Increasing the level of moniliformin in the diets resulted in a linear decrease in weight gain. Overall mortality of fish was 4% and not related to treatment effects. Hematocrit was significantly ($P < 0.05$) lowered by 60-mg moniliformin/kg diet or 40-mg fumonisin B_1/kg diet. Dose-dependent increases in serum pyruvate concentration and ratio of free sphinganine to free sphingonine were obtained with increasing concentration of dietary moniliformin and fumonisin B_1, respectively. Mean serum pyruvate level was significantly ($P < 0.05$) higher in fish fed the diet containing 60-mg moniliformin/kg diet. Addition of fumonisin B_1 (40 mg/kg) to the diet containing 40-mg moniliformin/kg significantly increased the serum pyruvate level above that of the control. Also, the lowest concentration of fumonisin B_1 (20 mg/kg diet) significantly ($P < 0.05$) increased the ratio of sphingolipids. Combinations of moniliformin and fumonisin B_1 at levels of 20:40 and 40:40 mg/kg diet did not significantly change the effect of fumonisin B_1 on the ratio of sphingolipids. The only tissue lesions observed in liver and heart were smaller nuclei of cells in livers of fish fed diets containing the two highest levels of moniliformin and the combinations of the two toxins.

Pepeljnjak *et al.* (2003) observed a reduction in body weight gain in common carp fed 0.5 and 5.0 mg/kg FB_1 for 42 d, and a higher incidence of the bacterial infection erythrodermatitis cyprini in the group receiving 5.0 mg/kg FB_1. In both treatment groups, FB_1 caused changes in red blood cell parameters and platelet count and dose-dependent changes in the biochemical profile.

Petrinec *et al.* (2004) fed one-year-old carp rations containing 100 or 10 mg/kg of FB_1 . The histology of fish showed that blood vessels, liver, exocrine and endocrine pancreas, excretory and hematopoietic kidney, heart and brain were sensitive to both levels of FB_1 and the rodlet cell frequency was increased in and around damaged tissues.

Gbore *et al.* (2010) used fingerlings of Clarias gariepinus to evaluate the effect of dietary fumonisin B1 (FB1), a mycotoxin produced by Fusarium verticillioides, on growth, haematological and serum biochemical parameters. The fingerlings were sorted, weighed and randomly stocked in 16 plastic tanks at the rate of 20 fingerlings

per tank. Fusarium-cultured maize grains containing FB1 were used to formulate three diets containing approximately 5.0, 10.0 and 15.0 mg FB1/kg, constituting diets 2, 3, and 4 respectively. These three diets, plus diet 1, which contained non-Fusarium cultured maize grains that served as the control, were used in a 6-week feeding trial. The final weight gains by the fingerlings were significantly ($P < 0.05$) influenced by FB1. The final weights of the fingerlings fed diets 2, 3 and 4 ranged from 70.07 to 87.10% of the controls. The haematocrit, erythrocytes, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the serum protein constituents (total protein, albumin and globulin) values significantly ($P < 0.05$) decreased, while the leucocytes, MCV and MCH increased significantly ($P < 0.05$) with increase in the dietary FB1. The total serum protein values of the fingerlings fed diets 2, 3 and 4 were 34.53, 39.42 and 50.17% lower than the total serum protein values of those fed the control diet. These results indicate that Fusarium-contaminated diets containing about 5.0 mg or more FB1/kg reduced weight gain and significantly altered haematological parameters and serum protein constituents in the fingerlings. These may have a significant impact on physiological activities and may be vital in immunosuppression in the fingerlings with a strong negative impact on subsequent performance of the fish

Gaecia (2013) evaluated the growth performance, feed intake, mortality and liver histopathology of juvenile salmon exposed to FB1 doses 0, 1, 5, 10 or 20 mg/kg feed. The hypothesis was that FB1 ingestion would reduce salmon growth, feed intake and would produce liver damage. At the end of the 10-week experiment no differences in the evaluated parameters were found. Species-specific differences in vulnerability because of variations in toxin metabolism could explain the results. However, due to the slow growth of fish during the trial additional research to confirm the results were suggested.

Rodríguez-Cervantes *et al.* (2013) detected FBs in tilapia feed ranging from 0.148 to 2.587 mg/kg, while Greco et al. (**2015**) observed levels in rainbow trout feed below their limit of detection (0.222 mg/kg). Despite FBs being the most prevalent mycotoxin in grains (the most common ingredient in commercial aquafeed), the overall concentration is low and does not represent a threat to fish.

Nácher-Mestre *et al.* (2015) analyzed alternative feed ingredients and complete feed for Atlantic salmon and found that the total concentration of $FB_1 + FB_2 + FB_3$ ranged from 0.112 to 0.754 mg/kg.

5. Deoxynivalenol (DON)

- DON, also known by the name vomitoxin, is a type B trichothecene mycotoxin produced by *Fusarium graminearum* and *Fusarium culmorum*. DON is the most prevalent trichothecene contaminant in cereal crops such as wheat, barley, and maize (**Marin *et al.*, 2013**) and is the most economically important mycotoxin (**Wegulo, 2012**).
- Fish in aquaculture are commonly exposed to DON in feed containing wheat (**Pietsch *et al.*, 2013**).
- The response of juvenile rainbow trout to diets containing DON-contaminated corn after 4 wk of feed intake was described by **Woodward *et al.* (1983)**.

- Histopathological examination recorded morphological changes in the liver, including subcapsular edema, hemorrhages, and fatty infiltration of hepatocytes, while hemorrhages were found in the intestinal tract (**Hooft et *al.*, 2011**).
- Differences in DON sensitivity between fish species could be caused by a difference in the ability of intestinal microbes to transform DON to the less toxic de-epoxy DON (**Guan *et al.*, 2009**).

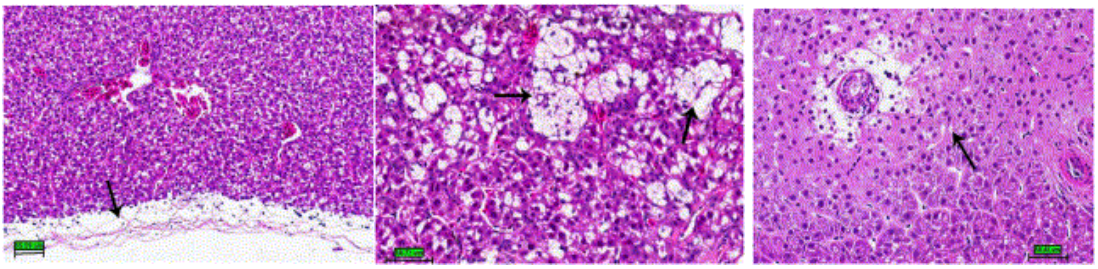
Reports:

Woodward *et al.* (1983) conducted a study to obtain information as to the response and sensitivity of rainbow trout (*Salmo gairdneri*) to diets containing vomitoxin-contaminated corn. Feed refusal occurred when diets contained 20 μg/g vomitoxin or more, but the trout recovered rapidly when subsequently fed a diet containing no detectable toxin. Diets containing graded levels of vomitoxin, incresing from 1.0 to 13.0 μg/g, caused progressively greater depression in 4-week liveweight gains of juvenile trout. The depression in weight gain ranged from 12% to 92% of the control value and resulted from an adverse effect on both feed intake and feed conversion efficiency. Emesis was not observed in this work. The results demonstrate that rainbow trout are highly sensitive to dietary vomitoxin.

Guan *et al.* (2009) screened digesta of 62 fishes from nine species for their ability to transform 4-deoxynivalenol (DON). Liquid chromatography-mass spectrometry was used to determine the reduction of DON concentrations and structures of DON-transformation products. The microbial community from one catfish *Ameiurus nebulosus*, namely microbial culture C133, completely transformed DON to deepoxy DON (dE-DON) at 15 °C in full medium after 96 h incubation. Various media and culture conditions were tested to evaluate their effect on DON transformation. Microbial culture C133 maintained high transformation ability over a broad range of temperatures from 4 to 25 °C and pH values from 4.5 to 10.4. The transformation of DON to dE-DON was enhanced in a rich medium such as full medium, nutrient broth and corn meal broth. Microbial culture C133 was then tested for its ability to transform other trichothecene mycotoxins; most of the toxins were transformed to deacetyl and/or deepoxy products. This is the first report on trichothecene transformation by microbes from the intestinal tract of fish.

Hooft and Elmor (2011) investigated the effects of feeding six diets containing low, graded levels of **DON** from two naturally contaminated sources of corn on the performance, health and apparent nutrient digestibility of rainbow trout. Feeding diets with increasing levels of DON (0.3, 0.8, 1.4, 2.0 and 2.6 ppm) for eight weeks to rainbow trout (initial weight = 24 g/fish) resulted in significant linear or quadratic decreases in feed intake, weight gain, growth rate (expressed as thermal-unit growth coefficient, TGC), feed efficiency (FE, gain:feed), retained nitrogen (RN), recovered energy (RE), energy retention efficiency (ERE), and nitrogen retention efficiency (NRE). Fish pair-fed the control diet (0.3 ppm DON) had significantly higher TGC ($P < 0.01$), FE ($P < 0.0001$) and whole body crude protein (CP) concentration ($P < 0.01$) compared to their counterparts fed the diet containing 2.6 ppm DON. No significant differences ($P > 0.05$) were observed in the apparent digestibility coefficients (ADC) of CP and gross energy (GE) of fish fed diets containing 0.3 (control) to 2.0 ppm DON. In addition, some morphological changes of the liver were

noted in fish fed the diet containing 2.6 ppm DON. These results suggest that, relative to other species, rainbow trout are extremely sensitive to DON from naturally contaminated grains and that the effects of DON on rainbow trout are not simply related to a reduction of feed intake, but rather, are due to metabolic effects. More research is required to identify the specific mechanism(s) of toxicity of DON in rainbow trout



Liver of rainbow traut exposed to DON 1.4 ppm showing congestion, Liver of rainbow traut fed DON 2.6 DON ppm **Hooft and Elmor (2011)**

Sanden *et al.* (2012) investigated the effects of feeding six diets spiked with increasing levels of DON for 45 days to zebrafish (Danio rerio) on performance and liver gene biomarkers. In addition long term effects on fecundity, offspring larvae swimming activity and global DNA methylation in embryos were investigated. Zebrafish performance was not affected. Liver CYP1A mRNA levels were significantly higher in fish fed 2.0 ppm DON compared to the control group, 0.1, 0.5 and 1.5 ppm group. Gene transcripts of CuZn SOD and Cyclin G1 increased with increasing content of dietary DON. The percentage of 5-methylcytosine in embryos did not differ and was 7.0-7.1% across the groups. Fecundity showed a biphasic response pattern. Interestingly, fish fed 1.5 ppm DON had 22% higher fecundity compared to control. A trend towards increased larvae swimming activity was seen in the high DON group. Our data suggest that DON is detoxified in the liver through the phase 1 system resulting in a disturbance in the oxidative balance. We do not know if effects observed on fecundity and larvae swimming activity are attributed to a direct interaction of DON with the reproductive organ or secondary to the maternal/paternal liver oxidative imbalance.

Pietsch *et al.* (2013) reported for the first time the occurrence of **DON and ZEN** in samples of commercial fish feed designed for nutrition of cyprinids collected from central Europe. A maximal DON concentration of 825 μg kg^{-1} feed was found in one feed whereas average values of 289 μg kg^{-1} feed were noted. ZEN was the more prevalent mycotoxin but the concentrations were lower showing an average level of 67.9 μg kg^{-1} feed.

Matejova *et al.* (2014) recorded that, after 23-d exposure to DON in a concentration of 2.0 mg/kg, rainbow trout showed severe hyaline droplet degeneration in tubular epithelial cells of the renal tubules in the caudal kidney. Although no significant changes in biometric parameters were recorded, significant changes in hematological parameters, such as lower mean corpuscular hemoglobin values, and biochemical parameters, such as a decrease in glucose, cholesterol, and ammonia, were observed.

Manning *et al.* (2014) fed Channel catfish practical corn-soybean meal diets for 10 weeks that contained various weighed amounts of ground, dried field corn

contaminated with 20 mg deoxynivalenol (DON) kg^{-1}. Weighed amounts of DON corn were blended with weighed amounts of ground, clean corn that contained no DON (0 mg kg^{-1}) to yield five diets that had 0, 2.5, 5.0, 7.5 and 10.0 mg DON kg^{-1} of diet. Results show that catfish fed diets that contained DON for 7 weeks did not experience lower weight gains or poorer feed conversion ratios that were significantly ($P > 0.05$) different from control-fed fish. Mortality of catfish during the 21-day post-challenge period indicate that catfish fed diets containing DON-contaminated corn that provided at least 5.0 mg DON kg^{-1} of diet had significantly ($P < 0.05$) lower mortality than catfish fed the control diet or the diet that provided 2.5 mg DON kg^{-1} of diet. The presence of DON-contaminated corn in the experimental diets did not significantly ($P > 0.05$) alter fish body weight gains and appeared to provide a protective effect for channel catfish challenged with the pathogenic bacterium *Edwardsiella ictaluri*.

Pietsch *et al.* (2014a) presented results from a feeding trial with carp (*Cyprinus carpio* L.) using three different concentrations of DON (352 μg kg^{-1}, 619 μg kg^{-1}, and 953 μg kg^{-1} final feed, respectively) which are comparable to levels found in commercial fish feeds. Effects on growth and mass of fish were not observed during this 6 weeks lasting experiment. Only marginal DON concentrations were found in muscle and plasma samples. Blood parameters were not influenced although smaller erythrocytes occurred in fish treated with 352 μg kg^{-1} DON. Analysis of antioxidative enzymes in erythrocytes showed increased superoxid dismutase and catalase activities in fish fed the low-dose feed. Immunosuppressive effects of DON were confirmed whereby cytotoxic effects on immune cells only partly explained the impairment of innate immune responses. Exact polarization of the immune system into pro-inflammatory or anti-inflammatory responses due to DON exposure should be clarified in further experiments, especially since the current results raise concern about impaired immune function in fish raised in aquaculture.

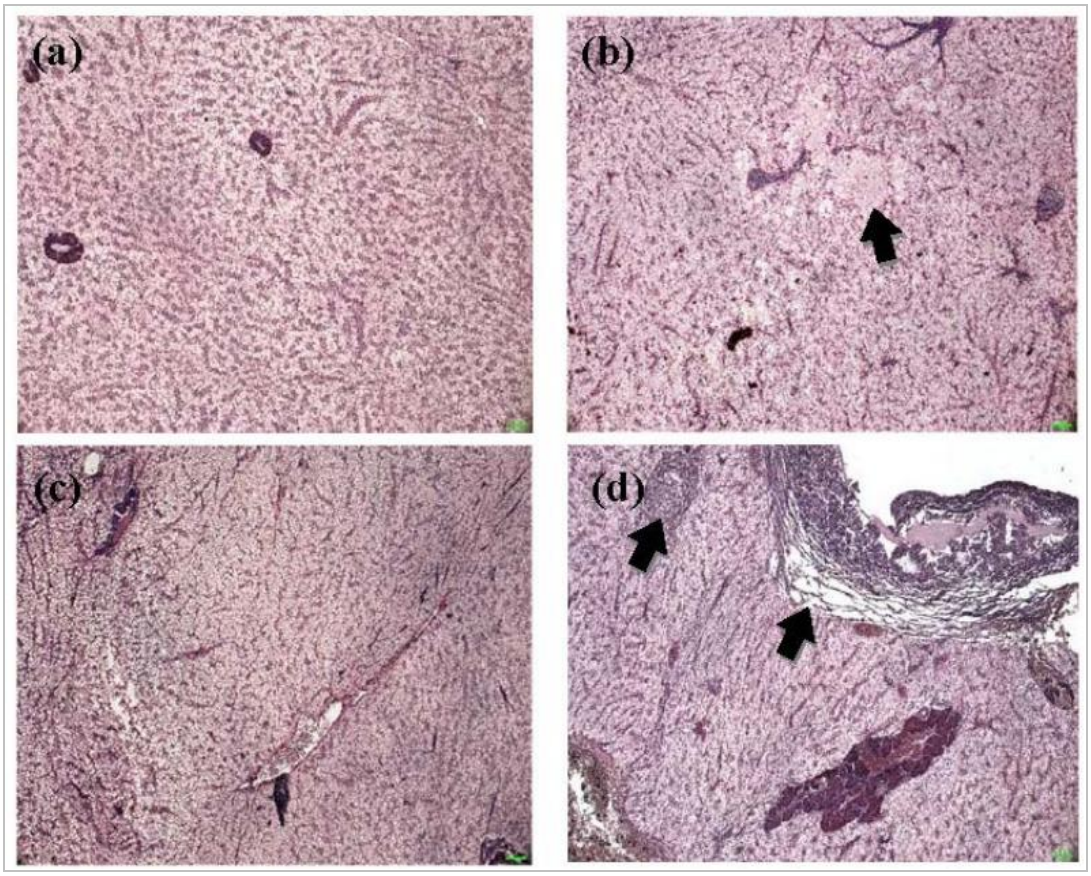
Pietsch *et al.* (2014b) investigated possible metabolization of ZEN in fish cell lines suggesting that mainly glucuronidation takes place. It demonstrates that concentrations up to 20,000 ng ml(-1) ZEN are capable of influencing cell viability in permanent fish cell cultures in a dose-response manner with different response patterns between the five tested cell lines, whereby lysosomes appeared to be the main target of ZEN. ZEN toxicity is often discussed in the context of oxidative stress. Our study shows a biphasic response of the cell lines when reactive oxygen species (ROS) production is monitored. Damage in cells was observed by measuring lipid peroxidation, DNA strand breaks, and alterations of intracellular glutathione levels. Metabolization of ZEN, especially at concentrations above 7500 ng ml(-1) ZEN, does not prevent cytotoxicity. ZEN as an estrogenic compound may involve processes mediated by binding to estrogen receptors (ER). Since one cell line showed no detectable expression of ER, an ER-mediated pathway seems to be unlikely in these cells. This confirms a lysosomal pathway as a main target of ZEN in fish cells.

Pietsch *et al.* (2014c) investigated the effects of DON on carp (*Cyprinus carpio* L.) at concentrations representative for commercial fish feeds. Experimental feeding with 352, 619 or 953 μg DON kg^{-1} feed resulted in unaltered growth performance of fish during six weeks of experimentation, but increased lipid peroxidation was observed in liver, head kidney and spleen after feeding of fish with the highest DON concentration. These effects of DON were mostly reversible by two weeks of feeding

the uncontaminated control diet. Histopathological scoring revealed increased liver damage in DON-treated fish, which persisted even after the recovery phase. At the highest DON concentration, significantly more fat, and consequently, increased energy content, was found in whole fish body homogenates. This suggests that DON affects nutrient metabolism in carp. Changes of lactate dehydrogenase (LDH) activity in kidneys and muscle and high lactate levels in serum indicate an effect of DON on anaerobic metabolism. Serum albumin was reduced by feeding the medium and a high dosage of DON, probably due to the ribotoxic action of DON. Thus, the present study provides evidence of the effects of DON on liver function and metabolism

Pietsch *et al.* (2015) examined the time course of innate immune responses of carp to orally administered DON. Changes in mRNA levels of immune genes in different organs (head kidney, trunk kidney, spleen, liver, and intestine) were observed indicating immune-modulating properties of DON. The immune-modulatory effects during the acute phase of DON exposure were characterized by the activation of both pro- and anti-inflammatory cytokines and enzymes in carp. The subchronic responses to DON were characterized by activation of arginases culminating in increased arginase activity in head kidney leukocytes after 26 days of DON treatment. These results suggest profound effects of this mycotoxin on fish in aquaculture

Tola *et al.* (2015) conducted an 8-week feeding trial to examine effects of wheat naturally contaminated with *Fusarium* mycotoxins (deoxynivalenol, **DON** 41 $mg \cdot kg^{-1}$) on growth performance and selected health indices of red tilapia (*Oreochromis niloticus* × *O. mossambicus*; initial weight = 4.3 g/fish). Five experimental diets were formulated by replacement of clean wheat with naturally contaminated wheat resulting in graded levels of DON and zearalenone (ZEN) (Diet 1 0.07/0.01, Diet 2 0.31/0.09, Diet 3 0.50/0.21, Diet 4 0.92/0.37 and Diet 5 1.15/0.98 $mg \cdot kg^{-1}$). Groups of 50 fish were randomly allocated into each of 20 aquaria and fed to near-satiety for eight weeks. Growth rate, feed intake and feed efficiency of fish fed the experimental diets decreased linearly with increasing levels of *Fusarium* mycotoxins ($p < 0.05$). Although growth depression was associated with feeding diets naturally contaminated with *Fusarium* mycotoxins, especially DON, no biochemical and histopathological parameters measured in blood and liver appeared affected by *Fusarium*mycotoxin concentrations of diets ($p > 0.05$). Though there was no clear evidence of overt DON toxicity to red tilapia, it is recommended that feed ingredients should be screened for *Fusarium* mycotoxin contamination to ensure optimal growth performance.



Light microscopy (×250, H & E stain) of the liver of red tilapia fed experimental diets for eight weeks. (**a**) Liver of red tilapia fed control diet (0.07 mg DON kg^{-1}); (**b**) distribution of focal necrosis (arrow) in liver of red tilapia fed Diet 3 (0.50 mg DON kg^{-1}); (**c**) cytoplasmic vacuolation in liver of red tilapia fed Diet 2 (0.31 mg DON kg^{-1}) and (**d**) subcapsular edema (arrow) in liver of red tilapia fed Diet 2 (0.31 mg **Tola *et al.* (2015)**

6. T-2 toxin

- The T-2 toxin is a fungal metabolite produced by a number of *Fusarium* spp., that is, *F. sporotrichioides*, *Fusarium equiseti*, *F. langsethiae*, *F. acuminatum*, and *F. poae*, which infect grains such as maize, barley, wheat, and oats. It is known as the most potent myelotoxin and hematotoxin.
- The T-2 toxin inhibits protein synthesis, which is particularly apparent in cells with a high rate of turnover, such as those in bone marrow and the epithelial cells of the digestive tract.
- Consumption of feed containing 0.63 mg/kg or more T-2 toxin caused significant reductions in body weight gain in channel catfish after 2 wk
- Significantly poorer FCRs were observed at concentrations of 5.0 mg/kg.
- Catfish fed 1.3, 2.5, and 5.0 mg/kg of T-2 toxin had significantly lower hematocrit values, possibly explained by dose-dependent depletion of hematopoietic cells in the head kidney.
- Carp fed with the T-2 toxin-contaminated diet displayed significantly lower body weight at the end of the experiment as well as changes in oxidative status, including an increase in glutathione (GSH) concentration and glutathione-peroxidase activity in the liver during the first week, followed by a

small decrease in the second week that again increased over the following weeks.

Reports:

Marasas *et al.* (1969) administered T-2 toxin derived from ***F. tricinctum*** to the trout feed pellets. Mature fish survived acute doses of T-2 higher than the single LC for fingerlings (6.1 mg/kg), although doses of 8 mg/kg severely damged the intestinal tracts og the fish.

Smalley (1973) mentioned that at LD of T-2 for trout, severe oedema and fluid accumulation in the body cavity and behind the eyes were produced in addition to the loss of the intestinal mucosa.

Poston *et al.* (1982) conducted a 16-wk feeding study to evaluate the chronic toxicity of graded levels (0, 1.0, 2.5.5, 10 and 15 mg/kg of chemically pure dietary T-2 toxin (4,15-diacetoxy-8-(3-methylbutyryloxy)-12,13-epoxy-Δ^9-tricothecen-3-ol) in 1-g rainbow trout, *Salmo gairdneri*, held in 9°C single-passage well water. Levels of T-2 toxin > 2.5 mg/kg depressed growth, efficiency of feed use, hematocrit, blood hemoglobin concentration and feed acceptance, and caused a transitory edema in a dose-dependent manner. Growth of trout fed a semipurified diet containing the toxin was described by the function: $Y = 0.265 + 142.075\ e^{(0.029X}{}_1 - 1.554x_2{}^{3.7})$, where Y = gain as percentage starting weight per wk; X_1 is time in wk and $0 \leqslant X_1 \leqslant 16$; and X_2 is T-2 content of diet in mg/kgand $0 \leqslant X_2 \leqslant 15$. Exposure of fish to T-2 toxin did not affect activity of intestinal lumen chymoirypsin or trypsin, nitrogen digestibility or metabolizabte energy. Feeding of 15 mg/kg T-2 toxin to adult trout caused hemorrhaging in the intestines and regurgitation of subsequently intubated feed regardless of T-2 loxin content.

Kravchenko*et al.* (1989) tested T-2 toxin on the activity of enzymes of xenobiotic metabolism in carp. Glutathione transferase activity increased moderatly, whereas the activity of lysosomal enzymes increased drastically (2-11 fold) and alkaline phosphatase activity increased 2-fold.

Manning *et al.* (2003) reported that T-2 toxin was responsible for significant reduction in growth, significantlly poor feed conversion, adversely affected hematocrit value, low survivability and histopathological anomalies of stomach and kidneys in juvenile channel catfish

Supamattaya *et al*. (2006) reported that in white shrimp growth was significantly reduced by T-2 toxin at 0.1 ppm while for black tiger shrimp reduced growth was observed at levels of 2.0 ppm. The presence of T-2 toxin at 1.0-2.0 ppm produced atrophic changes and severe degeneration of hepatopancreas tissue, inflamation and loose contact of hemopoietic tissue and lymphoid organ on black tiger and white shrimp after feeding for 10 weeks and 8 week respectively (Fig 1). The same pathology was found in shrimp received 1.0 ppm zearalenone. It was concluded by the authors that white tiger shrimp are more sensitive to mycotoxins then black tiger shrimp.

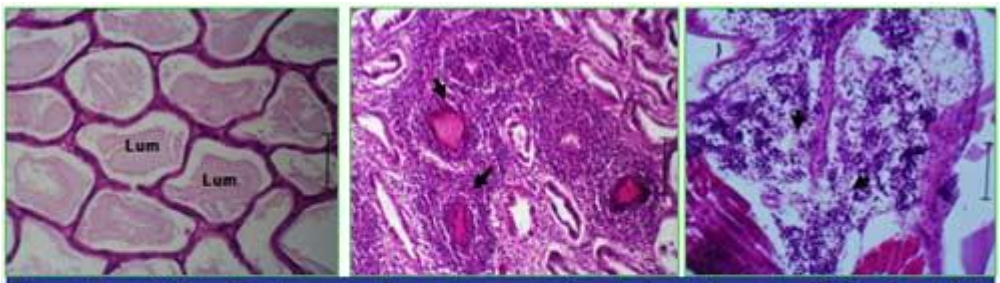


Figure 1 – a) Atrophic changes of hepatopancreatic tubule produced by T-2 toxin at 1.0 ppm for 8 weeks. b Tubular degeneration and encapsulation were observed in white shrimp fed zearalenone at 1.0 ppm for 8 weeks. C. Loose contact and necrosis of hemopoietic tissue affected by T-2 toxin at 2.0 ppm in white shrimp (source Supamattaya et al., 2006).

Yuan *et al.* (2014) exposed Zebrafish embryos to different concentrations of T-2 toxin at 4-6 hours post fertilization (hpf) stage of development, and were observed for different developmental toxic effects at 24, 48, 72, and 144 hpf. Exposure to 0.20 μmol/L or higher concentrations of T-2 toxin significantly increased the mortality and malformation rate such as tail deformities, cardiovascular defects and behavioral changes in early developmental stages of zebrafish. T-2 toxin exposure resulted in significant increases in reactive oxygen species (ROS) production and cell apoptosis, mainly in the tail areas, as revealed by Acridine Orange staining at 24 hpf. In addition, T-2 toxin-induced severe tail deformities could be attenuated by co-exposure to reduced glutathione (GSH). T-2 toxin and GSH co-exposure induced a significant decrease of ROS production in the embryos. The overall results demonstrate that T-2 toxin is able to produce oxidative stress and induce apoptosis, which are involved in the developmental toxicity of T-2 toxin in zebrafish embryos.

7. Moniliformin (MON)

- MON is a secondary metabolite of several *Fusarium* spp., particularly *F. moniliforme* and *F. proliferatum*. Both these species also host FBs, suggesting that there is the potential for co-contamination of grains with both MON and FB_1 (Manning and Abbas **2012**).
- MON toxicity is based on disruption of the pyruvate metabolism because of inhibition of pyruvate dehydrogenase and subsequent pyruvate accumulation in the tissues of the affected animal (Thiel **1978**; Gathercole et al. **1986**).

Reports:

Goel *et al.* (1994) evaluated the effect of *F. moniliforme* toxins on sphingolipids in year-2 channel catfish. In a 12-week feeding trial, four groups of catfish per treatment were fed pelleted balanced diets containing *F. moniliforme* cultured corn. The fumonisin B_1 (FB_1) concentrations in diets were 0.3 (control), 2.5, 5, 10, 20, 40, 80 and 240 mg/kg. The free sphinganine to free sphingosine ratio was significantly ($P < 0.05$) elevated (with exception of brain) at 10, 20, 40 and 80 mg FB_1 per kg diet in kidney, serum, liver and muscle, respectively. The increase in free sphingolipid ratios

observed were found to be due to increases in the levels of free sphinganine in tissues. These results demonstrate that a mode of action of *F. moniliforme* toxins in catfish is similar to other species (ponies, pigs, rats), and is suggestive of fumonisin toxicity. It also demonstrated the potential diagnostic value of ratios of free sphingolipids in catfish.

Lumlertdacha *et al.* (1995) fed year-1 (average initial weight 1.2 g) and year-2 channel catfish (average initial weight 31 g) were fed diets containing various amounts of *Fusarium moniliforme* corn culture to provide 0.3 (control), 20, 80, 320, or 720 mg of fumonisin B_1 (FB_1)/kg of diet for 10 and 14 weeks, respectively. Year-1 fish fed 20 mg or more of FB_1/kg of diet gained significantly less weight than the control and those fed 80 mg or more of FB_1/kg of diet had significantly lower hematocrits and red and white blood cell counts than those fed lower doses. Mortality among year-1 fish fed 80 mg or less of FB_1/kg of diet was not significantly different from controls but over 70% of fish fed 320 or 720 mg of FB_1/kg of diet died during the experiment compared to 0% in controls. Year-2 fish fed 80 mg or more of FB_1/ kg of diet gained significantly less weight than fish fed lower amounts of fumonisin. Dietary concentrations of 320 mg of FB_1/kg caused significantly lower hematocrit and red cell counts, and higher white cell counts. There were no significant differences in mortalities among year-2 fish fed 80 mg or less of FB_1/kg of diet, but over 50% of the fish fed 320 mg or more of FB_1/kg diet died from *Cytophaga columnaris* infection. Fish fed the two highest doses of FB_1 reduced their food consumption after 1 week and lost weight during the feeding trial. Small (2- to 4-mm diameter) white foci of subcapsular adipocyte hyperplasia were observed in the livers of year-1 and year-2 channel catfish fed 20 mg or more of FB_1/kg of diet. Livers of year-1 and year-2 channel catfish fed 20 mg or more of FB_1/kg of diet had swollen hepatocytes with lipid-containing vacuoles, lymphocyte infiltration, and scattered necrotic hepatocytes. These results indicate that diets containing *Fusarium moniliforme* culture material with FB_1 concentrations of 20 mg/kg or above are toxic to year-1 and year-2 channel catfish.

Yildirim *et al.* (2000) investigated growth, histological lesions, and biochemical changes in channel catfish *Ictalurus punctatus* fed various concentrations of moniliformin with or without fumonisin B_1. Channel catfish (average initial weight, 1.5 g) were fed diets formulated to contain 0, 20, 40, 60, and 120 mg moniliformin/kg; 0, 20, and 40 mg fumonisin B_1/kg, or two combinations of moniliformin and fumonisin B_1 for 10 wk. Fish fed diets with the lowest concentration of moniliformin or fumonisin B_1(20 mg/kg diet) had significantly ($P < 0.05$) less weight gain than the control fish. Increasing the level of moniliformin in the diets resulted in a linear decrease in weight gain. Overall mortality of fish was 4% and not related to treatment effects. Hematocrit was significantly ($P < 0.05$) lowered by 60-mg moniliformin/kg diet or 40-mg fumonisin B_1/kg diet. Dose-dependent increases in serum pyruvate concentration and ratio of free sphinganine to free sphingonine were obtained with increasing concentration of dietary moniliformin and fumonisin B_1, respectively. Mean serum pyruvate level was significantly ($P < 0.05$) higher in fish fed the diet containing 60-mg moniliformin/kg diet. Addition of fumonisin B_1 (40 mg/kg) to the diet containing 40-mg moniliformin/kg significantly increased the serum pyruvate level above that of the control. Also, the lowest concentration of fumonisin B_1 (20 mg/kg diet) significantly ($P < 0.05$) increased the ratio of sphingolipids. Combinations of moniliformin and fumonisin B_1 at levels of

20:40 and 40:40 mg/kg diet did not significantly change the effect of fumonisin B_1 on the ratio of sphingolipids. The only tissue lesions observed in liver and heart were smaller nuclei of cells in livers of fish fed diets containing the two highest levels of moniliformin and the combinations of the two toxins.

Nguyen *et al.* (2003) evaluated responses of Nile tilapia in terms of growth, histological anomalies, and biochemical changes to subchronic and toxic concentrations of fumonisin B_1 (FB_1) and moniliformin (MON) under controlled environmental conditions. Nile tilapia fingerlings (2.7 g) were fed diets containing 0, 10, 40, 70, 150 mg/kg of either FB_1 or MON for 8 weeks. These mycotoxins were obtained from *Fusarium moniliforme* or *Fusarium proliferatum* culture materials, respectively. Among tilapia fed diets containing MON, fish fed either 70 or 150 mg MON/kg diet had significantly ($P<0.05$) lower mean weight gains than the control fish. However, tilapia fed diets containing FB_1 at levels of 40 mg/kg or higher had significantly lower mean weight gains than the control fish. Mortality was low; differences in percent survival among diets were not observed. Hematocrit was significantly reduced only in fish fed diets containing 150 mg of FB_1 or MON/kg diet. Serum pyruvate levels were significantly higher than control fish for all tilapia fed MON. The ratio between free sphinganine and free sphingosine (SA/SO) in liver increased significantly in fish fed the diet containing 150 mg FB_1/kg. No histopathological lesions were observed in tilapia fed diets containing either MON or FB_1. Responses of Nile tilapia in this study to dietary FB_1 and MON demonstrate that both mycotoxins are toxic to tilapia and could reduce the productivity of this fish.

Tuan *et al.* (2003) studied the effects of MON (and MON and FB_1 in combination) on Nile tilapia, finding that consumption of feed containing 70 or 150 mg/kg MON caused significantly lower mean weight gain, with significantly reduced hematocrit values at 150 mg/kg MON. Levels of serum pyruvate were significantly higher in all tilapia fed MON (10, 40, 70, and 150 mg/kg). This study also showed that FB_1 is more toxic to tilapia than MON, as FB_1 suppressed fish growth earlier and at lower concentrations than MON.

8. Zearalenone

- ZON is a mycotoxin produced by several *Fusarium* spp. (particularly *F. graminearum*, but also *F. culmorum*, *Fusarium cerealis*, *F. equiseti*, *F. verticillioides*, and *Fusarium incarnatum*) and is an abundant contaminant of maize. These molds also produce small amounts of a number of related metabolites, α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL) being the most important derivatives (Richardson *et al.* **1985**).
- ZON and its metabolites α-ZOL and β-ZOL are estrogenic compounds that imitate natural estrogens, with α-ZOL having a higher estrogenic potential than ZON and β-ZOL because of a greater binding affinity to estrogen receptors (Hagler et al. **1979**; Fitzpatrick et al. **1989**; Le Guevel and Pakdel **2001**).
- The oestrogenic potency of ZON and its metabolites has been evaluated in Atlantic salmon by injecting 1.0 and 10.0 mg/kg i.p. and compared to fish

injected with estradiol-17β (5.0 mg/kg). Exposure to ZON and α-ZOL (but not β-ZOL) induced a dose-dependent increase in vitellogenin and greater expression of zona radiata protein (*Zr*-protein) in plasma, suggesting that α-ZOL has greater estrogenic potential than ZON, and that *Zr*-protein may be a more sensitive biomarker than vitellogenin (**Arukwe *et al.* 1999**).

- A slight tendency toward prolonged clotting time and lowered iron concentrations in the liver and ovary after exposing juvenile rainbow trout to 10 mg/kg ZON i.p. for 24, 72, and 168 h was observed by **Woźny et al. (2012).**
- ZON concentrations in commercial fish feed for cyprinids in Central Europe was assessed by Pietsch *et al*. (**2013**), while Greco *et al*. (**2015**) examined samples of rainbow trout feed in Argentina. Observed concentrations did not exceed an average level of 0.068 mg/kg (Central Europe) and 0.088 mg/kg (Argentina), suggesting that ZEN poses no threat to fish under aquaculture.

Reports:

Vanyi *et al.* (1974) studied the effects of zearalenone (ZON) on carp. Carp was fed with maize groats containing 1,000 ppm ZON and consumed 2-3 % of their body weight. In the testicles of treated fish severe degeneration of the caniculi was found. The alterations due to the toxin was reversible.

Arukwe *et al.* (1999) found that - zearalenol and ZON possess estrogenic potencies that are approximately 50% to that of estradiol-17ß. They concluded that blood analysis of vitellogenin and eggshell zona radiate (ZR) – proteins levels provides a suitable in vivo fish model for assessing the estrogenic potencies of ZON and its metabolites.

Celius *et al.* (2000) also came to the same conclusion that trout ZR – gene and proteins provide a sensitive biomarker for assessing oestrogenic activity of ZON .

Woźny *et al.* (2013) reported the concentrations of ZON in selected organs of rainbow trout (part of the dorsal white muscleswith skin, the ovary, the liver and gallbladder, and the caudal part of the intestine with its content) that were purchased from three commercial fish farms in north-eastern Poland. ZON was not detected in the trouts' muscles, and in the liver and the intestines only trace amounts of the mycotoxin were found (b2.0 μg·kg−1). Interestingly, the highest concentrations of ZON were found in the fish's ovaries (up to 7.1 μg·kg−1). Additional analyses of system (surface) water and fish feed samples from the farms indicate that animal feedmay be a possible source of ZON contamination (concentration up to 81.8 μg·kg−1). They concluded that ZON contamination may pose little health risk (if any) to the consumers of the fish. However, accumulation of this mycotoxin in the ovaries may be a concern for the aquaculture industry. Further research should evaluate the scale of this problemand answer whether the concentrations of ZON found in feed affect fish production, especially reproduction.

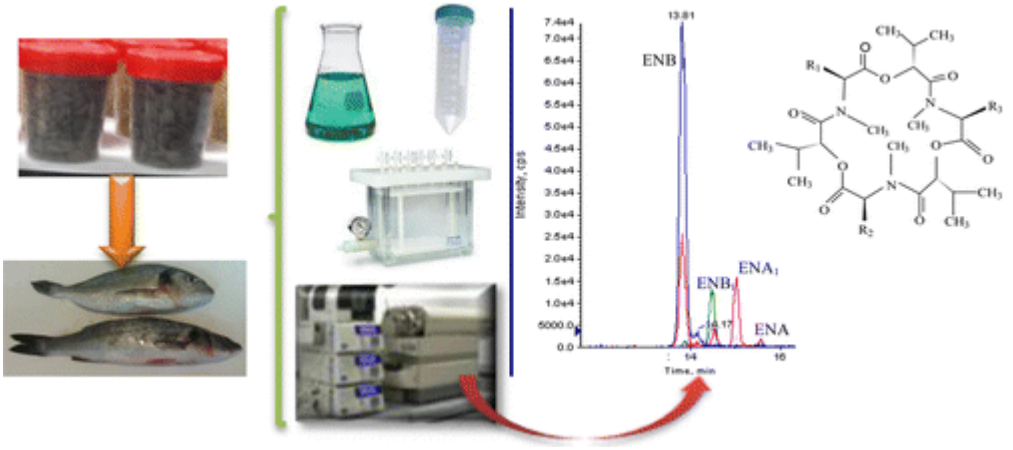
Schwartz *et al.* (2011) investigated the consequences of continuous long-term ZON exposure, including a subsequent depuration period, as well as transgenerational

effects of F0 short-term exposure on F1 generation. Effects on growth, reproduction activity, physiology, and morphology of zebrafish (Danio rerio) were examined in a 182 day live-cycle experiment. Life-long exposure to ZON for 140 days increased wet weight, body length, and condition factor of female fish at 1000 ng/L, and sex ratio was shifted toward female from 320 ng/L ZON. Only females at 1000 ng/L ZON revealed a 1.5-fold induction of plasma vitellogenin (VTG). Relative fecundity at 1000 ng/L recovered significantly during the depuration period. An increased condition factor in adult female F1 fish implies that exposure of F0 generation to 1000 ng/L ZON affected growth of F1 generation. A negative correlation between relative fecundity in the F1 generation (all groups exposed to 320 ng/L ZON) and the nominal ZON concentrations of the F0 exposure might indicate an influence of F0 exposure on reproductive performance of F1 generation. No exposure scenario affected fertility, hatch, embryo survival, and gonad morphology of zebrafish. Evaluating the environmental relevance of this data, the risk for fish to be harmed by exposure to ZON solely seems rather marginal, but ZON might contribute to the overall estrogenicity in the environment.

Pietsch *et al.* (2015) investigated the effects of dietary exposure to ZON on carp (*Cyprinus carpio* L.). ZON at three different concentrations (low dose: 332 µg kg^{-1}, medium dose: 621 µg kg^{-1} and high dose: 797 µg kg^{-1} final feed, respectively) was administered to juvenile carp for four weeks. Additional groups received the mycotoxin for the same time period but were fed with the uncontaminated diet for two more weeks to examine the reversibility of the ZON effects. No effects on growth were observed during the feeding trial, but effects on haematological parameters occurred. In addition, an influence on white blood cell counts was noted whereby granulocytes and monocytes were affected in fish treated with the medium and high dose ZON diet. In muscle samples, marginal ZON and α-zearalenol (α-ZEL) concentrations were detected. Furthermore, the genotoxic potential of ZON was confirmed by analysing formation of micronuclei in erythrocytes. In contrast to previous reports on other fish species, estrogenic effects measured as vitellogenin concentrations in serum samples were not increased by dietary exposure to ZON. This is probably due to the fact that ZON is rapidly metabolized in carp.

9. Enniatins (ENs) and beauvericin (BEA)

Tolosa *et al.* (2014) developed. a new analytical method for the simultaneous determination of enniatins (ENs) and beauvericin (BEA) in fish feed and fish tissues by liquid chromatography coupled to mass spectrometry with linear ion trap (LC-MS/MS-LIT).Results showed that the developed method is precise and sensitive. The presence of emerging Fusarium mycotoxins, ENs and BEA, was determined in samples of aquaculture fish and feed for farmed fish, showing that all feed samples analyzed were contaminated with mycotoxins, with 100% coexistence. In aquacultured fish samples, the highest incidence was found in edible muscle and liver. As for the exposure assessment calculated, it was found that average consumer intake was lower than tolerable daily intake (TDI) values for other Fusarium mycotoxins.



A new analytical method for the simultaneous determination of enniatins (ENs) and beauvericin (BEA) in fish feed and fish tissues by liquid chromatography coupled to mass spectrometry with linear ion trap (LC-MS/MS-LIT) was developed. Results showed that the developed method is precise and sensitive. The presence of emerging *Fusarium* mycotoxins, ENs and BEA, was determined in samples of aquaculture fish and feed for farmed fish, showing that all feed samples analyzed were contaminated with mycotoxins, with 100% coexistence. In aquacultured fish samples, the highest incidence was found in edible muscle and liver. As for the exposure assessment calculated, it was found that average consumer intake was lower than tolerable daily intake (TDI) values for other *Fusarium* mycotoxins.

10. Cyclopiazonic acid

Jantrarotai and Lovell (1990) mentioned that the 96-h median lethal dose (LD50; dose that is lethal to 50% of test organisms) of cyclopiazonic acid (CPA) injected intraperitoneally (IP) into channel catfish *Ictalurus punctatus* (average weight, 19 g) was 2.82 mg/kg of body weight, with a 95% confidence interval of 2.483.12 mg/kg. The acute effects of CPA were characteristic of a neurotoxin. Some fish injected with CPA doses of 2.40 mg/kg of body weight or higher showed severe convulsions, tetany, and death within 30 min postinjection. There were no lesions in the organs of the moribund fish examined grossly and histologically. Cyclopiazonic acid fed for 10 weeks at a concentration of 100 µg/kg of diet had a growth-suppressing effect ($P < 0.05$) on channel catfish (average weight, 7.5 g), and a concentration of 10,000 µg/kg caused accumulation of proteinaceous granules in renal tubular epithelium and necrosis of gastric glands. Cyclopiazonic acid had no effects on hematocrit, hemoglobin concentration, and erythrocyte and leukocyte counts ($P > 0.05$).

Lovell (1992) described signs of cyclopiazonic acid (CPA) toxication in channel catfish fed on 100 ppb CPA as reduced growth rate. The highest concentration (10 ppm) caused necrosis of the gastric glands. The IP LD_{50} for CPA was 2.82 mg/Kg. The effects of CPA were characteristic of a neurotoxin. Fish showed severe convulsions. So, CPA is more toxic to catfish than AFB_1. The fact that CPA and AFB_1 are found under similar conditions, often in combination with $AFB_1$and often more frequently, indicates that CPA may be a serious contaminant in fish feeds. Cyclopiazonic acid fed for 10 weeks at a concentration of 100 µg/kg of diet had significantly growth-suppressing effect on catfish and a concentration of 10 mg/kg caused accumulations of proteinaceous granules in renal tubular epithelium and necrosis of gastric glands.

11. Citrinin

Sahoo *et al*. (1999) carried out a preliminary experiment to evaluate the toxic effect of citrinin in rohu (Labeo rohita) fingerlings by intraperitoneally injecting two doses of citrinin (12.5 and 25.0mg/kg body weight). The toxin treated fish showed damage to the kidney, liver and intestine along with clinical signs of depigmentation and congestion of caudal fins and mortality. The causes of mortality were suggestive of acute nephrotoxic and hepatotoxic effects of citrinin in fish model.

Wu *et al.* (2012) applied zebrafish embryos to investigate the developmental toxicity of CTN on embryonic kidney. In the presence of CTN, the gross morphology of kidneys from embryos with green fluorescent kidney (wt1b:GFP) was not apparently altered. Histological analysis of CTN-treated embryos indicated cystic glomerular and tubular lesions. From the view point of renal function, dextran clearance abilities of embryos exposed to CTN were significantly reduced. The damaged renal function caused by CTN could be partially rescued by the administration of pentoxifylline, suggesting the reduction of glomerular blood flow contributes to CTN-induced renal dysfunction. Additionally, CTN induced the expression of proinflammation genes, including COX2a, TNF-α and IL-1β, but failed to modify the levels and distribution of wt1a transcript and Na(+)/K(+)-ATPase protein. In summary, CTN and PAT caused profound nephrotoxicity in histological structure and biological function of zebrafish embryos; the inflammatory pathway and blood rheology may involve in CTN-induced renal impairment.

12. Patulin

Wu *et al.* (2012) applied zebrafish embryos to investigate the developmental toxicity of PAT on embryonic kidney. In the presence of PAT, the gross morphology of kidneys from embryos with green fluorescent kidney (wt1b:GFP) was not apparently altered. Histological analysis of PAT-treated embryos indicated a disorganized arrangement of renal cells.

Nguyen (2014) attempted to evaluate whether the adult zebrafish is a good neuro-behavioral screening model to assess the neurotoxicity of patulin (PAT), a fungal mycotoxin found in apple juice. This preliminary study provided an experimental framework to investigate potential treatment intervention for prevention against behavioral disruptions caused by PAT and related mycotoxins. He observed that as the concentration of PAT increased, the locomotor activity seemed to increase. Compared to the DMSO control, 5 μg PAT-infected fish seemed to be the most effective dose in the experiment. Moreover, the ceiling limit of PAT dose seemed to establish at 10 μg (or 1 μg/μl PAT/DMSO). From the data collected, he postulated that differences in fish weight and the high instability of PAT in solution might contribute to its overall toxicity. Thus, a weight-dependent dose injection method for individual fish, and a special attention to the chemical stability are recommended. Due to limited number of subjects tested, further research is warranted to confirm the possibility of utilizing adult zebrafish in studying toxicity of PAT.

13. Ergot Alkaloids

- Alkaloids derived from species of the genus *Claviceps:*
  - *Claviceps purpurea*
  - *Claviceps fusiformis*
  - *Claviceps paspali*
  - *Claviceps africana*
- The alkaloids comprise 3groups:
  - the amino acid alkaloids typified by ergotamine,
  - the dihydrogenated amino acid alkaloids such asdihydroergotamine,
  - the amine alkaloids such as ergonovine.
- Ergot poisoning may result from accidental ingestion of contaminated grain.

- Ergot-rye concentrations of 30 and 50% in carp caused circulatory failure in the organs and tissues, dystrophia of gill lamellae, and occurrence of cellular polymorphonuclear subepithelial infiltrates in the renal tubuli.
- Short-term action of ergot induced a typical intoxication reaction, with 60% mortality and serious organ and tissue damage.

Reports:

Svobodova *et al.* (1981) investigated the effect of EAs in fish. A 15-wk comparative feeding trial on common carp with 4 and 14% of rye-ergot in the feed was performed by. They observed no significant difference between test groups in almost all characteristics assessed (mortality, pathoanatomic findings, condition, hematological and biochemical parameters, health condition). Furthermore, analysis of muscles and the hepatopancreas of experimental fish for EA residues proved negative. On the other hand, histopathological examination of the organs and tissues found circulatory disorders (passive congestion) in all parenchymatous organs in experimental carp. Quantitative and qualitative differences were not dependent on percentage content of ergot in feed. Higher concentrations of ergot resulted in a more pronounced desquamation and a higher mucus content in the intestinal mucosa and submucosa.

Svobodova *et al.* (1983) studied the effects of short-term oral administration of EAs on the health and behavior of carp, as well as any pathoanatomic or histopathological changes. The results showed that 4 and 10% ergot-rye in the feed had no clinical or pathoanatomic effect on carp but that concentrations of 30 and 50% caused circulatory failure in the organs and tissues, dystrophia of gill lamellae, and occurrence of cellular polymorphonuclear subepithelial infiltrates in the renal tubuli. Short-term action of ergot-rye alone induced a typical intoxication reaction, with 60% mortality and serious organ and tissue damage.

Mycotoxins in fish feeds

- Mycotoxin-contaminated fish feed is a widespread problem, especially in tropical regions and developing countries/
- Presence of *Fusarium* mycotoxins, trichothecenes (deoxynivalenol (DON) and T-2 toxin), fumonisins (FUM) and zearalenone (ZEA) in contaminated fish feeds can cause adverse effects:
    - Trichothecenes cause reduced feed intake and growth rates, performance reduction, immune impairment and organ lesions.
    - Zearalenone can have estrogenic effects, and fumonisins can cause reduce growth and increased liver glycogen. Rainbow trout is also very sensitive to DON present in naturally-contaminated grains.
    - Fumonisin toxicity disrupts sphingolipid metabolism, which provokes abnormal higher levels of sphinganine accumulation in different tissues, including the liver.
    - Fumonisin B1 promotes aflatoxin B1 and *N*-methyl-*N′*-nitro-nitrosoguanidine-initiated liver tumors in rainbow trout.
    - T-2 toxin reduces feed consumption and growth and lowers the hematocrit and blood hemoglobin in rainbow trout at levels higher than 2.5 ppm.
    - T-2 toxin levels above 10 ppm have caused gastrointestinal bleeding and regurgitation in adult trout.

• Occurrence of mycotoxins in fish feeds

Toxigenic fungi and their toxins are found often in various feeds of plant and animal origins including *Aspergillus flavus, A. niger, Mucor,* and *Pencillium* . The following Table illustrates some Egyptian aquafeeds and their mycotoxins content (Abdelhamid, 1980, 1983a - e, 1985, 1990, 2000b & 2005 and Abdelhamid *et al., 1996*)**:**

Feeds	**Mycotoxins**
Bone meal	Vomitoxin and Zearalenone
Cottonseed meal, bran	Aflatoxin-B_1, Citrinin, Ochratoxin-A, Vomitoxin, and Zearalenone
Grains	Aflatoxin-B_1 & G1, Citrinin and Ochratoxin-A
Maize	Aflatoxin-B_1, Fumonisins, Ochratoxin-A and Vomitoxin
Maize flour, beans	Aflatoxins, Cyclopiazonic acid, Patulin and Griseofulvin
Maize, peanut meal, sunflower meal, sorghum, wheat	Aflatoxins, Cyclopiazonic acid, Ochratoxin-A, and Zearlenone
Maize, Peanut oil	Aflatoxin-B1
Milk products	Aflatoxins-B_1, B_2. M1 and Patulin
Peanut, rice	Cyclopiazonic acid

Rice bran	Aflatoxin-B_1, Ochratoxin-A, Citrinin, Vomitoxin, Cyclopiazonic acid and Moniliformine

- **Mycotoxin residues in fish**
  - Residues of aflatoxin B_1 (AFB_1) were detected in fish muscle under experimental conditions (**Hussain et al. 1993; El-Sayed and Khalil 2009; Huang et al. 2011; Nomura et al. 2011**),
  - **Deng et al. (2010) and Svobodova and Piskac (1980)** noted residues in fish muscle and in the liver or hepatopancreas.
  - **Abdel-Wahaab et al. (2005)** reported residues of sterigmatocystin in edible tissue of Nile tilapia, *Oreochromis niloticus*, following intragastric dosing, was observed by
  - **Woźny et al. (2013)** found **t**race amounts of ZON in ovaries (but not muscle) of rainbow trout from Polish fish farms, where a possible source of the ZON was thought to be animal feed
  - **Tolosa et al. (2014)** found 65% of muscle samples aquacultural fish positive for enniatin B1[EN] and 50% positive for EN B1.

Reports:

Wu (1999) detected residues of AFB_1, OCTA , and FB_1 in flesh and other tissues of channel catfish soon after the fish consumed these mycotoxins in their diets. The rate at which they were retained in the tissues varied among mycotoxins and tissues. Net absorption coefficients were relatively high, being 83.5, 83.7, and 87.8%, respectively

Yildirim *et al.* (2000) reported that combinations of moniliformin (MON) and FB_1 at levels of 20:40 and 40:40 mg/kg diet did not significantly change the effeect of FB_1 on the ratio of sphingolipids. The only tissue lesions observed in liver and heart were smaller nuclei of cells in livers of fish fed diets containing the two highest levels of MON (60 & 120 mg/kg) and the combinations of the two toxins

Hashimoto *et al.* (2003) evaluated the risk of mycotoxin contamination (aflatoxin and fumonisin) in 42 feed samples, belonging to five commercial industries, and used in fishing activity of the Region of Londrina-PR. The aflatoxin levels ranged from non-detectable to 15.60 ng/g, where 61.90% showed < 4ng/g levels, which are in accordance with the Brazilian guideline (20 ng/g). Related to fumonisin (n.d. to 11,22¼g/g), 76.20% samples were into the levels < 4¼g/g. There was no significant difference between pellet and extruded feeds concerning mycotoxin contamination (p>0,05), but the aflatoxin/fumonisin co-occurrence in 23.8% feed samples suggested risk of toxic synergism, emphasizing the importance of mycotoxin monitoring in fish feeding quality. Taking into account the continuous renewal of feed in the fishing ones, there is low possibility of aflatoxin/fumonisin production due the storage, therefore the critical point should be targeted on crude material at field to pre-processing stage, independently of mark or nutritional differences.

Tuan *et al.* (2003) fed Nile tilapia fingerlings (2.7g) on diets containing 0,10,40,70,or 150 mg/kg of either FB_1 or MON for 8 weeks. Fish fed MON (either at 70 or 150 mg/kg) or FB_1 (at 40 mg/kg or higher) had significantly lower weight gain than the control. Results of hematocrit, serum pyruvate, and sphinganine/sphingosne ratio in liver demonstrated that both mycotoxins were toxic to tilapia.

Santos *et al.* (2010) mentioned that a recent survey on the occurrence of mycotoxins in feed components showed that analyzed samples from different regions were contaminated with one (74%) or more (40%) mycotoxins. Despite good screening programs, selection of high quality raw materials and feed ingredients and good storage conditions it is very difficult to guarantee the absence of mycotoxins in aquaculture feeds. Therefore it is urgent to find suitable ways to face the problem through an effective management of the risks posed by mycotoxins contaminations. The current paper presents an overview of the effects of mycotoxins on fish and shrimp performance and the occurrence of these mycotoxins in feed components.

Alinezhad *et al.* (2011) investigated the mycobiota and natural occurrence of aflatoxin B_1 (AFB_1) in pellet feed and feed ingredients used in a feed manufacturing plant for rainbow trout nutrition. The samples were cultured on the standard isolation media for 2 weeks at 28 ºC. $AFB_1$was detected using high performance liquid chromatography (HPLC). Based on the results obtained, a total of 109 fungal isolates were identified of which *Aspergillus* was the prominent genus (57.0%), followed by *Penicillium* (12.84%), *Absidia* (11.01%) and *Pseudallscheria* (10.10%). The most frequent *Aspergillus* species was *A. flavus* (60.66%) isolated from all feed ingredients as well as pellet feed. Among 37 *A. flavus* isolates, 19 (51.35%) were able to produce AFB_1 on YES broth in the range of 10.2 to 612.8 µg/g fungal dry weight. HPLC analysis of trout feed showed that pellet feed and all feed ingredients tested except gluten were contaminated with different levels of AFB_1 in the range of 1.83 to 67.35 µg/kg. Unacceptable levels of AFB_1 were reported for feed including soybean, fish meal and wheat. These results indicated the importance of AF contamination of trout feed in amounts higher than the acceptable level as a risk factor for fish farming production.

Cardoso Filho (2011) performed a study to determine the occurrence of fungi and aflatoxins in fish feeds. He analyzed 36 samples of feed for fish, with two protein compositions (juvenile/fattening) and two forms of use (sealed/open). Aspergillus and Penicillium species were counted, isolated and identified, the toxic capacity of Flavi strains was measured and aflatoxins in the feed were researched. The mean fungal counts ranged from 2.96 to 4.00 log10 CFU/g and there was no significant difference between treatments. The most isolated species were Aspergillus flavus. It was concluded that the feeds studied had high fungal counts; the isolated Aspergillus flavus strains were not producers of aflatoxin; and aflatoxin was not detected in the feed samples analyzed,

Hashem (2011) evaulated contamination of fish grown in aquacultures with potentially mycotoxin-producing microfungi. Five fishes including **Nile tilapia**, African catfish, Tilapia zilli, Bony bream and Thinlip Mullet species were collected from different aquacultures distributed in Delta region, Egypt. From each fish species, at least 10 random samples were subjected for the fungal analysis. The most common isolated fungi were tested for their potentiality to produce mycotoxins ***in vitro*.** Detection of the mycotoxins was carried out by thin layer chromatography compared

to mycotoxin standards. Results showed that 21 fungal species were isolated from five fish species. The highest number of species (15) was isolated from African catfish, but the lowest number (6 species) was isolated from Bony bream. The most common fungal species isolated from these fish species were; *Paecilomyces lilacinus*, *P. variotii* and *Phoma herbarum*. Mycotoxin producing fungi including ***Aspergillus flavus,*** *A. clavatus, A. ochraceous, A. parasiticus, A. sydowii, A. terreus, A. versicolor Penicillium chrysogenum* and *Trichoderma viride* were recovered. Production of aflatoxin B1, B2 and G1, strigmatocystin ochratoxin and T2-toxin by these fungal species was approved. Most species were found to produce aflatoxin and stregmatocystin while ochratoxin was produced only by *A. sydowii* and *A. versicolor*. This study proved the infection of aquacultures' fishes with mycotoxin-producing fungi.

Abdual-shahid *et al.* (2013) provides a brief review of approaches for the early detection of fungi and their metabolites in feed of fish from some Baghdad farms. During a mycological analysis of complete feed mixes(15 samples), a total of five genera of moulds were identified. *Penicillium* spp. was present in considerably more samples than any other genus 36.4%, followed by the genera *Fusarium* spp.24.5%. Other fungi from the genera *Aspergillus* spp. 20%, *Mucor* spp. 11.1% and *Alternaria* spp. 8% were represented in a smaller amount. The mycotoxinsdeoxynivalenol and zearalenone were detected. Deoxynivalenol was detected in 10 samples in the concentration range 0.25–2.5 mg/kg. Zaralenone were detected in 8 samples in the concentration range 0.2–5.0 mg/kg.Thesefindings indicate that there may be a risk for animal exposure to mycotoxins through the consumption of moldy infected feeds.

Barbosa *et al.* (2013) determined species of the fungal genera *Aspergillus*, *Fusarium*, and *Penicillium* and fumonisin B_1 (FB_1), aflatoxin B_1 (AFB_1), and ochratoxin A (OTA) contamination from feed intended for fish farms. A total of 60 samples were sampled from tilapia farms in the Rio de Janeiro State, Brazil. The quantitative enumeration of fungi as colony-forming units per gram of feed (CFU/g) was performed using the surface spread method in different culture media. The results were expressed as fungal isolation frequency and relative density. Fungal total counts ranged from $<1 \times 10^2$ to 4.7×10^4 CFU/g. *Fusarium* counts were not observed. Among toxigenic genera, *Aspergillus* (68%) was the most prevalent, followed by *Penicillium* species (60%). *Aspergillus niger* (36%), *Aspergillus flavus* (35%), and *Penicillium citrinum* (71%) were the most prevalent species. A high percentage of samples (98%) were contaminated with FB_1 levels, while 55% and 3.3% were contaminated with $AFB_1$and OTA, respectively. The simultaneous occurrence of these mycotoxins emphasizes the need for further research in the area to better assess the risk to the health of fish farms and their implications for the health of consumers of this meat.

Pietsch *et al.* (2013) reported for the first time the occurrence of DON and ZEN in samples of commercial fish feed designed for nutrition of cyprinids collected from central Europe. A maximal DON concentration of 825 $\mu g\ kg^{-1}$ feed was found in one feed whereas average values of 289 $\mu g\ kg^{-1}$ feed were noted. ZEN was the more prevalent mycotoxin but the concentrations were lower showing an average level of 67.9 $\mu g\ kg^{-1}$ feed

Embaby *et al.* (2015) analyzed feedstuff used for fish nutrition in Egypt for fungal

flora and natural incidence of selected mycotoxins. Seven fungal species belonging to four fungal genera were isolated and identified from these samples. These genera were Asperigillus (*A. Flavus, A. parasiticus, A. niger* and *A. ochraceous*), *Penicillum, Fusarium* and *Alternaria* spp. , of which six fungal isolates associated fish feeds were found to be produce one or more mycotoxin, i. e. aflatoxins, ochratoxin A (OTA) and fumonisin B1 (FB1

Table 5: Mycotoxins contaminated fish feed samples

Sample		Type of		Mycotoxin conc. (ng/ml)				
No	Location	fungi	mycotoxin	AFG_1	AFB_1	AFG_2	AFB_2	Total
1	Sharkiya	*A. flavus*	Aflatoxin Afs	ND	1.5	ND	ND	1.5
2	Cairo			ND	1.4	ND	ND	1.4
3				ND	.05	ND	ND	0.05
4	Qalubiya			ND	40.0	ND	ND	40.0
5	Qalubiya			ND	0 .018	ND	ND	0.018
6	Qalubiya	*A. parasiticus*		0.027	1.91	0.038	0.018	2.155
7	Sharkiya	*Fusarium* sp.	Fumonisin FB_1	0.412				0.412
8	Qalubiya	*Penicillium* sp.	Ochratoxin OTA	0.03				0.03
9	Cairo			0.025				0.025
10	Sharkiya	*A. ochraceous*		125.92				125.92
11				4.44				4.44

Greco *et al.* (2015) analyzed samples of rainbow trout feed with the aim to determine the mycobiota composition and the co-occurrence of mycotoxins. A total of 28 samples of finished rainbow trout feed from hatcheries in the provinces of Río Negro and Neuquén, Argentina, were studied. Fungal counts were obtained on three culture media in the ranges of <10 to 4.2×10^4 CFU/g on Dichloran Rose Bengal Chloramphenicol Agar (DRBC), <10 to 5.1×10^4 CFU/g on Dichloran Chloramphenicol Peptone Agar (DCPA) and <10 to 3.6×10^4 CFU/g on Dichloran 18% Glycerol Agar (DG18). The most frequent mycotoxigenic fungi were *Eurotium* (frequency (Fr) 25.0%), followed by *Penicillium* (Fr 21.4%) and *Aspergillus* (Fr 3.6%). The most prevalent mycotoxigenic species were *E. repens* (Fr 21.4%) and *E. rubrum* (Fr 14.3%). All samples were contaminated with mycotoxins: 64% samples were contaminated with T-2 toxin (median 70.08 ppb), 50% samples with zearalenone (median 87.97 ppb) and aflatoxins (median 2.82 ppb), 25% with ochratoxin A (median 5.26 ppb) and 3.57% samples with deoxynivalenol (median 230 ppb). Eight samples had a fumonisins contamination level below the limit of detection. Co-occurrence of six mycotoxins was determined in 7% of the samples

Sample	Genus Profile	Species Profile	Mycotoxin Profile					
1	*Cladosporium* yeast	*C. cladosporioides* ---	---	---	---	OTA	T-2	---
2	*Cladosporium*	*C. cladosporioides*	---	---	---	OTA *	T-2	---
3	*Aspergillus* *Cladosporium* yeast, others	*A. versicolor* *C. cladosporioides* ---	---	DON *	---	OTA	T-2	---
4	*Cladosporium*	*C. cladosporioides*	AFs *	DON *	FUM *	---	T-2	ZEA
5	*Cladosporium*	*C. cladosporioides*	---	DON *	FUM *	OTA	T-2	ZEA
6	*Cladosporium* yeast	*C. cladosporioides* ---	AFs	DON *	FUM *	---	T-2	ZEA
7	*Cladosporium* *Trichoderma*	*C. cladosporioides* *T. harzianum*	AFs	DON *	FUM *	OTA *	T-2	ZEA
8	*Cladosporium* yeast, others	*C. cladosporioides* ---	AFs	DON *	FUM *	---	T-2	ZEA
9	*Cladosporium* others	*C. cladosporioides* ---	AFs *	DON *	FUM *	---	T-2	ZEA
10	*Mucor* *Penicillium*	*Mucor* sp. *P. crustosum* *P. corylophilum*	---	DON *	---	OTA	T-2	ZEA *
11	---	---	---	DON *	---	OTA	T-2	ZEA
12	*Cladosporium* *Penicillium* yeast, others	*C. cladosporioides* *C. herbarum* *P. expansum*	AFs	DON *	---	OTA *	T-2 *	ZEA *
13	*Cladosporium* yeast	*C. cladosporioides* ---	AFs	DON *	---	OTA *	T-2 *	---
14	Yeast	---	AFs	---	---	OTA *	T-2	ZEA *
15	*Cladosporium*	*C. cladosporioides*	AFs *	---	---	OTA	T-2	ZEA *
16	*Mucor*	*Mucor* sp.	AFs	---	FUM *	OTA	T-2	ZEA *
17	Yeast	---	AFs	DON *	FUM *	OTA *	T-2	ZEA *
18	*Penicillium*	*P. nalgiovense*	AFs	DON *	---	---	T-2	ZEA *
19	*Cladosporium* *Eurotium* *Mucor* yeast	*C. cladosporioides* *E. repens* *Mucor* sp. ---	AFs	DON	---	---	T-2	ZEA *
20	*Cladosporium* *Penicillium* yeast	*C. cladosporioides* *P. nalgiovense* ---	AFs	DON *	---	---	T-2	ZEA *
21	*Eurotium* yeast	*E. rubrum* ---	---	---	---	OTA *	T-2 *	ZEA *
22	*Mucor* *Penicillium*	*Mucor* sp. *P. nalgiovense*	AFs *	---	---	OTA *	---	ZEA
23	Yeast	---	---	---	---	---	---	ZEA
24	*Cladosporium* *Eurotium* *Penicillium*	*C. cladosporioides* *E. repens*, *E. rubrum* *P. chrysogenum*	---	---	---	---	---	ZEA
25	*Eurotium*	*E. repens*, *Eurotium* sp.	AFs	---	---	---	---	ZEA
26	*Eurotium*	*E. repens*	AFs	---	---	---	---	ZEA
27	*Eurotium*	*E. repens*, *E. rubrum*	---	---	---	OTA *	---	ZEA
28	*Eurotium*	*E. repens*, *E. rubrum*	AFs	---	---	---	---	ZEA

AFs: aflatoxins; DON: deoxynivalenol; FUM: fumonisins; OTA: ochratoxin A; T-2: T-2 toxin; ZEA: zearalenone;
* contamination observed below the value set as the LOD (limit of detection).

Co-occurrence of mycotoxins in rainbow trout feed samples

Sample	Mycotoxins (ppb)											
	AFs		DON		FUM		OTA		T-2		ZEA	
1	<1.7 [a]	0 [b]	<222 [a]	0 [b]	<222 [a]	0 [b]	5.23	-	68.03	-	<50	0 [b]
2	<1.7 [a]	0 [b]	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	3.5 [c]	75.99	-	<50	0 [b]
3	<1.7 [a]	0 [b]	<222 [a]	165 [c]	<222 [a]	0 [b]	5.26	-	83.87	-	<50	0 [b]
4	<1.7 [a]	1.3 [c]	<222 [a]	178 [c]	<222 [a]	190 [c]	<5 [a]	0 [b]	62.57	-	67.98	-
5	<1.7 [a]	0 [b]	<222 [a]	207 [c]	<222 [a]	205 [c]	8.79	-	60.95	-	56.91	-
6	2.7	-	<222 [a]	195 [c]	<222 [a]	209 [c]	<5 [a]	0 [b]	104.44	-	95.53	-
7	1.78	-	<222 [a]	179 [c]	<222 [a]	222 [c]	<5 [a]	4.9 [c]	105.99	-	88.7	-
8	1.97	-	<222 [a]	169 [c]	<222 [a]	208 [c]	<5 [a]	0 [b]	104.22	-	52.65	-
9	<1.7 [a]	1.5 [c]	<222 [a]	155 [c]	<222 [a]	222 [c]	<5 [a]	0 [b]	63.71	-	67.3	-
10	<1.7 [a]	0 [b]	<222 [a]	184 [c]	ND	0	5.14	-	75.35	-	<50	32.0 [c]
11	<1.7 [a]	0 [b]	<222 [a]	156 [c]	<222 [a]	0 [b]	5.52	-	102.69	-	62.83	-
12	2.58	-	<222 [a]	210 [c]	<222 [a]	0 [b]	<5 [a]	3.7 [c]	<50	50 [c]	<50	20.4 [c]
13	2.79	-	<222 [a]	150 [c]	<222 [a]	0 [b]	<5 [a]	4.1 [c]	<50	50 [c]	<50	0 [b]
14	2.97	-	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	3.5 [c]	57.16	-	<50	22.6 [c]
15	<1.7 [a]	1.7 [c]	<222 [a]	0 [b]	<222 [a]	0 [b]	6.28	-	60.87	-	<50	35.0 [c]
16	4.19	-	<222 [a]	0 [b]	<222 [a]	191 [c]	5.23	-	75.23	-	<50	32.6 [c]
17	3.87	-	<222 [a]	205 [c]	<222 [a]	187 [c]	<5 [a]	3.6 [c]	71.05	-	<50	42.1 [c]
18	2.85	-	<222 [a]	164 [c]	<222 [a]	0 [b]	<5 [a]	0 [b]	57.44	-	<50	33.7 [c]
19	3.09	-	230	-	<222 [a]	0 [b]	<5 [a]	0 [b]	60.00	-	<50	39.5 [c]
20	2.77	-	<222 [a]	205 [c]	<222 [a]	0 [b]	<5 [a]	0 [b]	69.11	-	<50	24.3 [c]
21	ND	0	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	5.0 [c]	<50	50 [c]	<50	50.0 [c]
22	<1.7 [a]	1.7 [c]	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	4.8 [c]	ND	0	147.45	-
23	ND	0	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	0 [b]	ND	0	159.76	-
24	ND	0	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	0 [b]	ND	0	102.81	-
25	1.87	-	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	0 [b]	ND	0	95.77	-
26	7.05	-	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	0 [b]	ND	0	71.53	-
27	ND	0	<222 [a]	0 [b]	<222 [a]	0 [b]	<5 [a]	5.0 [c]	ND	0	110.26	-
28	8.91	-	<222 [a]	0 [b]	ND	0	<5 [a]	0 [b]	ND	0	87.24	-
Median [d]	2.82		230		---		5.26		70.08		87.97	
LOD *	<1.7		222		222		5		<20		29	
LOQ *,+	---		222		---		---		50		50	

* ppb (according to the manufacturer's provided information); + limit of quantification; [a] detected below the LOD (limit of detection); [b] estimated concentrations below LOD/√2 were assumed to be zero; [c] concentrations calculated by estimation with the Rida Soft win software; for statistical purposes results >LOD/√2 were considered [47]; [d] median of positive excludes results of estimated concentrations; - values above the LOD were not estimated.

Co-occurrence of at least two out of six mycotoxins was recorded in 93% (26/28) of samples analyzed. Co-occurrence of six mycotoxins was determined in 7% (2/28) of the samples.

Goncalves *et al.* (2016) analysed over a 1-year period, 41 samples of finished aquaculture feed, both shrimp and fish, within the scope of BIOMIN mycotoxin survey programme. The samples were tested for aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A. Samples were sourced in Asia (31 samples) and Europe (10 samples) from fish/shrimp farms or feed producers. The values detected pose a risk for several important aquaculture species, assuming single mycotoxin contamination, that is excluding possible additive and synergetic effects between mycotoxins. Co-occurrence of mycotoxins in feeds may induce synergistic effects and increase the negative impact of mycotoxins in aquatic-farmed species at lower levels than when present in single contamination. This review gives an overview of the different mycotoxins and revises the effects of mycotoxins in aquatic species. Additionally, it reports the levels of mycotoxins in aquafeeds in 2014 and compares detected levels with possible negative effects in fish and shrimp. As it is highlighted by the results of the survey, the risk of co-occurrence is high and the knowledge on the effects of multimycotoxins contamination in aquatic species is basically none

Control of mycotoxins in fish feeds

Soliman *et al.* (1998) found that the presence of Fix-A-tox in the contaminated diet led to a significant decreases in aflatoxin residue ($p < 0.05$) in *O. niloticus* fish. Also, recently Abdelhamid *et al*. (2004d) found that AFB_1 levels were reduced by going on the freezing time of the fish samples in all treatments of aflatoxicated fish. As well as, they reported that addition of 1% egg shell and 2% shrimp wastes to aflatoxicated diets led to adsorptive effects of the dietary aflatoxin and reduced its residue in fish carcass. However, in the present results the effects of ginger and aspirin may be due to their adsorbative characteristics as mentioned before, so prevent or reduce absorption of AFB_1 and hence there were no AFB_1 residues in the fish body and muscles.

Hussein *et al.* (2000) reported that *Nigella sativa* seeds reduced the negative effect of 1.0µg AFB_1/kg BW on internal organs indices of *O. niloticus* fish. Also, Abdelhamid *et al.* (2004 b &d) reported that the best feed additives led to significant overcoming the aflatoxic symptoms on organs indices were egg shell and clay, respectively. Also, they added that the effects of either adsorbents namely, egg shells and shrimp wastes at levels of 1 and 2%, respectively, were useful to reduce the toxic effects of AFB_1 on *O. niloticus* fish via adsorbing the toxin from the fish diets. On the other side, Abdelhamid *et al*. (2002a) found that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce aflatoxicosis symptoms. As well as,

Ellis *et al.* (2000) proved that 2% bentonite in trout diets contaminated with AFB_1 20 µg/kg significantly reduced the amount of AFB_1 absorbed from the digestive system following ingestion.

Sahoo and Mukherjee (2001) reported that feeding of glucan to AFB_1 – induced immunocomromised fish for 7 days significantly raised the degree of resistsnce against *A. hydrophila* challenge and the non-specific immunity level.

Srour (2004) concluded that dietary Biogen® supplementation to OCTA-contaminated diets of Nile tilapia improved all negative effects of OCTA on fish performance and feed utilization.

Abdelhamid *et al*. (2002-b) confirmed that Biogen® supplementation to the aflatoxic diet was not useful and did not completely recover the irreversible toxic effects of AFB_1 on Nile tilapia fish. So, they recommend hygienic control of aqua feeds during buying, transportation, storage and feeding to prevent fungal invasion and mycotoxin production.

Abdelhamid *et al*. (2003 and 2004 a&b) tested some natural materials (clay, egg shells, shrimp shells and betaine) to their effects on AFB_1-contaminated diet of tilapia fish. They found that the best feed additives led to significant overcoming the aflatoxic symptoms (on growth, mortality, feed utilization, organs indices, carcass composition and blood enzymes) were egg shell and clay, respectively. The obtained results showed that AFB_1 led to severe clinical lesions and postmortem symptoms of the aflatoxicated fish, significant ($p \leq 0.01$) decrease in growth performance parameters, survival rate, feed intake and nutrients utilization of fish, dry matter, crude protein and energy content of fish carcass, hemoglobin concentration, red blood cells count and uric acid. As well as, decrease in dry matter and increase in ether extract of the fish liver (fatty liver) were recorded. However, AFB_1 caused significant

($p \leq 0.01$) increase in mortality rate, organs indices, feed conversion ratio, fat and ash contents of fish carcass, white blood cells count, alkaline phosphatase, glutamic oxaloacetic transferase and glutamic pyruvic transferase activities in aflatoxicated fish. On the other side, residues of AFB_1 (ppb) were found in the whole body of the aflatoxicated fish directly at the end of the experiment and tended to decrease after freezing periods. As well as, severe histological alterations were recorded in livers, kidneys, intestines and gills of the aflatoxicated fish. Also, the results indicated that the effects of either adsorbents namely, egg shells and shrimp wastes at levels of 1 and 2%, respectively, were useful to reduce the toxic effects of AFB_1 on *O. niloticus* fish via adsorbing the toxin from the fish diets.

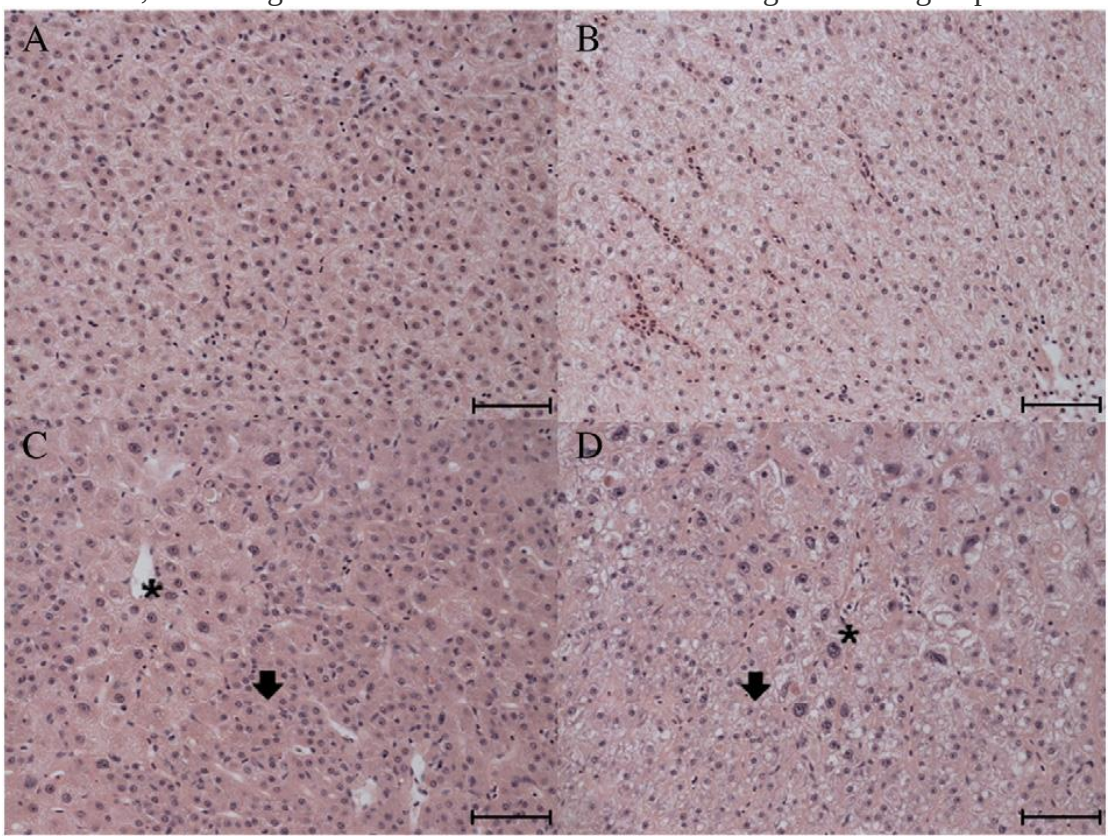
Abdel-Wahhab *et al*. (2005) showed that the Egyptian montmorillonite (EM) at levels of 0.5,1,2, and 4 mg/l had a high capacity of adsorbing STC at different concentrations (5,10, and 50 µg/ml aqueous solution) tested. The adsorption ranged from 93.1 to 97.8%. Nile tilapia fish received an intragastric dose of EM in corn oil (0.5 mg/kg body weight) with (1.6 µg/kg body weight) or without STC twice a week for 4 weeks. The results revealed that STC was toxic and clastogenic to fish as indicated by the significant decrease of body weight and the increase in the frequencies of micronucleated red blood cells and chromosomal aberrations in the kidney compared with the group treated with STC alone. So, it could be concluded that EM was safe and successful in the prevention of STC toxicity and clastogenicity.

Zaki *et al.* (2008) conducted a study to evaluate the ability of Fix in Toxin 0.2 % and Nigella sativa oil 1% to diminish the clinical signs of aflatoxicosis in Tilapia Zilli fish. 60 Tilapia Zilli fish were divided into three groups, 20 fish for each group: Group 1 served as control and will be fed on commercial fish diet. Group 2 were be supplied by Aflatoxin contaminated ration with corn 80 ug toxin /kg ration. Group 3 were be supplied by aflatoxin contaminated ration with corn 80 ug toxin/kg ration and treated with 0.2 % Fix in Toxin and 1 % Nigella sativa oil injected daily I/P. Analysis of hematological parameters, clinical chemistry revealed significant differences between the control groups and the aflatoxicotic groups. administration of Fix in Toxin 0.2% and Nigella sativa oil injection 1% of body weight reduced the aflatoxicosis in liver and kidney by improving all liver and kidney enzymes.The dietary HSCAS clay remedy is novel, inexpensive and easily disseminated and proves its efficacy in diminishing the clinical signs of aflatoxicosis in fish, where it acts as an alfatoxin enterosorbant that tightly and selectively binds the poison in the gastrointestinal tract of the fish, decreasing their bioavailability and associated toxicities. In addition the Nigella sativa oil has a synergistic effect with Fix in Toxin in diminishing aflatoxicosis in fish.

Abdelaziz, M., *et al.* (2010) detected total aflatoxin and ochratoxin in 3 naturally contaminated fish feed samples using immune-affinity method. The results revealed that the average levels of aflatoxins in the 3 examined samples were (15, 22, and 12 µg/kg) respectively while the average levels of ochratoxins were (15, 6, and 6 µg/kg). The results of determination of the effects of clay as a mycotoxin binder on the health status and performance of *Oreochromis niloticus* in comparing with a control group revealed that the survival rate in control group was 81% after the end of the culture season. The results also revealed that the survival rate in group 2 which received clay treated feed was 86%.. Higher performance parameters were recorded in group 2 that received feeds treated with clay which reflected in the total production which reaches

1,646.47 kg while in the control pond, the total production was 1,308.36 kg.

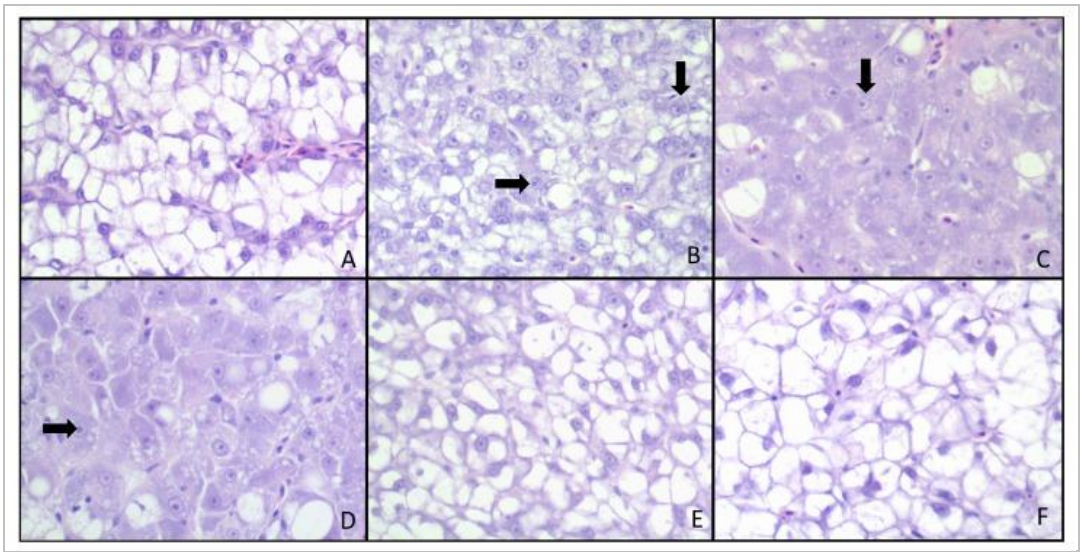
Zychowski *et al.* (2013a) evaluated the ability of NovaSil (NS) clay to sorb and mitigate the toxic effects of aflatoxin B_1 (AFB_1) in Nile tilapia (*Oreochromis niloticus*). Growth performance, targeted innate immunological function, intestinal microbial community and histology were evaluated after feeding tilapia diets with or without $AFB_1$and/or NS for 10 weeks. Aflatoxin B_1 at concentrations of 1.5 and 3.0 ppm significantly ($P < 0.05$) decreased weight gain, feed efficiency, hepatosomatic index and macrophage extracellular superoxide anion production in tilapia, regardless of NS addition to the diet. The overall results regarding the efficacy of NS were mixed; however, there was a trend ($P = 0.157$) towards AFB_1-toxicity prevention in regards to macrophage extracellular superoxide anion production. Additionally, when 0.5 and 1% NS was included in diets containing 1.5 ppm AFB_1, total histopathological score was lowered; however, this protective effect was not evident when fish were exposed to 3.0 ppm AFB_1. Denaturing gradient gel electrophoresis was performed to assess the effects of both AFB_1 and NS on gut microbiota, but no significant differences were found among treatment groups



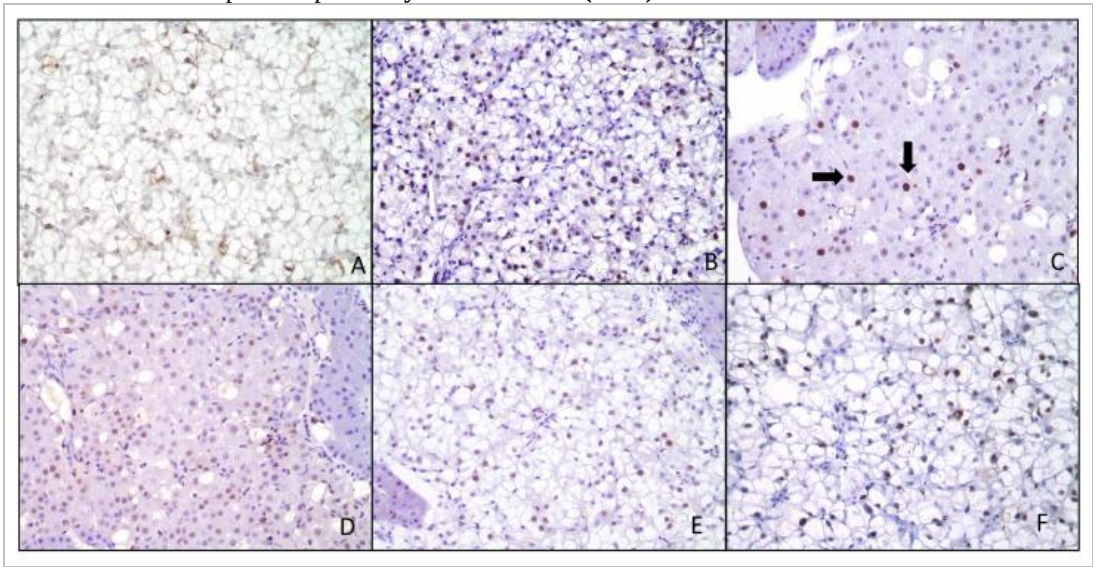
Microphotographs of liver sections. Nile tilapia (Oreochromis niloticus) were fed combinations of aflatoxin B1 (AFB1) and NovaSil (NS) (400× magnification, H/E staining). 0 ppm AFB1+0% NS (A) and 0 ppm AFB1+0.5%NS (B) treatments resulted in normal histological structure, whereas fish fed 1.5 ppm AFB1+0.5% NS (C) and 1.5 ppm AFB1 (D) showed marked cellular pleomorphism, characterized by zones of enlarged, stellated or spindle-shaped hepatocytes (*) surrounded by small polyhedric-shaped hepatocytes (arrow). Increased fatty degeneration was observed in fish fed 1.5 ppm AFB1 (D). Scale bar=50 μm. Results were based on two fish from each of the three replicate groups (n=6). **Zychowski *et al.* (2013)**

Zychowski *et al.* (2013b) designed a study to: (1) evaluate AFB_1 impact on cultured red drum, *Sciaenops ocellatus*, over the course of seven weeks; and (2) assess NS supplementation as a strategy to prevent aflatoxicosis. Fish were fed diets containing

0, 0.1, 0.25, 0.5, 1, 2, 3, or 5 ppm AFB_1. Two additional treatment groups were fed either 5 ppm AFB_1 + 1% NS or 5 ppm AFB_1 + 2% NS. Aflatoxin B_1 negatively impacted red drum weight gain, survival, feed efficiency, serum lysozyme concentration, hepatosomatic index (HSI), whole-body lipid levels, liver histopathological scoring, as well as trypsin inhibition. NovaSil inclusion in AFB_1-contaminated diets improved weight gain, feed efficiency, serum lysozyme concentration, muscle somatic index, and intraperitoneal fat ratios compared to AFB_1-treated fish. Although not significant, NS reduced AFB_1-induced histopathological changes in the liver and decreased Proliferating Cell Nuclear Antigen (PCNA) staining. Importantly, NS supplementation improved overall health of AFB_1-exposed red drum.



Liver histopathology in AFB_1-exposed red drum. Liver sections were stained with hematoxylin and eosin. Treatments were as follows: (**A**) 0 ppm AFB_1 (**B**) 1 ppm AFB_1 (**C**) 3 ppm (**D**) 5 ppm AFB_1 (**E**) AFB_1 + 1% NS and (**F**) 5 ppm AFB_1 + 2% NS. Marked pleomorphism, megalokaryosis with prominent nucleoli (arrows) and loss of hepatocellular cytoplasmic macrovacuolation was observed in the treatment groups that received large amounts of aflatoxin (**B**,**C**,**D**). Although not significant, inclusion of NS resulted in decreased histopathological scores attributable to increased cytoplasmic vacuolation and reduced cellular pleomorphism **Zychowski *et al.* (2013)**



Proliferating Cell Nuclear Antigen (PCNA) positive cells in red drum hepatocytes. Liver sections were stained with PCNA (arrows) and hematoxylin counterstain. Treatments were as follows: (**A**) 0 ppm

AFB_1 (**B**) 1 ppm AFB_1 (**C**) 3 ppm AFB_1 (**D**) 5 ppm AFB_1 (**E**) 5 ppm AFB_1 + 1% NS (**F**) 5 ppm AFB_1 + 2% NS. Although not significant, inclusion of NS resulted in a decrease of PCNA-positive hepatocytes. Reduction in cell proliferation suggests that NS afforded some protection from AFB_1 toxicity and cellular proliferation. **Zychowski *et al.* (2013)**

References:

1. Abdelaziz M, A Wael anwer2 Abeer Hamada abdelrazek. Field Study on the mycotoxin binding effects of clay in Oreochromis niloticus feeds and their impacts on the health status throughout the culture season.ibc, 2010
2. *Abdelhamid, A.M.* Effect of Sterigmatocystin contaminated diets on fish performance [1988]
3. Abdel-Wahhab MA , Hasan AM, Aly SE, Mahrous KF. Adsorption of sterigmatocystin by montmorillonite and inhibition of its genotoxicity in the Nile tilapia fish (Oreachromis nilaticus). Mutat Res. 2005 Apr 4;582(1-2):20-7. Epub 2005 Jan 26.
4. Abdual-shahid, D. K., Oday S. Abbas** ZahidE. Mohammad. ISOLATION AND CHARACTERIZATION OF FUNGI AND MYCOTOXINS (DEOXYNIVALENOL AND ZARALENONE) IN FISH FEED FROM BAGHDAD CITY. Diyala Agricultural Sciences Journal, 5(2) 38 – 44 ,2013
5. Alinezhad S., Tolouee M., Kamalzadeh A., Motalebi A.A., Nazeri M., Yasemi M., Shams-Ghahfarokhi M., Tolouei R., Razzaghi-Abyaneh M. Mycobiota and aflatoxin B1 contamination of rainbow trout (*Oncorhinchus mykiss*) feed with emphasis to *Aspergillus* section *Flavi*. I. J. Fish. Sci. 2011;10:363–374.
6. Almeida I.F.M., Martins H.M.L., Oliveira Santos S.M., Freitas M.S., Nunes da Costa J.M.G., d Almeida Bernardo F.M. Mycbiota and aflatoxin B1 in feed for farmed sea bass (*Dicentrarchus labax*) Toxins.2011;3:163–171
7. Arana S., Alves V.A.F., Sabino M., Tabata Y.A., Nonogaki S., Zaidan Dagli M.L., Hernández Blazquez F.J. Immunohistochemical evidence for myofibroblast like cells associated with liver injury induced by aflatoxin B1 in rainbow trout (*Oncorhynchus mykiss*) J. Comp. Pathol. 2014;150:258–265
8. Arukwe A., Grotmol T., Haugen T.B., Knudsen F.R., Goksoyr A. A fish model for assessing the *in vivo*estrogenic potency of the mycotoxin zearalenone and its metabolites. Sci. Total Environ. 1999;236:153–161.
9. Ashley L.M., Halver J.E. Multiple metastasis of rainbow trout hepatoma. Trans. Am. Fish. Soc.1963; 92:365–371.
10. Ashley, L.M., Halver, J.E. and Wogan, G.N. (1964) Hepatoma and aflatoxicosis in trout. Fed. Proc., 23, p. 105
11. Ashley, L.M. (1970) Pathology of fish fed aflatoxins and other antimetabolites, in S.F. Snieszko (ed.), A Symposium on Diseases of Fishes and Shellfishes, American Fisheries Society, New York, pp. 366-79
12. Barbosa T.S., Pereyra C.M., Soleiro C.A., Dias E.O., Oliveira A.A., Keller K.M., Silva P., Cavaglieri L.R., Rosa C. Mycobiota and mycotoxins present in finished fish feeds from farms in the Rio de Janeiro State, Brazil. Aquat. Res. 2013;5:3.
13. Bailey G.S., Williams D.E., Wilcox J.S., Loveland P.M., Coulombe R.A., Hendricks J.D. Aflatoxin B1 carcinogenesis and its relation to DNA adduct formation and adduct persistence in sensitive and resistant salmonid fish. Carcinogenesis. 1988;9:1919–1926.
14. Bauer, D. H., D. J. Lee, and R. O. Sinnhuber. 1969. Acute toxicity of aflatoxins B_1 and G_1 in the rainbow trout (*Salmo gairdneri*). *Toxicology and Applied Pharmacology* 15:415–419.
15. Bautista MN, Lavilla-Pitogo CR, Subosa PF, Begino ET: **Aflatoxin B_1 contamination of shrimp feeds and its effect on growth and hepatopancreas and pre-adult *Penaeus monodom* .** *J Sci Food Agri* 1994, **65:** 5–11.
16. Bintvihok, A., Ponpornpisit, A., Tangtrongpiros, J., Panichkriangkrai, W., Rattanapanee, R., Doi, K., Kumagai, S., 2003. Aflatoxin contamination in shrimp feed and effects of aflatoxin addition to feed on shrimp production. J. Food Prot. 66 (5), 882–885.
17. *Boonyaratpalin, M., Supamattaya, K., Verakunpiriya, V., Suprasert, D., 2001. Effects of aflotoxin B1 on growth performance, blood components, immune function and histopatological changes in black tiger shrimp (Paneus monodon Fabricius). Aquac. Res. 32 (suppl. 1), 388-398.*

18. Brown DW, McCoy CP, Rottinghaus GE: **Experimental feeding of *Fusarium moniliforme* culture material containing fumonisin B_1 to channel catfish, (*Ictalurus punctatus*).** *J Vet Diagn Invest* 1994,**6**(1):123–124.
19. Cagauan, A.G., Tayaban, R.H., Somga, J.R., Bartolome, R.M., 2004. Effect of aflatoxin-contaminated feeds in Nile tilapia (Oreochromis niloticus L.). In: Remedios, R.B., Mair, G.C., Fitzsimmons, K. (Eds.), Proceedings of the Sixth International Symposium on Tilapia in Aquaculture, pp. 172–178.
20. Cardoso Filho F.D.C. Master's Thesis. Universidade Federal do Piauí; Teresina, Brasil: 2011. Monitoramento de fungos toxigenicos e aflatoxinas em raçoes utilizadas na piscicultura em Teresina, Piauí, Brasil.
21. Carlson D.B., Williams D.E., Spitsbergen J.M., Ross P.F., Bacon C.W., Filmre I.M., Riley R.T. Fumonisin B1 promotes aflatoxin B1 and *N*-methyl-*N′*-nitro-nitrosoguanidine-initiated liver tumors in ranbow trout. Toxicol. Appl. Pharm. 2001;172:29–36
22. CarmichaeL J.W. (1966) Cerebral mycetomata of trout due to a phialophora-like fungus. Sabouraudia, 5, pp. 12n--3
23. Chàvez-Sànchez, M. C., C. A. Martinez-Palacios, and I. Osorio-Moreno. 1994. Pathological effects of feeding young *Oreochromis niloticus* diets supplemented with different levels of aflatoxin B_1. *Aquaculture* 127:49–60.
24. Coates, J.A., Potts, T.J. and Wilcke, H.L. (1967) Interim hepatoma research report. Trout Hepatoma Research Conference Papers. US Fish and Wildl. Serv., Res. Rep. 70, pp. 34—8
25. De Pedro, N., M. L. Pinillos, A. I. Valenciano, M. Alonso-Bedate, and M. J. Delgado. 1998. Inhibitory effect of serotonin on feeding behavior in goldfish: involvement of CRF. *Peptides* 19:505–511.
26. Deng, S.X., Tian, L.X., Liu, F.J., Jin, S.J., Liang, G.Y., Yang, H.J., Du, Z.Y., Liu, Y.J., 2010. Toxic effects and residue of aflatoxin B1 in tilapia (Oreochromis niloticus × O. aureus) during long-term dietary exposure. Aquaculture 307, 233–240
27. Döll S., Valenta H., Baardsen G., Möller P., Koppe W., Stubhaug I., Dänicke S. Effects of Increasing Concentrations of Deoxynivalenol, Zearalenone and Ochratoxin A in Diets for Atlantic Salmon (*Salmo salar*) on Performance, Health and Toxin Residues; Proceedings of 33rd Mycotoxin Workshop; Freising, Germany. 30 May–1 June 2011.
28. Doster R.C., Sinnhuber R.O., Wales J.H. Acute intraperitoneal toxicity of ochratoxins A and B in rainbow trout (*Salmo gairdneri*) Food Cosmet. Toxicol. 1972;10:85–92
29. El-Sayed, Y. S., R. H. Khalil, and T. T. Saad. 2009. Acute toxicity of ochratoxin-A in marine water-reared sea bass (*Dicentrarchus labrax* L.). *Chemosphere* 75:878–882.
30. Embaby E. M., Nahed M. Ayaat, 3Mona M. Abd El-Galil, 4Nasser Allah Abdel-Hameid and Mona M. Gouda. Mycoflora and Mycotoxin Contaminated Chicken and Fish Feeds. Middle East Journal of Applied Sciences: 05, : 04, 2015, 1044-1054
31. Fuchs, R., L. E. Appelgren, and K. Hult. 1986. Distribution of 14C-ochratoxin A in the rainbow trout (*Salmo gairdneri*). *Acta Pharmacologica et Toxicologica* 59:220–227.
32. Garcia, E. C. (2013). Effects of fumonisin B1 on performance of juvenile Baltic salmon (*Salmo salar*). MS Thesis. University of Jyväskylä
33. Gbore, F.A., Adewole, A.M., Oginni, O., Oguntolu,M.G., Bada, A.M., Akele, O., 2010. Growth performance, haematology and serum biochemistry of African catfish (Clarias gariepinus) fingerlings fed graded levels of dietary fumonisin B1. Mycotoxin Res. 26, 221–227.
34. Goel, S., S. D. Lenz, S. Lumlertdacha, R. T. Lovell, R. A. Shelby, M. Li, R. T. Riley, and B. W. Kemppainen. 1994. Sphingolipid levels in catfish consuming *Fusarium moniliforme* corn culture material containing fumonisins. *Aquatic Toxicology* 30:285–294.
35. Goldblatt L.A. Significance of aflatoxin in foods; Proceedings of the 80th Annual Conference of the Association of Food and Drug Officials; Atlanta, GA, USA. 22 June 1976; pp. 191–201.
36. Goncalves, R. A.., Karin Naehrer and Goncalo A. Santos. Occurrence of mycotoxins in commercial aquafeeds in Asia and Europe: a real risk to aquaculture? Reviews in Aquaculture (2016) 0, 1–18
37. Gonçalves-Nunes, E. M. C., Maria M. Gomes-Pereira, Amilton P. Raposo-Costa, Carlos A. da Rocha-Rosa, Carina M. Pereyra, Rodrigo M. Calvet, Ana L. Alves-Marques, Francisco Cardoso-Filho & Maria C. Sanches-Muratori. Screening of

aflatoxin B1 and mycobiota related to raw materials and finished feed destined for fish. Lat. Am. J. Aquat. Res., 43(3): 595-600, 2015

38. Greco, M., A. Pardo, and G. Pose. 2015. Mycotoxigenic fungi and natural co-occurrence of mycotoxins in rainbow trout (*Oncorhynchus mykiss*) feeds. *Toxins* 7:4595–4609.
39. Guan, S., He, J., Young, J.C., Zhu, H., Li, X.Z., Ji, C., Zhou, T., 2009. Transformation of trichothecene mycotoxins by microorganisms from fish digesta. Aquaculture 290, 290–295.
40. Halver. J.E. (1965) Aflatoxicosis and rainbow trout hepatoma. in G.N. Wogan (ed.), Mycotoxins in Foodstuffs, MIT Press, Cambridge, Mass., pp. 209--34
41. Halver, J.E. (1967) Crystalline aflatoxin and other vectors for trout hepatoma, in J.E. Halver and I.A. Mitchell (eds.), Trout Hepatoma Research Conference Papers, Res. Rep. 70, BSFW, GPO. Washington DC, pp. 78--102
42. Halver, J. E. (1969) Aflatoxicoses and trout hepatoma, in L.A. Goldblatt (cd.), Aflatoxins, Academic Press, New York, pp. 265-306
43. Hashem, M. (2011). **Isolation of Mycotoxin-producing Fungi from Fishes Growing in Aquacultures.** Research Journal of Microbiology, 6,12,862-872
44. Han, D., S. Xie, X. Zhu, Y. Yang & Z. Guo. 2009. Growth and hepatopancreas performances of gibel carp fed diets containing low levels of aflatoxin B1. Aquacult. Nutr., 16: 335-342.
45. Hashimoto E.H., Santos M.A., Ono E.Y.S., Hayashi C., Bracarense A.P.F., Hirooka E.Y. Bromatology and fumonisin and aflatoxin contamination aquaculture feed of the region of Londrina, State of Paraná, Brazil. Semin. Cienc. Agrar. 2003;24:123–132.
46. Hooft J.M., Elmor A.H.I., Encarnaçao P., Bureau D.P. Rainbow trout (*Oncorhynchus mykiss*) is extremely sensitive to te feed-borne *Fusarium* mycotoxin deoxynivalenol (DON) Aquaculture.2011;311:224–232.
47. Jakic-Dimic, D., S. Jeremic, K. Nesic & V. Radosavljevic. 2005. The influence of mycotoxins in food on fish health status. The first scientific meeting mycology, mycotoxicology and mycoses held from April 2005. Novi Sad., (109): 73-79.
48. Jantrarotai, W. and R.T. Lovell. 1990. Subchronic toxicity of aflatoxin B_1 to channel catfish. *Journal of Aquatic Animal Health* 2: 248–254
49. Loveland, P.M., J.E. Nixon, and G.S. Bailey. 1984. Glucuronides in rainbow trout (*Salmo gairdneri*) injected with [^{3}H] aflatoxin $B_1$and effects dietary beta-naphthoflavone. *Comparative Biochemistry and Physiology C, Comparative Pharmacology* 78: 13–19.
50. Lumlertdacha, S. and R.T. Lovell. 1995. Fumonisin-contaminated dietary corn reduced survival and antibody production by channel catfish challenged with *Edwardsiella ictaluri*. *Journal of Aquatic Animal Health* 7: 1–8.
51. Lumlertdacha, S., R.T. Lovell, R.A. Shelby, S.D. Lenz, and B.W. Kemppainen. 1995. Growth, hematology, and histopathy of channel catfish, *Ictalurus punctatus*, fed toxins from *Fusarium moniliforme*. *Aquaculture* 130: 201–218.
52. Mahfouz, M. E., Ahmed H. Sherif. A multiparameter investigation into adverse effects of aflatoxin on *Oreochromis niloticus* health status. The Journal of Basic & Applied Zoology Volume 71, August 2015, Pages 48–59
53. Mahrous, K. F., Wagdy Khalil Bassaly Khalil, Mahmoud Aly Mahmoud, Assessment of toxicity and clastogenicity of sterigmatocystin in Egyptian Nile tilapia. African Journal of Biotechnology Vol. 5 (12), pp. 1180-1189, 16 June 2006
54. Manning, B.B., 2001. Mycotoxins in fish feeds. In Nutrition and Fish Health. Lim, C. & Webster, C.D. Eds). Food Products Press. New York. 365
55. Manning B.B., Li M.H., Robinson E.H., Gaunt P.S., Camus A.L., Rottinghaus G.E. Response of channel catfish *Ictalurus punctatus* to diets containing T-2 toxin. J. Aquat. Anim. Health. 2003;15:230–239.
56. Manning, B.B., Ulloa, R.M., Li, M.H., Robinson, E.H., Rottinghaus, G.E., 2003. Ochratoxin A fed to channel catfish (Ictalurus punctatus) causes reduced growth and lesions of hepatopancreatic tissue. Aquaculture 219, 739–750

57. Manning, B.B., M.H. Li, and E.H. Robinson. 2005. Aflatoxins from moldy corn cause no reductions in channel catfish *Ictalurus punctatus* performance. *Journal of World Aquaculture Society* 36: 59–67.
58. Manning, B.B., H.K. Abbas, D.J. Wise, and B.C. Peterson. 2011. Channel Catfish, *Ictalurus punctatus*, fed diets containing aflatoxin from moldy corn do not experience increased mortality after challenge with *Edwardsiella ictaluri*. *Journal of World Aquaculture Society* 42(4): 598–602.
59. Manning B.B., Abbas H.K. The effect of *Fusarium* mycotoxins deoxynivalenol, fumonisin, and moniliformin from contaminated mouldy grains on aquaculture fish. Toxin Rev. 2012;31:11–15
60. Manning, B.B., Abbas, H.A., Wise, D.J., Greenway, T., 2014. The effect of feeding diets containing deoxynivalenol contaminated corn on channel catfish (Ictalurus punctatus) challenged with Edwardsiella ictaluri. Aquac. Res. 45, 1782–1786.
61. Matejova, I., Svobodova, Z., Vakula, J., Mares, J. and Modra, H. (2016), Impact of Mycotoxins on Aquaculture Fish Species: A Review. J World Aquacult Soc. doi:10.1111/jwas.12371
62. Mohapatra, S., Sahu, N.P., Pal, A.K., Prusty, A.K., Kumar, V., Shivendra, K., 2011. Haematoimmunology and histo-architectural changes in Labeo rohita fingerlings: effect of dietary aflatoxin and mould inhibitor. Fish Physiol. Biochem. 37, 177–186.
63. Mohebbi, Asma Hosseini, Rahim Tahmasebi, Mehdi Mohammadi. Aflatoxins in Tissues and Diets of Farmed White Shrimp (Litopenaeus vannamei) Gholamhossein Environmental Studies of Persian Gulf 1(2) (2014) 117-125
64. Náscher-Mestre, J., Beltrán, E., Pérez-Sánches, J., Silva, J., Karalazos, V., Hernández, F., Berntssen, M.H.G., 2015. Occurrence and potential transfer of mycotoxins in gilthead sea bream and Atlantic Salmon by use of novel alternative feed ingredients. Chemosphere 128, 314–320.

65. Ngethe, S., Tor Einar Horsbergb, Eric Mitemaa and Ktistian Ingebrigtsenb. Species differences in hepatic concentration of orally administered 3H-AFB1 between rainbow trout (Oncorhynchus mykiss) and tilapia (Oreochromis niloticus). Aquaculture, 114 (1993) 355-358
66. Nguyen AT, Manning BB, Lovell RT, Rottinghaus GE: **Responses of Nile tilapia (*Oreochromis niloticus*) fed diets containing different concentrations of moniliformin or fumonisin B_1.** *Aquaculture* 2003, **217:** 515–528
67. Nguyen, Q. (2014) Patulin Toxicity in Adult Zebrafish. http://patulinzebrafish blog.tumblr.com/pat
68. Nomura, H., Ogiso, M., Yamashita, M., Takaku, H., Kimura, A., Chikasou, M., Nakamura, Y., Fujii, S., Watai, M., Yamada, H., 2011. Uptake by dietary exposure and elimination of aflatoxins in muscle and liver of rainbow trout (Oncorhynchus mykiss). J. Agric. Food Chem. 59, 5150–5158.
69. Ottinger CA, Kaattari SL. 1998. Sensitivity of rainbow trout leucocytes to aflatoxin B1. Fish Shellfish Immunol. 8: 515-5
70. Pietsch C., Bucheli T.D., Wettstein F.E., Burkhardt-Holm P. Frequent biphasic cellular responses of permanent fish cell cultures to deoxynivalenol (DON) Toxicol. Appl. Pharm. 2011;256:24–34
71. Pietsch, C.; Kersten, S.; Burkhardt-Holm, P.; Valenta, H.; Danicke, S. Occurrence of deoxynivalenol and zearalenone in commercial fish feed: An initial study. *Toxins* 2013, *5*, 184–192.
72. Pietsch, C., Michel, C., Kersten, S., Valenta, H., Dänicke, S., Schulz, C., Kloas,W., Burkhardt-Holm, P., 2014a. In vivo effects of deoxynivalenol (DON) on innate immune responses of carp (Cyprinus carpio L.). Food Chem. Toxicol. 68, 44–52a.
73. Pietsch, C., Noser, J., Wettstein, F.E., Burkhardt-Holm, P., 2014b. Unraveling the mechanisms involved in zearalenone mediated toxicity in permanent fish cell cultures. Toxicon 88, 44–61b.
74. Pietsch, C., Schulz, C., Rovira, P., Kloas, W., Burkhardt-Holm, P., 2014c. Organ damage and hepatic lipid accumulation in carp (Cyprinus carpio L.) after feed-borne exposure to the mycotoxin, deoxynivalenol (DON). Toxins 6, 756–778c.
75. Pietsch, C., Katzenback, B.A., Garcia-Garcia, E. et al. Mycotoxin Res (2015a) 31: 151. Acute and subchronic effects on immune responses of carp (*Cyprinus carpio* L.) after exposure to deoxynivalenol (DON) in feed. Mycotoxin Research August 2015, Volume 31, Issue 3, pp 151–164

76. Pietsch C, Kersten S, Valenta H, et al. Effects of Dietary Exposure to Zearalenone (ZEN) on Carp (*Cyprinus carpio* L.). Battilani P, ed. *Toxins*. 2015b;7(9):3465-3480.
77. Santacroce, M.P., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G., Crescenzo, G., 2008. Aflatoxins in aquatic species: metabolism, toxicity and perspectives. Rev. Fish Biol. Fish. 18, 99–130.
78. Poston H.A., Coffin J.L., Combs G.F. Biological effects of dietary T-2 toxin on rainbow trout, *Salmo gairdneri*. Aquat. Toxicol. 1982;2:79–88.
79. Price, e.R. (1981) A study of aflatoxin in tilapia feedstuffs. MSc dissertation. University of Stirling, V + 51 pp
80. Rajeev Raghavan, P., X. Zhu, W. Lei, D. Han, Y. Yang, S. Xi. Low levels of Aflatoxin B_1 could cause mortalities in juvenile hybrid msturgeon, *Acipenser ruthenus* ♂×*A. baeri*♀. Aquqaculture Nutr. 17, I 2, 2011, 39– 47
81. Rasmussen H.B., Larsen K., Hald B., Moeller B., Elling F. Outbreak of liver cell carcinoma among saltwater reared rainbow trout (*Salmo gairdneri*) in Denmark. Dis. Aquat. Organ. 1986;1:191–196.
82. Ruiz Pérez A., Paasch Martínez L., Adamede Paasch P., Rosiles Martínez R. Hepatic neoplasia in the rainbow trout (*Salmo gairdneri*) bred in El Zarco Fish Hatchery, Federal District. Veterinaria. 1984;15:255–261.
83. Sahoo PK; Mukherjee SC; Mohanty S; Dey S; Nayak SK. A preliminary study of acute citrinin toxicity in rohu (Labeo rohita) fingerlings. *Indian Journal of Microbiology, Immunology and Infectious Diseases. 1999 Jan-Jun; 20(1): 62-4*
84. Sahoo, P.K., Mukherjee, S.C., 2001. Immunosuppressive effects of aflatoxin B1 in Indian major carp (Labeo rohita). Comp. Immunol. Microbiol. Infect. Dis. 24, 143–149.
85. Samuel, T. O. and Odunigba, O. aflatoxins associated with storage fungi in fish feed. *Ife Journal of Science* vol. 17, no. 2 (2015)
86. Sanden, M., Jorgensen, S., Hemre, G.I., Ornsrud, R., Sissener, N.H., 2012. Zebrafish (Danio rerio) as a model for investigating dietary toxic effects of deoxynivalenol contamination in aquaculture feeds. Food Chem. Toxicol. 50, 4441–4448.
87. Santacroce M.P., Conversano M.C., Casalino E., Lai O., Zizzadoro C., Centoducati G., Crescenzo G. Aflatoxins in aquatic species: Metabolism, toxicity and perspectives. Rev. Fish B Woodward B., Young L.G., Lun A.K. Vomitoxin in diets for rainbow trout (*Salmo gairdneri*) Aquaculture. 1983;35:93–101
88. Santos, G. et al. 2010. Mycotoxins in aquaculture: Occurrence in feeds components and impact on animal performance. En: Cruz-Suarez, L.E., Ricque Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J. (Eds), Avances en Nutrición Acuícola X - Memorias del Décimo Simposio Internacional de Nutrición Acuícola, 8-10 de Noviembre, San Nicolás de los Garza, N. L., México. ISBN 978-607-433-546-0. Universidad Autónoma de Nuevo León, Monterrey, México, pp. 502-513.
89. Schoenhard GL, Hendricks JD, Nixon JE, Lee DJ, Wales JH, Sinnhuber RO, Pawlowski NE. Aflatoxicol-induced hepatocellular carcinoma in rainbow trout (Salmo gairdneri) and the synergistic effects of cyclopropenoid fatty acids. Cancer Res. 1981 Mar;41(3):1011-4.
90. Schwartz, P., Bucheli, T.D., Wettstein, F.E., Burkhardt-Holm, P., 2011. Life-cycle exposure to the estrogenic mycotoxin zearalenone affects zebrafish (Danio rerio) development and reproduction. Environ. Toxicol. 276–289.
91. Selim, K.M., El-Hofy, H., Khalil, R.H., 2014. The efficacy of three mycotoxin adsorbents to alleviate aflatoxin B1 induced toxicity in Oreochromis niloticus. Aquac. Int. 22, 523–540.
92. Supamattaya, K., Bundit, O., Boonyarapatlin, M., Schatzmayr, G., Chittiwan, V., 2005. Effects of ochratoxin A and deoxynivalenol on growth performance and immuno-physiological parameters in black tiger shrimp (Panaeus monodon). Songklanakarin J. Sci. Technol., 27 (suppl 1), 91-99.
93. Supamattaya, K., Bundit, O., Boonyarapatlin, M., Schatzmayr, G., 2006. Effects of Mycotoxins T-2 and Zearalenone on growth performance immuno-ohysiological parameters and histological changes in Black tiger shrimp (Penaeus monodon) and white shrimp (Litopenaeus vannamei). XII International Symposium of Fish Nutrition & Feeding. May 28 – June 1. Biarritz, France.
94. Tola S, Bureau DP, Hooft JM, et al. Effects of Wheat Naturally Contaminated with *Fusarium* Mycotoxins on Growth Performance and Selected Health Indices of Red

Tilapia (*Oreochromis niloticus* × *O. mossambicus*). Dänicke S, ed. *Toxins*. 2015;7(6):1929-1944.

95. Tolosa, J., Font, G., Mañes, J., Ferrer, E., 2014. Natural occurrence of emerging Fusarium mycotoxins in feed and fish from aquaculture. J. Agric. Food Chem. 62 (51), 12462–12470.
96. Tuan, N.A., Grizzle, J.M., Lovell, R.T., Manning, B.B., Rottinghaus, G.E., 2002. Growth and hepatic lesions of Nile tilapia (Oreochromis niloticus) fed diets containing aflatoxin B1. Aquaculture 212, 311–319.
97. Tuan, N.A., Manning, B.B., Lovell, R.T., Rottinghaus, G.E., 2003. Responses of Nile tilapia (Oreochromis niloticus) fed diets containing different concentrations of moniliformin or fumonisin B1. Aquaculture 217, 515–528.
98. Varior, S. Biochemical and histopathological effects of aflatoxin on *oreochromis mossambicus* (peters), ph.d. Thesis, Cochin university of science and technology, kocill-16, kerala March 2003
99. Wales, l.H. and Sinnhuber, RO. (1966) An early hepatoma epizootic in rainbow trout, Salmo gairdnerii. Calif. Fish and Game, 52, pp. 85-91
100. Wales, 1.H. (1967) Degeneration and regeneration of liver parenchyma accompanying hepatomagenesis. Trout Hepatoma Research Conference Papers. US Fish and Wildl. Serv., Res. Rep., 70, pp. 56-9
101. Wales, l.H. (1970) Hepatoma in rainbow trout, in S.F. Snieszko (ed.), A Symposium on Diseases of Fishes and Shellfishes, American Fisheries Society, New York, pp. 351-65
102. Wales, 1.H. (1979) Induction of hepatoma in rainbow trout Salmo gairdneri Richardson by the egg bath technique. 1. Fish Dis., 2, pp. 563-6
103. Wolf, H. and Jackson, E.W. (1963) Hepatoma in rainbow trout: descriptive and experimental epidemiology. Science, 142, pp. 676--8
104. Wang, X., Yizhen Wang , Yongjin Li , Ming Huang , Yang Gao , Xianfeng Xue , Haiwen Zhang , Pedro Encarnação , Gonçalo A Santos , Rui A Gonçalves. Response of yellow catfish *(Pelteobagrus fulvidraco)* to different dietary concentrations of aflatoxin B1 and evaluation of an aflatoxin binder in offsetting its negative effects. Cienc. mar vol.42 no.1 Ensenada mar. 2016
105. Wolf, H. and Jackson, E.W. (1967) Hepatoma in salmonids: the role of cottonseed products and species differences. Trout Hepatoma Research Conference Papers. US Fish and Wildl. Servo Res. Rep., 70, pp. 29-33
106. Wood, E.M., Yasutake, W.T. and Lenihan, W.C. (1955) A mycosis like granuloma of fish. 1. Infect. Dis., 97, pp. 262-7
107. Woodward, *B., L.G. Young, and A.K. Lun. 1983. Vomitoxin in diets for rainbow trout (*Salmo gairdneri*).* Aquaculture *35: 93–101.*
108. Woźny, M., Obremski, K., Jakimiuk, E., Gusiatin, M., Brzuzan, P., 2013. Zearalenone contamination in rainbow trout farms in north-eastern Poland. Aquaculture 416–417, 209–211.
109. Wu TS, Yang JJ, Yu FY, Liu BH. Evaluation of nephrotoxic effects of mycotoxins, citrinin and patulin, on zebrafish (Danio rerio) embryos. Food Chem Toxicol. 2012 Dec;50(12):4398-404.
110. Wunder W., Korn H. Aflatoxin cancer (hepatoma) in the liver of the rainbow trout (*Salmo irideus*) Zool. Beitr. 1982;28:99–109.
111. Yildirim, M., B. Manning, R.T. Lovell, J.M. Grizzle, and G.E. Rottinghaus. 2000. Toxicity of moniliformin and fumonisin B_1 fed singly and combination in diets for channel catfish. *Journal of World Aquaculture Society* 31: 599–608.
112. Yuan, G.,Wang, Y., Yuan, X., Zhang, T., Zhao, J., Huang, L., Peng, S., 2014. T-2 toxin induces developmental toxicity and apoptosis in zebrafish embryos. J. Environ. Sci. 26, 917–925.
113. Zaki MS, Sharaf NE, Rashad H, Mastala SO, Fawz QM. 2008. Diminution of aflatoxicosis in tilapia nilotica fish by dietary supplementation with fix in toxin and *Nigella sativa* oil. Am.-Euras. J. Agric. Environ. Sci. 3: 211-215.
114. Zychowski KE, Pohlenz C, Mays T, Romoser A, Hume M, Buentello A, Gatlin III DM, Phillips TD. 2013a. The effect of NovaSil dietary supplementation on the growth and health

performance of Nile tilapia *(Oreochromis niloticus)*fed aflatoxin-B1 contaminated feed. Aquaculture 376-379: 117-123.

115. Zychowski KE, Hoffmann AR, Ly HJ, et al. The Effect of Aflatoxin-B_1 on Red Drum (*Sciaenops ocellatus*) and Assessment of Dietary Supplementation of NovaSil for the Prevention of Aflatoxicosis. *Toxins*. 2013b;5(9):1555-1573.