Reproductive Biology of the Thumbprint Emperor, *Lethrinus harak* (Forsskal 1775), using histological ultra-structural characteristic in gonads along Sudanese Coastal Waters

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Abstract

Lethrinus harak (Forsskal 1775) are very common species of the family Lethrinidae in Sudanese Red Sea Coast. Some aspects of the reproductive biology and histology of the gonads of *Lehtrinus harak* were studied between 2008 and 2009. The present study described six maturity stages for gonad development based on external features and histological study of males/females. Males of *Lethrinus harak* attain maturity stages at a relatively bigger size than females (at 22.5 cm for males and 21.7 cm for females). *Lethrinus harak* has a prolonged spawning season extending from October to April with peaks in April.

Keywords: Lethrinus harak, Reproduction, histology, Abu Hashish, Red Sea

DOI: 10.7176/JBAH/9-4-10

1. Introduction

The study of reproduction and egg production increases our knowledge about the state of a stock and improves standard assessments of many commercially valuable fish species. *Lethrinus harak* belongs to Family lethrinidae which is one of the commercially important fish groups along the Sudanese Red Sea Coast and widely distributed in Sudanese Red Sea Coast. There are at least four species of the genus Lethrinus common in the Sudanese waters, all of which come under the common Arabic name of Sha'oor, and are one of the most common local commercial fishes and are found throughout the year Abu Gideiri (1984).

There are no biological studies in detail such as studying reproduction and management of fishing in most fishes of Red Sea especially Sudanese Coast. Therefore, this study is an important matter. The aim of this study to collected main base line information on reproductive performance of *Lethrinus harak* during development in order to investigate the reproductive biology of *L. harak*, including its spawning season, gonad maturity stages, sex ratio, and the size at which50% of fish attain first sexual maturity which a future support mariculture and on fisheries management. Hilborn. *et al.* (1992) and Pauly (1994) reported that growth and mortality model parameter estimates are required for analytical fisheries management.

Hunter *et al.*, (1992) reported that description of reproductive strategies and the assessment of fecundity is fundamental topics in the study of the biology and population dynamics of fish species. Studies on reproduction, including the assessment of size at maturity, fecundity, duration of the reproductive season, daily spawning behaviour and spawning fraction, permit quantification of the reproductive capacity of individual fish. (Kraus, *et al.*, 2002). Filho, (1989) reported that knowledge about the gonadal development of marine fish species is important information to maintain the fishery stocks. The data required to provide such information is typically gathered through the examination and classification of gonads into developmental stages so that parameters such as reproductive period, spawning frequency, size at sexual maturity and sex ratios can be determined. Many studies have been conducted on the genus Lethrinus such as some aspect of the reproductive biology of *Lethrinus harak* (Ntiba, 1995; Kulmiye *et al.*, 2002and Ebisawa, 2006); histological studies on *Lethrinus nebulosus* (Loubens, 1980); size and age at sexual maturity (Hilomen, 1997 and Lassi, 2003) and some biological aspects of *Lethrinus mahsena* (Abu Degoon, 2005).

2. Material and Methods

Collection of specimen:

Total of 358 specimens of *Lethrinus harak* was collected monthly and randomly from the Fish Commercial Centre at Abu Hashish Fish Central Market-Port Sudan during the period from June 2008 to May 2009 The total length (TL),was measured to the nearest 0.1 cm and the total weight (TW), and gonad weight (GW) were weighed to the nearest 0.01 grams in an electronic balance and age estimates using the scales.



Reproduction:

Reproduction information were obtained through the examination of the gonad of Lethrinus harak fish.

Length and age at First Maturity:

The length at first maturity was defined at the length at which fish attain maturity. Stage (IV) was considered as the mature stage to enable determination of the minimum size at which fish attain sexual maturity. It was estimated according to Soondron *et al.* (1998).

The Percentage of Mature Fish:

The Percentage of Mature Fish (%MF) was a determination for the combined sexes of the stage (IV and V) using the following equation:

MF%=[Number of mature fish /Total number of in the sample] ×100

Histological processing:

Whole gonads removed from each individual were blotted dry, weighed to the nearest 0.001 g, sexed and staged macroscopically, and immediately fixed in Bouin's solution for 24 hours for histological processing. Sections of gonads embedded with paraffin wax were mounted on glass slides and stained with Haematoxylin and Eosin (Harris, 1900). These sections were viewed under a high powered microscope to confirm macroscopic sexes and stages.

3. Results

Maturity stages:

The gonads of 358 *L. harak* individuals were examined. These individuals ranged in size from 14.3 to 35.7cm. The description of maturity stages of *Lethrinus harak* in Table 1, while mature ovaries and testes are shown in Fig. 1 and 2.

	Testes	Ovary				
Maturity	External Feature	External Feature				
Stage 1 Immature	Long, slender and thread like translucent	Long, slender and thread like				
_	structures occupying about 33% of the	structures, red colour also occupying				
	abdominal cavity	33% of the abdominal cavity				
Developing	Ribbon-like structures slightly bigger	Firm and like with slight increase in				
	than stage 1, grayish-white in colour	size pink in colour and 50% of cavity				
	occupying 50% of cavity	oocytes not discernible.				
Maturing	Broad and thick dark white in in colour,	Board and thick occupying 70% of the				
	blood vessels visible externally milt	abdominal cavity red or reddish				
	oozes out from cut surfaces and	brown. Cavity red or reddish brown.				
	occupying 70% of the abdominal cavity	ernally. Oocytes visible through the				
		ovary wall				
Ripe	Further increase size occupying 90% of	Distended and occupying 90% of the				
	the abdominal cavity White in colour.	abdominal cavity. Vessels				
	Milt oozes out on slight pressure.	disappearing, oocytes can be seen				
		clearly through the ovary wall.				
Running	Fully distended, occupying almost all the	Fully distended with granular surface				
	abdominal cavity exudes milt on slight	occupying almost all the abdominal				
	pressure.	cavity.				
Spent	Shrunken and flaccid, walls are harder	Ovary is not fully empty. Residual				
	and wrinkled. No milt oozes out on	oocytes present flaccid and red in				
	pressure and blood vessels visible	colour and Ovary wall is thick.				
	externally.					

Table 1. Description of maturity stages of Lethrinus harak gonads.





Fig (1) mature ovary of *Lethrinus harak*



Fig (2) mature testes of Lethrinus harak

Length and age at first sexual maturity:

To determine the average size at which 50% of *Lethrinus harak* males and females attain first sexual maturity (fish in stage 2 of gonad development and above were considered mature). Fish belonging to this species attains their first sexual maturity at 22.5 cm for males and 21.7 cm for females (Table 2 and Fig. 3).

Table 2. Age, mean length and percentage of mature fish used to determine length and age at first maturity (L_{T50}) for *Lethrinus harak*.

Females				Males					
Age group	Mean length (cm)	Frequency	Cumulative Frequency	% of Mature fish	Age group	Mean length (cm)	Frequency	Cumulative frequency	% of Mature fish
Ι	14.83	0	0	0.00	Ι	14.3	0	0	0.00
II	19.96	26	26	19.70	II	20.16	9	9	7.56
III	23.39	78	104	78.79	III	23.5	75	84	70.59
IV	27.41	17	121	91.67	IV	27.2	21	105	88.24
V	35.04	11	132	100.00	V	35.7	14	119	100.
Total		132					119		

Age I = 0 +, Age II = 1 +, etc





Fig. 3: Shows the percentage of mature males and females for *Lethrinus harak* with total length, arrows show Length at first sexual maturity.

Histological aspect of gonads development:

The histological aspect of ovary development and microscopic characteristics were summarized in (Plates 1-17),

and (Plates 18-28) of females and males respectively.

The histological aspect of testes development and microscopic characteristics was summarized in (Plates 18-28), of males for *Lethrinus harak*.



Plate 1 (Whole section, x = 4)





Plate 2 (Enlarged section, x = 10)

Plate (1)and (2) transverse section through the immature ovary (undifferentiated) dominated will afew primary oocytes (Po) around the central cavity(CA) of the connective tissue(CT) in this stage the gonad wall (GW) is very thick, (stained with Hematoxyline and Eosin).



Plate 3 (Whole section, x = 4)



Plate 4 (Enlarged section, x = 10)

Plate (3) and (4) transverse section through the immature ovary showing gonad wall (GW) is very thick, and a nest of immature oocytes (IO) or oogonia and early perinucleolus (EP). Immature oocytes in this stages were small spherical cells present either solitary or in a cluster of the cells founded embedded in the ovigerous lamella, and the ovarian cavity (CA) is much reduced around the connective tissue, (stained with Hematoxyline and Eosin).



Plate 5 (whole section)



Plate 6 (Enlarged section)

Plate Fig (5) and (6) transverse section through immature ovary were `containing nest of chromatin nucleolus, oogonia (O) (primary oocytes)

(po) or immature oocytesw (IO); Early perinucleolus(EP) around the cavity(CA) and connective tissue (CT). Showed that oocytes appear small in size and nucleus disappearing or very small. The ovarian cavity is much reduced, (stained with Hematoxyline and Eosin).



Plate 7 (Whole section, x = 10)

Plate (7) transverse section through the developing ovary from immature to maturing there have much connective tissue (CT) between follicles, gonad wall (GW) still is thick. Large circular nucleus (N) is seen with a number of small nucleoli (Nu). In this stage, the young oocytes increase in size, early perinucleolus; extensive cytoplasm(CY)



the late perinuleolus(LP) spherical in shape, the oocytes(O) have yolk (Y).Many oocytes are observed, egg follicles started to appear in number, (stained with Hematoxyline and Eosin).



Plate 8 (Whole section, x = 4)



Plate 9 (Enlarged section, x = 10)

Plate (8) and (9) transverse section through the maturing ovary the gonad wall (GW)is thick, as the growth of the oocytes(O) progress they increase in size, and surrounded with follicular epithelial (FE). In this stage having early perinucleolus (EP), late perinucleolus, and secondary oocytes (SO), (stained with Hematoxyline and Eosin).





Plate 10 (Whole section, x = 4)



Plate 11 (Enlarged section, x = 10)

Plate (10) and (11) transverse section through the second stage maturing ovary showing the beginning atresia hypertrophic folliculars cells and material yolk(Y) and zona radiate (ZR). Mature oocyte (MO) with a follicular epithelial layer(FE). The cytoplasm is basophilic and occupies the greater part of the ovum the nucleus (N) is round in shape, the nucleoli increase in in number and become smaller size and locate near the periphery of the nucleus, (stained with Hematoxyline and Eosin).





Plate 12 (Whole section, x = 4)





Plate (12) and (13) transvere section through maturing ovary (near mature) the ovary is filled with oocytes (O) different stage of yolk (Y) deposition, nucleus (N) in central position, the mature oocytes (MO) have thecal cells (Th), granulosa(G) zona radiata (ZR), oocyte bounded by a distinct follicular epithelial(FE). The yolk granules appeared as small spheres in the inner part of cytoplasm around the nucleus, the oocyte membrane in thick ness and composed of three layer outer(Th) middle (G) inner (ZR), (stained with Hematoxyline and Eosin).





Fig 14 (Whole section, x = 4)



Plate 15 (Enlarged section, x = 10)

Plate (14) and (15) transverse section through the full mature ovary the ovary is filled with fully ripe oocytes (RO) and having small distinguisted spheres of yolk depositions, oocytes size and become oval in shape, the three layer thecal (Th), granulosa (G), zona radiata (ZR), appeared in clear, and spherical teriary yolk (TY), the ovary composes beside the eggs wich had reached final maturation, few small eggs with different diameters as well as some oocytes in atretic state, (stained with Hematoxyline and Eosin).





Plate 16 (Whole section, x = 10)





Plate (16) and(17) transverse section of spawning ovary showing spherical tertiary yolk (TY) and oval ripe oocyte (Ro). The ovary full mature oocyte(FMO), the structure of an unfertilized fish egg nucleus (N), micropyle (M), chorion (Ch), yolk (Y), oil globules (Og), vitelline membrance(Vm), perivitelline space(Ps), theca(Th), granulose (G), cytoplasm(Cy), yolk nucleus(YN).the granulosa cells are hypertrophied, and thecal cells transform into macrophages to invade the oocyte contents, The spawning ovary were observed most months of the year but peak in other from October to April for *L. harak* (stained with Hematoxyline and Eosin).





Plate 18 (Whole section, x = 10)

Plate (18) transverse section through the immature testes, showing a preponderance of spermatogonia (Sg), there are the primary germ cells. They undergo repeated mitotic divisions to form a large number of spermatogonia. It the first spermatogonial stage in the testes. The gonad wall (GW) is thick, and inter lobular connective tissue (CT), and cavity (CA). The seminiferous lobules at immature stage composed of the germ cells which are in active. They designate spermatogonium at a different stage, (stained with Hematoxyline and Eosin).



Plate 19(Whole section, x = 4)



Plate 20(Enlarged section, x = 10)

Plate (19) and(20) transverse section through immature testes contained fibrous connective tissue (CT), thick gonad wall (GW), the cavity (CA), and spermatogonia(sg). In this stage, the formed spermatogonium is nearly round the nucleus (N) of which have a much greater there is one nucleolus that lies in the center of some cells but others located in the periphery of the nucleus (stained with Hematoxyline and Eosin).



Plate 21 (Whole section, x = 4)



Plate 22 (Enlarged section, x = 10)

Plate (21)and(22) transverse section through the immature testes were containing nest of spermatogonia (Nt Sg), and the gonad wall (GW) still very thick, many cavity (CA) and connective tissue (CT), visible among spermatogonia (Sg) in lobules, nucleus (N) nucleolus (Nu). The chromatin material is arranged in the peripheral part of the nuclear membrane, the cytoplasm appeared lightly eosinophilic while the nucleoplasm was slightly basophilic.



Plate 23 (Whole section, x = 10)

Plate (23) transverse section through the developing teses showing spermatogonia (Sg) with multiplying another type of spermatocytes is primary spermatocytes (PSg) secondary spermatocytes (SSg), in this stage the gonad wall (GW) become thin and many cavity (CA) among connective tissue (CT) and tubule wall (TW) appears. As spermatogenesis proceeded, spermatogonia divided and multiplied to give the primary spermatocytes, the lobule exhibited the presence of spermatogonia and primary spermatocytes, the latter appeared smaller than



spermatogonia, the cell out-line are not well defined the nucleus is spherical and measured this stage is characterized by condensed chromatin material of their nuclei (stained with Hematoxyline and Eosin).



Plate 24 (Whole section, x = 10)

Plate (24) transverse section through the maturing testes showing the primary spermatocytes (PS) multiplied and divided to produce the secondary spermatocytes (SS). These cells were formed by the first meiotic division of the primary spermatocyte, the secondary spermatocyte has a short duration and rapidly divided into spermatid (ST). The cell wall of second spermatocytes becomes indistinct. The nucleus is spherical, the chromatin material become either polarized to one pole of the nucleus or deeply condensed with the transluescent centre. In this stage the gonad wall (GW) still thin and testicular lobe comprised of seminiferous fibrous connective tissue (CT), the tubule wall (TW) is appeared (stained with Hematoxyline and Eosin).



Plate 25 (Whole section, x = 10)

Plate (25) transverse section through the nearly ripe testes (last maturing) showing active spermatogenesis throughout the testes, nest primary spermatcytes (SP), a nest of secondary spermatocytes (SS). These cells were formed by the second meiotic division of secondary spermatocytes the spermatid (ST) appeared smaller than the primary and secondary spermaticytes are recognized by indistinct cell outlines. According to the degree of maturation, the spermatid is either grouped inside a cyst or distributed throughout the central lumen of the lobules. Spermatid developed to spermatozoa (SZ) at this stage and have many inter lobular connective tissue (CT) and gonad wall become very thin (stained with Hematoxyline and Eosin).





Plate 26 (Whole section, x = 10)

Plate (26) transverse section through the mature testes showing degenerative change of the seminiferous tubles expressed by damage of their membranes spermatozoa (SZ), in this stage the spermatid (ST) still appearing and the gonad wall (GW) still very thin. The spermatozoa (SZ) were distinguished by small round heads and slender, elongated tails. The chromatin material was condensed in the head of spermatozoa, the active spermatozoa migrated towards the centre of the lumen of the lobule (stained with Hematoxyline and Eosin).



Plate 27 (Whole section, x = 10)

Plate (27) transverse section through the full mature testes all the lobules is filled with free spermatozoa (SZ), with saw spermatids (ST) at the end of spermiogenesis are visible next to the lobule wall ,tubule wall (TW) and connective tissue (CT), (stained with Hematoxyline and Eosin).



Plate 28 (Whole section, x = 10)

Plate (28) transverse section through the spawning testes (oozing) the all tastes filled with spermatozoa (SZ) and some of connective tissue (CT) or blood vessels. In this stage showing reduction in size of lobules leaving space between them, decrease of the intensity of spermatozoa (SZ), and thick inter lobular connective tissue (CT). The spawning testes were observed most months of the year but peak in other from March until November for *L. harak* (stained with Hematoxyline and Eosin).

4. Discussion

Lethrinus harak was economical important fish species, beside its ease adaptability for culture an increasing interest has been given to its propagation and productivity on Red Sea Coast. The gross morphological criteria used to distinguish the six maturity stages assigned to gonad development, together with the cyclical changes shown by the gonad-index, to provide reliable evidence of spawning for *Lethrinus harak* in shore waters. The occurrence of high percentages of individual fishes with running gonads (stage5) in the samples in, at least, eight consecutive months from October to April and the subsequent emergence of spent fish one month after the October peak through April suggests that *L. harak* population at the Red Sea coast has a prolonge spawning season extending from October to April with two peaks occurring in October and February.

Unfortunately, no published data on reproduction of *L. harak* are available in the literature. However, the information available for other lethrinid species can be used for comparison. Wassef & Bawazeer (1992) found that the longnose emperor, *L. elongatus*, in the Red Sea has a protracted spawning season spanning four months (May–August). Kuo & Lee (1990) reported that the common porgy, *L. nebulosus*, also has a prolonged spawning season extending from September to February in the Northwestern Shelf of Australia. Nzioka (1979), examining the gonads of East African reef fishes, postulates two spawning seasons for some lethrinid species in September/ October and January/February.

The present data and histological study on length and age at first maturity for *L. harak* showed the male and females attain first sexual maturity (fish in stage 2 of gonad development and above were considered mature). It was found that fish lengths smaller than 19.5 cm and 18cm are always immature. The first sexual maturity at 21.5 cm for males, and 22.5 cm for females both sexes at the second year for life. These findings are in general in agreement with Ntiba., *et al* (1995) in study of *L. harak* in Kenyan water; Shakeel and Ahmed (1996) stated that, the minimum size allowed to be caught for *E. areolatus* in Malé, Maldives is 25 cm. McIlwain *et al.* (2006) in their study on *L. nebulosus* in the Arabian Sea, Sultanate of Oman found that, the immature individuals constituted more than 40% of the that, the age at first maturity of *L. nebulosus* was 1.58 year which corresponds to length 29.5 cm in the coastal areas of Mauritius. The length at first sexual maturity of *L. nebulosus* in Arab Emirates in front of Abu Dhabi was determined by Grandcourt *et al.* (2003), as 28.6 cm for males and 31.3 cm for females. While, Grandcourt *et al.* (2006) found that, the length at first sexual maturity of *L. nebulosus* in the Southern Arabian Gulf was 27.6 cm for males and 28.6 cm for females.

The Plates (from 1 to 17) show the histological changes (development) in ovaries during the reproductive cycle for the females of *L. harak*. Oogenesis in fish are known to undergo a sequence of external and internal changes, these changes in the oocytes development have been detected in various fish species. According to various authors, the course of development of oocytes have been divided into stages, phases or periods in order to differentiate the gradual changes in their peculiarities (Guraya et al. 1975 & Matsuyama et al. 1991).

It is well known that the growth of oocytes takes place at two development phases namely the primary growth phase and secondary growth phase (i.e. vitellogenic oocyte). The primary growth phase two species under the present study includes only an immature oocyte which can be divided into three subdivision or phases. Initially, the early young oocyte characterized by having a large nucleus containing one large nucleolus. The early stage of young oocyte species under the study is similar to prematuration period. These results are in agreement with Zaki *et al.* (1986), synapsis – period of Latif and Sandy (1973), immaturation period of Assem (1992 and 1995) and chromatin – nucleolus stage of El Gamal (1997) on *Cyprinus carpio* study. The late immature oocyte stage of the present study is similar to protoplasmic growth of Latif and Sandy (1973) and the perinucleolus stage of Mousa (1994 and 2002) and El Gamal (1997) on *Cyprinus carpio* study.

In the present results, the perinucleolus stage undergoes a gradual increase in the oocyte size and in number of nucleoli of the nucleus. During the late perinucleolus stage a basophilic organelle appears in the cytoplasm (yolk nucleus). The yolk nucleus can be termed as, achroplasm, crop vitelline or Balbiani bodies (Zaki, *et al.*, 1991).

The second growth phase (i.e. vitellogenic oocyte) includes vacuolization of cytoplasm and yolk deposition. In

some other teleosts, the vitellogenic stages were divided into phases vacuolization and yolk deposition as described by Zaki *et al.*, (1986) and Ashour et al. (1990). However, in some other fishes, there were four stages: vesicle stages, primary yolk granules stages, secondary yolk granules stage and tertiary yolk granules stage as described by (Khoo, 1979; Mousa, 1994 and 2002 and El Gamal 1997) on the ovary of many other fishes. On the contrary of many other fishes, the yolk depositions first appeared in the peripheral cytoplasm, thereafter scattered towards the center of ooyte as those described by (Zaki *et al.*, 1986; Zaki and El Gharabawy 1991; El Gamal, 1997 and Mousa, 2002).

Recent electron microscopical studies revealed that the yolk nucleus was not a homogenous structure, and it was composed of various cellular organelles such as mitochondria, smooth endoplasmic reticulum, multivesicular bodies and lipid granules (Wallace and Selmon, 1981). At the end of the perinucleolus stage and at the vesicles stage the follicular epithelium appeared surrounding the oocytes. On further growth of oocytes, these follicular epithelial cells formed a layer coating the oocytes.

It was believed that the prementioned follicular cells play an important role in active transport of proteins and other nutrients from blood to oocytes during vitellogenesis as reported by Norrevang (1968). Guraya *et al.*, (1975) claimed that follicle cells and oocytes are considered to play an important role in the cytoplasm structures was proved to synthesize sexual steroids by follicular theca envelope. In the present study the zona radiata layer contains microvillar processes pass through pore canals. The microvilli are thought to be the site of substance exchange between the follicle cells and the oocyte (Mastsuyama *et al.*, 1991).

For the nearly ripe stage the present results revealed the presence of few spermatogonia and spermatocytes showing moderate quantity of spermatozoa. These results confirm with most teleosts as reported by Ghabrial (1990) and El-Gohary (2001) in *Oreochromis nilotica*. Ripe stages show a markeds dilation of seminiferous lobules containing a lot of sperms. Also the present study revealed that the spawning stage similar to the ripe stage show a decrease in the size of lobules due to discharge of considerable amounts of spermatozoa. This stage extended through the period of many months through the year for *L. harak*.

The complication of the process of spermatogenesis and the character of the discharge of the sexual products are relative to the asynchronism in the reproduction of the primary spermatocyte as reported by Koppel (1955). Also this asynchronism may be due to the progress of spermatogenesis wave along the different parts of the testes (Butskaya-1955). Adaptation of prolonged and continuous spawning is characterized by fractional discharge of the sperm cells. The prolonged spawning is enhanced by the presence of different individual caught at the same period exhibit different spermatogenic activities and the spermatozoa are discharged gradually from the semineferous lobules and the reduced size of testes, so the specific characteristic of spermatogenesis is related to the type of spawning depending on the character of spawning in female (Zaki *et al.*, 1986; Assem, 1999 and El-ghamaly, 2001).

5. Conclusion

The results of the first length and age of maturity for the *L. harak* was extremely at the 21.5 cm for males and 22.5 cm for females both sexes at second year.

The histological and morphological characters of ovaries *L. harak* have indicated that the oocyte pass through six successive stages of sexual maturation stage 1, immature oocyte (resting stage); stage 11, Vacuolization of the cytoplasm (preparatory stage); stage 111, beginning of yolk deposition (maturing stage); stage 1v, nearly mature (last maturing); stage v, the maturation of oocytes (spawning stage) and stage v1, egg resorption (postpawning stage).

Acknowledgement: Authors acknowledge Sayed Yosif from Khartoum University for photo the slides and the Faculty of Marine Sciences and Fisheries, Red Sea University.

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