Pseudo-nitzschia (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms

AURÉLIE LELONG[1], HÉLÈNE HÉGARET[1], PHILIPPE SOUDANT[1] AND STEPHEN S. BATES[2]*

[1]*LEMAR UMR 6539, IUEM-UBO, Place Nicolas Copernic, Plouzané, France*
[2]*Fisheries and Oceans Canada, Gulf Fisheries Centre, Moncton, NB, Canada E1C 9B6*

Pseudo-nitzschia is a globally distributed diatom genus, some species of which produce domoic acid (DA), the neurotoxin that causes amnesic shellfish poisoning. This toxin killed at least three humans in 1987, launching numerous studies concerning the identification, distribution, ecology and physiology of *Pseudo-nitzschia* spp. Since previous reviews in 1998, knowledge has been gained about the fate of DA, including its accumulation by marine animals and its degradation by light and bacteria. Molecular techniques and more precise microscopy have enabled the description of new *Pseudo-nitzschia* species, 15 since 2002, including ones that are cryptic and pseudo-cryptic. An increasing number of the 37 identified species, including oceanic and coastal species, have been studied in laboratory culture. The sexual reproduction of 14 species has been documented. Fourteen species have now been shown to be toxigenic, although some strains are not always toxic under the testing conditions. The biotic and abiotic factors that modify DA production are reviewed, with a focus on how new discoveries have changed our original hypotheses about control mechanisms. Recent studies confirm that silicate and phosphate limitation trigger DA production. However, stress by low concentrations of iron or high concentrations of copper are newly discovered triggers, suggesting a trace-metal chelation role for DA. Organic sources of nitrogen (urea and glutamine), as well as changes in pH, CO_2, salinity and bacterial concentration, also enhance DA production. Laboratory and field studies sometimes give divergent results for conditions that are conducive to toxin production. Gaps in knowledge include further information about the whole genome of *Pseudo-nitzschia* (including sexual stages), mechanisms of DA production and decline, presence or absence of a resting stage, heterotrophic ability, impact of viruses and fungi, and a more complete description of the ecological and physiological roles of DA.

CONTENTS

* Corresponding author (Stephen.Bates@dfo-mpo.gc.ca).

INTRODUCTION

Diatoms of the *Pseudo-nitzschia* genus (Heterokonta, Bacillariophyceae) were first described by H. Peragallo (1897–1908), before Hustedt included them in the *Nitzschia* genus in 1958. Hasle (1994) then redescribed *Nitzschia* as a specific genus: *Pseudo-nitzschia* (summarized by Bates 2000). Contrary to *Nitzschia*, the long and narrow *Pseudo-nitzschia* cells form chains ('stepped colonies') by slightly overlapping their cell tips (Figs 1–3), among other small differences (Hasle 1994).

Interest in the *Pseudo-nitzschia* genus increased after 1987, when the first amnesic shellfish poisoning (ASP) event occurred, caused by human consumption of blue mussels (*Mytilus edulis*) containing the neurotoxin domoic acid (DA) (Bates *et al.* 1989). This event resulted in at least three deaths of elderly people and over 100 illnesses (reviewed by Bates *et al.* 1998; Pulido 2008; Trainer *et al.* 2008). The toxin was traced to a bloom of *Pseudo-nitzschia multiseries*, upon which the mussels had been feeding. This was the first time that a diatom was shown to produce a neurotoxin. Until this event DA was not considered as a toxin, although it was known as an anthelmintic treatment used by Japanese to rid young children of intestinal worms (Wright *et al.* 1989). However, an order-of-magnitude-lower concentration was used compared with the amount that the affected adults had consumed during the toxic mussel event.

Interest further increased when DA caused the death of seabirds (Fritz *et al.* 1992; Work *et al.* 1993) and marine mammals (Scholin *et al.* 2000), thus the alternative name domoic acid poisoning (DAP), and after it was found to be transferred up the food web by various vectors (see below, and summarized by Bargu *et al.* in press). Fortunately, no known human deaths have occurred since the original 1987 mussel poisoning incident, although consumers in France were affected when they ate uninspected shellfish (*Donax trunculus*) in 2000 (Thébaud *et al.* 2005). This is because surveillance programs worldwide now monitor the concentration of *Pseudo-nitzschia* species in seawater or the presence of DA in the flesh of molluscan shellfish and finfish destined for human consumption (Anderson *et al.* 2001). The harvesting and sale of seafood products is prohibited when the internationally accepted regulatory limit of 20 µg DA g^{-1} wet weight of tissue is attained (Wekell *et al.* 1994). These monitoring programs have taken advantage of research findings, since 1987, that have shed some light on which *Pseudo-nitzschia* species produce DA, the physical factors that may control bloom dynamics and location, and the environmental factors that are conducive for toxin production.

The goal of this review is to highlight some of the more recent important research advances regarding *Pseudo-nitzschia* biology and DA production in the context of previous paradigms. Updates since Bates (1998) and Bates *et al.* (1998) will be given on the chemistry of DA, fate of DA, new toxigenic species of *Pseudo-nitzschia*, distribution of these species and their blooms, biology of *Pseudo-nitzschia*, environmental factors conducive for *Pseudo-nitzschia* growth, and biotic and abiotic factors that trigger DA production. Other reviews have covered the occurrence of *Pseudo-nitzschia* species on the US west coast up to 1996

(Fryxell *et al.* 1997), the physiological ecology of *Pseudo-nitzschia* species up to 1998 (Bates 1998; Bates *et al.* 1998), general aspects (Todd 1993; Mos 2001; Jeffery *et al.* 2004), the ecology of this diatom genus (Bates & Trainer 2006), the molecular basis of DA toxicity (Ramsdell 2007), toxicologic pathology (Pulido 2008), *Pseudo-nitzschia* biology, including oceanographic factors that lead to toxic blooms (Trainer *et al.* 2008), risks of DA to wildlife (Bejarano *et al.* 2008b), molecular approaches for identifying *Pseudo-nitzschia* spp. and assessing its physiology (Kudela *et al.* 2010), the neurotoxicity of DA (Costa *et al.* 2010), vectors of DAP (Bargu *et al.* 2011a, b) and human exposure risks (Grant *et al.* 2010; Lefebvre & Robertson 2010). Additional literature is found in an updated, searchable compilation of DA and *Pseudo-nitzschia* references (Bates 2011).

DOMOIC ACID

General characteristics

Domoic acid is a water-soluble (Falk *et al.* 1991) amino acid of 311 Da, containing three carboxyl groups, and is an analogue of L-glutamic acid, a neurotransmitter, and of kainic acid (Falk *et al.* 1989). It has several geometrical isomers (isodomoic acids A, B, C, D, E, F, G and H) and the diastereoisomer epidomoic acid (Fig. 7) (de la Iglesia *et al.* 2008). Some of these are produced by *Chondria armata* (Maeda *et al.* 1986; Zaman *et al.* 1997), *Nitzschia navis-varingica* (Kotaki *et al.* 2005; Romero *et al.* 2011), *P. australis* (Holland *et al.* 2005; Rhodes *et al.* 2006) and *P. seriata* (Hansen *et al.* 2011). Others are found in molluscan shellfish, sometimes as degradation products (Wright *et al.* 1990; Vale & Sampayo 2001; Holland *et al.* 2003; Rhodes *et al.* 2004; Costa *et al.* 2005b; Holland *et al.* 2005), including photodegradation (see below). Epi-DA is a product of heat degradation (Thomas *et al.* 2008; McCarron *et al.* 2011). Isodomoic acids C (Clayden *et al.* 2005), G and H (Ni *et al.* 2009; Denmark *et al.* 2011), and B, E and F (Lemière *et al.* 2011) have been synthesized; DA has not yet been synthesized.

DA biosynthesis requires high levels of ATP (Pan *et al.* 1996a) and its pathway in *Pseudo-nitzschia* has only partially been resolved (Douglas *et al.* 1992; Thessen 2007). After ingestion, it can bind to *N*-methyl-D-aspartate receptors in the central nervous system with a coefficient 3 times greater than that of kainic acid and 100 times greater than that of glutamic acid (Teitelbaum *et al.* 1990). Because DA is not released by the neuron, in contrast to glutamic acid, depolarization is longer than it should be, thus increasing intraneuronal calcium concentration. Calcium-dependent enzyme activity is maintained, resulting in neuron swelling and then death. Neurons situated in the hippocampus (where memories are consolidated) are affected in mammals (Pulido 2008), leading to anterograde amnesia or short-term memory loss, hence the name ASP. The intoxication is followed by diverse symptoms, from gastric (nausea, diarrhea, gastroenteritis, cramps) and confusion in the first 24 h, to neurological (ataxia, headaches, breathing difficulties, disorientation, dizziness, memory loss) and sometimes coma in the 48 h after
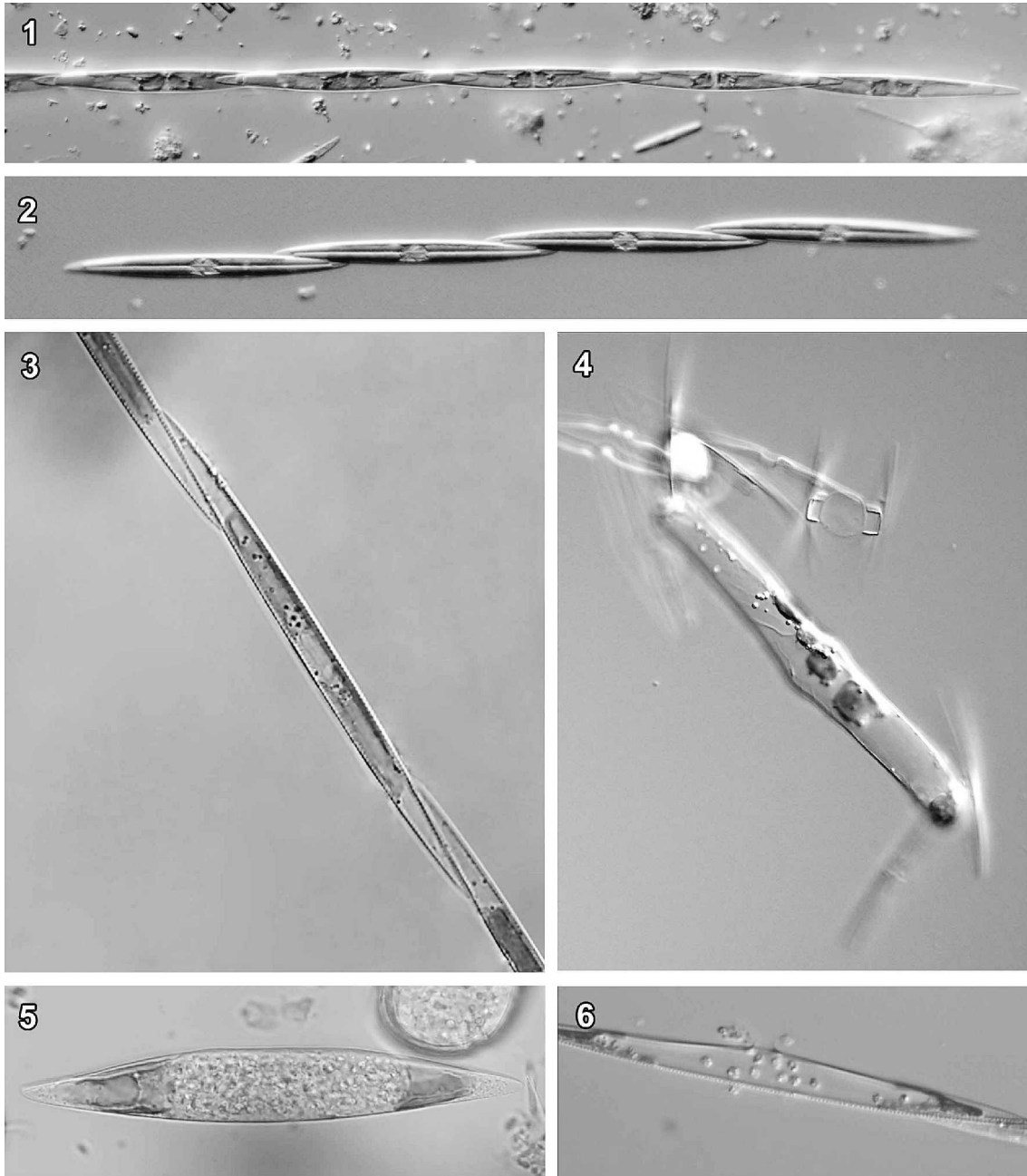
Figs 1–6. *Pseudo-nitzschia* species. Photo credit: Karie Holtermann and E. Virginia Armbrust (University of Washington, Seattle, WA, USA).

Fig. 1. Valve view of a *P. australis* chain, showing cells attached by their overlapping apices, sampled during a toxic bloom (Cabrillo Beach, CA, USA; 09 March 2011). Note that the chain is slightly curved.

Fig. 2. Girdle view of a *P. multiseries* chain in culture; the cells are in the initial stages of dividing (strain originated from Goleta Beach, CA, USA; 10 February 2011).

Fig. 3. Close-up of a *P. multiseries* cell in a chain (Goleta Beach, CA, USA; 10 February 2011).

Fig. 4. Auxospore of *P. australis*; observed after crossing two cultures containing cells of opposite mating type. Note empty parental frustules on upper left of auxospore; the frustules are short as a result of continuous vegetative division and slightly deformed, an artifact sometimes seen after prolonged growth in culture; differential interference microscopy (DIC).

Fig. 5. Girdle view, showing a swollen, fungal-infected *P. pungens* cell containing oomycete zoospores (Goleta Beach, CA, USA; 10 February 2011); DIC.

Fig. 6. A fungal-infected *P. pungens* cell, showing discharging zoospores (Hood Canal, WA, USA; March–April 2007); DIC.

intoxication (Teitelbaum *et al.* 1990). Death may then occur. There is still no antidote to ASP.

Symptoms depend on the quantity of DA ingested and on the health of the affected person. Elderly people and those with impaired renal function or a compromised blood–brain barrier are the most sensitive to DA intoxication. DA is more toxic when consumed with the shellfish than is pure DA (Novelli *et al.* 1992) because of DA
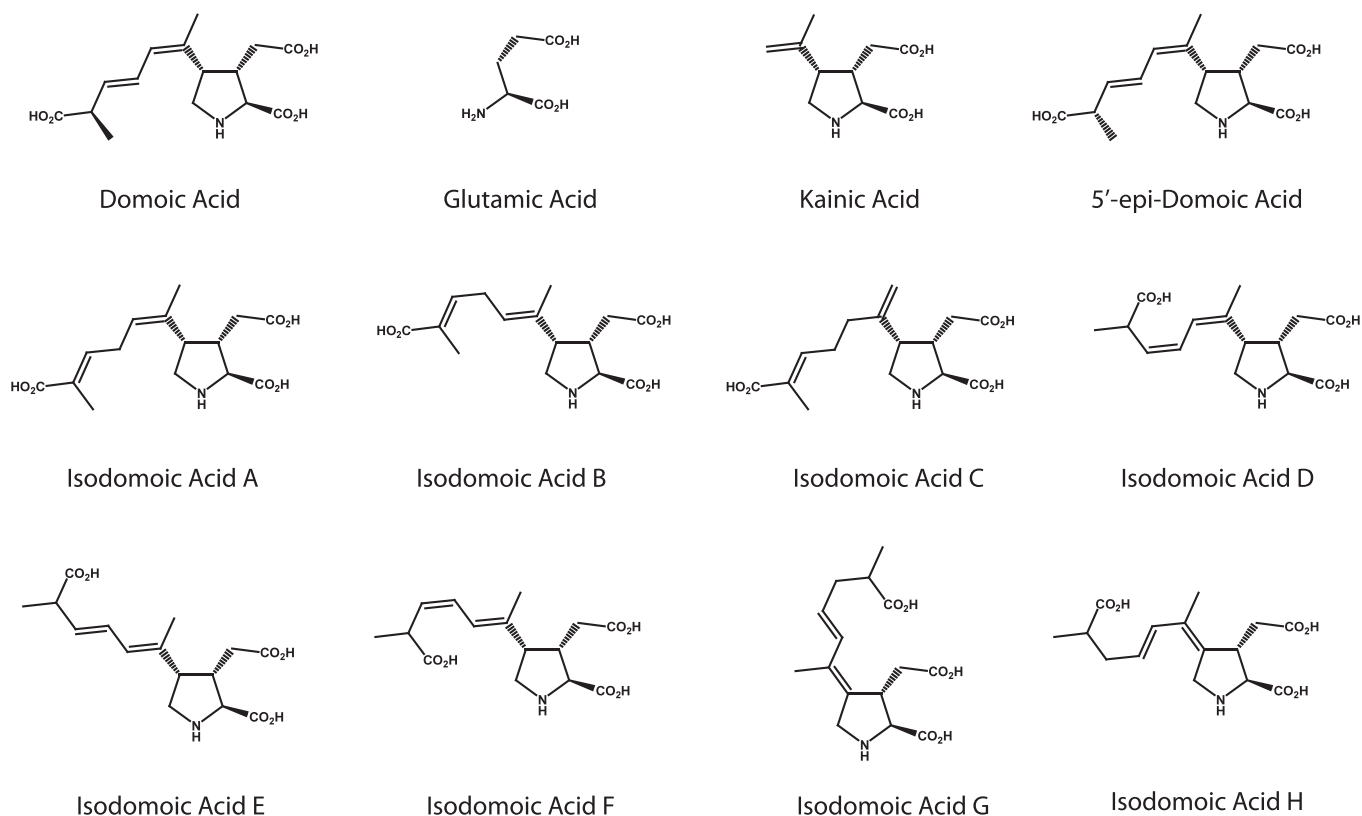
Domoic Acid

Glutamic Acid

Kainic Acid

5'-epi-Domoic Acid

Isodomoic Acid A

Isodomoic Acid B

Isodomoic Acid C

Isodomoic Acid D

Isodomoic Acid E

Isodomoic Acid F

Isodomoic Acid G

Isodomoic Acid H

Fig. 7. Molecular structure of domoic acid and its isomers, glutamic acid and kainic acid (Courtesy of M. Quilliam, NRCC).

potentiation, caused by the high concentrations of glutamic and aspartic acids in shellfish tissues. DA isomers bind less strongly to kainate receptors and are therefore less toxic than DA, with iso-F 2.7-fold less potent, iso-C, -D and -E 23 to 29-fold less potent and iso-B more than 95-fold less potent than DA (Sawant *et al.* 2007, 2010; Munday *et al.* 2008). Munday *et al.* (2008) and Sawant *et al.* (2007) found that iso-A was less toxic than DA, whereas Sawant *et al.* (2010) reported that iso-A is not significantly different from DA; however, different techniques were used.

Removal and degradation

DA does not accumulate in the water column because the low quantities produced are diluted into the vast oceans or sink to the depths while still within intact *Pseudo-nitzschia* cells (Sekula-Wood *et al.* 2009, 2011; Silver *et al.* 2010). Less than 20% of the available DA was adsorbed onto humic acids in the colloidal phase, and adsorption onto natural seawater particles and suspended sediment was negligible ($< 5\%$), but there were no losses onto suspensions of clay minerals (Lail *et al.* 2007); isomeric DA behaved similarly. Bacterial and photodegradation are the more important pathways for its elimination (see below).

DEGRADATION/STABILITY UNDER DIFFERENT CONDITIONS: Interest in the stability of DA stems from the desire to: (1) prevent DA degradation during transport and storage of shellfish samples prior to DA measurement in biotoxin monitoring programs; (2) develop efficient methods to extract DA from toxic cells and shellfish tissues; and (3)

prevent DA degradation in certified reference materials (CRMs) of aqueous solutions and tissue samples used to calibrate instruments.

Stability studies showed that DA plus epi-DA extracted from king scallops (*Pecten maximus*) into aqueous methanol degraded by $\sim 15\%$ and $\sim 45\%$ in the whole-animal extracts and gonad extracts, respectively, over a 2-wk period (Smith *et al.* 2006). Indeed, methanol is known to degrade DA (Vale & Sampayo 2001). Extracts should not be stored in methanol but rather in citric acid buffer, which was shown to be stable over a 3-mo period.

For DA extraction, exposure time to high temperature and the extraction solvent used are critical in determining the stability of DA. The original Association of Official Analytical Chemists-approved method for DA determination used the paralytic shellfish poisoning toxin acid extraction method, i.e. homogenizing the sample for 5 min in boiling 0.1 N HCl. However, this acidic medium (but not the boiling) results in a 5–10% loss in DA, so it has been replaced by an aqueous methanolic (1:1) extraction solution (summarized in Quilliam 2003). Regarding temperature stability, boiling Dungeness crabs (*Cancer magister*) in fresh or salt water for 20 min did not degrade the DA, although its concentration was reduced by 67–71% because the hydrophilic toxin leached from the shellfish tissues into the surrounding water (Hatfield *et al.* 1995). Boiling whole cultures of *Pseudo-nitzschia* (cells plus medium) in test tubes for 3 min efficiently extracted DA from toxic diatom cells, with no significant loss of toxin compared with the control (Bajarias *et al.* 2006). Such extracted material was stable at

room temperature for 10 d; it is then suitable for international transport, in contrast to whole-culture samples, which must be kept at a low temperature during transport. Steaming mussels (*M. edulis*) over boiling water for 10 min, or autoclaving mussel tissue at 121°C for 15 min, did not result in a reduction in the DA concentration, indicating that such processing or cooking is ineffective for eliminating DA before consumption (McCarron & Hess 2006).

For preparation of CRMs, studies have shown that DA is relatively stable (< 1% degradation) in an aqueous acetonitrile solution at 20°C and pH 5–7 for 9 mo, although a 12% loss of material was observed at 50°C after 8 mo (Thomas *et al.* 2008); extremes of pH (2 or 12) and exposure to oxygen also result in DA degradation (Quilliam 2003). DA can be stored in darkness at 4°C, ideally at pH 5–7 under nitrogen or argon, for up to a year; long-term storage is best at −80°C (Quilliam 2003). The stability of DA isomers is similar to that of DA (M. Quilliam, personal communication). Gamma-irradiation was tested as a way to prevent bacterial degradation and therefore to stabilize DA in aqueous and shellfish tissue CRMs (McCarron *et al.* 2007a). However, although irradiation sterilized the samples, it completely decomposed the DA in aqueous solutions and reduced the DA concentration by 40–100% in shellfish tissue, depending on the irradiation dose. Addition of antibiotics, in combination with an antioxidant addition (McCarron *et al.* 2007b) and freeze-drying the standards (McCarron *et al.* 2007c), proved to be the most effective in stabilizing the CRMs.

Freezing Dungeness crabs for 90 d at −23°C only resulted in redistributing the toxin within different tissues (Hatfield *et al.* 1995). Likewise, Leira *et al.* (1998) found that freezing the crabs for 180 d resulted in a net transfer of DA from the hepatopancreas to the rest of the body.

BACTERIAL DEGRADATION: Initial studies showed that bacteria isolated from seawater and sediments (Stewart *et al.* 1998) or from *P. multiseries* cultures (Bates *et al.* 2004) seemed unable to degrade DA. This is supported by Pan *et al.* (2001), who found no degradation of pure DA added to the cell-free filtrate of *P.* sp. cf. *pseudodelicatissima*. However, Hagström *et al.* (2007) found that the presence of high concentrations of bacteria did result in DA degradation, but at different rates depending on their source; bacteria from *P. multiseries* cultures were unable to, whereas bacteria concentrated from the bloom location were capable. Stewart (2008) found that the decline in DA added to a bacterial growth medium was due to bacteria, including *Alteromonas* sp. and *Moraxella* sp. (see below), originating from a *P. multiseries* culture. As well, bacteria of the genera *Alteromonas* and *Pseudomonas*, isolated from blue mussels (*M. edulis*) and softshell clams (*Mya arenaria*), were able to degrade DA and were thought to be partly responsible for the more rapid depuration of DA in these molluscan shellfish (Stewart *et al.* 1998). These bacteria were thought to originate from the *Pseudo-nitzschia* cells that the shellfish had consumed (Stewart 2008). Interestingly, sea scallops (*Placopecten magellanicus*) and red mussels (*Modiolus modiolus*), which do not readily depurate the toxin, only occasionally yielded bacteria with this capability (Stewart *et al.* 1997).

PHOTODEGRADATION: This was first discovered when dilute aqueous solutions of DA were exposed to ultraviolet (UV) light (253.7 nm) for 15 min (Wright *et al.* 1990). Later, exposure of DA in cell-free growth media to the fluorescent light normally used for growth studies showed a 68% decline in DA concentration after 12 d; those kept in darkness displayed a 17% decline (Bates *et al.* 2004). These results suggest that DA production in cultures grown under these same conditions may have been underestimated, and that control flasks may be required. DA photodegradation also occurred in artificial seawater, natural seawater and deionized water, most rapidly during the first 5 min of exposure, at wavelengths < 370 nm and in full-spectrum light, but not in darkness; the addition of iron, which presumably chelated the DA, enhanced the photodegradation in deionized water but not in artificial seawater (Bates *et al.* 2003). Similar results were found by Bouillon *et al.* (2006), with an exponential loss of DA when irradiated with simulated sunlight; there was no loss in dark controls. Radiation in the UV-B (280–320 nm) was most effective, whereas visible light had no apparent effect. Isodomoic acids D, E and F were produced, and these could be further photodegraded, or be regenerated to DA (Bouillon *et al.* 2008). Addition of humic material did not affect the photodegradation rate, showing that DA is most likely photodegraded via a direct photochemical pathway (Bouillon *et al.* 2006). In contrast, Fisher *et al.* (2006) found that iron and dissolved organic matter did prompt DA photodegradation, which was believed to be an indirect, not a direct, photochemical process. These discrepancies are attributed to differences in experiment design, illustrating that results of such experiments should be interpreted with caution. Modelling showed that DA photodegradation is limited to the first few centimetres or metres of the water column (Bouillon *et al.* 2006). These results indicate that photochemical removal of dissolved DA by sunlight is an important sink for DA in natural waters on a timescale of days. Its significance relative to bacterial degradation, adsorption onto particles or dilution still requires quantification in the field.

There is concern that dissolved or particulate DA could be problematic in certain local waters, e.g. where seawater is used for desalination (Bandala *et al.* 2009; Caron *et al.* 2010) or in biotoxin depuration facilities (Djaoued *et al.* 2009). Methods for photodegrading DA have therefore been developed, although never used in a field situation. Such methods use advanced oxidation processes that include a combination of cobalt, peroxymonosulfate, hydrogen peroxide or UV light (Bandala *et al.* 2009), or UV light and the photocatalyst titanium dioxide (TiO_2) on thin films (Djaoued *et al.* 2008, 2009).

PSEUDO-NITZSCHIA SPP.

General characteristics

Members of the *Pseudo-nitzschia* genus are pennate diatoms with a longitudinal symmetry, in contrast to centric diatoms that exhibit a radial symmetry. They are distinguished, in part, from the genus *Nitzschia* by their ability to form chains (Hasle 1994), with the exception of *Pseudo-nitzschia*
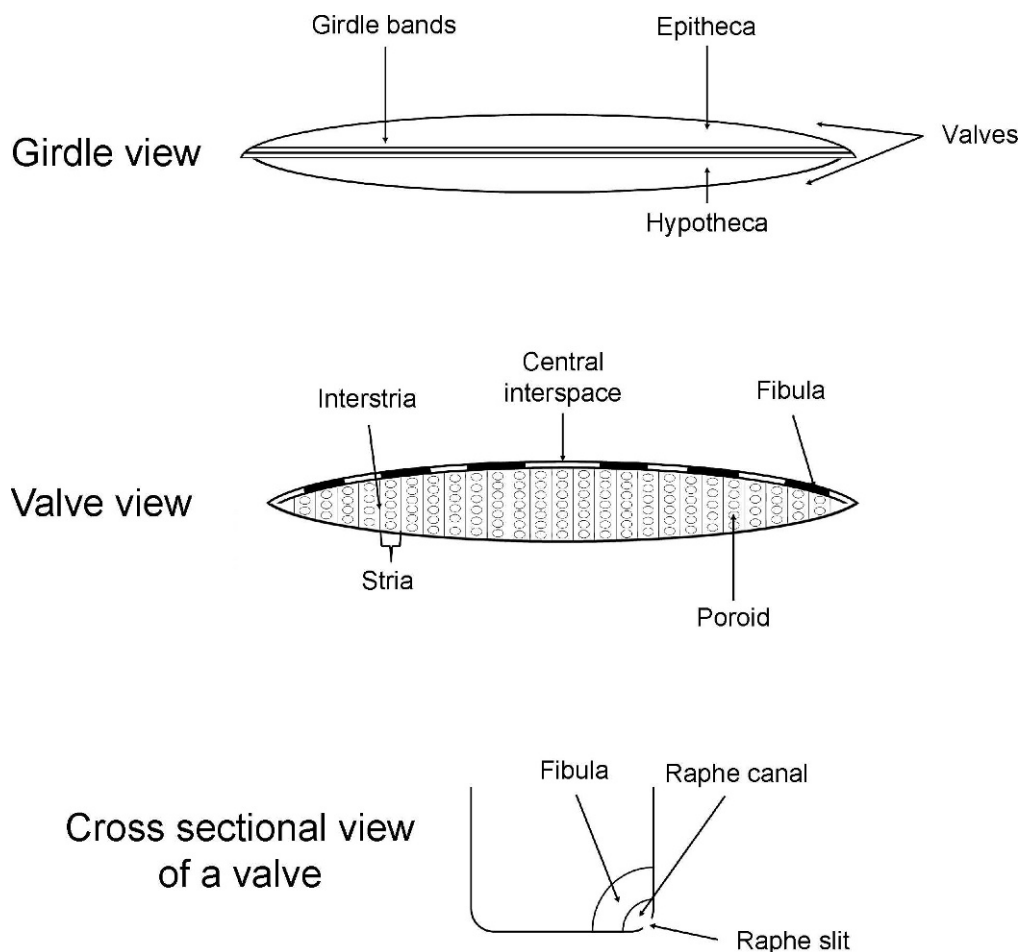
Fig. 8. Silica frustule of *Pseudo-nitzschia* sp. shown in three different views. The cell in valve view is made purposely larger than actual *Pseudo-nitzschia* cells to show poroids and striae. Redrawn from Thessen (2007).

americana, which can be present as single cells (Hernández-Becerril 1998; Orlova & Shevchenko 2002; Stonik *et al.* 2011) or as a chain of cells (Kaczmarska *et al.* 2005b; Nézan *et al.* 2007); *P. antarctica* also forms solitary cells (Scott & Thomas 2005), but this species has rarely been observed and its status requires clarification. Chain length is somewhat dependent on the species, but more likely on the degree of turbulence and the nutritional state of the cells. For *P. multiseries*, nutrient-replete, exponentially growing cells have the longest chains, which usually separate into single cells and sink when nutrient depleted, as during the stationary phase (personal observation; Fryxell *et al.* 1990). This same pattern of chain formation was also reported for *P. brasiliana* (Lundholm *et al.* 2002b). Lundholm & Moestrup (2002) reported that *P. galaxiae* lost its ability to form chains in culture and was found as single cells, even in exponentially growing cultures. Chains of young *P. pungens* (Chepurnov *et al.* 2005), *P. australis* (Fig. 1) and *P. multiseries* (personal observation) in culture and field material exhibit a curvature because the apical axes of adjacent cells deviate by ~ 1°, where one cell attaches to the next. Long chains reaching hundreds of cells, can form spirals with a diameter of ~ 0.5 cm (Fryxell *et al.* 1990).

All species display the same general morphology. As all diatoms, they have a cell wall, called a frustule, made of silicic

acid [$Si(OH)_4$]. This frustule is composed of two valves, or thecae, that fit tightly one inside the other, like a pillbox. The hypotheca is the smaller valve and its edges fit inside the epitheca (Fig. 8). A slit, called a raphe, runs all along each valve and is reinforced inside the cell by silica bridges, called fibulae (Amato *et al.* 2007; Trainer *et al.* 2008). In some species, the raphe is interrupted by a central interspace. Ribs, called interstriae, are separated by striae on the underside of each valve (Figs 8, 9). Piercing these striae are poroids (Figs 8, 9), which allow exchanges between inside the cell and the exterior medium, through the otherwise solid frustule. Numbers and spacing of fibulae, striae and poroids are species dependent and allow a morphological species determination (Hernández-Becerril 1998; Lundholm *et al.* 2002b, 2003; Orlova & Shevchenko 2002; Kaczmarska *et al.* 2005b; Amato *et al.* 2007; Almandoz *et al.* 2008). However, these morphometrics may vary from one strain to another, even for the same species, depending on the environment (Orlova & Shevchenko 2002; Lundholm *et al.* 2003; Amato *et al.* 2007; Trainer *et al.* 2008; Hansen *et al.* 2011). In the case of *P. galaxiae*, the large differences in cell shape and length are caused by variable extensions of the rostra (Cerino *et al.* 2005).

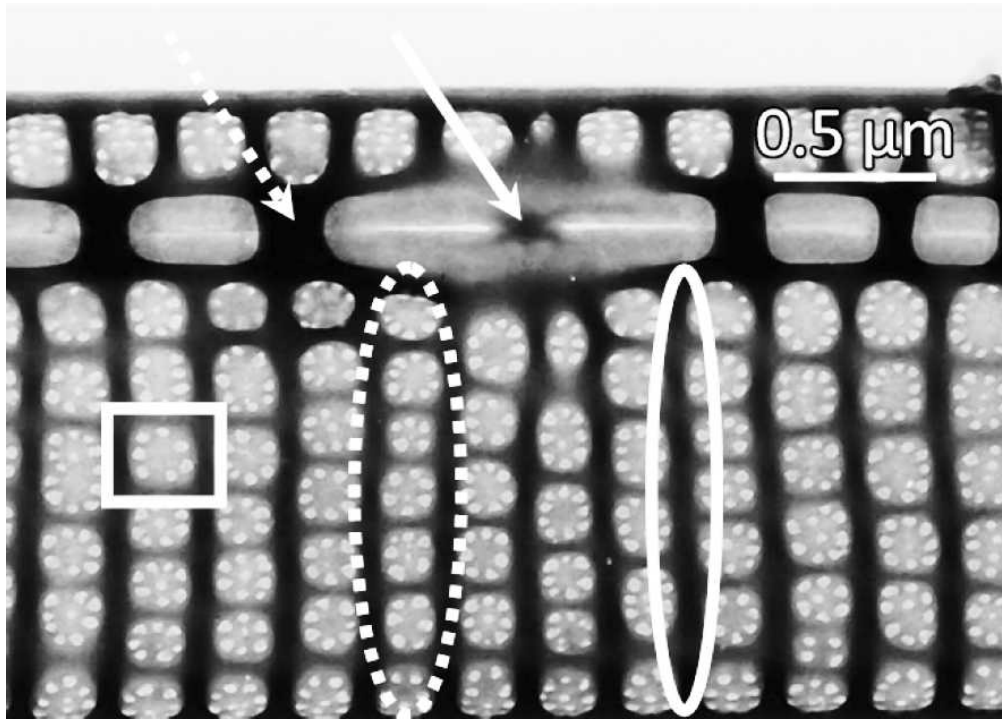The cause of 'lobed', 'undulate' or 'sickle-shaped' cell deformities (Mengelt & Prézelin 2002), most often found in

Fig. 9. Transmission electron microscope image of *Pseudo-nitzschia calliantha*. Dotted arrow indicates the fibulae, solid arrow the wider separation of the two central fibulae (= central interspace), dotted ellipse a stria, solid ellipse an interstria and the square shows a poroid, with the structural pattern of the poroid hymen.

older cultures (Amato *et al.* 2005; Li *et al.* 2005; Hagström *et al.* 2010) but also reported in some field samples (discussed in Bates *et al.* 1998), is still not fully explained. Deformed cells, characterized as 'boomerang' or 'handle bar' shaped, have also been found at depths of 150 m in the Santa Barbara Channel, California, USA, as well as in surface samples associated with toxic *P. pseudodelicatissima*-complex species (C. Anderson, personal communication). Once present, these deformities are propagated because they form the template for subsequent cells produced by vegetative cell division. This is a mechanism that could (although rarely) lead to 'abrupt cell size reduction' (Chepurnov *et al.* 2005). It starts with the appearance of the small bulge on one side of the valve and a constriction, which can get progressively deeper, on the other side, leading to the cell separating in two. Likewise, it is still not known what causes the appearance of 'stacked' (or 'ribbon-shaped') colonies (e.g. Lundholm *et al.* 2002b), although this, plus cell deformities, may be an artefact, at least in cultures, e.g. caused by prolonged silicate limitation. Finally, it is still not known if these variants affect cell physiology, including DA production. They may or may not be associated with the gradual decline in DA production as a culture ages (see below), because not all cultures display deformed cells.

Presently, 37 species of *Pseudo-nitzschia* have been described (Table 1). More recent additions include: *P. sinica* (Qi *et al.* 1994), *P. multistriata* (Takano 1995), *P. galaxiae* (Lundholm & Moestrup 2002), *P. micropora* (Priisholm *et al.* 2002), *P. brasiliana*, *P. linea* (Lundholm *et al.* 2002b), *P. calliantha*, *P. caciantha* (Lundholm & Moestrup 2002), *P. antarctica* (Marchant & Thomas 2005),

P. obtusa (Hasle & Lundholm 2005), *P. decipiens*, *P. dolorosa* (Lundholm *et al.* 2006), *P. roundii* (Hernández-Becerril & Díaz-Almeyda 2006), *P. mannii* (Amato & Montresor 2008), *P. arenysensis* (Quijano-Scheggia *et al.* 2009b), *P. hasleana* and *P. fryxelliana* (Lundholm *et al.* in press).

Identification

Precise determination of *Pseudo-nitzschia* species identity by light microscopy is difficult, or impossible. Most of the above frustule morphometrics needed for species determination are visible only by scanning or transmission electron microscopy, with the exception of cryptic species (see below). Nevertheless, cell shape, length and width can be determined by light microscopy, although cell length is the least diagnostic character because it decreases with each cell division. Thus, a crude categorization of field samples can be made by separating *Pseudo-nitzschia* species into two distinct groups, on the basis of cell width: (1) *seriata* group, containing wide species (> 3 μm width) and (2) *delicatissima* group (< 3 μm width) (Hasle & Syvertsen 1997) (Table 1). Sometimes three groups are made: (1) *multiseries/pungens* group, (2) *australis/fraudulenta/heimii* group and (3) *pseudodelicatissima/delicatissima* group (Trainer *et al.* 2008). *Pseudo-nitzschia americana* is separated from the other groups as it is different, with short (< 37 μm), rounded cells that may or may not form chains (see above), and because its cell width overlaps that of both the *seriata* and the *delicatissima* groups (Table 1).

Early descriptions were based on morphology, but it is becoming increasingly important to supplement this with

Table 1. List of described *Pseudo-nitzschia* species and their distribution (see also Fig. 6 and the listings for each species in http://diatoms. lifedesks.org/pages/990, and Hasle 2002). Species were identified by electron microscopy or molecular techniques. 'Group' refers to a classification based on the width of the cell as seen by light microscopy: *seriata* group > 3 μm width; *delicatissima* group < 3 μm width (Hasle & Syvertsen 1997); 'Neither' indicates that the species cell width spans both groups. Cell widths (μm) are the minimum and maximum values found in the references listed for each species. 'Toxicity' indicates if toxin analyses have been carried out: 'yes' = the species produces domoic acid (only the first reference demonstrating toxicity is given); 'no' = values are below the limit of detection; 'yes/no' = not all strains show toxicity; 'not tested' = species not yet tested.

Species	Group (cell width)	Toxicity	References for toxicity	Geographical distribution	References for distribution
P. americana (Hasle) Fryxell	neither (2.5–4.5)	no		Australia, Bay of Fundy, Bermuda, Brazil, California, Chile, Costa Rica, France (Atlantic), Gulf of Mexico, Japan, Malaysia, Mexico (Pacific), Monterey Bay, Narragansett Bay, New Zealand, North Carolina, North Sea, NW Africa, Oregon, Peru, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk), Skagerrak, South Africa, Thailand, Uruguay, Vietnam, Washington	Miller & Kamykowski 1986; Rhodes 1998; Villac *et al.* 1993; Hallegraeff 1994; Hernández-Becerril 1998; Gailhard *et al.* 2002; Lundholm *et al.* 2002b; Orlova & Shevchenko 2002; Kaczmarska *et al.* 2005b; Nézan *et al.* 2007; Álvarez *et al.* 2009; Churro *et al.* 2009; Del Rio *et al.* 2010; Stonik *et al.* 2011
P. antarctica Manguin	*seriata* (3–4)	not tested		Terre Adélie (Antarctica)	Scott & Thomas 2005
P. arenysensis Quijano-Scheggia, Garcés, Lundholm	*delicatissima* (1.6–2.5)	no		France (Atlantic), Gulf of Naples, Mexico (Gulf of Mexico), Spain (Mediterranean)	Quijano-Scheggia *et al.* 2009b; Orive *et al.* 2010; Lundholm *et al.* in press
P. australis Frenguelli	*seriata* (4.6–10.0)	yes/no	Fritz *et al.* 1992; Garrison *et al.* 1992	Alaska, Argentina, Australia, Brazil, Chile, France (Atlantic), Ireland, Mexico (Pacific), Monterey Bay, Namibia, New Zealand, Peru, Portugal, Russian Arctic seas (White Sea, Barents Sea), Scotland, South Africa, Spain (Atlantic, Mediterranean), Uruguay, US west coast	Buck *et al.* 1992; Fritz *et al.* 1992; Bates *et al.* 1993a, 1998; Hernández-Becerril 1998; Rhodes 1998; Hasle 2002; Suárez-Isla *et al.* 2002; Lundholm *et al.* 2003; Procopiak *et al.* 2006; Almandoz *et al.* 2007; Howard *et al.* 2007; Álvarez *et al.* 2009; Churro *et al.* 2009; Orive et al. 2010; Quijano-Scheggia *et al.* 2010
P. brasiliana Lundholm, Hasle & G.A. Fryxell	*delicatissima* (1.8–3.4)	yes/no	Sahraoui *et al.* in press	Brazil, Gulf of Mexico, Gulf of Panama, Indonesia, Korea, Malaysia, Mexico (Pacific), Spain (Mediterranean), Thailand, Tunisia, Vietnam	Lundholm *et al.* 2002b; Quijano-Scheggia *et al.* 2005, 2010; Thessen *et al.* 2005; Villac *et al.* 2005; Amato & Montresor 2008; Sahraoui *et al.* in press
P. caciantha Lundholm, Moestrup & Hasle	*delicatissima* (2.7–3.5)	no		Greece, Gulf of Naples, Mexico (Gulf of Mexico), New Zealand, Russia (NW Sea of Japan, Sea of Okhotsk), Spain (Mediterranean), Thailand	Lundholm *et al.* 2003; Amato *et al.* 2007; Amato & Montresor 2008; Quijano-Scheggia *et al.* 2010; Stonik *et al.* 2011; L. Rhodes, personal communication
P. calliantha Lundholm, Moestrup & Hasle	*delicatissima* (1.1–2.6)	yes/no	Martin *et al.* 1990	Adriatic Sea, Argentina, Australia, Beaufort Sea, Bermuda, Brazil, Chesapeake Bay, Chile, Denmark, France (Mediterranean), Germany (Kiel Bay), Greece, Gulf of Mexico, Gulf of Naples, Gulf of St. Lawrence, North Atlantic, Norway, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk), Russian Arctic seas (White Sea, Barents Sea), Scotland, Slovenia, Spain (Atlantic, Mediterranean), Tunisia, Turkey (Black Sea), Vietnam	Martin *et al.* 1990; Bargu *et al.* 2002a; Lundholm *et al.* 2003; Thessen *et al.* 2005; Procopiak *et al.* 2006; Almandoz *et al.* 2007; Amato *et al.* 2007; Besiktepe *et al.* 2008; Thessen & Stoecker 2008; Álvarez *et al.* 2009; Churro *et al.* 2009; Sahraoui *et al.* 2009; Del Rio *et al.* 2010; Moschandreou *et al.* 2010; Quijano-Scheggia *et al.* 2010; Stonik *et al.* 2011; L. Rhodes, personal communication

Table 1. Continued

Species	Group (cell width)	Toxicity	References for toxicity	Geographical distribution	References for distribution
P. cuspidata (Hasle) Hasle	*delicatissima* (1.0–2.0)	yes/no	Bill *et al.* 2005	Australia, Brazil, Canary Islands, Chesapeake Bay, Hong Kong, Korea, Mexico (Gulf of Mexico), Portugal, Spain (Atlantic), Thailand, US west coast, Vietnam, West Africa (Mauritania)	Hasle 1965; Fraga *et al.* 1998; Cho *et al.* 2001; Priisholm *et al.* 2002; Lundholm *et al.* 2003; Bill *et al.* 2005; Amato *et al.* 2007; Doan-Nhu *et al.* 2008; Thessen & Stoecker 2008; Churro *et al.* 2009; Trainer *et al.* 2009a; L. Rhodes, personal communication
P. decipiens Lundholm & Moestrup	*delicatissima* (1.4–2.5)	no		Black Sea, Canary Islands, Italy (Tyrrhenian Sea), Mexico (Gulf of Mexico), Portugal	Lundholm *et al.* 2006; Amato & Montresor 2008; Congestri *et al.* 2008
P. delicatissima[1] (Cleve) Heiden	*delicatissima* (1.0–2.4)	yes/no	Smith *et al.* 1991	Adriatic Sea, Australia, Azores, Brazil, California, Chile, China, Denmark, France (Atlantic), Greece, Greenland, Gulf of Naples, Gulf of St. Lawrence, Ireland, Japan, Mexico (Pacific, Gulf of Mexico), Morocco, New Zealand, North Sea, Norway, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk, Baltic Sea), Scotland, South Africa, Spain (Atlantic, Mediterranean), Sweden, Thailand, US northeast coast, US west coast, Vietnam, West Africa (Mauritania)	REPHY; Hasle 1965, 2002; Hasle *et al.* 1996; Bates *et al.* 1998; Parsons *et al.* 1999; Lundholm *et al.* 2006; Procopiak *et al.* 2006; Kaczmarska *et al.* 2007; Amato & Montresor 2008; Quijano-Scheggia *et al.* 2008; Vershinin & Orlova 2008; Trainer *et al.* 2009b; Moschandreou *et al.* 2010; Stonik *et al.* 2011
P. dolorosa Lundholm & Moestrup	*seriata* (2.5–3.0)	no		Australia, Drake Passage, Greece, Gulf of Naples, Ireland, Monterey Bay, Portugal, subarctic Pacific (Ocean Station Papa)	Ferrario *et al.* 2004; Lundholm *et al.* 2006; Amato *et al.* 2007; McDonald *et al.* 2007; Marchetti *et al.* 2008; Churro *et al.* 2009; Moschandreou *et al.* 2010; Quijano-Scheggia *et al.* 2010; L. Rhodes, personal communication; G. Hallegraeff, personal communication
P. fraudulenta (Cleve) Hasle	*seriata* (4.0–8.0)	yes/no	Rhodes *et al.* 1998	Argentina, Australia, Azores, Bay of Fundy, Bering Sea, Brazil, Chesapeake Bay, Chile, Denmark, France (Atlantic), Greece, Gulf of Alaska, Gulf of Maine, Gulf of Naples, Gulf of St. Lawrence, Hong Kong, Ireland, Japan, Korea, Mexico (Pacific), Morocco (Atlantic), New Zealand, Norway, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk, Baltic Sea), Scotland, South Africa, Spain (Atlantic), Uruguay, US central and northeast coasts, US west coast	Hallegraeff 1994; Hasle *et al.* 1996; Bates *et al.* 1998; Rhodes 1998; Hasle 2002; Lundholm *et al.* 2003; Kaczmarska *et al.* 2005b; Almandoz *et al.* 2007; Quijano-Scheggia *et al.* 2008, 2010; Thessen & Stoecker 2008; Vershinin & Orlova 2008; Trainer *et al.* 2009a; Churro *et al.* 2009; Moschandreou *et al.* 2010; Orive *et al.* 2010; Silver *et al.* 2011; Stonik *et al.* 2011
P. fryxelliana Lundholm	*delicatissima* (2.1–2.5)	not tested		Washington	Lundholm *et al.* in press

Table 1. Continued

Species	Group (cell width)	Toxicity	References for toxicity	Geographical distribution	References for distribution
P. galaxiae Lundholm & Moestrup	*delicatissima* (1.0–1.8)	yes/no	Cerino *et al.* 2005	Australia, France (Atlantic), Greece, Gulf of Naples, Mexico (Gulf of Mexico), Spain (Mediterranean)	Lundholm & Moestrup 2002; Lundholm *et al.* 2003; Cerino *et al.* 2005; Trainer *et al.* 2008; Moschandreou *et al.* 2010; Orive *et al.* 2010; Quijano-Scheggia *et al.* 2010
P. granii (Hasle) Hasle	*delicatissima* (1.3–1.8)	yes/no	Trick *et al.* 2010[2]	Korea, Russian Arctic seas (White Sea, Barents Sea), subarctic Pacific (Ocean Station Papa)	Cho *et al.* 2001; El-Sabaawi & Harrison 2006; Marchetti *et al.* 2008; Vershinin & Orlova 2008; Trick *et al.* 2010
P. hasleana Lundholm	*delicatissima* (1.5–2.8)	no		Japan, NE Pacific (off Washington), Spain (Atlantic), Washington	Lundholm *et al.* in press
P. heimii Manguin	*seriata* (3.5–6.0)	no		Argentina, Drake Passage, Monterey Bay, New Zealand, Norway, Russia (Sea of Okhotsk), Skagerrak, Subarctic Pacific (Ocean Station Papa), Thailand, US west coast, Weddell Sea (Antarctica)	Hasle *et al.* 1996; Fryxell *et al.* 1997; Rhodes 1998; Ferrario *et al.* 2004; Almandoz *et al.* 2007, 2008; Marchetti *et al.* 2008; Trainer *et al.* 2009a; Stonik *et al.* 2011
P. inflatula (Hasle) Hasle	*delicatissima* (1.3–2.0)	no		Denmark, Italy (Tyrrhenian Sea), Mexico (Pacific), Monterey Bay, NE subarctic Pacific, Thailand, Vietnam	Hasle 1965; Fryxell *et al.* 1997; Priisholm *et al.* 2002; Skov *et al.* 2004; Hernández-Becerril & Díaz-Almeyda 2006; Congestri *et al.* 2008
P. linea Lundholm, Hasle & G.A. Fryxell	*delicatissima* (1.8–2.2)	not tested		Brazil, Costa Rica, Gulf of Mexico, Narragansett Bay, Spain (Mediterranean)	Lundholm *et al.* 2002a, b; Quijano-Scheggia *et al.* 2010; Fernandes & Brandini 2010
P. lineola (Cleve) Hasle	*delicatissima* (2.0–2.7)	no		Argentina, Australia, Denmark, Drake Passage, Mexico (Pacific), NE Pacific (off Washington), US west coast, Weddell Sea (Antarctica)	Hasle 1965; Hallegraeff 1994; Fryxell *et al.* 1997; Hernández-Becerril 1998; Ferrario *et al.* 2004; Almandoz *et al.* 2007, 2008; Trainer *et al.* 2009b; Lundholm *et al.* in press
P. mannii Amato & Montresor	*delicatissima* (1.7–2.6)	not tested		Adriatic Sea, Greece, Gulf of Naples, Spain (Mediterranean)	Almandoz *et al.* 2008; Amato & Montresor 2008; Moschandreou *et al.* 2010; Quijano-Scheggia *et al.* 2010; Z. Ljubešić, personal communication
P. micropora Priisholm, Moestrup & Lundholm	*delicatissima* (1.3–2.0)	not tested		Thailand, Vietnam	Priisholm *et al.* 2002; Skov *et al.* 2004; Lundholm *et al.* 2003
P. multiseries (Hasle) Hasle	*seriata* (3.5–4.8)	yes	Bates *et al.* 1989	Algeria, Argentina, Baltic Sea, Bay of Fundy, Bering Sea, Brazil, British Columbia, Chesapeake Bay, China, Denmark, France (Atlantic), Greece, Gulf of Maine, Gulf of Mexico, Gulf of St. Lawrence, Hong Kong, Ireland, Japan, Korea, Monterey Bay, New Zealand, Norway, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk, Baltic Sea), Scotland, Spain (Atlantic), Uruguay, US northeast coast, US west coast	Bates *et al.* 1989, 1998; Hasle *et al.* 1996; Pan *et al.* 1996a; Rhodes 1998; Kotaki *et al.* 1999; Hasle 2002; Kaczmarska *et al.* 2005b; Procopiak *et al.* 2006; Hagström *et al.* 2007; Thessen & Stoecker 2008; Vershinin & Orlova 2008; Moschandreou *et al.* 2010; Stonik *et al.* 2011

Table 1. Continued

Species	Group (cell width)	Toxicity	References for toxicity	Geographical distribution	References for distribution
P. multistriata (Takano) Takano	neither (2.5–3.7)	yes/no	Rhodes *et al.* 2000	Australia, Brazil, China, France (Atlantic), Greece, Gulf of Mexico, Gulf of Naples, Japan, Korea, New Zealand, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk), Spain (Atlantic, Mediterranean), Thailand, Uruguay, Vietnam	Takano 1995; Hasle 2002; Orsini *et al.* 2002; Skov *et al.* 2004; Lundholm *et al.* 2003; Quijano-Scheggia *et al.* 2005, 2008; Thessen *et al.* 2005; Churro *et al.* 2009; D'Alelio *et al.* 2009; Méndez & Ferrario 2009; Moschandreou *et al.* 2010; Orive *et al.* 2010; Stonik *et al.* 2011
P. obtusa (Hasle) Hasle & Lundholm	*seriata* (2.9–5.0)	no		Alaska, Barents Sea, Canadian Arctic, Denmark, Greenland, Gulf of St. Lawrence, Hudson Strait, Ireland, Newfoundland, Norway, Russia (Sea of Okhotsk, Kamchatka)	Hasle 2002; Hasle & Lundholm 2005; Stonik *et al.* 2011
P. prolongatoides[3] (Hasle) Hasle	*delicatissima* (0.5–2.5)	not tested		Gulf of Mexico, Weddell Sea (Antarctica)	Almandoz *et al.* 2008; Krayevsky *et al.* 2009
P. pseudodelicatissima[3] (Hasle) Hasle	*delicatissima* (1.1–2.1)	yes/no	Lundholm *et al.* 1997	Australia, Bay of Fundy, Chile, China, Croatia, Denmark, France (Atlantic), Greece, Gulf of Maine, Gulf of Mexico, Gulf of Naples, Iceland, Ireland, Mexico (Gulf of Mexico), New Zealand, Norway, Portugal, Russia (Black Sea, Baltic Sea), Russian Arctic seas (White Sea), Scotland, Spain (Atlantic Mediterranean), Thailand, US west coast, Vietnam	Martin *et al.* 1990; Bates *et al.* 1993a; Hasle *et al.* 1996; Fraga *et al.* 1998; Rhodes 1998; Pan *et al.* 2001; Hasle 2002; Lundholm *et al.* 2003; Kaczmarska *et al.* 2005b; Churro *et al.* 2009; Moschandreou *et al.* 2010; Orive *et al.* 2010; Ljubešić *et al.* 2011
P. pungens (Grunow ex Cleve) Hasle	*seriata* (2.2–5.4)	yes/no	Rhodes *et al.* 1996	Argentina, Australia, Bay of Fundy, Bering Sea, Brazil, British Columbia, Caribbean Sea, Chesapeake Bay, Chile, China, Denmark, Ecuador, France (Atlantic, Mediterranean), Greece, Gulf of Mexico, Gulf of St. Lawrence, Hong Kong, Indian Ocean, Indonesia, Ireland, Japan, Korea, Mexico (Pacific, Gulf of Mexico), Morocco, Mozambique, New Zealand, Norway, Peru, Portugal, Russia (NW Sea of Japan, Sea of Okhotsk, Bering Sea, Black Sea, Baltic Sea), Russian Arctic seas (White Sea), Scotland, Spain (Atlantic, Mediterranean), Thailand, US east coast, US west coast, Vietnam, West Africa	Forbes & Denman 1991; Bates *et al.* 1993a, 1998; Hallegraeff 1994; Hasle *et al.* 1996; Rhodes *et al.* 1998; Hasle 2002; Lundholm *et al.* 2003; Kaczmarska *et al.* 2005b; Procopiak *et al.* 2006; Almandoz *et al.* 2007; Thessen & Stoecker 2008; Moschandreou *et al.* 2010; Orive *et al.* 2010; Stonik *et al.* 2011
P. pungiformis (Hasle) Hasle	*seriata* (4.0–5.0)	not tested		Monterey Bay	Hasle *et al.* 1996; Fryxell *et al.* 1997
P. roundii Hernández-Becerril	*seriata* (4.6–6.5)	not tested		Mexico (Pacific)	Hernández-Becerril & Díaz-Almeyda 2006

Table 1. Continued

Species	Group (cell width)	Toxicity	References for toxicity	Geographical distribution	References for distribution
P. seriata (Cleve) H. Peragallo	*seriata* (4.6–8.0)	yes/no	Lundholm *et al.* 1994	Argentina (Beagle Channel), Baltic Sea, Barents Sea, Bay of Fundy, Canadian Arctic (Resolute Bay), Chesapeake Bay, Denmark, Greece, Greenland, Gulf of St. Lawrence, Iceland, Ireland, Newfoundland, North Sea, Norway, Russia (NW Sea of Japan, Sea of Okhotsk), Russian Arctic seas (White Sea, Barents Sea), Scotland, US northeast coast	Bates *et al.* 1998; Lundholm *et al.* 1994; Hasle *et al.* 1996; Hasle 2002; Lundholm *et al.* 2003; Fehling *et al.* 2004; Kaczmarska *et al.* 2005b; Davidson & Fehling 2006; Procopiak *et al.* 2006; Almandoz *et al.* 2009; Ignatiades & Gotsis-Skretas 2010; Stonik *et al.* 2011; Hansen *et al.* 2011
P. sinica Qi, Ju & Lei	neither (2.5–5.0)	not tested		China, Thailand, Vietnam	Qi *et al.* 1994; Priisholm *et al.* 2002; Li *et al.* 2005; Doan-Nhu *et al.* 2008
P. subcurvata (Hasle) Fryxell	*delicatissima* (1.5–2.0)	no		Argentina, Drake Passage (Antarctica), Gulf of Mexico, Weddell Sea (Antarctica)	Fryxell *et al.* 1991; Bates *et al.* 1993a; Almandoz *et al.* 2007, 2008; Aké-Castillo & Okolodkov 2009
P. subfraudulenta (Hasle) Hasle	*seriata* (5.0–7.0)	no		Australia, Chile, China, France (Mediterranean), Greece, Gulf of Mexico, Gulf of Naples, Gulf of Panama, Japan, Korea, Mexico (Pacific, Gulf of Mexico), Monterey Bay, Northwest Africa, Vietnam	Hasle 1965, 2002; Skov *et al.* 2004; Thessen *et al.* 2005; Hernández-Becerril & Díaz-Almeyda 2006; Álvarez *et al.* 2009; Moschandreou *et al.* 2010
P. subpacifica (Hasle) Hasle	*seriata* (5.0–7.0)	no		Australia, Bay of Fundy, California, Chesapeake Bay, China, France (Atlantic), Gulf of Mexico; Gulf of Panama, Ireland, Korea, Monterey Bay, Northwest Africa, Portugal, Spain (Atlantic), Washington	Hasle 1965; Hallegraeff 1994; Fryxell *et al.* 1997; Fraga *et al.* 1998; Cho *et al.* 2001; Lundholm *et al.* 2003; Kaczmarska *et al.* 2005b; Thessen & Stoecker 2008; Churro *et al.* 2009; Orive *et al.* 2010
P. turgidula (Hasle) Hasle	neither (2.5–5.0)	yes	Rhodes *et al.* 1996	Argentina, Australia, Barents Sea, Bay of Fundy, China, Drake Passage, Monterey Bay, New Zealand, Scotland, Subarctic Pacific, Weddell Sea (Antarctica)	Hallegraeff 1994; Rhodes *et al.* 1996; Hasle 2002; Ferrario *et al.* 2004; Almandoz *et al.* 2007, 2008; Leandro *et al.* 2010a; Trick *et al.* 2010
P. turgiduloides Hasle	*delicatissima* (1.7–2.5)	no		Argentina, Drake Passage, Ross Sea, Weddell Sea (Antarctica)	Lundholm *et al.* 2003; Ferrario *et al.* 2004; Almandoz *et al.* 2007, 2008

[1] Some species may be *P. arenysensis* (*sensu* Quijano-Scheggia *et al.* 2009).
[2] Shipboard batch culture of natural seawater containing *P. granii* as the only species of *Pseudo-nitzschia*.
[3] May be multiple species within the *P. pseudodelicatissima* complex (*sensu* Lundholm *et al.* 2003; Amato & Montresor 2008), if reports are earlier than 2003.

molecular studies, as well as with mating studies, especially because of the number of cryptic and pseudo-cryptic species being identified (see below). As well, growth temperature has been shown to affect cell morphology, e.g. for *P. multiseries* (Lewis *et al.* 1993) and *P. seriata* (Lundholm *et al.* 1994; Fehling *et al.* 2004; Hansen *et al.* 2011), further confusing species identification. We must still not lose sight of the importance of training qualified taxonomists to work alongside those using molecular approaches.

Molecular studies involve sequence analyses of the nuclear-encoded large-subunit (LSU) ribosomal DNA (rDNA) (Stehr *et al.* 2002; Amato *et al.* 2007; McDonald *et al.* 2007; Thessen *et al.* 2009; Moschandreou *et al.* 2010) or of the internal transcribed spacer (ITS1 or ITS2) regions (e.g. Lundholm *et al.* 2003, 2006; Orsini *et al.* 2004; Amato *et al.* 2007; Kaczmarska *et al.* 2008; Casteleyn *et al.* 2009b; Thessen *et al.* 2009; Andree *et al.* 2011), the mitochondrion-encoded cytochrome *c* oxidase 1 (Kaczmarska *et al.* 2008; Lundholm *et al.* in press) or the chloroplast-encoded large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*L) (Amato *et al.* 2007; Casteleyn *et al.* 2009b, 2010; Lundholm *et al.* in press) or small (*rbc*S) (Delaney *et al.* in press) gene. Such molecular information has been used to develop an automated ribosomal intergenic spacer analysis approach to identify *Pseudo-nitzschia* species rapidly in environmental samples (Hubbard *et al.* 2008; Marchetti *et al.*

2008). McDonald *et al.* (2007) identified different species and pseudo-cryptic species in the natural environment by amplifying LSU fragments, which appeared to be more reliable than microscopic observations. Microsatellite markers, requiring isolates in culture, have been developed for *P. pungens* (Evans & Hays 2004), *P. multiseries* (Evans *et al.* 2004), *P. multistriata* (Tesson *et al.* 2011) and *P. australis* (N. Adams, personal comunication). These can be used to help determine if *Pseudo-nitzschia* populations are made up of cryptic species, different varieties of the same species or contain hybrid forms of species varieties (reviewed in Trainer *et al.* in press).

Phylogenetic trees on the basis of LSU rDNA sequence analysis have proven that *Pseudo-nitzschia* is a paraphyletic genus (Lundholm *et al.* 2002b). Often, the above groupings on the basis of cell width are consistent with those shown in phylogenetic trees (e.g. Cerino *et al.* 2005). The taxonomic status and phylogeny of *P. americana*, however, is still not clear because it is uncertain if the original sequences submitted came from the single-celled or chain-forming strains (Lundholm *et al.* 2002a; Orsini *et al.* 2002).

On the basis of molecular evidence and careful light microscopy examination of existing species, some of these species have recently been emended and several new ones defined (included in Table 1). It is likely that new species will continue to be discovered in this way, thus increasing the known species diversity (Medlin & Kooistra 2010; Lundholm *et al.* in press). *Pseudo-nitzschia pseudodelicatissima* was split into three species: *P. calliantha*, *P. pseudodelicatissima* and *P. caciantha*, and *P. cuspidata* was redefined (Lundholm *et al.* 2003). The genotype identified as *P. calliantha*2 (Amato *et al.* 2007) was named as a new species, *P. mannii*, within the *P. pseudodelicatissima/cuspidata* complex (Amato & Montresor 2008). Furthermore, a recent more thorough examination of the above narrow (~ 1.5–2.5 μm) species has revealed even greater diversity, with the identification of two new species: *P. hasleana* and *P. fryxelliana* (Lundholm *et al.* in press). Reports of DA production by strains identified as *P. pseudodelicatissima* before 2003 should therefore be questioned. *Pseudo-nitzschia seriata* f. *obtusa* was raised in rank to *P. obtusa* (Hasle & Lundholm 2005). *Pseudo-nitzschia delicatissima* was redefined and split into two new species: *P. dolorosa* and *P. decipiens* (Lundholm *et al.* 2006). Later, the *P. delicatissima* del1 strain of Amato *et al.* (2007) was named a new species, *P. arenysensis* (Quijano-Scheggia *et al.* 2009b). The detection of three ITS1 fragments, which differed from any known species, were assumed to correspond to uncultivated, and therefore not yet discovered, *Pseudo-nitzschia* species (Hubbard *et al.* 2008).

The molecular approach has also allowed the discovery of cryptic species, i.e. those that are morphologically identical, or too similar to be distinguished, but genetically different, so they are reproductively isolated from other members of their own 'species'. For example, Amato *et al.* (2007) showed that *P. pseudodelicatissima* is in fact a group of five species (*P. cuspidata*, *P. calliantha*, *P. calliantha*2, *P. caciantha* and *P. pseudodelicatissima*) and that *P. delicatissima* is a group of three species (*P. delicatissima*, *P. delicatissima*2 and *P. dolorosa*). Pseudo-cryptic species are likewise genetically distinct but also have minor ultrastructural differences that

are difficult to detect (Amato *et al.* 2005). McDonald *et al.* (2007) found three new genotypes among the *Pseudo-nitzschia* by studying subtle morphological differences and LSU rDNA sequences: two were from within the *galaxiae* clade and one was possibly of an undescribed *delicatissima*-like cell. Such studies document the process of speciation and may also eventually help to explain the existence of toxic and nontoxic strains of presumably the same species.

Using both nuclear-encoded rDNA ITS and plastid-encoded *rbc*L sequences, three distinct clades (I–III) of *P. pungens* have been revealed that are also distinguishable by subtle differences in frustule ultrastructure (Casteleyn *et al.* 2010). The clades differ in their geographical distribution: clade I (*P. pungens* var. *pungens*) has a cosmopolitan distribution in temperate waters of the Atlantic and Pacific oceans; clade II (*P. pungens* var. *cingulata*), originally described by Villac & Fryxell (1998), has only been found in the northeastern Pacific, where it co-occurs with clade I; and clade III (*P. pungens* var. *aveirensis*), described by Churro *et al.* (2009), occurs in the tropical to warm-temperate waters of the Atlantic and Pacific oceans. Clades I and II are sexually compatible in culture (Casteleyn *et al.* 2008), and hybrids have been found in the field (Casteleyn *et al.* 2009a; Holtermann *et al.* 2010). So far, the correlation between the reported abilities of some strains of *P. pungens* to produce small quantities of DA (e.g. < 0.2 pg DA cell^{-1}) (see Bates *et al.* 1998; Calu *et al.* 2009) and the clade to which they belong has not been investigated. These studies support the existence of factors that limit gene flow in a presumably cosmopolitan species, and how this can lead to speciation.

Reproduction

Pseudo-nitzschia spp. exhibit asexual and sexual reproduction. In asexual reproduction, the parental hypotheca (the smaller theca) becomes the epitheca (the larger theca) of the daughter cell. A new hypotheca will then be formed within that smaller epitheca. Thus, the cells become smaller each time they divide. Because of the rigid frustule, sexual reproduction is generally required to restore the largest cell size of *Pseudo-nitzschia* spp. (Fig. 10). Unusually, Pan *et al.* (2001) observed a vegetative cell enlargement of *P.* cf. *pseudodelicatissima* on five occasions in monocultures. Sexual reproduction (also called auxosporulation) occurs when daughter cells reach a lower size threshold (Davidovich & Bates 1998; Hiltz *et al.* 2000; Amato *et al.* 2005) as a result of continued vegetative cell division (except for the unusual abrupt reduction in cell length; Chepurnov *et al.* 2005). This threshold, however, is not universal and is species dependent, often having a wide size range, e.g. for *P. delicatissima*, 20–90% of the maximal cell length (Amato *et al.* 2005); for *P. multiseries*, 23–70% (Hiltz *et al.* 2000; Bates & Davidovich 2002); 20–60% for *P. pungens* (Chepurnov *et al.* 2005); and 20–90% for *P. delicatissima* (Amato *et al.* 2005). In a field study, the upper size thresholds for sexual induction were ~ 62% and 75% of the maximum size for *P. pungens* and *P. australis*, respectively (Holtermann *et al.* 2010). Below a given size window, cells can no longer reproduce sexually and will eventually die.

To date, sexual reproduction has been reported, in laboratory cultures, for 14 *Pseudo-nitzschia* species and one
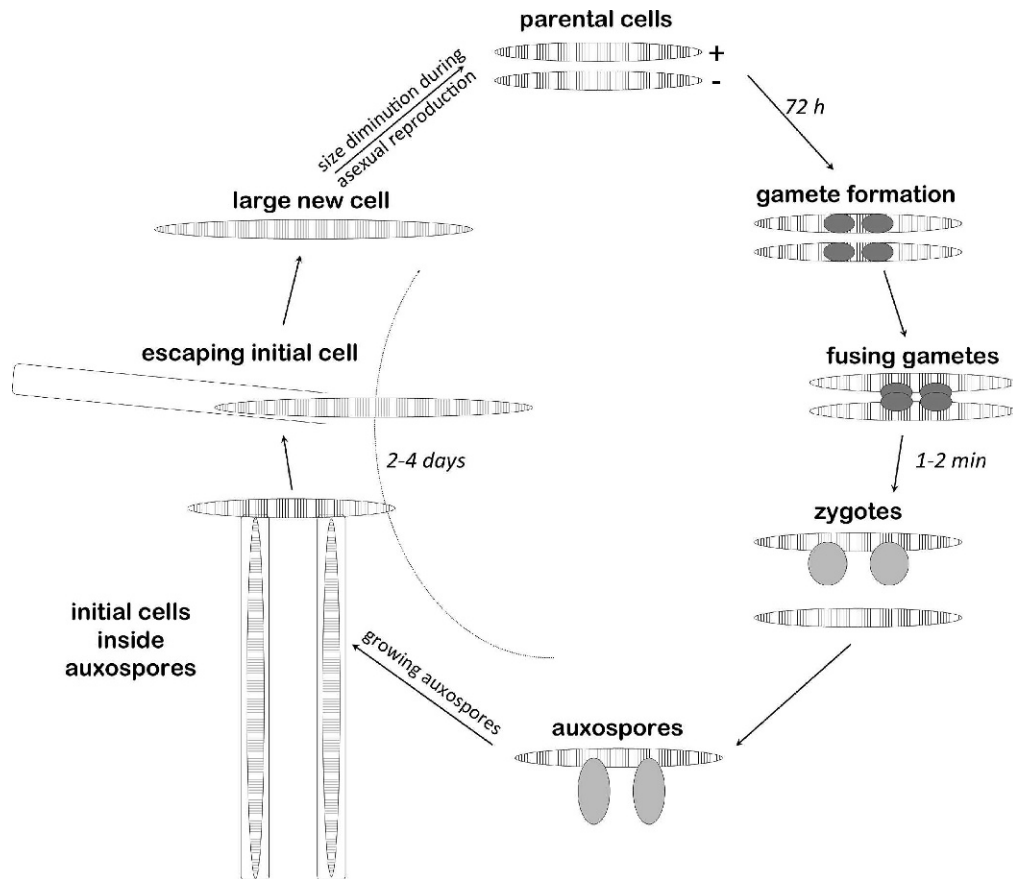
Fig. 10. Sexual reproduction of *Pseudo-nitzschia* sp., carried out to restore the large cell size, after size diminution as a result of asexual reproduction (vegetative cell division).

variety: *P. arenysensis* (Quijano-Scheggia *et al.* 2009b), *P. australis* (Holtermann *et al.* 2010), *P. brasiliana* (Quijano-Scheggia *et al.* 2009a), *P. calliantha* (Amato *et al.* 2007; Lundholm *et al.* in press), *P. cuspidata* (Lundholm *et al.* in press), *P. delicatissima* (Amato *et al.* 2005, 2007; Kaczmarska *et al.* 2008; Quijano-Scheggia *et al.* 2009b), *P. dolorosa* (Amato *et al.* 2007), *P. fraudulenta* (Chepurnov *et al.* 2004), *P. mannii* (Amato & Montresor 2008), *P. multiseries* (Davidovich & Bates 1998; Hiltz *et al.* 2000; Kaczmarska *et al.* 2000), *P. multistriata* (D'Alelio *et al.* 2009), *P. pseudodelicatissima* (Davidovich & Bates 1998; which may be *P. cuspidata*; Lundholm *et al.* 2003), *P. pseudodelicatis-sima sensu stricto* (Amato *et al.* 2007), *P. pungens* (Chepurnov *et al.* 2004, 2005; Casteleyn *et al.* 2008), *P. pungens* var. *aveirensis* (Churro *et al.* 2009) and *P. subcurvata* (Fryxell *et al.* 1991). Mating compatibility studies are increasingly being used to confirm species differences (Davidovich & Bates 1998; Amato *et al.* 2007; Casteleyn *et al.* 2008; Orlova *et al.* 2008; Quijano-Scheggia *et al.* 2009b; Lundholm *et al.* in press).

Most *Pseudo-nitzschia* species are heterothallic, requiring two parental cells (gametangia) of opposite mating type, a '+' and a '−' (Chepurnov *et al.* 2005; D'Alelio *et al.* 2009). Exceptions are *P. brasiliana*, which is homothallic, i.e. it does not require the opposite mating type (Quijano-Scheggia *et al.* 2009a), and *P. subcurvata*, which produced auxospores in a clonal culture and is therefore also apparently homothallic (Fryxell *et al.* 1991). After several hours, the + and − gametangia find each other and align themselves in a parallel fashion, without mucilage or any physical link. Sexual reproduction may occur between two single cells or between a cell in a chain and a single cell. Gametogenesis starts and two identical nonflagellated, spherical gametes appear from each gametangium. This is a cis-anisogamy, i.e. the + and − gametes do not behave in the same way: each of the two slightly mobile (active) + gametes fuse with the two immobile − (passive) gametes that are directly opposite to them, but not necessarily at the same time. Gamete fusion (plasmogamy) occurs within 1–2 min, resulting in the formation of a spherical zygote for each pair of gametes. Each zygote, which remains attached to the − parental frustule, expands to form an elongated auxospore, which is surrounded by a perizonium (Fig. 4). The initial cell develops inside the auxospore and, after 2–4 d when it reaches the maximum cell length for the species, it escapes by breaking through the tips of the perizonium (Davidovich & Bates 1998; Amato *et al.* 2005; D'Alelio *et al.* 2009). The initial cell does not have the same morphology as the vegetative cell (Kaczmarska *et al.* 2000). Curiously, initial cells of *P. multiseries*, at least, must be isolated from the parental cell mixture for them to survive (personal observation). After the first cell division, the morphology begins to appear like the normal vegetative cells.

The sexual stages are fragile and easily broken during the sampling, rendering them difficult to observe (Davidovich & Bates 1998; Mann & Bates 2001). Furthermore,

identification of these different sexual stages is difficult. Molecular probes must still be developed for identifying the sex of the cell, sexually induced cells, as well as the sexual stages (Mann & Bates 2001). In the ocean, only 9–14% of the cells, depending on the species, would be able to reproduce at the same time (Sarno *et al.* 2010). Nevertheless, two massive sexual reproduction events have recently been observed, for the first time, in natural populations (Holtermann *et al.* 2010; Sarno *et al.* 2010). These field studies, and that of D'Alelio *et al.* (2010), show a period between cycles of sexual reproduction (2–3 yr) similar to that found in culture studies (Davidovich & Bates 1998).

Questions remain as to how cells of opposite mating type find each other, and when they do, what triggers them to initiate the steps in sexual reproduction. There is some evidence that a compound is released by *P. multiseries* cells when cells of opposite mating type are placed together in 14–40 ml of medium (Haché 2000; Bates & Davidovich 2002). When cell-free filtrate collected from the medium containing the mating cells was added to other pairs of cells of opposite mating type, the number of gametes produced increased. Furthermore, some compound in that filtrate caused cells to produce gametes even in absence of any cells of the opposite mating type. Could that compound be a pheromone? The coincidence of auxosporulation occurring at the same time and location as high DA concentrations in razor clams at Kalaloch Beach (Washington, USA) (Holtermann *et al.* 2010) also raises the question about a possible relationship between sexual reproduction and production of DA.

One conclusion from the above studies is that defining a species from morphology only is inadequate; combining morphology and genetic sequencing is better (Lundholm & Moestrup 2002; Lundholm *et al.* 2002a, b, 2003, 2006), and adding mating compatibility (Amato *et al.* 2007; Lundholm *et al.* in press) provides the most complete picture (Mann 1999). For example, strains of opposite mating type and grouped together on the basis of their ITS2 sequences, which also gave the same morphological features, produced viable offspring when crossed, whereas crosses between strains of different ITS2 sequences failed to mate successfully (Amato *et al.* 2007).

Producers of DA

Domoic acid was first discovered in the red alga *Chondria armata* (Takemoto & Daigo 1958), and is also produced by two other pennate diatoms, in addition to certain *Pseudonitzschia* species: *Amphora coffeaeformis* from Canada (although this finding has been questioned; Bates 2000), and *N. navis-varingica* from Southeast Asia (Kotaki *et al.* 2000). In the Philippines, strains of *N. navis-varingica* from one region only produced DA, although elsewhere some isolates did produce isodomoic acids A and B (Bajarias *et al.* 2006; Kotaki *et al.* 2006).

Of the 37 species of *Pseudo-nitzschia*, 14 have been reported to produce DA, although not all strains are toxic under the testing conditions (Table 1; Bates *et al.* 1998; Thessen *et al.* 2009). *Pseudo-nitzschia* cf. *granii* is the most recent addition to the list of toxigenic species, although toxicity was demonstrated only in a shipboard continuous culture of a natural seawater sample at Ocean Station PAPA (OSP) (Trick *et al.* 2010; see below), not in a clonal isolate. In contrast, Guannel *et al.* (2011) determined that an isolate of *P. granii* from OSP was undectable for DA. They did report, however, relatively high levels of cellular DA (13.48 pg DA cell^{-1}) in an unidentified new species of *Pseudo-nitzschia* (*P.* sp. 233, strain PNWH20 233), isolated from Sequim Bay, Washington (USA). Ten species have not yet been tested for toxigenicity: *P. antarctica*, *P. fryxelliana*, *P. linea*, *P. lineola*, *P. mannii*, *P. micropora*, *P. prolongatoides*, *P. pungiformis*, *P. roundii* and *P. sinica*. Some researchers believe that all species of *Pseudo-nitzschia* will prove to be toxigenic, given the proper growth conditions and sensitive-enough detection protocols, although perhaps not at levels great enough to generate toxicity at higher trophic levels under all conditions (Parsons *et al.* 1999; Wells *et al.* 2005).

Most toxic species are coastal, with cellular DA values on the order of < 1 to ~ 100 pg cell^{-1} (although usually < 100 pg cell^{-1}), depending on the species, cell size and growth conditions (laboratory studies summarized by Trainer *et al.* 2008; field studies summarized by Caron *et al.* 2010). For example, the large (73–129 μm long, 6.0–7.8 μm wide) *P. australis* contained a maximum of 78 pg DA cell^{-1} (Trainer *et al.* 2000), and the small (10–82 μm long, 1.0–1.8 μm wide) *P. galaxiae*, when toxic, contained only 0.36 fg DA cell^{-1} (Cerino *et al.* 2005). Other examples of cellular DA values, calculated from total abundances of *Pseudo-nitzschia* spp. and particulate DA concentrations in the field, are: 2.7 pg cell^{-1} (Adams *et al.* 2000), 88 pg cell^{-1} (Anderson *et al.* 2009) and 117 pg cell^{-1} (Schnetzer *et al.* 2007); 43 pg cell^{-1} was reported for *P. australis* (Howard *et al.* 2007).

There are few oceanic species: *P. turgidula*, *P. heimii*, *P. inflatula*, *P. granii* and *P. prolongatoides*, although the first three are also found along some coasts (Rhodes *et al.* 1996; Priisholm *et al.* 2002; Hernández-Becerril & Díaz-Almeyda 2006; Congestri *et al.* 2008). Previously, none of the species isolated from mid-ocean waters was reported to produce DA at detectable levels (Marchetti *et al.* 2008); *P. prolongatoides* has not yet been tested (Table 1). However, a recent study carried out in the eastern subarctic Pacific (at OSP) has shown exceedingly low levels of DA in seawater (0.1 pg DA ml^{-1}) and in *P.* cf. *granii* cells (4 ag DA cell^{-1}) from a shipboard growth experiment that used a natural seawater sample (Trick *et al.* 2010). By comparison, coastal waters contained much greater concentrations, ranging from 0.15–9.39 ng DA ml^{-1} in San Francisco Bay (Howard *et al.* 2007) to 136 ng DA ml^{-1} in Washington State (Trainer *et al.* 2007). The above oceanic studies are among many that have been carried out to examine the effects of adding iron to stimulate the growth of iron-limited phytoplankton in the high-nitrate, low-chlorophyll (HNLC) regions of mid-oceans. It has been proposed that the phytoplankton growth stimulated by this artificial fertilization could result in a drawdown of atmospheric CO_2 concentrations and hence a slowing of global warming, although the effectiveness of this approach has recently been questioned (Hamme *et al.* 2010). Moreover, it has raised concerns because numerous tests have resulted in favouring the growth of *Pseudo-nitzschia* spp. (summarized
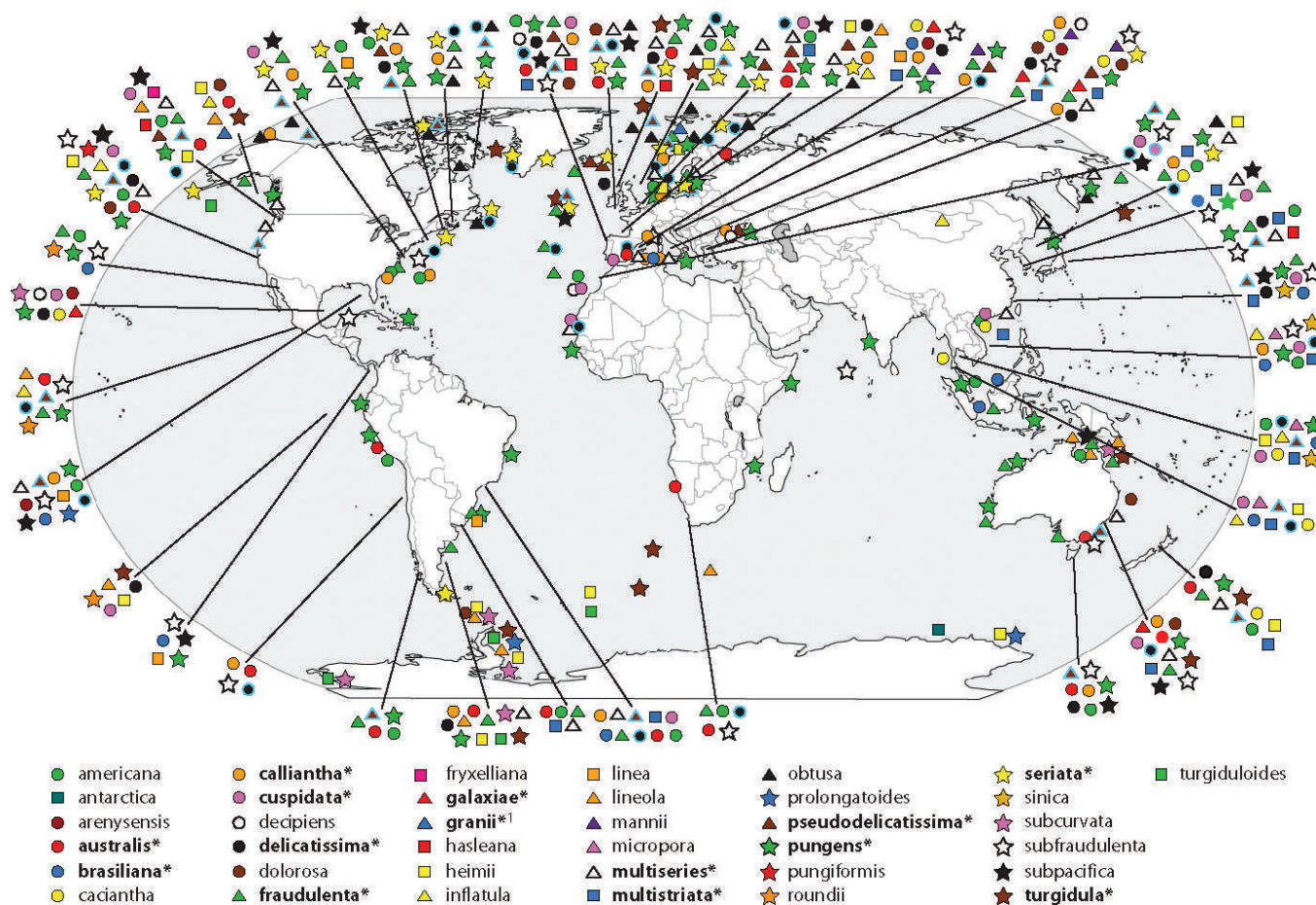
● americana	● **calliantha***	■ fryxelliana	■ linea	▲ obtusa	☆ **seriata***	■ turgiduloides
■ antarctica	● **cuspidata***	▲ **galaxiae***	▲ lineola	★ prolongatoides	☆ sinica
● arenysensis	○ decipiens	▲ **granii*¹**	▲ mannii	★ **pseudodelicatissima***	★ subcurvata
● **australis***	● **delicatissima***	■ hasleana	▲ micropora	★ **pungens***	☆ subfraudulenta
● **brasiliana***	● dolorosa	■ heimii	△ **multiseries***	★ pungiformis	★ subpacifica
○ caciantha	▲ **fraudulenta***	▲ inflatula	■ **multistriata***	★ roundii	★ **turgidula***

Fig. 11. World distribution of *Pseudo-nitzschia* spp. (see also Table 1). Toxigenic species are in bold and shown with an *; note that only certain strains of these species are toxigenic at some locations.[1] Toxicity determined from a shipboard continuous culture of a natural seawater sample containing *P.* cf. *granii* as the only species of *Pseudo-nitzschia* (Trick *et al.* 2010). Symbols outlined in blue indicate that the report was made before major taxonomic revisions were made for *P. delicatissima* and *P. pseudodelicatissima*, although updates were made when available. Modified and updated from Thessen (2007). A color version of this map is available online.

by Trick *et al.* 2010). Furthermore, Trick *et al.* (2010) showed that iron addition stimulated not only *Pseudo-nitzschia* spp. growth, but also the production of DA by *P.* cf. *granii* (see above) and *P. turgidula* (see more on iron stimulation, below). Although the amounts of toxin produced are low (4 ag DA cell^{-1} and 0.3 fg DA cell^{-1} for *P.* cf. *granii* and *P. turgidula*, respectively), there are still unknown consequences of this to the ocean ecosystem.

Blooms

Diatoms, including *Pseudo-nitzschia* spp., often bloom in upwelling zones, where currents allow them to remain in the upper, sunlit water column and to take advantage of the rich nutrients coming from the ocean depths. For example, *Pseudo-nitzschia* blooms are common along the west coast of the United States (Horner *et al.* 2000; Trainer *et al.* 2000; Kudela *et al.* 2005; Anderson *et al.* 2006), where river runoff can also supply nutrients (Schnetzer *et al.* 2007), although runoff is not always implicated (Kudela *et al.* 2004a). Worldwide, *Pseudo-nitzschia* blooms are also common along the west coast of continents because of upwelling as well as to circulation patterns caused by seafloor and coastal topographies (Trainer *et al.* 2008). This can cause them to

be retained for long periods under conditions that are favourable for elevated DA production, e.g. in the Juan de Fuca eddy region off the coasts of Washington (USA) and British Columbia (Canada) (Trainer *et al.* 2009a) and in the eddy of the Santa Barbara Channel, California (USA) (Anderson *et al.* 2006). Otherwise, *Pseudo-nitzschia* spp. can be found in other coastal or mid-oceanic waters (Trainer *et al.* 2008), as well as in cold (polar) (Orlova & Shevchenko 2002; Almandoz *et al.* 2008), temperate (Amato *et al.* 2005) or tropical/subtropical waters (Hernández-Becerril 1998; Hasle 2002) (Table 1). *Pseudo-nitzschia* spp. are found on all continents, including Antarctica, although no toxigenic species have yet been found there (Fig. 11). Since last reported in 2007, the number of species and locations documented has increased; compare the distribution map in Thessen (2007) with that shown in Fig. 11. The increases are for all continents except Antarctica, but it appears that they are usually associated with locations where research institutions devote particular attention to this genus.

The increased interest directed at *Pseudo-nitzschia* spp., and the latest naming of new species, has resulted in their recent discovery in new locations (Table 2). Some species are described as being cosmopolitan: *P. australis*, *P. delicatissima*, *P. fraudulenta*, *P. multiseries*, *P. pseudodelica-*

Table 2. New records for *Pseudo-nitzschia* spp. at different locations of the world, since 2002.

Species	Location	Reference
P. americana	Russia (NW Sea of Japan, Sea of Okhotsk)	Orlova & Shevchenko 2002
	Bay of Fundy	Kaczmarska *et al.* 2005b
	Atlantic coast of France	Nézan *et al.* 2007
	Portugal	Churro *et al.* 2009
	Denmark and Scandinavia	Lundholm *et al.* 2010
P. brasiliana	Mediterranean coast of Spain	Quijano-Scheggia *et al.* 2005
	Tokyo Bay, Japan	Yap-Dejeto *et al.* 2010
	Eastern Russian seas	Stonik *et al.* 2011
	Bizerte Lagoon, Tunisia	Sahraoui *et al.* in press
P. caciantha	Tokyo Bay, Japan	Yap-Dejeto *et al.* 2010
P. calliantha	Turkish coast of the Black Sea	Bargu *et al.* 2002a
	southern Adriatic coastal waters	Caroppo *et al.* 2005
	Chesapeake Bay	Thessen & Stoecker 2008
	Black Sea[1]	Besiktepe *et al.* 2008
	Bizerte Lagoon, Tunisia	Sahraoui *et al.* 2009
	northern Chile	Álvarez *et al.* 2009
	Portugal	Churro *et al.* 2009
	Gulf of Mexico	Del Rio *et al.* 2010
	Tokyo Bay, Japan	Yap-Dejeto *et al.* 2010
P. cuspidata[2]	coastal Washington State	Trainer *et al.* 2009b
	Portugal	Churro *et al.* 2009
P. delicatissima	southern Adriatic coastal waters	Caroppo *et al.* 2005
	Greece	Moschandreou *et al.* 2010
P. dolorosa[3]	Drake Passage	Ferrario *et al.* 2004
	Greece	Moschandreou *et al.* 2010
P. fraudulenta	Bay of Fundy	Kaczmarska *et al.* 2005b
	Russian waters of the Japan Sea	Stonik *et al.* 2008
	Greece	Moschandreou *et al.* 2010
P. galaxiae	Greece, Spain (Mediterranean Sea)	Moschandreou *et al.* 2010; Quijano-Scheggia *et al.* 2010
	France (Atlantic)	Orive *et al.* 2010
	Tokyo Bay, Japan	Yap-Dejeto *et al.* 2010
P. heimii	Argentina	Almandoz *et al.* 2007
	Denmark and Scandinavia	Lundholm *et al.* 2010
P. inflatula	Mexico (Pacific)	Hernández-Becerril & Díaz-Almeyda 2006
	Tyrrhenian Sea (Italy)	Congestri *et al.* 2008
P. linea	Mediterranean Sea (Spain)	Quijano-Scheggia *et al.* 2010
	Paraná coast, Brazil	Fernandes & Brandini 2011
P. lineola	Argentina	Almandoz *et al.* 2007
P. mannii	Greece	Moschandreou *et al.* 2010
P. multiseries	Bay of Fundy	Kaczmarska *et al.* 2005b
P. multistriata	Mediterranean coast of Spain	Quijano-Scheggia *et al.* 2005
	Uruguay	Méndez & Ferrario 2009
	Portugal	Churro *et al.* 2009
	Greece	Moschandreou *et al.* 2010
	Sea of Okhotsk (Russia)	Stonik *et al.* 2011
P. pseudodelicatissima	Denmark and Scandinavia	Lundholm *et al.* 2010
	Greece	Moschandreou *et al.* 2010
P. pungens	Bay of Fundy	Kaczmarska *et al.* 2005b
P. seriata	west coast of Greenland	Hansen *et al.* 2011
	Eastern Russian seas	Stonik *et al.* 2011
	Greece	Moschandreou *et al.* 2010
P. subfraudulenta	northern Chile	Álvarez *et al.* 2009
	Greece	Moschandreou *et al.* 2010
P. subpacifica	Bay of Fundy	Kaczmarska *et al.* 2005b
	Portugal	Churro *et al.* 2009
P. turgidula	Bay of Fundy	Leandro *et al.* 2010a
P. turgiduloides	Argentina	Almandoz *et al.* 2007

[1] Lundholm *et al.* (2003) previously reported that the *P. pseudodelicatissima* used by Davidovich & Bates (1998) and isolated from the Black Sea was actually *P. calliantha.*
[2] Previously reported as *P.* cf. *pseudodelicatissima* in these waters (e.g. Marchetti *et al.* 2004).
[3] Indicated as 'one strain similar to *P. dolorosa*' by Moschandreou *et al.* (2010).

tissima and *P. pungens* (Hasle 2002; Lundholm & Moestrup 2002), but Table 1 and Fig. 11 suggest that most of the others could also be in that category, except for: *P. antarctica*, *P. arenysensis*, *P. fryxelliana*, *P. granii*, *P. mannii*, *P. micropora*, *P. prolongatoides*, *P. pungiformis*, *P. roundii*, *P. subcurvata* and *P. turgiduloides*, which have a more restricted distribution, including polar regions. As well, some of the cosmopolitan species may actually be more diverse, if cryptic species are considered. It is not yet clear if the distribution of the noncosmopolitan species is in fact caused by an inability to grow in a broad spectrum of conditions, or if they are truly stenohaline or stenothermal

(see below). Interestingly, all of noncosmopolitan species have so far been shown to be nontoxic (Table 1), which supports the hypothesis that the cosmopolitan species are toxigenic (Hasle 2002). Curiously, although *P. australis* is listed as a cosmopolite (Hasle 2002), it is absent from the northwestern Atlantic (east coasts of the United States and Canada). A worldwide distribution may be explained by ballast water transport, relocation of aquacultured bivalve molluscs and a greater adaptive ability of some species (Zhang & Dickman 1999; Burkholder *et al.* 2007; Hégaret *et al.* 2008). Two recent reports of an unexpected presence of a *Pseudo-nitzschia* species require further investigation: *P.* cf. *subcurvata* (identified by light microscopy only, so not shown in Fig. 11), normally in polar regions (Fryxell *et al.* 1991; Bates *et al.* 1993a; Almandoz *et al.* 2007, 2008), was reported along the Mexican coast of the Gulf of Mexico (Aké-Castillo & Okolodkov 2009), and *P. seriata*, normally in the North Atlantic Ocean (Hasle 2002; Hasle & Lundholm 2005) and recently in eastern Russian seas (Stonik *et al.* 2011), was reported in the Beagle Channel of Argentina (Almandoz *et al.* 2009), although it exhibited only two rows of poroids. It should be pointed out that a strain of *Pseudo-nitzschia* isolated from Puget Sound, Washington (USA) was reported to be *P. seriata* on the basis of its morphometrics and ITS1 sequences (Hubbard *et al.* 2008).

Pseudo-nitzschia spp. are a frequent part of the phyto-plankton community. Several species of *Pseudo-nitzschia* often grow at the same time and location, e.g. in the Bay of Fundy (Canada), where seven species are found, among them five potentially toxic (Kaczmarska *et al.* 2005b) (Table 1). Nine species are found in Monterey Bay, California, two of which are toxigenic (*P. australis*, *P. multiseries*; Kudela *et al.* 2004b) and one (*P. turgidula*) potentially so. Seven species are found in coastal Washington waters; all except possibly *P.* cf. *heimii* are toxigenic (Stehr *et al.* 2002). Likewise, seven *Pseudo-nitzschia* spp. are in Chesapeake Bay (Marshall *et al.* 2005; Thessen & Stoecker 2008), only one of which (*P. subpacifica*) has never been proven to be toxigenic. Ten species of *Pseudo-nitzschia* grow in the Gulf of Naples, although some during different seasons (Orsini *et al.* 2002; Lundholm *et al.* 2003; Cerino *et al.* 2005; Amato *et al.* 2007; McDonald *et al.* 2007; D'Alelio *et al.* 2009). Only *P. multistriata* (Orsini *et al.* 2002) and *P. galaxiae* (Cerino *et al.* 2005) are toxigenic, but with low cellular DA content (0.8 ag DA cell^{-1} for *P. galaxiae*) because of their small cell size, thus possibly explaining the absence of any ASP events in Italian waters. Six species were documented over an entire year in Normandy (France), with *P. australis* as the most important toxigenic diatom (Klein *et al.* 2010).

Where there are sufficient long-term data on *Pseudo-nitzschia* spp. and accompanying environmental informa-tion, it has been possible to develop predictive models of toxigenic *Pseudo-nitzschia* blooms, e.g. the northwest coast of the Iberian Peninsula, Spain (Corchado *et al.* 2004); Monterey Bay [Lane *et al.* 2009; although the presence of *P. australis* in thin layers (McManus *et al.* 2008) is one challenge for applying the model]; Santa Barbara Channel, California (Anderson *et al.* 2009); Chesapeake Bay (Anderson *et al.* 2010); and Lisbon Bay, Portugal (Palma *et al.* 2010). Improvements are still required to predict more

accurately the timing of a bloom's appearance and its intensity. Commonalities of the above locations are that they are either in upwelling zones or in coastal bays, with high levels of macronutrients and perhaps trace metals (Trainer *et al.* 2008, and see below).

Shifts in the relative abundance of *Pseudo-nitzschia* spp. have been documented on seasonal, decadal and centennial timescales. Different species of *Pseudo-nitzschia* have unique seasonal distributions, often linked to temperature and salinity (Klein *et al.* 2010), although some may be present all year at the same location (Hasle *et al.* 1996). Blooms mainly occur between January and May in European waters, rarely during summer (Hasle *et al.* 1996); in the fall in eastern North America (Bates *et al.* 1998); early summer (Trainer *et al.* 2002) or early fall in Washington State (Trainer *et al.* 2010); or late spring in Southern California (Anderson *et al.* 2006, 2009) and the Pacific Mexican coast (García-Mendoza *et al.* 2009). There is sometimes a seasonal succession of *Pseudo-nitzschia* spp., e.g. *P. pungens* has tended to bloom in late summer waters, before *P. multiseries* in eastern and western Canadian waters, and in the Gulf of Mexico (Bates *et al.* 1998). In Scottish waters (Fehling *et al.* 2006), blooms of *P. pungens*, *P. pseudodelicatissima* and *P. australis* are more likely to occur during the warm season, whereas blooms of *P. multiseries* tend to occur during the cold season (late fall and spring). In the Bay of Naples, blooms of *P. galaxiae* occur between February and November, with higher concentrations in May and August (Cerino *et al.* 2005). Numbers of *Pseudo-nitzschia* species in Peter the Great Bay (eastern Russia) peak at the summer and fall (Stonik *et al.* 2011). In the Argentine Sea, *P. pungens* and *P. australis* reach high densities at warm temperatures (15.8°C), high salinities [33.8 practical salinity units (psu)] and low nutrient concentrations (Almandoz *et al.* 2007). *Pseudo-nitzschia heimii*, *P. lineola*, *P. turgidula* and *P. turgiduloides* are more restricted in distribution and reach lower cell densities. They are associated with low salinity (32.45 psu) and cold (8.8°C) waters that are rich in nutrients (Almandoz *et al.* 2007). In contrast to the above, *P. pungens* was present all year in Normandy (France), indicating that it exhibited lower environmental constraints than the other local *Pseudo-nitzschia* species (Klein *et al.* 2010).

On a decadal timescale, there was a shift in the Skagerrak from *P. multiseries* during 1967–1968 to *P. pungens* in the 1970s and 1980s. This was speculated to be caused by warmer waters that favoured the latter (Hasle 1995), in agreement with the above seasonal patterns. In the southern Gulf of St. Lawrence (Bates *et al.* 1998), and perhaps elsewhere in the world, *P. multiseries* abundance has also drastically decreased since *c.* 1990, to the point that it has become more difficult, recently, to obtain fresh isolates of this species in the field (personal observation). Further work is required to determine if this decline correlates with the worldwide increasing trend in sea surface temperatures over the past century, which is also linked to the global decline of phytoplankton biomass (Boyce *et al.* 2010). A study of species composition along the California coast during 2000–2006 discovered a decrease in *P. multiseries* and *P. australis*, as well as a significant decline in shellfish contaminated with DA, after 2004, when the species composition shifted toward toxic *Alexandrium* and *Dino-*

physis (Jester *et al.* 2009). However, after 2007, toxigenic *Pseudo-nitzschia* spp. again became dominant, with the exception of *P. multiseries*, along with a recurrence of DA contamination (Jester *et al.* 2009).

On a centennial timescale, one dominant species has been shown to replace another. The sediment record showed that *P. multiseries* in Mariager Fjord (Denmark) made up > 90% of the *Pseudo-nitzschia* cells from 1905 to 1947, but then it decreased dramatically to ~ 5% in 2002 (Lundholm *et al.* 2010). It was replaced by *P. pungens*, which increased in relative abundance from < 1% in the period 1905–1947 to 63% in *c.* 1964. Consistent with the above, because *P. pungens* tends to be favoured by warmer temperatures in the field (but supported by only some laboratory experiments; see below), Lundholm *et al.* (2010) hypothesized that the increase in seawater temperature observed over the last century could have affected the competitive balance between the two species.

Pseudo-nitzschia spp. are sometimes a large proportion of the total diatom biomass. The contribution of the *seriata* group species reached 77% in western Scottish waters; that for the *delicatissima* group was 14% (Fehling *et al.* 2006). In those waters, there was a negative correlation between *Pseudo-nitzschia* abundance and concentrations of nitrogen, phosphorus and silicon, with a depletion of each before the bloom reached its maximum. Each species, whatever its group, can be linked to different physicochemical factors (Kaczmarska *et al.* 2007; Schnetzer *et al.* 2007; Almandoz *et al.* 2008). For example, in Scottish waters, the *seriata* group species are linked to temperature, whereas the *delicatissima* group species are linked to salinity and the presence of ammonium (Fehling *et al.* 2006). Photoperiod can influence the timing of *Pseudo-nitzschia* blooms and showed a strong positive correlation with their presence (Fehling *et al.* 2006). It also affects sexual reproduction (see above; Hiltz *et al.* 2000).

Pseudo-nitzschia spp. may be located throughout the water column or concentrated in layers. Healthy, motile chains of *P. fraudulenta* were found in thin layers at depths of ~ 5 m and ~ 24 m, in an estuarine fjord (Washington State); these were likely advected from the surface (Rines *et al.* 2002). High concentrations of *P. australis* were found in a narrow band at 10–20-m depth, in Monterey Bay, California (Ryan *et al.* 2005). Thin layers of *P.* cf. *pseudodelicatissima* and *P. australis*, likely senescent and therefore containing DA, were also found at 10–20 m, in a Galician Ría, Spain (Velo-Suárez *et al.* 2008). Such layers may be missed by conventional monitoring methods. All were just above or within the pycnocline and may be displaced vertically during downwelling events, allowing them to contaminate benthic organisms (Velo-Suárez *et al.* 2008). Single cells, apparently dead, were found in sediment traps at 10–16 m in the Gulf of Mexico (Dortch *et al.* 1997). Cellular DA was found in sediment traps below the euphotic zone in coastal California waters (Trainer *et al.* 2000). Particulate DA, chlorophyll-containing cells and even intact chains were found sinking at depths of up to 800 m off the coast of Southern California (Schnetzer *et al.* 2007; Sekula-Wood *et al.* 2009, 2011). Such cells may be a source of DA that contaminates benthic communities (Kvitek *et al.* 2008; see below). These cells may also be a seed population if

brought to the surface during the next upwelling event (Trainer *et al.* 2000; Mengelt & Prézelin 2002).

PHYSICAL PARAMETERS AFFECTING GROWTH AND TOXICITY

Growth phase

DA production is associated with different phases of the *Pseudo-nitzschia* growth cycle, perhaps linked to changes in physicochemical parameters of the surrounding medium caused by the cells' growth. Early studies with *P. multiseries* and a Danish strain of *P. seriata* showed that DA production started in late-exponential phase and continued more rapidly during the stationary phase (summarized by Bates 1998). This was confirmed by Fehling *et al.* (2004) in another strain of *P. seriata*. Likewise, *P. cuspidata* (Trainer *et al.* 2009b), *P. fraudulenta* and *P. calliantha* (Thessen *et al.* 2009) produced most of its DA during the stationary phase, although some was reported during the exponential phase. Interestingly, *N. navis-varingica* showed this same pattern (Kotaki *et al.* 2000). In contrast to the above, DA was produced by *P.* sp. cf. *pseudodelicatissima* during the early exponential phase (Pan *et al.* 2001). Few studies have since been carried out to examine the timing of DA production in batch culture, and existing results are sometimes contradictory. In the case of *P. pseudodelicatissima*, this may perhaps be explained by the recent finding that this species is actually composed of at least seven species (see above), some of which may behave differently. For example, the *P.* sp. cf. *pseudodelicatissima* mentioned above could be identified as either *P. pseudodelicatissima* or *P. cuspidata* (Lundholm *et al.* 2003). Likewise, the true identity of '*P. pseudodelicatissima*' from Washington State coastal waters is not known with certainty; it produced DA steadily through the exponential phase, and then production increased dramatically during the late-stationary phase (Adams *et al.* 2000). Garrison *et al.* (1992) showed that *P. australis* produced DA during most of the exponential phase but not during stationary phase. In contrast, Cusack *et al.* (2002) documented that *P. australis* began DA production in late-exponential phase and it continued into stationary phase. Interestingly, under conditions of silicate or phosphate limitation, the cells release increasing proportions of produced DA into the growth medium during stationary phase (reviewed by Bates 1998). Additional species require study to verify these patterns. In the end, it must still be resolved if changes in DA production over the growth cycle are due to some intrinsic alteration in the cells' physiology during growth, or to a specific nutrient limitation per se (see below).

Over a longer timescale, the viability and toxicity of *P. multiseries* (Bates 1998), *P. australis* (Rhodes *et al.* 2004) and perhaps other species as well decline over a period of several years in culture. For several strains of *P. multiseries*, the decrease in cellular toxicity was exponential and was associated with a reduction in cell length over a 2.7-yr period, resulting in a positive linear relationship between cellular DA and cell volume (Mafra *et al.* 2009a). As discussed above, a cell decreases to a species-specific length before it is capable of rejuvenating its large cell size via sexual
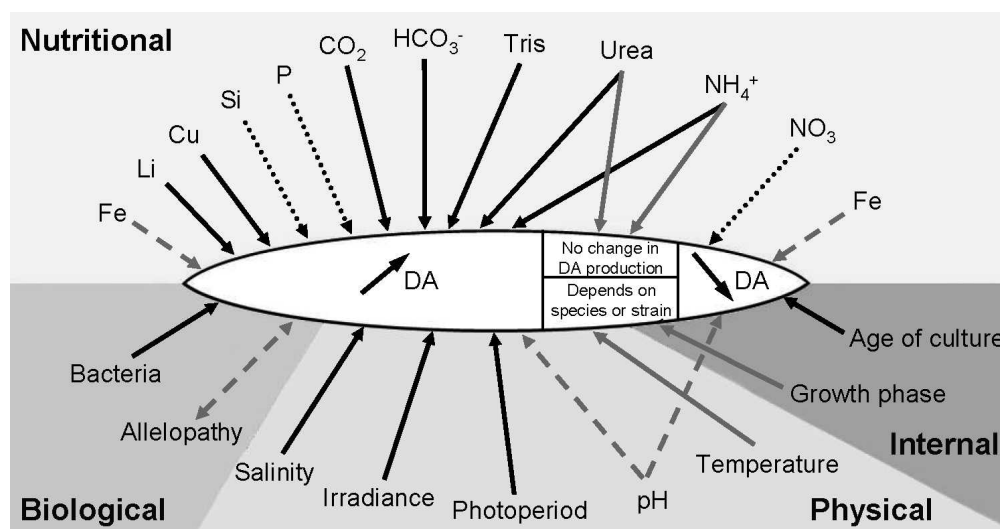
Fig. 12. Synthesis of all the factors (nutritional, internal, physical or biological) studied and their impacts on domoic acid (DA) production (increase or decrease). Black arrow = effect of increasing the parameter; black dotted arrow = effect of decreasing the parameter; grey dashed arrow = conflicting results found. Grey arrow = result depends on species or strain, or no change observed in DA production.

reproduction. Large cells, just after sexual reproduction, produced more DA than their smaller-celled parents, once they had first divided several times (Bates *et al.* 1999). Some, but not all, offspring of *P. cuspidata* were also more toxic than their parents (Lundholm *et al.* in press). There may thus be a relationship between cell toxicity and readiness to undergo sexual reproduction, but this has not been proven. Interestingly, the cellular DA content of *P.* sp. cf. *pseudodelicatissima* increased by an order of magnitude after 'cell enlargement' (apparently not related to sexual reproduction) (Pan *et al.* 2001). The decline in ability to produce DA may also be related to a gradual decline in cellular chlorophyll *a*, which provides photosynthetic energy required for DA biosynthesis (Pan *et al.* 1998), although this requires more study. Another hypothesis is that it could be related to changes in the bacterial assemblage growing with *Pseudo-nitzschia* (Bates 1998). Stewart (2008) proposed that the presence of DA-degrading bacteria could explain the decline over months in culture. Differences were found in the bacterial community composition over time in culture, although for nontoxic *P. pungens*, which supports this possibility (Sapp *et al.* 2007). On the other hand, Guannel *et al.* (2011) found no shifts in bacterial assemblages in a *Pseudo-nitzschia* culture over its initial 9 mo (see below).

Results of any study that used only one strain of a given *Pseudo-nitzschia* species to evaluate a factor affecting DA production must be interpreted with caution, because of the high variability among strains of the same species (Bates *et al.* 1999; Kudela *et al.* 2004b; Thessen *et al.* 2009; Amato *et al.* 2010; Lundholm *et al.* in press). This was the case in most of the studies below, unless otherwise indicated. A summary of factors affecting DA production is given in Table 3 and Fig. 12.

Temperature

Laboratory studies, up to about 1998, on the effects of temperature on DA production, growth and photosynthesis of *P. multiseries* are reviewed in Bates (1998); *P.*

pseudodelicatissima (Lundholm *et al.* 1997) was not included in that review. Additional species have since been studied: *P. cuspidata*, *P. granii* and *P. pungens* (Table 4), but this is still a small fraction of the 37 known species. As well, only one strain of each species has been studied.

Acclimatization time (~ 7 d: Miller & Kamykowski 1986; Lundholm *et al.* 1997; 10 generations: El-Sabaawi & Harrison 2006), interactions between temperature and salinity (Miller & Kamykowski 1986; Lundholm *et al.* 1997; Doan-Nhu *et al.* 2008), as well as irradiance (El-Sabaawi & Harrison 2006) must be considered when temperature characteristics are measured. This is because growth at different temperatures is salinity dependent. For example, *P. cuspidata* tolerated a wider range of temperatures when grown at its optimum salinity (30 psu) (Doan-Nhu *et al.* 2008). Likewise, *P. pseudodelicatissima* achieved the highest growth rate at 25°C when measured at its optimum salinity for growth (25‰), although higher temperatures were not tested (Lundholm *et al.* 1997). Furthermore, this species reached a lower temperature limit for growth (5°C) at this optimum salinity than at any other salinity. Similar findings were reported for *P. americana*, whose optimum growth temperature (25 ± 2.5°C) was salinity dependent (Miller & Kamykowski 1986). This species was unable to grow at a low salinity (8‰), even at its optimum growth temperature.

The growth responses from individual laboratory experiments tend to support the temperature ranges found in the waters from which the *Pseudo-nitzschia* spp. were isolated. For example, the ability of *P. multiseries* to grow at a lower temperature than *P. pungens* in culture (Table 4) can explain its higher cell concentration at lower temperatures in the fall and spring in Chinhae Bay (South Korea) (Cho *et al.* 2001). Likewise, *P. multiseries* was more likely to be abundant at lower temperatures than *P. pungens* in Prince Edward Island (Canada) and elsewhere (Bates *et al.* 1998). The growth response of *P. cuspidata* at high temperatures (Table 4) and salinities (Table 5) may account for its presence under these conditions in Nha Trang Bay

Table 3. Summary of factors affecting the production of domoic acid (DA) by *Pseudo-nitzschia* spp. in culture; modified and updated from Mafra (2009).

Factor	Species	Effect on DA production (range in growth parameter)	Reference
Physicochemical			
Temperature	P. seriata	DA higher at 4°C than at 15°C; 1.0–33.6 pg cell^{-1}, 0.31–1.6 pg cell^{-1}, respectively	Lundholm *et al.* 2004
	P. multiseries	increased DA (5–25°C); 100 times more at 25°C	Lewis *et al.* 1993
Salinity	P. multiseries	increased DA (10–40 psu); three to four times more at highest salinity	Doucette *et al.* 2008
Irradiance	P. multiseries	increased DA (35–130 µmol photons m^{-2} s^{-1}); seven times more DA at highest irradiance	Bates 1998
	P. australis	increased DA (11.7 and 115 µmol photons m^{-2} s^{-1}); 24–130 times more at the higher irradiance	Cusack *et al.* 2002
Photoperiod	P. seriata	increased DA (18:6 L:D cycle compared with 9:15 L:D); 1.5 times more with longest photoperiod	Fehling *et al.* 2005
pH	P. multiseries	increased DA at higher pH (7.9–8.9); 74 times more at highest pH	Lundholm *et al.* 2004; Trimborn *et al.* 2008
	P. multiseries	increased DA at lower pH (8.38–7.94); 1.4–5 times more at lower pH	Sun *et al.* 2011
Nutritional			
Silica	P. multiseries	produced DA under Si limitation	Bates *et al.* 1991; Pan *et al.* 1996b, c; Kudela *et al.* 2004b; Hagström *et al.* 2010
	P. sp. cf. *pseudodelicatissima*	produced DA under Si limitation	Pan *et al.* 2001
	P. australis	produced DA under Si limitation	Cusack *et al.* 2002
	P. cuspidata	produced DA under Si limitation	Trainer *et al.* 2009a
	P. seriata	produced DA under Si limitation	Fehling *et al.* 2004; Kudela *et al.* 2004b
Phosphorus	P. multiseries	produced DA under P limitation	Bates *et al.* 1991; Pan *et al.* 1996a
	P. seriata	produced DA under P limitation	Fehling *et al.* 2004
Nitrate	P. multiseries	no detectable DA under N deficiency	Bates *et al.* 1991
	P. multiseries	low DA in N-limited chemostat cultures; 1000 times less than in Si-limited chemostats	Kudela *et al.* 2004b
	P. multiseries	low DA in N-deficient batch culture; 2.5 times less than urea, 15 times less than nitrate, but reported only on 1 d	Calu *et al.* 2009
	P. australis	increased DA (50 µM added)	Howard *et al.* 2007
Ammonium	P. multiseries	increased DA (110–440 µM added); two to four times more than at 55 µM and the same concentration of nitrate	Bates *et al.* 1993b
	P. multiseries	increased DA in some strains (88 µM added)	Thessen *et al.* 2009
	P. calliantha	increased DA in some strains (88 µM added)	Thessen *et al.* 2009
	P. fraudulenta	increased DA in some strains (88 µM added)	Thessen *et al.* 2009
	P. australis	increased DA (10 µM added)	Howard *et al.* 2007
Urea	P. australis	increased DA (20 µM added); two times more DA than nitrate, three times more than ammonium and control treatments	Howard *et al.* 2007
	P. multiseries	increased DA (220 µM added); ~ two times more than nitrate on day 8 in batch culture, then the same	Calu *et al.* 2009
	P. pungens	increased DA (220 µM added); ~ two times more than nitrate in chemostat culture	Calu *et al.* 2009
	P. multiseries	increased in some strains (88 µM added)	Thessen *et al.* 2009
	P. calliantha	increased in some strains (88 µM added)	Thessen *et al.* 2009
	P. fraudulenta	increased in some strains (88 µM added)	Thessen *et al.* 2009
Tris buffer	P. multiseries	increased DA (2.1–8.2 mM added); two to three times more than the control with no Tris added	Douglas *et al.* 1993
Bicarbonate	P. multiseries	increased DA (1–2 mM added); 4.8 times and 11.5 times more when supplemented with 1 and 2 mM bicarbonate, respectively	Bates & Léger 2006
Carbon dioxide	P. multiseries	increased DA (220–730 ppm added); 1.4 times and 5.0 times more when supplemented with 400 ppm and 730 ppm CO_2, respectively	Sun *et al.* 2011
Lithium	P. multiseries	increased DA (386 µM added); 1.7 times compared with control	Subba Rao *et al.* 1998

Table 3. Continued

Factor	Species	Effect on DA production (range in growth parameter)	Reference
Iron	*P. multiseries*; *P. australis*	increased DA under Fe limitation, during late-exponential phase	Rue & Bruland 2001; Maldonado *et al.* 2002; Wells *et al.* 2005
	P. multiseries	decreased DA under Fe deficiency, during stationary phase	Bates *et al.* 2001
Copper	*P. multiseries*; *P. australis*	increased DA at high Cu concentration, during late-exponential phase	Rue & Bruland 2001; Maldonado *et al.* 2002; Wells *et al.* 2005
Biological			
Allelopathy	–	not studied specifically to test for DA production	Subba Rao *et al.* 1995; Lundholm *et al.* 2005b
Bacteria	*P. multiseries*	DA increased in the presence of bacteria	Douglas *et al.* 1993; Bates *et al.* 1995a, b, 2004; Kobayashi *et al.* 2009

(Vietnam) (Doan-Nhu *et al.* 2008). Nevertheless, *P. cuspidata* was also found in colder (12 to 15°C) upwelling waters of coastal Washington State (Trainer *et al.* 2009b). Thus, it appears that the origin of the isolate may be important, and generalizations about growth temperature should be made with caution.

As well, because *Pseudo-nitzschia* spp. have such a broad tolerance for temperature, found in nature from −1.5 to at least 30°C (summarized by Bates *et al.* 1998), temperature can be used only in a general way to elucidate species distributions. The cosmopolite nature of several *Pseudo-nitzschia* spp. (Table 1; Hasle 2002) may be explained in part by their wide temperature tolerance. In contrast, polar, tropical and oceanic species likely have different temperature optima and a narrower tolerance; e.g. *P. granii*, the only polar *Pseudo-nitzschia* species studied, has a lower optimum temperature (∼ 14°C) than the other species (Table 4) and is also found in colder waters (Table 1). Its ability to grow at lower temperatures is related to an increased cellular chlorophyll *a* level (El-Sabaawi & Harrison 2006).

As more growth temperature data are obtained from laboratory studies, it is becoming clearer that there is a ∼ 10°C overlap in the temperature tolerance of some *Pseudo-nitzschia* spp. (Table 4). It has thus become more difficult to correlate seawater temperature with species succession (see above). Nevertheless, temperature has been correlated with the presence of certain species in the field, e.g. a negative correlation for *P. calliantha* in the southern Adriatic Sea (Italy) (Caroppo *et al.* 2005) and Chesapeake Bay (USA) (Thessen & Stoecker 2008), and a positive correlation for *delicatissima* group species in Bizerte Lagoon (Tunisia) (Sahraoui *et al.* 2009) and for *P. americana* and *P. australis* in Normandy (France) (Klein *et al.* 2010). These findings often simply reflect that the species is found under summer or winter conditions.

Bates (1998) summarized earlier studies for five species of *Pseudo-nitzschia*, showing that DA production generally increases with increasing temperature, as expected. No further studies have been carried out to advance this area of research.

Salinity

Up to 1998, three species had been studied with respect to salinity tolerance for growth (Bates 1998): *P. multiseries*, *P.*

pungens and *P. pseudodelicatissima*. Since then, additional data have become available for these species, and three more *Pseudo-nitzschia* spp. have been studied (Table 5): *P. delicatissima*, *P. cuspidata* and *P. multistriata*; *P. americana* (Miller & Kamykowski 1986) and *P. pseudodelicatissima* (Lundholm *et al.* 1997) were studied earlier but were not included in Bates (1998). All are estuarine/coastal species. Most studies were carried out at one temperature only, although as the above has shown, temperature and salinity interact to affect the growth response. For example, *P. cuspidata* can tolerate a wider range of salinities when grown at higher temperatures (Doan-Nhu *et al.* 2008), illustrating the importance of including several temperatures when studying the growth response to salinity. As well, the acclimation time to the different salinities before the growth measurements is important (Thessen *et al.* 2005). For example, a gradual acclimation resulted in a wider salinity growth range for *P. pungens* and *P. multistriata* than when the cells were shocked with a sudden change in salinity (Villac *et al.* 2004), although abrupt salinity changes may indeed occur in nature, e.g. at the mouth of a river. An ability to persist at low salinities, even without growing, is also an important aspect that is often not considered in these laboratory studies (Thessen *et al.* 2005).

The new studies add increasing evidence that these species are euryhaline and halotolerant. In the natural environment they are able to grow at salinities ranging from 1 to 39.8 psu (Thessen *et al.* 2005), e.g. with a maximum abundance between 10 and 20 psu in the Chesapeake Bay (Anderson *et al.* 2010) and 22 and 28 psu in Louisiana–Texas coastal waters (Thessen *et al.* 2005). The salinity range was slightly higher (26–32 psu) in Alabama coastal waters, where the maximum in the mean population density of *Pseudo-nitzschia* spp. occurred at 30.1 ± 3.2 psu (Liefer *et al.* 2009). When all the laboratory studies are examined there appears to be little difference between most species with respect to salinity, because of the broad range in salinity tolerance for each species (Table 5). Exceptions are *P. delicatissima*, which can grow at lower salinities and *P. cuspidata*, which grows less well at low salinities.

The salinity ranges determined in these studies are often broader than those actually found where the species are growing in nature, especially at the high end. Thus, one cannot necessarily use these laboratory studies to define when and where a particular species will bloom. Neverthe-

Table 4. Summary of temperature data (°C), showing the minimum (Min), maximum (Max) and the optimum temperature at which the *Pseudo-nitzschia* spp. are able to grow in culture. The location of each isolate, as well as the salinity (‰ or psu) used for the measurements, are also shown. n/a = data not available. A < or > indicates that the minimum or maximum temperature, respectively, that allows growth was not reached.

Species	Location of isolate	Salinity	Min	Max	Optimum	Reference
P. americana	Cape Fear River estuary, North Carolina, USA	26	15	> 32	25 ± 2.5	Miller & Kamykowski 1986
P. cuspidata	Nha Trang Bay, Vietnam	25–35	< 20	> 30	30	Doan-Nhu *et al.* 2008
P. granii	Ocean Station Papa, NE subarctic Pacific	n/a	< 8	20	~ 14	El-Sabaawi & Harrison 2006
P. multiseries	Pomquet Harbour, Nova Scotia, Canada	n/a	< 5	> 25	20–25	Lewis *et al.* 1993
	Chinhae Bay, South Korea	30	10	> 25	20	Cho *et al.* 2001
P. pseudodelicatissima[1]	Limfjord, Denmark	25	5	> 25	> 25	Lundholm *et al.* 1997
P. pungens	Chinhae Bay, South Korea	30	15	> 25	20	Cho *et al.* 2001
	Nha Trang Bay, Vietnam	26	< 26	> 26	26	Doan-Nhu *et al.* 2008

[1] May be multiple species within the *P. pseudodelicatissima* complex (*sensu* Lundholm *et al.* 2003; Amato & Montresor 2008).

less, salinity has been shown to be an important parameter, among several others, that correlates with the presence of *Pseudo-nitzschia* spp. Several studies have shown significant positive correlations between salinity and the appearance of species or groups of species, e.g. *P. delicatissima* (Caroppo *et al.* 2005) and the *delicatissima* group (Fehling *et al.* 2006; Sahraoui *et al.* 2009). As well, certain *Pseudo-nitzschia* spp. were associated with specific salinity ranges in the Chesapeake Bay (Thessen & Stoecker 2008), and stenohaline species were found in two different bays in Vietnam (Doan-Nhu *et al.* 2008).

Only one study (Doucette *et al.* 2008) has examined the effects of salinity on DA production. When acclimated to different salinities, the cell division rate of *P. multiseries* was greatest at the three highest salinities tested (20, 30 and 40 psu) but declined by about half at the lowest salinity (10 psu). Cellular DA and toxin production were also maximal at the highest salinities (30 and 40 psu). DA production declined significantly (three- to sevenfold) in cells adapted to lower salinities (10 and 20 psu). The authors suggested that *P. multiseries* was able to maintain a high growth rate at 20 psu, but at the expense of being able to produce DA at elevated levels. They hypothesized that energy generated from photosynthesis may have been diverted from DA production to the maintenance of an osmotic balance, required to survive at the low salinity. Their findings suggested that DA levels should be greatest in higher-salinity coastal waters compared with low-salinity estuaries, which was consistent with their field observations along the Louisiana coast. In spite of the above finding that DA production was greatest at the highest salinities, there is still no proof that DA is acting as an osmolyte (Bates 1998). There are also no further studies advancing how taurine (Jackson *et al.* 1992), sorbitol (Stewart *et al.* 1997) or other such compounds may play this role for *P. multiseries* growing at high salinities.

Irradiance

Earlier research on *P. multiseries* (reviewed by Bates 1998; Bates *et al.* 1998) showed that photosynthesis saturated at 100–600 μmol photons m^{-2} s^{-1} and growth at 80–200 μmol photons m^{-2} s^{-1}. The photosynthesis of *P. americana* saturated at 140 μmol photons m^{-2} s^{-1} (Miller & Kamykowski 1986). Growth rates of the oceanic *P. granii* increased when the irradiance was increased from 20 to 150 μmol photons m^{-2} s^{-1}, and photosystem II activity increased under the high irradiance (El-Sabaawi & Harrison 2006). The growth of *P. australis* saturated at 100 μmol photons m^{-2} s^{-1} (Cochlan *et al.* 2008). Surprisingly, there is still a paucity of studies on irradiance–growth–photosynthesis relationships for *Pseudo-nitzschia* spp.

Metabolic energy derived via photosynthesis is essential for DA production (Bates *et al.* 1991; Pan *et al.* 1998) and thus a minimum irradiance is necessary to satisfy this energy requirement. For *P. multiseries*, at least, this is 100 μmol photons m^{-2} s^{-1} or greater (reviewed by Bates 1998); this should also be determined for other species. Cultures of *P. australis* produced 24–130 times more DA when grown at 115 compared with 12 μmol photons m^{-2} s^{-1} (Cusack *et al.* 2002). DA is produced under continuous irradiance (e.g. Kudela *et al.* 2004b) as well as under different photoperiods, although comparisons have not been made to determine the optimum irradiance condition. However, Fehling *et al.* (2005) determined that cultures grown under a long photoperiod [18:6 light:dark (L:D)] had a higher division rate than those under a short photoperiod (9:15 L:D). As well, greater amounts of DA were produced under the long photoperiod, in support of the requirement for photosynthetic energy to produce DA; interestingly, more DA was released from the cells under the short photoperiod.

Pseudo-nitzschia multiseries cells are capable of surviving for up to 6 wk in darkness, whether or not they are given dissolved organic nitrogen in the form of glutamate plus glutamine (Mengelt & Prézelin 2002). This dark survival ability, however, does not necessarily provide them with a competitive advantage because other diatoms may survive for longer periods in darkness. Moreover, the time required to resume rapid growth upon return to the light was on the order of weeks. Thus, dark survival and upwelling of a seed population from the depths were deemed less likely mechanisms for initiating blooms. The heterotrophic ability of *Pseudo-nitzschia* spp., which would allow dark survival, has not yet been studied sufficiently (see below).

There is an apparent contradiction in the requirement for light in DA production. No DA was produced by *P. multiseries* during the dark cycle in a batch culture (Bates *et al.* 1991). In contrast, cellular DA did increase during the

Table 5. Summary of salinity data, showing the minimum (Min), maximum (Max) and the optimum salinity at which the *Pseudo-nitzschia* spp. are able to grow in culture. The location of each isolate and the temperatures (Temp) at which the salinity-growth experiments were carried out are also shown. A < or > indicates that the minimum or maximum salinity, respectively, that allows growth was not reached.

Species	Location of isolate	Temp (°C)	Min	Max	Optimum	Reference
P. americana	Cape Fear River estuary, North Carolina, USA	25	< 8	> 32	26	Miller & Kamykowski 1986
P. cuspidata	Nha Trang Bay, Vietnam	20	25	30	30	Doan-Nhu *et al.* 2008
	Nha Trang Bay, Vietnam	25	25	35	25–35	Doan-Nhu *et al.* 2008
	Nha Trang Bay, Vietnam	30	25	35	25–35	Doan-Nhu *et al.* 2008
P. delicatissima[1]	nearshore coastal Louisiana, USA	24.5	6.25	> 45	15–40	Thessen *et al.* 2005
	Terrebonne Bay, Louisiana, USA	24.5	6.25	> 45	10–30	Thessen *et al.* 2005
P. multiseries	Galveston, Texas, USA	20	21	> 34	25–28	Reap 1991
	Pomquet Harbour, Nova Scotia, Canada	15	15	> 48	30–45	Jackson *et al.* 1992
	Chinhae Bay, South Korea	20	20	> 50	20–40	Cho *et al.* 2001
	Santa Cruz, California, USA	19	10	40	15–40	Thessen *et al.* 2005
	Monterey Bay, California, USA	19	7	> 45	25–30	Thessen *et al.* 2005
	Monterey Bay, California, USA	19	10	40	20–40	Doucette *et al.* 2008
P. multistriata	Guanabara Bay, Brazil	22–23	< 15	> 40	25–40	Villac *et al.* 2004
P. pseudodelicatissima[2]	Limfjord, Denmark	25	12	> 35	25	Lundholm *et al.* 1997
	offshore coastal Louisiana	24.5	13	> 45	25–40	Thessen *et al.* 2005
	offshore coastal Louisiana	24.5	13	> 45	25–40	Thessen *et al.* 2005
	Bermuda	24.5	13	> 45	15–30	Thessen *et al.* 2005
P. pungens	Galveston, Texas, USA	20	21	> 34	25–28	Reap 1991
	Brudenell River, Prince Edward Island, Canada	15	< 6	30	15–30	Jackson *et al.* 1992
	Chinhae Bay, South Korea	20	10	> 50	20–30	Cho *et al.* 2001
	Guanabara Bay, Brazil	22–23	< 15	> 40	20–40	Villac *et al.* 2004
	Nha Trang Bay, Vietnam	26	20	35	25–35	Doan-Nhu *et al.* 2008

[1] May be multiple species within the *P. delicatissima* complex (*sensu* Lundholm *et al.* 2006; Quijano-Scheggia *et al.* 2009a, 2010).
[2] May be multiple species within the *P. pseudodelicatissima* complex (*sensu* Lundholm *et al.* 2003; Amato & Montresor 2008).

dark cycle when this species was grown in a silicon-limited chemostat culture (Bates 1998). Further research is required to resolve this.

Except for one study on UV irradiation (reviewed in Bates 1998), light quality has otherwise not been studied. Subsequent research has shown that UV-A exposure enhanced the primary production of *P. australis*, in contrast to the surrounding phytoplankton community in the Santa Barbara Channel (California, USA), perhaps via a photoprotective or photorepair mechanism (Gorga *et al.* 2002). UV-B exposure, however, inhibited primary production, as expected. Nevertheless, the enhancement by UV-A was still thought to provide a competitive advantage, especially at depths where UV-A and photosynthetically active radiation are high but UV-B is absent.

pH

The decline in ocean pH, due to atmospheric CO_2 increase or to respiration, as well as an increase in pH due to photosynthesis, affects phytoplankton by altering the inorganic carbon species available for uptake during photosynthesis (see below). *Pseudo-nitzschia multiseries* is capable of using both CO_2 and HCO_3^- (Trimborn *et al.* 2008), which gives it an advantage at both low and high pH. Despite the long-term ocean acidification accompanying global climate change, a more immediate interest is the high pH reached at the end of intense blooms or in batch

cultures, which results in a decrease in CO_2 available for photosynthesis. When cultures reached a pH > 8.6, the division rate of *P. multiseries* slowed, then stopped (Lundholm *et al.* 2004). Because the cultures were not limited by light, vitamins, nitrate, silicate or phosphate, the authors argued that the pH specifically, and not inorganic carbon, was the factor that limited growth at stationary phase. DA was detected simultaneously with the rise in pH; thus they further argued that elevated pH, rather than silicate or phosphate limitation, was the trigger for DA production under their growth conditions.

Trimborn *et al.* (2008) found that *P. multiseries* cellular DA increased more than 70-fold with an increase in pH (i.e. 1.9 pg DA cell^{-1} at pH 7.9; 4.2 pg cell^{-1} at pH 8.4 and 140 pg cell^{-1} at pH 8.9; note that the latter cellular DA value is the highest ever reported for *P. multiseries*), thus supporting the results of Lundholm *et al.* (2004). Both papers speculated that the increased cellular DA may be due to a change in internal pH, thus favouring DA biosynthesis. If validated by others, this finding adds one more trigger for DA production, in addition to silicate and phosphate limitation and trace metals (see below). Contrary to the above studies, an earlier study showed no significant effect of pH on division rates of *P. multiseries* and *P. pungens* when grown at pH 5–9 (Cho *et al.* 2001). A recent study by Sun *et al.* (2011) also showed contrary results, i.e. an increase in cellular DA (from 2.0 to 10.0 pg cell^{-1}, i.e. fivefold) when the pH was decreased (from 8.38 to 7.95), in

this case because of addition of CO_2 (see below). Clearly, additional experiments are required to resolve these different findings about the effects of pH on growth, carbon limitation and DA production. This is also important because of the direct effect of pH on trace-metal availability (see below), one factor in bloom development (Hutchins *et al.* 1998).

NUTRITIONAL PARAMETERS AFFECTING GROWTH AND TOXICITY

Macronutrients

Nutrient enrichment of seawater has led to increases in *Pseudo-nitzschia* spp. abundance and may be responsible for an increase in harmful algal blooms (HABs) worldwide (Anderson *et al.* 2008; Heisler *et al.* 2008). Sometimes, however, links to eutrophication are not easily made, e.g. when there is a negative correlation between *Pseudo-nitzschia* abundance and ambient nutrient concentration (Dortch *et al.* 1997; Schnetzer *et al.* 2007). This may be caused by an immediate drawdown of nutrients by the developing bloom and may therefore obscure the true, longer-term relationship. When cells become nutrient limited they can no longer divide but are still able to photosynthesize and acquire energy. This energy may be used for the production of secondary metabolites, including DA (Pan *et al.* 1998).

SILICON AND PHOSPHORUS: Early studies showed that DA production by *P. multiseries* is triggered by silicate and phosphate limitation in cultures (Pan *et al.* 1996a, b, c; reviewed by Bates 1998). Later studies have confirmed this for silicate (Kudela *et al.* 2004b; Lundholm *et al.* 2004) and phosphate (Pan *et al.* 1996a; Hagström *et al.* 2010; Sun *et al.* 2011). DA production by *P. seriata* is similarly triggered (Fehling *et al.* 2004). Growth of *P. australis* in f/2 medium containing low concentrations of silicate also resulted in DA production (Cusack *et al.* 2002), as was also found for *P.* sp. cf. *pseudodelicatissima* (Pan *et al.* 2001), which could be identified as either *P. pseudodelicatissima* or *P. cuspidata* (Lundholm *et al.* 2003), and *P. cuspidata* (Trainer *et al.* 2009b). Studies are still required to determine if any other species are similarly triggered. Mathematical models have described the growth and DA production of *P. seriata* under limitations by silicate and phosphate (Davidson & Fehling 2006). It is possible, however, that other limiting factors (e.g. carbon, trace metals; see below) are responsible; these would still slow cell division, thus permitting DA to accumulate within the cells. The exact mechanism that triggers DA production nevertheless remains elusive.

Phosphorus limitation decreased the chlorophyll *a* content of *P. multiseries* (Pan *et al.* 1996a), which runs counter to an energy requirement for the observed increase in DA production. Silicon limitation, on the other hand, increased the chlorophyll *a* content of *P. multiseries* (Pan *et al.* 1996a) and decreased its efficiency of photosynthesis, as measured by variable fluorescence (Kudela *et al.* 2004b). In contrast to other diatoms, the lipid content of *P.*

multiseries decreased, rather than increased, in response to silicon deficiency during stationary phase (Parrish *et al.* 1991). This was thought to be caused by light limitation in the dense cultures, but an alternative explanation is that shared precursors, such as acetyl coenzyme A, are channelled into DA rather than lipid synthesis at that time (Pan *et al.* 1998).

NITROGEN: In mesocosm experiments, species of the genus *Pseudo-nitzschia* responded to nitrate addition (Carter *et al.* 2005; Claquin *et al.* 2010). In the Gulf of Mexico, the abundance of wide *Pseudo-nitzschia* species ('*pungens*' group) and thin species ('*delicatissima*' group) has increased since the 1960s and 1980s, respectively (Parsons *et al.* 2002). A positive correlation between nitrate flow and *Pseudo-nitzschia* concentration suggested the Mississippi River as the nitrogen source. Nitrate from submarine groundwater discharge (as distinct from surface runoff or river discharge) is thought to have created a 'hot spot' for *Pseudo-nitzschia* spp. growth in coastal Alabama (USA) waters (Liefer *et al.* 2009; MacIntyre *et al.* 2011).

Nitrogen is indispensable to amino acid synthesis, thus to cell growth and division. As DA is an amino acid, it requires nitrogen to be synthesized. Early studies (Bates 1998; Pan *et al.* 1998), and later ones (Fehling *et al.* 2004; Kudela *et al.* 2004b) showed that N limitation resulted in diminished *P. multiseries* cell yield and no detectable DA production. However, some studies showed that low levels (~ 0.2 pg DA cell^{-1}) were produced (Kudela *et al.* 2004b; Calu *et al.* 2009). *Pseudo-nitzschia multiseries* can grow on multiple sources of nitrogen, both inorganic (nitrate, ammonium) (Thessen *et al.* 2009) and organic (urea, glutamine) (Hillebrand & Sommer 1996; Calu *et al.* 2009; Thessen *et al.* 2009), as can *P. delicatissima* (Ilyash *et al.* 2007, Loureiro *et al.* 2009b) and *P. australis* (Howard *et al.* 2007). *Pseudo-nitzschia delicatissima* took up ammonium more readily than urea but was able to grow on urea when other nitrogen sources were low, giving comparable photosynthetic rates with either substrate (Loureiro *et al.* 2009b). The growth rate of *P. delicatissima* was similar when nitrogen was provided as urea, nitrate or ammonium, or as an undefined form in high-molecular-weight organic matter. *Pseudo-nitzschia multiseries* is sensitive to high concentrations of ammonium, compared with *Skeletonema costatum*; four strains showed growth or photosynthetic impairment at concentrations > 220 µM (Bates *et al.* 1993b). At the same time, DA production was enhanced two- to fourfold.

Interpretation of results about the ability of different species of *Pseudo-nitzschia* to take up and grow on different nitrogen sources must consider the finding that there is great interstrain variability, even if the strains are isolated from the same water sample. For example, of the five *P. fraudulenta* strains studied, two grew fastest on nitrate and ammonium, two on ammonium and one on urea (Thessen *et al.* 2009). One strain of *P. calliantha* grew fastest on nitrate and ammonium and the other on ammonium. The one commonality of all three species (*P. multiseries*, *P. fraudulenta* and *P. calliantha*) was that they all had higher growth rates on ammonium and lower growth rates on urea.

The effect of nitrogen source on DA production is just as unpredictable, with some strains of each species producing

more DA when grown with nitrate, ammonium or urea, and others showing no variation in toxicity with nitrogen source (Thessen *et al.* 2009). Cellular DA levels were highest in *P. multiseries* and only near the detection limit in toxic *P. fraudulenta* and *P. calliantha*. Intraspecific variation in toxin production was greater than the interspecific variation and could be caused by bacteria or genetics (or both).

The following studies included only one strain of a given species. *Pseudo-nitzschia australis* showed a preference for taking up nitrate, followed by glutamine, ammonium and urea (Cochlan *et al.* 2008). Growing *P. australis* with urea resulted in two times more DA than with nitrate and three times more than with ammonium or the control (Howard *et al.* 2007). The ability to produce DA when grown with glutamine was not tested. Growth of one strain of axenic *P. multiseries* with glutamate resulted in a higher cell number and 34 times more cellular DA than the axenic control, although this was still less than the xenic (containing bacteria) control (Lyons 2002); the results could not be replicated using another strain. Similar to *P. australis*, *P. multiseries* and *P. pungens* also produced more DA when grown with urea than with nitrate in batch and chemostat cultures (Calu *et al.* 2009). Curiously, the finding of cellular DA in *P. pungens* (up to 0.2 pg DA cell^{-1}), which would be the first report for a European strain, was not discussed.

Artificial sources of nitrogen must also be considered when carrying out experiments. Adding 2.1–8.2 mM Tris buffer, a primary amine, to cultures of *P. multiseries* (three different strains) enhanced DA production by two- to threefold, perhaps by providing a nitrogen source or acting indirectly on the cells, rather than by affecting pH (Douglas *et al.* 1993).

Taken together, these results indicate the ability of different *Pseudo-nitzschia* species to take advantage of the wide variety of nitrogen sources available in different environments. Of special concern is that urea enhanced the toxicity of *P. australis* (Howard *et al.* 2007) and *P. multiseries* (Calu *et al.* 2009), although more variable results were found for two other strains of *P. multiseries* and strains of *P. calliantha* and *P. fraudulenta* (Thessen *et al.* 2009). Nevertheless, the use of urea has increased threefold over the last 4 decades in certain agricultural areas of the world (Glibert *et al.* 2006), making this nutrient especially problematic.

ORGANIC NUTRIENTS AND HETEROTROPHIC ABILITY: Some organic sources of nitrogen were discussed above, but other studies have also suggested a heterotrophic ability for *Pseudo-nitzschia* spp. Addition of sewage effluent to microcosms resulted in the dominance of *P. multiseries* or *P. pungens* over other phytoplankton (Pan & Subba Rao 1997). After treating the effluent with UV light to break down organic material, *Pseudo-nitzschia* spp. no longer dominated, suggesting that members of this genus had been taking advantage of the presence of organic material for growth. Loureiro *et al.* (2009a) later demonstrated that addition of dissolved organic matter to seawater samples resulted in an increase in *Pseudo-nitzschia* spp., although not in chlorophyll *a*, suggesting that the cells fulfilled their nutritional needs via assimilation of the organic matter.

The heterotrophic capacity of *P. multiseries* has not been well studied. Acetate (labeled with ^{13}C or ^{14}C) was used in DA biosynthesis studies (Douglas *et al.* 1992; Ramsey *et al.* 1998) and has thus been indirectly shown to be taken up by the diatom. Although gluconic acid/gluconolactone apparently increased DA production in axenic cultures (see below), uptake of the compound was not demonstrated. The uptake of other organic substrates and their potential effects on DA production was investigated by Lyons (2002). No growth or DA production occurred when a strain of axenic *P. multiseries* was placed in darkness for 35 d in media supplemented with 28 mM glucose, acetate, gluconic acid/gluconolactone or glutamate, which argues against its ability for heterotrophy. However, other concentrations of these organic substrates should be tested, using several strains and different acclimation times, before coming to a solid conclusion about its heterotrophic ability.

Tang *et al.* (2010) recently determined that *P. pungens* requires cobalamin (B$_{12}$), biotin (vitamin B$_7$) and thiamin (vitamin B$_1$), whereas *P. multiseries* (two strains) requires only cobalamin (B$_{12}$), indicating different auxotrophic abilities. The significance of this difference is not yet known, especially with respect to the ability to produce DA.

CARBON: In most culture studies, with *P. multiseries* at least, nearly all of the DA is produced postexponential phase. At that time, the concentration of CO_2 is decreased because of photosynthetic uptake, the pH is high and the carbonate system is shifted toward higher proportions of bicarbonate and carbonate. Although *P. multiseries* is capable of using both of these forms of inorganic carbon (Trimborn *et al.* 2008), total inorganic carbon (TIC) becomes low. It is therefore possible that the available TIC concentration may modify DA biosynthesis during the stationary phase. This hypothesis was tested by amending medium f/2 (containing 1.9 mM TIC) with sodium bicarbonate to give 2.8 and 3.7 mM TIC (Bates & Léger 2006). These additions resulted in 4.8 times and 11.5 times more cellular DA, respectively, than the unamended control. The significant linear relationship between cellular DA concentration and initial TIC concentration suggested carbon limitation of DA biosynthesis. Likewise, agitation on a rotary shaker table as well as bubbling with air can increase DA production (unpublished results; Mafra 2009), another indication of possible inorganic carbon limitation. The bicarbonate addition experiments also showed a positive relationship between DA levels and pH, in support of Lundholm *et al.* (2004) and Trimborn *et al.* (2008) (see above).

Interestingly, results from a recent study showed that increasing the partial pressure (Pa) of CO_2 (pCO_2) in phosphorus limited *P. multiseries* semicontinuous cultures stimulated DA production 1.4-fold when supplemented with ~ 41 Pa [400 parts per million (ppm)] and fivefold with ~ 74 Pa (730 ppm) CO_2 (Sun *et al.* 2011). The differences in pH were small when CO_2 was added (8.38 to 7.94), but the greatest DA production occurred at the lowest pH, in contrast to Lundholm *et al.* (2004) and Trimborn *et al.* (2008). Surprisingly, even though the CO_2 addition increased the growth rate and DA production, carbon limitation was believed not to have been alleviated by the CO_2 addition (Sun *et al.* 2011). This is because the photosynthetic rates were lower in the phosphorus-limited

culture, which had the highest DA production, than in the phosphorus-replete culture, where DA production was lowest. As well, *P. multiseries* has a high affinity for CO_2 (Trimborn *et al.* 2008), which argued against the likelihood of inorganic carbon limitation. Nevertheless, results of Bates & Léger (2006) do support this possibility (see above). The ratio of carbon fixation to the degree of nutrient stress may be an important determinant of DA production (Sun *et al.* 2011). Clearly, additional research is required to verify the effects of pH and carbon limitation on toxin production and to understand mechanisms of action.

Trace-metal nutrients

LITHIUM: The first study conducted with trace metals and *Pseudo-nitzschia* concerned lithium, which was shown to stimulate DA production in *P. multiseries* (Subba Rao *et al.* 1998). Lithium was found at concentrations of 1.5 to 47.8 µM in Cardigan Bay, Prince Edward Island (Canada) during the DA episode of 1987–1988, presumably from waters flowing over an adjacent dump. Enrichment of a culture with 386 µM lithium, although substantially more than was found in the original episode, resulted in a higher cellular DA level (230 fg cell^{-1} on day 17) than in the control (135 fg cell^{-1} on day 25); released DA was also substantially higher. Because silicate and phosphate remained at nonlimiting concentrations, it was believed that lithium was responsible for enhancing DA biosynthesis, perhaps by increasing the levels of cyclic adenosine monophosphate (cf. Pan *et al.* 1998).

IRON AND COPPER: At least three situations can be described whereby toxic *Pseudo-nitzschia* blooms occur along the west coast of the United States in relation to differing concentrations of macronutrients, iron and copper. First, toxic blooms in the Juan de Fuca eddy (discussed above, Marchetti *et al.* 2004; Trainer *et al.* 2009a, b) occurred in waters that were not limited by silicate or phosphate. Instead, the lowest iron concentrations (< 0.5 nM) in the eddy were found where particulate DA was the highest (Trainer *et al.* 2009a), suggesting that iron limitation, not macronutrients, was responsible for triggering DA production. Second, toxic blooms occurred along the California coast where iron was not limiting, but neither were silicate and phosphate (Hutchins & Bruland 1998; Trainer *et al.* 2000; Kudela *et al.* 2004a). What then, could trigger DA production? Ladizinsky (2003) found high correlations between DA accumulation and elevated concentrations of copper from anthropogenic sources, suggesting that copper could have triggered DA production (see below). Third, areas of southern California experienced toxic blooms in the presence of low concentrations of macronutrients (Schnetzer *et al.* 2007; Seeyave *et al.* 2009), which may have been caused by the concurrent growth of the phytoplankton population; unfortunately, these studies did not report information about iron or copper.

The presence of both iron-replete and iron-limiting upwelling waters in California may be explained by the coastal bathymetry (Bruland *et al.* 2001). Those waters with a narrow continental shelf, thus depriving them of iron from sediments, and with no riverine iron inputs (e.g. Big Sur), can have limiting concentrations of iron similar to the mid-oceanic HNLC waters. Other upwelling areas (e.g. Peru) are also characterized by such 'coastal' HNLC waters (Eldridge *et al.* 2004). Iron bioavailability is further reduced in these waters by its complexation with strong organic ligands released by microbes (Wells *et al.* 2005). On the other hand, coasts with wide continental shelves and shallow bays with freshwater inputs (e.g. Monterey Bay) have high concentrations of iron that do not limit phytoplankton growth (Hutchins *et al.* 1998), although copper concentrations may also be high and potentially toxic (Ladizinsky 2003). The presence of toxigenic *Pseudo-nitzschia* spp. in waters that have both high and low iron and macronutrient concentrations indicates that there are multiple conditions, including macronutrients, iron and copper, that are conducive to these blooms. Given that macronutrients could not always explain the distribution of *Pseudo-nitzschia* spp. and the physiology of DA production, we now focus attention on the potential role of trace metals, particularly iron and copper, in this respect.

Interestingly, the addition of iron to mid-ocean HNLC waters that are limited by iron often results in the increased growth of pennate diatoms, including *Pseudo-nitzschia* spp. (as discussed above). Transition zones ('ecotones'), where iron-poor, nitrate-rich oceanic HNLC waters are transported toward iron-rich, nitrate-poor coastal waters, also promote the growth of *Pseudo-nitzschia* spp., as documented in the northeast Pacific Ocean (Ribalet *et al.* 2010). This indicates that low numbers of these species are always present in these waters, so they must have particular adaptations for low iron conditions. Furthermore, such seed populations are capable of responding rapidly to periodic inputs of iron from aeolian deposition or intermittent upwelling and mixing (Marchetti *et al.* 2006b; Silver *et al.* 2010).

Several strategies are used to survive at low ambient iron levels. In the case of pennate diatoms, oceanic *Pseudo-nitzschia* species have significantly lower iron cell quotas and higher iron-use efficiencies than the coastal *P. multiseries* (Marchetti *et al.* 2006a). Growth rates were therefore not as reduced when iron concentrations were low. The oceanic *P. granii* uses the iron-concentrating protein ferritin to enhance its storage of iron (Marchetti *et al.* 2009). This ability to sequester iron from sporadic inputs of this metal allowed *P. granii* to maintain near-maximum growth rates in culture, even at low iron concentrations. Although the coastal species *P. multiseries* also exhibits the ability to produce ferritin, it has only a twofold range in ferritin transcript abundance compared with a 20-fold range in *P. granii*. On the basis of ferritin sequence phylogenies, Marchetti *et al.* (2009) argue that this allowed *P. granii* to grow at low ambient iron concentrations and ultimately to radiate into open ocean waters.

Similar to other pennate diatoms (Leynaert *et al.* 2004), iron depletion in *Pseudo-nitzschia* spp. leads to a decrease in cell volume (by decreasing the cell width), cellular chlorophyll *a*, carbon, nitrogen and silicon cell quotas, maximum photochemical yield and growth rate (Marchetti & Harrison 2007). A decrease in cell size results in an increase in the cells' surface-to-volume ratio, which could increase the number of receptors available to scavenge the

Table 6. Comparison of domoic acid (DA) production by *Pseudo-nitzschia multiseries* growing in synthetic seawater with sufficient iron or with limited/depleted iron. n.d. = not determined.

Iron condition	Growth phase	Cellular DA (pg cell^{-1})	Dissolved DA production (fg cell^{-1} h^{-1})	Cellular DA production (fg cell^{-1} h^{-1})	Total DA production (fg cell^{-1} h^{-1})	Reference
Sufficient	stationary	40[1]	n.d.	n.d.	63.0	Bates *et al.* 2001
Limited		4[1]	n.d.	n.d.	4.7	
Sufficient	exponential	0.021	1.6	0.61	2.2	Maldonado *et al.*
Depleted		0.011	12.3	0.24	12.5	2002

[1] Includes dissolved DA.

limiting concentrations of iron (Eldridge *et al.* 2004). Iron-deficient *Pseudo-nitzschia* spp. cells were also characterized by elevated Si:N ratios, which increased twofold in the case of *P.* cf. *calliantha* (Marchetti & Harrison 2007).

A final strategy may involve DA as a chelator of iron and copper. The correlation between DA production and the presence of low concentrations of iron (Trainer *et al.* 2009a, b) or high concentrations of copper (Ladizinsky 2003) in field studies supports the hypothesis that one metabolic role of DA in *Pseudo-nitzschia* spp. may be to chelate iron and copper, for survival in low-iron or high-copper environments. This would be similar to the strategy of siderophore production by cyanobacteria during iron depletion or limitation to enhance their access to this metal (Wilhelm & Trick 1994), or metallothionein production to alleviate copper toxicity. There are some architectural similarities between the phytosiderophore mugenic acid and DA (Rue & Bruland 2001), which supports a chelation role of DA. Trace-metal chelation is possible because of the three carboxyl groups in the DA molecule (Bates *et al.* 2001). Values for the conditional stability constant of DA binding to iron and copper indicate that environmental concentrations of DA can compete for these trace metals with natural ligands in the seawater (Rue & Bruland 2001). The hypothesis was therefore put forward that DA production may be tied closely to the acquisition of iron or the detoxification of copper in marine waters.

A test of this hypothesis (Maldonado *et al.* 2002) showed that iron-deficient *P. multiseries* cells released DA more rapidly into the medium when grown with low levels of iron compared with iron-sufficient cells during the exponential phase of a semicontinuous culture (Table 6). Because 95% of the DA produced ended up in the medium, less DA remained within the iron-deficient cells than in the iron-sufficient cells. Nevertheless, the total DA production (intracellular DA plus extracellular release) was about six times faster for the iron-deficient than iron-sufficient cells (Table 6). Furthermore, addition of DA to the growth medium resulted in a threefold increase in iron uptake. When stressed by high copper levels, the cells released ∼ 20 times more DA than the control. Similar results were found for *P. australis* producing DA (Ladizinsky 2003) and isodomoic C (Rhodes *et al.* 2004, 2006). It was thus argued that DA was produced and then released as a trace-metal chelator. This strategy would allow *Pseudo-nitzschia* cells to acquire iron at low concentrations by scavenging it, or to alleviate copper toxicity by chelation, which would reduce its bioavailability. Laboratory and field studies support these findings for copper (Ladizinsky 2003).

Further work carried out by some of the coauthors of the Maldonado *et al.* (2002) study extended these findings, but also revealed that the mechanisms could be much more complex (Wells *et al.* 2005). First, *P. multiseries*, *P. australis* and *P. fraudulenta* had low growth rates as well as low cellular DA concentrations only during an initial period of adaptation to iron-depleted media. As in Maldonado *et al.* (2002), this was interpreted to be caused by a greater release of DA from the cells to chelate the low levels of available iron. However, after acclimation, as shown by the resumption of a high growth rate (requiring about eight transfers), less DA was released because the cells were able to satisfy their iron requirement by accessing the DA-chelated iron; cellular DA then increased. Second, copper deficiency increased DA per cell by 20-fold, compared with a doubling when iron was the only limiting trace metal. Third, addition of dissolved DA increased growth rates in low-copper culture media, as well as in an iron-stressed Juan de Fuca eddy population composed mainly of *Pseudo-nitzschia* spp., to the same extent as did iron addition. Fourth, addition of ferrichrome, a nonmarine siderophore, to this field population to further increase iron limitation resulted in a three- to fourfold increase in cellular DA, but this was not observed when copper was also added. Taken together, these results were interpreted as showing a synergy between iron and copper with regard to controlling DA production. This led Wells *et al.* (2005) to develop a hypothesis that incorporated a role for DA and copper in alleviating iron limitation in *Pseudo-nitzschia* spp. It is based on the copper-dependent, high-affinity iron acquisition system of the yeast, *Saccharomyces cerevisiae*. They hypothesized that *Pseudo-nitzschia* cells actively release DA under low-iron conditions to first facilitate copper acquisition. This copper is then used to induce a high-affinity transport system that would allow it to access iron bound to strong ligands such as siderophores and therefore to alleviate the cells' iron deficiency. Wells *et al.* (2005) argue that without sufficient iron and copper, *Pseudo-nitzschia* will become more toxic by increasing its DA production rate to obtain the low levels of these trace metals via released DA. This hypothesis relies on the presence of a multicopper iron oxidase (Peers *et al.* 2005; Wells *et al.* 2005), as is found on the membrane of *S. cerevisiae* cells. The oxidase reoxidizes Fe(II) to Fe(III), making it available to the Fe(III) high-affinity transporters. It is found in the oceanic *Thalassiosira oceanica*, and a putative version of it was found in *Thalassiosira pseudonana* (Maldonado *et al.* 2006). Its presence, however, has not been verified in *P. multiseries*. Among other approaches, this will be deter-

mined when the whole-genome study of *P. multiseries*, now underway, is completed.

In contrast to the results of Maldonado *et al.* (2002) and Wells *et al.* (2005), which have been interpreted to mean that iron-stressed cells increase their DA production to alleviate iron limitation, Bates *et al.* (2001) had earlier found that total DA production by *P. multiseries* decreased, not increased, during iron limitation; i.e. the cellular DA levels and production rates were ~ 10-fold lower in iron-limited cells than in iron-sufficient cells during the stationary phase (Table 6). This decrease was attributed to a reduction in the iron-limited cells' ability to take up nitrogen and to produce sufficient photosynthetic energy, two processes that require iron and are also essential for DA production. It should be noted, however, that Bates *et al.* (2001) measured only total DA production (DA in the cells plus medium), and not dissolved DA, which means that direct comparisons cannot be made with the dissolved fraction in Maldonado *et al.* (2002). Nevertheless, differences in the results for total DA production can be compared and may perhaps be reconciled by noting that the cells used by Bates *et al.* (2001) were in stationary phase, whereas those used by Maldonado *et al.* (2002) were in exponential phase. Thus, the stationary-phase cells produced more DA whether or not they were iron limited, compared with the exponential-phase cells, as expected (Table 6). It could also be argued that the stationary-phase cells tested by Bates *et al.* (2001) were more iron stressed (= 'iron limited') than those used by Maldonado *et al.* (2002), which were in exponential growth and therefore less severely stressed (= 'iron depleted'). This could mean that the iron-depleted cells still had access to sufficient iron, via the hypothesized DA chelation mechanism, to function normally. In contrast, the stationary-phase, iron-limited cells may have depleted the available iron, thus impairing their ability to take up and metabolize nitrogen and to synthesize sufficient amounts of chlorophyll, and ultimately, of DA. The results suggest that DA production and release as a strategy to scavenge limiting concentrations of iron is useful only if the cells are mildly stressed by low concentrations of iron, and not if they are more severely iron limited. It then becomes important to know, e.g. for modeling or monitoring purposes, what state the cells are in within the spectrum of iron depletion to limitation, to predict if they will be capable of producing larger or smaller amounts of DA. This information may be gained, for example, by using variable fluorescence measurements (Bates *et al.* 2001; Kudela *et al.* 2004b). The finding of particulate DA at several depths in unamended HNLC waters at OSP (Trick *et al.* 2010) suggests that these waters were not severely iron limited.

The combined results of all previous studies with nutrients and trace metals suggest that DA would accumulate in the cells when triggered by silicate or phosphate limitation, but only when iron is still in excess. If iron also becomes limiting, then cellular DA levels would decrease because an increasing proportion of the cellular DA would be released by the cells. Maldonado *et al.* (2002) argue that this could account for the large variability in cellular DA levels observed during *Pseudo-nitzschia* blooms in coastal waters. It could also explain why little DA is produced by exponentially growing, nonlimited cells in batch cultures, whereas large amounts are produced during early to mid-stationary phase when limited by silicate or phosphate, but not by iron. In late-stationary phase, cellular DA decreases and increasing amounts of DA are released into the medium, perhaps triggered by iron limitation. To validate this hypothesis, measurements of DA production with respect to macronutrient and iron concentrations must be made during the entire growth cycle in batch culture, from exponential to late-stationary phase. It follows that increasing the concentration of iron, and possibly even of copper, in the growth medium may result in greater DA production and less release of DA from the cells.

Clearly, many physical factors are able to influence DA production, and others will likely be found. Ultimately, the only commonality among them may be how the factor affects gene expression within the DA biosynthetic pathway, which still requires fuller elucidation.

BIOLOGICAL PARAMETERS AFFECTING GROWTH AND TOXICITY

The appearance of *Pseudo-nitzschia* blooms is linked to numerous biotic factors, some of which are species specific. This makes it a challenge to understand bloom dynamics, which is essential to forecast bloom timing, intensity and location, and to predict cellular toxicity. Nevertheless, the growth of *Pseudo-nitzschia* with other phytoplankton and with bacteria may provide some clues.

Association with other phytoplankton

Most *Pseudo-nitzschia* spp. cells form chains that are free-living in the water column (e.g. Rines *et al.* 2002) or on the sediment surface (Dortch *et al.* 1997). Several examples, however, show *Pseudo-nitzschia* spp. living amongst colonies of other diatoms or other unicellular, photosynthetic algae: *P. pseudodelicatissima* in *Chaetoceros socialis* colonies (Gailhard *et al.* 2002; Rines *et al.* 2002); *P. americana* (Lundholm *et al.* 2002b; Nézan *et al.* 2007; Stonik *et al.* 2011) and *P. linea* (Lundholm *et al.* 2002b; Quijano-Scheggia *et al.* 2010) as single cells on *Chaetoceros* and *Odontella* spp. colonies; *P. delicatissima* as single cells in *Phaeocystis* colonies (Lundholm *et al.* 2002a; Sazhin *et al.* 2007); and *P.* cf. *granii* var. *curvata* in *Phaeocystis pouchetii* colonies (Sazhin *et al.* 2007). The advantages of these associations are unknown, but the colonies could provide a growth substrate or organic compounds, including protective aldehydes, that could benefit the colonies and the *Pseudo-nitzschia* cells. Note that the above identifications of *P. pseudodelicatissima* and *P. delicatissima* may in fact be any of the several new species named in the *P. pseudodelicatissima/cuspidata* complex (Lundholm *et al.* 2003) or the *P. delicatissima* complex (Lundholm *et al.* 2006), respectively (see above). Recently, the sexual pairing between parental cells of *P. australis* or *P. pungens* was observed on dense colonies of surf-zone diatoms (Holtermann *et al.* 2010). In that case, it is possible that the sexualized *Pseudo-nitzschia* cells (see above) could be taking advantage of the surf-zone diatom substrate to move toward each other.

Allelopathy

As with many other phytoplankton species (Granéli & Hansen 2006), it is reasonable to assume that *Pseudo-nitzschia* may have allelopathic effects on sympatric species. However, thus far, no studies have been able to demonstrate allelopathic interactions between *Pseudo-nitzschia* and any other algal species. Early trials showed no influence of DA addition on diatom (*C. gracilis*, *Skeletonema costatum*) growth (Windust 1992). Subba Rao *et al.* (1995) reported an allelopathic interaction between toxic *P. multiseries* and the diatom *Rhizosolenia alata* in a mixed culture. However, the results show that increasing the proportion of *R. alata*, or its filtrate, resulted in a decrease in *P. multiseries* growth, not the other way around. Thus, it was not proven that *P. multiseries* produced an allelopathic compound. Likewise, Lundholm *et al.* (2005b) later confirmed that DA addition had no effect on the growth of nine different phytoplankton species, nor did toxic *P. multiseries* have an effect on four algal species in mixed-batch culture experiments.

A recent meta-analysis of possible allelopathic effects on HAB species (but not *Pseudo-nitzschia*) found that, in the few studies that included low cell numbers, none detected any allelopathic effects (Jonsson *et al.* 2009). Modelling showed that the concentrations of such compounds in the bulk seawater would be too low to be effective. They therefore doubted that allelopathy is a mechanism in HAB formation. Nevertheless, their modelling also showed an order-of-magnitude-higher concentration of a putative allelopathic compound immediately around the cell, which opens the possibility of such effects during cell-to-cell encounters. For example, Olson & Lessard (2010) argued that the localized high DA concentrations within the diffusion zone of a cell, in contrast to its concentration in the bulk seawater, could play a role as a microzooplankton grazing deterrent (but see below). Likewise, the proximity of the cells within thin layers may facilitate the usefulness of any allelopathic compound or increase the likelihood of cell-to-cell encounter, leading to sexual reproduction.

Bacteria

Amongst other biotic factors is the existence of bacteria, which have a complex relationship with HAB species. This includes both positive and negative effects on algal growth, as well as a role in phycotoxin production (reviewed by Kodama *et al.* 2006). Bacteria are capable of lysing some diatoms, although there is no solid proof that bacteria have killed *Pseudo-nitzschia* spp. There is only the observation that bacteria attached to *P.* sp. cf. *pseudodelicatissima* appeared to coincide with the lysis of the diatom cells (Pan *et al.* 2001). As well, empty frustules of *P. multiseries* in late stationary phase were packed with bacteria, but this may have occurred after cell death (Kaczmarska *et al.* 2005a). Otherwise, positive effects have been documented. The presence of bacteria in *P. multiseries* cultures increased the cells' growth rate and longevity relative to axenic cultures (Douglas *et al.* 1993), although another study found that axenic and xenic *P. multiseries* cultures had the same growth rates (Kobayashi *et al.* 2009).

Bacteria isolated from *P. multiseries* (Kaczmarska *et al.* 2005a) and *P. pungens* (Sapp *et al.* 2007) cultures were found to be members of the Alphaproteobacteria, Gammaproteobacteria and Bacteroidetes. Another study, using automated ribosomal intergenic spacer analysis (ARISA) profiling, reported Flavobacteria, Alphaproteobacteria and Gammaproteobacteria in both *P. multiseries* and *P. multiseries* (Guannel *et al.* 2011). More specifically, *Alteromonas*, *Moraxella* and *Spirosoma*-like bacteria were reported in other *P. multiseries* cultures (Stewart *et al.* 1997). All bacteria found are Gram negative (Bates *et al.* 1995a).

Depending on the study, the bacterial composition in *Pseudo-nitzschia* cultures shifted, or not, over time in culture. A shift in the bacterial community within a *P. pungens* culture was observed 4–12 mo after isolating the cells (Sapp *et al.* 2007). Moreover, the bacterial assemblage in the freshly isolated culture was different from that in the waters from which the diatoms were originally isolated. In contrast, Guannel *et al.* (2011), using ARISA profiling, found that two strains of nontoxic *P. delicatissima* did not exhibit any shifts in bacterial assemblages over 9–14 mo in culture, nor did toxic *P. multiseries* and *P.* sp. 233 over a 2-mo period. Moreover, some of the assemblages in the cultures were similar to those found in field samples where the diatoms originated, again, contrary to Sapp *et al.* (2007). Interestingly, Guannel *et al.* (2011) found, using ARISA profiling, no differences in bacterial community composition between the exponential and stationary phases for all *Pseudo-nitzschia* strains tested, including the five toxic strains. As well, the composition of the attached and free-living communities (defined operationally, on the basis of what passed through or was retained on filters) did not differ significantly for 9 of the 11 *Pseudo-nitzschia* strains assessed. These findings argue against the hypothesis that bacterial composition is in part responsible for the decline in toxicity in culture (see above) or for the differences in toxicity between exponential-phase and stationary-phase cultures. However, their study did report differences between toxigenic and nontoxigenic *Pseudo-nitzschia* species. Toxigenic strains hosted fewer bacterial ARISA operational taxonomic units, in comparison with nontoxigenic strains. Although both *P. multiseries* (toxigenic) and *P. delicatissima* (nontoxigenic) cultures hosted members of the Gammaproteobacteria and Flavobacteria, 16S rDNA sequencing revealed that the specific bacteria coexisting with each *Pseudo-nitzschia* strain differed on a finer taxonomic scale, i.e. *P. multiseries* hosted members of the order *Oceanospirillales*, whereas *P. delicatissima* hosted *Alteromonadales*. The results of Guannel *et al.* (2011) provided evidence for their hypothesis that DA plays a role in structuring bacterial community composition, i.e. that the growth environment may become enriched with DA-utilizing, DA-tolerant or DA-intolerant bacteria in response to exposure to DA.

The existence of intracellular bacteria in *P. multiseries* is debated, with some authors not finding any (Bates *et al.* 1995b) and others reporting molecular evidence of them (Kobayashi *et al.* 2003). In the latter case, Kobayashi *et al.* (2003) argued that the intracellular bacteria originated from the culture medium, although it was not clear how the *P. multiseries* cells acquired them. Extracellular bacteria may

be either epiphytic or free living. Pan *et al.* (2001) reported an increase in bacterial abundance during the stationary phase of *P.* sp. cf. *pseudodelicatissima*, with the majority of them attached to the cell surface. In *P. multiseries* cultures, only ~ 40% of cells carried epiphytic bacteria (one to five per cell), but their number and the diversity of morphotypes increased during growth in batch culture (Kaczmarska *et al.* 2005a), in contrast to the above ARISA results (Guannel *et al.* 2011). Two subclones of *P. multiseries* from the same parent stock, but grown under slightly different conditions in a different laboratory, developed different epiphytic bacterial assemblages (Kaczmarska *et al.* 2005a).

The presence of bacteria in *Pseudo-nitzschia* cultures has raised considerable interest because several research groups have independently demonstrated that axenic cultures produce less DA than xenic cultures, and that bacteria can enhance DA production (Douglas *et al.* 1993; Bates *et al.* 1995a, b; Kotaki *et al.* 1999; Kobayashi *et al.* 2003). Not all bacteria enhanced DA production, but a wide variety from different locations was capable of it. Axenic cultures produced 2- to 95-fold less DA than xenic cultures, and readdition of the bacteria restored DA production (Douglas *et al.* 1993; Bates *et al.* 1995a, b). There is still no proof that bacteria themselves are able to produce DA (Bates *et al.* 2004). DA production by *N. navis-varingica* was also enhanced by bacteria (Kotaki *et al.* 2000), and bacteria were suggested to play a role in the isodomoic acid composition in this species (Kotaki *et al.* 2008). The effect of epiphytic bacteria, specifically, on DA production has yet to be determined, although the *P. multiseries* strain that had the greatest number and diversity of epiphytic bacteria also had the highest DA production (Kaczmarska *et al.* 2005a). Notwithstanding the above results of Guannel *et al.* (2011), the effects of free-living and attached bacteria, their abundance and composition must be considered, along with the abiotic factors discussed above, in studies of toxin production.

It is still not known how the bacteria enhance DA production. One hypothesis is that some bacteria have an 'antagonistic relationship' with *Pseudo-nitzschia* cells, which then respond by producing DA (Kaczmarska *et al.* 2005a). That DA may, however, be beneficial or not to the *Pseudo-nitzschia* cells (Guannel *et al.* 2011). Another hypothesis involves the production of gluconic acid/gluconolactone from glucose by certain bacteria, including *Alteromonas* sp. (Osada & Stewart 1997). This compound is a 'powerful sequestering agent' that can tie up nutrients, and may therefore be a competitor of DA, also a chelating agent (although for trace metals). Adding gluconic acid/gluconolactone to an axenic culture of *P. multiseries* enhanced DA production in a dose-dependent manner (Osada & Stewart 1997; Stewart 2008). It was argued that DA could be produced by the diatom as an external chemical scavenger to counter nutrient stress during the stationary phase (Stewart 2008). However, it was not specified which nutrients the gluconic acid/gluconolactone scavenged, whereas there is evidence (see above) that DA is a chelator for copper and iron (Maldonado *et al.* 2002). Furthermore, when gluconic acid/gluconolactone was added to a xenic culture, DA production was not enhanced, but rather decreased; no explanation could be found for

this incongruity (Stewart 2008). Thus, the gluconic acid/gluconolactone hypothesis requires further verification.

Another hypothesis is that the bacteria may be supplying nitrogenous compounds or other precursors that are used directly in DA production, or indirectly as 'elicitors' of toxin production (Bates 1998). Three of four bacteria isolated from a toxic *P. multiseries* culture produced *N*-acyl homoserine lactones (N-AHLs), a group of chemical signal molecules used in quorum sensing (Johnston *et al.* 2001). The authors postulated that N-AHLs may be a possible route by which bacteria influence diatom toxin production. Adding bacterial extracts to axenic cultures did not affect DA production, suggesting that a dynamic interaction is required between the diatom and bacterial cells (Bates 1998). This is supported by Kobayashi *et al.* (2009), who suspended an axenic *P. multiseries* culture, contained within a cellophane tube, into a xenic culture. They determined that the cellular DA level of the cells within the tube was much lower than that of the cells outside the tube. This led them to conclude that direct contact between living bacteria and *P. multiseries* cells is necessary for producing high levels of DA in this diatom species. Clearly, bacteria are playing an important but complex role in toxin production, but the details of this are still elusive.

Viruses and fungi

Viruses are known to infect diatoms, and these are mainly species specific (e.g. Nagasaki *et al.* 2005). To date, viruses have only been hypothesized to infect *Pseudo-nitzschia* spp., although research is underway to study this (Carlson *et al.* 2009). Recently, parasitic oomycetes (Figs 5, 6) and chytrids were found to infect *P. pungens* in Canada and elsewhere (Hanic *et al.* 2009). Their role in controlling *Pseudo-nitzschia* bloom dynamics and toxicity should be considered.

IMPACTS OF DOMOIC ACID

Since 1987, numerous observations have been made about how DA-producing blooms affect shellfish, fish, seabirds and marine mammals (Table 7). For example, blue mussels (*Mytilus edulis*) and clams (*Mya arenaria*) from the Bay of Fundy (Canada) became contaminated during July to October 1988, after filtrating toxic *P. pseudodelicatissima* (Martin *et al.* 1990) [the identity of this diatom may be *P. calliantha*, but this is still in dispute (Lundholm *et al.* 2003)]. High DA concentrations are often observed in crabs and mussels from the US west coast (Horner *et al.* 1997). In contrast to most molluscan shellfish (see below), other animals are negatively affected. From 1989 to 1991, hundreds of brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) died in Monterey Bay (California) after ingesting contaminated anchovies (Work *et al.* 1993). Seabirds were also affected in 1996 in Mexico after ingesting anchovies and sardines contaminated by toxic *Pseudo-nitzschia australis* (Sierra-Beltrán *et al.* 1997). The DA contamination of seabirds is perhaps better known than people think. Indeed, the Alfred Hitchcock film ''The Birds'' (1963) may have been inspired

from a real event of birds becoming violent with humans after consuming fish contaminated with DA, near Santa Cruz, California (Dybas 2004).

Marine mammals are also heavily affected. In 1998, > 400 sea lions (*Zalophus californianus*) that had fed on contaminated anchovies died in California (Scholin *et al.* 2000; Howard *et al.* 2007; reviewed by Bargu *et al.* 2011a, b). Surviving sea lions exhibited neurological dysfunction, including head waving, ataxia and abnormal behavior, similar to that shown by DA-intoxicated mice. Identical syndromes were previously observed in sea lions and sea otters in 1978, 1986, 1988 and 1992 (Scholin *et al.* 2000). The cause of sea lion deaths and strandings is complex, involving a combination of environmental and physiological factors in addition to toxic *Pseudo-nitzschia* blooms (Bargu *et al.* 2010). DA later caused the death of minke whales (*Balaenoptera acutorostrata*) in Southern California (Fire *et al.* 2010), and of pygmy and dwarf sperm whales (*Kogia* spp.) in southeastern and mid-Atlantic US waters (Fire *et al.* 2009). It also contaminated North Atlantic right whales (*Eubalaena glacialis*) in the Bay of Fundy (Leandro *et al.* 2010a); a copepod (*Calanus finmarchicus*) was the potential vector (Leandro *et al.* 2010b).

DA is accumulated in the digestive gland and tissues of filter-feeders as a result of their feeding on toxic *Pseudo-nitzschia* cells. There is no evidence that DA may be accumulated directly from the seawater. The mechanics and ability of molluscan shellfish and zooplankton to filter-feed on DA-producing *Pseudo-nitzschia* cells have been studied by few researchers. Detailed information is available only for oysters (*Crassostrea virginica*) and mussels (*M. edulis*) feeding on *P. multiseries* (Mafra *et al.* 2009a, b, 2010). Oysters filtered fewer cells when fed with *P. multiseries* as the only food source, compared with a mixture of *P. multiseries* and nontoxic algae, and produced pseudofeces when a threshold concentration of *Pseudo-nitzschia* was reached. There was a selective rejection of *Pseudo-nitzschia* cells into pseudofeces when these cells were mixed with flagellates, but not when mixed with other diatoms. Rejection can also occur on the gills. As a consequence of these selective mechanisms, oysters accumulate lower concentrations of DA than do other shellfish, including mussels (Mafra *et al.* 2009a). Interestingly, the presence of DA played no role in the oysters' selection process; rather, other intrinsic properties of diatoms in general were believed to be responsible. A similar conclusion was reached for microzooplankton feeding on *Pseudo-nitzschia* cells: the presence of DA or toxic *Pseudo-nitzschia* cells had no effect on microzooplankton grazing or growth (Olson & Lessard 2010). Likewise, this study showed that a diatom-consuming dinoflagellate did not feed on toxic or nontoxic *Pseudo-nitzschia* cells, indicating the involvement of factors other than DA. These, and previous studies (e.g. Lincoln *et al.* 2001; Tester *et al.* 2001), indicate that DA *per se* plays no role as a grazing deterrent, in contrast to an earlier hypothesis (Bates *et al.* 1989).

With the exception of Pacific oysters (*Crassostrea gigas*) (Jones *et al.* 1995a, b), bivalves are very resistant to DA, even at concentrations as high as 790 μg g^{-1}, as seen in healthy mussels of the original 1987 ASP event (Bates *et al.* 1989). As with DA-resistant Pacific razor clams (*Siliqua patula*), they may have proteins that sequester DA or mute

the receptors, thus limiting DA fixation (Trainer & Bill 2004). Shellfish do not have a nervous system as developed as that in birds and mammals, which may explain the differences in susceptibility. DA affects the behavior of anchovies (Lefebvre *et al.* 2001), krill (Bargu *et al.* 2006), sea lions (Goldstein *et al.* 2008; Bargu *et al.* 2011b), and northern fur seals (*Callorhinus ursinus*) (Lefebvre *et al.* 2010) via nervous system dysfunction. DA also has genotoxic effects on fish, such as the Nile tilapia (*Oreochromis niloticus*) (Cavas & Konen 2008). The leopard shark (*Triakis semifasciata*) is so far the only vertebrate unaffected by DA (Schaffer *et al.* 2006). These sharks posses the molecular targets for DA, but are resistant to it when injected with high doses.

DA can also accumulate to high concentrations in other benthic organisms during toxic *Pseudo-nitzschia* blooms (Kvitek *et al.* 2008). This may be another source of contamination for predators like seabirds, sea lions and fish. Contaminated fish have been caught and consumed by recreational anglers in southern California, posing an additional risk to humans (Vigilant & Silver 2007; Mazzillo *et al.* 2010). Consumption of contaminated menhaden (*Brevoortia patronus*) is yet another potential vector of DA to humans (Del Rio *et al.* 2010).

The effects of long-term consumption of DA by humans and animals are unknown, although evidence is slowly being gathered (Pulido 2008; Lefebvre & Robertson 2010). Low, chronic doses of DA given to rats (Truelove *et al.* 1996) or monkeys (Truelove *et al.* 1997) did not induce clinical or histopathology abnormalities. However, chronic, sublethal exposure of DA to sea lions led to epilepsy (Ramsdell & Stafstrom 2009). Moreover, prenatal exposure of rats to DA at mid-gestation caused learning and memory impairments that persisted into adulthood (Levin *et al.* 2006). DA can cross the placenta, accumulating in the amniotic fluid and entering the brain tissue of prenatal rats (Maucher & Ramsdell 2007). A 5-yr prospective epidemiological cohort study of American Indians has been initiated to determine if the DA levels in razor clams in the Pacific Northwest of the United States are placing them at risk of illness (Grattan *et al.* 2009).

Some countries/locations, such as Japan (Kotaki *et al.* 1999), Australia (Takahashi *et al.* 2007), Chile (Suárez-Isla *et al.* 2002), Chesapeake Bay (Thessen & Stoecker 2008), the Gulf of Naples (Orsini *et al.* 2002; Cerino *et al.* 2005) and the Gulf of Mexico (Liefer *et al.* 2009; Thessen *et al.* 2010; MacIntyre *et al.* 2011), have detected DA produced by toxic species of *Pseudo-nitzschia*; however, there have so far been no resulting serious ecosystem or human-health-related consequences. This may be because toxic cell numbers (Thessen & Stoecker 2008) or cellular DA concentrations (Orsini *et al.* 2002; Cerino *et al.* 2005) are too low, the species present are not suitable for being grazed upon (Thessen *et al.* 2010) or conditions are not conducive to DA production (see above). Indeed, not all strains of the same *Pseudo-nitzschia* species are toxic (Bates *et al.* 1998; Bates 2000), and other strains produce DA in variable amounts (Kudela *et al.* 2004b). This variable toxicity within a species may be explained by genetic factors, instrumentation that is not sensitive enough to detect the DA, misidentification of the species or use of growth conditions that do not trigger DA production.

Table 7. Chronology of domoic acid contamination involving various animals used for human consumption or not ([1]).

Location	Year	Affected animal Animal group	Affected animal Species	*Pseudo-nitzschia* species implicated	Reference
Prince Edward Island, Canada	1987	shellfish	*Mytilus edulis*	*P. multiseries*	Bates *et al.* 1989
Prince Edward Island, Canada	1988 to 1989	shellfish	*Mytilus edulis*	*P. multiseries* or *P. pungens*	Bates *et al.* 1998
Bay of Fundy, Canada	1988	shellfish	*Mya arenaria, Mytilus edulis, Volsella modiolus, Placopecten magellanicus*	*P. pseudodelicatissima* or *P. calliantha*	Martin *et al.* 1990
California, USA	1989 to 1991	fish	*Poeobius meseres*	*P. australis*	Buck *et al.* 1992
Washington & Oregon coasts, USA	1991	shellfish, crabs	*Siliqua patula, Cancer magister*	*P. australis*?	Horner & Postel 1993
California, USA	1991 to 1992	fish	*Engraulis mordax*	*P. australis*	McGinness *et al.* 1995
California, USA	1991	birds	*Pelecanus occidentalis,*[1] *Phalacrocorax penicillatus*[1]	*P. australis*	Fritz *et al.* 1992; Work *et al.* 1993
California, USA	1991 to 1993	shellfish	*Siliqua patula*	unknown	Wekell *et al.* 1994
Washington, USA	1992	shellfish	*Mytilus edulis, Crassostrea gigas*	unknown	Horner & Postel 1993
Denmark	1992	shellfish	*Mya arenaria, Mytulis edulis*	*P. pseudodelicatissima*	Lundholm & Skov 1993
New Zealand	1993 to 1996	shellfish	not specified	*P. australis*	Rhodes 1996
Baja California peninsula, Mexico	1996	birds, fish	*Pelecanus occidentals,*[1] *Scomber japonicus*	unknown	Sierra-Beltrán *et al.* 1997
Portugal	1997 to 2000	shellfish	*Mytilus edulis, Cerastoderma edule, Scrobicularia plana, Venerupis pullastra, Ostrea edulis, Ensis* spp., *Ruditapes decussata*	*P. australis*-like	Vale & Sampayo 2001
Atlantic coast, USA	1997 to 2005	marine mammals	*Kogia breviceps,*[1] *K. Sima*[1]	not directly linked	Fire *et al.* 2009
California, USA	1998	fish, marine mammals	*Engraulis mordax, Zalophus californianus*[1]	*P. australis*	Lefebvre *et al.* 1999
Washington, USA	1998	shellfish	*Siliqua patula*	*P. pseudodelicatissima*	Adams *et al.* 2000
California, USA	1998	marine mammals	*Zalophus californianus*[1]	*P. australis*	Scholin *et al.* 2000
California, USA	1998 to 2006	marine mammals	*Zalophus californianus*[1]	unknown	Bejarano *et al.* 2008a
Scotland	1999	shellfish	*Pecten maximus*	*P. australis*	Campbell *et al.* 2001
Ireland	1999	shellfish	*Pecten maximus, Mytilus edulis, Crassostrea edulis, Ensis siliqua*	*P. australis*	James *et al.* 2005
Brittany, France	1999	shellfish	*Donax trunculus*	*P. multiseries*	REPHY
California, USA	1999 to 2000	shellfish, crabs	*Mytilus edulis, Emerita analoga*		Ferdin *et al.* 2002
France	1999 to 2000	shellfish	*Mytilus galloprovincialis, Ruditapes decussatus, Donax trunculus*	*P. pseudodelicatissima* or *P. multiseries*	Amzil *et al.* 2001
California, USA	2000	fish	*Citharichthys sardidus, Scomber japonicus, Thunnas alalunga, Eopsetta jordani, Atherinopsis califoniensis, Hyperprosopon argenteum, Engraulis mordax, Sardinops saga*	*P. australis*	Lefebvre *et al.* 2002a, b
California, USA	2000	krill	*Euphausia pacifica*[1]	*P. australis*	Bargu *et al.* 2002b
California, USA	2000	squid	*Loligo opalescens*[1]	*P. australis*	Bargu *et al.* 2008
France	2000	shellfish	*Donax trunculus*	unknown	Thébaud *et al.* 2005
Prince Edward Island, Canada	2000	shellfish	*Mytilus edulis*	*P. multiseries*	Bates & Richard 2000
Argentina	2000	fish, shellfish	*Mytilus edulis, Engraulis anchoita*	*P. australis*	Negri *et al.* 2004
California, USA	2000 to 2001	benthic species	*Emerita analoga, Urechis caupo, Citharichthys sordidus, Nassarius fossatus, Pagurus samuelis, Neotrypaea californiensis, Dendraster excentricus, Olivella biplicata*[1]	*P. australis*	Kvitek *et al.* 2008
Portugal	2000 to 2001	fish, shellfish	*Sardina pilchardus, Engraulis mordax, Mytilus edulis, Sardinops sagax, Cerastoderma edule, Venerupis pullastra, Ruditapes decussate, Crassostrea japonica, Ensis* spp., *Solen* spp.	unknown	Costa & Garrido 2004

Table 7. Continued

Location	Year	Affected animal Animal group	Species	*Pseudo-nitzschia* species implicated	Reference
California, USA	2001	fish	*Genyonemus lineatus, Leptocottus armatus*	*P. australis*	Fire & Silver 2005
California, USA	2002	marine mammals	*Zalophus californianus,*[1] *Delphinus capensis,*[1] *Delphinus delphis*[1]	unknown	de la Riva *et al.* 2009
Monterey Bay, California, USA	2002	squid	*Loligo opalescens*[1]	*P. australis*	Bargu *et al.* 2008
France	2002	shellfish	*Donax trunculus*	unknown	Thébaud *et al.* 2005
Portugal	2002	crab	*Polybius henslowii*	not directly linked	Costa *et al.* 2003
California, USA	2002 to 2003	fish	*Citharichthys sordidus, Eopsetta exilis, Eopsetta jordan, Psettichthys melanostictus, Errex zachirus, Microstomus pacificus, Pleuronectes vetulus, Pleuronicthys decurrens, Hippoglossus stenolepis*	*P. australis*	Vigilant & Silver 2007
Portugal	2002 to 2003	fish	*Sardina pilchardus*	*P. australis*	Costa & Garrido 2004
Greece	2002 to 2003	shellfish	*Mytilus galloprovincialis, Venus verucosa*	unknown	Kaniou-Grigoriadou *et al.* 2005
Ireland	2003	shellfish	*Pecten maximus*	unknown	Bogan *et al.* 2007
Washington, USA	2003	shellfish	*Mytilus edulis*	*P. australis*	Bill *et al.* 2004
Portugal	2003 to 2004	cephalopods, fish	*Octopus vulgaris, Sepia officinalis, Eledone moschata, Eledone cirrhosa*	not directly linked	Costa *et al.* 2004, 2005a, b
California, USA	2003 to 2004	crabs, fish	*Pleuroncodes planipes, Scomber japonicus, Trachurus symmetricus, Citharichthys sordidus, Zaniolepis latipinnis*	*P. multiseries*	Busse *et al.* 2006
Isle of Man	2003 to 2004	shellfish	*Pecten maximus*	unknown	Bogan *et al.* 2007
California, USA	2003 to 2004	fish	*Errex zachirus, Microstomus pacificus, Pleuronectes vetulus, Pleuronectes decurrens, Genyonemus fineatus, Gymnocanthus tricupsis*	*P. australis*-like	Trainer *et al.* 2008
Florida, USA	2004	marine mammals	*Tursiops truncatus*[1]	unknown	NMFS 2004
Australia	2004	shellfish	*Saccostrea glomerata, Modiolus proclivis, Donax deltoides*	unknown	Takahashi *et al.* 2007
Mexico	2004	fish, marine mammals, birds	*Delphinus capensis,*[1] *D. delphis,*[1] *Zalophalus californianus,*[1] *Pelecanus occidentalis,*[1] *Sardinops* spp.	unknown	Sierra-Beltrán *et al.* 2005
Chile	2004	shellfish, tunicate	*Mytilus chilensis, Aulacomya ater, Protothaca thaca, Pyura chilensis*[1]	*P. australis*	López-Rivera *et al.* 2009
West coast, France	2004	shellfish	*Pecten maximus*	*P. australis* or *P. multiseries*	Nézan *et al.* 2010
Vietnam	2004 to 2005	shellfish	*Spondylus versicolor, Spondylus cruentus*	unknown	Ha *et al.* 2006; Dao *et al.* 2009
Denmark	2005	shellfish	*Mytilus edulis*	*P. seriata*	Lundholm *et al.* 2005a
Bay of Fundy, Canada	2005 to 2006	marine mammal	*Eubalaena glacialis*[1]	*P. seriata, P. cuspidata, P. delicatissima*	Leandro *et al.* 2010a
California, USA	2005 to 2009	marine mammal	*Callorhinus ursinus*[1]	*Pseudo-nitzschia* spp.	Lefebvre *et al.* 2010
Japan & Thailand	2006	shellfish	*Spondylus* spp.	unknown	Takata *et al.* 2009
Korea	2006 to 2007	shellfish	*Mactra veneriformis, Peronidia venulosa*	unknown	Choi *et al.* 2009
Croatia	2006 to 2008	shellfish	*Mytilus galloprovincialis, Ostrea edulis, Pecten jacobaeus, Flexopecten proteus*	unknown	Ujević *et al.* 2010
California, USA	2007	marine mammal	*Balaenoptera acutorostrata*[1]	*P. australis*	Fire *et al.* 2010
Angola	2007	shellfish	*Dosinia orbignyi, Venerupis corrugata, Mactra glabrata*	unknown	Blanco *et al.* 2010
West coast, France	2007	shellfish	*Pecten maximus*	unknown	REPHY
Louisiana, USA	2007 to 2008	fish	*Brevoortia patronus*	unknown	Del Rio *et al.* 2010
Scotland	2008 to 2009	marine mammal	*Phoca vitulina*[1]	unknown	Hall & Frame 2010
West coast, France	2010	shellfish	*Pecten maximus*	*P. australis*	Nézan *et al.* 2010

GAPS IN KNOWLEDGE

The genus *Pseudo-nitzschia* has been the main subject of numerous studies since its discovery as a DA producer in 1988, yet there is still much to learn, analyze and understand. Some of the gaps in knowledge previously identified (Bates 1998) have been at least partially filled: 'triggers of DA production, other than silicon and phosphorus limitation'; 'role of trace metals (especially iron) in mediating DA production and *Pseudo-nitzschia* growth'; 'details of the life cycle of *Pseudo-nitzschia* species' (although not in relation to DA production); 'physiological studies of *Pseudo-nitzschia* species other than *P. multiseries*'; and 'identification of other producers of DA'. However, the remaining gaps, along with some new ones identified below, still remain. Knowledge gaps specific to life-cycle events are outlined in Mann & Bates (2001).

Only a few papers have investigated, specifically, the intraspecific variability in *Pseudo-nitzschia* physiology, including toxin production (e.g. Bates *et al.* 1999; Kudela *et al.* 2004b; Thessen *et al.* 2009; Amato *et al.* 2010) and association with bacteria (Guannel *et al.* 2011). However, because of the magnitude of the differences among strains, future studies should consider multiple strains to arrive at more solid conclusions and generalities about a given species. Molecular markers appropriate for detecting intraspecific variability, including use of microsatellite analysis, could be applied to these culture studies, in addition to their current application in field studies. They could also be relevant for investigating the progeny obtained during mating studies.

Physiological and ecological roles of DA production

After 23 years of research, the role of DA is still not known with certainty, other than its possible advantages for *Pseudo-nitzschia* in chelating iron and copper (Wells *et al.* 2005); these finding, however, require corroboration by other laboratories. Some of the roles hypothesized by Bates (1998) have been addressed, but require more study. For example, the 'osmolyte hypothesis', whereby DA could serve as an osmolyte in response to increasing salinity, has not been fully addressed. Although DA production was greatest at elevated salinities (Doucette *et al.* 2008), there was no proof that DA was acting as an osmolyte. The 'antifeedant hypothesis', whereby DA could act as a grazing deterrent, is not supported by the more recent research (Lincoln *et al.* 2001; Tester *et al.* 2001; Mafra *et al.* 2009a; Olson & Lessard 2010). Likewise, the 'allelopathy hypothesis', whereby DA could be deleterious to other phytoplankton, is not supported by evidence (Lundholm *et al.* 2005b). The 'excretion hypothesis', whereby DA may be produced and released as a mechanism to get rid of 'excess' photosynthetic energy when cells are no longer able to grow optimally, remains to be addressed.

Presence/absence of a resting stage

Centric diatoms form resting spores, which are very different from vegetative cells in appearance and physiology. Some pennate diatoms form resting stages, which have undergone physiological and cytoplasmic changes but remain morphologically similar to the vegetative cells of the species. In either case, these forms enable the cells to survive during unfavourable conditions (McQuoid & Hobson 1996). Amato *et al.* (2005) hypothesized that *Pseudo-nitzschia* spp. may have a 'quiescent phase' during which cell growth is reduced. However, there is contradictory information regarding the existence of resting stages in *Pseudo-nitzschia* spp. McQuoid & Godhe (2004) indicated that *Pseudo-nitzschia* spp. are not known to form a resting stage, and none was found in sediments. On the other hand, Orlova & Morozova (2009) provide evidence of *Pseudo-nitzschia* sp. 'resting cells' in recent sediments of Peter the Great Bay (eastern Russia). Knowing if such a resting stage exists is important for understanding bloom formation and disappearance. From where do cells that initiate new blooms originate, and what is their physiological condition? A resting stage could enhance the survival of *Pseudo-nitzschia* from one growth season to another, regardless of the conditions. It could also decrease the number of cell divisions over a year, allowing them to survive for a longer period without undergoing sexual reproduction.

Genomics of sexual stages

When cells are undergoing sexual reproduction, it is quite easy to identify the different stages in cultures (Davidovich & Bates 1998). However, this is more difficult in field samples, where *Pseudo-nitzschia* spp. sexual events have only been observed twice (Holtermann *et al.* 2010; Sarno *et al.* 2010). In natural seawater, sexual stages represent only a low percentage of total cell numbers (Mann 1988; Sarno *et al.* 2010), plus they are a challenge to collect, as they might be destroyed during sampling. This makes it difficult to identify the sexual stages and to carry out experiments on them. Therefore, little is known about these stages and their genomics. Sexual reproduction allows genetic recombination between strains, but it is not known how sexual reproduction is regulated, how it might be related to DA production, what determines the production of 'male' or 'female' cells (i.e. what the genes are that determine mating types), how cells of opposite mating type find each other (involvement of pheromones?) and how genetic recombination occurs within the *Pseudo-nitzschia* genus. This information would help to understand the genetic structure of *Pseudo-nitzschia* populations, how new species appear and why different strains of the same species can produce, or not, DA. One way to identify the sexual stages and cells of opposite mating type is to develop genetic probes against them (Mann & Bates 2001).

Whole genome of *Pseudo-nitzschia* spp. and complete DA biosynthetic pathway

Although numerous studies on *Pseudo-nitzschia* biology have been published over the last few years, the genome of *Pseudo-nitzschia* spp. still remains incomplete. Comparisons between the genomes of toxigenic and apparently nontoxic *Pseudo-nitzschia* species are also lacking. Such studies are required to determine why different strains of the same species are not always toxic. The whole-genome

sequencing of *P. multiseries* is still ongoing (Parker *et al.* 2009). When completed, this will help to understand *Pseudo-nitzschia* physiology and how cells adapt to such a broad range of environmental parameters. It will also provide more information about which genes are involved in DA production (Boissonneault 2004). This will allow the development of molecular probes to identify which species at least have the genes for DA production, and when and why the cells become toxic. Furthermore, sequencing the whole genome may help to better assess the role of DA. Nevertheless, knowing the whole genome of *Pseudo-nitzschia* is not enough; the biosynthetic pathway of DA is still incompletely known (Pan *et al.* 1998; Thessen 2007). The regulation of the enzymes involved in this pathway remains unresolved and the relationship between DA biosynthesis and the cell division cycle (Bates 1998) requires clarification. Moreover, it is still not known where DA is stored within cells. The new molecular tools being developed (summarized by Kudela *et al.* 2010) should help to answer some of these questions.

Mechanisms of DA production and decline

DA production is modulated by factors other than various environmental parameters. Indeed, the amount of DA produced by *P. multiseries* decreases with the age of the culture, as does cell size. Is there a causal relationship between cell volume and the ability to produce DA? Otherwise, no one has been able to explain this decrease in toxicity. The mechanisms leading to an increase in DA production, when triggered by silicon or phosphorus limitation and iron deficiency/iron excess, remain unknown and are likely different from each other. Do these limitations induce DA production to contend with an unfavourable environment, or is it an indirect consequence of these limitations? Is there a synergy between macro- and micronutrient limitation in modulating DA production? Knowledge of the complete biosynthetic pathway and of the whole genome would help to analyze gene expression under these limitations in relation to DA production.

Biological control of DA production and of bloom dynamics

Although *Pseudo-nitzschia* spp. do not appear to produce allelopathic compounds, other phytoplankton species may still affect DA production. As well, competition for resources or grazing by predators may be other factors that modulate DA production. Of the biotic factors, only bacteria have been studied so far and these have been shown to enhance DA production. However, there is still incomplete knowledge about which groups of bacteria may be more conducive to enhancing DA production, and about how closely associated the bacteria must be to achieve this. The hypothesis concerning the bacterial production of gluconic acid/gluconolactone, as a chelator that prompts *Pseudo-nitzschia* to produce DA to counter this competition (Stewart *et al.* 1997), requires validation. Finally, studies are needed to determine how bacteria boost DA production.

The role of viruses in controlling *Pseudo-nitzschia* bloom dynamics or modifying cell physiology to trigger DA production is just in its early stage of study (Carlson *et al.* 2009). Only one study has been published that describes oomycete and chytrid fungal parasite infections of *P. pungens*, although the phenomenon is known to be more widespread (Hanic *et al.* 2009). It is not known which parasite species are responsible, or how many other *Pseudo-nitzschia* species may be affected. The potential importance of fungal parasites in controlling *Pseudo-nitzschia* bloom dynamics and toxin production is unknown. Sequencing the genes of parasites would help to identify their life stages and to quantify their prevalence and infection rates. These tasks are so far hampered by the inability to grow the fungal–*Pseudo-nitzschia* pair in culture.

Heterotrophic ability

Conflicting or incomplete evidence, presented above (Pan & Subba Rao 1997; Lyons 2002; Mengelt & Prézelin 2002), indicates that more research is required before solid conclusions can be reached regarding the heterotrophic ability of *Pseudo-nitzschia* spp. Factors to be considered when carrying out such experiments include: the concentration of each organic substrate, the pH of the medium after addition of the organics, preincubation for different times with several substrates to possibly induce uptake and use of both axenic and xenic controls. As well, care must be taken to avoid bacterial contamination in the treatments containing the organics. Tests are required for photoheterotrophy (or photo-organotrophy; ability to use organic molecules as the only carbon source and solar radiation as the energy source), carried out in the light, and for chemoheterotrophy (or chemo-organotrophy; ability to use organic compounds both as a source of carbon and as a source of energy), carried out in darkness.

Resolution of conflicting findings

Several conflicting findings require resolution, including the ability of toxigenic *Pseudo-nitzschia* spp. to produce DA in darkness (Bates *et al.* 1991; Bates 1998), the finding of different responses to nitrogen when different strains of the same *Pseudo-nitzschia* species are studied (Lyons 2002; Thessen *et al.* 2009) and the effects of pH on DA production (Lundholm *et al.* 2004; Trimborn *et al.* 2008; Sun *et al.* 2011), including its mechanisms of action.

Ability to predict blooms reliably

These gaps in knowledge concerning the biology of *Pseudo-nitzschia* and its production of DA make it difficult to predict blooms and their toxicity reliably, especially at a local scale. Recent models of *Pseudo-nitzschia* bloom dynamics (Anderson *et al.* 2009; Lane *et al.* 2009; Palma *et al.* 2010) are a step in the right direction, but more exact data and additional parameters are required to improve their accuracy. In particular, trace metals, biotic factors and species composition (or even the morphology-based groups) have yet to be incorporated. Results of further experimental work and field sampling will slowly fill in these gaps, resulting in more reliable predictive models at different temporal and geographic scales.

SUMMARY

Recent scientific breakthroughs have revealed new information about *Pseudo-nitzschia* species identification, molecular biology, physiology, toxicity and distribution. Molecular methods are now used regularly to examine phylogenetic relationships among *Pseudo-nitzschia* species and to assist in species identification in field samples and cultures; this has allowed the discovery of new species, some of which are cryptic or pseudo-cryptic. From 1993 to 2011, 17 new species have been described; 15 of these are since 2002. When possible, these identifications have been supported by mating studies, but this approach is only recent. Fourteen *Pseudo-nitzschia* spp. have now been shown to be toxigenic in culture.

Although most of the attention still remains focused on *P. multiseries*, and marginally on *P. pungens*, *P. seriata* or *P. australis*, other coastal and oceanic species have recently been included in experimental studies: *P. pseudodelicatissima*, *P. delicatissima*, *P. brasiliana*, *P. multistriata*, *P. calliantha*, *P. cuspidata*, *P. granii* and *P. fraudulenta*. The diversity of species studied gives a broader view of the differences between species, including coastal and oceanic, regarding toxicity and factors controlling DA production.

New triggers or enhancers of DA production have recently been found, in addition to the already known triggers caused by silicon or phosphorus limitation. Iron deficiency or copper excess are believed to enhance DA production and release from the cells, because of the ability of DA to chelate these trace metals. Chelation would render low concentrations of iron more bioavailable to the cells, or protect the cells from potentially toxic, high concentrations of copper. High salinities (30–40 psu) enhanced DA production by *P. multiseries*, the only species studied so far. The role of inorganic carbon, whose concentrations are controlled by CO_2 addition or removal, remains unclear because of conflicting evidence. For example, DA production was reported to be enhanced by both high and low pH, and was also shown to be limited, or not, by TIC concentration.

Organic sources of nitrogen, i.e. glutamine and urea, were shown to enhance DA production, relative to inorganic nitrogen sources, by several species of *Pseudo-nitzschia*, although there are many inter- and intraspecies variations. Such information will affect the design of field studies, as well as decisions about which nutrients are measured and which nutrient parameters are included in models. The importance of a relationship between iron and copper, as well as between macro- and micronutrients, was also revealed. Additional complexities in the interactions of bacteria with *Pseudo-nitzschia* cells in relation to enhancing DA production were discovered. Laboratory studies should include more than one strain of *Pseudo-nitzschia* because of intraspecies variations in the response to environmental factors affecting DA production.

An increasing number of field studies have been conducted to find links between the occurrence, abundance, succession and toxicity of *Pseudo-nitzschia* spp. and environmental parameters at a local scale. These have demonstrated the challenge in determining the trigger for DA production (i.e. silicon, phosphorus or iron deficiency, copper excess, presence of organic nitrogen), which may vary depending on location. Experiments and field sampling have shown that artificial or natural fertilization of HNLC regions of open oceans with iron selects for *Pseudo-nitzschia* spp., including at least two toxigenic species (*P. turgidula* and *P.* cf. *granii*). This is the first time that DA production has been demonstrated for species growing in open ocean waters.

Contamination of diverse animal genera by DA has been documented worldwide and has led to the closures of shellfish-harvesting areas. All trophic levels, from zooplankton to marine mammals and birds, accumulate DA, although its consequences on ecosystem function have been only partially elucidated.

A major advance has been the knowledge gained about the sexual reproduction of *Pseudo-nitzschia* species. First demonstrated in the laboratory in 1998 with *P. multiseries* and *P. pseudodelicatissima* (which may be *P. calliantha*), it has now been documented in 14 *Pseudo-nitzschia* species. This advancement has allowed the production of new large cells for further laboratory study and the confirmation that strains are members of the same species. Only rudimentary information is available about its implications for the toxicity of cells and its significance for bloom dynamics, factors important for incorporating into mathematical models. Recently, sexual reproduction was observed for the first time in the field. Further advances will be gained by studying the molecular biology of sexual stages.

ACKNOWLEDGEMENTS

REFERENCES

ADAMS N.G., LESOING M. & TRAINER V.L. 2000. Environmental conditions associated with domoic acid in razor clams on the Washington coast. *Journal of Shellfish Research* 19: 1007–1015.

AKÉ-CASTILLO J.A. & OKOLODKOV Y.B. 2009. *Pseudo-nitzschia subcurvata* (Bacillariophyceae) in the Gulf of Mexico? *Harmful Algae News* 40: 6–7.

ALMANDOZ G.O., FERRARIO M.E., FERREYRA G.A., SCHLOSS I.R., ESTEVES J.L. & PAPARAZZO F.E. 2007. The genus *Pseudo-nitzschia* (Bacillariophyceae) in continental shelf waters of Argentina (Southwestern Atlantic Ocean, 38–55° S). *Harmful Algae* 6: 93–103.

ALMANDOZ G.O., FERREYRA G.A., SCHLOSS I.R., DOGLIOTTI A.I., RUPOLO V., PAPARAZZO F.E., ESTEVES J.L. & FERRARIO M.E. 2008. Distribution and ecology of *Pseudo-nitzschia* species (Bacillariophyceae) in surface waters of the Weddell Sea (Antarctica). *Polar Biology* 31: 429–442.

ALMANDOZ G.O., HERNANDO M. & FERRARIO M.E. 2009. SEM observations of *Pseudo-nitzschia* from Beagle Channel: *P. seriata* in the southern hemisphere? *Harmful Algae News* 39: 6–7.

ÁLVAREZ G., URIBE E., QUIJANO-SCHEGGIA S., LÓPEZ-RIVERA A., MARIÑO C. & BLANCO J. 2009. Domoic acid production by

Pseudo-nitzschia australis and *Pseudo-nitzschia calliantha* isolated from North Chile. *Harmful Algae* 8: 938–945.

AMATO A. & MONTRESOR M. 2008. Morphology, phylogeny, and sexual cycle of *Pseudo-nitzschia mannii* sp. nov. (Bacillariophyceae): a pseudo-cryptic species within the *P. pseudodelicatissima* complex. *Phycologia* 47: 487–497.

AMATO A., ORSINI L., D'ALELIO D. & MONTRESOR M. 2005. Life cycle, size reduction patterns, and ultrastructure of the pennate planktonic diatom *Pseudo-nitzschia delicatissima* (Bacillariophyceae). *Journal of Phycology* 41: 542–556.

AMATO A., KOOISTRA W., GHIRON J.H.L., MANN D.G., PROSCHOLD T. & MONTRESOR M. 2007. Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist* 158: 193–207.

AMATO A., LUDEKING A. & KOOISTRA W.H.C.F. 2010. Intracellular domoic acid production in *Pseudo-nitzschia multistriata* isolated from the Gulf of Naples (Tyrrhenian Sea, Italy). *Toxicon* 55: 157–161.

AMZIL Z., FRESNEL J., LE GAL D. & BILLARD C. 2001. Domoic acid accumulation in French shellfish in relation to toxic species of *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima*. *Toxicon* 39: 1245–1251.

ANDERSON C.R., BRZEZINSKI M.A., WASHBURN L. & KUDELA R. 2006. Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. *Marine Ecology Progress Series* 327: 119–133.

ANDERSON C.R., SIEGEL D.A., KUDELA R.M. & BRZEZINSKI M.A. 2009. Empirical models of toxigenic *Pseudo-nitzschia* blooms: potential use as a remote detection tool in the Santa Barbara Channel. *Harmful Algae* 8: 478–492.

ANDERSON C.R., SAPIANO M.R.P., PRASAD M.B.K., LONG W., TANGO P.J., BROWN C.W. & MURTUGUDDE R. 2010. Predicting potentially toxigenic *Pseudo-nitzschia* blooms in the Chesapeake Bay. *Journal of Marine Systems* 83: 127–140.

ANDERSON D.M., ANDERSEN P., BRICELJ V.M., CULLEN J.J. & RENSEL J.E. 2001. Monitoring and management strategies for harmful algal blooms in coastal waters, APEC #201-MR-01.1, Asia Pacific Economic Program, Singapore, and Intergovernmental Oceanographic Commission Technical Series No. 59, Paris.

ANDERSON D.M., BURKHOLDER J.M., COCHLAN W.P., GLIBERT P.M., GOBLER C.J., HEIL C.A., KUDELA R.M., PARSONS M.L., RENSEL J.E.J., TOWNSEND D.W., TRAINER V.L. & VARGO G.A. 2008. Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. *Harmful Algae* 8: 39–53.

ANDREE K.B., FERNÁNDEZ-TEJEDOR M., ELANDALOUSSI L.M., QUIJANO-SCHEGGIA S., SAMPEDRO N., GARCÉS E., CAMP J. & DIOGÈNE J. 2011. Quantitative PCR coupled with melt curve analysis for detection of selected *Pseudo-nitzschia* spp. (Bacillariophyceae) from the NW Mediterranean Sea. *Applied and Environmental Microbiology*. (in press).

BAJARIAS F.A., KOTAKI Y., RELOX J.R. JR., ROMERO M.L.J., FURIO E.F., LUNDHOLM N., KOIKE K., FUKUYO Y. & KODAMA M. 2006. Screening of diatoms producing domoic acid and its derivatives in the Philippines. *Coastal Marine Science* 30: 121–129.

BANDALA E.R., BRITO L. & PELAEZ M. 2009. Degradation of domoic acid toxin by UV-promoted Fenton-like processes in seawater. *Desalination* 245: 135–145.

BARGU S., KORAY T. & LUNDHOLM N. 2002a. First report of *Pseudo-nitzschia calliantha* Lundholm, Moestrup and Hasle 2003, a new potentially toxic species from Turkish coasts. *European Union Journal of Fisheries & Aquatic Sciences* 19: 479–483.

BARGU S., POWELL C.L., COALE S.L., BUSMAN M., DOUCETTE G.J. & SILVER M.W. 2002b. Krill: a potential vector for domoic acid in marine food webs. *Marine Ecology Progress Series* 237: 209–216.

BARGU S., LEFEBVRE K. & SILVER M.W. 2006. Effect of dissolved domoic acid on the grazing rate of krill *Euphausia pacifica*. *Marine Ecology Progress Series* 312: 169–175.

BARGU S., POWELL C.L., WANG Z.H., DOUCETTE G.J. & SILVER M.W. 2008. Note on the occurrence of *Pseudo-nitzschia australis* and domoic acid in squid from Monterey Bay, CA (USA). *Harmful Algae* 7: 45–51.

BARGU S., SILVER M., GOLDSTEIN T., ROBERTS K. & GULLAND F. 2010. Complexity of domoic acid-related sea lion strandings in Monterey Bay, California: foraging patterns, climate events, and toxic blooms. *Marine Ecology Progress Series* 418: 213–222.

BARGU S., GOLDSTEIN T., ROBERTS K., LI C. & GULLAND F. 2011a. *Pseudo-nitzschia* blooms, domoic acid, and related California sea lion strandings in Monterey Bay, California. *Marine Mammal Science*. (in press).

BARGU S., SMITH E. & OZHAN K. 2011b. Toxic diatom *Pseudo-nitzschia* and its primary consumers (vectors). In: *The diatom world* (Ed. by J. Seckbach & P. Kociolek), pp. 493–512. Koeltz Scientific Books, Koenigstein, Germany.

BATES S.S. 1998. Ecophysiology and metabolism of ASP toxin production. In: *Physiological ecology of harmful algal blooms* (Ed. by D.M. Anderson, A.D. Cembella & G.M. Hallegraeff), pp. 405–426. Springer-Verlag, Heidelberg.

BATES S.S. 2000. Domoic-acid-producing diatoms: another genus added! *Journal of Phycology* 36: 978–983.

BATES S.S. 2011. Domoic acid and *Pseudo-nitzschia* references. Fisheries and Oceans Canada website, available at http://www2. glf.dfo-mpo.gc.ca/os/aes-sae/dapr-radp/.

BATES S.S. & RICHARD D.J.A. 2000. Shellfish harvest area closure due to domoic acid – Mill river, Prince Edward Island. *Harmful Algae News* 21: 6–7.

BATES S.S. & DAVIDOVICH N.A. 2002. Factors affecting the sexual reproduction of diatoms, with emphasis on *Pseudo-nitzschia* spp. In: *LIFEHAB workshop: life history of microalgal species causing harmful algal blooms* (Ed. by E. Garcés, A. Zingone, B. Dale, M. Montresor & B. Reguera), pp. 31–36. Commission of the European Community.

BATES S.S. & LÉGER C. 2006. Effect of bicarbonate addition on domoic acid production by *Pseudo-nitzschia multiseries* in batch culture. *12th International Conference on Harmful Algae* 132, (Abstract).

BATES S.S. & TRAINER V.L. 2006. The ecology of harmful diatoms. In: *Ecology of harmful algae* (Ed. by E. Granéli & J.T. Turner), pp. 81–93. Springer-Verlag, Berlin.

BATES S.S., BIRD C.J., DE FREITAS A.S.W., FOXALL R., GILGAN M., HANIC L.A., JOHNSON G.R., McCULLOCH A.W., ODENSE P., POCKLINGTON R., QUILLIAM M.A., SIM P.G., SMITH J.C., SUBA RAO D.V., TODD E.C.D., WALTER J.A. & WRIGHT J.L.C. 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* 46: 1203–1215.

BATES S.S., DE FREITAS A.S.W., MILLEY J.E., POCKLINGTON R., QUILLIAM M.A., SMITH J.C. & WORMS J. 1991. Controls on domoic acid production by the diatom *Nitzschia pungens* f. *multiseries* in culture: nutrients and irradiance. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1136–1144.

BATES S.S., LÉGER C., KEAFER B.A. & ANDERSON D.M. 1993a. Discrimination between domoic-acid-producing and nontoxic forms of the diatom *Pseudonitzschia pungens* using immunofluorescence. *Marine Ecology Progress Series* 100: 185–195.

BATES S.S., WORMS J. & SMITH J.C. 1993b. Effects of ammonium and nitrate on growth and domoic acid production by *Nitzschia pungens* in batch culture. *Canadian Journal of Fisheries and Aquatic Sciences* 50: 1248–1254.

BATES S.S., DOUGLAS D.J., DOUCETTE G.J. & LÉGER C. 1995a. Effects of reintroducing bacteria on domoic acid production by axenic cultures of the diatom *Pseudonitzschia pungens* f. *multiseries*. In: *Harmful marine algal blooms* (Ed. by P. Lassus, G. Arzul, E. Erard, P. Gentien & C. Marcaillou), pp. 401–406. Lavoisier Publishing, Paris.

BATES S.S., DOUGLAS D.J., DOUCETTE G.J. & LÉGER C. 1995b. Enhancement of domoic acid production by reintroducing bacteria to axenic cultures of the diatom *Pseudo-nitzschia multiseries*. *Natural Toxins* 3: 428–435.

BATES S.S., GARRISON D.L. & HORNER R.A. 1998. Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. In: *Physiological ecology of harmful algal*

blooms (Ed. by D.M. Anderson, A.D. Cembella & G.M. Hallegraeff), pp. 267–292. Springer-Verlag, Heidelberg.

BATES S.S., HILTZ M.F. & LÉGER C. 1999. Domoic acid toxicity of large new cells of *Pseudo-nitzschia multiseries* resulting from sexual reproduction. In: *Proceedings of the Sixth Canadian Workshop on Harmful Marine Algae* (Ed. by J.L. Martin & K. Haya), pp. 21–26. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2261.

BATES S.S., LÉGER C., SATCHWELL M.F. & BOYER G.L. 2001. The effects of iron on domoic acid production by *Pseudo-nitzschia multiseries*. In: *Harmful algal blooms 2000* (Ed. by G.M. Hallegraeff, S.I. Blackburn, C.J. Bolch & R.J. Lewis), pp. 320–323. Intergovernmental Oceanographic Commission of UNESCO, Paris.

BATES S.S., LÉGER C., WELLS M.L. & HARDY K. 2003. Photodegradation of domoic acid. In: *Proceedings of the Eighth Canadian Workshop on Harmful Marine Algae* (Ed. by S.S. Bates), pp. 30–35. *Canadian Technical Report of Fisheries and Aquatic Sciences* 2498.

BATES S.S., GAUDET J., KACZMARSKA I. & EHRMAN J.M. 2004. Interaction between bacteria and the domoic-acid-producing diatom *Pseudo-nitzschia multiseries* (Hasle) Hasle; can bacteria produce domoic acid autonomously? *Harmful Algae* 3: 11–20.

BEJARANO A.C., GULLAND F.M., GOLDSTEIN T., ST LEGER J., HUNTER M., SCHWACKE L.H., VAN DOLAH F.M. & ROWLES T.K. 2008a. Demographics and spatio-temporal signature of the biotoxin domoic acid in California sea lion (*Zalophus californianus*) stranding records. *Marine Mammal Science* 24: 899–912.

BEJARANO A.C., VAN DOLAH F.M., GULLAND F.M., ROWLES T.K. & SCHWACKE L.H. 2008b. Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: a review. *Human and Ecological Risk Assessment* 14: 544–567.

BESIKTEPE S., RYABUSHKO L., EDIGER D., YIMAZ D., ZENGINER A., RYABUSHKO V. & LEE R. 2008. Domoic acid production by *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle (Bacillariophyta) isolated from the Black Sea. *Harmful Algae* 7: 438–442.

BILL B.D., COX F.H., HORNER R.A., BORCHERT J.A. & TRAINER V.L. 2004. The first closure of shellfish harvesting due to domoic acid in Puget Sound, Washington, USA. *African Journal of Marine Science* 28: 435–440.

BILL B., LUNDHOLM N., CONNELL L., BAUGH K.A. & TRAINER V.L. 2005. Domoic acid in *Pseudo-nitzschia cuspidata* from Washington State coastal waters. In: *Proceedings of the 3rd Symposium on Harmful Algae in the U.S.,* Alismor, CA. 77 pp. (Abstract).

BLANCO J., LIVRAMENTO F. & RANGEL I.M. 2010. Amnesic shellfish poisoning (ASP) toxins in plankton and molluscs from Luanda Bay, Angola. *Toxicon* 55: 541–546.

BOGAN Y.M., KENNEDY D.J., HARKIN A.L., GILLESPIE J., VAUSE B.J., BEUKERS-STEWART B.D., HESS P. & SLATER J.W. 2007. Variation in domoic acid concentration in king scallop (*Pecten maximus*) from fishing grounds around the Isle of Man. *Harmful Algae* 6: 81–92.

BOISSONNEAULT K.R. 2004. Gene discovery and expression profiling in the toxin-producing marine diatom, *Pseudo-nitzschia multiseries* (Hasle) Hasle. Massachusetts Institute of Technology and the Wood Hole Oceanographic Institution Joint Program on Biological Oceanography, Cambridge, MA, USA. 181 pp.

BOUILLON R.C., KNIERIM T.L., KIEBER R.J., SKRABAL S.A. & WRIGHT J.L.C. 2006. Photodegradation of the algal toxin domoic acid in natural water matrices. *Limnology and Oceanography* 51: 321–330.

BOUILLON R.C., KIEBER R.J., SKRABAL S.A. & WRIGHT J.L.C. 2008. Photochemistry and identification of photodegradation products of the marine toxin domoic acid. *Marine Chemistry* 110: 18–27.

BOYCE D.G., LEWIS M.R. & WORM B. 2010. Global phytoplankton decline over the past century. *Nature (London)* 466: 591–596.

BRULAND K.W., RUE E.L. & SMITH G.J. 2001. Iron and macronutrients in California coastal upwelling regimes: implications for diatom blooms. *Limnology and Oceanography* 46: 1661–1674.

BUCK K.R., UTTALCOOKE L., PILSKALN C.H., ROELKE D.L., VILLAC M.C., FRYXELL G.A., CIFUENTES L. & CHAVEZ F.P. 1992. Autoecology of the diatom *Pseudonitzschia australis*, a domoic acid producer, from Monterey Bay, California. *Marine Ecology Progress Series* 84: 293–302.

BURKHOLDER J.M., HALLEGRAEFF G.M., MELIA G., COHEN A., BOWERS H.A., OLDACH D.W., PARROW M.W., SULLIVAN M.J., ZIMBA P.V., ALLEN E.H., KINDER C.A. & MALLIN M.A. 2007. Phytoplankton and bacterial assemblages in ballast water of U.S. military ships as a function of port of origin, voyage time, and ocean exchange practices. *Harmful Algae* 6: 486–518.

BUSSE L.B., VENRICK E.L., ANTROBUS R., MILLER P.E., VIGILANT V., SILVER M.W., MENGELT C., MYDLARZ L. & PRÉZELIN B.B. 2006. Domoic acid in phytoplankton and fish in San Diego, CA, USA. *Harmful Algae* 5: 91–101.

CALU G., MARTIN-JEZEQUEL V., LEFAU E., SECHET V., LASSUS P., WEIGEL P. & AMZIL Z. 2009. The influence of nitrogen speciation on growth and toxicity of *Pseudo-nitzschia multiseries* and *P. pungens* in batch and continuous cultures. In: *7th International Conference on Molluscan Shellfish Safety* (Ed. by P. Lassus), 7 pp. Éditions Quæ, Nantes, France.

CAMPBELL D.A., KELLY M.S., BUSMAN M., BOLCH C.J., WIGGINS E., MOELLER P.D.R., MORTON S.L., HESS P. & SHUMWAY S.E. 2001. Amnesic shellfish poisoning in the king scallop, *Pecten maximus*, from the west coast of Scotland. *Journal of Shellfish Research* 20: 75–84.

CARLSON M., JOHNSON B. & ROCAP G. 2009. Viral–phytoplankton interactions: characterization and ecology of viruses infecting the diatom *Pseudo-nitzschia*. In: *5th Symposium on Harmful Algae in the U.S.,* Ocean Shores, WA. 101 pp. (Abstract).

CARON D.A., GARNEAU M.E., SEUBERT E., HOWARD M.D.A., DARJANY L., SCHNETZER A., CETINIC I., FILTEAU G., LAURI P., JONES B. & TRUSSELL S. 2010. Harmful algae and their potential impacts on desalination operations off southern California. *Water Research* 44: 385–416.

CAROPPO C., CONGESTRI R., BRACCHINI L. & ALBERTANO P. 2005. On the presence of *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle and *Pseudo-nitzschia delicatissima* (Cleve) Heiden in the Southern Adriatic Sea (Mediterranean Sea, Italy). *Journal of Plankton Research* 27: 763–774.

CARTER C.M., ROSS A.H., SCHIEL D.R., HOWARD-WILLIAMS C. & HAYDEN B. 2005. In situ microcosm experiments on the influence of nitrate and light on phytoplankton community composition. *Journal of Experimental Marine Biology and Ecology* 326: 1–13.

CASTELEYN G., CHEPURNOV V.A., LELIAERT F., MANN D.G., BATES S.S., LUNDHOLM N., RHODES L., SABBE K. & VYVERMAN W. 2008. *Pseudo-nitzschia pungens* (Bacillariophyceae): a cosmopolitan diatom species? *Harmful Algae* 7: 241–257.

CASTELEYN G., ADAMS N.G., VANORMELINGEN P., DEBEER A.-E., SABBE K. & VYVERMAN W. 2009a. Natural hybrids in the marine diatom *Pseudo-nitzschia pungens* (Bacillariophyceae): genetic and morphological evidence. *Protist* 160: 343–354.

CASTELEYN G., EVANS K.M., BACKELJAU T., D'HONDT S., CHEPURNOV V., SABBE K. & VYVERMAN W. 2009b. Lack of population genetic structuring in the marine planktonic diatom *Pseudo-nitzschia pungens* (Bacillariophyceae) in a heterogeneous area in the Southern Bight of the North Sea. *Marine Biology* 156: 1149–1158.

CASTELEYN G., LELIAERT F., BACKELJAU T., DEBEER A.E., KOTAKI Y., RHODES L., LUNDHOLM N., SABBE K. & VYVERMAN W. 2010. Limits to gene flow in a cosmopolitan marine planktonic diatom. *Proceedings of the National Academy of Sciences of the United States of America* 107: 12952–12957.

CAVAS T. & KONEN S. 2008. In vivo genotoxity testing of the amnesic shellfish poison (domoic acid) in piscine erythrocytes using the micronucleus test and the comet assay. *Aquatic Toxicology* 90: 154–159.

CERINO F., ORSINI L., SARNO D., DELL'AVERSANO C., TARTAGLIONE L. & ZINGONE A. 2005. The alternation of different morphotypes in the seasonal cycle of the toxic diatom *Pseudo-nitzschia galaxiae*. *Harmful Algae* 4: 33–48.

CHEPURNOV V.A., MANN D.G., SABBE K. & VYVERMAN W. 2004. Experimental studies on sexual reproduction in diatoms. *International Review of Cytology* 237: 91–154.

CHEPURNOV V.A., MANN D.G., SABBE K., VANNERUM K., CASTELEYN G., VERLEYEN E., PEPERZAK L. & VYVERMAN W. 2005. Sexual reproduction, mating system, chloroplast dynamics

and abrupt cell size reduction in *Pseudo-nitzschia pungens* from the North Sea (Bacillariophyta). *European Journal of Phycology* 40: 379–395.

CHO E.S., KOTAKI Y. & PARK J.G. 2001. The comparison between toxic *Pseudo-nitzschia multiseries* (Hasle) Hasle and non-toxic *P. pungens* (Grunow) Hasle isolated from Jinhae Bay, Korea. *Algae* 16: 275–285.

CHOI K.D., LEE J.S., LEE J.O., OH K.S. & SHIN I.S. 2009. Investigation of domoic acid in shellfish collected from Korean fish retail outlets. *Food Science and Biotechnology* 18: 842–848.

CHURRO C.I., CARREIRA C.C., RODRIGUES F.J., CRAVEIRO S.C., CALADO A.J., CASTELEYN G. & LUNDHOLM N. 2009. Diversity and abundance of potentially toxic *Pseudo-nitzschia* Peragallo in Aveiro coastal lagoon, Portugal and description of a new variety, *P. pungens* var. *aveirensis* var. nov. *Diatom Research* 24: 35–62.

CLAQUIN P., LONGPHUIRT S.N., FOUILLARON P., HUONNIC P., RAGUENEAU O., KLEIN C. & LEYNAERT A. 2010. Effects of simulated benthic fluxes on phytoplankton dynamic and photosynthetic parameters in a mesocosm experiment (Bay of Brest, France). *Estuarine, Coastal and Shelf Science* 86: 93–101.

CLAYDEN J., KNOWLES F.E. & BALDWIN I.R. 2005. The synthesis of (−)-isodomoic acid C. *Journal of the American Chemical Society* 127: 2412–2413.

COCHLAN W.P., HERNDON J. & KUDELA R.M. 2008. Inorganic and organic nitrogen uptake by the toxigenic diatom *Pseudo-nitzschia australis* (Bacillariophyceae). *Harmful Algae* 8: 111–118.

CONGESTRI R., POLIZZANO S. & ALBERTANO P. 2008. Toxic *Pseudo-nitzschia* populations from the Middle Tyrrhenian Sea (Mediterranean Sea, Italy). In: *Algal toxins: nature, occurrence, effect and detection* (Ed. by V. Evangelista, L. Barsanti, A.M. Frassanito, V. Passarelli & P. Gualtieri), pp. 197–210. NATO Science for Peace and Security. Series A: Chemistry and Biology, Springer, the Netherlands.

CORCHADO J.M., TORRES J.M. & SACAI CAUDRADO M.M. 2004. Forecasting red tides caused by *Pseudo-nitzschia* spp. using an artificial intelligence model. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 531–533. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

COSTA L.G., GIORDANO G. & FAUSTMAN E.M. 2010. Domoic acid as a developmental neurotoxin. *Neurotoxicology* 31: 409–423.

COSTA P.R. & GARRIDO S. 2004. Domoic acid accumulation in the sardine *Sardina pilchardus* and its relationship to *Pseudo-nitzschia* diatom ingestion. *Marine Ecology Progress Series* 284: 261–268.

COSTA P.R., RODRIGUES S.M., BOTELHO M.J. & SAMPAYO M.A.M. 2003. A potential vector of domoic acid: the swimming crab *Polybius henslowii* Leach (Decapoda-brachyura). *Toxicon* 42: 135–141.

COSTA P.R., ROSA R. & SAMPAYO M.A.M. 2004. Tissue distribution of the amnesic shellfish toxin, domoic acid, in *Octopus vulgaris* from the Portuguese coast. *Marine Biology* 144: 971–976.

COSTA P.R., ROSA R., PEREIRA J. & SAMPAYO M.A.M. 2005a. Detection of domoic acid, the amnesic shellfish toxin, in the digestive gland of *Eledone cirrhosa* and *E. moschata* (Cephalopoda, Octopoda) from the Portuguese coast. *Aquatic Living Resources* 18: 395–400.

COSTA P.R., ROSA R., DUARTE-SILVA A., BROTAS V. & SAMPAYO M.A.M. 2005b. Accumulation, transformation and tissue distribution of domoic acid, the amnesic shellfish poisoning toxin, in the common cuttlefish, *Sepia officinalis*. *Aquatic Toxicology* 74: 82–91.

CUSACK C.K., BATES S.S., QUILLIAM M.A., PATCHING J.W. & RAINE R. 2002. Confirmation of domoic acid production by *Pseudo-nitzschia australis* (Bacillariophyceae) isolated from Irish waters. *Journal of Phycology* 38: 1106–1112.

D'ALELIO D., AMATO A., LUEDEKING A. & MONTRESOR M. 2009. Sexual and vegetative phases in the planktonic diatom *Pseudo-nitzschia multistriata*. *Harmful Algae* 8: 225–232.

D'ALELIO D., D'ALCALÁ M.R., DUBROCA L., SARNO D., ZINGONE A. & MONTRESOR M. 2010. The time for sex: a biennial life cycle in a marine planktonic diatom. *Limnology and Oceanography* 55: 106–114.

DAO H.V., TAKATA Y., OMURA T., SATO S., FUKUYO Y. & KODAMA M. 2009. Seasonal variation of domoic acid in a bivalve *Spondylus versicolor* in association with that in plankton samples in Nha Phu Bay, Khanh Hoa, Vietnam. *Fisheries Science* 75: 507–512.

DAVIDOVICH N.A. & BATES S.S. 1998. Sexual reproduction in the pennate diatoms *Pseudo-nitzschia multiseries* and *P. pseudodelicatissima* (Bacillariophyceae). *Journal of Phycology* 34: 126–137.

DAVIDSON K. & FEHLING J. 2006. Modelling the influence of silicon and phosphorus limitation on the growth and toxicity of *Pseudo-nitzschia seriata*. *African Journal of Marine Science* 28: 357–360.

DE LA IGLESIA P., GIMÉNEZ G. & DIOGÈNE J. 2008. Determination of dissolved domoic acid in seawater with reversed-phase extraction disks and rapid resolution liquid chromatography tandem mass spectrometry with head-column trapping. *Journal of Chromatography A* 1215: 116–124.

DE LA RIVA G.T., JOHNSON C.K., GULLAND F.M.D., LANGLOIS G.W., HEYNING J.E., ROWLES T.K. & MAZET J.A.K. 2009. Association of an unusual marine mammal mortality event with *Pseudo-nitzschia* spp. blooms along the southern California coastline. *Journal of Wildlife Diseases* 45: 109–121.

DEL RIO R., BARGU S., BALTZ D., FIRE S., PETERSON G. & WANG Z.H. 2010. Gulf menhaden (*Brevoortia patronus*): a potential vector of domoic acid in coastal Louisiana food webs. *Harmful Algae* 10: 19–29.

DENMARK S.E., HUNG-CHANG LIU J. & MUHUHI J.M. 2011. Stereocontrolled total syntheses of isodomoic acids G and H via a unified strategy. *Journal of Organic Chemistry* 76: 201–215.

DJAOUED Y., THIBODEAU M., ROBICHAUD J., BALAJI S., PRIYA S., TCHOUKANOVA N. & BATES S.S. 2008. Photocatalytic degradation of domoic acid using nanocrystalline TiO_2 thin films. *Journal of Photochemistry and Photobiology A – Chemistry* 193: 271–283.

DJAOUED Y., ROBICHAUD J., THIBODEAU M., BALAJI S., TCHOUKANOVA N. & BATES S.S. 2009. Photocatalytic properties of nanocrystalline titanium dioxide films in the degradation of domoic acid in aqueous solution: potential for use in molluscan shellfish biotoxin depuration facilities. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment* 26: 248–257.

DOAN-NHU H., NGUYEN THI M.A. & NGUYEN-NGOC T.G. 2008. *Pseudo-nitzschia* in south-central coastal waters of Vietnam: growth and occurrence related to temperature and salinity. In: *Proceedings of the 12th International Conference on Harmful Algae* (Ed. by Ø. Moestrup), pp. 29–32. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, Copenhagen.

DORTCH Q., ROBICHAUX R., POOL S., MILSTED D., MIRE G., RABALAIS N.N., SONIAT T.M., FRYXELL G.A., TURNER R.E. & PARSONS M.L. 1997. Abundance and vertical flux of *Pseudo-nitzschia* in the northern Gulf of Mexico. *Marine Ecology Progress Series* 146: 249–264.

DOUCETTE G.J., KING K.L., THESSEN A.E. & DORTCH Q. 2008. The effect of salinity on domoic acid production by the diatom *Pseudo-nitzschia multiseries*. *Nova Hedwig, Beiheft* 133: 31–46.

DOUGLAS D.J., RAMSEY U.P., WALTER J.A. & WRIGHT J.L.C. 1992. Biosynthesis of the neurotoxin domoic acid by the marine diatom *Nitzschia pungens* forma *multiseries*, determined with [^{13}C]-labeled precursors and nuclear magnetic resonance. *Journal of the Chemical Society. Chemical Communications* 1992: 714–716.

DOUGLAS D.J., BATES S.S., BOURQUE L.A. & SELVIN R.C. 1993. Domoic acid production by axenic and non-axenic cultures of the pennate diatom *Nitzschia pungens* f. *multiseries*. In: *Toxic phytoplankton blooms in the sea* (Ed. by T.J. Smayda & Y. Shimizu), pp. 595–600. Elsevier Science Publishers B.V., Amsterdam.

DYBAS C.L. 2004. Harmful algal blooms: biosensors provide new ways of detecting and monitoring growing threat in coast waters. *BioScience* 53: 918–923.

EL-SABAAWI R. & HARRISON P.J. 2006. Interactive effects of irradiance and temperature on the photosynthetic physiology of

the pennate diatom *Pseudo-nitzschia granii* (Bacillariophyceae) from the northeast subarctic Pacific. *Journal of Phycology* 42: 778–785.

ELDRIDGE M.L., TRICK C.G., ALM M.B., DITULLIO G.R., RUE E.L., BRULAND K.W., HUTCHINS D.A. & WILHELM S.W. 2004. Phytoplankton community response to a manipulation of bioavailable iron in HNLC waters of the subtropical Pacific Ocean. *Aquatic Microbial Ecology* 35: 79–91.

EVANS K.M. & HAYES P.K. 2004. Microsatellite markers for the cosmopolitan marine diatom *Pseudo-nitzschia pungens. Molecular Ecology Notes* 4: 125–126.

EVANS K.M., BATES S.S., MEDLIN L.K. & HAYES P.K. 2004. Microsatellite marker development and genetic variation in the toxic marine diatom *Pseudo-nitzschia multiseries* (Bacillariophyceae). *Journal of Phycology* 40: 911–920.

FALK M., WALTER J.A. & WISEMAN P.W. 1989. Ultraviolet spectrum of domoic acid. *Canadian Journal of Chemistry* 67: 1421–1425.

FALK M., SETO P.F. & WALTER J.A. 1991. Solubility of domoic acid in water and in nonaqueous solvents. *Canadian Journal of Chemistry* 69: 1740–1744.

FEHLING J., DAVIDSON K., BOLCH C.J. & BATES S.S. 2004. Growth and domoic acid production by *Pseudo-nitzschia seriata* (Bacillariophyceae) under phosphate and silicate limitation. *Journal of Phycology* 40: 674–683.

FEHLING J., DAVIDSON K. & BATES S.S. 2005. Growth dynamics of non-toxic *Pseudo-nitzschia delicatissima* and toxic *P. seriata* (Bacillariophyceae) under simulated spring and summer photoperiods. *Harmful Algae* 4: 763–769.

FEHLING J., DAVIDSON K., BOLCH C. & TETT P. 2006. Seasonality of *Pseudo-nitzschia* spp. (Bacillariophyceae) in western Scottish waters. *Marine Ecology Progress Series* 323: 91–105.

FERNANDES L.F. & BRANDINI F.P. 2010. The potentially toxic diatom *Pseudo-nitzschia* H. Peragallo in the Paraná and Santa Catarina States, Southern Brazil. *Iheringia. Série Botânica* 65: 47–62.

FERDIN M.E., KVITEK R.G., BRETZ C.K., POWELL C.L., DOUCETTE G.J., LEFEBVRE K.A., COALE S. & SILVER M.W. 2002. *Emerita analoga* (Stimpson) – possible new indicator species for the phycotoxin domoic acid in California coastal waters. *Toxicon* 40: 1259–1265.

FERRARIO M., LICEA S., BALESTRINI C.F. & FERREYRA G. 2004. Species of *Pseudo-nitzschia* in the Drake Passage (54–61°S to 46–64°W), pp. 434–436. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

FIRE S.E. & SILVER M.W. 2005. Domoic acid in the Santa Cruz wharf fishery. *California Fish and Game* 91: 179–192.

FIRE S.E., WANG Z., LEIGHFIELD T.A., MORTON S.L., MCFEE W.E., MCLELLAN W.A., LITAKER R.W., TESTER P.A., HOHN A.A., LOVEWELL G., HARMS C., ROTSTEIN D.S., BARCO S.G., COSTIDIS A., SHEPPARD B., BOSSART G.D., STOLEN M., DURDEN W.N. & VAN DOLAH F.M. 2009. Domoic acid exposure in pygmy and dwarf sperm whales (*Kogia* spp.) from southeastern and mid-Atlantic U.S. waters. *Harmful Algae* 8: 658–664.

FIRE S.E., WANG Z.H., BERMAN M., LANGLOIS G.W., MORTON S.L., SEKULA-WOOD E. & BENITEZ-NELSON C.R. 2010. Trophic transfer of the harmful algal toxin domoic acid as a cause of death in a minke whale (*Balaenoptera acutorostrata*) stranding in southern California. *Aquatic Mammals* 36: 342–350.

FISHER J.M., REESE J.G., PELLECHIA P.J., MOELLER P.L. & FERRY J.L. 2006. Role of Fe(III), phosphate, dissolved organic matter, and nitrate during the photodegradation of domoic acid in the marine environment. *Environmental Science & Technology* 40: 2200–2205.

FORBES J.R. & DENMAN K.L. 1991. Distribution of *Nitzschia pungens* in coastal waters of British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 960–967.

FRAGA S., ALVEREZ M.J., MÍGUEZ Á., FERNÁNDEZ M.L., COSTAS E. & LÓPEZ-RODAS V. 1998. *Pseudo-nitzschia* species isolated from Galician waters: toxicity, DNA content and lectin binding assay. In: *Harmful algae* (Ed. by B. Reguera, J. Blanco, M.L. Fernández

& T. Wyatt), pp. 270–273. Xunta de Galicia and the Intergovernmental Oceanographic Commission of UNESCO, Paris.

FRITZ L., QUILLIAM M.A., WRIGHT J.L.C., BEALE A.M. & WORK T.M. 1992. An outbreak of domoic acid poisoning attributed to the pennate diatom *Pseudo nitzschia australis. Journal of Phycology* 28: 439–442.

FRYXELL G.A., REAP M.E. & VALENCIC D.L. 1990. *Nitzschia pungens* Grunow f. *multiseries* Hasle: observations of a known neurotoxic diatom. *Nova Hedwigia, Beiheft* 100: 171–188.

FRYXELL G.A., GARZA S.A. & ROELKE D.L. 1991. Auxospore formation in an Antarctic clone of *Nitzschia subcurvata* Hasle. *Diatom Research* 6: 235–245.

FRYXELL G.A., VILLAC M.C. & SHAPIRO L.P. 1997. The occurrence of the toxic diatom genus *Pseudo-nitzschia* (Bacillariophyceae) on the West Coast of the USA, 1920–1996: a review. *Phycologia* 36: 419–437.

GAILHARD I., GROS P., DURBEC J.P., BELIAEFF B., BELIN C., NÉZAN E. & LASSUS P. 2002. Variability patterns of microphytoplankton communities along the French coasts. *Marine Ecology Progress Series* 242: 39–50.

GARCÍA-MENDOZA E., RIVAS D., OLIVOS-ORTIZ A., ALMAZÁN-BECERRIL A., CASTAÑEDA-VEGA C. & PEÑA-MANJARREZ J.L. 2009. A toxic *Pseudo-nitzschia* bloom in Todos Santos Bay, northwestern Baja California, Mexico. *Harmful Algae* 8: 493–503.

GARRISON D.L., CONRAD S.M., EILERS P.P. & WALDRON E.M. 1992. Confirmation of domoic acid production by *Pseudo-nitzschia australis* (Bacillariophyceae) cultures. *Journal of Phycology* 28: 604–607.

GLIBERT P.M., SEITZINGER S., HEIL C.A., BURKOLDER J.M., PARROW M.W., CODISPOTI L.A. & KELLY V. 2006. Escalating worldwide use of urea – a global change contributing to coastal eutrophication. *Oceanography* 18: 198–208.

GOLDSTEIN T., MAZET J.A.K., ZABKA T.S., LANGLOIS G., COLEGROVE K.M., SILVER M., BARGU S., VAN DOLAH F., LEIGHFIELD T., CONRAD P.A., BARAKOS J., WILLIAMS D.C., DENNISON S., HAULENA M. & GULLAND F.M.D. 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. *Proceedings of the Royal Society B – Biological Sciences* 275: 267–276.

GORGA J.J., MENGELT C. & PRÉZELIN B.B. 2002. UV-B inhibition of *Pseudo-nitzschia australis*: primary production is minimized by UV-A enhancement effects. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 385–387. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

GRANÉLI E. & HANSEN P.J. 2006. Allelopathy in harmful algae: a mechanism to compete for resources? In: *Ecology of harmful algae* (Ed. by E. Granéli & J.T. Turner), pp. 189–201. Springer-Verlag, Berlin.

GRANT K.S., BURBACHER T.M., FAUSTMAN E.M. & GRATTAN L. 2010. Domoic acid: neurobehavioral consequences of exposure to a prevalent marine biotoxin. *Neurotoxicology and Teratology* 32: 132–141.

GRATTAN L., ROBERTS S., TRAINER V., BOUSHEY C., BURBACHER T., GRANT K., TRACY K. & MORRIS G. 2009. Domoic acid neurotoxicity in American Indians: baseline characteristics of the coastal cohort. *Neurotoxicology and Teratology* 31: NBTS12.

GUANNEL M.L., HORNER-DEVINE M.C. & ROCAP G. 2011. Bacterial community composition differs with species and toxigenicity of the diatom *Pseudo-nitzschia. Aquatic Microbial Ecology* 64: 117–133.

HA D.V., TAKATA Y., SATO S., FUKUYO Y. & KODAMA M. 2006. Domoic acid in a bivalve *Spondylus cruentus* in Nha Trang Bay, Khanh Hoa Province, Vietnam. *Coastal Marine Science* 30: 130–132.

HACHÉ S. 2000. Détermination d'un signal (phéromone) produit par la diatomée toxique *Pseudo-nitzschia multiseries* ayant un rôle dans la communication sexuelle. 29 pp. Université de Moncton, Moncton, New Brunswick, Canada.

HAGSTRÖM J.A., GRANÉLI E., MANEIRO I., BARREIRO A., PETERMANN A. & SVENSEN C. 2007. Release and degradation

of amnesic shellfish poison from decaying *Pseudo-nitzschia multiseries* in presence of bacteria and organic matter. *Harmful Algae* 6: 175–188.

HAGSTRÖM J.A., GRANÉLI E., MOREIRA M.O.P. & ODEBRECHT C. 2010. Domoic acid production and elemental composition of two *Pseudo-nitzschia multiseries* strains, from the NW and SW Atlantic Ocean, growing in phosphorus- or nitrogen-limited chemostat cultures. *Journal of Plankton Research* 33: 297–308.

HALL A.J. & FRAME E. 2010. Evidence of domoic acid exposure in harbour seals from Scotland: a potential factor in the decline in abundance? *Harmful Algae* 9: 489–493.

HALLEGRAEFF G.M. 1994. Species of the diatom genus *Pseudo-nitzschia* in Australian waters. *Botanica Marina* 37: 397–411.

HAMME R.C., WEBLEY P.W., CRAWFORD W.R., WHITNEY F.A., DEGRANDPRE M.D., EMERSON S.R., ERIKSEN C.C., GIESBRECHT K.E., GOWER J.F.R., KAVANAUGH M.T., PENA M.A., SABINE C.L., BATTEN S.D., COOGAN L.A., GRUNDLE D.S. & LOCKWOOD D. 2010. Volcanic ash fuels anomalous plankton bloom in subarctic northeast Pacific. *Geophysical Research Letters* 37: L19604.

HANIC L.A., SEKIMOTO S. & BATES S.S. 2009. Oomycete and chytrid infections of the marine diatom *Pseudo-nitzschia pungens* (Bacillariophyceae) from Prince Edward Island, Canada. *Botany* 87: 1096–1105.

HANSEN L.R., SOYLU S., KOTAKI Y., MOESTRUP Ø. & LUNDHOLM N. 2011. Toxin production and temperature-induced morphological variation of the diatom *Pseudo-nitzschia seriata* from the Arctic. *Harmful Algae* 10: 689–696.

HASLE G.R. 1965. *Nitzschia* and *Fragilariopsis* species studied in the light and electron microscopes. II. The group *Pseudo-nitzschia*. *Skrifter utgitt av Det Norske Vidensk-Akademi I. Mat. -Nat. Klasse, Ny Series* 18: 1–45.

HASLE G.R. 1994. *Pseudo-nitzschia* as a genus distinct from *Nitzschia* (Bacillariophyceae). *Journal of Phycology* 30: 1036–1039.

HASLE G.R. 1995. *Pseudo-nitzschia pungens* and *P. multiseries* (Bacillariophyceae): nomenclatural history, morphology, and distribution. *Journal of Phycology* 31: 428–435.

HASLE G.R. 2002. Are most of the domoic acid-producing species of the diatom genus *Pseudo-nitzschia* cosmopolites? *Harmful Algae* 1: 137–146.

HASLE G.R. & LUNDHOLM N. 2005. *Pseudo-nitzschia seriata* f. *obtusa* (Bacillariophyceae) raised in rank based on morphological, phylogenetic and distributional data. *Phycologia* 44: 608–619.

HASLE G.R. & SYVERTSEN E.E. 1997. Marine diatoms. In: *Identifying marine phytoplankton* (Ed. by C.R. Tomas), pp. 5–385. Academic Press, San Diego.

HASLE G.R., LANGE C.B. & SYVERTSEN E.E. 1996. A review of *Pseudo-nitzschia*, with special reference to the Skagerrak, North Atlantic, and adjacent waters. *Helgolander Meeresuntersuchungen* 50: 131–175.

HATFIELD C.L., GAUGLITZ E.J., BARNETT H.J., LUND J.A.K., WEKELL J.C. & EKLUND M. 1995. The fate of domoic acid in Dungeness crab (*Cancer magister*) as a function of processing. *Journal of Shellfish Research* 14: 359–363.

HÉGARET H., SHUMWAY S.E., WIKFORS G.H., PATE S. & BURKHOLDER J.M. 2008. Potential transport of harmful algae via relocation of bivalve molluscs. *Marine Ecology Progress Series* 361: 169–179.

HEISLER J., GLIBERT P.M., BURKHOLDER J.M., ANDERSON D.M., COCHLAN W., DENNISON W.C., DORTCH Q., GOBLER C.J., HEIL C.A., HUMPHRIES E., LEWITUS A., MAGNIEN R., MARSHALL H.G., SELLNER K., STOCKWELL D.A., STOECKER D.K. & SUDDLESON M. 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae* 8: 3–13.

HERNÁNDEZ-BECERRIL D.U. 1998. Species of the planktonic diatom genus *Pseudo-nitzschia* of the Pacific coasts of Mexico. *Hydrobiologia* 379: 77–84.

HERNÁNDEZ-BECERRIL D.U. & DÍAZ-ALMEYDA E.M. 2006. The *Nitzschia bicapitata* group, new records of the genus *Nitzschia*, and further studies on species of *Pseudo-nitzschia* (Bacillariophyta) from Mexican Pacific coasts. *Nova Hedwigia, Beiheft* 130: 293–306.

HILLEBRAND H. & SOMMER U. 1996. Nitrogenous nutrition of the potentially toxic diatom *Pseudonitzschia pungens* f. *multiseries* Hasle. *Journal of Plankton Research* 18: 295–301.

HILTZ M.F., BATES S.S. & KACZMARSKA I. 2000. Effect of light:dark cycles and cell apical length on the sexual reproduction of *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture. *Phycologia* 39: 59–66.

HOLLAND P.T., McNABB P., RHODES L.L., SELWOOD A.I. & NEIL T. 2003. Amnesic shellfish poisoning toxins in New Zealand shellfish – detection of a novel domoic acid isomer. In: *Molluscan shellfish safety* (Ed. by A. Villalba, B. Reguera, J.L. Romalde & R. Beiras), pp. 29–42. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO, Paris.

HOLLAND P.T., SELWOOD A.I., MOUNTFORT D.O., WILKINS A.L., McNABB P., RHODES L.L., DOUCETTE G.J., MIKULSKI C.M. & KING K.L. 2005. Isodomoic acid C, an unusual amnesic shellfish poisoning toxin from *Pseudo-nitzschia australis*. *Chemical Research in Toxicology* 18: 814–816.

HOLTERMANN K.E., BATES S.S., TRAINER V.L., ODELL A. & ARMBRUST E.V. 2010. Mass sexual reproduction in the toxigenic diatoms *Pseudo-nitzschia australis* and *P. pungens* (Bacillariophyceae) on the Washington coast, USA. *Journal of Phycology* 46: 41–52.

HORNER R.A. & POSTEL J.R. 1993. Toxic diatoms in western Washington waters (US west coast). *Hydrobiologia* 269: 197–205.

HORNER R.A., GARRISON D.L. & PLUMLEY F.G. 1997. Harmful algal blooms and red tide problems on the U.S. west coast. *Limnology and Oceanography* 42: 1076–1088.

HORNER R.A., HICKEY B.M. & POSTEL J.R. 2000. *Pseudo-nitzschia* blooms and physical oceanography off Washington state, USA. *South African Journal of Marine Science* 22: 299–308.

HOWARD M.D.A., COCHLAN W.P., LADIZINSKY N. & KUDELA R.M. 2007. Nitrogenous preference of toxigenic *Pseudo-nitzschia australis* (Bacillariophyceae) from field and laboratory experiments. *Harmful Algae* 6: 206–217.

HUBBARD K.A., ROCAP G. & ARMBRUST E.V. 2008. Inter- and intraspecific community structure within the diatom genus *Pseudo-nitzschia* (Bacillariophyceae). *Journal of Phycology* 44: 637–649.

HUSTEDT F. 1958. Phylogenetische Untersuchungen an Diatomeen. *Österreichische Botanische Zeitschrift* 105: 193–211.

HUTCHINS D.A. & BRULAND K.W. 1998. Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. *Nature (London)* 393: 561–564.

HUTCHINS D.A., DITULLIO G.R., ZHANG Y. & BRULAND K.W. 1998. An iron limitation mosaic in the California upwelling regime. *Limnology and Oceanography* 43: 1037–1054.

IGNATIADES L. & GOTSIS-SKRETAS O. 2010. A review on toxic and harmful algae in Greek coastal waters (E. Mediterranean Sea). *Toxins* 2: 1019–1037.

ILYASH L.V., BELEVICH T.A., ULANOVA A.Y. & MATORIN D.N. 2007. Fluorescence parameters of marine plankton algae at the assimilation of organic nitrogen. *Moscow University Biological Sciences Bulletin* 62: 111–116.

JACKSON A.E., AYER S.W. & LAYCOCK M.V. 1992. The effect of salinity on growth and amino acid composition in the marine diatom *Nitzschia pungens*. *Canadian Journal of Botany* 70: 2198–2201.

JAMES K.J., GILLMAN M., AMANDI M.F., LÓPEZ-RIVERA A., PUENTE P.F., LEHANE M., MITROVIC S. & FUREY A. 2005. Amnesic shellfish poisoning toxins in bivalve molluscs in Ireland. *Toxicon* 46: 852–858.

JEFFERY B., BARLOW T., MOIZER K., PAUL S. & BOYLE C. 2004. Amnesic shellfish poison. *Food and Chemical Toxicology* 42: 545–557.

JESTER R., LEFEBVRE K., LANGLOIS G., VIGILANT V., BAUGH K. & SILVER M.W. 2009. A shift in the dominant toxin-producing algal species in central California alters phycotoxins in food webs. *Harmful Algae* 8: 291–298.

JOHNSTON M., GALLACHER S., SMITH E.A. & GLOVER L.A. 2001. Detection of *N*-acyl homoserine lactones in marine bacteria associated with production and biotransformation of sodium channel blocking toxins and the microflora of toxin-producing phytoplankton. In: *Harmful algal blooms 2000* (Ed. by G.M.

Hallegraeff, S.I. Blackburn, C.J. Bolch & R.J. Lewis), pp. 375–378. Intergovernmental Oceanographic Commission of UNESCO, Paris.

Jones T.O., Whyte J.N.C., Ginther N.G., Townsend L.D. & Iwama G.K. 1995a. Haemocyte changes in the Pacific oyster, *Crassostrea gigas*, caused by exposure to domoic acid in the diatom *Pseudonitzschia pungens* f. *multiseries*. *Toxicon* 33: 347–353.

Jones T.O., Whyte J.N.C., Townsend L.D., Ginther N.G. & Iwama G.K. 1995b. Effects of domoic acid on hemolymph pH, pCO_2 and pO_2 in the Pacific oyster, *Crassostrea gigas* and the California mussel, *Mytilus californianus*. *Aquatic Toxicology* 31: 43–55.

Jonsson P.R., Pavia H. & Toth G. 2009. Formation of harmful algal blooms cannot be explained by allelopathic interactions. *Proceedings of the National Academy of Sciences of the United States of America* 106: 11177–11182.

Kaczmarska I., Bates S.S., Ehrman J.M. & Léger C. 2000. Fine structure of the gamete, auxospore and initial cell in the pennate diatom *Pseudo-nitzschia multiseries* (Bacillariophyta). *Nova Hedwigia* 71: 337–357.

Kaczmarska I., Ehrman J.M., Bates S.S., Green D.H., Léger C. & Harris J. 2005a. Diversity and distribution of epibiotic bacteria on *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture, and comparison with those on diatoms in native seawater. *Harmful Algae* 4: 725–741.

Kaczmarska I., LeGresley M.M., Martin J.L. & Ehrman J. 2005b. Diversity of the diatom genus *Pseudo-nitzschia* Peragallo in the Quoddy Region of the Bay of Fundy, Canada. *Harmful Algae* 4: 1–19.

Kaczmarska I., Martin J.L., Ehrman J.M. & LeGresley M.M. 2007. *Pseudo-nitzschia* species population dynamics in the Quoddy Region, Bay of Fundy. *Harmful Algae* 6: 861–874.

Kaczmarska I., Reid C., Martin J.L. & Moniz M.B.J. 2008. Morphological, biological and molecular characteristics of *Pseudo-nitzschia delicatissima* from the Canadian Maritimes. *Botany* 86: 763–772.

Kaniou-Grigoriadou I., Mouratidou T. & Katikou P. 2005. Investigation on the presence of domoic acid in Greek shellfish. *Harmful Algae* 4: 717–723.

Klein C., Claquin P., Bouchart V., Le Roy B. & Véron B. 2010. Dynamics of *Pseudo-nitzschia* spp. and domoic acid production in a macrotidal ecosystem of the Eastern English Channel (Normandy, France). *Harmful Algae* 9: 218–226.

Kobayashi K., Kobiyama A., Kotaki Y. & Kodama M. 2003. Possible occurrence of intracellular bacteria in *Pseudo-nitzschia multiseries*, a causative diatom of amnesic shellfish poisoning. *Fisheries Science* 69: 974–978.

Kobayashi K., Takata Y. & Kodama M. 2009. Direct contact between *Pseudo-nitzschia multiseries* and bacteria is necessary for the diatom to produce a high level of domoic acid. *Fisheries Science* 75: 771–776.

Kodama M., Doucette G.J. & Green D.H. 2006. Relationships between bacteria and harmful algae. In: *Ecology of harmful algae* (Ed. by E. Granéli & J.T. Turner), pp. 243–255. Springer-Verlag, Berlin.

Kotaki Y., Koike K., Sato S., Ogata T., Fukuyo Y. & Kodama M. 1999. Confirmation of domoic acid production of *Pseudo-nitzschia multiseries* isolated from Ofunato Bay, Japan. *Toxicon* 37: 677–682.

Kotaki Y., Koike K., Yoshida M., Thuoc C.V., Huyen N.T.M., Hoi N.C., Fukuyo Y. & Kodama M. 2000. Domoic acid production in *Nitzschia* sp. (Bacillariophyceae) isolated from a shrimp-culture pond in Do Son, Vietnam. *Journal of Phycology* 36: 1057–1060.

Kotaki Y., Furio E.F., Satake M., Lundholm N., Katayama T., Koike K., Fulgueras V.P., Bajarias F.A., Takata Y., Kobayashi K., Sato S., Fukuyo Y. & Kodama M. 2005. Production of isodomoic acids A and B as major toxin components of a pennate diatom *Nitzschia navis-varingica*. *Toxicon* 46: 946–953.

Kotaki Y., Furio E.F., Bajarias F.A., Satake M., Lundholm N., Koike K., Sato S., Fukuyo Y. & Kodama M. 2006. New stage of the study on domoic acid-producing diatoms – a finding of *Nitzschia navis-varingica* that produces domoic acid derivatives as major toxin components. *Coastal Marine Science* 30: 116–120.

Kotaki Y., Lundholm N., Katayama T., Furio E.F., Romero M.L., Relox J.R., Yasumoto T., Naoki H., Hirose M.Y., Thanh T.D., Thuoc C.V., Huyen N.T.M., Thu P.T., Takata Y., Kodama M. & Fukuyo Y. 2008. ASP toxins of pennate diatoms and bacterial effects on the variation in toxin composition. In: *Proceedings of the 12th International Conference on Harmful Algae* (Ed. by Ø. Moestrup), pp. 300–302. International Society for the Study of Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, Copenhagen.

Krayevsky D.M., Meave del Castillo E., Zamudio E., Norris J.N. & Frederique S. 2009. Diatoms (Bacillariophyta) of the Gulf of Mexico. In: *Gulf of Mexico origin, waters, and biota* (Ed. by D.L. Felder & D.K. Camp), Vol. 1, Biodiversity, pp. 155–186. Texas A&M University Press, College Station.

Kudela R., Cochlan W. & Roberts A. 2004a. Spatial and temporal patterns of *Pseudo-nitzschia* species in central California related to regional oceanography. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 347–349. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

Kudela R., Roberts A. & Armstrong M. 2004b. Laboratory analyses of nutrient stress and toxin accumulation in *Pseudo-nitzschia* species from Monterey Bay, California. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 136–138. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

Kudela R., Pitcher G., Probyn T., Figueiras F., Moita T. & Trainer V. 2005. Harmful algal blooms in coastal upwelling systems. *Oceanography* 18: 184–197.

Kudela R.M., Howard M.D.A., Jenkins B.D., Miller P.E. & Smith G.J. 2010. Using the molecular toolbox to compare harmful algal blooms in upwelling systems. *Progress in Oceanography* 85: 108–121.

Kvitek R.G., Goldberg J.D., Smith G.J., Doucette G.J. & Silver M.W. 2008. Domoic acid contamination within eight representative species from the benthic food web of Monterey Bay, California, USA. *Marine Ecology Progress Series* 367: 35–47.

Ladizinsky N. 2003. *The influence of dissolved copper on the production of domoic acid by* Pseudo-nitzschia *species in Monterey Bay, California: laboratory experiments and field observations*. MS thesis. California State University, Monterey Bay. 68 pp.

Lail E.M., Skrabal S.A., Kieber R.J., Bouillon R.C. & Wright J.L.C. 2007. The role of particles on the biogeochemical cycling of domoic acid and its isomers in natural water matrices. *Harmful Algae* 6: 651–657.

Lane J.Q., Raimondi P.T. & Kudela R.M. 2009. Development of a logistic regression model for the prediction of toxigenic *Pseudo-nitzschia* blooms in Monterey Bay, California. *Marine Ecology Progress Series* 383: 37–51.

Leandro L.F., Rolland R.M., Roth P.B., Lundholm N., Wang Z.H. & Doucette G.J. 2010a. Exposure of the North Atlantic right whale *Eubalaena glacialis* to the marine algal biotoxin, domoic acid. *Marine Ecology Progress Series* 398: 287–303.

Leandro L.F., Teegarden G.J., Roth P.B., Wang Z.H. & Doucette G.J. 2010b. The copepod *Calanus finmarchicus*: a potential vector for trophic transfer of the marine algal biotoxin, domoic acid. *Journal of Experimental Marine Biology and Ecology* 382: 88–95.

Lefebvre K.A. & Robertson A. 2010. Domoic acid and human exposure risks: a review. *Toxicon* 56: 218–230.

Lefebvre K.A., Powell C.L., Busman M., Doucette G.J., Moeller P.D.R., Sliver J.B., Miller P.E., Hughes M.P., Singaram S., Silver M.W. & Tjeerdema R.S. 1999. Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. *Natural Toxins* 7: 85–92.

LEFEBVRE K.A., DOVEL S.L. & SILVER M.W. 2001. Tissue distribution and neurotoxic effects of domoic acid in a prominent vector species, the northern anchovy *Engraulis mordax*. *Marine Biology* 138: 693–700.

LEFEBVRE K.A., BARGU S., KIECKHEFER T. & SILVER M.W. 2002a. From sanddabs to blue whales: the pervasiveness of domoic acid. *Toxicon* 40: 971–977.

LEFEBVRE K.A., SILVER M.W., COALE S.L. & TJEERDEMA R.S. 2002b. Domoic acid in planktivorous fish in relation to toxic *Pseudo-nitzschia* cell densities. *Marine Biology* 140: 625–631.

LEFEBVRE K.A., ROBERTSON A., FRAME E.R., COLEGROVE K.M., NANCE S., BAUGH K.A., WIEDENHOFT H. & GULLAND F.M.D. 2010. Clinical signs and histopathology associated with domoic acid poisoning in northern fur seals (*Callorhinus ursinus*) and comparison of toxin detection methods. *Harmful Algae* 9: 374–383.

LEIRA F.J., VIEITES J.M., BOTANA L.M. & VYEITES M.R. 1998. Domoic acid levels of naturally contaminated scallops as affected by canning. *Journal of Food Science* 63: 1081–1083.

LEMIÈRE G., SEDEHIZADEH S., TOUEG J., FLEARY-ROBERTS N. & CLAYDEN J. 2011. A general synthetic approach to the amnesic shellfish toxins: total synthesis of (−)-isodomoic acid B, (−)-isodomoic acid E and (−)-isodomoic acid F. *Chemical Communications* 47: 3745–3747.

LEVIN E.D., PANG W.G., HARRISON J., WILLIAMS P., PETRO A. & RAMSDELL J.S. 2006. Persistent neurobehavioral effects of early postnatal domoic acid exposure in rats. *Neurotoxicology and Teratology* 28: 673–680.

LEWIS N.I., BATES S.S., McLACHLAN J.L. & SMITH J.C. 1993. Temperature effects on growth, domoic acid production, and morphology of the diatom *Nitzschia pungens* f. *multiseries*. In: *Toxic phytoplankton blooms in the sea* (Ed. by T.J. Smayda & Y. Shimizu), pp. 601–606. Elsevier Science Publishers B.V., Amsterdam.

LEYNAERT A., BUCCIARELLI E., CLAQUIN P., DUGDALE R.C., MARTIN-JÉZÉQUEL V., PONDAVEN P. & RAGUENEAU O. 2004. Effect of iron deficiency on diatom cell size and silicic acid uptake kinetics. *Limnology and Oceanography* 49: 1134–1143.

LI A., YU R., WANG Y., YAN T. & ZHOU M. 2005. Morphological and toxicity characteristics of *Pseudo-nitzschia pungens* strain PP0201-01 isolated from the East China Sea. *Chinese Journal of Oceanology and Limnology* 23: 418–426.

LIEFER J.D., MACINTYR H.L., NOVOVESKÁ L., SMITH W.L. & DORSEY C.P. 2009. Temporal and spatial variability in *Pseudo-nitzschia* spp. in Alabama coastal waters: a ''hot spot'' linked to submarine groundwater discharge? *Harmful Algae* 8: 706–714.

LINCOLN J.A., TURNER J.T., BATES S.S., LÉGER C. & GAUTHIER D.A. 2001. Feeding, egg production, and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-nitzschia pungens*. *Hydrobiologia* 453: 107–120.

LJUBEŠIĆ Z., BOSAK S., VILIČIĆ D., BOROJEVIĆ K.K., MARIĆ D., GODRIJAN J., UJEVIĆ I., PEHAREC P. & ĐAKOVAC T. 2011. Ecology and taxonomy of potentially toxic *Pseudo-nitzschia* species in Lim Bay (north-eastern Adriatic Sea). *Harmful Algae* 10: 713–722.

LÓPEZ-RIVERA A., PINTO M., INSINILLA A., ISLA B.S., URIBE E., ALVAREZ G., LEHANE M., FUREY A. & JAMES K.J. 2009. The occurrence of domoic acid linked to a toxic diatom bloom in a new potential vector: the tunicate *Pyura chilensis* (Piure). *Toxicon* 54: 754–762.

LOUREIRO S., GARCÉS E., FERNÁNDEZ-TEJEDOR M., VAQUÉ D. & CAMP J. 2009a. *Pseudo-nitzschia* spp. (Bacillariophyceae) and dissolved organic matter (DOM) dynamics in the Ebro Delta (Alfacs Bay, NW Mediterranean Sea). *Estuarine, Coastal and Shelf Science* 83: 539–549.

LOUREIRO S., JAUZEIN C., GARCÉS E., COLLOS Y., CAMP J. & VAQUÉ D. 2009b. The significance of organic nutrients in the nutrition of *Pseudo-nitzschia delicatissima* (Bacillariophyceae). *Journal of Plankton Research* 31: 399–410.

LUNDHOLM N. & MOESTRUP Ø. 2002. The marine diatom *Pseudo-nitzschia galaxiae* sp. nov. (Bacillariophyceae): morphology and phylogenetic relationships. *Phycologia* 41: 594–605.

LUNDHOLM N., SKOV J., POCKLINGTON R. & MOESTRUP Ø. 1994. Domoic acid, the toxic amino acid responsible for amnesic

shellfish poisoning, now in *Pseudo nitzschia seriata* (Bacillariophyceae) in Europe. *Phycologia* 33: 475–478.

LUNDHOLM N., SKOV J., POCKLINGTON R. & MOESTRUP Ø. 1997. Studies on the marine planktonic diatom *Pseudo-nitzschia*. 2. Autecology of *P. pseudodelicatissima* based on isolates from Danish coastal waters. *Phycologia* 36: 381–388.

LUNDHOLM N., DAUGBJERG N. & MOESTRUP Ø. 2002a. Phylogeny of the Bacillariaceae with emphasis on the genus *Pseudo-nitzschia* (Bacillariophyceae) based on partial LSU rDNA. *European Journal of Phycology* 37: 115–134.

LUNDHOLM N., HASLE G.R., FRYXELL G.A. & HARGRAVES P.E. 2002b. Morphology, phylogeny and taxonomy of species within the *Pseudo-nitzschia americana* complex (Bacillariophyceae) with descriptions of two new species, *Pseudo-nitzschia brasiliana* and *Pseudo-nitzschia linea*. *Phycologia* 41: 480–497.

LUNDHOLM N., MOESTRUP Ø., HASLE G.R. & HOEF-EMDEN K. 2003. A study of the *Pseudo-nitzschia pseudodelicatissimal cuspidata* complex (Bacillariophyceae): what is *P. pseudodelicatissima*? *Journal of Phycology* 39: 797–813.

LUNDHOLM N., HANSEN P.J. & KOTAKI Y. 2004. Effect of pH on growth and domoic acid production by potentially toxic diatoms of the genera *Pseudo-nitzschia* and *Nitzschia*. *Marine Ecology Progress Series* 273: 1–15.

LUNDHOLM N., ANDERSEN P., JØRGENSEN K., RING THORBJØRNSEN B., CEMBELLA A. & KROCK B. 2005a. Domoic acid in Danish blue mussels due to a bloom of *Pseudo-nitzschia seriata*. *Harmful Algae News* 29: 8–10.

LUNDHOLM N., HANSEN P.J. & KOTAKI Y. 2005b. Lack of allelopathic effects of the domoic acid-producing marine diatom *Pseudo-nitzschia multiseries*. *Marine Ecology Progress Series* 288: 21–33.

LUNDHOLM N., MOESTRUP Ø., KOTAKI Y., HOEF-EMDEN K., SCHOLIN C. & MILLER P. 2006. Inter- and intraspecific variation of the *Pseudo-nitzschia delicatissima* complex (Bacillariophyceae) illustrated by rRNA probes, morphological data and phylogenetic analyses. *Journal of Phycology* 42: 464–481.

LUNDHOLM N., CLARKE A. & ELLEGAARD M. 2010. A 100-year record of changing *Pseudo-nitzschia* species in a sill-fjord in Denmark related to nitrogen loading and temperature. *Harmful Algae* 9: 449–457.

LUNDHOLM N., BATES S.S., BAUGH K.A., BILL B., CONNELL L., LÉGER C. & TRAINER V.L. . Cryptic and pseudo-cryptic diversity in diatoms – with descriptions of *Pseudo-nitzschia hasleana* sp. nov. and *P. fryxelliana* sp. nov. *Journal of Phycology*. (in press)

LYONS D. 2002. Effect of organic enrichment on growth and domoic acid production by axenic cultures of the pennate diatom *Pseudo-nitzschia multiseries*. 94 pp. Mount Allison University, Sackville, New Brunswick, Canada.

MACINTYRE H.L., STUTES A.L., SMITH W.L., DORSEY C.P., ABRAHAM A. & DICKEY R.W. 2011. Environmental correlates of community composition and toxicity during a bloom of *Pseudo-nitzschia* spp. in the northern Gulf of Mexico. *Journal of Plankton Research* 33: 273–295.

MAEDA M., KODAMA T., TANAKA T., YOSHIZUMI H., TAKEMOTO T., NOMOTO K. & FUJITA T. 1986. Structures of isodomoic acids A, B and C, novel insecticidal amino-acids from the red alga *Chondria armata*. *Chemical and Pharmaceutical Bulletin* 34: 4892–4895.

MAFRA L.L. JR. 2009. *Comparative domoic acid kinetics in oysters (Crassostrea virginica) and mussels (Mytilus edulis) feeding on* Pseudo-nitzschia *multiseries: implications for species-specific management.* PhD thesis. Dalhousie University, Halifax, Nova Scotia, Canada. 233 pp.

MAFRA L.L. JR., BRICELJ V.M., OUELLETTE C., LÉGER C. & BATES S.S. 2009a. Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. I. Filtration and pseudofeces production. *Aquatic Biology* 6: 201–212.

MAFRA L.L. JR., BRICELJ V.M. & WARD J.E. 2009b. Mechanisms contributing to low domoic acid uptake by oysters feeding on *Pseudo-nitzschia* cells. II. Selective rejection. *Aquatic Biology* 6: 213–226.

MAFRA L.L. JR., BRICELJ V.M., OUELLETTE C. & BATES S.S. 2010. Feeding mechanics as the basis for differential uptake of the neurotoxin domoic acid by oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*. *Aquatic Toxicology* 97: 160–171.

MALDONADO M.T., HUGHES M.P., RUE E.L. & WELLS M.L. 2002. The effect of Fe and Cu on growth and domoic acid production by *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis*. *Limnology and Oceanography* 47: 515–526.

MALDONADO M.T., ALLEN A.E., CHONG J.S., LIN K., LEUS D., KARPENKO N. & HARRIS S.L. 2006. Copper-dependent iron transport in coastal and oceanic diatoms. *Limnology and Oceanography* 51: 1729–1743.

MANN D.G. 1988. Why didn't Lund see sex in *Asterionella*? A discussion of the diatom life cycle in nature. In: *Algae and the aquatic environment* (Ed. by F.E. Round), pp. 383–412. Biopress, Bristol, UK.

MANN D.G. 1999. The species concept in diatoms. *Phycologia* 38: 437–495.

MANN D.G. & BATES S.S. 2001. Life cycles in diatoms; gaps in knowledge. In: *Life history of microalgal species causing harmful blooms* (Ed. by E. Garcés, A. Zingone, B. Dale, M. Montresor & B. Reguera), pp. 117–120. Commission of the European Community, Calviá, Majorca, Spain.

MARCHANT H.J. & THOMAS D.P. 2005. Diatoms. In: *Antarctic marine protists* (Ed. by H.J. Marchant & J.J. Scott), pp. 13–201. Australian Biological Resources Study, Canberra and Australian Antarctic Division, Hobart.

MARCHETTI A. & HARRISON P.J. 2007. Coupled changes in the cell morphology and the elemental (C, N, and Si) composition of the pennate diatom *Pseudo-nitzschia* due to iron deficiency. *Limnology and Oceanography* 52: 2270–2284.

MARCHETTI A., TRAINER V.L. & HARRISON P.J. 2004. Environmental conditions and phytoplankton dynamics associated with *Pseudo-nitzschia* abundance and domoic acid in the Juan de Fuca eddy. *Marine Ecology Progress Series* 281: 1–12.

MARCHETTI A., MALDONADO M.T., LANE E.S. & HARRISON P.J. 2006a. Iron requirements of the pennate diatom *Pseudo-nitzschia*: comparison of oceanic (high-nitrate, low-chlorophyll waters) and coastal species. *Limnology and Oceanography* 51: 2092–2101.

MARCHETTI A., SHERRY N.D., KIYOSAWA H., TSUDA A. & HARRISON P.J. 2006b. Phytoplankton processes during a mesoscale iron enrichment in the NE subarctic Pacific: Part I – Biomass and assemblage. *Deep-Sea Research. Part II* 53: 2095–2113.

MARCHETTI A., LUNDHOLM N., KOTAKI Y., HUBBARD K., HARRISON P.J. & ARMBRUST E.V. 2008. Identification and assessment of domoic acid production in oceanic *Pseudo-nitzschia* (Bacillariophyceae) from iron-limited waters in the northeast subarctic Pacific. *Journal of Phycology* 44: 650–661.

MARCHETTI A., PARKER M.S., MOCCIA L.P., LIN E.O., ARRIETA A.L., RIBALET F., MURPHY M.E.P., MALDONADO M.T. & ARMBRUST E.V. 2009. Ferritin is used for iron storage in bloom-forming marine pennate diatoms. *Nature* 457: 467–470.

MARSHALL H.G., BURCHARDT L. & LACOUTURE R. 2005. A review of phytoplankton composition within Chesapeake Bay and its tidal estuaries. *Journal of Plankton Research* 27: 1083–1102.

MARTIN J.L., HAYA K., BURRIDGE L.E. & WILDISH D.J. 1990. *Nitzschia pseudodelicatissima* – a source of domoic acid in the Bay of Fundy, eastern Canada. *Marine Ecology Progress Series* 67: 177–182.

MAUCHER J.M. & RAMSDELL J.S. 2007. Maternal–fetal transfer of domoic acid in rats at two gestational time points. *Environmental Health Perspectives* 115: 1743–1746.

MAZZILLO F.F., POMEROY C., KUO J., RAMONDI P.T., PRADO R. & SILVER M.W. 2010. Angler exposure to domoic acid via consumption of contaminated fishes. *Aquatic Biology* 9: 1–12.

MCCARRON P. & HESS P. 2006. Tissue distribution and effects of heat treatments on the content of domoic acid in blue mussels, *Mytilus edulis*. *Toxicon* 47: 473–479.

MCCARRON P., KOTTERMAN M., DE BOER J., REHMANN N. & HESS P. 2007a. Feasibility of gamma irradiation as a stabilisation technique in the preparation of tissue reference materials for a range of shellfish toxins. *Analytical and Bioanalytical Chemistry* 387: 2487–2493.

MCCARRON P., BURRELL S. & HESS P. 2007b. Effect of addition of antibiotics and an antioxidant on the stability of tissue reference materials for domoic acid, the amnesic shellfish poison. *Analytical and Bioanalytical Chemistry* 387: 2495–2502.

MCCARRON P., EMTEBORG H. & HESS P. 2007c. Freeze-drying for the stabilisation of shellfish toxins in mussel tissue (*Mytilus edulis*) reference materials. *Analytical and Bioanalytical Chemistry* 387: 2475–2486.

MCCARRON P., EMTEBORG H., NULTY C., RUNDBERGET T., LOADER J.I., TEIPEL K., MILES C.O., QUILLIAM M.A. & HESS P. 2011. A mussel tissue certified reference material for multiple phycotoxins. Part 1: design and preparation. *Analytical and Bioanalytical Chemistry* 400: 821–833.

MCDONALD S.M., SARNO D. & ZINGONE A. 2007. Identifying *Pseudo-nitzschia* species in natural samples using genus-specific PCR primers and clone libraries. *Harmful Algae* 6: 849–860.

MCGINNESS K.L., FRYXELL G.A. & MCEACHRAN J.D. 1995. *Pseudonitzschia* species found in digestive tracts of northern anchovies (*Engraulis mordax*). *Canadian Journal of Zoology* 73: 642–647.

MCMANUS M.A., KUDELA R.M., SILVER M.W., STEWARD G.F., DONAGHAY P.L. & SULLIVAN J.M. 2008. Cryptic blooms: are thin layers the missing connection? *Estuaries and Coasts* 31: 396–401.

MCQUOID M.R. & GODHE A. 2004. Recruitment of coastal planktonic diatoms from benthic versus pelagic cells: variations in bloom development and species composition. *Limnology and Oceanography* 49: 1123–1133.

MCQUOID M.R. & HOBSON L.A. 1996. Diatom resting stages. *Journal of Phycology* 32: 889–902.

MEDLIN L.K. & KOOISTRA W.H.C.F. 2010. Methods to estimate the diversity in the marine photosynthetic protist community with illustrations from case studies: a review. *Diversity* 2: 973–1014.

MÉNDEZ S. & FERRARIO M. 2009. First report of *Pseudo-nitzschia multistriata* in Uruguay – January 2009. *Harmful Algae News* 40: 5–6.

MENGELT C. & PRÉZELIN B.B. 2002. Dark survival and subsequent light recovery for *Pseudo-nitzschia multiseries*. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 388–390. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

MILLER R.L. & KAMYKOWSKI D.L. 1986. Effects of temperature, salinity, irradiance and diurnal periodicity on growth and photosynthesis in the diatom *Nitzschia americana* – light-saturated growth. *Journal of Phycology* 22: 339–348.

MOS L. 2001. Domoic acid: a fascinating marine toxin. *Environmental Toxicology and Pharmacology* 9: 79–85.

MOSCHANDREOU K.K., PAPAEFTHIMIOU D., KATIKOU P., KALOPESA E., PANOU A. & NIKOLAIDIS G. 2010. Morphology, phylogeny and toxin analysis of *Pseudo-nitzschia pseudodelicatissima* (Bacillariophyceae) isolated from the Thermaikos Gulf, Greece. *Phycologia* 49: 260–273.

MUNDAY R., HOLLAND P.T., MCNABB P., SELWOOD A.I. & RHODES L.L. 2008. Comparative toxicity to mice of domoic acid and isodomoic acids A, B and C. *Toxicon* 52: 954–956.

NAGASAKI K., TOMARU Y., TAKAO Y., NISHIDA K., SHIRAI Y., SUZUKI H. & NAGUMO T. 2005. Previously unknown virus infects marine diatom. *Applied and Environmental Microbiology* 71: 3528–3535.

NEGRI R.M., MONTOYA N.G., CARRETO J.I., AKSELMAN R. & INZA D. 2004. *Pseudo-nitzschia australis*, *Mytilus edulis*, *Engraulis anchoita* and domoic acid in the Argentine Sea. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 139–141. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

NÉZAN E., CHOMÉRAT N. & CRASSOUS M.P. 2007. First record of diatom *Pseudo-nitzschia americana* in French waters. *Harmful Algae News* 33: 18–19.

NÉZAN E., CHOMÉRAT N., BILIEN G., BOULBEN S., DUVAL A. & RYCKAERT M. 2010. *Pseudo-nitzschia australis* on French Atlantic coast—an unusual toxic bloom. *Harmful Algae News* 41: 1–2.

NI Y., KASSAB R.M., CHEVLIAKOV M.V. & MONTGOMERY J. 2009. Total syntheses of isodomoic acids G and H: an exercise in tetrasubstituted alkene synthesis. *Journal of the American Chemical Society* 131: 17714–17718.

NMFS. 2004. Interim report on the bottlenose dolphin (*Tursiops truncatus*) unusual mortality event along the Panhandle of Florida, March–April 2004.

NOVELLI A., KISPERT J., FERNÁNDEZ-SÁNCHEZ M.T., TORRE-BLANCA A. & ZITKO V. 1992. Domoic acid-containing toxic mussels produce neurotoxicity in neuronal cultures through a synergism between excitatory amino acids. *Brain Research* 577: 41–48.

OLSON M.B. & LESSARD E.J. 2010. The influence of the *Pseudo-nitzschia* toxin, domoic acid, on microzooplankton grazing and growth: a field and laboratory assessment. *Harmful Algae* 9: 540–547.

ORIVE E., LAZA-MARTINEZ A., SEOANE S., ALONSO A., ANDRADE R. & MIGUEL I. 2010. Diversity of *Pseudo-nitzschia* in the Southeastern Bay of Biscay. *Diatom Research* 25: 125–145.

ORLOVA T.Y. & MOROZOVA T.V. 2009. Resting stages of microalgae in recent marine sediments of Peter the Great Bay, Sea of Japan. *Russian Journal of Marine Biology* 35: 313–322.

ORLOVA T.Y. & SHEVCHENKO O.G. 2002. The first finding of *Pseudo-nitzschia americana* (Bacillariophyta) in Russian seas. *Russian Journal of Marine Biology* 28: 336–339.

ORLOVA T.Y., STONIK I.V., AIZDAICHER N.A., BATES S.S., LÉGER C. & FEHLING J. 2008. Toxicity, morphology and distribution of *Pseudo-nitzschia calliantha*, *P. multistriata* and *P. multiseries* (Bacillariophyta) from the northwestern Sea of Japan. *Botanica Marina* 51: 297–306.

ORSINI L., SARNO D., PROCACCINI G., POLETTI R., DAHLMANN J. & MONTRESOR M. 2002. Toxic *Pseudo-nitzschia multistriata* (Bacillariophyceae) from the Gulf of Naples: morphology, toxin analysis and phylogenetic relationships with other *Pseudo-nitzschia* species. *European Journal of Phycology* 37: 247–257.

ORSINI L., PROCACCINI G., SARNO D. & MONTRESOR M. 2004. Multiple rDNA ITS-types within the diatom *Pseudo-nitzschia delicatissima* (Bacillariophyceae) and their relative abundances across a spring bloom in the Gulf of Naples. *Marine Ecology Progress Series* 271: 87–98.

OSADA M. & STEWART J.E. 1997. Gluconic acid/gluconolactone: physiological influences on domoic acid production by bacteria associated with *Pseudo-nitzschia multiseries*. *Aquatic Microbial Ecology* 12: 203–209.

PALMA S., MOURINO H., SILVA A., BARAO M.I. & MOITA M.T. 2010. Can *Pseudo-nitzschia* blooms be modeled by coastal upwelling in Lisbon Bay? *Harmful Algae* 9: 294–303.

PAN Y.L. & SUBBA RAO D.V. 1997. Impacts of domestic sewage effluent on phytoplankton from Bedford Basin, eastern Canada. *Marine Pollution Bulletin* 34: 1001–1005.

PAN Y.L., SUBBA RAO D.V. & MANN K.H. 1996a. Changes in domoic acid production and cellular chemical composition of the toxigenic diatom *Pseudo-nitzschia multiseries* under phosphate limitation. *Journal of Phycology* 32: 371–381.

PAN Y.L., SUBBA RAO D.V., MANN K.H., BROWN R.G. & POCKLINGTON R. 1996b. Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. 1. Batch culture studies. *Marine Ecology Progress Series* 131: 225–233.

PAN Y.L., SUBBA RAO D.V., MANN K.H., LI W.K.W. & HARRISON W.G. 1996c. Effects of silicate limitation on production of domoic acid, a neurotoxin, by the diatom *Pseudo-nitzschia multiseries*. 2. Continuous culture studies. *Marine Ecology Progress Series* 131: 235–243.

PAN Y., BATES S.S. & CEMBELLA A.D. 1998. Environmental stress and domoic acid production by *Pseudo-nitzschia*: a physiological perspective. *Natural Toxins* 6: 127–135.

PAN Y.L., PARSONS M.L., BUSMAN M., MOELLER P.D.R., DORTCH Q., POWELL C.L. & DOUCETTE G.J. 2001. *Pseudo-nitzschia* sp. cf. *pseudodelicatissima* – a confirmed producer of domoic acid from the northern Gulf of Mexico. *Marine Ecology Progress Series* 220: 83–92.

PARKER M.S., SCHRUTH D., BERTHIAUME C., IVERSON V., ROCAP G., JENKINS B., BATES S.S. & ARMBRUST E.V. 2009. Genome sequencing of the toxin-producing diatom *Pseudo-nitzschia multiseries*. In: *5th Symposium on Harmful Algae in the U.S.*, Ocean Shores, WA. 157 pp. (Abstract).

PARRISH C.C., DE FREITAS A.S.W., BODENNEC G., MACPHERSON E.J. & ACKMAN R.G. 1991. Lipid composition of the toxic marine diatom, *Nitzschia pungens*. *Phytochemistry* 30: 113–116.

PARSONS M.L., SCHOLIN C.A., MILLER P.E., DOUCETTE G.J., POWELL C.L., FRYXELL G.A., DORTCH Q. & SONIAT T.M. 1999. *Pseudo-nitzschia* species (Bacillariophyceae) in Louisiana coastal waters: molecular probe field trials, genetic variability, and domoic acid analyses. *Journal of Phycology* 35: 1368–1378.

PARSONS M.L., DORTCH Q. & TURNER R.E. 2002. Sedimentological evidence of an increase in *Pseudo-nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. *Limnology and Oceanography* 47: 551–558.

PEERS G., QUESNEL S.A. & PRICE N.M. 2005. Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. *Limnology and Oceanography* 50: 1149–1158.

PERAGALLO H. & PERAGALLO M. 1897–1908. *Diatomées de France* (Ed. by M.J. Tempère), Micrographe-Éditeur, Grez-sur-Loing, France, 492 pp.

PRIISHOLM K., MOESTRUP Ø. & LUNDHOLM N. 2002. Taxonomic notes on the marine diatom genus *Pseudo-nitzschia* in the Andaman Sea near the island of Phuket, Thailand, with a description of *Pseudo-nitzschia micropora* sp. nov. *Diatom Research* 17: 153–175.

PROCOPIAK L.K., FERNANDES L.F. & MOREIRA FILHO H. 2006. Marine and estuarine diatoms (Bacillariophyta) from Parana, southern Brazil: check-list with emphasis on harmful species. *Biota Neotrop* 6, (3), On-line version ISSN 1676-0603. [In Portugese.]

PULIDO O.M. 2008. Domoic acid toxicologic pathology: a review. *Marine Drugs* 6: 180–219.

QI Y., WANG J. & ZHENG L. 1994. The taxonomy and bloom ecology of *Pseudo-nitzschia* on the coasts of China. In: *IOC-WESTPAC Third International Scientific Symposium* (Ed. by A. Nontji, S. Soemodihardjo, A.G. Iladude, D. Setiapermana, D.P. Praseno, M.K. Moosa & O.S.R. Ongkosongo), pp. 88–95, Bali, Indonesia.

QUIJANO-SCHEGGIA S., GARCÉS E., VAN LENNING K., SAMPEDRO N. & CAMP J. 2005. First detection of diatom *Pseudo-nitzschia brasiliana* (nontoxic) and its relative *P. multistriata* (presumably toxic) in the NW Mediterranean Sea. *Harmful Algae News* 29: 5.

QUIJANO-SCHEGGIA S., GARCÉS E., SAMPEDRO N., VAN LENNING K., FLO E., ANDREE K., FORTUÑO J.M. & CAMP J. 2008. Identification and characterisation of the dominant *Pseudo-nitzschia* species (Bacillariophyceae) along the NE Spanish coast (Catalonia, NW Mediterranean). *Scientia Marina* 72: 343–359.

QUIJANO-SCHEGGIA S., GARCÉS E., ANDREE K., FORTUÑO J.M. & CAMP J. 2009a. Homothallic auxosporulation in *Pseudo-nitzschia brasiliana* (Bacillariophyta). *Journal of Phycology* 45: 100–107.

QUIJANO-SCHEGGIA S., GARCÉS E., LUNDHOLM N., MOESTRUP Ø., ANDREE K. & CAMP J. 2009b. Morphology, physiology, molecular phylogeny and sexual compatibility of the cryptic *Pseudo-nitzschia delicatissima* complex (Bacillariophyta), including the description of *P. arenysensis* sp. nov. *Phycologia* 48: 492–509.

QUIJANO-SCHEGGIA S., GARCÉS E., ANDREE K.B., DE LA IGLESIA P., DIOGÈNE J., FORTUÑO J.M. & CAMP J. 2010. *Pseudo-nitzschia* species on the Catalan coast: characterization and contribution to the current knowledge of the distribution of this genus in the Mediterranean Sea. *Scientia Marina* 74: 395–410.

QUILLIAM M.A. 2003. Chemical methods for domoic acid, the amnesic shellfish poisoning (ASP) toxin. In: *Manual on harmful marine microalgae*, Monographs on Oceanographic Methodology (Ed. by G.M. Hallegraeff, D.M. Anderson & A.D. Cembella), pp. 247–266. Intergovernmental Oceanographic Commission (UNESCO), Paris.

RAMSDELL J.S. 2007. The molecular and integrative basis to domoic acid toxicity. In: *Phycotoxins: chemistry and biochemistry* (Ed. by L. Botana), pp. 223–250. Blackwell Publishing Professional, Cambridge, MA.

RAMSDELL J.S. & STAFSTROM C.E. 2009. Rat kainic acid model provides unexpected insight into an emerging epilepsy syndrome in sea lions. *Epilepsy Currents* 9: 142–143.

RAMSEY U.P., DOUGLAS D.J., WALTER J.A. & WRIGHT J.L.C. 1998. Biosynthesis of domoic acid by the diatom *Pseudo-nitzschia multiseries*. *Natural Toxins* 6: 137–146.

REAP M.E. 1991. *Nitzschia pungens* Grunow f. *multiseries* Hasle: growth phases and toxicity of clonal cultures isolated from Galveston Bay, Texas. 78 pp. MS thesis. Texas A&M University.

REPHY. Réseau de Surveillance du Phytoplancton et des Phycotoxines, IFREMER, France.

RHODES L. 1996. New Zealand update on HABs, 1992–1996. *Harmful Algae News* 15: 10.

RHODES L.L. 1998. Identification of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae) in New Zealand coastal waters, using lectins. *New Zealand Journal of Marine and Freshwater Research* 32: 537–544.

RHODES L., WHITE D., SYHRE M. & ATKINSON M. 1996. *Pseudo-nitzschia* species isolated from New Zealand coastal waters: domoic acid production in vitro and links with shellfish toxicity. In: *Harmful and toxic algal blooms* (Ed. by T. Yasumoto, Y. Oshima & Y. Fukuyo), pp. 155–158. Intergovernmental Oceanographic Commission of UNESCO, Paris.

RHODES L., SCHOLIN C., GARTHWAITE I., HAYWOOD A. & THOMAS A. 1998. Domoic acid producing *Pseudo-nitzschia* species educed by whole cell DNA probe-based and immunochemical assays. In: *Harmful algae* (Ed. by B. Reguera, I. Blanco, M.L. Fernádez & T. Wyatt), pp. 274–277. Xunta de Gilicia and the Intergovernmental Oceanographic Commission of UNESCO, Paris.

RHODES L.L., ADAMSON J. & SCHOLIN C. 2000. *Pseudo-nitzschia multistriata* (Bacillariophyceae) in New Zealand. *New Zealand Journal of Marine and Freshwater Research* 34: 463–467.

RHODES L., HOLLAND P.A.J., SELWOOD A. & MCNABB P. 2004. Mass culture of *Pseudo-nitzschia australis* for production of a new isomer of domoic acid. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 125–127. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

RHODES L., SELWOOD A., MCNABB P., BRIGGS L., ADAMSON J., VAN GINKEL R. & LACZKA O. 2006. Trace metal effects on the production of biotoxins by microalgae. *African Journal of Marine Science* 28: 393–397.

RIBALET F., MARCHETTI A., HUBBARD K.A., BROWN K., DURKIN C.A., MORALES R., ROBERT M., SWALWELL J.E., TORTELL P.D. & ARMBRUST E.V. 2010. Unveiling a phytoplankton hotspot at a narrow boundary between coastal and offshore waters. *Proceedings of the National Academy of Sciences of the United States of America* 107: 16571–16576.

RINES J.E.B., DONAGHAY P.L., DEKSHENIEKS M.M., SULLIVAN J.M. & TWARDOWSKI M.S. 2002. Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. *Marine Ecology Progress Series* 225: 123–137.

ROMERO M.L.J., KOTAKI Y., LUNDHOLM N., THOHA H., OGAWA H., RELOX J.R., TERADA R., TAKEDA S., TAKATA Y., HARAGUCHI K., ENDO T., LIM P.-T., KODAMA M. & FUKUYO Y. 2011. Unique amnesic shellfish toxin composition found in the South East Asian diatom *Nitzschia navis-varingica*. *Harmful Algae* 10: 456–462.

RUE E. & BRULAND K. 2001. Domoic acid binds iron and copper: a possible role for the toxin produced by the marine diatom *Pseudo-nitzschia*. *Marine Chemistry* 76: 127–134.

RYAN J.P., CHAVEZ F.P. & BELLINGHAM J.G. 2005. Physical–biological coupling in Monterey Bay, California: topographic influences on phytoplankton ecology. *Marine Ecology Progress Series* 287: 23–32.

SAHRAOUI I., HLAILI A.S., MABROUK H.H., LÉGER C. & BATES S.S. 2009. Blooms of the diatom genus *Pseudo-nitzschia* H. Peragallo in Bizerte Lagoon (Tunisia, SW Mediterranean). *Diatom Research* 24: 175–190.

SAHRAOUI I., BATES S.S., BOUCHOUICHA D., HADJ MABROUK H. & SAKKA HLAILI A. 2011. Toxic and potentially toxic *Pseudo-nitzschia* populations in Bizerte Lagoon (Tunisia, SW Mediterranean) during 2006–7, and first report of domoic acid production by *Pseudo-nitzschia brasiliana* Lundholm, Hasle & G.A. Fryxell. *Diatom Research* (in press).

SAPP M., WICHELS A. & GERDTS G. 2007. Impacts of cultivation of marine diatoms on the associated bacterial community. *Applied and Environmental Microbiology* 73: 3117–3120.

SARNO D., ZINGONE A. & MONTRESOR M. 2010. A massive and simultaneous sex event of two *Pseudo-nitzschia* species. *Deep-Sea Research. Part II* 57: 248–255.

SAWANT P.M., WEARE B.A., HOLLAND P.T., SELWOOD A.I., KING K.L., MIKULSKI C.M., DOUCETTE G.J., MOUNTFORT D.O. & KERR D.S. 2007. Isodomoic acids A and C exhibit low KA receptor affinity and reduced *in vitro* potency relative to domoic acid in region CA1 of rat hippocampus. *Toxicon* 50: 627–638.

SAWANT P.M., TYNDALL J.D.A., HOLLAND P.T., PEAKE B.M., MOUNTFORT D.O. & KERR D.S. 2010. *In vivo* seizure induction and affinity studies of domoic acid and isodomoic acids-D, -E and -F. *Neuropharmacology* 59: 129–138.

SAZHIN A.F., ARTIGAS L.F., NEJSTGAARD J.C. & FRISCHER M.E. 2007. The colonization of two *Phaeocystis* species (Prymnesiophyceae) by pennate diatoms and other protists: a significant contribution to colony biomass. *Biogeochemistry* 83: 137–145.

SCHAFFER P., REEVES C., CASPER D.R. & DAVIS C.R. 2006. Absence of neurotoxic effects in leopard sharks, *Triakis semifasciata*, following domoic acid exposure. *Toxicon* 47: 747–752.

SCHNETZER A., MILLER P.E., SCHAFFNER R.A., STAUFFER B.A., JONES B.H., WEISBERG S.B., DIGIACOMO P.M., BERELSON W.M. & CARON D.A. 2007. Blooms of *Pseudo-nitzschia* and domoic acid in the San Pedro Channel and Los Angeles harbor areas of the Southern California Bight, 2003–2004. *Harmful Algae* 6: 372–387.

SCHOLIN C.A., GULLAND F., DOUCETTE G.J., BENSON S., BUSMAN M., CHAVEZ F.P., CORDARO J., DELONG R., DE VOGELAERE A., HARVEY J., HAULENA M., LEFEBVRE K., LIPSCOMB T., LOSCUT-OFF S., LOWENSTINE L.J., MARIN R. III, MILLER P.E., MCLELLAN W.A., MOELLER P.D.R., POWELL C.L., ROWLES T., SILVAGNI P., SILVER M., SPRAKER T., TRAINER V. & VAN DOLAH F.M. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature* (London) 403: 80–84.

SCOTT F.J. & THOMAS D.P. 2005. Diatoms. In: *Antarctic marine protists* (Ed. by F.J. Scott & H.J. Marchant), pp. 13–201. Australian Biological Resources Study, Canberra and Australian Antarctic Division, Hobart.

SEEYAVE S., PROBYN T.A., PITCHER G.C., LUCAS M.I. & PURDIE D.A. 2009. Nitrogen nutrition in assemblages dominated by *Pseudo-nitzschia* spp., *Alexandrium catenella* and *Dinophysis acuminata* off the west coast of South Africa. *Marine Ecology Progress Series* 379: 91–107.

SEKULA-WOOD E., SCHNETZER A., BENITEZ-NELSON C.R., ANDERSON C., BERELSON W.M., BRZEZINSKI M.A., BURNS J.M., CARON D.A., CETINIC I., FERRY J.L., FITZPATRICK E., JONES B.H., MILLER P.E., MORTON S.L., SCHAFFNER R.A., SIEGEL D.A. & THUNELL R. 2009. Rapid downward transport of the neurotoxin domoic acid in coastal waters. *Nature Geoscience* 2: 272–275.

SEKULA-WOOD E., BENITEZ-NELSON C., MORTON S., ANDERSON C., BURRELL C. & THUNELL R. 2011. *Pseudo-nitzschia* and domoic acid fluxes in Santa Barbara Basin (CA) from 1993 to 2008. *Harmful Algae* 10: 567–575.

SIERRA-BELTRÁN A.P., PALAFOX-URIBE M., GRAJALES-MONTIEL J., CRUZ-VILLACORTA A. & OCHOA J.L. 1997. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. *Toxicon* 35: 447–453.

SIERRA-BELTRÁN A.P., CORTÉS-ALTAMIRANO R., GALLO-REYNOSO J.P., LICEA-DURAN S. & ÉGIDO-VILLARREAL J. 2005. Is *Pseudo-nitzschia pseudodelicatissima* toxin the principal cause of sardines, dolphins, sea lions and pelicans mortality in 2004 in Mexico? *Harmful Algae News* 29: 6–8.

SILVER M.W., BARGU S., COALE S.L., BENITEZ-NELSON C.R., GARCIA A.C., ROBERTS K.J., SEKULA-WOOD E., BRULAND K.W. & COALE K.H. 2010. Toxic diatoms and domoic acid in natural and iron-enriched waters of the oceanic Pacific. *Proceedings of the National Academy of Sciences of the United States of America* 107: 20762–20767.

SKOV J., TON T.P. & DO T.B.L. 2004. Bacillariophyceae. In: *Potentially toxic microalgae of Vietnamese waters* (Ed. by J.

Larsen & N.L. Nguyen), pp. 23–51. *Opera Botanica* Vol. 140, Council for Nordic Publications in Botany, Copenhagen.

SMITH E.A., PAPAPANAGIOTOU E.P., BROWN N.A., STOBO L.A., GALLACHER S. & SHANKS A.M. 2006. Effect of storage on amnesic shellfish poisoning (ASP) toxins in king scallops (*Pecten maximus*). *Harmful Algae* 5: 9–19.

SMITH J.C., PAULEY K., CORMIER P., ANGUS R., ODENSE P., O'NEIL D., QUILLIAM M.A. & WORMS J. 1991. Population dynamics and toxicity of various species of *Dinophysis* and *Nitzschia* from the southern Gulf of St. Lawrence. In: *Proceedings of the Second Canadian Workshop on Harmful Marine Algae* (Ed. by D.C. Gordon Jr), 25 pp. *Canadian Technical Report of Fisheries and Aquatic Sciences* 1799 [Abstract].

STEHR C.M., CONNELL L., BAUGH K.A., BILL B.D., ADAMS N.G. & TRAINER V.L. 2002. Morphological, toxicological, and genetic differences among *Pseudo-nitzschia* (Bacillariophyceae) species in inland embayments and outer coastal waters of Washington State, USA. *Journal of Phycology* 38: 55–65.

STEWART J.E. 2008. Bacterial involvement in determining domoic acid levels in *Pseudo-nitzschia multiseries* cultures. *Aquatic Microbial Ecology* 50: 135–144.

STEWART J.E., MARKS L.J., WOOD C.R., RISSER S.M. & GRAY S. 1997. Symbiotic relations between bacteria and the domoic acid-producing diatom *Pseudo-nitzschia multiseries* and the capacity of these bacteria for gluconic acid/gluconolactone formation. *Aquatic Microbial Ecology* 12: 211–221.

STEWART J.E., MARKS L.J., GILGAN M.W., PFEIFFER E. & ZWICKER B.M. 1998. Microbial utilization of the neurotoxin domoic acid: blue mussels (*Mytilus edulis*) and soft shell clams (*Mya arenaria*) as sources of the microorganisms. *Canadian Journal of Microbiology* 44: 456–464.

STONIK I.V., ORLOVA T.Y. & BEGUN A.A. 2008. Potentially toxic diatoms *Pseudo-nitzschia fraudulenta* and *P. calliantha* from Russian waters of East Japan Sea and Sea of Okhotsk. *Ocean Science Journal* 43: 25–30.

STONIK I.V., ORLOVA T.Y. & LUNDHOLM N. 2011. Diversity of *Pseudo-nitzschia* from the Western North Pacific. *Diatom Research* 26: 121–134.

SUÁREZ-ISLA B.A., LÓPEZ A., HERNÁNDEZ C., CLÉMENT A. & GUZMÁN L. 2002. Impacto económico de las floraciones de microalgas nocivas en Chile y datos recientes sobre la ocurrencia de veneno amnésico de los mariscos. In: *Floraciones algales nocivas en el cono Sur Americano* (Ed. by E.A. Sar, M.E. Ferrario & B. Reguera), pp. 257–268. Instituto Español de Oceanografía, Madrid.

SUBBA RAO D.V., PAN Y. & SMITH J.S. 1995. Allelopathy between *Rhizosolenia alata* (Brightwell) and the toxigenic *Pseudonitzschia pungens* f. *multiseries* (Hasle). In: *Harmful marine algal blooms* (Ed. by P. Lassus, G. Arzul, E. Erard, P. Gentien & C. Marcaillou-Le Baut), pp. 681–686. Lavoisier Publishing, Paris.

SUBBA RAO D.V., PAN Y. & MUKHIDA K. 1998. Production of domoic acid by *Pseudo-nitzschia multiseries* Hasle, affected by lithium. *Marine Ecology* 19: 31–36.

SUN J., HUTCHINS D.A., FENG Y., SEUBERT E.L., CARON D.A. & FU F.-X. 2011. Effects of changing pCO_2 and phosphate availability on domoic acid production and physiology of the marine harmful bloom diatom *Pseudo-nitzschia multiseries*. *Limnology and Oceanography* 56: 829–840.

TAKAHASHI E., YU Q., EAGLESHAM G., CONNELL D.W., MCBROOM J., COSTANZO S. & SHAW G.R. 2007. Occurrence and seasonal variations of algal toxins in water, phytoplankton and shellfish from North Stradbroke Island, Queensland, Australia. *Marine Environmental Research* 64: 429–442.

TAKANO H. 1995. *Pseudo-nitzschia multistriata* (Takano) Takano, a new combination for the pennate diatom *Nitzschia multistriata* Takano. *Diatom, The Japanese Journal of Diatomology* 10: 73–74.

TAKATA Y., SATO S., HA D.V., MONTOJO U.M., LIRDWITAYAPRASIT T., KAMOLSIRIPICHAIPORN S., KOTAKI Y., FUKUYO Y. & KODAMA M. 2009. Occurrence of domoic acid in tropical bivalves. *Fisheries Science* 75: 473–480.

TAKEMOTO T. & DAIGO K. 1958. Constituents of *Chondria armata*. *Chemical and Pharmaceutical Bulletin* 6: 578–580.

TANG Y.Z., KOCH F. & GOBLER C.J. 2010. Most harmful algal bloom species are vitamin B_1 and B_{12} auxotrophs. *Proceedings of the National Academy of Sciences of the United States of America* 107: 20756–0761.

TEITELBAUM J., CARPENTER S. & CASHMAN N.R. 1990. Neurologic sequelae after ingestion of mussels contaminated with domoic acid. *New England Journal of Medicine* 323: 1632–1633.

TESSON S.V.M., BORRA M., KOOISTRA W.H.C.F. & PROCACCINI G. 2011. Microsatellite primers in the planktonic diatom *Pseudo-nitzschia multistriata* (Bacillariophyceae). *American Journal of Botany* 98: e33–e35.

TESTER P.A., PAN Y., DOUCETTE G.J. & SCHOLIN C.A. 2001. Accumulation of domoic acid activity in copepods. In: *Harmful algal blooms 2000* (Ed. by G.M. Hallegraeff, S.I. Blackburn, C.J. Bolch & R.J. Lewis), pp. 418–421. Intergovernmental Oceanographic Commission of UNESCO, Paris.

THÉBAUD O., VÉRON G. & FIFAS S. 2005. Incidences des épisodes d'efflorescence de micro algues toxiques sur les écosystèmes et sur les pêcheries de coquillages en baie de Douarnenez. Ifremer. Available at http://archimer.ifremer.fr.doc.2005.rapport-1262.pdf.

THESSEN A.E. 2007. Taxonomy and ecophysiology of *Pseudo-nitzschia* in the Chesapeake Bay. PhD thesis. University of Maryland, College Park, MD. 231 pp.

THESSEN A.E. & STOECKER D.K. 2008. Distribution, abundance and domoic acid analysis of the toxic diatom genus *Pseudo-nitzschia* from the Chesapeake Bay. *Estuaries and Coasts* 31: 664–672.

THESSEN A.E., DORTCH Q., PARSONS M.L. & MORRISON W. 2005. Effect of salinity on *Pseudo-nitzschia* species (Bacillariophyceae) growth and distribution. *Journal of Phycology* 41: 21–29.

THESSEN A.E., BOWERS H.A. & STOECKER D.K. 2009. Intra- and interspecies differences in growth and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. *Harmful Algae* 8: 792–810.

THESSEN A.E., SONIAT T.M., DORTCH Q. & DOUCETTE G.J. 2010. *Crassostrea virginica* grazing on toxic and non-toxic diatoms. *Toxicon* 55: 570–579.

TODD E.C.D. 1993. Domoic acid and amnesic shellfish poisoning – a review. *Journal of Food Protection* 56: 69–83.

THOMAS K., TREMBLAY M.-L., WALTER J.A. & QUILLIAM M.A. 2008. NRC CRM-DA-f, a certified calibration solution reference material for domoic acid. CRMP Technical Report CRM-DA-f-20071205, June 2008. Available at http://www.nrc-cnrc.gc.ca/obj/imb-ibm/doc/DA-fCertificateENGFeb2010.pdf

TRAINER V.L. & BILL B.D. 2004. Characterization of a domoic acid binding site from Pacific razor clam. *Aquatic Toxicology* 69: 125–132.

TRAINER V.L., ADAMS N.G., BILL B.D., STEHR C.M., WEKELL J.C., MOELLER P., BUSMAN M. & WOODRUFF D. 2000. Domoic acid production near California coastal upwelling zones, June 1998. *Limnology and Oceanography* 45: 1818–1833.

TRAINER V.L., HICKEY B.M. & HORNER R.A. 2002. Biological and physical dynamics of domoic acid production off the Washington coast. *Limnology and Oceanography* 47: 1438–1446.

TRAINER V.L., COCHLAN W.P., ERICKSON A., BILL B.D., COX F.H., BORCHERT J.A. & LEFEBVRE K.A. 2007. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. *Harmful Algae* 6: 449–459.

TRAINER V.L., HICKEY B.M. & BATES S.S. 2008. Toxic diatoms. In: *Oceans and human health: risks and remedies from the sea* (Ed. by P.J. Walsh, S.L. Smith, L.E. Fleming, H. Solo-Gabrielle & W.H. Gerwick), pp. 219–237. Elsevier Science Publishers, New York.

TRAINER V.L., HICKEY B.M., LESSARD E.J., COCHLAN W.P., TRICK C.G., WELLS M.L., MACFADYEN A. & MOORE S.K. 2009a. Variability of *Pseudo-nitzschia* and domoic acid in the Juan de Fuca eddy region and its adjacent shelves. *Limnology and Oceanography* 54: 289–308.

TRAINER V.L., WELLS M.L., COCHLAN W.P., TRICK C.G., BILL B.D., BAUGH K.A., BEALL B.F., HERNDON J. & LUNDHOLM N. 2009b. An ecological study of a massive bloom of toxigenic *Pseudo-nitzschia cuspidata* off the Washington State coast. *Limnology and Oceanography* 54: 1461–1474.

TRAINER V.L., PITCHER G.C., REGUERA B. & SMAYDA T.J. 2010. The distribution and impacts of harmful algal bloom species in eastern boundary upwelling systems. *Progress in Oceanography* 85: 33–52.

TRAINER V.L., BATES S.S., LUNDHOLM N., THESSEN A.E., ADAMS N.G., COCHLAN W.P. & TRICK C.G. 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. *Harmful Algae.* (in press)

TRICK C.G., BILL B.D., COCHLAN W.P., WELLS M.L., TRAINER V.L. & PICKELL L.D. 2010. Iron enrichment stimulates toxic diatom production in high-nitrate, low-chlorophyll areas. *Proceedings of the National Academy of Sciences of the United States of America* 107: 5887–5892.

TRIMBORN S., LUNDHOLM N., THOMS S., RICHTER K.U., KROCK B., HANSEN P.J. & ROST B. 2008. Inorganic carbon acquisition in potentially toxic and non-toxic diatoms: the effect of pH-induced changes in seawater carbonate chemistry. *Physiologia Plantarum* 133: 92–105.

TRUELOVE J., MUELLER R., PULIDO O. & IVERSON F. 1996. Subchronic toxicity study of domoic acid in the rat. *Food and Chemical Toxicology* 34: 525–529.

TRUELOVE J., MUELLER R., PULIDO O., MARTIN L., FERNIE S. & IVERSON F. 1997. 30-day oral toxicity study of domoic acid in cynomolgus monkeys: lack of overt toxicity at doses approaching the acute toxic dose. *Natural Toxins* 5: 111–114.

UJEVIĆ I., NINČEVIĆ-GLADAN Ž., ROJE R., SKEJIĆ S., ARAPOV J. & MARASOVIĆ I. 2010. Domoic acid – a new toxin in the Croatian Adriatic shellfish toxin profile. *Molecules* 15: 6835–6849.

VALE P. & SAMPAYO M.A.M. 2001. Domoic acid in Portuguese shellfish and fish. *Toxicon* 39: 893–904.

VELO-SUÁREZ L., GONZÁLEZ-GIL S., GENTIEN P., LUNVEN M., BECHEMIN C., FERNAND L., RAINE R. & REGUERA B. 2008. Thin layers of *Pseudo-nitzschia* spp. and the fate of *Dinophysis acuminata* during an upwelling-downwelling cycle in a Galician Ria. *Limnology and Oceanography* 53: 1816–1834.

VERSHININ A.O. & ORLOVA T.Y. 2008. Toxic and harmful algae in the coastal waters of Russia. *Oceanology* 48: 524–537.

VIGILANT V.L. & SILVER M.W. 2007. Domoic acid in benthic flatfish on the continental shelf of Monterey Bay, California, USA. *Marine Biology* 151: 2053–2062.

VILLAC M.C. & FRYXELL G.A. 1998. *Pseudo-nitzschia pungens* var. *cingulata* var. nov. (Bacillariophyceae) based on field and culture observations. *Phycologia* 37: 269–274.

VILLAC M.C., ROELKE D.L., VILLAREAL T.A. & FRYXELL G.A. 1993. Comparison of two domoic acid-producing diatoms – a review. *Hydrobiologia* 269: 213–224.

VILLAC M.C., MATOS M.G., SANTOS V.S., RODRIGUES A.W. & VIANA S.C. 2004. Composition and distribution of *Pseudo-nitzschia* from Guanabara Bay, Brazil: the role of salinity, based on field and culture observations. In: *Harmful algae 2002* (Ed. by K.A. Steidinger, J.H. Landsberg, C.R. Tomas & G.A. Vargo), pp. 56–58. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, Paris.

VILLAC M.C., MELO S., MENEZES M. & RIVERA D. 2005. *Pseudo-nitzschia brasiliana* (Bacillariophyceae), an opportunistic diatom on the coast of the state of Rio de Janeiro, Brazil. Atlântica, Río Grande. *Atlantica* 27: 139–145.

WEKELL J.C., GAUGLITZ E.J., BARNETT H.J., HATFIELD C.L. & EKLUND M. 1994. The occurrence of domoic acid in razor clams (*Siliqua patula*), Dungeness crab (*Cancer magister*), and anchovies (*Engraulis mordax*). *Journal of Shellfish Research* 13: 587–593.

WELLS M.L., TRICK C.G., COCHLAN W.P., HUGHES M.P. & TRAINER V.L. 2005. Domoic acid: the synergy of iron, copper, and the toxicity of diatoms. *Limnology and Oceanography* 50: 1908–1917.

WILHELM S.W. & TRICK C.G. 1994. Iron-limited growth of cyanobacteria – multiple siderophore production is a common response. *Limnology and Oceanography* 39: 1979–1984.

WINDUST A. 1992. *The response of bacteria, microalgae, and zooplankton to the diatom* Nitzschia pungens *forma* multiseries, *and its toxic metabolite domoic acid.* MS thesis. Dalhousie University, Halifax, Nova Scotia, Canada. 107 pp.

WORK T.M., BARR B., BEALE A.M., FRITZ L., QUILLIAM M.A. & WRIGHT J.L.C. 1993. Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. *Journal of Zoo and Wildlife Medicine* 24: 54–62.

WRIGHT J.L.C., BOYD R.K., DE FREITAS A.S.W., FALK M., FOXALL R.A., JAMIESON W.D., LAYCOCK M.V., MCCULLOCH A.W., MCINNES A.G., ODENSE P., PATHAK V.P., QUILLIAM M.A., RAGAN M.A., SIM P.G., THIBAULT P., WALTER J.A., GILGAN M., RICHARD D.J.A. & DEWAR D. 1989. Identification of domoic acid, a neuroexcitatory amino-acid, in toxic mussels from eastern Prince Edward Island. *Canadian Journal of Chemistry* 67: 481–490.

WRIGHT J.L.C., FALK M., MCINNES A.G. & WALTER J.A. 1990. Identification of isodomoic acid D and two new geometrical isomers of domoic acid in toxic mussels. *Canadian Journal of Chemistry* 68: 22–25.

YAP-DEJETO L.G., OMURA T., NAGAHAMA Y., NAGAHAMA Y. & FUKUYO Y. 2010. Observations of eleven *Pseudo-nitzschia* species in Tokyo Bay, Japan. *La Mer (La Société Franco-Japonaise d'Océanographie, Tokyo)* 48: 1–16.

ZAMAN L., ARAKAWA O., SHIMOSU A., ONOUE Y., NISHIO S., SHIDA Y. & NOGUCHI T. 1997. Two new isomers of domoic acid from a red alga, *Chondria armata. Toxicon* 35: 205–212.

ZHANG F.Z. & DICKMAN M. 1999. Mid-ocean exchange of container vessel ballast water. 1: Seasonal factors affecting the transport of harmful diatoms and dinoflagellates. *Marine Ecology Progress Series* 176: 243–251.