

1    **Pseudo-nitzschia, Nitzschia, and domoic acid: new research since 2011**

2

3    Stephen S. Bates <sup>a,\*</sup>, Katherine A. Hubbard <sup>b,c</sup>, Nina Lundholm <sup>d</sup>, Marina Montresor <sup>e</sup> and Chui  
4    Pin Leaw <sup>f</sup>

5

6

7    <sup>a</sup> *Fisheries and Oceans Canada, Gulf Fisheries Centre, P.O. Box 5030, Moncton, New  
8    Brunswick, Canada E1C 9B6*

9    <sup>b</sup> *Fish and Wildlife Research Institute (FWRI), Florida Fish and Wildlife Conservation  
10   Commission (FWC), 100 Eighth Avenue SE, St. Petersburg, FL 33701 USA*

11   <sup>c</sup> *Woods Hole Center for Oceans and Human Health, Woods Hole Oceanographic Institution,  
12   266 Woods Hole Road, Woods Hole, MA, 02543 USA*

13   <sup>d</sup> *Natural History Museum of Denmark, University of Copenhagen, Sølvgade 83S, DK-1307  
14   Copenhagen K, Denmark*

15   <sup>e</sup> *Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy*

16   <sup>f</sup> *Bachok Marine Research Station, Institute of Ocean and Earth Sciences, University of Malaya,  
17   16310 Bachok, Kelantan, Malaysia*

18

19

20

21

22

23

24   Keywords: Diatom phylogeny, Diatom taxonomy, Amnesic shellfish poisoning, Bacteria,  
25   Diatom sexual reproduction, Domoic acid, Harmful algal bloom, *Nitzschia*, *Pseudo-nitzschia*,  
26   Zooplankton

27

28   \* Corresponding author. Tel.: +1 902-431-4243.

29   E-mail address: [stephen.bates@dfo-mpo.gc.ca](mailto:stephen.bates@dfo-mpo.gc.ca) (S.S. Bates).

30   Conflicts of interest: none.

## 31 ABSTRACT

32 Some diatoms of the genera *Pseudo-nitzschia* and *Nitzschia* produce the neurotoxin domoic acid  
33 (DA), a compound that caused amnesic shellfish poisoning (ASP) in humans just over 30 years  
34 ago (December 1987) in eastern Canada. This review covers new information since two previous  
35 reviews in 2012. *Nitzschia bizertensis* was subsequently discovered to be toxigenic in Tunisian  
36 waters. The known distribution of *N. navis-varingica* has expanded from Vietnam to Malaysia,  
37 Indonesia, the Philippines and Australia. Furthermore, 15 new species (and one new variety) of  
38 *Pseudo-nitzschia* have been discovered, bringing the total to 52. Seven new species were found  
39 to produce DA, bringing the total of toxigenic species to 26.

40 We list all *Pseudo-nitzschia* species, their ability to produce DA, and show their global  
41 distribution. A consequence of the extended distribution and increased number of toxigenic  
42 species worldwide is that DA is now found more pervasively in the food web, contaminating  
43 new marine organisms (especially marine mammals), affecting their physiology and disrupting  
44 ecosystems.

45 Recent findings highlight how zooplankton grazers can induce DA production in *Pseudo-*  
46 *nitzschia* and how bacteria interact with *Pseudo-nitzschia*. Since 2012, new discoveries have  
47 been reported on physiological controls of *Pseudo-nitzschia* growth and DA production, its  
48 sexual reproduction, and infection by an oomycete parasitoid. Many advances are the result of  
49 applying molecular approaches to discovering new species, and to understanding the population  
50 genetic structure of *Pseudo-nitzschia* and mechanisms used to cope with iron limitation. The  
51 availability of genomes from three *Pseudo-nitzschia* species, coupled with a comparative  
52 transcriptomic approach, has allowed advances in our understanding of the sexual reproduction  
53 of *Pseudo-nitzschia*, its signaling pathways, its interactions with bacteria, and genes involved in  
54 iron and vitamin B<sub>12</sub> and B<sub>7</sub> metabolism.

55 Although there have been no new confirmed cases of ASP since 1987 because of monitoring  
56 efforts, new blooms have occurred. A massive toxic *Pseudo-nitzschia* bloom affected the entire  
57 west coast of North America during 2015–2016, and was linked to a ‘warm blob’ of ocean water.  
58 Other smaller toxic blooms occurred in the Gulf of Mexico and east coast of North America.  
59 Knowledge gaps remain, including how and why DA and its isomers are produced, the world  
60 distribution of potentially toxigenic *Nitzschia* species, the prevalence of DA isomers, and  
61 molecular markers to discriminate between toxigenic and non-toxigenic species and to discover  
62 sexually reproducing populations in the field.

### 63     **1. Introduction**

64     Just over thirty years ago (December 17, 1987), domoic acid (DA) was discovered to be the toxin  
65     that killed at least three elderly people and sickened over another 100 others who consumed  
66     mussels (*Mytilus edulis*) from Prince Edward Island (PEI), eastern Canada (Bates et al., 1989;  
67     Hanic, 2014). Since then, no additional deaths have been confirmed due to Amnesic Shellfish  
68     Poisoning (ASP), the syndrome caused by DA, although the prevalence of DA, its isomers and  
69     toxic diatoms, has increased worldwide. This is because of increased monitoring, research and  
70     awareness, but is most certainly also due to limited medical awareness of ASP.

71         Here, we highlight the literature published after the reviews of Lelong et al. (2012a) and  
72     Trainer et al. (2012), focusing on diatoms that produce DA, i.e. *Pseudo-nitzschia* spp., *Nitzschia*  
73     *navis-varingica* and *N. bizertensis*. Literature not emphasized by those reviews is also discussed.  
74     This review also builds on other reviews of *Pseudo-nitzschia* and DA, from 1998 to 2017 (Table  
75     1). Those reviews provide background information on the discovery of DA and its physiological  
76     effects on fish, birds, mammals and humans. We will not discuss the chemistry, mechanisms of  
77     action and pharmacology of DA, nor seafood safety aspects of ASP, which have been reviewed  
78     elsewhere (Rossini and Hess, 2010; Pulido, 2014a,b, 2016; Tasker, 2014, 2016; Vale, 2014;  
79     Ramsdell and Gulland, 2014; La Barre et al., 2014; Novelli et al., 2014; Angus, 2015; Sitprija  
80     and Sitprija, 2016; McPartlin et al., 2016; Hallegraeff, 2017; Lahvis, 2017).

81

### 82     **2. *Nitzschia navis-varingica***

#### 83     *2.1. Western Pacific distribution*

84         The DA-producing pennate diatom *Nitzschia navis-varingica* (Lundholm and Moestrup,  
85     2000) was first described from shrimp-culture ponds in Vietnam in 1997 (Kotaki et al., 2000),  
86     although no shrimp were reported to be toxic. After *Pseudo-nitzschia*, this is the second diatom  
87     genus confirmed to produce DA. There is uncertainty about the report of another diatom,  
88     *Halamphora coffeaeformis*, described to produce DA (discussed in Bates, 2000). Current  
89     information on the geographical distribution of *N. navis-varingica* indicates that it is apparently  
90     restricted to the Western Pacific region, but not limited to the tropics (Kotaki et al., 2008; Tan et  
91     al., 2016b) (Table 2; Fig. 1). The species has been recorded between 40°N and 40°S, in Australia  
92     (Higgins et al., 2003), Japan (Kotaki et al., 2004, 2008) and Southeast Asia, including Vietnam  
93     (Kotaki et al., 2000; Lundholm and Moestrup, 2000), Thailand (Romero et al., 2008), Malaysia

94 (Thoha et al., 2012; Suriyanti and Usup, 2015; Tan et al., 2016b), Indonesia (Romero et al.,  
95 2011; Thoha et al., 2012), and the Philippines (Kotaki et al., 2004, 2005, 2006, 2008; Bajarias et  
96 al., 2006; Romero et al., 2012). The current pan-western-Pacific distribution of *N. navis-*  
97 *varingica* may be the result of an under-recorded presence in the Atlantic and other regions. For  
98 example, Bellinger and Hagerthey (2010) reported very small amounts of DA in periphyton  
99 samples collected in the Florida Everglades, USA, where several unidentified *Nitzschia* species  
100 were present. So far, the toxin-producing organism has never been identified. Nevertheless, this  
101 suggests that *N. navis-varingica* or the other toxin-producing *Nitzschia* species, *N. bizertensis*,  
102 may be more widely distributed or that other toxigenic *Nitzschia* species are yet to be discovered.

103 *Nitzschia navis-varingica* is commonly associated with benthic brackish ecosystems (e.g.  
104 Kotaki et al., 2004, 2005; Romero et al., 2012; Tan et al., 2016b). It is euryhaline and frequently  
105 found in estuaries and mangrove forests. Tan et al. (2016b) recorded contrasting salinity regimes  
106 where the species was found, ranging from 5–29 (Tan et al., 2016b); the type strain (VSP974-1)  
107 was maintained at a salinity of 33 (Lundholm and Moestrup, 2000).

108

## 109 2.2. Production of domoic acid

110 DA production by *N. navis-varingica* has been confirmed in clonal cultures by several  
111 analytical methods (Table 2). The total cellular DA content in cultures ( $0.04\text{--}15.3\text{ }\mu\text{g cell}^{-1}$ ) is  
112 comparable to that of toxigenic *Pseudo-nitzschia* species (Trainer et al., 2012). Early studies only  
113 included analyses for DA (Kotaki et al., 2004, 2005, 2008), but isomers of DA were included  
114 later on (e.g. Kotaki et al., 2005). It was then found that, in some locations, no DA was detected  
115 in the cells, but rather they contained the DA isomers isodomoic acid A (IA) and/or isodomoic  
116 acid B (IB) (Table 2). Trace amounts of isodomoic acid D (ID), isodomoic acid E (IE) and  
117 diastereoisomer epi-domoic acid (epi-DA) were detected in some strains from the Philippines  
118 (Romero et al., 2011).

119 Five types of toxin profiles have been found in *N. navis-varingica*: DA, DA-IB, IA-IB,  
120 IB and DA-IA-IB (Romero et al., 2012; Tan et al., 2016b), apparently varying depending on the  
121 location (Fig. 1). Malaysian strains belong to the toxin profile types DA, DA-IB, DA-IA-IB and  
122 DA-IA, the Philippines strains exhibit types DA-IB, DA-IA-IB, IB and IA-IB, and strains from  
123 Japan, Indonesia, Thailand and Vietnam exhibit only type DA-IB (Romero et al., 2012; Tan et  
124 al., 2016b). There is a tendency for the ratio of IB to DA to be higher in isolates from the

southernmost areas of eastern Asia (Kotaki et al., 2008). These, and other isomers, have previously been found in shellfish extracts and the red alga *Chondria armata* (reviewed in La Barre et al., 2014), but *Pseudo-nitzschia* species and *Nitzschia bizertensis* (Section 3) also contain isomers. Isodomoic acid was first reported in some strains of *P. australis* (Rhodes et al., 2003). *Pseudo-nitzschia seriata* produces IA and IB (Hansen et al., 2011), and these isomers make up 5–6% of the total DA in *P. multiseries* and 4–12% in *P. cf. delicatissima* (Kotaki et al., 2008). IA and IB are minor components of *N. bizertensis* (Bouchouicha Smida et al., 2014) (Section 3.1). So far, it appears that there is more heterogeneity in DA isomers from *N. navis-varingica* than from *Pseudo-nitzschia* or *N. bizertensis*, but this may reflect the restricted information on species other than *N. navis-varingica*.

Like *Pseudo-nitzschia*, there is strain-specificity in toxin production for *N. navis-varingica*. Although most strains produce DA, IA or IB, non-toxic strains have been reported from the Philippines (Kotaki et al., 2005, 2006) and Malaysia (Suriyanti and Usup, 2015). Likewise, the toxin profile of *N. navis-varingica* has been shown to be a stable character, as identical profiles were detected in both parent and daughter strains (Romero et al., 2011), suggesting a genetic control of toxigenesis. However, the genetic basis of DA production in both toxicogenic *Pseudo-nitzschia* and *Nitzschia* species remains to be elucidated.

There are few studies on the physiology and DA production of *N. navis-varingica*. In batch culture experiments, as for most *Pseudo-nitzschia* species, DA is produced during the late exponential phase and the maximum cellular DA level is during the early stationary phase (Kotaki et al., 2000). Maximum cellular DA has, however, also been observed during the lag phase of batch cultures, when cell division is stalled, as has also been observed in *P. multiseries*. The toxin profile remained constant throughout the growth phases, but the composition changed (Tan et al., 2016b). As in toxicogenic *Pseudo-nitzschia* species (Lelong et al., 2012a, 2014) (Section 9.2.5), bacterial communities in cultures of *N. navis-varingica* can influence DA production (Kotaki et al., 2000, 2008). Furthermore, bacteria may also influence its toxin profile (Kotaki et al., 2008), as a change in toxin profile from DA-IB to IA-IB was found once the strain was made axenic (Romero et al., 2008).

153

### 154 **3. *Nitzschia bizertensis***

#### 155 *3.1. Morphology, distribution, toxicity*

156        The second DA-producing *Nitzschia* species was discovered in the Bizerte Lagoon  
157 (Mediterranean Sea, Tunisia) and isolated during a presumed *Pseudo-nitzschia* bloom, where DA  
158 was detected in shellfish (Bouchouicha Smida et al., 2014, 2015b). The cells were originally  
159 considered to belong to *Pseudo-nitzschia*, due to the morphologically similar slender and linear-  
160 lanceolate cells with two chloroplasts, one in each end (Bouchouicha Smida et al., 2014).  
161 Detailed electron microscopic and phylogenetic analyses, however, revealed it to be a *Nitzschia*  
162 species, although not closely related to *N. navis-varingica* (Section 2). This suggests either that  
163 the ability to produce DA evolved several times during evolution and/or was lost multiple times,  
164 or simply that it has not been explored in great detail in other *Nitzschia* species (Bouchouicha  
165 Smida et al., 2014).

166        The cellular toxin content of *N. bizertensis* is similar to that of several *Pseudo-nitzschia*  
167 species that produce lower amounts of DA (Trainer et al., 2012). In batch cultures, levels were  
168 1.3–9.7 ng DA l<sup>-1</sup>, corresponding to 7–36 fg DA cell<sup>-1</sup>, analyzed using ELISA tests, FMOC-LC-  
169 FLD or LC-MS/MS (Bouchouicha Smida et al., 2014; Sakka Hlaili et al., 2016). Similar to *N.*  
170 *navis-varingica*, *N. bizertensis* also produces IA and IB, although they make up a minor  
171 component (< 0.6%) of the toxin profile. For example, in strain BD2, the relative amounts of  
172 DA, IA and IB were 0.2, 0.05 and 0.02 fg cell<sup>-1</sup>, respectively (Bouchouicha Smida et al., 2014).  
173 As in several *Pseudo-nitzschia* species and in *N. navis-varingica*, some strains of *N. bizertensis*  
174 do not produce detectable levels of DA or isodomoic acid.

175        The species has so far only been recorded from the Bizerte Lagoon, where it was found  
176 throughout the year and at several locations (in the lagoon and in the canal leading to the  
177 lagoon), at temperatures of 14–28 °C and salinities of 34–40 (Bouchouicha Smida et al., 2014). It  
178 might be distributed more broadly, but because light microscopy cannot separate it from *Pseudo-*  
179 *nitzschia*, electron microscopy or molecular data are required to determine its distribution.

180        *Nitzschia bizertensis* has bloomed frequently in the lagoon (Bouchouicha-Smida et al.,  
181 2015b; Sakka Hlaili et al., 2016). During a bloom in August 2008, *N. bizertensis* reached  
182 densities up to 3 x 10<sup>6</sup> cells l<sup>-1</sup>, contributing 45% of the total phytoplankton abundance, and toxic  
183 strains were isolated (Bouchouicha-Smida et al., 2015b). In at least one incidence, toxic *N.*  
184 *bizertensis* strains were isolated at the same location and time that mussels (*Mytilus*  
185 *galloprovincialis*) contained DA (Bouchouicha-Smida et al., 2015b). Otherwise, when DA was  
186 found in mussels and oysters (*Ostrea edulis*) at 0.25–0.86 and 0.42–1.04 µg DA g<sup>-1</sup> tissue,

187 respectively, it was hypothesized, but not confirmed, that *N. bizertensis* was the culprit organism.  
188 Currents in the lagoon are considered to play a key role in transporting blooms of toxic *N.*  
189 *bizertensis* to shellfish areas (Bouchouicha-Smida et al., 2015b). It may also be spread during  
190 transfer of shellfish, as *N. bizertensis* has been shown to survive and be capable of producing DA  
191 after being filtered by mussels and ejected in biodeposits (Bouchouicha-Smida et al., 2015a).

192

#### 193 **4. *Pseudo-nitzschia* spp.**

##### 194 *4.1. New species*

195 Lelong et al. (2012a) reported 37 species of *Pseudo-nitzschia*, of which 14 were  
196 toxigenic; eight species had not yet been tested for the ability to produce DA. Since then, 15 new  
197 species have been described: *P. abreensis*, *P. arctica*, *P. batesiana*, *P. bipertita*, *P. bucculenta*, *P.*  
198 *circumpora*, *P. fukuyoi*, *P. hallegraeffii*, *P. kodamae*, *P. limii*, *P. lundholmiae*, *P. nanaoensis*, *P.*  
199 *plurisecta*, *P. sabit* and *P. simulans*. This brings the total to 52 species, plus a new variety (*P.*  
200 *cuspidata* var. *manzanillensis*; Rivera-Vilarelle et al., 2018). Of the total, 26 (51%) species are  
201 toxigenic (Table 3), although 24 of them have strains that did not produce detectable DA; some  
202 of these were even isolated from the same locations. For example, only one strain out of five of  
203 the newest species, *P. simulans*, produced low amounts of DA (1.05–1.54 fg DA cell<sup>-1</sup>); they  
204 were all isolated from Chinese waters, although at different locations and seasons (Li et al.,  
205 2017). This is the first toxigenic diatom species reported from Chinese waters. As for *N. navis-*  
206 *varingica* and *N. bizertensis*, it may turn out that some of the species that are non-detectable for  
207 DA may in fact produce isomers of DA, as these have so far only been tested for in *P. australis*,  
208 *P. multiseries*, *P. seriata* and *P. cf. delicatissima* (Section 2.2). Several species that have been  
209 reported to be exclusively toxic or non-toxic have had only a few strains tested. Perhaps all  
210 strains of *Pseudo-nitzschia* species will eventually prove to be DA producers, given proper  
211 conditions; elucidation of the biosynthesis pathway for DA, development of molecular probes  
212 targeting involved genes, and further genomic investigations will help to address this. Five  
213 species (*P. antarctica*, *P. prolongatoides*, *P. pungiformis*, *P. roundii* and *P. sinica*) have yet to be  
214 tested for their ability to produce DA (Table 3). Indeed, there is no physiological nor molecular  
215 information about any of these species, only about their distribution (Lelong et al., 2012a).  
216 Updated information on DA-producing species is reported in the IOC UNESCO Taxonomic  
217 Reference List of Harmful Micro Algae (Lundholm, 2017) and Wikipedia

218 (<https://en.wikipedia.org/wiki/Pseudo-nitzschia>), where newly described species can also be  
219 found.

220 Some of the new species are the result of continued detailed morphological and  
221 molecular studies of species in the *delicatissima* and *pseudodelicatissima* complexes, as also  
222 reviewed in Lelong et al. (2012a). For example, the following new species and one new variety  
223 belong to the *pseudodelicatissima* complex and generally have similar morphological and  
224 molecular characteristics: *P. arctica* (Percopo et al., 2016), *P. abreensis*, *P. plurisecta* (Orive et  
225 al., 2013), *P. cuspidata* var. *manzanillensis* (Rivera-Vilarelle et al., 2018), *P. batesiana*, *P.*  
226 *fukuyoi*, *P. lundholmiae* (Lim et al., 2013), *P. bipertita*, *P. limii* (Teng et al., 2016), *P.*  
227 *circumpora* (Lim et al., 2012a) and *P. kodamae* (Teng et al., 2014a). The only new species that  
228 belongs to the *delicatissima* complex is *P. sabit* (Teng et al., 2015).

229

#### 230 4.2. World distribution

231 Supporting previous microscopy findings that the *Pseudo-nitzschia* genus is  
232 cosmopolitan (Hasle, 2002), a recent global metabarcoding study (targeting V9-18S rRNA),  
233 found this genus to account for 4.4% of the diatom ribotype sequences and the highest number of  
234 operational taxonomic units (OTUs) amidst the pennate diatoms worldwide (Malviya et al.,  
235 2016). All reports of *Pseudo-nitzschia* species since the reviews by Lelong et al. (2012a) and  
236 Trainer et al. (2012), even if the species had previously been reported at a location, are  
237 summarized in Table 3 and mapped in Fig. 2. Given the prevalence of electron microscopy and  
238 molecular methods for species identification (Section 10), we did not consider any of the recent  
239 publications that identified *Pseudo-nitzschia* species based only on light microscopy. Table 4 is a  
240 subset of Table 3 and lists new records for *Pseudo-nitzschia* spp. in different countries. Note that  
241 previous reports of some species in Australia are erroneous, such that *P. turgidula* is actually *P.*  
242 *dolorosa* (Ajani et al., 2016) and *P. pseudodelicatissima* is now *P. cuspidata* (Ajani et al., 2013b,  
243 2016).

244 The number of locations around the world where *Pseudo-nitzschia* species have been  
245 reported for the first time has increased since the previous reviews. These include new areas of  
246 the Arctic (Baffin Bay, Barrow Strait, Chukchi Sea), Angola, Pakistan, Singapore, and Ukraine.  
247 Most of the new knowledge comes from Malaysia, where a concerted effort, using transmission  
248 electron microscopy (TEM) and molecular methods, has described eight new species (Tables 3,

249 4): *P. batesiana*, *P. bipertita*, *P. circumpora*, *P. fukuyoi*, *P. kodamae*, *P. limii*, *P. lundholmii* and  
250 *P. sabit*. An additional 22 *Pseudo-nitzschia* species have been reported in Malaysian waters. It  
251 should be pointed out that it is challenging to distinguish closely related pseudo-cryptic species  
252 in the *delicatissima* and *pseudodelicatissima* complexes based on morphology only. For  
253 example, in Malaysian waters, it cannot yet be confirmed if *P. arenysensis* or *P. delicatissima* is  
254 present (Teng et al., 2013). Due to general taxonomical problems, the identity of *P. cuspidata*  
255 versus *P. pseudodelicatissima* requires further study, as they are morphologically and genetically  
256 (ITS rDNA) similar and presently not delineated. Likewise, *P. delicatissima* could not be  
257 distinguished from *P. cf. arenysensis* in the Adriatic Sea (Pugliese et al., 2017) and in Morocco  
258 (Mediterranean Sea) (Rijat Leblad et al., 2013).

259 Other countries with new reports of *Pseudo-nitzschia* species since Lelong et al. (2012a)  
260 and Trainer et al. (2012) include (Table 4): China (16 species); Morocco (12 species); Italy (8  
261 species); Australia (7 species); Japan (7 species); Pacific Mexico (7 species); Spain (7 species);  
262 Tunisia (7 species); Namibia (6 species); Singapore (6 species); Angola (5 species); Philippines  
263 (5 species); Turkey (4 species); Ukraine (4 species); Greece (3 species); Tunisia (3 species);  
264 North Sea (2 species); Argentina (2 species); Uruguay (2 species); US west coast (2 species),  
265 plus several other countries with one species (Table 4).

266 Thus, *Pseudo-nitzschia* species are now found in all oceans of the world (Fig. 2),  
267 including the Arctic and Antarctic. One of the newest species is *P. arctica*, from the Barrow  
268 Strait, Beaufort Sea, Baffin Bay and Greenland (Percopo et al., 2016). Previously, toxicogenic *P.*  
269 *seriata* and *P. obtusa* were documented from the Arctic (west coast of Greenland) (Hansen et al.,  
270 2011, Harðardóttir et al., 2015). Tested strains of *P. arctica* proved negative for DA, but the  
271 Arctic is becoming more accessible due to global warming, so the presence of toxic diatoms  
272 must be followed with vigilance (Lefebvre et al., 2016). In the meantime, virtually nothing is  
273 known about the Antarctic species *P. antarctica*, including its potential toxicity. Another  
274 Antarctic species, *P. subcurvata*, tested negative for DA (Fryxell et al., 1991; Kang et al., 1993).  
275 Such information is essential, given the importance of the food webs within polar ecosystems.

276 The world distribution of the cosmopolitan species *P. pungens* (Hasle, 2002), and its  
277 morphological varieties, is becoming clearer. Based on scanning electron microscopy (SEM),  
278 observations of the valve structure and cingular bands, Villac and Fryxell (1998) revealed a new  
279 variety of *P. pungens* from Monterey Bay, California, i.e. *P. pungens* var. *cingulata*. A decade

280 later, *P. pungens* var. *aveirensis* was described from the coastal lagoon of Ria de Aveiro  
281 (Portugal), based on differences in valve width, densities of striae, fibulae, poroids and  
282 morphology of the girdle band striae (copulae), as well as molecular differences (Churro et al.,  
283 2009). Thus, the originally described *P. pungens* was designated as the nominal variety, i.e. *P.*  
284 *pungens* var. *pungens*. Insights into the evolution and phylogeny of the varieties of *P. pungens*  
285 have since been inferred from the nuclear-encoded large subunit (LSU) ribosomal DNA gene,  
286 the internal transcribed spacer (ITS) region of rDNA, the secondary structure of the ITS2  
287 transcript, and the chloroplast-encoded *rbcL* gene (summarized in Lim et al., 2014b).

288 Using the ITS rDNA region, Casteleyn et al. (2008, 2010) recognized three clades of *P.*  
289 *pungens*: clade I (var. *pungens*) has a cosmopolitan distribution, found in the North Sea, the  
290 eastern and western North Atlantic (Spain, Canada), the NW and South Pacific (Japan, New  
291 Zealand) and the NE Pacific (Washington State), and now elsewhere (Tables 3, 4); clade II (var.  
292 *cingulata*) was only found in the NE Pacific (California, Washington State), and now elsewhere  
293 (Tables 3, 4); and clade III (var. *aveirensis*) was found in geographically widely separated areas  
294 (Vietnam, China, Gulf of Mexico), and now elsewhere (Tables 3, 4). Evidence from  
295 morphological and mating studies supports these clades. Further studies on the ITS region  
296 revealed that clade III comprised strains resembling *P. pungens* var. *pungens* (which is also  
297 found in clade II) and var. *aveirensis*, and confirmed that hybrids between *P. pungens* var.  
298 *pungens* and var. *cingulata* co-occurred in clades I and II (Lim et al., 2014b; Kim et al., 2018).  
299 Later, three subgroups of clade III were distinguished, with populations reported for the first time  
300 in Korean coastal waters and the East China Sea, as well as in the Philippines (Kim et al., 2015,  
301 2017, 2018). Furthermore, physiological differences among the clades were found with respect  
302 to salinity and temperature tolerances, providing support for differences in their global  
303 distribution (Kim et al., 2015, 2018). Thus, ecological barriers (e.g. differences in water  
304 temperature, salinity) could have allowed subsequent genetic differentiation of the populations of  
305 var. *pungens* north and south of the tropics, perhaps connected by the thermohaline circulation of  
306 the global “conveyor belt” (Lim et al., 2014b). This hypothesis is supported by the finding of a  
307 hybrid zone for clades I and III in southern coastal waters of Korean (Kim et al., 2018). Another  
308 hypothesis for the global diversification of *P. pungens* could be the formation of the Isthmus of  
309 Panama ~3 Mya, which could have separated *P. pungens* into Atlantic and Pacific populations

310 (Lim et al., 2014b). The sterol and fatty acid composition of *P. pungens* was described by Stonik  
311 et al. (2016).

312 Since the review by Lelong et al. (2012a), molecular and morphological information has  
313 identified *P. pungens* var. *pungens* (clade I) on the Catalan coast of Spain (Quijano-Scheggia et  
314 al., 2010), in the Adriatic Sea (Penna et al., 2013), Australia (Ajani et al., 2013b), Brazil  
315 (Cavalcante, 2011), Greece (Moschandrou et al., 2012), Korea, East China Sea (Kim et al.,  
316 2015, 2018) and Malaysia (Lim et al., 2012b,c; Teng et al., 2014b; Tan et al., 2015) (Table 4).  
317 *Pseudo-nitzschia pungens* var. *cingulata* (clade II) had previously been identified in Monterey  
318 Bay (Villac and Fryxell, 1998), Brazil (Fernandes and Brandini, 2010) and Washington State  
319 (Kim et al., 2015). It is now also present in the Lüderitz upwelling cell and the northern  
320 Benguela upwelling system of Namibian waters (Gai et al., 2018). *Pseudo-nitzschia pungens* var.  
321 *aveirensis* (clade III) has now been found in Korea, East China Sea, Philippines (Kim et al.,  
322 2015, 2017, 2018), Malaysia (Lim et al., 2014b), Mexico (Pacific) (Rivera-Vilarelle et al., 2013),  
323 Morocco (Mediterranean Sea) (Rijat Leblad et al., 2013) and Turkey (Black Sea) (Baytut, 2013;  
324 Baytut et al., 2013) (Table 4).

325 Few toxicity studies have indicated which variety of *P. pungens* was used, making it  
326 difficult to draw conclusions about the toxicity of varieties. Nevertheless, var. *pungens* is the  
327 only variety that was shown to contain strains that are both non-toxic (Lim et al., 2010, 2014b;  
328 Quijano-Scheggia et al., 2010) and toxic, although only one out of nine strains of var. *pungens*  
329 from Greek coastal waters was toxigenic (Moschandrou et al., 2012). The only study of clade II  
330 *P. pungens* var. *cingulata* (Villac and Fryxell, 1998), and three studies of clade III *P. pungens*  
331 var. *aveirensis* (Churro et al., 2009; Lim et al., 2010, 2014b), showed that these varieties did not  
332 produce DA (Table 3). Several studies have examined the population structure of this species  
333 (Section 8.2).

334 Several reports of *Pseudo-nitzschia* spp. were not considered because no information at  
335 the species level was provided (e.g. Downes-Tettmar et al., 2012; Widiarti et al., 2013; Rachman  
336 and Thoha, 2014; Timmerman et al., 2014; Bazzoni et al., 2015, 2016; Al Muftah et al., 2016;  
337 Shanks et al., 2016; Belhaouari et al., 2017; Drakulović et al., 2017; Dursun et al., 2017; Louw et  
338 al., 2017). Likewise, we did not include publications that categorized field samples based on cell  
339 width: *seriata* group (>3 µm width) and *delicatissima* group (<3 µm width) (see Lelong et al.,

340 2012a). This categorization is not useful scientifically, as it gives no species-specific information  
341 and several species have a valve width that overlaps 3  $\mu\text{m}$ .

342

## 343 **5. Unusual new events**

### 344 *5.1. Gulf of Mexico*

345 *Pseudo-nitzschia* blooms occur commonly in the Gulf of Mexico and associated estuarine  
346 waters, often in spring or fall, and can reach cell concentrations up to  $10^7 \text{ cells l}^{-1}$  (reviewed in  
347 O'Dea, 2012; Lelong et al., 2012a; Trainer et al., 2012; Parsons et al., 2013;  
348 <http://myfwc.com/research/redtide/monitoring/database/>). At least 17 species have been  
349 identified in US and Mexican waters (Delaney, 2010; Teng et al., 2015).

350 While blooms in coastal embayments in Alabama and in the eastern Gulf of Mexico are  
351 thought to be linked to anthropogenic nutrient inputs (reviewed in Trainer et al., 2012), little is  
352 known about the mechanisms underlying blooms in Florida (O'Dea, 2012), nor in other coastal  
353 and possible offshore regions. This is due to the near ubiquitous presence of multiple *Pseudo-*  
354 *nitzschia* species in monitoring samples, the occurrence of blooms in geographically distant and  
355 environmentally distinct locations across the Gulf of Mexico and, accordingly, the prohibitive  
356 costs associated with monitoring species and toxicity.

357 In June 2009, a toxic bloom dominated (>90%) by *P. subfraudulenta* and other species  
358 (Liefer et al., 2013) occurred along Alabama's shoreline, reaching  $5.05 \times 10^6 \text{ cells l}^{-1}$ , with up to  
359  $0.54 \mu\text{g l}^{-1}$  particulate DA and  $3.34 \mu\text{g l}^{-1}$  dissolved DA measured in seawater, with trophic  
360 transfer to fish documented. Interestingly, except for Teng et al. (2016), *P. subfraudulenta* is not  
361 known to be toxic (Table 3). Previously (May 2005), higher DA levels ( $8.03 \mu\text{g l}^{-1}$ ) were  
362 observed along Alabama's coast, although the *Pseudo-nitzschia* species was not reported  
363 (MacIntyre et al., 2011). Another bloom ( $4.31 \times 10^7 \text{ cells l}^{-1}$ ) in southwest Florida, in October  
364 2009, coincided with particulate and total DA seawater concentrations of up to 10.9 and  $47.0 \mu\text{g}$   
365  $\text{l}^{-1}$ , respectively (O'Dea, 2012), although the species composition was again not determined.

366 Prior to 2013, all Gulf of Mexico shellfish tested were below the regulatory limit for DA  
367 ( $20 \mu\text{g g}^{-1}$  tissue). Then, three closures occurred due to DA: May–July 2013, October–December  
368 2014, and July–September 2017, all in Saint Joseph Bay, Florida, a small embayment in  
369 Florida's Panhandle region. In late April 2013, high *Pseudo-nitzschia* cell densities ( $1.4 \times 10^7$   
370  $\text{cells l}^{-1}$ ) were observed, and HPLC screening of eastern oysters (*Crassostrea virginica*) collected

371 on 6 May revealed 76 µg DA g<sup>-1</sup> tissue (Table 5) (almost 4-fold higher than the action limit),  
372 initiating the first closure due to DA (O'Dea et al., 2013). Other shellfish species, including bay  
373 scallops (*Argopecten irradians*) and pen shells (*Atrina rigida*) collected on 14 May, also  
374 exceeded the action limit (28.2 µg DA g<sup>-1</sup> and 52.1 µg DA g<sup>-1</sup> tissue, respectively) during this  
375 event (Table 5). Electron microscopy (SEM and TEM) and genetic analyses (ITS rRNA  
376 sequencing and ARISA [Automated Ribosomal Intergenic Spacer Analysis]; Section 10.2.2.2)  
377 indicated a near monospecific bloom of *P. cuspidata*. The bloom declined in late May, shellfish  
378 toxin concentrations fell below the action limit by late June, and shellfish harvest areas reopened  
379 in early July. High *Pseudo-nitzschia* spp. cell counts (>6 x 10<sup>6</sup> cells l<sup>-1</sup>) were again observed in  
380 late October 2014. Subsequent testing of eastern oysters (22 µg DA g<sup>-1</sup> tissue) resulted in a  
381 second closure, starting on 23 October and ending in early December. Genetic testing (ARISA,  
382 ITS1 sequencing) indicated that a mixed species assemblage, including *P. cuspidata*, was  
383 observed at the time of shellfish collection (Hubbard et al., 2015). A third closure in Saint Joseph  
384 Bay started in July 2017, when the *Pseudo-nitzschia* cell abundance reached 4.0 x 10<sup>6</sup> cells l<sup>-1</sup>,  
385 and whole scallops and pen shells tested over the regulatory limit (maximum levels observed by  
386 LC-MS/MS were 61 µg DA g<sup>-1</sup> and 663 µg DA g<sup>-1</sup>, respectively; unpubl. data). Using ARISA  
387 and SEM, a mixed *Pseudo-nitzschia* species assemblage, dominated by a member of the *P.*  
388 *pseudodelicatissima* species complex, was identified during the early stages of the closure  
389 (Hubbard et al., 2017). Toxin levels in scallops then decreased to <20 µg DA g<sup>-1</sup>, permitting a  
390 recreational scallop season opening by 23 September. However, the area remained closed to  
391 commercial harvests until November 2017, due to the persistence of higher DA concentrations in  
392 pen shells.  
393

### 394 5.2. Southeast Florida

395 Between November 2014 and April 2015, strandings of small juvenile green sea turtles  
396 (*Chelonia mydas*) increased across southeast Florida (Flewelling et al., 2015). Most affected  
397 individuals displayed neurological symptoms. As part of the investigation, >50 turtles were  
398 analyzed for HAB toxins. DA was detected in most turtles that had died and in some of the live  
399 turtles. Concentrations ranged from very low (<1 ng DA ml<sup>-1</sup> plasma) to a maximum of 29 µg  
400 DA g<sup>-1</sup> in intestinal contents (Table 5). Intestinal and fecal material was further examined for a  
401 subset of four DA-positive turtles, using a combination of light and electron microscopy (SEM)

402 and genetic approaches (DNA sequencing/ARISA). Although few pennate cells resembling  
403 *Pseudo-nitzschia* were observed by microscopy (and none definitively confirmed), genetic  
404 approaches confirmed the presence of *P. cuspidata*, as well as other *Pseudo-nitzschia* spp.  
405 (unpubl. data).

406

#### 407 5.3. North American west coast bloom

408 In 2015, an unprecedented toxic *Pseudo-nitzschia* bloom event occurred along the North  
409 American west coast. This bloom was unique in that it was spatially extensive and long-lasting.  
410 It was initiated nearly synchronously along the entire coast in late spring/early summer (April–  
411 June), with the onset of seasonal upwelling, and endured through the end of 2015 (Du et al.,  
412 2016; McCabe et al., 2016; McKibben et al., 2017; Ryan et al., 2017). Both DA toxicity and cell  
413 abundance peaked at the beginning of the event when toxic *P. australis* dominated (Bowers et  
414 al., 2015; McCabe et al., 2016). Cell and DA concentrations, as well as cellular DA quotas, were  
415 anomalously high given prior regional observations (Bowers et al., 2015; Smith et al., 2015;  
416 Ryan et al., 2017). For example, particulate DA exceeded previously reported values in  
417 California ( $31.7 \mu\text{g DA l}^{-1}$  associated with  $>2 \times 10^6 Pseudo-nitzschia$  cells  $\text{l}^{-1}$  in Monterey Bay).  
418 After the peak, cell and/or toxin concentrations varied, as did species assemblages, with some  
419 communities dominated by *P. fraudulenta* or the *P. pseudodelicatissima* complex. Nevertheless,  
420 toxic species (including *P. australis*) and persisting DA concentrations were observed through  
421 November in some locations (e.g. Bowers et al., 2015; Du et al., 2016, 2018; Haigh et al., 2016;  
422 Johnson et al., 2016; McCabe et al., 2016; Russell, 2016; Ryan et al., 2017).

423 The cause of such a spatially and temporally extensive and unusually toxic bloom is  
424 undoubtedly complex, and has been examined both locally and at the regional scale (Cavole et  
425 al., 2016; Du et al., 2016; McCabe et al., 2016; Ryan et al., 2017; Zhu et al., 2017b;  
426 <https://www.nationalgeographic.com/magazine/2016/09/warm-water-pacific-coast-algae-nino/>).  
427 This event occurred during a northeast Pacific warm anomaly (late 2013 to 2016), when surface  
428 water temperatures were on average  $2.5^\circ\text{C}$  warmer (nicknamed “the Blob”) (Cavole et al., 2016;  
429 Di Lorenzo and Mantua, 2016; McCabe et al., 2016; Gentemann et al., 2017) and nutrient  
430 concentrations (silicic acid and nitrate) in surface and subsurface waters were ~50% lower than  
431 long-term regional means (Ryan et al., 2017). *Pseudo-nitzschia australis* isolates collected  
432 during the event were able to grow ( $\sim 0.5\text{--}1.5 \text{ doublings d}^{-1}$ ) across a broad temperature range (4–

433 20 °C), and displayed maximum growth at 17–18 °C (McCabe et al., 2016). However, the onset  
434 of the 2015 bloom, as well as the peak bloom toxicity, occurred in cooler waters (<14 °C)  
435 associated with the transition to upwelling conditions (McCabe et al., 2016; Ryan et al., 2017)  
436 that temporarily eroded much of the warming signature related to the multi-year anomaly (Haigh  
437 et al., 2016; Gentemann et al., 2017). Hotspots for bloom initiation along this coast include  
438 offshore sites and/or subsurface layers (Kudela et al., 2015), although lower DA concentrations  
439 were found in San Francisco Bay (Peacock et al., 2018). The intermittent weak to moderate  
440 upwelling that followed bloom initiation in April and May was suspected to play a role in the  
441 subsequent blooms and/or the maintenance of toxic *Pseudo-nitzschia* species and toxicity in  
442 nearshore waters (i.e. where bivalve resources also commonly occur) throughout the summer and  
443 fall (Du et al., 2016; McCabe et al., 2016; Ryan et al., 2017).

444 High *P. australis* cell counts ( $10^5$ – $10^6$  cells L<sup>-1</sup>) observed during peak toxicity were  
445 associated with some of the highest cellular DA quotas reported for California (with a maximum  
446 of >100 pg DA cell<sup>-1</sup>; Ryan et al., 2017). Ryan et al. (2017) further explored a 24-year record of  
447 nutrient stoichiometry in Monterey Bay to better understand potential mechanisms underlying  
448 bloom toxicity. Amidst lower nutrient concentrations observed during the 2014–2016 warm  
449 anomaly, the most severe depletion of silicic acid relative to nitrate was observed in 2015, and in  
450 particular, during the most toxic part of the bloom in April–June 2015. Other laboratory and field  
451 studies have similarly shown that cellular DA quotas increase for *Pseudo-nitzschia* isolates  
452 grown under silicic acid-limited conditions (reviewed in Lelong et al., 2012a; Trainer et al.,  
453 2012). Other local to regional features likely played a role in setting the stage for anomalous  
454 nutrient stoichiometry prior to the onset of toxicity in 2015, such as early season diatom blooms  
455 and stratification prior to the onset of upwelling along the Oregon and Washington coast (Du et  
456 al., 2016). Based on microscope observations, Du et al. (2016) also suspected that a sexual event  
457 occurred during the first toxic bloom peak in May, which could have contributed to the high DA  
458 concentrations observed during 2015 event. This had been shown previously along the  
459 Washington coast for *P. australis* (Holtermann et al., 2010). The high cellular DA quotas  
460 observed during the bloom could therefore potentially be attributed to a combination of  
461 physicochemical conditions favorable for bloom development (e.g. anomalous temperatures;  
462 McKibben et al., 2017) and retention, as well as the anomalous nutrient stoichiometry prior to,  
463 during and following the onset of seasonal upwelling.

464       Trophic transfer of DA was extensively documented throughout and following the  
465 event's peak toxicity. Shellfish harvest closures from California (USA) to British Columbia  
466 (Canada) first occurred in late April and early May for razor clams (*Siliqua patula*), mussels,  
467 and/or other bivalves (Haigh et al., 2016; McCabe et al., 2016; <https://www.cdph.ca.gov/>). In  
468 June and July, as bivalve toxicity levels temporarily decreased in some areas (e.g. Haigh et al.,  
469 2016), closures were nevertheless expanded to include other recreational and commercial  
470 fisheries, including anchovies and sardines, as well as Dungeness crabs (*Cancer magister*) and  
471 rock crabs (*Cancer antennarius*). In Oregon, this represented the first DA-related closure to the  
472 Dungeness crab fishery (Du et al., 2016), which closed again in 2017  
473 (<http://www.oregon.gov/oda/programs/foodsafety/shellfish/pages/shellfishclosures.aspx>). The  
474 reopening of some fisheries was delayed into 2016 (Haigh et al., 2016;  
475 [http://wdfw.wa.gov/fishing/shellfish/razorclams/domoic\\_levels.html](http://wdfw.wa.gov/fishing/shellfish/razorclams/domoic_levels.html)), resulting in extensive  
476 economic losses and a federal disaster declaration in CA. Seabird and marine mammal strandings  
477 and mortalities started to occur in May (Di Liberto, 2015;  
478 [http://www.nmfs.noaa.gov/pr/health/mmume/large\\_whales\\_2015.html](http://www.nmfs.noaa.gov/pr/health/mmume/large_whales_2015.html)) and DA was detected in  
479 whales, dolphins/porpoises, harbor seals, fur seals, and sea lions (Table 5) (McCabe et al., 2016).  
480 DA poisoning was confirmed for sea lions from California to Washington State (McCabe et al.,  
481 2016), with seizures in sea lions reported in Washington State for the first time. A concurrent  
482 large whale Unusual Mortality Event (UME) declared for the Western Gulf of Alaska (USA)  
483 began in late May. A mortality of common murres (*Uria aalge*) occurred in California during  
484 August–October in California, resulting from starvation and secondarily from DA poisoning  
485 (Gibble et al., 2018); 24 out of 29 tested birds contained detectable levels of DA (Table 5). A  
486 die-off of common murres also occurred in Alaska in early June. Additional large whale  
487 mortalities in British Columbia and Alaska occurred through July and August, involving >30  
488 marine mammals representing at least four species of whales  
489 ([http://www.nmfs.noaa.gov/pr/health/mmume/faqs\\_2015\\_large\\_whale.html](http://www.nmfs.noaa.gov/pr/health/mmume/faqs_2015_large_whale.html)); no definitive cause  
490 of the UME was identified, although DA played a contributory role.  
491

#### 492 5.4. US east coast and Canadian blooms

493       There have been few closures due to DA on the east coast of North America since 2002,  
494 when shellfish harvesting in most of the southern Gulf of St. Lawrence (Canada) was closed

495 because of DA produced by *P. seriata* (reviewed in Trainer et al., 2012). Levels of DA  
496 exceeding the closure limit have nevertheless since been found in sea scallops (*Placopecten*  
497 *magellanicus*) on the Quebec shore of the Gulf of St. Lawrence in 2004, 2005 and 2008  
498 (Fernandes et al., 2014). The entire SW coast of New Brunswick, on the Bay of Fundy (Canada),  
499 to the US border was then closed for 10 days during August to September, 2008 (Fernandes et  
500 al., 2014). Unfortunately, the *Pseudo-nitzschia* species responsible for the DA was not  
501 determined.

502 Toxigenic *Pseudo-nitzschia* species continue to be found in the Gulf of Maine (e.g. Ellis,  
503 2009; Resedarits, 2011; Mogensen, 2014; Fernandes et al., 2014). Fernandes et al. (2014) and  
504 prior reports summarized therein identify 14 species of *Pseudo-nitzschia* (Table 3), including a  
505 novel form, *P. sp. GOM*, later classified by Teng et al. (2014a) as *P. plurisecta*. Isolates from  
506 seven regional species, including *P. plurisecta*, have been shown to produce DA (Table 3).  
507 Subsequent studies (Ellis, 2009; Resedarits, 2011; Mogensen, 2014; Hubbard et al., 2015)  
508 identified the persistence and succession of toxic species in the Gulf of Maine using a DNA  
509 fingerprinting approach (ARISA; Section 10.2.2.2) to characterize the *Pseudo-nitzschia*  
510 community in nearshore (2008, 2010, 2013) and/or offshore waters, including Georges Bank  
511 (2008, 2010). Particulate DA was confirmed in each study using the Biosense ASP ELISA, or  
512 the Jellett Scotia Rapid Testing ASP test kit and LC-MS/MS. Correlations were observed  
513 between toxigenic *P. seriata*, *P. plurisecta* and DA, as well as between environmental and  
514 biological factors and DA.

515 Although precautionary closures have occurred previously in the Gulf of Maine, based on  
516 rapid screening for DA in phytoplankton and/or shellfish tissue samples, the first closure  
517 associated with exceeding the action limit of 20 µg DA g<sup>-1</sup> tissue did not occur until 2016. In late  
518 September 2016, a harvest closure coincided with a recall of certain mussels, quahogs, clams and  
519 European oysters, issued by the Maine Department of Marine Resources (DMR), after shellfish  
520 samples from some areas exceeded 100 µg DA g<sup>-1</sup> of tissue (Lewis et al., 2017). The highest  
521 concentration of *Pseudo-nitzschia* (species not indicated) was 206,000 cells l<sup>-1</sup>, at Jonesport,  
522 Maine. In October 2016, the Canadian Food and Inspection Agency closed a number of shellfish  
523 harvesting areas on both sides of the Bay of Fundy (Canada) after DA levels reached 30 µg DA  
524 g<sup>-1</sup> tissue. The presence of *P. australis* was confirmed by SEM in Canada (J. Martin, pers.  
525 commun.) and by SEM and molecular (ITS1 rDNA sequencing) data in US waters (Tables 3, 4).

526 This is highly unusual, as this species has never before been reported on the east coast of North  
527 America. Also in October 2016, Massachusetts and Rhode Island implemented precautionary  
528 ASP closures, a first for both states. These closures were warranted based on the concurrent  
529 events in Maine/Canada, high counts of *Pseudo-nitzschia* cells, and positive DA results from  
530 phytoplankton and shellfish samples screened with the Scotia Rapid Testing ASP test kit,  
531 although values in shellfish were subsequently determined to be below the action limit. During  
532 the Rhode Island bloom (26 September to 24 October 2016), *Pseudo-nitzschia* spp. counts  
533 reached a maximum ( $2 \times 10^6$  cells l<sup>-1</sup>) in lower Narragansett Bay. A second toxigenic *Pseudo-*  
534 *nitzschia* bloom and shellfish closure occurred in Rhode Island during March 2017 (Borkman et  
535 al., 2017), when DA levels reached 32 µg DA g<sup>-1</sup> tissue  
536 ([http://www.providencejournal.com/news/20170315/clam-mussel-toxin-levels-decrease-but-](http://www.providencejournal.com/news/20170315/clam-mussel-toxin-levels-decrease-but-shellfishing-ban-remains-in-ri-waters)  
537 [shellfishing-ban-remains-in-ri-waters](#)) and *P. australis* was again observed (Hubbard et al.,  
538 2017).

539 For the second year in a row, sections of eastern Maine were again closed starting in mid-  
540 September 2017, because of elevated concentrations of DA  
541 ([http://www.pressherald.com/2017/09/14/algae-bloom-forces-suspension-of-shellfishing-in-](http://www.pressherald.com/2017/09/14/algae-bloom-forces-suspension-of-shellfishing-in-parts-of-down-east-maine/)  
542 [parts-of-down-east-maine/](#)). The DMR issued a recall of mussels harvested near Mount Desert  
543 Island between September 10–14, 2017 ([http://www.pressherald.com/2017/09/15/maine-issues-](http://www.pressherald.com/2017/09/15/maine-issues-mussel-recall-because-of-harmful-algae-bloom/)  
544 [mussel-recall-because-of-harmful-algae-bloom/](#)). Closures persisted into December 2017, and  
545 were then extended, for the first time, into western Maine, when DA concentrations  
546 briefly exceeded the quarantine level at some harvest sites  
547 (<http://www.pressherald.com/2017/12/06/toxic-bloom-closes-casco-bay-shellfish-industry/>). The  
548 closure in Casco Bay extended into 2018, and these recent events may be related to changing  
549 conditions in the Gulf of Maine ([https://www.smithsonianmag.com/smart-news/toxic-algae-](https://www.smithsonianmag.com/smart-news/toxic-algae-closes-important-maine-shellfish-region-180967753/)  
550 [closes-important-maine-shellfish-region-180967753/](#)).

551

## 552 **6. Domoic acid in the food web**

553 Since the review of Trainer et al. (2012), research has continued to reveal the extent to  
554 which DA produced by toxigenic *Pseudo-nitzschia* species enters the food web and impacts  
555 ecosystems. In particular, the toxic bloom on the west coast of North America in 2015 (Section  
556 5.3) allowed an examination of data from before, during, and after that event. The results suggest

557 that DA is ubiquitous in the food web during these massive blooms and as well as more  
558 “normal” periods (Kudela et al., 2017). This is one area of research showing a great number of  
559 discoveries, especially for marine mammals (Table 5). Interestingly, there are no new reports of  
560 DA in marine birds, whose contamination had previously been associated with behavioral  
561 changes and mortalities (Trainer et al., 2012).

562

### 563 *6.1. Molluscs and echinoderms*

564 Bivalve shellfish are the primary vector for potentially causing ASP in humans (Lelong et  
565 al., 2012a; Trainer et al., 2012). Thus, traditional food products, such as Mediterranean mussels  
566 (*Mytilus galloprovincialis*), brown mussels (*Perna perna*), Asian green mussels (*P. viridis*), pearl  
567 oysters (*Pinctada fucata*), black-scar oysters (*Crassostrea lugubris*), and surf clams (*Mesodesma*  
568 *donacium*) (Reizopoulou et al., 2012; Álvarez et al., 2015; Veschaisit et al., 2017) continue to be  
569 a source of DA contamination (Table 5). DA was also discovered in benthic scavengers (e.g.  
570 whelks, sea snails), as well as in benthic sea cucumbers and brittle stars (Reizopoulou et al.,  
571 2012) (Table 5); these are first reports of DA in those invertebrates. The finding of DA in seven  
572 primary and secondary consumer species in coastal waters of Alabama (Gulf of Mexico) is the  
573 first demonstration of trophic transfer of DA in that region (Liefer et al., 2013).

574 Previous reports of DA in cephalopods are reviewed in Lopes et al. (2013). Highest  
575 values are in the digestive gland (e.g. 75.91 µg DA g<sup>-1</sup> in the common cuttlefish *Sepia officinalis*;  
576 Lopes et al., 2018) (Table 5), but studies have found, for the first time, that DA can also  
577 accumulate in the brain, up to 0.29 µg DA g<sup>-1</sup> in the common cuttlefish (*Sepia officinalis*) and  
578 2.14 µg DA g<sup>-1</sup> in the common octopus (*Octopus vulgaris*) (Lopes et al., 2018) (Table 5). In  
579 contrast, there was no detectable DA in the brain nor digestive gland of myopsid and  
580 ommastrephid squids, which are pelagic and may be less prone to feed on contaminated fish and  
581 thus do not accumulate DA (Lopes et al., 2018). Cuttlefish and octopus, in contrast, are benthic  
582 and obtain their DA from consuming contaminated bivalve shellfish after blooms of toxic  
583 *Pseudo-nitzschia*. Furthermore, once these cephalopods accumulate DA, it is retained and not  
584 easily eliminated, contrary to what happens in most bivalve species. These animals may be able  
585 to tolerate chronic exposure, but given their complex central nervous system, further studies are  
586 needed to determine if they are neurally or behaviourally impaired by DA (Lopes et al., 2018).

587 DA was found for the first time in Humboldt squid (*Dosidicus gigas*) during a toxic  
588 *Pseudo-nitzschia* bloom (species not indicated) in the Southern California Bight (Mazzillo et al.,  
589 2011), and again a year later in British Columbia (Canada) (Braid et al., 2012) (Table 5). This  
590 secondary predator, sometimes commercially harvested, could have accumulated DA from fish  
591 that it preyed upon. It is possible that DA could have been responsible for strandings of this  
592 squid. The neurotoxin has also been reported, for the first time, in four species of wild-caught  
593 abalone in Australia (Malhi et al., 2014) (Table 5).

594

### 595 6.2. Fish

596 Although DA has previously been reported in fish (Trainer et al., 2012), this toxin has  
597 now been found in 16 new species (Table 5). Many are a food source for human consumption,  
598 including cod (*Gadhus morhua*) (Jensen et al., 2015). Although most fish were below the limit  
599 for preventing consumption (20 µg DA g<sup>-1</sup> tissue), others had elevated levels, e.g. the Pacific  
600 sardine (*Sardinops sagax*; 72.2 µg DA g<sup>-1</sup>, stomach; Stauffer et al., 2012) and plaice  
601 (*Pleuronectes*; 177.4 µg DA g<sup>-1</sup>, whole tissue; Jensen et al., 2015).

602

### 603 6.3. Marine mammals

604 After DA was first discovered in stranded California sea lions (*Zalophus californianus*) in  
605 1998, 11 other marine mammals were subsequently reported to be contaminated by the toxin  
606 (reviewed in Trainer et al., 2012). Its presence has continued to be documented in some of these,  
607 plus seven other marine mammals (Table 5). These include seals, walruses, sea otters, porpoises,  
608 dolphins and a variety of whales. The presence of DA in harbour seals (*Phoca vitulina*) in  
609 California (McHuron et al., 2013; Rust et al., 2014), Scotland (Jensen et al., 2015) and Alaska  
610 (Lefebvre et al., 2016) is a recent discovery. These findings are evidence that DA is becoming  
611 more pervasive in the oceans.

612 Exposure of marine mammals to DA could be the cause of the decline of some marine  
613 mammals, such as the southern right whale (*Eubalaena australis*; D'Agostino et al., 2015, 2017;  
614 Wilson et al., 2016), beluga whales (*Delphinapterus leucas*; Burek-Huntington et al., 2015) and  
615 harbor seals (*Phoca vitulina*; Jensen et al., 2015). DA intoxication could also be implicated in the  
616 mass strandings of cetaceans, e.g. in Tasmania (Australia) (Bengtson Nash et al., 2017). As in  
617 humans, the clinical signs indicative of DA toxicosis include disorientation, seizures, and

618 uncoordinated movements, which are supported by histopathology that reveals the presence of  
619 brain lesions in the hippocampus, consistent with DA toxicosis (e.g. McHuron et al., 2013).  
620 These behavioral changes, seizures and brain lesions can also be caused by a repetitive exposure  
621 to sub-lethal concentrations of the toxin (Cook et al., 2015). Because marine mammals are  
622 dynamic foragers that rely on flexible navigation, impaired spatial memory may affect their  
623 survival in the wild (Cook et al., 2015, 2016).

624 Information has now been gathered on the presence of DA in the blood, urine, feces, bile,  
625 milk, amniotic fluid, fetal meconium, stomach and tissues of these marine mammals (Table 5).  
626 There is thus evidence that DA can be transferred *in utero* to the developing fetus of marine  
627 mammals (McHuron et al., 2013; Lefebvre et al., 2016), as in rodents (Ramsdell and Gulland,  
628 2014). Likewise, DA in milk can be transferred to young suckling California sea lions (Rust et  
629 al., 2014). Nursing marine mammals may thus be exposed continually to a low dose of DA for  
630 the duration of their nursing period, resulting in cumulative effects.

631 Of particular concern is the presence of DA in 13 marine mammal species living in  
632 Alaskan Subarctic and Arctic ecosystems, much farther north than expected (Lefebvre et al.,  
633 2016) (Table 5; Fig. 3). Given the current trend of decreasing sea ice, warming ocean waters and  
634 increased light penetration into the water column, this will extend the season favorable for  
635 growth of toxicogenic *Pseudo-nitzschia* cells in the Arctic. This is expected to increase the risk to  
636 marine mammal health and may pose a risk to the local Arctic human populations that, to a large  
637 degree, depend on marine food sources.

638

## 639 **7. Sexual reproduction in *Pseudo-nitzschia***

640 In the last few decades, observations or detailed descriptions have been provided of the  
641 life cycle of 14 *Pseudo-nitzschia* species (Table 6) (reviewed in Lelong et al., 2012a; Trainer et  
642 al., 2012; Montresor et al., 2016). Almost all these species have a heterothallic mating system,  
643 i.e. two strains of opposite mating type must be co-cultured to induce the formation of sexual  
644 stages. As in most other diatoms, the sexual phase of *Pseudo-nitzschia* has the additional  
645 function of counteracting the progressive cell size reduction that occurs during mitotic division.  
646 A consequence of this size reduction is that the population will have a progressively lower  
647 average cell size (cell length in the case of *Pseudo-nitzschia*) and a progressively larger standard

648 deviation. The most common way that this miniaturization process can be counteracted is by  
649 undergoing sexual reproduction.

650       Laboratory experiments demonstrated that sex can only occur when cells are below a  
651 species-specific size threshold, and, in heterothallic species like *Pseudo-nitzschia*, when two  
652 strains of opposite mating type (Section 7.1) are placed in the same culture vessel. Successful  
653 sexual reproduction can also only be achieved, as documented in *P. multiseries*, *P.*  
654 *pseudodelicatissima* (Davidovich and Bates, 1998) and *P. multistriata* (Scalco et al., 2014),  
655 when crossing parental strains that are growing exponentially. This apparently differs from what  
656 was reported for unicellular microalgae belonging to other lineages, where sex is a response to  
657 stress conditions, e.g. nitrogen starvation in *Chlamydomonas* (Goodenough et al., 2007), and  
658 oxidative stress in *Volvox* (Nedelcu et al., 2004). Scalco et al. (2014) showed that sex also  
659 requires a threshold cell concentration to start, thus suggesting that in *P. multistriata* pheromones  
660 may mediate the perception of opposite mating types, as reported for two benthic diatoms (Sato  
661 et al., 2011; Gillard et al., 2013). Moreover, the onset of the sexual phase in *P. multistriata* was  
662 accompanied by vegetative cells stopping their growth (Scalco et al., 2014), as is also likely the  
663 case in other *Pseudo-nitzschia* species.

664

### 665 7.1. Sexual cycle

666       The general scheme of the sexual cycle in *Pseudo-nitzschia* species includes (Fig. 4): the  
667 pairing of gametangia and the formation of gametes following meiosis; the conjugation of  
668 gametes of opposite mating type; and the formation of diploid auxospores (two auxospores are  
669 produced by one pair of gametangia), as first described by Davidovich and Bates (1998).  
670 Auxospores do not have the rigid siliceous frustule and are thus capable of elongating, thanks to  
671 the production of circular perizonial bands. The large initial cell is produced within the  
672 auxospore, and when it exits, restores the large initial cell size.

673       Because there is no way to distinguish the cells or gametangia of opposite mating type,  
674 we ‘conventionally’ define them as follows (Montresor et al., 2016): mating type minus (MT–) is  
675 the strain whose gametangial frustule carries the auxospores. Mating type plus (MT+) produces  
676 two gametes that move by amoeboid action toward the other two sessile gametes in the MT–  
677 gametangium that aligns parallel to it. To identify the mating type of a set of clonal *Pseudo-*  
678 *nitzschia* strains, a matrix of crosses is carried out and successful sexual stages are recorded in

679 the crosses with the different mating types (e.g. Quijano-Scheggia et al., 2009; Lundholm et al.,  
680 2012). When crosses are carried out using strains with different cell size, it is possible to identify  
681 the strain that holds the auxospores, i.e. MT-. Exceptions to the heterothallic scheme are *P.*  
682 *brasiliiana* and *P. subcurvata*, in which the formation of sexual stages has been reported in a  
683 clonal strain, thus suggesting that these species have a homothallic mating system (Fryxell et al.,  
684 1991; Quijano-Scheggia et al., 2009).

685 Cytological details of the sexual cycle are available for *P. multistriata*, where nuclear  
686 staining allowed the progression of the two-step meiotic process to be visualized (Scalco et al.,  
687 2016). Meiosis occurred asynchronously in the two mating types, with MT+ starting earlier. In  
688 both mating types, one of the two nuclei originating from meiosis II degenerates, so that the final  
689 products of meiosis are two gametes per gametangium. Gamete formation takes about two hours  
690 to complete, while gamete conjugation is fast, being completed in 1–2 minutes. The pattern of  
691 chloroplast segregation has implications for the inheritance of plastid genomes, including the  
692 *rbcL* gene used for phylogenetic studies. In diatoms, chloroplast inheritance depends on their  
693 segregation pattern that occurs first during gamete formation and then during the first mitotic  
694 division of the initial cell. *Pseudo-nitzschia* species have two chloroplasts, and each gamete of *P.*  
695 *multistriata* inherits a copy of both chloroplasts from the gametangial cell. It follows that the  
696 auxospore has four chloroplasts (a copy of each chloroplast from the parental cells). Segregation  
697 during the first mitotic division of the initial cell was studied in *P. arenysensis* (reported as *P.*  
698 *delicatissima* in Levialdi Ghiron et al., 2008), where it was shown that chloroplasts segregate  
699 stochastically. Evidence was provided by the fact that, out of 96 F1 strains produced by the  
700 cross, 16 strains retained two paternal plastids, 20 had two maternal plastids and the remaining  
701 60 had one maternal and one paternal plastid. The study took advantage of sexually compatible  
702 *P. arenysensis* strains with two slightly different haplotypes for the plastid-encoded *rbcL* gene  
703 region. Recombination between plastids is rare but well documented in both plants and algae and  
704 has been reported in *P. multistriata* and *P. fraudulenta* (D'Alelio and Ruggiero, 2015).

705

## 706 7.2. Molecular aspects

707 The availability of the *P. multistriata* genome (Section 8.1), coupled with a  
708 transcriptomic approach, made it possible to study the signaling pathways employed in the early  
709 phases of sexual reproduction (Basu et al., 2017). Strains of opposite mating type were placed in

710 different compartments of an apparatus that allowed free exchange of the medium but no  
711 physical contact between cells. Flow cytometry analyses demonstrated that cell growth stopped  
712 during sexual reproduction. Changes in gene expression in the two mating types (MT+ and MT–)  
713 were demonstrated by analyzing their transcriptomes, compared to the control, in which they  
714 were grown in single culture. These changes were induced by the perception of putative  
715 chemical cues derived from the mating partner. Gene expression changes were stronger in MT–  
716 cells, suggesting that this mating type may be responsible for secreting a chemical signal related  
717 to the initiation of the sexual phase. Moreover, cells of both mating types in the experimental  
718 setup arrested their cell cycle in the G1 phase, a mechanism also recorded in yeast and in the  
719 benthic pennate diatom *Seminavis robusta* (Moeys et al., 2016). This was interpreted as a cell  
720 synchronization mechanism in response to pheromones. Cyclins and genes encoding nutrient  
721 transporters for silicate, ammonium, and nitrate/nitrite were also down-regulated in both mating  
722 types of *P. multistriata* once the sexual phase was initiated, suggesting that cells can modulate  
723 nutrient uptake during this time. Genes encoding for proteins of the cohesin complex and for  
724 meiosis (*Rad51-A1*, *Rad51-C*, *Smc5*, and *Smc*) were up-regulated, indicating preparation for  
725 meiosis (Patil et al., 2015). The expression of several genes specific for the two mating types  
726 highlighted the involvement of different pathways in response to pheromones. During the initial  
727 phases of sexual reproduction, a considerable percentage of differentially expressed genes were  
728 specific to *P. multistriata* (35%) or present only in Bacillariales, i.e. those genes were also  
729 present in the genomes of *P. multiseries* and *Fragilariaopsis cylindrus*. This is consistent with the  
730 evolution of species-specific mechanisms to attract and mate with the right partner, for those  
731 diatoms characterized by a heterothallic mating system.

732 A comparison with other diatom genomes showed that many genes differentially  
733 expressed during the early phases of sexual reproduction were predicted to be *P. multistriata*-  
734 specific (35%) or gene gain events in Bacillariales (20%) (Basu et al., 2017). An in-depth  
735 clustering of the *P. multistriata* genes with a broad range of prokaryotes and eukaryotes showed  
736 that most non-orphan genes differentially expressed during sex were specific to diatoms, a  
737 further proof of the uniqueness of the diatom life cycle.

738

739 *7.3. Field observations*

740 As illustrated above, sex is a fundamental phase of the life cycle of *Pseudo-nitzschia*.  
741 However, there is still limited evidence for the occurrence of sex in the natural environment.  
742 This is in part because of the infrequency of the event and the difficulty in identifying sexual  
743 stages in phytoplankton samples (Mann, 1988). Massive production of sexual stages by *P.*  
744 *australis* and *P. pungens* was reported along the Washington State coast (Holtermann et al.,  
745 2010). Mating cells of both species were attached to surf-zone diatoms, and auxospores and  
746 initial cells were recorded during the bloom that lasted about three weeks. At about the same  
747 time, a high percentage of sexual stages was reported during a bloom of *P. cf. delicatissima* and  
748 *P. cf. calliantha* in the Gulf of Naples (Mediterranean Sea) (Sarno et al., 2010). In both studies, a  
749 clear fingerprint of large-sized cells was also recorded in the natural population, providing  
750 further evidence that a sexual event had occurred. Indeed, cell size has been used as a proxy for  
751 the occurrence of sex in natural diatom populations (e.g. Mann, 1988). This approach was  
752 applied to *P. multistriata*, in which cell size has been measured over 10 years at the Long-Term  
753 Ecological Research (LTER) sampling station MareChiara in the Gulf of Naples, providing an  
754 example of a fine-tuned organization of the life cycle of this diatom (D'Alelio et al., 2010). This  
755 species has a distinctive, slightly sigmoid cell shape, so can be easily identified by light  
756 microscopy. It regularly blooms in summer-autumn and large-sized cohorts of cells were  
757 recorded every two years. The biennial recurrence of sex was further supported by the results of  
758 a modelling approach, in which the observed pattern was reproduced only when 1) cell growth  
759 rate varied through the year, and 2) cell concentration was still high. The model also predicted  
760 that *P. multistriata* would become locally extinct if sexual reproduction did not occur within four  
761 years.

762

## 763 **8. Genomic and population genetic approaches**

### 764 *8.1. Genomic insights into Pseudo-nitzschia*

765 The availability of ‘omic’ resources (genomic, transcriptomic, proteomic) and genomic  
766 tools for an increasing number of phytoplankton species allows one to address questions related  
767 to the molecular mechanisms that regulate their physiology, life cycle traits, ability to perceive  
768 environmental signals, and interactions with other components of the planktonic community (e.g.  
769 Lu et al., 2016; Moeys et al., 2016).

770           Genomic resources are now available for two *Pseudo-nitzschia* species that produce DA:  
771       1) *P. multiseries* (<http://genome.jgi.doe.gov/Psemu1/Psemu1.home.html>), for which the  
772       mitochondrial (Yuan et al., 2016) and plastidial (Cao et al., 2016) genomes are also available,  
773       and 2) *P. multistriata* (Basu et al., 2017) with a fully annotated nuclear genome and extensive  
774       transcriptomic datasets. Partial genomic sequencing is available for *P. australis* (Stone, 2017).  
775       The genome size of *P. multiseries* is 219 Mbp and 59 Mbp for *P. multistriata* (Basu et al., 2017).  
776       That of *P. australis* is even larger (~900 Mbp), based on the draft assembly, but includes  
777       bacterial reads and large repeated elements and heterozygous regions (Stone, 2017); further work  
778       is required to confirm this.

779           For *P. multistriata*, a protocol has been implemented for genetic transformation, using the  
780       biolistic method and fluorescent tagging. This has shown that the transgene can be transferred to  
781       the progeny after sexual crosses with a wild type strain (Sabatino et al., 2015). Transcriptomic  
782       resources for several *Pseudo-nitzschia* species have been produced within the Marine Microbial  
783       Eukaryote Transcriptome Sequencing Project (Keeling et al., 2014) (e.g. Patil et al., 2015), and  
784       will enable comparative approaches to assess functional differences (e.g. Di Dato et al., 2015).

785           The analysis of the sequenced genome of *P. multistriata* (Basu et al., 2017) showed the  
786       presence of conserved noncoding elements, which can have a regulatory function, and of  
787       transposable element activity. The study included an in-depth phylogenomic approach to assess  
788       the turnover of gene families among Stramenopiles and to identify putative horizontal gene  
789       transfer events from bacteria to diatoms.

790           A comparative study of five diatom genomes (*Phaeodactylum tricornutum*, *Thalassiosira*  
791       *pseudonana*, *Fragilaropsis cylindrus*, *Pseudo-nitzschia multistriata* and *P. multiseries*) and one  
792       transcriptome (*Seminavis robusta*) allowed the identification of 15 meiotic-specific genes in  
793       diatoms (Patil et al., 2015). A number of genes within the ‘meiosis toolkit’ of eukaryotes,  
794       including the disrupted meiotic cDNA (*DMC1*) gene (which is essential for meiotic  
795       recombination), could not be detected in the diatom genomes. Moreover, five out of the 15  
796       diatom-specific genes were recorded in all genomes, including those of *P. tricornutum* and *T.*  
797       *pseudonana*, species for which the sexual phase has not been reported. Gene expression analyses  
798       carried out during the meiotic phase of *S. robusta* and *P. multistriata* confirmed that five genes  
799       (*RAD21*, *SPO11-2*, *RAD51-A*, *RAD51-B* and *RAD51-C*) were upregulated during meiosis. These

800 genes may represent candidate markers to track the occurrence of sexual reproduction in the  
801 natural environment.

802 Analysis of the transcriptomes of *P. multistriata*, *P. delicatissima* and *P. arenysensis*  
803 revealed an overall similarity among these species but also interesting species-specific transcripts  
804 (Di Dato et al., 2015). An example is the detection of nitric oxide synthase (PmNOS) in *P.*  
805 *multistriata*, an enzyme reported for the first time in diatoms and whose predicted protein  
806 structure has domains identical to the metazoan NOS pathway. SLC6-sodium- and chloride-  
807 dependent amino acid transporters were recorded in toxigenic *P. multistriata*. These enzymes are  
808 similar to those that transport kainoid molecules (to which DA belongs), and could therefore be  
809 involved in the transport of DA. Interestingly, Pazos et al. (2017) found a transcript of this  
810 transporter family that was up-regulated in the digestive gland of the mussel *Mytilus*  
811 *galloprovincialis* that had fed on DA-producing *P. australis*, suggesting a mechanism involved  
812 in the depuration of DA. Using cDNA microarray technology and reverse transcription  
813 quantitative polymerase chain reaction (RT-qPCR), Boissonneault et al. (2013) studied *P.*  
814 *multiseries* under conditions that induced high and low DA production. They identified a set of  
815 reference genes for these experimental conditions, as well as a number of genes that were  
816 upregulated when high amounts of DA were produced. These could be candidate genes for  
817 probing the ability of a species to produce DA.

818 Taking advantage of the whole genome sequences and gene expression libraries of the  
819 above four diatoms (*P. multiseries*, *T. pseudonana*, *P. tricornutum* and *F. cylindrus*), Port et al.  
820 (2013) used a comparative genomics approach to search for evidence of a G protein-coupled  
821 receptor (GPCR) signaling pathway in these diatoms. GPCR signaling pathways play an  
822 essential role in signal transmission and response to external stimuli in mammalian cells.  
823 Interestingly, there was evidence of these pathway proteins in all four diatoms. Furthermore,  
824 transcriptional data indicated that several of the putative GPCR sequences were expressed in  
825 these diatoms under various stress conditions in culture. This may thus be part of a ‘stress  
826 surveillance system’ functioning in cell-cell communication within diatom populations to detect  
827 damaged or stressed cells resulting from grazing or other physical stressors. The availability of  
828 the complete genome sequence of *P. multiseries*, and of other diatoms, also allowed the  
829 discovery that they did not contain sequences coding for profilin (Aumeier et al., 2015). This is  
830 an essential actin-binding protein that takes part in regulation of the cytoskeleton, suggesting that

831 diatoms use a different mechanism. Finally, gene variants associated with adaptive phenotypes  
832 that are positively selected (e.g. for pressures due to nutrient limitation and sea-surface  
833 temperature) were studied in *P. multistriata*, *P. multiseries* and *P. australis* (Koester et al.,  
834 2013).

835 Certain *Pseudo-nitzschia* species are present in open-ocean and Antarctic waters that  
836 experience severe Fe limitation (e.g. Marchetti et al., 2008, 2014b, 2018; Russo et al., 2015).  
837 Genomic approaches were used to gain information about the molecular mechanisms used by  
838 these species to cope with this limitation. Adaptations to low-Fe environments include  
839 production of the Fe-storage protein ferritin and of the non-ferrous electron carriers plastocyanin  
840 and flavodoxin (Groussman et al., 2015). Gene transcripts for plastocyanin were detected in *P.*  
841 *granii* (Marchetti et al., 2015). The gene encoding a ferritin-like protein was first detected in *P.*  
842 *australis*, *P. granii* and *P. multiseries*, as well as in the cold-water diatom *Fragilariopsis*  
843 (Marchetti et al., 2009). A subsequent study indicated that ferritin is more widespread in diatoms  
844 than originally suspected, including *Pseudo-nitzschia* species (e.g. *P. pungens* var. *pungens*, *P.*  
845 *pungens* var. *cinctula*, *P. delicatissima*, *P. arenysensis*, *P. heimii*, *P. australis*) and other  
846 pennate and centric diatom genera (Groussman et al., 2015). Following Fe enrichment, oceanic  
847 *Pseudo-nitzschia* (species not indicated) showed distinct differential expression of gene products  
848 involved in nitrogen assimilation, photosynthetic carbon fixation, and vitamin production (Cohen  
849 et al., 2017b). Genes expressed differentially include ferritin (for *P. granii*; Cohen et al., 2018), a  
850 metal transporter, a putative bicarbonate transporter, and an NADPH-dependent glutamate  
851 synthase (Cohen et al., 2017b). These findings explain how some *Pseudo-nitzschia* species are  
852 able to sequester and store large amounts of Fe during pulses of Fe input, and then use it to  
853 support growth when Fe concentrations become low. Pfaffen et al. (2013) studied the kinetics of  
854 Fe binding onto ferritin derived from *P. multiseries*. Later, Pfaffen et al. (2015) showed that *P.*  
855 *multiseries* ferritin does not serve as an Fe-storage molecule, in contrast to other organisms, but  
856 rather may have an Fe buffering function by holding Fe at the ferroxidase center.

857 Another physiological adaptation to Fe-poor conditions is represented by  
858 proteorhodopsins (PR). These are transmembrane proteins that function as light-driven proton  
859 pumps, and provide an alternative source of energy to the cells. An overexpression of PR-like  
860 transcripts was detected in an Fe-limited natural community compared to Fe-amended conditions  
861 (Marchetti et al., 2012). Furthermore, a PR-like gene was recorded in the transcriptome of *P.*

862 *granii*, a species recorded in high-nutrient, low-chlorophyll (HNLC) regions of the ocean, where  
863 the biomass of diatoms is low in spite of high concentrations of macronutrients. This finding  
864 suggests that rhodopsin can provide energy to diatom cells when photosynthesis is compromised  
865 by low Fe availability (Marchetti et al., 2015). Using a comparative transcriptomic approach,  
866 coupled with laboratory and field experiments, Marchetti et al. (2017) identified two genes that  
867 track the Fe status of oceanic *P. granii*: *ISIP2a*, which encodes for an Fe-concentrating protein,  
868 and *FTN*, which encodes for the Fe storage protein ferritin. The *ISIP2a:FTN* transcript ratio  
869 reflects the Fe status of cells, where a high ratio indicates Fe limitation. This ratio has the  
870 potential to be applied to other ecologically relevant species and thus to become a molecular  
871 indicator for the Fe status of diatoms in natural populations.

872 Using transcriptomic analysis, Ellis et al. (2017) determined that *P. granii* from the  
873 Northeast Pacific Ocean (Ocean Station Papa) expressed the methionine synthase enzyme gene,  
874 *METH*. This is consistent with the fact that this diatom requires vitamin B<sub>12</sub>, which is a cofactor  
875 in one form of the *METH* enzyme that catalyzes the synthesis of the amino acid methionine.  
876 Furthermore, it did not have a functional *METE* gene (also absent in the *P. multiseries* genome),  
877 which synthesizes vitamin B<sub>12</sub>-independent methionine synthase (*METE*) that does not require  
878 vitamin B<sub>12</sub> to produce methionine. This is in contrast to another oceanic diatom, *Fragilariaopsis*  
879 *cylindrus*, which expresses *METE* and does not require vitamin B<sub>12</sub>. Likewise, expression of the  
880 biotin (B<sub>7</sub>) synthase gene (*BIOB*) in *P. granii* decreased 2-fold under Fe limitation, suggesting  
881 that the Fe status in Northeast Pacific Ocean waters may affect B<sub>7</sub> production in diatoms (Cohen  
882 et al., 2017a, 2017b). Furthermore, Fe enrichment of those waters may result in an increased  
883 demand for B<sub>12</sub>, which may then become limiting. These findings illustrate the interplay between  
884 Fe and vitamins in the selection of certain diatoms in oceans that have low concentrations of Fe  
885 and B vitamins.

886 Using a proteomics approach and analysis of synchronized cells, Xu et al. (2014)  
887 identified 48 proteins that were differentially expressed during cell division of *P. multiseries*.  
888 The most important up-regulated proteins were: clathrin, ATP-binding cassette protein, cofilin, a  
889 tubulin-specific chaperone protein, and dynein, all of which are involved in bio-silification.  
890 Proteins involved in chlorophyll ATP synthesis and lipid biosynthesis were also up-regulated in  
891 response to bio-silification. Protein profiling and expression data thus improved our

892 understanding of the molecular mechanisms underlying silicon cell wall formation in *P.*  
893 *multiseries*.

894

895 *8.2. Population genetic structure of Pseudo-nitzschia*

896 The increasing number of known *Pseudo-nitzschia* species is a result of studies  
897 combining detailed ultrastructural analyses with the use of various molecular markers (Section  
898 10.2). However, the genetic structure of relatively few of these species has been studied at the  
899 intraspecific level. Considerable genetic variation has been recorded in several microalgae, but  
900 the mechanisms that originate this diversity are poorly known (reviewed in Rengefors et al.,  
901 2017). The diversity and complexity of life cycles, the frequency of sexual reproduction (and  
902 thus genetic recombination) in organisms that increase their number asexually, and selective  
903 mechanisms resulting from environmental conditions are factors that play a role in shaping the  
904 population genetic structure of unicellular microalgae. Population genetics provides the  
905 conceptual framework and methodology to assess spatial and temporal patterns of diversity of  
906 species and populations. It thus helps us to understand the potential for phytoplankton species to  
907 evolve and adapt in response to environmental change.

908 Polymorphic microsatellite loci were used to study the population genetics of *P.*  
909 *australis*, *P. pungens* and *P. multistriata*. The microsatellite markers for *P. australis*, a species  
910 implicated as the causative organism in several DA events on the US west coast (Section 5.3),  
911 showed that there was a lack of cryptic diversity in Pacific Northwest *P. australis* (Adams et al.,  
912 2017). This suggests that *P. australis* at that location may consist of a single well-mixed coastal  
913 population. A similar conclusion was reached about *P. pungens* var. *pungens* (clade I)  
914 populations in the North Sea (Casteleyn et al., 2009). A later study carried out on this clade  
915 showed that allopatric speciation can occur in unicellular microalgae characterized by huge  
916 population sizes and apparently unlimited dispersal. Significant genetic structure was in fact  
917 recorded among *P. pungens* populations collected at a global scale (Casteleyn et al., 2010).  
918 Isolates grouped according to their geographic origin, suggesting that gene flow between the  
919 sampling locations is limited and that geographic structuring is possible for highly dispersed  
920 unicellular microalgae. Oceanic current patterns, as well as land masses, can hence represent  
921 barriers to gene flow and large-scale dispersal may date back to the Pleistocene, when the three  
922 main clades of *P. pungens* diverged (Casteleyn et al., 2010; Lim et al., 2014b).

923        The population genetic structure of *P. multistriata* was studied over four years (using 525  
924 genotyped strains collected at 22 sampling points) at the LTER station MareChiara in the Gulf of  
925 Naples, where the species is regularly recorded in summer-autumn (Tesson et al., 2014). Using  
926 microsatellite genotyping, distinct populations were detected over time. One dominated the first  
927 year, followed by a second population the subsequent two years. Then, the first population  
928 apparently dominated again in the fourth year. Populations were capable of interbreeding, and a  
929 considerable number of strains had the allelic signature of both populations, defining them as  
930 ‘putative hybrids’.

931        The ‘genetic proof’ of sexual reproduction (Section 7) was obtained for *P. multistriata* by  
932 analyzing the inheritance pattern of the microsatellite alleles in 246 F1-generation strains  
933 obtained from crosses between parental strains of known genotype (Tesson et al., 2013). Results  
934 generally followed the expected Mendelian inheritance patterns, except for a low percentage  
935 (8.7%) that deviated from it, suggesting the occurrence of mutations during sexual reproduction.  
936 Numerous mitotic mutations were also found in clonal strains of *P. multistriata* maintained in  
937 culture for 3 to 16 months (Tesson et al., 2013). These results suggest that genotypic diversity of  
938 natural populations may be partially attributed to mutations during both the sexual and the  
939 vegetative phases of the life cycle.

940        A further population genetic study was carried out on *P. multistriata* in the Gulf of  
941 Naples over two consecutive years to genotype isolates with a high number (22) of microsatellite  
942 markers (Ruggiero et al., 2018). A massive clonal expansion event (the production of daughter  
943 cells all arising originally from a single clone) – never reported for a marine phytoplankton  
944 species up to now – was detected during the bloom season of the first year. At that time, a few  
945 genotypes, reciprocally differing by a few alleles, started diverging clonally and eventually a  
946 single one became dominant. Linkage disequilibrium values (a parameter that quantifies the level  
947 of random association of alleles) were low during the pre- and post-bloom phase and in all  
948 samples collected during the second year. This suggests that free recombination occurred in the  
949 population during these periods. Both sex and vegetative growth thus interplay in shaping intra-  
950 specific diversity in this planktonic pennate diatom. Moreover, the combined analysis of the data  
951 produced in this study, and those by Tesson et al. (2014), showed that the genetic fingerprint of  
952 *P. multistriata* changed over time, with intermittent periods of weak and strong diversification  
953 related to the temporary predominance of clonal expansions over sexual recombination.

954

955 **9. New knowledge of *Pseudo-nitzschia***956 *9.1. Zooplankton interactions with Pseudo-nitzschia*

957       Zooplankton are an essential component in marine food webs, providing energy and  
958 nutrients to higher trophic levels. With regard to toxic phytoplankton, they may also serve as  
959 vectors for transferring toxins up the food web. Whether or not DA functions as a deterrent  
960 against grazing zooplankton has previously been reviewed (Lelong et al., 2012a). If blooms of  
961 toxic diatoms negatively affect zooplankton grazing rates, it may have profound effects on the  
962 food web, resulting in negative top-down control and thus alter the transfer of energy to higher  
963 trophic levels. However, if DA does not function as a grazing deterrent, then high levels of toxin  
964 may be accumulated in the zooplankton, with negative toxic effects on organisms in the food  
965 web. Interactions between *Pseudo-nitzschia* and their grazers are thus relevant to explore, but  
966 surprisingly few studies have been conducted.

967

968 *9.1.1. Effects on zooplankton*

969       Most studies exploring zooplankton grazing on *Pseudo-nitzschia* show no negative effect  
970 on the grazing rate of different grazers, such as the copepods *Calanus finmarchicus*, *C. glacialis*,  
971 *C. hyperboreus*, *Calanus* copepodite stages, *Acartia clausi*, *A. tonsa* and *Temora longicornis*, the  
972 krill *Euphausia pacifica* and different heterotrophic dinoflagellate species of the genus  
973 *Protoperidinium* (Table 7). Several older studies used only short-term experiments (hours), but  
974 more recent longer-term studies (8–10 days) confirm the lack of grazing deterrence, including on  
975 copepodites (Harðardóttir et al., 2015). However, two studies, one on *E. pacifica* grazing on  
976 toxic *P. multiseries* (Bargu et al., 2003) and one on *C. finmarchicus* and *C. hyperboreus* grazing  
977 on toxic *P. seriata* (Tammilehto et al., 2012) showed an effect on the grazing pattern. In both  
978 instances, the grazing became interrupted, as opposed to a more constant grazing when feeding  
979 on non-toxic diatoms. The copepods grazed initially for a number of hours, as expected, and then  
980 stopped feeding for several hours, after which they resumed grazing. This could be related to a  
981 threshold for DA, which may result in grazing inhibition for a period when the threshold is  
982 surpassed (Bargu et al., 2003; Tammilehto et al., 2012). Further studies on grazing patterns are  
983 needed to pursue this.

Given a choice, zooplankton will avoid toxic *Alexandrium* cells (Selander et al., 2006). Copepods, however, do not discriminate between toxic and non-toxic/less toxic *Pseudo-nitzschia* cells. This is supported by studies on different copepods (*C. finmarchicus*, *T. longicornis*, *A. clausi*, *A. tonsa*) as well as *Calanus* copepodite stages (Lincoln et al., 2001; Tester et al., 2001; Maneiro et al., 2005; Leandro et al., 2010a; Tammilehto et al., 2012; Harðardóttir et al., 2015).

But does dissolved DA in the surrounding water affect zooplankton? Dissolved DA has shown no lethal effect on the copepod *Calanus glacialis* when exposed to concentrations of 0.05–50 µg DA ml<sup>-1</sup>, whereas smaller copepods died, i.e. *T. longicornis* and *Pseudocalanus acuspes* (LC<sub>50</sub> = 135 and 37.5 µg DA ml<sup>-1</sup>, respectively; Windust, 1992) and *Tigriopus californicus* (LC<sub>50</sub> = 2.68 µg DA ml<sup>-1</sup>; Shaw et al., 1997) (Table 7). Furthermore, Shaw et al. (1997) reported that DA deterred feeding at 3.24 µg DA ml<sup>-1</sup> and experiments using krill (*E. pacifica*) showed that dissolved DA (0.4 µg DA ml<sup>-1</sup>) added to non-toxic food resulted in significantly reduced grazing rates (Bargu et al., 2006). These levels of dissolved DA are, however, above those found in the ocean (e.g. 0.0033 µg ml<sup>-1</sup> [Liefer et al., 2013]; 0.0080 µg ml<sup>-1</sup> [MacIntyre et al., 2011]; 0.00124 µg ml<sup>-1</sup>; [Trainer et al., 2009]), even during blooms; an exception is >0.1 µg ml<sup>-1</sup>, a new record (Kudela et al., 2015). Lethal effects of dissolved DA are therefore presently not considered ecologically relevant.

1001

### 1002 9.1.2. Zooplankton as vectors in the food web

1003 In the field, DA has been detected in zooplankton (Table 7), e.g. in krill (*E. pacifica*) 1004 offshore from Monterey Bay (at 0–44 µg DA g<sup>-1</sup>) at the same time that cellular DA levels in 1005 *Pseudo-nitzschia* spp. were at 0–24 pg DA cell<sup>-1</sup> (Bargu et al., 2002b). DA in krill showed the 1006 same temporal variation as did cellular DA in *Pseudo-nitzschia*. A potential vector to be 1007 considered for transferring DA to zooplankton is “marine snow” (Schnetzer et al., 2017). 1008 Zooplankton (species not identified) and copepods (>150 µm) in Thailand contained 0.04–0.21 1009 ng DA l<sup>-1</sup> and 0.11 ng copepod<sup>-1</sup>, respectively (Veschasit et al., 2017). A field study found that 1010 all zooplankton samples (n=32; either copepods or krill) from Georges Bank, Roseway Basin 1011 (USA) and the Bay of Fundy (Canada) contained 0.02–0.17 µg DA g<sup>-1</sup> tissue (Leandro et al., 1012 2010b). Samples were dominated by *C. finmarchicus*, the predominant food source for the 1013 northern right whale (*Eubalaena glacialis*), which was also shown to contain both DA and 1014 remnants of *C. glacialis* in the feces. The transfer of DA from *Pseudo-nitzschia* via copepods to

1015 whales may contribute to the failed recovery of the *E. glacialis* population, as the right whales  
1016 are exposed to DA for up to six months annually (Leandro et al., 2010b). Findings were similar  
1017 for the southern right whale (*Eubalaena australis*), where DA was also detected in their food  
1018 source, copepods (D'Agostino et al., 2017), and *P. australis* frustules were found in their feces  
1019 (D'Agostino et al., 2015, 2017).

1020 The levels of DA found in the field are generally lower than those found in grazing  
1021 experiments, where a wide variation in DA content has been found in copepods, copepodites and  
1022 rotifers (Table 7). The higher levels of DA in laboratory experiments reflect that most of these  
1023 experiments were performed using monoclonal cultures, and thus higher DA levels are  
1024 anticipated than in the field. The experiment levels are, however, relevant for understanding  
1025 interactions, as well as for modelling a worst-case scenario for an almost monoclonal toxic  
1026 bloom, as the one in PEI, Canada in 1987 (reviewed in Trainer et al., 2012).

1027 Copepods retain from 5–50% of the ingested DA, depending on the copepod species  
1028 (Table 7). In spite of DA being hydrophilic, copepods accumulate DA in their body tissues (e.g.  
1029 Tammilehto et al., 2012; Miesner et al., 2016) and thus retain DA for a period of time, probably  
1030 days (Leandro et al., 2010a). DA is excreted from copepods mainly via fecal pellets, while  
1031 excretion via eggs in females is 30–60 times lower (Miesner et al., 2016). The period of time that  
1032 zooplankton remain toxic after grazing ceases requires further exploration in order to model the  
1033 risk for bioaccumulating DA.

1034

### 1035 9.1.3. Induction of domoic acid production by zooplankton grazers

1036 Grazing copepods can induce an increase in the production of DA by *P. seriata*  
1037 (Harðardóttir et al., 2015; Tammilehto et al., 2015). The induction has so far been shown for  
1038 three *Calanus* species (*C. finmarchicus*, *C. glacialis* and *C. hyperboreus*), as well as for *Calanus*  
1039 copepodites, but is probably a more general phenomenon. Brine shrimp (*Artemia salina*) and a  
1040 euryhaline rotifer (*Brachionus plicatilis*) increased the DA production of *P. fukuyoi* in a mixed  
1041 culture by 4.7–28.5 times (Huang et al., 2017c).

1042 Toxin production can be up to 130 times higher than in the non-grazing control, an  
1043 increase similar to that caused by the depletion of silicate or phosphate (reviewed in Lelong et  
1044 al., 2012a; Trainer et al., 2012; Lundholm and Harðardóttir, 2017). Copepods can induce DA  
1045 production in exponentially growing cultures, in contrast to nutrient-depletion, which primarily

1046 has an effect on DA production during the stationary phase (Trainer et al., 2012). Grazing  
1047 copepods can induce toxin production not only in previously known toxic species/strains like *P.*  
1048 *seriata*, but also in a species previously considered to be non-toxic; i.e. *P. obtusa* was shown to  
1049 produce DA for the first time in these grazing experiments (Harðardóttir et al., 2015). Thus,  
1050 current evidence points to an innate ability of at least some previously non-toxic *Pseudo-*  
1051 *nitzschia* strains to produce DA, but perhaps only when modulated by certain external biological  
1052 interactions (cf. Woods, 2016). It must still be determined if grazing by other copepods, or even  
1053 other grazers, can induce DA production, and if all *Pseudo-nitzschia* species can produce DA  
1054 when induced by grazers. For example, a recent study showed that brine shrimp and copepods  
1055 (*Paracalanus crassirostris*) do not induce DA production in a newly discovered non-toxic  
1056 *Pseudo-nitzschia nanaoensis* species from coastal waters of China (Li et al., 2018).

1057 It has generally been difficult to correlate the high levels of DA in the field to  
1058 environmental parameters (Trainer et al., 2012). Likewise, the cellular DA content of cells in a  
1059 bloom is sometimes higher than that of cells isolated into culture from the same bloom. This has  
1060 made it difficult to predict consistently with any confidence the toxicity of *Pseudo-nitzschia*  
1061 blooms. It would be interesting to determine if including copepod grazing would increase the  
1062 prediction capabilities.

1063 It has been repeatedly shown that DA production decreases over time when toxic *Pseudo-*  
1064 *nitzschia* isolates remain in culture (Lelong et al., 2012a). This has been explained as being due  
1065 to a decrease in cell size over time and thus a lower toxin content per cell, or to changes over  
1066 time in bacterial composition in cultures, as it is known that bacteria affect toxin production  
1067 (Section 9.2.5). A lack of grazers in diatom cultures maintained in the laboratory may also  
1068 explain the decrease in toxin production. For example, a strain of *P. seriata* kept in culture for  
1069 several years exhibited a steady decrease in toxin production until DA was below the detection  
1070 level (Tammilehto et al., 2015). However, exposure to grazers revived toxin production,  
1071 indicating that the culture had not completely lost its ability to produce DA.

1072 We presently have no solid evidence that DA functions as a grazing deterrent;  
1073 zooplankton grazing rates are not negatively affected during blooms of toxic diatoms, as opposed  
1074 to blooms of, e.g. the toxic dinoflagellate *Alexandrium*. However, the grazing zooplankton have  
1075 a toxin-inducing effect on *Pseudo-nitzschia*. The result is that, in the presence of grazers, the

1076 remaining *Pseudo-nitzschia* cells will accumulate high levels of DA. This, plus the fact that  
1077 zooplankton are vectors of DA, will have negative effects on organisms in the food web.

1078

1079 *9.2. Controls on growth and domoic acid production*

1080 Laboratory studies have shown that abiotic (Si and P limitation, inorganic and organic N,  
1081 salinity, irradiance, temperature, pH/pCO<sub>2</sub>) and biotic (bacteria) factors enhance DA production  
1082 by various *Pseudo-nitzschia* species (reviewed in Lelong et al., 2012a; Trainer et al., 2012). Van  
1083 Meerssche and Pinckney (2017) summarized the correlations between some of these abiotic  
1084 factors, *Pseudo-nitzschia*, and dissolved DA in field studies. New studies have provided  
1085 additional information about the above factors, including copepod grazing as a DA enhancer  
1086 (Table 8). Moreover, further advances were made by studying interactive effects of various  
1087 abiotic factors (although not always in relation to DA production), e.g. Cu and Si (Fuentes and  
1088 Wikfors, 2013); Cu and Fe (Lelong et al., 2013); pCO<sub>2</sub> and silicate (Tatters et al., 2012); pCO<sub>2</sub>  
1089 and Fe (Sugie and Yoshimura, 2013); temperature and irradiance (Thorel et al., 2014);  
1090 temperature and Fe (Zhu et al., 2016); temperature and pCO<sub>2</sub> (Zhu et al., 2017a); temperature  
1091 and solar ultraviolet radiation (Chen et al., 2018); irradiance and N substrate (Auro and Cochlan,  
1092 2013); nitrate, silicate and phosphate (Lema et al., 2017); different N sources (Radan and  
1093 Cochlan, 2018); and temperature, nitrate/urea and pCO<sub>2</sub> (Tatters et al., 2018) (Table 8). It must  
1094 be noted, however, that although better than studying individual parameters, the interaction of  
1095 two parameters may still neglect accompanying changes in other factors, e.g. Fe availability  
1096 when interactions between temperature and pCO<sub>2</sub> are examined.

1097 Field studies continue to show correlations between the presence of *Pseudo-nitzschia* and  
1098 various environmental parameters (Husson et al., 2016; Thorel et al., 2017; Pednekar et al.,  
1099 2018), as well as a correlation between *Pseudo-nitzschia* toxicity and limiting concentrations of  
1100 both silicate and phosphate (Thorel et al., 2017). Paredes-Banda et al. (2016) reviewed the  
1101 ecophysiological studies of *P. australis* isolated from Baja California (Mexico). A mechanistic  
1102 model was developed to explore abiotic factors that control the production of DA by *Pseudo-*  
1103 *nitzschia* (Terseleer et al., 2013). Model simulations showed that excess light, in combination  
1104 with Si or P limitation, favors DA production, provided that N is sufficient; this supports  
1105 previous laboratory studies and is consistent with DA being a secondary metabolite (reviewed in  
1106 Lelong et al., 2012a). In spite of the model not yet implementing the effects of inorganic vs.

1107 organic N, trace metals, salinity, temperature, pH, bacteria, or grazers, the simulations were  
1108 generally realistic.

1109

### 1110 9.2.1. Irradiance

1111 Sufficient irradiance is required for DA production (reviewed in Lelong et al., 2012b).  
1112 For example, the mechanistic model of Terseleer et al. (2013) used an irradiance of 120  $\mu\text{mol}$   
1113 photons  $\text{m}^{-2} \text{s}^{-1}$  in order to avoid light limitation of growth or toxin production; this compares to  
1114 the 100  $\mu\text{mol}$  photons  $\text{m}^{-2} \text{s}^{-1}$  discussed in Lelong et al. (2012a). However, an abrupt 4-fold  
1115 increase in irradiance, from 100 to 400  $\mu\text{mol}$  photons  $\text{m}^{-2} \text{s}^{-1}$ , resulted in a ~5-fold increase in DA  
1116 production by *P. australis* both 24 and 48 hours after the increase in irradiance to excess levels  
1117 (Table 8) (Woods, 2016). This increase in light intensity induced an oxidative stress response, as  
1118 evidenced by an increase in superoxide dismutase activity, which is an important antioxidant  
1119 defense. Furthermore, a similar 5-fold increase in DA production occurred after oxidative stress  
1120 was chemically induced by adding a sublethal dose of the herbicide methyl viologen. This was  
1121 accompanied by hydrogen peroxide production (an indicator of oxidative stress) and a marked  
1122 decrease in photosynthetic efficiency. It must be questioned, however, how DA production can  
1123 increase while photosynthetic efficiency decreased, given an inherent requirement of  
1124 photosynthetic energy to biosynthesize DA (Lelong et al., 2012a).

1125 Nevertheless, Woods (2016) also found a positive and significant correlation between  
1126 antioxidant capacity and the cellular DA content of *P. australis* and *P. multiseries*. This led to  
1127 the hypothesis that DA could act as an antioxidant to protect the cells from stress, e.g. exposure  
1128 to high irradiance levels. However, there was no effect of adding DA to a solution of superoxide  
1129 dismutase. Additionally, there was no quenching after DA was spiked into a solution of  
1130 hydrogen peroxide. Thus, the potential antioxidant function of DA was ruled out.

1131

### 1132 9.2.2. Growth phase in culture

1133 In the following, only differences from previous studies will be highlighted. In contrast to  
1134 other species of *Pseudo-nitzschia* (Lelong et al., 2012a), *P. australis* from the English Channel  
1135 produced DA under balanced growth and without nutrient limitation in a semi-continuous culture  
1136 (Thorel et al., 2014). It also produced DA during the exponential phase, in agreement with other  
1137 strains of *P. australis* (Lelong et al., 2012a; Martin-Jézéquel et al., 2015; Schnetzer et al., 2017;

1138 Wingert, 2017), but contrary to most other species of *Pseudo-nitzschia* that produce DA during  
1139 the stationary phase (Lelong et al., 2012a). The concentration of particulate DA reached a  
1140 maximum on ~day 45, during the formation of “marine snow” by *P. australis* in roller table  
1141 experiments (Schnetzer et al., 2017), providing evidence for mechanisms of particulate DA  
1142 transport to the sediments. *Pseudo-nitzschia* cf. *pseudodelicatissima* was previously shown to  
1143 also produce DA when growing exponentially (see Lelong et al., 2012a). However, the species  
1144 identity was uncertain, as it could have been *P. pseudodelicatissima* or *P. cuspidata*.  
1145 Interestingly, Auro and Cochlan (2013) reported that *P. cuspidata* produced more DA during the  
1146 exponential than stationary phase. Similarly, *P. seriata* and *P. obtusa* produce DA in exponential  
1147 growth phase (Tammilehto et al., 2012; Harðardóttir et al., 2015).

1148 Thorel et al. (2014) also observed higher values of cellular DA in *P. australis* at higher  
1149 growth rates, in contrast to other species (e.g. *P. multiseries* and *P. seriata*). Therefore, healthy  
1150 *P. australis* cells can also produce DA, and, in contrast to certain other species, nutrient stress is  
1151 not required; this suggests that the mechanism of DA production may be different among species  
1152 and potentially strains. These results are important for modeling DA production in the field, and  
1153 may explain the differing results when different *Pseudo-nitzschia* species are present.

1154

#### 1155 9.2.3. Nitrogen

1156 Studies continue to show the ability of different *Pseudo-nitzschia* species to grow and  
1157 produce DA when grown with different inorganic and organic forms of N (Table 8). Martin-  
1158 Jézéquel et al. (2015) are the first to demonstrate toxin production by *P. multiseries* growing on  
1159 arginine and glutamine, although *P. australis* did not produce DA on these substrates. Cultures  
1160 of *P. calliantha* grew significantly faster on urea than on  $\text{NO}_3^-$  or  $\text{NH}_4^+$  (Garali et al., 2016).  
1161 Auro and Cochlan (2013) found that *P. cuspidata* produced more DA when grown on  $\text{NO}_3^-$  or  
1162  $\text{NH}_4^+$  than on urea, at subsaturating irradiance levels. However, these results are contrary to  
1163 those of previous studies (reviewed in Lelong et al., 2012a) that reported higher DA production  
1164 when *P. australis* was grown on urea rather than on the other inorganic N substrates. This was  
1165 also recently observed for *P. multiseries* (Radan and Cochlan, 2018). Nevertheless, the differing  
1166 results show that there are still no consistent patterns for different N substrates. This may be  
1167 clarified by considering the N concentration, the interaction of N and irradiance, the growth  
1168 phase in culture, as well as inter- and intra-species differences. For example, Auro and Cochlan

1169 (2013) found that the affinity of *P. fryselliana* for different N substrates depended on their  
1170 concentration (Table 8). Furthermore, they reported that the differences in DA production by *P.*  
1171 *cuspidata* with the different N substrates and irradiances occurred only during the exponential  
1172 phase; there were no differences during the stationary phase, regardless of the irradiance level.  
1173 This was attributed to the fact that their growth medium was designed specifically so that  
1174 dissolved N became exhausted to induce the stationary phase, rather than Si or P (which, when  
1175 limiting, promote DA production) as in most other studies. DA production during the  
1176 exponential phase in the presence of N was also simulated in the model of Terseleer et al. (2013).  
1177 Thus, it may be necessary to re-evaluate the cause of DA production during the exponential vs  
1178 stationary phase, in order to determine if there is actually a species-specific effect, or if the  
1179 supply of N vs Si and P is more important.

1180

#### 1181 9.2.4. Trace metals

1182 Exposing *P. multiseries* to Cu did not increase DA production (Lelong et al., 2012b), in  
1183 contrast to previous studies (reviewed in Lelong et al., 2012a). Similarly, Cu addition did not  
1184 induce a non-toxic strain of *P. delicatissima* to produce DA (Lelong et al., 2012b). Furthermore,  
1185 co-limitation of Cu and Fe did not induce a non-toxic strain of *P. delicatissima* to produce DA  
1186 (Lelong et al., 2013). This is in contrast to the postulation that limitation by these trace metals  
1187 may cause all species of *Pseudo-nitzschia* to become toxigenic (Wells et al., 2005). Cu and Fe  
1188 limitation had opposite effects on photosynthetic parameters, which strongly suggests that the  
1189 main effect of Cu limitation was not to induce Fe limitation in *P. delicatissima* (Lelong et al.,  
1190 2013). This is also in contrast to findings of Wells et al. (2005), although they studied *P.*  
1191 *australis*, *P. multiseries* and *P. fraudulenta*. It is thus clear that the role of Cu in triggering DA  
1192 production, in protecting the cells from Cu toxicity, and in inducing Fe limitation requires further  
1193 assessment with additional species.

1194 The role of Fe in regulating the growth and DA production by *Pseudo-nitzschia* was  
1195 reviewed in Lelong et al. (2012a). Different conclusions were reached, perhaps depending on the  
1196 growth phase, species, and how Fe-depleted the cultures were. In further developing this line of  
1197 research, Sobrinho et al. (2017) determined that DA concentrations of *P. multiseries* were  
1198 significantly higher under the highest Fe concentration, indicating that Fe is required for toxin  
1199 synthesis. Furthermore, they found that similar exponential growth rates and stationary-phase

1200 cell concentrations were reached in cultures exposed to the different levels of Fe enrichment,  
1201 suggesting that the cells may not have been Fe limited, in spite of growing at a low Fe  
1202 concentration. These results support a previous study (Bates et al., 2001), although they provide  
1203 no evidence that DA is produced as a chelator to sustain *Pseudo-nitzschia* at low Fe  
1204 concentrations, as concluded in other studies (reviewed in Lelong et al., 2012a). These laboratory  
1205 studies nevertheless add to the evidence that some *Pseudo-nitzschia* species possess an effective  
1206 adaptation for growing in regions where Fe is limiting (Section 8.1).

1207 The interaction between Cu and Si in regulating Si uptake and DA production is also  
1208 important (Table 8). Responses of *P. multiseries* to Cu and Si when concentrations of both were  
1209 either high or low were amplified compared to the responses to the individual nutrients (Fuentes  
1210 and Wikfors, 2013). Highest Si uptake occurred when Si and Cu synergistically co-limited  
1211 growth. In contrast to other studies, there is no indication that Cu uses the silicic acid transport  
1212 site to enter the cell. Highest DA production occurred with an increase in Cu and Si, as well as  
1213 with low Si and sufficient Cu. Lowest DA production occurred when both Si and Cu were low,  
1214 perhaps because excess energy was used for Si uptake rather than for toxin production.  
1215 Interactions among factors are thus important for understanding DA production. Prince et al.  
1216 (2013) describe the complex interactions between Fe, DA and the growth of non-toxic *P.*  
1217 *delicatissima* in the presence of the diatom *Skeletonema marinoi* (Section 9.3).

1218

### 1219 9.2.5. *Bacteria*

1220 It is well-known that marine diatoms and bacteria have a complex interaction that  
1221 evolved during their existence in the same marine environment for millions of years. It is hence  
1222 not surprising that the interaction between toxic diatoms and bacteria is similarly complex. Our  
1223 knowledge of this interaction is still restricted, but recent advances have been made.

1224

#### 1225 9.2.5.1. *Bacterial community composition in different diatom species*

1226 Studies continue to show that there is a difference in bacterial communities between toxic  
1227 and non-toxic *Pseudo-nitzschia* species (Guannel et al., 2011; Lelong et al., 2014; Sison-Mangus  
1228 et al., 2014). Toxic diatoms are associated with a lower bacterial diversity than non-toxic  
1229 diatoms, as seen in both laboratory (Sison-Mangus et al., 2014) and field (Sison-Mangus et al.,  
1230 2016) studies. For example, 16S rDNA sequencing showed that a non-toxic *P. fraudulenta*

1231 bloom had a higher bacterial diversity than a toxic *P. australis* bloom (Sison-Mangus et al.,  
1232 2016). The differences were consistent throughout the different phases of the blooms.

1233 Several types of bacteria and complex bacterial communities are associated with toxic  
1234 diatoms (Lelong et al., 2014; Sison-Mangus et al., 2014, 2016; Amin et al., 2015; Hong et al.,  
1235 2015; Stone, 2017). Sequencing the microbial community in cultures of *P. fraudulenta*, *P.*  
1236 *multiseries* and *P. pungens*, for example, revealed 266 different OTUs belonging mainly to  $\alpha$ -  
1237 proteobacteria,  $\gamma$ -proteobacteria and Bacteroidetes (Sison-Mangus et al., 2014). Isolation of  
1238 bacteria from *P. multiseries* resulted in 49 different bacterial strains belonging to  $\beta$ -  
1239 proteobacteria (*Limnobacter*),  $\gamma$ -proteobacteria (*Marinobacter*),  $\alpha$ -proteobacteria (*Sulfitobacter*,  
1240 *Hyphomonas*), and Bacteroidetes (*Croceibacter*) (Amin et al., 2015). The diversity of bacteria in  
1241 cultures of *P. delicatissima* and *P. multiseries* was revealed by constructing 16S ribosomal  
1242 rDNA clone libraries; these showed bacteria belonging to  $\alpha$ -proteobacteria (*Sulfitobacter*,  
1243 *Roseobacter*),  $\gamma$ -proteobacteria and Bacteroidetes (Guannel et al., 2011). Earlier studies found  
1244 these same types of bacteria (reviewed in Lelong et al., 2012a, Stone, 2017). Furthermore, their  
1245 appearance in toxic *Pseudo-nitzschia* agrees with findings in other diatoms (Amin et al., 2012).  
1246 There is, however, no agreement as to whether the bacterial community may change over time.  
1247 Some studies found a shift in bacterial community over time in cultures of toxic diatoms  
1248 (reviewed in Lelong et al., 2012a), whereas Guannel et al. (2011) found no change over time, or  
1249 between the exponential and stationary growth phases.

1250 A consensus among studies is that the bacterial association is species specific. For  
1251 example, the bacterial community differed significantly among five *Pseudo-nitzschia* species: *P.*  
1252 *australis*, *P. multiseries*, *P. delicatissima*, *P. granii* and *P. pungens* (Guannel et al., 2011).  
1253 Similarly, bacterial community analyses showed that *P. australis*, *P. delicatissima* and *P.*  
1254 *fraudulenta* hosted distinctly different communities (Sison-Mangus et al., 2014). Different  
1255 bacterial communities were also found in non-toxic *P. delicatissima* and toxic *P. multiseries*  
1256 cultures (Lelong et al., 2014). Hence, there is increasing support that different species of *Pseudo-*  
1257 *nitzschia* have distinct bacterial communities.

1258 A recent study showed that three strains of the globally distributed flavobacterium  
1259 *Croceibacter atlanticus* were associated with three different cultures of *P. multiseries* collected  
1260 from different locations and maintained in culture for over 10 years; some were progeny of  
1261 parental cultures (van Tol et al., 2017). This indicates that it is a common, long-term member of

1262 the bacterial consortia associated with *P. multiseries* in culture. Similarly, sequence reads from  
1263 genomic sequencing of *P. australis* were mapped to ~80% of the *Rhodobacteraceae bacterium*  
1264 HTCC2083 genome, suggesting that this species associates with *P. australis* (Stone, 2017).  
1265 Interestingly, this high percentage of bacterial genome was found even after the *P. australis*  
1266 culture was treated with antibiotics for three months. It was therefore not clear if the *R.*  
1267 *bacterium* remained attached to the exterior of the *P. australis* cell (cf. Kaczmarśka et al., 2005),  
1268 or if its sequences were incorporated into the *P. australis* genome. The conclusion, however, is  
1269 that bacteria are intimately associated with these diatoms.

1270 The effect of bacteria on the growth characteristics of different *Pseudo-nitzschia* species  
1271 was assessed by using transplant experiments. Experiments exposing different strains of toxic  
1272 diatoms to strains or communities of bacteria from other diatom cultures resulted in significantly  
1273 different effects on the growth rates of the diatoms, either positive, negative or none, depending  
1274 on the bacterial or diatom species (Lelong et al., 2014; Sison-Mangus et al., 2014; Amin et al.,  
1275 2015; van Tol et al., 2017). Most often, naturally associated bacteria provided the diatoms with a  
1276 better growth environment (Sison-Mangus et al., 2014). Exposing diatoms to foreign harmful  
1277 bacteria (i.e. those from other cultures) showed that a natural bacterial community could even  
1278 protect the diatom host against the lytic effect of those bacteria (Sison-Mangus et al., 2014). For  
1279 example, the growth of axenic cultures of *P. multiseries* and *P. fraudulenta* declined within a few  
1280 hours after exposing them to different strains of the flavobacterium *C. atlanticus* (van Tol et al.,  
1281 2017). This antagonistic effect of *C. atlanticus* was unexpected, because this bacterium was  
1282 observed growing on the cell surface of both *Pseudo-nitzschia* species for a long period of time.  
1283 However, the authors noted that because axenic strains were used for the co-culturing  
1284 experiments, it was possible that other members of the pre-existing bacterial consortia from those  
1285 diatoms mitigated the impacts of *C. atlanticus*. This could explain how *C. atlanticus* remained so  
1286 long as a consistent member of the *P. multiseries* consortium without completely stopping the  
1287 diatom's growth; this may help to explain the variable growth and toxicity in field versus  
1288 cultivated populations.

1289 Each diatom is surrounded by a phycosphere, a thin layer of fluid known as the boundary  
1290 layer, where bacterial growth is stimulated by extracellular products from the alga (Bell and  
1291 Mitchell, 1972). The phycosphere could hence represent a place where diatoms 'cultivate'  
1292 bacterial communities by releasing nutrient-rich or other important substances, including DA.

1293 The bacteria may benefit the diatom either by providing more available sources of nutrients, or  
1294 by deterring other bacteria. At the same time, the bacteria may be able to exploit the diatoms by  
1295 causing stress or cell lysis (Amin et al., 2012). Hence, a host-specific bacterial community like  
1296 what has been shown in *Pseudo-nitzschia* could appear in the phycosphere, but also in the  
1297 surrounding water. *Pseudo-nitzschia* may harbor specific bacteria, e.g. as protection against  
1298 foreign bacteria, or as a source of remineralized nutrients to support growth. For example, of the  
1299 49 bacterial strains isolated from *P. multiseries* cultures, members of the genus *Sulfitobacter* had  
1300 the largest positive effect on the diatom's growth, sometimes in a strain-specific manner (Amin  
1301 et al., 2015). By studying gene expression of both *P. multiseries* and associated bacteria, Amin et  
1302 al. (2015) showed that they communicate via production and exchange of a number of different  
1303 chemical substances or cues. Bacteria and *Pseudo-nitzschia* are hence closely coupled and  
1304 interact in many and complex ways.

1305

#### 1306 9.2.5.2. Effect of bacteria on domoic acid production

1307 As reviewed in Lelong et al. (2012a), bacteria are implicated in the production of DA, as  
1308 axenic cultures of both *Pseudo-nitzschia* and *N. navis-varingica* produce less DA. However, in  
1309 contrast to this, axenic *P. fraudulenta* had a significantly higher DA content than the original  
1310 non-axenic culture (Sison-Mangus et al., 2014). This was attributed to the possibility that some  
1311 bacteria may have consumed the DA in the control culture, or else that the axenic condition itself  
1312 may have been stressful, and hence the diatom produced more DA. It seems that the effect of  
1313 bacteria on toxin production in *Pseudo-nitzschia* depends to a high degree on the bacterial strain.  
1314 Exposing *P. fraudulenta* and *P. multiseries* to 16 different bacterial strains showed that the effect  
1315 on DA production varied considerably depending on the bacterial strain, and that the effect also  
1316 varied between the two diatom strains (Sison-Mangus et al., 2014). There was a tendency for  
1317 only foreign bacteria to induce an increase in DA production. Similar to this being an anti-  
1318 bacterial defense mechanism (Kaczmarska et al., 2005), it was explained as a stress response by  
1319 the diatom having to cope with foreign bacteria (Lelong et al., 2014; Sison-Mangus et al., 2014).  
1320 Although foreign bacteria can induce increased DA production, transplanting foreign bacteria  
1321 into non-toxic strains of *P. delicatissima* (Lelong et al., 2014) and *P. pungens* (Sison-Mangus et  
1322 al., 2014) did not induce toxin production. This is in contrast to what was seen when non-toxic *P.*  
1323 *obtusa* was induced to produce DA in the presence of grazing copepods (Section 9.1.3); it should

1324 be noted that grazers may also be a source of a different microbial assemblage in these types of  
1325 experiments. The exact mechanisms of action for how bacteria, or grazing zooplankton, induce  
1326 toxicity remain elusive.

1327

1328 *9.3. Allelopathy*

1329 Previous studies provided no evidence that DA, nor the presence of *Pseudo-nitzschia*  
1330 cells, conferred any allelopathic effects on other phytoplankton species (reviewed in Bates, 1998;  
1331 Lelong et al., 2012a). This was also demonstrated in a field study (Van Meerssche and Pinckney,  
1332 2017). However, two recent studies show, for the first time, evidence of allelopathy in *Pseudo-*  
1333 *nitzschia* growing in culture. First, Xu et al. (2015) demonstrated that *P. pungens* significantly  
1334 reduced the cell concentration and growth rate in cultures of the dinoflagellate *Akashiwo*  
1335 *sanguinea*, the cryptophyte *Rhodomonas salina*, and the raphidophyte *Chattonella marina*,  
1336 although not of the armored dinoflagellate *Prorocentrum minimum* and the haptophyte  
1337 *Phaeocystis globosa*. As well, co-culture experiments using two strains of *P. multiseries* showed  
1338 the growth inhibition of *A. sanguinea*. Similar effects were seen when filtrates or sonicated  
1339 cultures were used, strongly suggesting the presence of unidentified allelochemicals. Because the  
1340 effects were seen with both a DA-producing (*P. multiseries*) and a non-toxic (*P. pungens*) strain,  
1341 the inhibition was not believed to be caused by DA (although the addition of DA was not tested).  
1342 Sobrinho et al. (2017) then demonstrated that the cell concentration of the pennate diatom  
1343 *Bacillaria* sp. was reduced by 50–70% when the dissolved cell contents from a *P. multiseries*  
1344 culture were added. Although the culture also contained a low concentration DA (52 ng DA ml<sup>-1</sup>  
1345 ), undetermined allelochemicals were again suspected to cause the growth inhibition. Adding  
1346 DA to co-cultures of *P. delicatissima* (non-toxic) and the diatom *Skeletonema marinoi* resulted in  
1347 a 38% reduction in *S. marinoi* cells and an increase in *P. delicatissima* cells by up to 17% under  
1348 Fe-replete conditions (Prince et al., 2013). It was concluded that DA can indirectly improve the  
1349 competitive ability of *Pseudo-nitzschia* spp. and that Fe is likely to be involved.

1350 It should be noted that some *Pseudo-nitzschia* species produce oxylipins (d’Ippolito et al.,  
1351 2009), and that a diet of *P. delicatissima* induced low hatching success and apoptosis in the  
1352 offspring of the copepod *Calanus helgolandicus* compared to control diets (Ianora and Miraldo,  
1353 2010).

1354

1355 9.4. *Oomycete parasitoids*

1356 A parasitoid infection of *P. pungens* from PEI was described by Hanic et al. (2009), and  
1357 photographs of what appears to be the same oomycete, but from Washington State and  
1358 California, are shown in Lelong et al. (2012a). A morphologically similar organism was again  
1359 isolated from infected *P. pungens* from PEI, as well as from Helgoland (Germany), and was  
1360 identified as a new genus and species, *Miracula helgolandica* (Buaya et al., 2017). Its phylogeny  
1361 was reconstructed based on partial nuclear small subunit ribosomal RNA (nrSSU) sequences.  
1362 However, the genus to which most other oomycetes belong, *Ectrogella*, has yet to be sequenced,  
1363 so further work is required to define its exact position phylogenetically. As well, fuller  
1364 sequencing is needed to better define *M. helgolandica*. Such work is underway, from oomycete  
1365 parasites isolated from four *Pseudo-nitzschia* species from the North Atlantic coast (Fig. 5)  
1366 (Garvetto et al., 2017). Their finding that the same oomycete infected both *P. australis* and  
1367 *Melosira* sp. supports the hypothesis of its lack of host specificity. The role of this oomycete in  
1368 controlling *Pseudo-nitzschia* bloom dynamics is still being investigated. The potential role of  
1369 chytrid parasites in affecting HAB dynamics, including *Pseudo-nitzschia*, is reviewed by  
1370 Gleason et al. (2015). Interestingly, Thorel et al. (2017) also mentioned a fungal infection of  
1371 *Pseudo-nitzschia* sp., although the prevalence of infected cells (<1%) may have been too low to  
1372 affect population dynamics (see Trainer et al., 2012).

1373

## 1374 10. Identification of *Pseudo-nitzschia* species

### 1375 10.1. Classical methods

1376 Morphology-based classical methods remain a vital component in *Pseudo-nitzschia*  
1377 taxonomy. However, identification and discovery of *Pseudo-nitzschia* species based upon  
1378 variations in frustule morphology require careful taxonomic assessment by electron microscopy.  
1379 There is thus a need to continue training classical taxonomists. Morphological characters of the  
1380 frustule and their morphometric data (including valve width and shape, densities of fibulae,  
1381 striae, and poroids, density of band striae in cingular bands, and stria and poroid structure) are  
1382 among the common taxonomically informative characters used, although some have debated the  
1383 reliability of some of these morphometrics (reviewed in Lelong et al., 2012a). Likewise, many  
1384 studies show high intraspecific variability in the morphometrics (Lundholm et al., 2002b; Cerino  
1385 et al., 2005) or overlapping data in different species (Lundholm et al., 2003, 2006). Additional

1386 character traits have been discovered and used in species delimitation. On that account, and  
1387 coupling with molecular approaches, the taxonomy of *Pseudo-nitzschia* has experienced an  
1388 explosion of new species discovery (Section 4.1).

1389 Poroid morphology is one example of a character that has been used to group species of  
1390 *Pseudo-nitzschia*. This character describes the size and shape of perforations within a poroid, the  
1391 division of poroids into sectors (or those simple poroids without sectors), and the presence of  
1392 central sectors (Fig. 6). Because poroid morphology is highly variable among species in the  
1393 genus, these character states have been employed successfully to distinguish many species, such  
1394 as *P. galaxiae* (Lundholm and Moestrup, 2002), *P. manni* (Amato and Montresor, 2008), *P.*  
1395 *circumpora* (Lim et al., 2012a) and *P. bipertita* (Teng et al., 2016). The height of poroids in the  
1396 proximal mantle has been used to distinguish *P. hasleana* (Lundholm et al., 2012), *P. kodamae*  
1397 (Teng et al., 2014a), and *P. limii* (Teng et al., 2016). Detailed structures of cingular bands  
1398 (valvocopula, band II and band III) are also used to distinguish varieties of *P. pungens* (Section  
1399 4.2).

1400 While the taxonomy of *Pseudo-nitzschia* has progressed, knowledge of how the  
1401 morphological characters or traits have evolved (character state evolution) is lacking, even  
1402 though several limited attempts have been conducted to assess their phylogenetics (cladistics)  
1403 (Orsini et al., 2002; Lim et al., 2013; Teng et al., 2013; Lim et al., 2018). A web-based  
1404 interactive identification key to species of *Pseudo-nitzschia* (cf. Teng et al., 2014c) uses 18  
1405 morpho-characters (numerical and multiple-state characters).

1406

## 1407 10.2. Molecular approaches

### 1408 10.2.1. Sequence analysis

1409 Multiple lines of evidence have often been used in the taxonomic circumscriptions of  
1410 diatoms, including genetic information coupled with phylogenetic hypotheses. Among the  
1411 genetic markers used in delimiting species of *Pseudo-nitzschia* are: nuclear-encoded ribosomal  
1412 RNA genes (SSU, LSU and ITS rDNAs); the mitochondrial encoded cytochrome *c* oxidase  
1413 subunit 1 (*cox1*) gene (Lundholm et al., 2012; Tan et al., 2015; Lim et al., 2018); the chloroplast  
1414 encoded RuBisCO large subunit (*rbcL*) gene (Amato et al., 2007; Lundholm et al., 2012;  
1415 D'Alelio and Ruggiero, 2015); and the RuBisCO small subunit (*rbcS*) gene (Delaney et al.,  
1416 2011). Each of these marker regions has advantages and disadvantages, and more studies are

needed to determine which region, or a combination thereof (Grbin et al., 2017), is best for delineating species. More than 4000 *Pseudo-nitzschia* sequences have been deposited in NCBI nucleotide databases, and rDNAs remain the widely used markers. To obtain informative characters in the genetic markers, various bioinformatics methods and algorithms have been applied to infer the phylogenetic frameworks, for example, simultaneously aligning sequences and secondary structures of ITS2 (Lim et al., 2013, 2018; Teng et al., 2015, 2016) or SSU rDNA (Lim et al., 2016) to guide orthologous alignments and to test phylogenetic hypotheses at the species level.

Compensatory base changes (CBCs) in the ITS2 transcript are also used to delimit species of *Pseudo-nitzschia* (e.g. Amato and Montresor, 2008; Quijano-Scheggia et al., 2009a; Teng et al., 2015; Percopo et al., 2016). The presence of CBCs facilitates the differentiation of biologically distinct but morphologically similar species. Likewise, the analysis of CBCs has been supported by mating experiments in some studies of *Pseudo-nitzschia* (Amato et al., 2007; Lundholm et al., 2012; Amato and Orsini, 2015; Teng et al., 2015). Even though ITS2 has been shown to exhibit a degree of intragenomic variation in protists (e.g. Thornhill et al., 2007), this has not yet been observed in *Pseudo-nitzschia*, supporting its suitability as a tool for delineating these species.

The *in situ* identification of *Pseudo-nitzschia* species can be greatly advanced with the application of high-throughput sequencing (HTS), also called next-generation sequencing (NGS) or massively parallel sequencing (MPS), of phylogenetic markers (metabarcoding) (Casabianca et al., 2017; Dzhembekova et al., 2017b; Nagai et al., 2017). The target of this approach is generally to characterize the diatom (e.g. Malviya et al., 2016) or whole protist communities. However, it is difficult to select a suitable marker for barcode identification. If the chosen marker is too variable, then diversity is overestimated. Furthermore, some level of intra- and interspecific hybridizations may occur in the genus (D'Alelio et al., 2009; Tesson et al., 2014; Amato and Orsini, 2015; D'Alelio and Ruggiero, 2015). Among the pitfalls are a lack of relevant sequence coverage for all species, the use of universal primer sets that have so far targeted regions that are less informative for *Pseudo-nitzschia* taxonomy, the requirement for curated reference sequences to interpret the huge number of sequences produced by HTS (e.g. Zimmermann et al., 2014), and issues with taxonomic assignments when defining the molecular OTUs. The latter, in many cases, stems from a redundancy in GenBank entries, such that there

1448 are either multiple entries for a single species (which is easier to track) or else multiple species  
1449 are entered with an identical sequence.

1450

1451 *10.2.2. Molecular tools*

1452 Several overviews discuss molecular tools available to identify and quantify harmful  
1453 phytoplankton species (Kudela et al., 2010; Danovaro et al., 2016; Medlin and Orozco, 2017;  
1454 Medlin, 2018). Among these are gene sequence-based hybridization methods, microarray  
1455 methods, ARISA, and qPCR.

1456

1457 *10.2.2.1. Gene sequence-based hybridization*

1458 Cell homogenate (sandwich hybridization assay; SHA) or whole cell (fluorescent *in situ*  
1459 hybridization; FISH) molecular genetic approaches can be used to identify *Pseudo-nitzschia*  
1460 species, either in the laboratory or *in situ*, e.g. using the Environmental Sample Processor  
1461 (Greenfield et al., 2006, 2008; Kudela et al., 2010; Doucette and Kudela, 2017; Bowers et al.,  
1462 2017 and references therein). These methods take advantage of large pools of cellular SSU  
1463 ribosomal RNA transcripts, thereby eliminating the need for a pre-amplification step. Recently,  
1464 the SHA and FISH probes were re-assessed and expanded, given previous intra-species genetic  
1465 variations and the discovery of new species (Bowers et al., 2017). New SHA probes were  
1466 developed to detect *P. arenysensis*, *P. fraudulenta*, *P. hasleana*, and *P. pungens*. As well,  
1467 updated cross-reactivity information was presented for historical SHA and FISH probes that  
1468 target *P. australis* and *P. multiseries*.

1469

1470 *10.2.2.2. Microarrays*

1471 Microarrays allow the simultaneous identification of multiple harmful species. This  
1472 technique is based on the design of a series of species- and genus-specific probes that are placed  
1473 on a microarray, and then hybridized with either RNA or DNA extracted from the environment  
1474 (or resulting from amplification). A broad set of probes for the SSU and LSU regions of various  
1475 *Pseudo-nitzschia* species was used on microarrays implemented within the MIDTAL EU project  
1476 (<http://www.midtal.com>) that included several other toxic phytoplankton species, as well as in  
1477 other projects (Ahn et al., 2006; Smith et al., 2012; Dittami et al., 2013; Kegel et al., 2013;  
1478 McCoy et al., 2013; Barra et al., 2014; Dhar et al., 2015; Medlin, 2018). The approach turned out

1479 to be challenging for identifying *Pseudo-nitzschia* species because of non-optimal probe  
1480 specificity when tested with RNA from field samples under standardized hybridization  
1481 conditions (Barra et al., 2013). Further refinements of the microarray design turned out to be  
1482 promising for some species (Medlin and Kegel, 2014). A DNA-based microarray was also  
1483 implemented, which was able to detect 12 species of *Pseudo-nitzschia* (Noyer et al., 2015).

1484

1485 *10.2.2.3. Automated Ribosomal Intergenic Spacer Analysis (ARISA)*

1486 *Pseudo-nitzschia* species commonly co-occur in the natural environment. To facilitate  
1487 species identification, a *Pseudo-nitzschia*-targeted Automated Ribosomal Intergenic Spacer  
1488 Analysis (ARISA) implements genus-specific primers with PCR to amplify a short, length-  
1489 variable region of the ITS1 (Hubbard et al., 2008; reviewed in Lelong et al., 2012a; Trainer et al.,  
1490 2012). This fingerprinting approach has been adapted to permit semi-quantitative comparisons of  
1491 *Pseudo-nitzschia* species assemblages across environmental samples (e.g. Hubbard, 2010;  
1492 Hubbard et al., 2014a,b; Carlson et al., 2016). Validation thus far has shown that the ARISA  
1493 assay is robust and approximates the relative abundance of ITS1 copies from each species  
1494 amplified, which varies not just with cellular abundance, but also reflects interspecific genome  
1495 size differences (Hubbard et al., 2014a). The ARISA primer set is not expected to amplify all  
1496 *Pseudo-nitzschia* species for which ITS1 sequence data are available and certain taxa with  
1497 distinct sequences share the same ITS1 amplicon size. Nevertheless, when applied in a given  
1498 region over space and/or time, it can typically resolve >10 unique taxa (Hubbard, 2010; Guannel  
1499 et al., 2015; Carlson et al., 2016).

1500 Taxonomic assignments associated with the fingerprints vary regionally, and it is  
1501 important to validate sequences with EM-identified species before incorporating ARISA into  
1502 monitoring and event response programs. Coupling ARISA with environmental sequencing has  
1503 proven informative for expanding knowledge of inter- and intra-specific diversity in *Pseudo-*  
1504 *nitzschia* communities, e.g. from coastal waters (Hubbard et al., 2008, 2014a; Carlson et al.,  
1505 2016) and offshore waters of the Pacific Northwest (Marchetti et al., 2008), the South Atlantic  
1506 (Guannel et al., 2015), Alaska (Glacier Bay) (Bailey, 2008; Bailey et al., 2009), Georges Bank  
1507 (Ellis, 2008; Ellis et al., 2009), and the Gulf of Mexico (Hubbard et al., 2014b, 2015).

1508 Population-level diversity may be reflected by insertions-deletions (e.g. *P. seriata*, *P.*  
1509 *cuspidata*) and/or nucleotide polymorphisms (Hubbard et al., 2008, 2015; Ellis et al., 2009). In

1510 the Gulf of Maine, Gulf of Mexico and the US west coast, ARISA has revealed dynamic changes  
1511 in *Pseudo-nitzschia* diversity associated with physicochemical environmental variability, in some  
1512 cases prior to and during DA events (e.g. Haring, 2010; Reserarts, 2011; Bowers et al., 2014,  
1513 2015; Mogensen, 2014; Flewelling et al., 2015; Hubbard et al., 2015; Carlson et al., 2016; Smith  
1514 et al., 2018). Although species-specific calibration to better reflect cellular abundance is still  
1515 cumbersome, ARISA is a rapid-screening tool that may be paired with quantitative species-  
1516 specific assays (e.g. qPCR, sandwich hybridization) as needed.

1517

#### 1518 10.2.2.4. Quantitative polymerase chain reaction (qPCR)

1519 qPCR is a sensitive molecular technique that has been developed and used to detect and  
1520 quantify species of *Pseudo-nitzschia* in natural assemblages (reviewed in Penna and Galluzzi,  
1521 2013; Penna et al., 2013; Adelfi et al., 2014). This technique monitors and records the  
1522 accumulation of amplified molecular fragments during PCR reactions (in real time) by  
1523 visualizing the amplification using intercalating nucleic acid stain (e.g. SYBR Green I) or  
1524 fluorophore-labeled oligonucleotide probes (e.g. hydrolysis TaqMan probe). Choice of assay  
1525 (target gene region, PCR chemistry, DNA/RNA isolation methods, choice of controls) affects the  
1526 levels of qPCR efficiency. Fitzpatrick et al. (2010) developed a genus-specific qPCR assay  
1527 targeting a gene region of SSU rRNA and applied it to detect eight species of *Pseudo-nitzschia* in  
1528 the environment. This assay, however, is less specific (as it also detects *Fragilariaopsis cylindrus*)  
1529 and requires additional verification of the existing *Pseudo-nitzschia* species in the environment  
1530 by electron microscopy. Estimation of species abundance by this method is thus less reliable  
1531 when species co-exist, as different species harbor a different rDNA copy number per cell (Penna  
1532 et al., 2013). Species-specific qPCR assays targeting ITS1 or ITS2 (Andree et al., 2011) exhibit  
1533 greater sensitivity. Quantitative reverse transcription PCR (qRT-PCR), targeting the *rbcS* mRNA  
1534 transcript of *P. multiseries*, has demonstrated good estimations of actual cell abundance in  
1535 natural samples, despite the variability of *rbcS* expressions (Delaney et al., 2011). Kim et al.  
1536 (2017, 2018) used qPCR to detect all three clades of *P. pungens* (Section 4.2). A multiplexing  
1537 qPCR approach, using post-PCR high resolution melting (HRM) curve analysis, was developed  
1538 to simultaneously detect *P. calliantha*, *P. pungens* and *P. delicatissima/P. cf. arenysensis*  
1539 (Pugliese et al., 2017). A stated advantage is that this approach can provide higher throughput

1540 and specificity at a lower cost and more quickly compared to microarrays, rDNA sequencing, or  
1541 single target qPCR.

1542

## 1543 **11. Questions and directions for future research**

1544 Much information has been gained since the reviews of Lelong et al. (2012a) and Trainer  
1545 et al. (2012) about new species of *Pseudo-nitzschia* and *Nitzschia* and their world distribution,  
1546 triggers of DA production, the interaction between bacteria and *Pseudo-nitzschia* species, the  
1547 prevalence of DA in the food web, impacts of DA on zooplankton, details of the sexual cycle,  
1548 and oomycete infections. Many of these advances have occurred because of the increased  
1549 application of molecular methods. Nevertheless, gaps in knowledge remain. Below are questions  
1550 remaining and suggestions for future research:

- 1551 • Large gaps remain in understanding the molecular mechanism(s) regulating DA  
1552 biosynthesis. Are the mechanisms the same in *Pseudo-nitzschia* and *Nitzschia*? Do all  
1553 species in these genera possess the genes of the metabolic pathway of DA production?  
1554 Use of the new ‘omics’, aimed at the universal detection of genes (genomics), mRNA  
1555 (transcriptomics), proteins (proteomics) and metabolites (metabolomics), may be of help  
1556 to understand the molecular pathway of DA production. Specific genes may require  
1557 activation under certain environmental (e.g. light, nutrients, pH) and biological (e.g.  
1558 presence of bacteria, zooplankton) conditions.
- 1559 • Understanding the pathway for DA production and its regulation will help to develop  
1560 methods that discriminate between DA producers and their non-toxic congeners. This  
1561 will allow addressing the question as to why some species, and even strains, produce DA,  
1562 whereas others do not. Sequencing the genomes of additional *Pseudo-nitzschia* species  
1563 would provide information to answer some of these questions.
- 1564 • The mechanisms of action to explain how bacteria enhance DA production are still  
1565 elusive. Do *Pseudo-nitzschia* cells contain intracellular bacteria? How do certain bacteria  
1566 become attached to *Pseudo-nitzschia* cells, and what is their role? Do any bacteria  
1567 produce DA autonomously? How much of the bacterial genome is integrated into the  
1568 *Pseudo-nitzschia* genome?
- 1569 • What other zooplankton, or other grazers, induce DA production? What are its  
1570 mechanisms of action? What are the grazing patterns of zooplankton exposed to toxic

- 1571           *Pseudo-nitzschia* species? How long do zooplankton remain toxic after grazing ceases?
- 1572           The presence of zooplankton and bacteria may potentially explain the intra-species
- 1573           variability in DA production.
- 1574         • Given the evidence of intraspecific diversity of toxin production, more genetically
  - 1575           characterized strains of different species should be tested. This will enable a more solid
  - 1576           basis for understanding intra- and inter-species differences. The growth phase in culture
  - 1577           should be taken into account when collecting samples for analysis. Standardized
  - 1578           protocols should be developed in order to test for triggers of DA production.
  - 1579         • More studies should be carried out to explore differences toxin composition, i.e. DA and
  - 1580           its isomers IA–IE, among strains of *Pseudo-nitzschia* and *Nitzschia*. Why are DA and its
  - 1581           isomers produced, and what is the metabolic cost?
  - 1582         • More *Nitzschia* species, and other genera in the Bacillariaceae, should be tested for the
  - 1583           ability to produce DA and its isomers. The original report that *Halamphora coffeaeformis*
  - 1584           is a DA producer (discussed in Bates, 2000) is still not confirmed.
  - 1585         • It is important to apply an integrated approach to species circumscription, coupling
  - 1586           morphological, genetic, life cycle and functional approaches. ‘Omics’ now provide the
  - 1587           tools to address these questions, e.g. metabolomics, mating systems and pre-/post-
  - 1588           fertilization barriers, and toxin production. Ideally, studies should be carried out on a
  - 1589           global scale to assess patterns of genetic structure of different species and/or populations.
  - 1590           *Pseudo-nitzschia* are ideal candidates to explore speciation mechanisms in marine
  - 1591           diatoms.
  - 1592         • Are there barcode sequences that can be used for species detection through high-
  - 1593           throughput sequencing? Curated reference sequences are required to interpret these data.
  - 1594           Many sequences in GenBank have not been updated with regard to species identification.
  - 1595         • Most studies have focused on temperate regions. However, recent investigations in
  - 1596           tropical areas (e.g. Malaysia) discovered several new species. Studies should also be
  - 1597           expanded polar regions, especially the Arctic, which is strongly impacted by climate
  - 1598           change. Africa is also a very sparsely studied region.
  - 1599         • It is important to maintain taxonomic expertise, so that researchers can couple traditional
  - 1600           morphological knowledge with advanced molecular tools, in order to improve our
  - 1601           understanding of the diversity and evolutionary history of these diatom species.

- 1602     • More ultrastructure and morphometric studies are required to find possible hidden  
1603        characters that may help to delineate species, especially closely related pseudo-cryptic  
1604        species, e.g. in the *delicatissima*, *pseudodelicatissima* and *americana* complexes. The  
1605        arrangement/pattern of sectors in a poroid could be a new informative character, but  
1606        additional approaches are required to resolve this.
- 1607     • Improved detection tools are required to study the biogeographical structure at the  
1608        population level of taxa. Some molecular markers may work only at a regional scale due  
1609        to genetic diversity amongst populations from different areas.
- 1610     • Laboratory and field studies should be coupled in order to better understand the  
1611        mechanisms leading to bloom formation and toxin production in the field. What is the  
1612        relationship between the seasonality of blooms of different species and the presence of  
1613        distinct bacterial populations?
- 1614     • Sexual reproduction plays a key role in the life cycle of *Pseudo-nitzschia* species,  
1615        although we have only limited evidence for this in the field. More information is required  
1616        to determine where it occurs, how frequently, and what the involvement of pheromones  
1617        may be. Genomic studies may provide molecular markers to address this question.
- 1618     • There is only limited information concerning the interactions between viruses and  
1619        *Pseudo-nitzschia* (Carlson et al., 2016). Further studies are also required on infections by  
1620        fungal parasites and how they may affect population dynamics or DA production. What  
1621        species of *Pseudo-nitzschia* can be infected by oomycete and chytrid parasitoids, and  
1622        what is their specificity? Is there a relationship between susceptibility to infection and the  
1623        ability to produce DA? Similar studies could be carried out with toxicogenic *Nitzschia*  
1624        species.
- 1625     • Given the conflicting reports of allelopathy in cultures of different *Pseudo-nitzschia*  
1626        species and strains, and of differing responses on different target phytoplankton,  
1627        additional studies on allelopathy, including the quantification and composition of  
1628        allelochemicals, are warranted.

1629

### 1630   **Acknowledgments**

1631   We thank Penelope Ajani (University of Technology Sydney) for providing information about  
1632   *Pseudo-nitzschia* species in Australian waters, Kathi A. Lefebvre and Su Kim (NOAA,

1633 Northwest Fisheries Science Center) for creating the modified Fig. 3, Sing Tung Teng  
1634 (Universiti Malaysia Sarawak) for creating Fig. 6, Holly A. Bowers (Monterey Bay Aquarium  
1635 Research Institute/Moss Landing Marine Laboratories) for comments, Deborah Robertson and  
1636 Emily Stone (Clark University) for information about the *P. australis* genome, Leanne  
1637 Flewelling and Sheila O'Dea (FWRI) for information about DA in the Gulf of Mexico, Steve  
1638 Archer (Bigelow Laboratory for Ocean Sciences), Kohl Kanwit and Bryant Lewis (ME DMR),  
1639 Anna Farrell (Mount Desert Island Biological Laboratory), Emily Denny, Maya Robert and Celia  
1640 Villac (FWRI) for Gulf of Maine bloom samples/data, and Claire Gachon (Scottish Marine  
1641 Institute), Raffaele Siano and Elisabeth Nézan (IFREMER, France) for information on *Pseudo-*  
1642 *nitzschia* oomycetes. Funding to N.L. was by the Danish Research Council, Grant DFF-1323-  
1643 00258. Funding to K.H was through the Woods Hole Center for Oceans and Human Health, the  
1644 US National Science Foundation (Grant OCE-1314642), and the National Institute of  
1645 Environmental Health Sciences (Grant 1P01ES021923-01).  
1646  
1647

- 1648     **References**
- 1649
- 1650     Adams, N.G., Schwenke, P., Smith, G.J., Trainer, V.L., 2017. Microsatellite markers for  
1651       population genetic applications in the domoic acid-producing diatom *Pseudo-nitzschia*  
1652       *australis* Frenguelli (Bacillariophyceae). *Protist* 168, 197–205.
- 1653     Ahn, S., Kulis, D.M., Erdner, D.L., Anderson, D.M., Walt, D.R., 2006. Fiber-optic microarray for  
1654       simultaneous detection of multiple harmful algal bloom species. *Appl. Environ. Microbiol.*  
1655       72, 5742–5749.
- 1656     Ajani, P., Murray, S., Hallegraeff, G., Brett, S., Armand, L., 2013a. First reports of *Pseudo-nitzschia*  
1657       *micropora* and *P. hasleana* (Bacillariaceae) from the Southern Hemisphere: morphological,  
1658       molecular and toxicological characterization. *Phycol. Res.* 61, 237–248.
- 1659     Ajani, P., Murray, S., Hallegraeff, G., Lundholm, N., Gillings, M., Brett, S., Armand, L., 2013b.  
1660       The diatom genus *Pseudo-nitzschia* (Bacillariophyceae) in New South Wales, Australia:  
1661       morphotaxonomy, molecular phylogeny, toxicity, and distribution. *J. Phycol.* 49, 765–785.
- 1662     Ajani, P., Kim, J.H., Han, M.S., Murray, S.A., 2016. The first report of the potentially harmful  
1663       diatom *Pseudo-nitzschia caciantha* from Australian coastal waters. *Phycol. Res.* 64, 312–317.
- 1664     Ajani, A., Verma, A., Lassudrie, M., Doblin, M.A., Murray, S.A., 2018. A new diatom species *P.*  
1665       *hallegraeffii* sp. nov. belonging to the toxic genus *Pseudo-nitzschia* (Bacillariophyceae)  
1666       from the East Australian Current. *PLoS ONE* 13, e0195622.
- 1667     Adelfi, M.G., Borra, M., Sanges, R., Montresor, M., Fontana, A., Ferrante, M.I., 2014. Selection  
1668       and validation of reference genes for qPCR analysis in the pennate diatoms *Pseudo-nitzschia*  
1669       *multistriata* and *P.* *J. Exp. Mar. Biol. Ecol.* 451, 74–81.
- 1670     Al Muftah, Selwood, A.I., Foss, A.J., Al-Jabri, H.M.S.J., Potts, M., Yilmaz, M., 2016. Algal toxins  
1671       and producers in the marine waters of Qatar, Arabian Gulf. *Toxicon* 122, 54–66.
- 1672     Almundoz, G.O., Fabro, E., Ferrario, M., Tillmann, U., Cembella, A., Krock, B., 2017. Species  
1673       occurrence of the potentially toxicogenic diatom genus *Pseudo-nitzschia* and the associated  
1674       neurotoxin domoic acid in the Argentine Sea. *Harmful Algae* 63, 45–55.
- 1675     Álvarez, G., Uribe, E., Quijano-Scheggia, S., López-Rivera, A., Mariño, C., Blanco J., 2009.  
1676       Domoic acid production by *Pseudo-nitzschia australis* and *Pseudo-nitzschia calliantha*  
1677       isolated from North Chile. *Harmful Algae* 8, 938–945.
- 1678     Álvarez, G., Uribe, E., Regueiro, J., Martin, H., Gajardo, T., Jara, L., Blanco, J., 2015. Depuration  
1679       and anatomical distribution of domoic acid in the surf clam *Mesodesma donacium*. *Toxicon*  
1680       102, 1–7.
- 1681     Amato, A., Montresor, M., 2008. Morphology, phylogeny and sexual cycle of *Pseudo-nitzschia*  
1682       *mannii* sp. nov. (Bacillariophyceae): a pseudo-cryptic species within the *P.*  
1683       *pseudodelicatissima* complex. *Phycologia* 47, 487–497.
- 1684     Amato, A., Orsini, L., 2015. Rare interspecific breeding in *Pseudo-nitzschia* (Bacillariophyceae).  
1685       *Phytotaxa* 217, 145–154.
- 1686     Amato, A., Orsini, L., D'Alelio, D., Montresor, M., 2005. Life cycle, size reduction patterns, and  
1687       ultrastructure of the pennate planktonic diatom *Pseudo-nitzschia delicatissima*  
1688       (Bacillariophyceae). *J. Phycol.* 41, 542–556.

- 1689 Amato, A., Kooistra, W.H.C.F., Levialdi Ghiron, J.H., Mann, D.G., Pröschold, T., Montresor, M.,  
1690 2007. Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist* 158,  
1691 193–207.
- 1692 Amato, A., Lüdeking, A., Kooistra, W.H.C.F., 2010. Intracellular domoic acid production in  
1693 *Pseudo-nitzschia multistriata* isolated from the Gulf of Naples (Tyrrhenian Sea, Italy).  
1694 *Toxicon* 55, 157–161.
- 1695 Amin, S.A., Parker, M.S., Armbrust, E.V., 2012. Interactions between diatoms and bacteria.  
1696 *Microbiol. Mol. Biol. Rev.* 76, 667–684.
- 1697 Amin, S.A., Hmelo, L.R., van Tol, H.M., Durham, B.P., Carlson, L.T., Heal, K.R., Morales,  
1698 R.L., Berthiaume, C.T., Parker, M.S., Djunaedi, B., Ingalls, A.E., Parsek, M.R., Moran,  
1699 M.A., Armbrust, E.V., 2015. Interaction and signalling between a cosmopolitan  
1700 phytoplankton and associated bacteria. *Nature* 522, 98–101.
- 1701 Andree, K.B., Fernández-Tejedor, M., Elandaloussi, L.M., Quijano-Scheggia, S., Sampedro, N.,  
1702 Garcés, E., Camp, J., Diogène, J., 2011. Quantitative PCR coupled with melt curve  
1703 analysis for detection of selected *Pseudo-nitzschia* spp. (Bacillariophyceae) from the NW  
1704 Mediterranean Sea. *Appl. Environ. Microbiol.* 77, 1651–1659.
- 1705 Angus, T.H., 2015. Examining the toxicity, exposure and regulatory approach to potential human  
1706 health risks of the algal toxin domoic acid. PhD Thesis, University of Massachusetts  
1707 Boston, 360 p.
- 1708 Arapov, J., Ujević, I., Pfannkuchen, D.M., Godrijan, J., Bakrač, A., Gladan, Ž.N., Marasović, I.,  
1709 2016. Domoic acid in phytoplankton net samples and shellfish from the Krka River  
1710 estuary in the Central Adriatic Sea. *Mediterr. Mar. Sci.* 17, 340–350.
- 1711 Arapov, J., Skejić, S., Bužančić, M., Bakrač, A., Vidjak, O., Bojanic, N., Ujević, I., Gladan,  
1712 Ž.N., 2017. Taxonomical diversity of *Pseudo-nitzschia* from the Central Adriatic Sea.  
1713 *Phycol. Res.* 65, 280–290.
- 1714 Aumeier, C., Polinski, E., Menzel, D., 2015. Actin, actin-related proteins and profilin in diatoms:  
1715 a comparative genomic analysis. *Mar. Genomics* 23, 133–142.
- 1716 Auro, M.E., Cochlan, W.P., 2013. Nitrogen utilization and toxin production by two diatoms of  
1717 the *Pseudo-nitzschia pseudodelicatissima* complex: *P. cuspidata* and *P. fryselliana*. *J.*  
1718 *Phycol.* 49, 156–169.
- 1719 Bailey, A., 2008. *Pseudo-nitzschia* species distributions in Glacier Bay, AK as measured by  
1720 automated ribosomal intergenic spacer analysis (ARISA). Senior Undergraduate Thesis,  
1721 University of Washington, 12 p.  
1722 <https://digital.lib.washington.edu/researchworks/handle/1773/3745>
- 1723 Bailey, A.J.S., Hubbard, K., Armbrust, E.V., 2009. Spatial distribution of *Pseudo-nitzschia* ITS1  
1724 types detected with ARISA in Glacier Bay, Alaska. Fifth Symposium on Harmful Algae in  
1725 the U.S., Ocean Shores, WA, p. 90.  
1726 <https://www.whoi.edu/fileserver.do?id=229605&pt=2&p=28786>
- 1727 Bajarias, F.F.A., Kotaki, Y., Relox, J.R., Jr., Romero, M.L.J., Furio, E.F., Lundholm, N., Koike,  
1728 K., Fukuyo, Y., Kodama, M., 2006. Screening of diatoms producing domoic acid and its  
1729 derivatives in the Philippines. *Coast. Mar. Sci.* 30, 121–129.

- 1730 Balzano, S., Percopo, I., Siano, R., Gourvil, P., Chanoine, M., Dominique, M., Vaulot, D., Sarno,  
1731 D., 2017. Morphological and genetic diversity of Beaufort Sea diatoms with high  
1732 contributions from the *Chaetoceros neogracilis* species complex. *J. Phycol.* 53, 161–187.
- 1733 Bargu, S., Koray, T., Lundholm, N., 2002a. First report of *Pseudo-nitzschia calliantha*  
1734 Lundholm, Moestrup & Hasle 2003, a new potentially toxic species from Turkish coasts.  
1735 *E.U. J. Fish. Aquat. Sci.* 19, 479–483.
- 1736 Bargu, S., Powell, C.L., Coale, S.L., Busman, M., Doucette, G.J., Silver, M.W., 2002b. Krill: a  
1737 potential vector for domoic acid in marine food webs. *Mar. Ecol. Prog. Ser.* 237, 209–216.
- 1738 Bargu, S., Marinovic, B., Mansergh, S., Silver, M., 2003. Feeding responses of krill to the toxin-  
1739 producing diatom *Pseudo-nitzschia*. *J. Exp. Mar. Biol. Ecol.* 284, 87–104.
- 1740 Bargu, S., Lefebvre, K., Silver, M.W., 2006. Effect of dissolved domoic acid on the grazing rate  
1741 of krill *Euphausia pacifica*. *Mar. Ecol. Prog. Ser.* 312, 169–175.
- 1742 Bargu, S., Smith, E., Ozhan, K., 2011. Toxic diatom *Pseudo-nitzschia* and its primary consumers  
1743 (vectors). In: Seckbach, J., Kociolek, P. [Eds.], *The Diatom World. (Cellular Origin, Life*  
1744 *in Extreme Habitats and Astrobiology, 19)*. Koeltz Scientific Books, Koenigstein,  
1745 Germany, pp. 491–512.
- 1746 Bargu, S., Baustian, M.M., Rabalais, N.N., Del Rio, R., Von Korff, B., Turner, R.E., 2016,  
1747 Influence of the Mississippi River on *Pseudo-nitzschia* spp. abundance and toxicity in  
1748 Louisiana coastal waters. *Estuar. Coasts* 39, 1345–1356.
- 1749 Barra, L., Ruggiero, M.V., Sarno, D., Montresor, M., Kooistra, W.H.C.F., 2013. Strengths and  
1750 weaknesses of microarray approaches to detect *Pseudo-nitzschia* species in the field.  
1751 *Environ. Sci. Pollut. Res.* 20, 6705–6718.
- 1752 Barra, L., Ruggiero, M.V., Chen, J., Kooistra, W.H.C.F., 2014. Specificity of LSU rRNA-  
1753 targeted oligonucleotide probes for *Pseudo-nitzschia* species tested through dot-blot  
1754 hybridisation. *Environ. Sci. Pollut. Res.* 21, 548–557.
- 1755 Basu, S., Patil, S., Mapleson, D., Russo, M.T., Vitale, L., Fevola, C., Maumus, F., Casotti, R.,  
1756 Mock, T., Caccamo, M., Montresor, M., Sanges, R., Ferrante, M.I., 2017. Finding a partner  
1757 in the ocean: molecular and evolutionary bases of the response to sexual cues in a  
1758 planktonic diatom. *New Phytol.* 215, 140–156.
- 1759 Bates, S.S., 1998. Ecophysiology and metabolism of ASP toxin production. In: Anderson, D.M.,  
1760 Cembella, A.D., Hallegraeff, G.M. [Eds.], *Physiological Ecology of Harmful Algal*  
1761 *Blooms*. Springer-Verlag, Heidelberg, pp. 405–426.
- 1762 Bates, S.S., 2000. Domoic-acid-producing diatoms: another genus added! *Journal of Phycology*  
1763 36, 978–983.
- 1764 Bates, S.S., Trainer, V.L., 2006. The ecology of harmful diatoms. In: Granéli, E., Turner, J.  
1765 [Eds.], *Ecology of Harmful Algae. Ecological Studies, Vol. 189*. Springer-Verlag,  
1766 Heidelberg, pp. 81–93.
- 1767 Bates, S.S., Bird, C.J., de Freitas, A.S.W., Foxall, R., Gilgan, M., Hanic, L.A., Johnson, G.R.,  
1768 McCulloch, A.W., Odense, P., Pocklington, R., Quilliam, M.A., Sim, P.G., Smith, J.C.,  
1769 Subba Rao, D.V., Todd, E.C.D., Walter, J.A., Wright, J.L.C., 1989. Pennate diatom

- 1770        *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern  
1771        Prince Edward Island, Canada. Can. J. Fish. Aquatic Sci. 46, 1203–1215.
- 1772        Bates, S.S., Garrison, D.L., Horner, R.A., 1998. Bloom dynamics and physiology of domoic-  
1773        acid-producing *Pseudo-nitzschia* species. In: Anderson, D.M., Cembella, A.D.,  
1774        Hallegraeff, G.M. [Eds.], Physiological Ecology of Harmful Algal Blooms. Springer-  
1775        Verlag, Heidelberg, pp. 267–292.
- 1776        Bates, S.S., Léger, C., Satchwell, M.F., Boyer, G.L., 2001. The effects of iron on domoic acid  
1777        production by *Pseudo-nitzschia multiseries*. In: Hallegraeff, G.M., Blackburn, S.I., Bolch,  
1778        C.J., Lewis, R.J. [Eds.], Harmful Algal Blooms 2000. Intergovernmental Oceanographic  
1779        Commission of UNESCO, Paris, pp. 320–323.
- 1780        Bates, S.S., Hubbard, K.A., Lundholm, N., Montresor, M., Leaw, C.P., 2018. Toxic and harmful  
1781        diatoms. In: Seckbach, J., Gordon, R. [Eds.], Diatoms: Fundamentals & Applications  
1782        [Volume 1 in the series: Diatoms: Biology & Applications, series editors: Richard Gordon  
1783        & Joseph Seckbach]. Wiley-Scrivener, Beverly, MA, USA. (in revision)
- 1784        Baytut, Ö., 2013. A study on the phylogeny and phylogeography of a marine cosmopolite diatom  
1785        from the southern Black Sea. Oceanol. Hydrobiol. Studies 42, 406–411.
- 1786        Baytut, Ö., Moestrup, Ø., Lundholm, N., Gönülol, A., 2013. Contributions to the diatom flora of  
1787        the Black Sea from ultrastructural and molecular studies: new records of *Skeletonema*  
1788        *marinoi*, *Pseudo-nitzschia pungens* var. *aveirensis* and *Chaetoceros tenuissimus* for the  
1789        marine flora of Turkey. Nova Hedwigia 96, 427–444.
- 1790        Bazzoni, A.M., Caddeo, T., Pulina, S., Padedda, B.M., Satta, C.T., Sechi, N., Lugliè, A., 2015.  
1791        Spatial distribution and multiannual trends of potentially toxic microalgae in shellfish  
1792        farms along the Sardinian coast (NW Mediterranean Sea). Environ. Monit. Assess. 187,  
1793        86.
- 1794        Bazzoni, A.M., Mudadu, A.G., Lorenzoni, G., Arras, I., Lugliè, A., Vivaldi, B., Cicotelli, V.,  
1795        Sanna, G., Tedde, G., Ledda, S., Alessio, E., Marongiu, E., Virgilio, S., 2016. Occurrence  
1796        of harmful algal species and shellfish toxicity in Sardinia (Italy). Ital. J. Food Saf. 5, 194–  
1797        199.
- 1798        Bejarano, A.C., Van Dola, F.M., Gulland, F.M., Rowles, T.K., Schwacke, L.H., 2008.  
1799        Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: a  
1800        review. Huma. Ecol. Risk Assess. 14, 544–567.
- 1801        Bell, W., Mitchell, R., 1972. Chemotactic and growth responses of marine bacteria to algal  
1802        extracellular products. Biol. Bull. 143, 265–277.
- 1803        Belhaouari, B., Setti, M., Kawther, A., 2017. Monitoring of phytoplankton on coast of Ténès  
1804        (Algeria). J. Water Sci. Environ. Technol. 2, 159–263.
- 1805        Bellinger, B.J., Hagerthey, S.E., 2010. Presence and diversity of algal toxins in subtropical  
1806        peatland periphyton: the Florida everglades, USA. J. Phycol. 46, 674–678.
- 1807        Bengtson Nash, S.M., Baddock, M.C., Takahashi, E., Dawson, A., Cropp, R., 2017. Domoic acid  
1808        poisoning as a possible cause of seasonal cetacean mass stranding events in Tasmania,  
1809        Australia. Bull. Environ. Contam. Toxicol. 98, 8–13.

- 1810 Ben Haddouch, A., Taleb, H., Elmortaji, H., Ben Brahim, S., Ennafah, B., Menchih, K., Boumaz,  
1811 A., Mzaki, F., Radi, A., Loutfi, M., 2016. Accumulation and tissue distribution of domoic  
1812 acid in the common cuttlefish, *Sépia officinalis* from the south Moroccan coast. Amer. Sci.  
1813 Res. J. Engin. Technol. Sci. 15, 252–264.
- 1814 Besiktepe, S., Ryabushko, L., Ediger, D., Yilmaz, D., Zenginer, A., Ryabushko, V., Lee, R.,  
1815 2008. Domoic acid production by *Pseudo-nitzschia calliantha* Lundholm, Moestrup et  
1816 Hasle (Bacillariophyta) isolated from the Black Sea. Harmful Algae 7, 438–442.
- 1817 Bill, B.D., Cochlan, W.P., Trainer, V.L., 2012. The effect of light on growth rate and primary  
1818 productivity in *Pseudo-nitzschia australis* and *Pseudo-nitzschia turgidula*. In: Pagou, P.,  
1819 Hallegraeff, G. [Eds.], Proceedings of the 14th International Conference on Harmful  
1820 Algae. International Society for the Study of Harmful Algae and Intergovernmental  
1821 Oceanographic Commission of UNESCO, Paris, pp. 78–80.
- 1822 Boissonneault, K.R., Henningsen, B.M., Bates, S.S., Robertson, D.L., Milton, S., Pelletier, J.,  
1823 Hogan, D.A., Housman, D.E., 2013. Gene expression studies for the analysis of domoic  
1824 acid production in the marine diatom *Pseudo-nitzschia multiseries*. BMC Molec. Biol. 14,  
1825 25.
- 1826 Borkman, D., Hannus, L., Scott, E., Liberti, A., Rodrigue, K., Leibovitz, H., 2017. Novel  
1827 toxicogenic *Pseudo-nitzschia* blooms in Narragansett Bay, Rhode Island. 9<sup>th</sup> US Symp.  
1828 HABs, Baltimore, Maryland. (Abstract)
- 1829 Bouchouicha Smida, D., Lundholm, N., Kooistra, W.H.C.F., Sahraoui, I., Valeria, M., Kotaki,  
1830 Y., Ellegaard, M., Lambert, C., Hadj Mabrouk, H., Sakka Hlaili, A., 2014. Morphology  
1831 and molecular phylogeny of *Nitzschia bizertensis* sp. nov. – A new domoic acid-producer.  
1832 Harmful Algae 32, 49–63.
- 1833 Bouchouicha-Smida, D., Bates, S.S., Lundholm, N., Lambert, C., Mabrouk, H.H., Sakka Hlaili,  
1834 A., 2015a. Viability, growth and domoic acid toxicity of the diatom *Nitzschia bizertensis*  
1835 following ingestion and gut passage in the mussel *Mytilus* sp. Mar. Biol. 162, 2513–2519.
- 1836 Bouchouicha-Smida, D., Lundholm, N., Sahraoui, I., Lambert, C., Hadj Mabrouk, H., Sakka  
1837 Hlaili, A., 2015b. Detection of domoic acid in *Mytilus galloprovincialis* and *Ostrea edulis*  
1838 linked to the presence of *Nitzschia bizertensis* in the Bizerte lagoon (SW Mediterranean).  
1839 Estuar. Coast. Shelf Sci. 5, 270–278.
- 1840 Bowers, H.A., Ryan, J.P., Zhang, Y., Hubbard, K., Smith, G.J., Woods, A., Negrey, K., Kudela,  
1841 R., and Scholin, C.A., 2014. Characterizing phytoplankton distributions using targeted  
1842 sampling by an autonomous underwater vehicle. 16<sup>th</sup> International Conference on Harmful  
1843 Algae, Wellington, New Zealand.
- 1844 Bowers, H., Woods, A., Ryan, J., Marin, R., Smith, G.J., Mikulski, T., Doucette, G., Hubbard,  
1845 K.A., Negrey, K., Kudela, R., Birch, J., Scholin, C., 2015. Unraveling diversity in *Pseudo-*  
1846 *nitzschia* species throughout a toxic event in Monterey Bay, California. Eighth Symposium  
1847 on Harmful Algae in the U.S., Long Beach, CA, p. 54.  
1848 <https://www.whoi.edu/fileserver.do?id=229004&pt=2&p=28786>
- 1849 Bowers, H.A., Marin, R. III, Birch, J.M., Scholin, C.A., Doucette, G.J., 2016. Recovery and  
1850 identification of *Pseudo-nitzschia* (Bacillariophyceae) frustules from natural samples  
1851 acquired using the environmental sample processor. J. Phycol. 52, 135–140.

- 1852 Bowers, H.A., Marin, R. III, Birch, J.M., Scholin, C.A., 2017. Sandwich hybridization probes for  
1853 the detection of *Pseudo-nitzschia* (Bacillariophyceae) species: an update to existing probes  
1854 and a description of new probes. *Harmful Algae* 70, 37–51.
- 1855 Braid, H.E., Deeds, J., DeGrasse, S.L., Wilson, J.J., Osborne, J., Hanner, R.H., 2012. Preying on  
1856 commercial fisheries and accumulating paralytic shellfish toxins: a dietary analysis of  
1857 invasive *Dosidicus gigas* (Cephalopoda Ommastrephidae) stranded in Pacific Canada.  
1858 *Mar. Biol.* 159, 25–31.
- 1859 Bresnan, E., Kraberg, A., Fraser, S., Brown, L., Hughes, S., Wiltshire, K.H., 2015. Diversity and  
1860 seasonality of *Pseudo-nitzschia* (Peragallo) at two North Sea time-series monitoring sites.  
1861 *Helgoland Mar. Res.* 69, 193–204.
- 1862 Bresnan, E., Fryer, R.J., Fraser, S., Smith, N., Stobo, L., Brown, N., Turrell, E., 2017. The  
1863 relationship between *Pseudo-nitzschia* (Peragallo) and domoic acid in Scottish shellfish.  
1864 *Harmful Algae* 63, 193–202.
- 1865 Buaya, A.T., Ploch, S., Hanic, L., Nam, B., Nigrelli, L., Kraberg, A., Thines, M., 2017.  
1866 Phylogeny of *Miracula helgolandica* gen. et sp. nov. and *Olpidiopsis drebesii* sp. nov.,  
1867 two basal oomycete parasitoids of marine diatoms, with notes on the taxonomy of  
1868 *Ectrogella*-like species. *Mycol. Prog.* 16, 1041–1050.
- 1869 Burek-Huntington, K.A., Dushane, J.L., Goertz, C.E.C., Measures, L.N., Romero, C.H., Raverty,  
1870 S.A., 2015. Morbidity and mortality in stranded Cook Inlet beluga whales *Delphinapterus*  
1871 *leucas*. *Dis. Aquat. Organ.* 114, 45–60.
- 1872 Busch, J.A., Andree, K.B., Diogène, J., Fernández-Tejedor, M., Toebe, K., John, U., Krock, B.,  
1873 Tillmann, U., Cembella, A.D., 2016. Toxigenic algae and associated phycotoxins in two  
1874 coastal embayments in the Ebro Delta (NW Mediterranean). *Harmful Algae* 55, 191–201.
- 1875 Bushati, M., Koni, E., Bregaj, M., Kashta, L., Miho, A., 2012. Horizontal structure of potentially  
1876 toxic algae and their toxins production. *J. Institut. Alb-Shkenca* 1, 13–19.
- 1877 Carlson, M.C.G., McCary, N.D., Leach, T.S., Rocap, G., 2016. *Pseudo-nitzschia* challenged with  
1878 co-occurring viral communities display diverse infection phenotypes. *Front. Microbiol.* 7,  
1879 527.
- 1880 Cao, M., Yuan, X.-L., Bi, G., 2016. Complete sequence and analysis of plastid genomes of  
1881 *Pseudo-nitzschia multiseries* (Bacillariophyta). *Mitochondrial DNA* 27, 2897–2898.
- 1882 Caroppo, C., Cerino, F., Auriemma, R., Cibic, T., 2016. Phytoplankton dynamics with a special  
1883 emphasis on harmful algal blooms in the Mar Piccolo of Taranto (Ionian Sea, Italy).  
1884 *Environ. Sci. Pollut. Res.* 23, 12691–12706.
- 1885 Casabianca, S., Cornetti, L., Capellacci, S., Vernesi, C., Penna, A., 2017. Genome complexity of  
1886 harmful microalgae. *Harmful Algae* 63, 7–12.
- 1887 Casteleyn, G., Chepurnov, V.A., Leliaert, F., Mann, D.G., Bates, S.S., Lundholm, N., Rhodes,  
1888 L., Sabbe, K., Vyverman, W., 2008. *Pseudo-nitzschia pungens* (Bacillariophyceae): a  
1889 cosmopolitan diatom species? *Harmful Algae* 7, 241–257.
- 1890 Casteleyn, G., Adams, N.G., Vanormelingen, P., Debeer, A.-E., Sabbe, K., Vyverman, W., 2009.  
1891 Natural hybrids in the marine diatom *Pseudo-nitzschia pungens* (Bacillariophyceae):  
1892 genetic and morphological evidence. *Protist* 160, 343–354.

- 1893 Casteleyn, G., Leliaert, F., Backeljau, T., Debeer, A.-E., Kotaki, Y., Rhodes, L., Lundholm, N.,  
1894 Sabbe, K., Vyverman W., 2010. Limits to gene flow in a cosmopolitan marine planktonic  
1895 diatom. Proc. Natl. Acad. Sci. USA 107, 12952–12957.
- 1896 Cavalcante, K.P., 2011. Taxonomia da diatomácea potencialmente tóxica *Pseudo-nitzschia*  
1897 Peragallo (Bacillariophyceae) em áreas de maricultura de Santa Catarina. M.Sc. Thesis,  
1898 Curitiba, Brazil, Universidade Federal do Paraná, 69 pp.
- 1899 Cavole, L.M., Demko, A.M., Diner, R.E., Giddings, A., Koester, I., Pagniello, C.M.L.S.,  
1900 Paulsen, M.-L., Ramirez-Valdez, A., Schwenck, S.M., Yen, N.K., Zill, M.E., Franks,  
1901 P.J.S., 2016. Biological impacts of the 2013–2015 warm-water anomaly in the Northeast  
1902 Pacific: winners, losers, and the future. Oceanography 29, 273–285.
- 1903 Cerino F., Orsini, L., Sarno, D., Dell’Aversano, C., Tartaglione, L., Zingone A., 2005. The  
1904 alternation of different morphotypes in the seasonal cycle of the toxic diatom *Pseudo-*  
1905 *nitzschia galaxiae*. Harmful Algae 4, 33–48.
- 1906 Chai, Y., Deng, W.-J., Qin, X., Xu, X., 2017. Occurrence of four species of algae in the marine  
1907 water of Hong Kong. Mar. Pollut. Bull. 124, 890–896.
- 1908 Chen, J., Wang, H., Yang, A.Q., Si, R.R., Guan, W.C., 2018. Short-term and diurnal temperature  
1909 changes alter the response of harmful algal blooms of *Pseudo-nitzschia pungens* to solar  
1910 ultraviolet radiation. New Zealand J. Mar. Freshwater Res. 52, 69–81.
- 1911 Chepurnov, V.A., Mann, D.G., Sabbe, K., 2004. Experimental studies on sexual reproduction in  
1912 diatoms. Int. Rev. Cytol. 237, 91–154.
- 1913 Chepurnov, V.A., Mann, D.G., Sabbe, K., Vannerum, K., Casteleyn, G., Verleyen, E., Peperzak,  
1914 L., Vyverman, W., 2005. Sexual reproduction, mating system, chloroplast dynamics and  
1915 abrupt cell size reduction in *Pseudo-nitzschia pungens* from the North Sea  
1916 (Bacillariophyta). Eur. J. Phycol. 40, 379–395.
- 1917 Churro, C.I., Carreira, C.C., Rodrigues, F.J., Craveiro, S.C., Calado, A.J., Casteleyn, G.,  
1918 Lundholm, N., 2009. Diversity and abundance of potentially toxic *Pseudo-nitzschia*  
1919 Peragallo in Aveiro coastal lagoon, Portugal and description of a new variety, *P. pungens*  
1920 var. *aveirensis* var. nov. Diatom Res. 24, 35–62.
- 1921 Cohen, N.R., Ellis, K.A., Burns, W.G., Lampe, R.H., Schuback, N., Johnson, Z., Sañudo-  
1922 Wilhelmy, S., Marchetti, A., 2017a. Iron and vitamin interactions in marine diatom  
1923 isolates and natural assemblages of the Northeast Pacific Ocean. Limnol. Oceanogr. 62,  
1924 2076–2096.
- 1925 Cohen, N.R., Ellis, K.A., Lampe, R.H., McNair, H., Twining, B.S., Maldonado, M.T., Brzezinski,  
1926 M.A., Kuzminov, F.I., Thamatrakoln, K., Till, C.P., Bruland, K.W., Sunda, W.G., Bargu, S.,  
1927 Marchetti, A., 2017b. Diatom transcriptional and physiological responses to changes in iron  
1928 bioavailability across ocean provinces. Front. Mar. Sci. 4, 360. doi: 10.3389/fmars.2017.00360
- 1929 Cohen, N.R., Mann, E., Stemple, B., Moreno, C.M., Rauschenberg, S., Jacquot, J.E., Sunda,  
1930 W.G., Twining, B.S., Marchetti, A., 2018. Iron storage capacities and associated ferritin  
1931 gene expression among marine diatoms. Limnol. Oceanogr. (in press)  
1932 <https://doi.org/10.1002/lno.1080>

- 1933 Cook, P.F., Reichmuth, C., Rouse, A.A., Libby, L.A., Dennison, S.E., Carmichael, O.T., Kruse-  
 1934 Elliott, K.T., Bloom, J., Singh, B., Fravel, V.A., Barbosa, L., Stuppino, J.J., Van Bonn,  
 1935 W.G., Gulland, F.M., Dickerson, D., Ranganath, C., 2015. Algal toxin impairs sea lion  
 1936 memory and hippocampal connectivity, with implications for strandings. *Science* 350,  
 1937 1545–1547.
- 1938 Cook, P.F., Reichmuth, C., Rouse, A., Dennison, S., Van Bonn, B., Gulland, F., 2016. Natural  
 1939 exposure to domoic acid causes behavioral perseveration in wild sea lions: neural  
 1940 underpinnings and diagnostic application. *Neurotoxicol. Teratol.* 57, 95–105.
- 1941 D'Agostino, V.C., Hoffmeyer, M.S., Almandoz, G.O., Sastre, V., Degrazi, M., 2015. Potentially  
 1942 toxic *Pseudo-nitzschia* species in plankton and fecal samples of *Eubalaena australis* from  
 1943 península Valdés calving ground, Argentina. *J. Sea Res.* 106, 39–43.
- 1944 D'Agostino, V.C., Degrazi, M., Sastre, V., Santinelli, N., Krock, B., Krohn, T., Dans, S.L.,  
 1945 Hoffmeyer, M.S., 2017. Domoic acid in a marine pelagic food web: exposure of southern  
 1946 right whales *Eubalaena australis* to domoic acid on the Península Valdés calving ground,  
 1947 Argentina. *Harmful Algae* 68, 248–257.
- 1948 D'Alelio, D., Ruggiero, M.V., 2015. Interspecific plastidial recombination in the diatom genus  
 1949 *Pseudo-nitzschia*. *J. Phycol.* 51, 1024–1028.
- 1950 D'Alelio, D., Ribera d'Alcalà, M., Dubroca, L., Sarno, D., Zingone, A., Montresor, M., 2010.  
 1951 The time for sex: a biennial life cycle in a marine planktonic diatom. *Limnol. Oceanogr.*  
 1952 55, 106–114.
- 1953 Danovaro, R., Carugati, L., Berzano, M., Cahill, A.E., Carvalho, S., Chenail, A., Corinaldesi, C.,  
 1954 Cristina, S., David, R., Dell'Anno, A., Dzhembekova, N., Garcés, E., Gasol, J.M., Goela,  
 1955 P., Féral, J.-P., Ferrera, I., Forster, R.M., Kurekin, A.A., Rastelli, E., Marinova, V., Miller,  
 1956 P.I., Moncheva, S., Newton, A., Pearman, J.K., Pitois, S.G., Reñé, A., Rodríguez-Ezpeleta,  
 1957 N., Saggiomo, V., Simis, S.G.H., Stefanova, K., Wilson, C., Martire, M.L., Greco, S.,  
 1958 Cochrane, S.K.J., Mangoni, O., Borja, A., 2016. Implementing and innovating marine  
 1959 monitoring approaches for assessing marine environmental status. *Front. Mar. Sci.* 3, 213.
- 1960 Dao, V.H., Lim, P.T., Ky, P.X., Takata, Y., Teng, S.T., Omura, T., Fukuyo, Y., Kodama, M.,  
 1961 2014. Diatom *Pseudo-nitzschia* cf. *caciantha* (Bacillariophyceae), the most likely source  
 1962 of domoic acid contamination in the thorny oyster *Spondylus versicolor* Schreibers 1793 in  
 1963 Nha Phu Bay, Khanh Hoa Province, Vietnam. *Asian Fish. Sci.* 27, 16–29.
- 1964 Dao, H.V., Phan, V.B., Teng, S.T., Uchida, H., Leaw, C.P., Lim, P.T., Suzuki, T., Pham, K.X.,  
 1965 2015. *Pseudo-nitzschia fukuyoi* (Bacillariophyceae), a domoic acid-producing species  
 1966 from Nha Phu Bay, Khanh Hoa Province, Vietnam. *Fish. Sci.* 81, 533–539.
- 1967 Davidovich, N.A., Bates, S.S., 1998. Sexual reproduction in the pennate diatoms *Pseudo-*  
 1968 *nitzschia multiseries* and *P. pseudodelicatissima* (Bacillariophyceae). *J. Phycol.* 34, 126–  
 1969 137.
- 1970 Delaney, J.A., 2010. Molecular detection of the toxic marine diatom *Pseudo-nitzschia*  
 1971 *multiseries*. Master's thesis, University of South Florida, 100 pp.
- 1972 Delaney, J.A., Ulrich, R.M., Paul, J.H., 2011. Detection of the toxic marine diatom *Pseudo-*  
 1973 *nitzschia multiseries* using the RuBisCO small subunit (*rbcS*) gene in two real-time RNA  
 1974 amplification formats. *Harmful Algae* 11, 54–64.

- 1975 Dhar, B.C., Cimarelli, L., Singh, K.S., Brandi, L., Brandi, A., Puccinelli, C., Marcheggiani, S.,  
1976 Spurio, R., 2015. Molecular detection of a potentially toxic diatom species. *Internat. J.*  
1977 *Environ. Res. Publ. Health* 12, 4921–4941.
- 1978 Di Dato, V., Musacchia, F., Petrosino, G., Patil, S., Montresor, M., Sanges, R., Ferrante, M.I.,  
1979 2015. Transcriptome sequencing of three *Pseudo-nitzschia* species reveals comparable  
1980 gene sets and the presence of nitric oxide synthase genes in diatoms. *Sci. Rep.* 5, 12329.
- 1981 Di Liberto, T., 2015. This summer's West Coast algal bloom was unusual. What would usual  
1982 look like? Online article published September 30, 2015. <https://www.climate.gov/news-features/event-tracker/summer%20%99s-west-coast-algal-bloom-was-unusual-what-would-usual-look>
- 1985 Di Lorenzo, E., Mantua, N., 2016. Multi-year persistence of the 2014/15 North Pacific marine  
1986 heatwave. *Nature Climate Change*. 6, 1042–1047.
- 1987 Dittami, S.M., Pazos, Y., Laspra, M., Medlin, L.K., 2013. Microarray testing for the presence of  
1988 toxic algae monitoring programme in Galicia (NW Spain). *Environ. Sci. Pollut. Res.* 20,  
1989 6778–6793.
- 1990 d'Ippolito, G., Lamari, N., Montresor, M., Romano, G., Cutignano, A., Gerecht, A., Cimino, G.,  
1991 Fontana, A., 2009. 15S-Lipoxygenase metabolism in the marine diatom *Pseudo-nitzschia*  
1992 *delicatissima*. *New Phytologist* 183, 1064–1071.
- 1993 Doucette, G.J., Kudela, R.M., 2017. *In situ* and real-time identification of toxins and toxin-  
1994 producing microorganisms in the environment. *Comp. Analyt. Chem.* 78, 411–443.
- 1995 Downes-Tettmar, N., Rowland, S., Widdicombe, C., Woodward, M., Llewellyn, C., 2013.  
1996 Seasonal variation in *Pseudo-nitzschia* spp. and domoic acid in the Western English  
1997 Channel. *Cont. Shelf Res.* 53, 40–49.
- 1998 Drakulović, D., Gvozdenović, S., Joksimović, D., Mandić, M., Pestorić, B., 2017. Toxic and  
1999 potentially toxic phytoplankton in the mussel and fish farms in the transitional area of  
2000 Montenegrin Coast (south-eastern Adriatic Sea). *Turkish J. Fish. Aquat. Sci.* 17, 885–900.
- 2001 Du, X., Peterson, W., Fisher, J., Hunter, M., Peterson, J., 2016. Initiation and development of a  
2002 toxic and persistent *Pseudo-nitzschia* bloom off the Oregon coast in spring/summer 2015.  
2003 *PLoS ONE* 11, e0163977.
- 2004 Du, X., Peterson, W.T., 2018. Phytoplankton community structure in 2011–2013 compared to  
2005 the extratropical warming event of 2014–2015. *Geophys. Res. Lett.* 45, 1534–1540.
- 2006 Dursun, F., Yurdun, T., Ünlü, S., 2016. The first observation of domoic acid in plankton net  
2007 samples from the Sea of Marmara, Turkey. *Bull. Environ. Contam. Toxicol.* 96, 70–75.
- 2008 Dursun, F., Ünlü, S., Tas, S., Yurdun, T., 2017. Domoic acid variations in response to  
2009 environmental conditions in an eutrophic estuary, Golden Horn (Turkey). *Turkish J. Fish.*  
2010 *Aquat. Sci.* 17, 653–662.
- 2011 Dursun, F., Ünlü, S., Yurdun, T., 2018. Determination of domoic acid in plankton net samples  
2012 from Golden Horn Estuary, Turkey, using HPLC with fluorescence detection. *Bull.*  
2013 *Environ. Contamin. Toxicol.* 100, 457–462.

- 2014 Dzhembekova, N., Atanasov, I., Ivanova, P., Moncheva, S., 2017a. New potentially toxic  
2015 *Pseudo-nitzschia* species (Bacillariophyceae) identified by molecular approach in the  
2016 Black Sea (Varna Bay). 17th International Multidisciplinary Scientific GeoConference  
2017 Surveying Geology and Mining Ecology Management Conference Proceedings. 17, 889–  
2018 896. (Abstract) DOI: 10.5593/sgem2017/31/S15.112  
2019 <https://sgemworld.at/sgemlib/spip.php?article10308>
- 2020 Dzhembekova, N., Urusizaki, S., Moncheva, S., Ivanova, P., Nagai, S., 2017b. Applicability of  
2021 massively parallel sequencing on monitoring harmful algae at Varna Bay in the Black Sea.  
2022 Harmful Algae 68, 40–51.
- 2023 Ellis, C., 2009. Transitions in *Pseudo-nitzschia* community structure at Georges Bank during  
2024 2008. Senior Undergraduate Thesis, University of Washington.
- 2025 Ellis, C.H., Hubbard, K.A., Fernandes, L.F., Keafer, B.A., Richlen, M., Erdner, D.L.,  
2026 McGillicuddy, D.J., Anderson, D.M., Armbrust, E.V., 2009. Diversity and distribution  
2027 patterns of *Pseudo-nitzschia* species off the United States NE coast during summer 2008.  
2028 Fifth Symposium on Harmful Algae in the U.S., Ocean Shores, WA, p. 114.  
2029 <https://www.whoi.edu/fileserver.do?id=229605&pt=2&p=28786>
- 2030 Ellis, K.A., Cohen, N.R., Moreno, C., Marchetti, A., 2017. Cobalamin-independent methionine  
2031 synthase distribution and influence on vitamin B12 growth requirements in marine  
2032 diatoms. Protist 168, 32–47.
- 2033 Ennaffah, B., Nafil, E., Chafik, A., 2012. First report of *Pseudo-nitzschia australis* on Moroccan  
2034 Atlantic coast and toxicity in Moroccan shellfish. Harmful Algae News, No. 21, p. 4.
- 2035 Facca, C., Bilaničovà, D., Pojana, G., Sfriso, A., Marcomini, A., 2014. Harmful algae records in  
2036 Venice Lagoon and in Po River Delta (Northern Adriatic Sea, Italy). Sci. World J. 2014,  
2037 Article ID 806032, 11 pp.
- 2038 Fernandes, L.F., Brandini, F.P., 2010. The potentially toxic diatom *Pseudo-nitzschia* H.  
2039 Peragallo in the Paraná and Santa Catarina States, Southern Brazil. Iheringia, Série Bot.  
2040 65, 47–62.
- 2041 Fernandes, L.F., Cavalcante, K.P., Proença, L.A.O., Schramm, M.A., 2013. Blooms of *Pseudo-*  
2042 *nitzschia pseudodelicatissima* and *P. calliantha*, and associated domoic acid accumulation  
2043 in shellfish from the South Brazilian coast. Diatom Res. 28, 381–393.
- 2044 Fernandes, L.F., Hubbard, K.A., Richlen, M., Smith, J., Bates, S.S., Ehrman, J., Léger, C.,  
2045 Mafra, L.L., Jr., Kulis, D., Quilliam, M., Erdner, D., Libera, K., McCauley, L., Anderson,  
2046 D.M., 2014. Diversity and toxicity of the diatom *Pseudo-nitzschia* Peragallo in the Gulf of  
2047 Maine, Northwestern, Atlantic Ocean. Deep-Sea Res. II 103, 139–162.
- 2048 Fire, S.E., Adkesson, M.J., Wang, Z., Jankowski, G., Cárdenas-Alayza, S., Broadwater, M.,  
2049 2017. Peruvian fur seals (*Arctocephalus australis* ssp.) and South American sea lions  
2050 (*Otaria byronia*) in Peru are exposed to the harmful algal toxins domoic acid and okadaic  
2051 acid. Mar. Mamm. Sci. 33, 630–644.
- 2052 Fitzpatrick, E., Caron, D.A., Schnetzer, A., 2010. Development and environmental application of  
2053 a genus-specific quantitative PCR approach for *Pseudo-nitzschia* species. Mar. Biol. 157,  
2054 1161–1169.

- 2055 Flewelling, L.J., Manire, C.A., Stacy, B., Foley, A., Rodriguez, M., Perry, S., Butts, R., Hubbard,  
2056 K.A., 2015. Domoic acid in stranded juvenile green sea turtles, *Chelonia mydas*, in  
2057 Southeast Florida. Eighth Symposium on Harmful Algae in the U.S., Long Beach, CA, p.  
2058 63. <https://www.whoi.edu/fileserver.do?id=229004&pt=2&p=28786>
- 2059 Fryxell, G.A., Garza, S.A., Roelke, D.L., 1991. Auxospore formation in an Antarctic clone of  
2060 *Nitzschia subcurvata* Hasle. Diatom Res. 6, 235–245.
- 2061 Fuentes, M.S., Wikfors, G.H., 2013. Control of domoic acid toxin expression in *Pseudo-*  
2062 *nitzschia multiseries* by copper and silica: relevance to mussel aquaculture in New  
2063 England (USA). Mar. Environ. Res. 83, 23–28.
- 2064 Gai, F.F., Hedemand, C.K., Louw, D.C., Grobler, K., Krock, B., Moestrup, Ø., Lundholm, N.,  
2065 Morphological, molecular and toxicogenic characteristics of Namibian *Pseudo-nitzschia*  
2066 species – including *Pseudo-nitzschia bucculenta* sp. nov. Harmful Algae (in revision)
- 2067 Gárate-Lizárraga, I., Poot-Delgado, C.A., Ramírez-Castillo, E.R., Páez-Hernández, M.H., 2013.  
2068 Proliferation of *Pseudo-nitzschia brasiliiana* and *P. cf. pseudodelicatissima*  
2069 (Bacillariophyceae) in the Estero Santa Cruz, northern Gulf of California, Mexico. Rev.  
2070 Biol. Mar. Oceanogr. 48, 393–399.
- 2071 García Blásquez, L.C.T., 2014. Veneno amnésico en especies de *Pseudo-nitzschia* en la costa  
2072 central del Perú y su riesgo para la acuicultura de pectínidos. Master's Thesis. Universidad  
2073 Nacional Agraria La Molina, Lima, Peru, 77 p.
- 2074 Garvetto, A., Nezan, E., Badis, Y., Bilien, G., Arce, P., Bresnan, E., Gachon, C.M.M., 2017.  
2075 Phylogenetic characterisation of oomycetes infecting toxic species of the marine diatom  
2076 *Pseudo-nitzschia*. Phycologia 56, 60. (Abstract)
- 2077 Geng, H.-X., Yu, R.-C., Chen, Z.-F., Peng, Q.-C., Yan, T., Zhou, M.-J., 2017. Analysis of sterols  
2078 in selected bloom-forming algae in China. Harmful Algae 66, 29–39.
- 2079 Gentemann, C.L., Fewings, M.R., García-Reyes, M., 2017. Satellite sea surface temperatures  
2080 along the West Coast of the United States during the 2014–2016 northeast Pacific marine  
2081 heat wave, Geophys. Res. Lett. 44, 312–319.
- 2082 Gibble, C., Duerr, R., Bodenstein, B., Lindquist, K., Lindsey, J., Beck, J., Henkel, L., Roletto, J.,  
2083 Harvey, J., Kudela, R., 2018. Investigation of a largescale common murre (*Uria aalge*)  
2084 mortality event in California in 2015. J. Wildlife Dis. (in press)  
2085 <https://doi.org/10.7589/2017-07-179>
- 2086 Gillard, J., Frenkel, J., Devos, V., Sabbe, K., Paul, C., Rempt, M., Inzé, D., Pohnert G,  
2087 Vuylsteke, M., Vyverman, W., 2013. Metabolomics enables the structure elucidation of a  
2088 diatom sex pheromone. Angew. Chem. 52, 854–857.
- 2089 Gleason, F.H., Jephcott, T.G., Küpper, F.C., Gerphagnon, M., Sime-Ngando, T., Karpov, S.A.,  
2090 Guillou, L., Van Ogtrop, F.F., 2015. Potential roles for recently discovered chytrid  
2091 parasites in the dynamics of harmful algal blooms. Fungal Biol. Rev. 29, 20–33.
- 2092 Goodenough, U., Lin, H., Lee, J.-H., 2007. Sex determination in *Chlamydomonas*. Semin. Cell  
2093 Dev. Biol. 18, 350–361.
- 2094 Grbin, D., Pfannkuchen, M., Babić, I., Mejdandžić, M., Mihanović, H., Pfannkuchen, D.M.,  
2095 Godrijan, J., Štefanić, P.P., Olujić, G., Ljubešić, Z., 2017. Multigene phylogeny and

- 2096 morphology of newly isolated strain of *Pseudo-nitzschia manni* Amato & Montresor  
2097 (Adriatic Sea). Diatom Res. 32, 127–131.
- 2098 Greenfield, D.I., Marin, R., Jensen, S., Massion, E., Roman, B., Feldman, J., Scholin, C.A.,  
2099 2006. Application of Environmental Sample Processor (ESP) methodology for quantifying  
2100 *Pseudo-nitzschia australis* using ribosomal RNA-targeted probes in sandwich and  
2101 fluorescent *in situ* hybridization formats. Limnol. Oceanogr. Meth. 4, 426–435.
- 2102 Greenfield, D.I., Marin, R., Doucette, G.J., Mikulski, C., Jones, K., Jensen, S., Roman, B.,  
2103 Alvarado, N., Feldman, J., Scholin, C., 2008. Field applications of the second- generation  
2104 Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006–2007.  
2105 Limnol. Oceanogr. Meth. 6, 667–679.
- 2106 GRIIS. The Global Register of Introduced and Invasive Species. IUCN SSC Invasive Species  
2107 Specialist Group. <http://griis.org/>
- 2108 Groussman, R.D., Parker, M.S., Armbrust, E.V., 2015. Diversity and evolutionary history of iron  
2109 metabolism genes in diatoms. PLoS ONE 10, e0129081.
- 2110 Grzebyk, D., Audic, S., Lasserre, B., Abadie, E., de Vargas, C., Bec, B., 2017. Insights into the  
2111 harmful algal flora in northwestern Mediterranean coastal lagoons revealed by  
2112 pyrosequencing metabarcodes of the 28S rRNA gene. Harmful Algae 68, 1–16.
- 2113 Guannel, M.L., Horner-Devine, M.C., Rocap, G., 2011. Bacterial community composition differs  
2114 with species and toxigenicity of the diatom *Pseudo-nitzschia*. Aquat. Microb. Ecol. 64,  
2115 117–133.
- 2116 Guannel, M.L., Haring, D., Twiner, M.J., Wang, Z., Noble, A.E., Lee, P.A., Saito, M.A., Rocap,  
2117 G., 2015. Toxigenicity and biogeography of the diatom *Pseudo-nitzschia* across distinct  
2118 environmental regimes in the South Atlantic Ocean. Mar. Ecol. Prog. Ser. 526, 67–87.
- 2119 Garali, S.M.B., Sahraoui, I., de la Iglesia, P., Chalghaf, M., Diogène, J., Ksouri, J., Sakka Hlaili,  
2120 A., 2016. Effects of nitrogen supply on *Pseudo-nitzschia calliantha* and *Pseudo-nitzschia*  
2121 cf. *seriata*: field and laboratory experiments. Ecotoxicology 25, 1211–1225.
- 2122 Hallegraeff, G.M., 2017. Marine phycotoxins and seafood safety. In: Witczak, A., Sikorski, Z.E.  
2123 [Eds.], Toxins and Other Harmful Compounds in Foods. CRC Press, Taylor & Francis  
2124 Group. Boca Raton, Florida, pp. 63–84.
- 2125 Haigh, N., Russell, T., Johnson, D., 2016. *Pseudo-nitzschia* species and domoic acid on the west  
2126 coast of Vancouver Island, British Columbia, in 2015. PICES 2016 Annual Meeting, Nov  
2127 2–13, 2016, San Diego, U.S.A. <http://meetings.pices.int/Publications/Presentations/PICES-2016/W2-Haigh.pdf>
- 2129 Hanic, L.A., 2014. The 1987 PEI toxic mussel episode – A personal perspective. Bates, S.S.  
2130 [Ed.] Unpublished document. 113 p. (PDF)
- 2131 Hanic, L.A., Sekimoto, S., Bates, S.S., 2009. Oomycete and chytrid infections of the marine  
2132 diatom *Pseudo-nitzschia pungens* (Bacillariophyceae) from Prince Edward Island, Canada.  
2133 Botany 87, 1096–1105.
- 2134 Hansen, L.R., Soylu, S.í, Kotaki, Y., Moestrup, Ø., Lundholm, N., 2011. Toxin production and  
2135 temperature-induced morphological variation of the diatom *Pseudo-nitzschia seriata* from  
2136 the Arctic. Harmful Algae 10, 689–696.

- 2137 Haring, D., 2010. *Pseudo-nitzschia* species distribution in estuaries of the Pacific Northwest  
2138 based on Automated Ribosomal Intergenic Spacer Analysis (ARISA). Senior  
2139 Undergraduate Thesis, University of Washington, 21 p.  
2140 <https://digital.lib.washington.edu/researchworks/handle/1773/16119>
- 2141 Harðardóttir, S., Pančić, M., Tammilehto, A., Krock, B., Møller, E.F., Nielsen, T.G., Lundholm,  
2142 N., 2015. Dangerous relations in the Arctic marine food web: interactions between toxin  
2143 producing *Pseudo-nitzschia* diatoms and *Calanus* copepodites. Mar. Drugs 13, 3809–3835.
- 2144 Hasle, G.R., 1974. Validation of the names of some marine planktonic species of *Nitzschia*  
2145 (Bacillariophyceae). Taxon 23, 425–428.
- 2146 Hasle, G.R., 2002. Are most of the domoic acid producing species of the diatom genus *Pseudo-*  
2147 *nitzschia* cosmopolites? Harmful Algae 1, 137–146.
- 2148 Hasle, G.R., Lundholm, N., 2005. *Pseudo-nitzschia seriata* f. *obtusa* (Bacillariophyceae) raised  
2149 in rank based on morphological, phylogenetic and distributional data. Phycologia 44, 608–  
2150 619.
- 2151 Hermann, G., Graham, J., Lacaze, J.-P., 2017. Microarray for the detection and quantification of  
2152 toxin-producing phytoplankton species in Scottish coastal waters. Scottish Mar.  
2153 Freshwater Sci. 8 (24). DOI: 10.7489/2019-1
- 2154 Hernández-Becerril, D.U., Villagrán-Lorenzana, H., 2017. Changes in phytoplankton species  
2155 composition during various algal blooms in bays of Manzanillo and Santiago, Colima,  
2156 Mexico (April-May, 2015). In: Proença, L.A.O., Hallegraeff, G.M. [Eds.], Marine and  
2157 Fresh-Water Harmful Algae. Proceedings of the 17<sup>th</sup> International Conference on Harmful  
2158 Algae. International Society for the Study of Harmful Algae, and Intergovernmental  
2159 Oceanographic Commission of UNESCO, pp. 62–64.
- 2160 Hiltz, M.F., Bates, S.S., Kaczmarśka, I., 2000. Effect of light:dark cycles and cell apical length  
2161 on the sexual reproduction of *Pseudo-nitzschia multiseries* (Bacillariophyceae) in culture.  
2162 Phycologia 39, 59–66.
- 2163 Huang, C.-X., Xu, G.-S., Li, Y., 2017a. The identification of *Pseudo-nitzschia decipiens* from  
2164 Daya Bay and analysis of its domoic acid production. Acta Hydrobiol. Sinica 41, 1118–  
2165 1125. (in Chinese)
- 2166 Huang, C.-X., Dong, H.-C., Li, Y., 2017b. Two new records of diatom genus *Pseudo-nitzschia*  
2167 and their domoic acid production in southeastern China coastal waters. Oceanol. Limnol.  
2168 Sinica 48, 767–776. (in Chinese)
- 2169 Huang, C.-X., Dong, H.-C., Wu, H.-Y., Tan, Z.-J., Li, Y., 2017c. *Pseudo-nitzschia fukuyoi*—a  
2170 new record of toxic *Pseudo-nitzschia* taxa from China. Oceanol. Limnol. Sinica 48, 1014–  
2171 1021. (in Chinese)
- 2172 Higgins, M.J., Sader, J.E., Mulvaney, P., Wetherbee, R., 2003. Probing the surface of living  
2173 diatoms with atomic force microscopy: the nanostructure and nanomechanical properties  
2174 of the mucilage layer. J. Phycol. 39, 722–734.
- 2175 Holtermann, K.E., Bates, S.S., Trainer, V.L., Odell, A., Armbrust, E.V., 2010. Mass sexual  
2176 reproduction in the toxicogenic diatoms *Pseudo-nitzschia australis* and *P. pungens*  
2177 (Bacillariophyceae) on the Washington coast. J. Phycol. 46, 41–52.

- 2178 Hong, Z., Lai, Q., Luo, Q., Jiang, S., Zhu, R., Liang, J., Gao, Y., 2015. *Sulfitobacter*  
2179 *pseudonitzschiae* sp. nov., isolated from toxic marine diatom *Pseudo-nitzschia multiseries*.  
2180 *Internat. J. System. Evolut. Microbiol.* 65, 1 95–100.
- 2181 Hoppe, C.J.M., Hassler, C.S., Payne, C.D., Tortell, P.D., Rost, B., Trimborn, S., 2013. Iron  
2182 limitation modulates ocean acidification effects on Southern Ocean phytoplankton  
2183 communities. *PLoS ONE* 8, e79890.
- 2184 Hubbard, K.A., 2010. Ecological investigations of genetic diversity in the diatom genus *Pseudo-*  
2185 *nitzschia*. PhD Thesis, University of Washington, Seattle, WA, 167 p.
- 2186 Hubbard, K.A., Rocap, G., Armbrust, E.V., 2008. Inter- and intraspecific community structure  
2187 within the diatom genus *Pseudo-nitzschia* (Bacillariophyceae). *J. Phycol.* 44, 637–649.
- 2188 Hubbard, K.A., Olson, C.E., Armbrust, E.V., 2014a. Molecular characterization of *Pseudo-*  
2189 *nitzschia* community structure and species ecology in a hydrographically complex  
2190 estuarine system (Puget Sound, Washington, USA). *Mar. Ecol. Prog. Ser.* 507, 39–55.
- 2191 Hubbard, K.A. O'Dea, S., Richlen, M.L., Anderson, D.M. McGillicuddy, D.J., Cembella, A.,  
2192 Chakraborty, S., Disney, J., Sirois, A., and Mogenson, H., 2014b. DNA fingerprinting of  
2193 eastern North American phytoplankton communities reveals complex structure within the  
2194 diatom genus *Pseudo-nitzschia*. Joint Aquatic Sciences Meeting, Portland, OR (oral).  
2195 <http://sgmeet.com/jasm2014/viewabstract.asp?AbstractID=15488>
- 2196 Hubbard, K.A., Bruzek, S.E., O'Dea, S., Farrell, A., Markley, L., Henschen, K., Keller-Abbe,  
2197 S.R., Keafer, B., Tilney, C.L., Fernandes, L.F., Richlen, M.L., Flewelling, L.J., Sirois, A.,  
2198 Townsend, D.W., Olson, C.E., Tuney, I., Resetarits, E.J., Disney, J., Armbrust, E.V.,  
2199 Anderson, D.M., McGillicuddy, D.J., 2015. Complex bloom dynamics revealed by genetic  
2200 characterization of US Atlantic and Gulf of Mexico *Pseudo-nitzschia* communities. Eighth  
2201 Symposium on Harmful Algae in the U.S., Long Beach, CA, p. 75.  
2202 <https://www.whoi.edu/fileserver.do?id=229004&pt=2&p=28786>
- 2203 Hubbard, K., Anderson, D., Archer, S., Berger, H., Brosnahan, M., Chadwick, C., Denny, E.,  
2204 Disney, J., Farrell, A., Granholm, A., Fleiger, J., Flewelling, L., Heil, C., Henschen, K.,  
2205 Kanwit, K., Kulic, D., Keafer, B., Keller Abbe, S., Lewis, B., Markley, L., Park, J.,  
2206 Petitpas, C., Racicot, E., Robert, M., Villac, C., McGillicuddy, D., 2017. Synergistic  
2207 characterization of *Pseudo-nitzschia* communities during unprecedented domoic acid  
2208 events. Ninth Symposium on Harmful Algae in the U.S., Baltimore, MD, p. 135.
- 2209 Husson, B., Hernández-Fariñas, T., Le Gendre, R., Schapira, M., Chapelle, A., 2016. Two  
2210 decades of *Pseudo-nitzschia* spp. blooms and king scallop (*Pecten maximus*)  
2211 contamination by domoic acid along the French Atlantic and English Channel coasts:  
2212 seasonal dynamics, spatial heterogeneity and interannual variability. *Harmful Algae* 51,  
2213 26–39.
- 2214 Ianora, A., Miraldo, A., 2010. Toxigenic effects of diatoms on grazers, phytoplankton and other  
2215 microbes: a review. *Ecotoxicology* 19, 493–511.
- 2216 Jensen, S.-K., Lacaze, J.-P., Hermann, G., Kershaw, J., Brownlow, A., Turner, A., Hall, A.,  
2217 2015. Detection and effects of harmful algal toxins in Scottish harbour seals and potential  
2218 links to population decline. *Toxicon* 97, 1–14.

- 2219 Johnson, D., Haigh, N., Russell, T., 2016. *Pseudo-nitzschia* species and domoic acid in southeast  
2220 Vancouver Island, November 2015 to July 2016. PICES 2016 Annual Meeting, Nov 2–13,  
2221 2016, San Diego, U.S.A. <http://meetings.pices.int/Publications/Presentations/PICES-2016/W2-Johnson.pdf>
- 2223 Kaczmarcka, I., Bates, S.S., Ehrman, J.M., Léger, C., 2000. Fine structure of the gamete,  
2224 auxospore and initial cell in the pennate diatom *Pseudo-nitzschia multiseries*. Nova  
2225 Hedwigia 71, 337–357.
- 2226 Kaczmarcka, I., Ehrman, J.M., Bates, S.S., Green, D.H., Léger, C., Harris, J., 2005. Diversity and  
2227 distribution of epibiotic bacteria on *Pseudo-nitzschia multiseries* (Bacillariophyceae) in  
2228 culture, and comparison with those on diatoms in native seawater. Harmful Algae 4, 725–  
2229 741.
- 2230 Kaczmarcka, I., Reid, C., Martin, J.L., Moniz, M.B.J., 2008. Morphological, biological, and  
2231 molecular characteristics of the diatom *Pseudo-nitzschia delicatissima* from the Canadian  
2232 Maritimes. Botany 86, 763–772.
- 2233 Kang, S-H., Fryxell, G.A., Roelke, D.L., 1993. *Fragilariopsis cylindrus* (Grunow) Krieger  
2234 compared with other species of the diatom family Bacillariaceae in Antarctic marginal ice  
2235 edge zones. Beih. Nova Hedwigia 106, 335–352.
- 2236 Keeling, P.J., Burki, F., Wilcox, H.M., Allam, B., Allen, E.E., Amaral-Zettler, L.A., Armbrust,  
2237 E.V., Archibald, J.M., Bharti, A.K., Bell, C.J., Beszteri, B., Bidle, K.D., Cameron, C.T.,  
2238 Campbell, L., Caron, D.A., Cattolico, R.A., Collier, J.L., Coyne, K., Davy, S.K.,  
2239 Deschamps, P., Dyhrman, S.T., Edvardsen, B., Gates, R.D., Gobler, C.J., Greenwood, S.J.,  
2240 Guida, S.M., Jacobi, J.L., Jakobsen, K.S., James, E.R., Jenkins, B., John, U., Johnson,  
2241 M.D., Juhl, A.R., Kamp, A., Katz, L.A., Kiene, R., Kudryavtsev, A., Leander, B.S., Lin,  
2242 S., Lovejoy, C., Lynn, D., Marchetti, A., McManus, G., Nedelcu, A.M., Menden-Deuer,  
2243 S., Miceli, C., Mock, T., Montresor, M., Moran, M.A., Murray, S., Nadathur, G., Nagai,  
2244 S., Ngam, P.B., Palenik, B., Pawlowski, J., Petroni, G., Piganeau, G., Posewitz, M.C.,  
2245 Rengefors, K., Romano, G., Rumpho, M.E., Rynearson, T., Schilling, K.B., Schroeder,  
2246 D.C., Simpson, A.G.B., Slamovits, C.H., Smith, D.R., Smith, G.J., Smith, S.R., Sosik,  
2247 H.M., Stief, P., Theriot, E., Twary, S., Umale, P.E., Vaulot, D., Wawrik, B., Wheeler,  
2248 G.L., Wilson, W.H., Xu, Y., Zingone, A., Worden, A.Z., 2014. The Marine Microbial  
2249 Eukaryote Transcriptome Sequencing Project (MMETSP): illuminating the functional  
2250 diversity of eukaryotic life in the oceans through transcriptome sequencing. PLoS Biol. 12,  
2251 doi: org/10.1371/journal.pbio.1001889.
- 2252 Kegel, J.U., Del Amo, Y., Costes, L., Medlin, L.K., 2013. Testing a microarray to detect and  
2253 monitor toxic microalgae in Arcachon Bay in France. Microarrays 2, 1–23.
- 2254 Kesici, K., Tüney, İ., Zeren, D., Güden, M., Sukatar, A., 2013. Morphological and molecular  
2255 identification of pennate diatoms isolated from Urla, İzmir, coast of the Aegean Sea.  
2256 Turkish J. Biol. 37, 530–537.
- 2257 Kim, J.H., Park, B.S., Kim, J.H., Wang, P., Han, M.S., 2015. Intraspecific diversity and  
2258 distribution of the cosmopolitan species *Pseudo-nitzschia pungens* (Bacillariophyceae):  
2259 morphology, genetics and ecophysiology of the three clades. J. Phycol. 51, 159–172.

- 2260 Kim, J.H., Kim, J.-H., Park, B.S., Wang, P., Patidar, S.K., Han, M.-S., 2017. Development of a  
2261 qPCR assay for tracking the ecological niches of genetic sub-populations within *Pseudo-*  
2262 *nitzschia pungens* (Bacillariophyceae). Harmful Algae 63, 68–78.
- 2263 Kim, J.H., Wang, P., Park, B.S., Kim, J.-H., Patidar, S.K., Han, M.-S., 2018. Revealing the  
2264 distinct habitat ranges and hybrid zone of genetic sub-populations within *Pseudo-nitzschia*  
2265 *pungens* (Bacillariophyceae) in the West Pacific area. Harmful Algae 73, 72–83.
- 2266 Koester, J.A., Swanson, W.J., Armbrust, E.V., 2013. Positive selection within a diatom species  
2267 acts on putative protein interactions and transcriptional regulation. Molec. Biol. Evol. 30,  
2268 422–434.
- 2269 Kotaki, Y., Koike, K., Yoshida, M., Thuoc, C.V., Huyen, N.T.M., Hoi, N.C., Fukuyo, Y.,  
2270 Kodama, M., 2000. Domoic acid production in *Nitzschia* sp. (Bacillariophyceae) isolated  
2271 from a shrimp-culture pond in Do Son, Vietnam. J. Phycol. 36, 1057–1060.
- 2272 Kotaki, Y., 2008. Ecobiology of ASP producing diatoms. In: Botana, L.M. [Ed.], Seafood and  
2273 Freshwater Toxins: Pharmacology, Physiology and Detection. CRC Press, Boca Raton,  
2274 FL, pp. 383–396.
- 2275 Kotaki, Y., Koike, K., Sato, S., Ogata, T., Fukuyo, Y., Kodama, M., 1999. Confirmation of  
2276 domoic acid production of *Pseudo-nitzschia multiseries* isolated from Ofunato Bay, Japan.  
2277 Toxicon 37, 677–682.
- 2278 Kotaki, Y., Lundholm, N., Onodera, H., Kobayashi, K., Bajarias, F.F.A., Furio, E.F., Iwataki,  
2279 M., Fukuyo, Y., Kodama, M., 2004. Wide distribution of *Nitzschia navis-varingica*, a new  
2280 domoic acid-producing benthic diatom found in Vietnam. Fish. Sci. 70, 28–32.
- 2281 Kotaki, Y., Furio, E.F., Satake, M., Lundholm, N., Katayama, T., Koike, K., Fulgueras, V.P.,  
2282 Bajarias, F.A., Takata, Y., Kobayashi, K., Sato, S., Fukuyo, Y., Kodama, M., 2005.  
2283 Production of isodomoic acids A and B as major toxin components of a pennate diatom  
2284 *Nitzschia navis-varingica*. Toxicon 46, 946–953.
- 2285 Kotaki, Y., Furio, E.F., Bajarias, F.A., Satake, M., Lundholm, N., Koike, K., Sato, S., Fukuyo,  
2286 Y., Kodama, M., 2006. New stage of the study on domoic acid-producing diatoms—a  
2287 finding of *Nitzschia navis-varingica* that produces domoic acid derivatives as major toxin  
2288 components. Coast. Mar. Sci. 30, 116–120.
- 2289 Kotaki, Y., Lundholm, N., Katayama, T., Furio, E.F., Romero, M.L., Relox, J.R., Yasumoto, T.,  
2290 Naoki, H., Hirose, M.Y., Thanh, T.D., Thuoc, C.V., Huyen, N.T.M., Thu, P.T., Takata, Y.,  
2291 Kodama, M., Fukuyo, Y., 2008. ASP toxins of pennate diatoms and bacterial effects on the  
2292 variation in toxin composition. In: Moestrup Ø. [Ed.], Proceedings of the 12th  
2293 International Conference on Harmful Algae. IOC of UNESCO, Copenhagen, Denmark,  
2294 pp. 300–302.
- 2295 Krock, B., Borel, C.M., Barrera, F., Tillmann, U., Fabro, E., Almandoz, G.O., Ferrario, M.,  
2296 Cardona, J.E.G., Koch, B.P., Alonso, C., Lara, R., 2015. Analysis of the hydrographic  
2297 conditions and cyst beds in the San Jorge Gulf, Argentina, that favor dinoflagellate  
2298 population development including toxigenic species and their toxins. J. Mar. Syst. 148,  
2299 86–100.

- 2300 Kudela, R.M., Howard, M.D.A., Jenkins, B.D., Miller, P.E., Smith, G.J., 2010. Using the  
2301 molecular toolbox to compare harmful algal blooms in upwelling systems. *Prog.*  
2302 *Oceanogr.* 85, 108–121.
- 2303 Kudela, R., Anderson, C., Birch, J.M., Bowers, H., Caron, D.A., Chao, Y., Doucette, G., Farrara,  
2304 J.D., 2015. Harmful algal bloom hotspots really are hot: a case study from Monterey Bay,  
2305 California. Abstract OS51C-02 presented at 2015 Fall Meeting, AGU, San Francisco,  
2306 Calif., 14–18 Dec.  
2307 [https://fallmeeting.agu.org/2015/files/2015/12/Kudela\\_AGU\\_PRESS.pdf](https://fallmeeting.agu.org/2015/files/2015/12/Kudela_AGU_PRESS.pdf)
- 2308 Kudela, R.M., Hayashi, K., Peacock, M., Gibble, C., Boyer, A., 2017. What's for dinner?  
2309 Persistent and extensive domoic acid contamination in the food web before, during, and  
2310 after the 2015 mega-bloom in California. 9<sup>th</sup> US Symp. HABs, Baltimore, Maryland.  
2311 (Abstract)
- 2312 La Barre, S., Bates, S.S., Quilliam M.A., 2014. Domoic acid. In: La Barre, S., Kornprobst, J.-M.  
2313 [Eds.], *Outstanding Marine Molecules: Chemistry, Biology, Analysis*. Wiley-VCH Verlag  
2314 GmbH & Co. KgaA, Weinheim, Germany, pp. 189–216.
- 2315 Lapworth, C.J., Hallegraeff, G.M., Ajani, P.A., 2001. Identification of domoic-acid producing  
2316 *Pseudo-nitzschia* species in Australian waters. In: Hallegraeff, G.M., Blackburn, S.I.,  
2317 Bolch, C.J., Lewis, R.J. [Eds.], *Harmful Algal Blooms 2000*. Intergovernmental  
2318 Oceanographic Commission of UNESCO, Paris, pp. 38–41.
- 2319 Lahvis, G.P., 2017. What California sea lions exposed to domoic acid might teach us about  
2320 autism: lessons for predictive and preventive medicine. *EPMA J.* 8, 229–235.
- 2321 Leandro, L.F., Teegarden, G.J., Roth, P.B., Wang, Z., Doucette, G.J., 2010a. The copepod  
2322 *Calanus finmarchicus*: a potential vector for trophic transfer of the marine algal biotoxin,  
2323 domoic acid. *J. Exp. Mar. Biol. Ecol.* 382, 88–95.
- 2324 Leandro, L.F., Rolland, R.M., Roth, P.B., Lundholm, N., Wang, Z., Doucette, G.J., 2010b.  
2325 Exposure of the North Atlantic right whale *Eubalaena glacialis* to the marine algal  
2326 biotoxin, domoic acid. *Mar. Ecol. Prog. Ser.* 398, 287–303.
- 2327 Lefebvre, K.A., Quakenbush, L., Frame, E., Burek Huntington, K., Sheffield, G., Stimmelmayr,  
2328 R., Bryan, A., Kendrick, P., Ziel, H., Goldstein, T., Snyder, J.A., Gelatt, T., Gulland, F.,  
2329 Dickerson, B., Gill, V., 2016. Prevalence of algal toxins in Alaskan marine mammals  
2330 foraging in a changing arctic and subarctic environment. *Harmful Algae* 55, 13–24.
- 2331 Lelong, A., Hégaret, H., Soudant, P., Bates, S.S., 2012a. *Pseudo-nitzschia* (Bacillariophyceae)  
2332 species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms.  
2333 *Phycologia* 51, 168–216.
- 2334 Lelong, A., Jolley, D.F., Soudant, P., Hégaret H., 2012b. Impact of copper exposure on *Pseudo-*  
2335 *nitzschia* spp. physiology and domoic acid production. *Aquat. Toxicol.* 118–119, 37–47.
- 2336 Lelong, A., Bucciarelli, E., Hégaret, H., Soudant, P., 2013. Iron and copper limitations  
2337 differently affect growth rates and photosynthetic and physiological parameters of the  
2338 marine diatom *Pseudo-nitzschia delicatissima*. *Limnol. Oceanogr.* 58, 613–623.

- 2339 Lelong, A., Hégaret, H., Soudant P., 2014. Link between domoic acid production and cell  
2340 physiology after exchange of bacterial communities between toxic *Pseudo-nitzschia*  
2341 *multiseries* and non-toxic *Pseudo-nitzschia delicatissima*. Mar. Drugs 12, 3587–3607.
- 2342 Lema, K.A., Latimier, M., Nézan, É., Fauchot, J., Le Gac, M., 2017. Inter and intra-specific  
2343 growth and domoic acid production in relation to nutrient ratios and concentrations in  
2344 *Pseudo-nitzschia*: phosphate an important factor. Harmful Algae 64, 11–19.
- 2345 Levialdi Ghiron, J.H., Amato, A., Montresor, M., Kooistra, W.C.H.F., 2008. Plastid inheritance  
2346 in the planktonic raphid pennate diatom *Pseudo-nitzschia delicatissima*  
2347 (Bacillariophyceae). Protist 159, 91–98.
- 2348 Lewis, B., MacLeod, J. Hubbard, K., 2017. The Gulf of Maine domoic acid event of 2016: an  
2349 emerging public health concern. 9<sup>th</sup> US Symp. HABs, Baltimore, Maryland. (Abstract)  
2350 <https://static1.squarespace.com/static/587536c8893fc0b475a16169/t/5a0b1fb3e4966b7f0218f293/1510678548010/9thHABstract+Book.pdf>
- 2352 Li, Y., Huang, C.X., Xu, G.S., Lundholm, N., Teng, S.T., Wu, H., Tan, Z., 2017. *Pseudo-*  
2353 *nitzschia simulans* sp. nov. (Bacillariophyceae), the first domoic acid producer from  
2354 Chinese waters. Harmful Algae 67, 119–130.
- 2355 Li, Y., Dong, H.C., Ten, S.T., Bates, S.S., Lim, P.T., 2018. *Pseudo-nitzschia nanaoensis* sp. nov.  
2356 (Bacillariophyceae), a novel species from the Chinese coast of the South China Sea. J.  
2357 Phycol. (in revision)
- 2358 Licea, R.L., Okolodkov, Y.B., Cortes-Almirano, R., 2017. Phytoplankton abundance and  
2359 distribution on the Yucatan shelf (June 1979 and April 1983). Novosti Sist. Nizsh. Rast.  
2360 51, 121–144.
- 2361 Liefer, J.D., Robertson, A., MacIntyre, H.L., Smith, W.L., Dorsey, C.P., 2013. Characterization  
2362 of a toxic *Pseudo-nitzschia* spp. bloom in the Northern Gulf of Mexico associated with  
2363 domoic acid accumulation in fish. Harmful Algae 26, 20–32.
- 2364 Lim, H.C., Su, S.N.P., Mohamed-Ali, H., Kotaki, Y., Leaw, C.P., Lim, P.-T., 2010. Toxicity of  
2365 diatom *Pseudo-nitzschia* (Bacillariophyceae) analyzed using high performance liquid  
2366 chromatography (HPLC). J. Sci. Technol. Tropics 6, S116–S119.
- 2367 Lim, H.C., Leaw, C.P., Su, S.N.P., Teng, S.T., Usup, G., Noor, N.M., Lundholm, N., Kotaki, Y.,  
2368 Lim, P.T., 2012a. Morphology and molecular characterization of *Pseudo-nitzschia*  
2369 (Bacillariophyceae) from Malaysian Borneo, including the new species of *Pseudo-*  
2370 *nitzschia circumpora* sp. nov. J. Phycol. 48, 1232–1247.
- 2371 Lim, H.C., Lim, P.T., Su, S.N.P., Teng, S.T., Leaw, C.P., 2012b. Genetic diversity of *Pseudo-*  
2372 *nitzschia brasiliiana* (Bacillariophyceae) from Malaysia. J. Appl. Phycol. 24, 1465–1475.
- 2373 Lim, H.C., Lim, P.T., Su, S.N.P., Kotaki, Y., Leaw, C.P., 2012c. Morphological observation of  
2374 two species of *Pseudo-nitzschia* (Bacillariophyceae). Coast. Mar. Sci. 35, 52–57.
- 2375 Lim, H.C., Lim, P.-T., Su, S.N.P., Teng, S.T., Leaw, C.-P., 2012d. Genetic diversity of *Pseudo-*  
2376 *nitzschia pungens* (Bacillariophyceae) in Borneo, Malaysia. Coast. Mar. Sci. 35, 58–63.
- 2377 Lim, H.G., Teng, S.T., Leaw, C.P., Lim, P.T., 2013. Three novel species in the *Pseudo-nitzschia*  
2378 *pseudodelicatissima* complex: *P. batesiana* sp. nov., *P. lundholmiae* sp. nov. and *P.*

- 2379        *fukuyoi* sp. nov. (Bacillariophyceae), from the Strait of Malacca, Malaysia. J. Phycol. 49,  
2380        902–916.
- 2381        Lim, H.C., Teng, S.T., Leaw, C.P., binti Kamarudin, S.Z., Lim, P.T., 2014a. Growth response of  
2382        *Pseudo-nitzschia circumpora* (Bacillariophyceae) to different salinities. In: Kim, H.G.,  
2383        Reguera, B., Hallegraeff, G.M., Lee, C.K., Han, M.S., Choi, J.K. [Eds.], Harmful Algae  
2384        2012, Proceedings of the 15<sup>th</sup> International Conference on Harmful Algae. International  
2385        Society for the Study of Harmful Algae, Maple Design, Busan, Korea, pp. 135–136.
- 2386        Lim, H.C., Lim, P.T., Teng, S.T., Bates, S.S., Leaw, C.P., 2014b. Genetic structure of *Pseudo-*  
2387        *nitzschia pungens* (Bacillariophyceae) populations: implications of a global diversification  
2388        of the diatom. Harmful Algae 37, 142–152.
- 2389        Lim, H.C., Teng, S.T., Leaw, C.P., Iwataki, M., Lim, P.T., 2014c. Phytoplankton assemblage of  
2390        the Merambong Shoal, Tebrau Straits with note on potentially harmful species. Malayan  
2391        Nat. J. 66, 198–211.
- 2392        Lim, H.G., Teng, S.T., Lim, P.T., Wolf, M., Leaw, C.P., 2016. 18S rDNA phylogeny of *Pseudo-*  
2393        *nitzschia* (Bacillariophyceae) inferred from sequence-structure information. Phycologia  
2394        55, 134–146.
- 2395        Lim, H.C., Tan, S.N., Teng, S.T., Lundholm, N., Orive, E., David, H., Quijano-Scheggia, S.,  
2396        Leong, S.C.Y., Wolf, M., Bates, S.S., Lim, P.T., Leaw, C.P., 2018. Phylogeny and species  
2397        delineation in the marine diatom *Pseudo-nitzschia* (Bacillariophyceae) using *cox1*, LSU  
2398        and ITS2 rDNA sequences: a perspective in character evolution. J. Phycol. 54, 234–248
- 2399        Lincoln, J.A., Turner, J.T., Bates, S.S., Léger, C., Gauthier, D.A., 2001. Feeding, egg production,  
2400        and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets  
2401        of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-*  
2402        *nitzschia pungens*. Hydrobiologia 453, 107–120.
- 2403        Lopes, V.M., Lopes, A.R., Costa, P., Rosa, R., 2013. Cephalopods as vectors of harmful algal  
2404        bloom toxins in marine food webs. Mar. Drugs 11, 3381–3409.
- 2405        Lopes, V.M., Rosa, R., Costa, P.R., 2018. Presence and persistence of the amnesic shellfish  
2406        poisoning toxin, domoic acid, in octopus and cuttlefish brains. Mar. Environ. Res. 133:  
2407        45–48.
- 2408        Lorrain, A., Paulet, Y.M., Chauvaud, L., Savoye, N., Nezan, E., Guerin, L., 2000. Growth  
2409        anomalies in *Pecten maximus* from coastal waters (Bay of Brest, France): relationship with  
2410        diatom blooms. J. Mar. Biol. Assoc. UK 80, 667–673.
- 2411        Louw, D.C., Doucette, G.J., Voges, E., 2017. Annual patterns, distribution and long-term trends  
2412        of *Pseudo-nitzschia* species in the northern Benguela upwelling system. J. Plank. Res. 39,  
2413        35–47.
- 2414        Louw, D.C., Doucette, G.J., Lundholm, N., 2018. Morphology and toxicity of *Pseudo-nitzschia*  
2415        species in the northern Benguela Upwelling System. Harmful Algae 75, 118–128.
- 2416        Lu, Y.M., Wohlrab, S., Groth, M., Glockner, G., Guillou, L., John, U., 2016. Transcriptomic  
2417        profiling of *Alexandrium fundyense* during physical interaction with or exposure to  
2418        chemical signals from the parasite *Amoebophrya*. Mol. Ecol. 25, 1294–1307.

- 2419 Lundholm, N. (Ed.), 2017. Bacillariophyceae. In: IOC-UNESCO Taxonomic Reference List of  
2420 Harmful Micro Algae. Accessed at <http://www.marinespecies.org/hab>.
- 2421 Lundholm, N., Moestrup, Ø., 2000. Morphology of the marine diatom *Nitzschia navis-varingica*  
2422 sp. nov., another producer of the neurotoxin domoic acid. J. Phycol. 36, 1162–1174.
- 2423 Lundholm, N., Moestrup, Ø., 2002. The marine diatom *Pseudo-nitzschia galaxiae* sp. nov.  
2424 (Bacillariophyceae): morphology and phylogenetic relationships. Phycologia 41, 594–605.
- 2425 Lundholm, N., Harðardóttir, S., 2017. Toxin production in lab-rat diatoms (e.g. *Pseudo-*  
2426 *nitzschia*) in the presence of copepods. Harmful Algae News No. 56, 5–6.
- 2427 Lundholm, N., Hasle, G.R., Fryxell, G.A., Hargraves, P.E., 2002a. Morphology, phylogeny and  
2428 taxonomy of species within the *Pseudo-nitzschia americana* complex (Bacillariophyceae)  
2429 with descriptions of two new species, *Pseudo-nitzschia brasiliiana* and *Pseudo-nitzschia*  
2430 *linea*. Phycologia 41, 480–497.
- 2431 Lundholm, N., Daugbjerg, N., Moestrup, Ø., 2002b. Phylogeny of the Bacillariaceae with  
2432 emphasis on the genus *Pseudo-nitzschia* (Bacillariophyceae) based on partial LSU rDNA.  
2433 Europ. J. Phycol. 37, 115–134.
- 2434 Lundholm, N., Moestrup, Ø., Hasle, G.R., Hoef-Emden, K., 2003. A study of the *Pseudo-*  
2435 *nitzschia pseudodelicatissima/cuspidata* complex (Bacillariophyceae): What is *P.*  
2436 *pseudodelicatissima*? J. Phycol. 39, 797–813.
- 2437 Lundholm, N., Moestrup, Ø., Kotaki, Y., Hoef-Emden, K., Scholin, C., Miller, P., 2006. Inter-  
2438 and intraspecific variation of the *Pseudo-nitzschia delicatissima* complex  
2439 (Bacillariophyceae) illustrated by rRNA probes, morphological data and phylogenetic  
2440 analyses. J. Phycol. 42, 464–481.
- 2441 Lundholm, N., Bates, S.S., Baugh, K.A., Bill, B.D., Connell, L.B., Léger, C., Trainer V.L., 2012.  
2442 Cryptic and pseudo-cryptic diversity in diatoms—with descriptions of *Pseudo-nitzschia*  
2443 *hasleana* sp. nov. and *P. fryxelliana* sp. nov. J. Phycol. 48, 436–454.
- 2444 Macintyre, H.L., Stutes, A.L., Smith, W.L., Dorsey, C.P., Abraham, A., Dickey, R.W., 2011.  
2445 Environmental correlates of community composition and toxicity during a bloom of  
2446 *Pseudo-nitzschia* spp. in the northern Gulf of Mexico. J. Plank. Res. 33, 273–295.
- 2447 Maeno, Y., Kotaki, Y., Terada, R., Cho, Y., Konoki, K., Yotsu-Yamashita, M., 2018. Six domoic  
2448 acid related compounds from the red alga, *Chondria armata*, and domoic acid biosynthesis  
2449 by the diatom, *Pseudo-nitzschia multiseries*. Sci. Rep. 8, 356.
- 2450 Malhi, N., Turnbull, A., Tan, J., Kiermeier, A., Nimmagadda, R., McLeod, C., 2014. A national  
2451 survey of marine biotoxins in wild-caught abalone in Australia. J. Food Prot. 77, 1960–  
2452 1967.
- 2453 Malviya, S., Scalco, E., Audic, S., Vincent, F., Veluchamy, A., Poulain, J., Wincker, P.,  
2454 Iudicone, D., de Vargas, C., Bittner, L., Zingone A., Bowler, C., 2016. Insights into global  
2455 diatom distribution and diversity in the world's ocean. Proc. Natl. Acad. Sci. USA 113,  
2456 E1516–E1525.
- 2457 Maneiro, I., Iglesias, P., Guisande, C., Riveiro, I., Barreiro, A., Zervoudaki, S., Granéli, E., 2005.  
2458 Fate of domoic acid ingested by the copepod *Acartia clausi*. Mar. Biol. 148, 123–130.

- 2459 Mann, D.G., 1988. Why didn't Lund see sex in *Asterionella*? A discussion of the diatom life  
2460 cycle in nature. In: Round, F.E. [Ed.] *Algae and the Aquatic Environment*. Biopress,  
2461 Bristol, pp. 385–412.
- 2462 Marchetti, A., 2005. Ecophysiological aspects of iron nutrition and domoic acid production in  
2463 oceanic and coastal diatoms of the genus *Pseudo-nitzschia*. PhD thesis. University of  
2464 British Columbia, Canada, 220 pp.
- 2465 Marchetti, A., Lundholm, N., Kotaki, Y., Hubbard, K., Harrison, P.J., Armbrust, E.V., 2008.  
2466 Identification and assessment of domoic acid production in oceanic *Pseudo-nitzschia*  
2467 (Bacillariophyceae) from iron-limited waters in the northeast subarctic Pacific. J. Phycol.  
2468 44, 650–661.
- 2469 Marchetti, A., Parker, M.S., Moccia, L.P., Lin, E.O., Arrieta, A.L., Ribalet, F., Murphy, M.E.P.,  
2470 Maldonado, M.T., Armbrust, E.V., 2009. Ferritin is used for iron storage in bloom-forming  
2471 marine pennate diatoms. Nature 456, 1–4.
- 2472 Marchetti, A., Schruth, D.M., Durkin, C.A., Parker, M.S., Kodner, R., Berthiaume, C.T., Morales,  
2473 R., Allen, A.E., Armbrust, E.V., 2012. Comparative metatranscriptomics identifies molecular  
2474 bases for the physiological responses of phytoplankton to varying iron availability. Proc.  
2475 Natl. Acad. Sci. USA 109, E317–E325.
- 2476 Marchetti, A., Catlett, D., Hopkinson, B.M., Ellis, K., Cassar, N., 2015. Marine diatom  
2477 proteorhodopsins and their potential role in coping with low iron availability. ISME J. 9,  
2478 2745–2748.
- 2479 Marchetti, A., Moreno, C.M., Cohen, N.R., Oleinikov, I., deLong, K., Twining, B.S., Armbrust,  
2480 V.E., Lampe, R.H., 2017. Development of a molecular-based index for assessing iron  
2481 status in bloom-forming pennate diatoms. J. Phycol. 53, 820–832.
- 2482 Markina, Z.V., Aizdaicher, N.A., 2016. The effect of lowered salinity of sea water on the growth  
2483 and photosynthetic pigment content in three strains of the microalgae *Pseudo-nitzschia*  
2484 *pungens* (Grunow ex. P.T. Cleve) Hasle, 1993 (Bacillariophyta). Russ. J. Mar. Biol. 42,  
2485 414–418.
- 2486 Martin-Jézéquel, V., Calu, G., Candela, L., Amzil, Z., Jauffrais, T., Séchet, V., Weigel, P., 2015.  
2487 Effects of organic and inorganic nitrogen on the growth and production of domoic acid by  
2488 *Pseudo-nitzschia multiseries* and *P. australis* (Bacillariophyceae) in culture. Mar. Drugs  
2489 13, 7067–7086.
- 2490 Mazzillo, F.F.M., Staaf, D.J., Field, J.C., Carter, M.L., Ohman, M.D., 2011. A note on the  
2491 detection of the neurotoxin domoic acid in beach-stranded *Dosidicus gigas* in the Southern  
2492 California Bight. CalCOFI Rep. 52, 109–115.
- 2493 McCoy, G.R., Touzet, N., Fleming, G.T.A., Raine, R., 2013. An evaluation of the applicability  
2494 of microarrays for monitoring toxic algae in Irish coastal waters. Environ. Sci. Pollut. Res.  
2495 20, 6751–6764.
- 2496 McKibben, S.M., Peterson, W., Wood, A.M., Trainer, V.L., Hunter, M., White, A.E., 2017.  
2497 Climatic regulation of the neurotoxin domoic acid. Proc. Natl. Acad. Sci. USA 114, 239–  
2498 244.

- 2499 Medlin, L., 2018. Mini review: diatom species as seen through a molecular window. *Braz. J. Bot.* (in  
2500 press) <https://doi.org/10.1007/s40415-018-0444-1>
- 2501 Medlin, L.K., Kegel, J.U., 2014. Validation of the detection of *Pseudo-nitzschia* spp. using  
2502 specific RNA probes tested in a microarray format: calibration of signal based on  
2503 variability of RNA content with environmental conditions. *Harmful Algae* 37, 183–193.
- 2504 Medlin, L.K., Orozco, J., 2017. Molecular techniques for the detection of organisms in aquatic  
2505 environments, with emphasis on harmful algal bloom species. *Sensors* 17, 1184;  
2506 doi:10.3390/s17051184
- 2507 Méndez, S.M., Ferrario, M., Cefarelli, A.O., 2012. Description of toxigenic species of the genus  
2508 *Pseudo-nitzschia* in coastal waters of Uruguay: morphology and distribution. *Harmful  
2509 Algae* 19, 53–60.
- 2510 McCabe, R.M., Hickey, B.M., Kudela, R.M., Lefebvre, K.A., Adams, N.G., Bill, B.D., Gulland,  
2511 F.M.D., Thomson, R.E., Cochlan, W.P., Trainer V.L., 2016. An unprecedented coastwide  
2512 toxic algal bloom linked to anomalous ocean conditions. *Geophys. Res. Lett.* 43, 10,366–  
2513 10,376.
- 2514 McHuron, E.A., Greig, D.J., Colegrove, K.M., Fleetwood, M., Spraker, T.R., Gulland, F.M.D.,  
2515 Harvey, J.T., Lefebvre, K.A., Frame, E.R., 2013. Domoic acid exposure and associated  
2516 clinical signs and histopathology in Pacific harbor seals (*Phoca vitulina richardii*).  
2517 *Harmful Algae* 23, 28–33.
- 2518 McPartlin, D.A., Lochhead, M.J., Connell, L.B., Doucette, G.J., O'Kennedy, R.J., 2016. Use of  
2519 biosensors for the detection of marine toxins. *Essays Biochem.* 60, 49–58.
- 2520 Miesner, A.K., Lundholm, N., Krock, B., Nielsen, T.G., 2016. The effect of *Pseudo-nitzschia*  
2521 *seriata* on grazing and fecundity of *Calanus finmarchicus* and *Calanus glacialis*. *J. Plank.  
2522 Res.* 38, 564–574.
- 2523 Moeys, S., Frenkel, J., Lembke, C., Gillard, J.T.F., Devos, V., Van den Berge, K., Bouillon, B.,  
2524 Huysman, M.J.J., De Decker, S., Scharf, J., Bones, A., Brembu, T., Winge, P., Sabbe, K.,  
2525 Vuylsteke, M., Clement, L., De Veylder, L., Pohnert, G., Vyverman, W., 2016. A sex-  
2526 inducing pheromone triggers cell cycle arrest and mate attraction in the diatom *Seminavis  
2527 robusta*. *Sci. Rep.* 6, 19252.
- 2528 Montresor, M., Vitale, L., D'Alelio, D., Ferrante, M.I., 2016. Sex in marine planktonic diatoms:  
2529 insights and challenges. *Perspect. Phycol.* 3, 61–75.
- 2530 Mogensen, H.M., 2014. Investigating complex phytoplankton dynamics: *Pseudo-nitzschia* spp.  
2531 diversity and toxicity in the nearshore Gulf of Maine. Honors Project, Department of  
2532 Biological Sciences, Smith College, Northampton, Massachusetts. 79 pp.
- 2533 Moschandrou, K.K., Baxevanis, A.D., Katikou, P., Papaefthimiou, D., Nikolaidis, G.,  
2534 Abatzopoulos, T.J., 2012. Inter- and intra-specific diversity of *Pseudo-nitzschia*  
2535 (Bacillariophyceae) in the northeastern Mediterranean. *Europ. J. Phycol.* 47, 321–339.
- 2536 Nagai, S., Urusizaki, S., Hongo, Y., Chen, H., Dzhembekova, N., 2017. An attempt to semi-  
2537 quantify potentially toxic diatoms of the genus *Pseudo-nitzschia* in Tokyo Bay, Japan by  
2538 using massively parallel sequencing technology. *Plank. Benthos Res.* 12, 248–258.

- 2539 Naz, T., Burhan, Z.-U.-N., Munir, S., Siddiqui, P.J.A., 2012. Taxonomy and seasonal  
2540 distribution of *Pseudo-nitzschia* species (Bacillariophyceae) from the coastal waters of  
2541 Pakistan. *Pakistani J. Bot.* 44, 1467–1473.
- 2542 Nedelcu, A.M., Marcu, O., Michod, R.E., 2004. Sex as a response to oxidative stress: a twofold  
2543 increase in cellular reactive oxygen species activates sex genes. *Proc. Roy. Soc. Lond. Ser.*  
2544 *B-Biol. Sci.* 271, 1591–1596.
- 2545 Novelli, A., Fernández-Sánchez, M.T., Pérez-Gómez, A., Cabrera-García, D., Lipsky, R.,  
2546 Marini, A.M., Salas-Puig, J., 2014. The mechanisms of action of domoic acid: from  
2547 pathology to physiology. In: Rossini, G.P. [Ed.], *Toxins and Biologically Active*  
2548 *Compounds from Microalgae. Volume 2. Biological Effects and Risk Management*. CRC  
2549 Press, Taylor & Francis Group, Boca Raton, FL, pp. 86–115.
- 2550 Noyer, C., Abot, A., Trouilh, L., Leberre, V.A., Dreanno, C., 2015. Phytochip: development of a  
2551 DNA-microarray for rapid and accurate identification of *Pseudo-nitzschia* spp. and other  
2552 harmful algal species. *J. Microbiol. Meth.* 112, 55–66.
- 2553 O'Dea, S.N., 2012. Occurrence, toxicity, and diversity of *Pseudo-nitzschia* in Florida coastal  
2554 waters. Master's Thesis, University of South Florida, 103 pp.  
2555 <http://scholarcommons.usf.edu/cgi/viewcontent.cgi?article=5383&context=etd>
- 2556 O'Dea, S.N., Flewelling, L.J., Wolny, J., Brame, J., Henschen, K., Scott, P., Hubbard, K.A.,  
2557 Wren, J., Jones, C., Knight, C., Brooks, C., 2013. Florida's first shellfish closure due to  
2558 domoic acid. *Seventh Symposium on Harmful Algae in the U.S.*, Sarasota, FL, p. 103.  
2559 <http://www.whoi.edu/fileserver.do?id=208984&pt=2&p=28786>
- 2560 Okolodkov, Y.B., Aké-Castillo, J.A., Campos-Bautista, G., Lara-Martínez, R., Jiménez-García,  
2561 L.F., 2015. Dinámica anual del género *Pseudo-nitzschia* (Bacillariophyceae) en el Sistema  
2562 Arrecifal Veracruzano. In: Granados-Barba, A., Ortiz-Lozano, L., Salas-Monreal, D.,  
2563 González-Gándara, C. [Eds.], *Aportes al Conocimiento del Sistema Arrecifal*  
2564 *Veracruzano: Hacia el Corredor Arrecifal del Suroeste del Golfo de México*. Universidad  
2565 Veracruzana, pp. 235–252.
- 2566 Olson, M.B., Lessard, E.J., 2010. The influence of the *Pseudo-nitzschia* toxin, domoic acid, on  
2567 microzooplankton grazing and growth: a field and laboratory assessment. *Harmful Algae*  
2568 9, 540–547.
- 2569 Orive, E., Pérez-Aicua, L., David, H., García-Etxebarria, K., Laza-Martínez, A., Seoane, S.,  
2570 Miguel, I., 2013. The genus *Pseudo-nitzschia* (Bacillariophyceae) in a temperate estuary  
2571 with description of two new species: *Pseudo-nitzschia plurisecta* sp. nov. and *Pseudo-*  
2572 *nitzschia abrensis* sp. nov. *J. Phycol.* 49, 1192–1206.
- 2573 Orsini, L., Sarno, D., Procaccini, G., Poletti, R., Dahlmann, J., Montresor, M., 2002. Toxic  
2574 *Pseudo-nitzschia multistriata* (Bacillariophyceae) from the Gulf of Naples: morphology,  
2575 toxin analysis and phylogenetic relationships with other *Pseudo-nitzschia* species. *Europ.*  
2576 *J. Phycol.* 37, 247–257.
- 2577 Paredes-Banda, P., Cruz-López, R., García-Mendoza, E., Santiago-Morales, I., Ruiz-de la Torre,  
2578 M.C., Murillo-Martínez, R., Peña-Manjarrez, J.L., Sánchez-Bravo, Y., Medina-Elizalde,  
2579 J., 2016. Estudios de ecofisiología sobre especies fitoplanctónicas nocivas en Baja  
2580 California. In: García-Mendoza, E., Quijano-Scheggia, S.I., Olivos-Ortiz, A., Núñez-

- 2581 Vázquez, E.J. [Eds.], Florecimientos Algales Nocivos en México. Centro de Investigación  
2582 Científica y de Educación Superior de Ensenada, Mexico, pp. 79–91.
- 2583 Parsons, M.L., Okolodkov, Y.B., Aké-Castillo, J.A., 2012. Diversity and morphology of the  
2584 species of *Pseudo-nitzschia* (Bacillariophyta) of the national park Sistema Arrecifal  
2585 Veracruzano. *Acta Bot. Mex.* 98, 51–72.
- 2586 Parsons, M.L., Dortch, Q., Doucette, G.J., 2013. An assessment of *Pseudo-nitzschia* population  
2587 dynamics domoic acid production in coastal Louisiana. *Harmful Algae* 30, 65–77.
- 2588 Patil, S., Moeys, S., von Dassow, P., Huysman, M.J.J., Mapleson, D., De Veylder, L., Sanges, R.,  
2589 Vyverman, W., Montresor, M., Ferrante, M.I., 2015. Identification of the meiotic toolkit in  
2590 diatoms and exploration of meiosis-specific SPO11 and RAD51 homologs in the sexual  
2591 species *Pseudo-nitzschia multistriata* and *Seminavis robusta*. *BMC Genomics* 16, 930.
- 2592 Pazos, A.J., Ventoso, P., Martínez-Escauriaza, R., Pérez-Parallé, M.L., Blanco, J., Triviño, J.C.,  
2593 Sánchez, J.L., 2017. Transcriptional response after exposure to domoic acid-producing  
2594 *Pseudo-nitzschia* in the digestive gland of the mussel *Mytilus galloprovincialis*. *Toxicon* 140,  
2595 60–71.
- 2596 Peacock, M.B., Gibble, C.M., Senn, D.B., Cloern, J.E., Kudela, R.M., 2018. Blurred lines: multiple  
2597 freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California.  
2598 *Harmful Algae* 73, 138–147.
- 2599 Pednekar, S.M., Bates, S.S., Kerkar, V., Prabhu Matondkar, S.G., 2018. Environmental factors  
2600 affecting the distribution of *Pseudo-nitzschia* in two monsoonal estuaries of western India  
2601 and effects of salinity on growth and domoic acid production by *P. pungens*. *Estuar. Coasts*  
2602 (in press) <https://doi.org/10.1007/s12237-018-0366-y>
- 2603 Penna, A., Galluzzi, L., 2013. The quantitative real-time PCR applications in the monitoring of  
2604 marine harmful algal bloom (HAB) species. *Environ. Sci. Pollut. Res.* 20, 6851–6862.
- 2605 Penna, A., Casabianca, S., Perini, F., Bastianini, M., Riccardi, E., Pigozzi, S., Scardi, M., 2013.  
2606 Toxic *Pseudo-nitzschia* spp. in the northwestern Adriatic Sea: characterization of species  
2607 composition by genetic and molecular quantitative analyses. *J. Plank. Res.* 35, 352–366.
- 2608 Percopo, I., Ruggiero, M.V., Balzano, S., Gourvil, P., Lundholm, N., Siano, R., Tammilehto, A.,  
2609 Vaulot, D., Sarno, D., 2016. *Pseudo-nitzschia arctica* sp. nov., a new cold-water cryptic  
2610 complex. *J. Phycol.* 52, 184–199.
- 2611 Pérez-Gómez, A., Tasker, R.A., 2014. Domoic acid as a neurotoxin. In: Kostrzewa, R.M. [Ed.],  
2612 *Handbook of Neurotoxicity*. Volume 1. Springer Science+Business Media, New York, pp.  
2613 399–419.
- 2614 Peteva, Z.V., Georgieva, S., Stancheva, M., Makedonski, L., 2017. Recreational angler exposure  
2615 to domoic acid via consumption of contaminated shellfish from the Black Sea, Bulgaria: a  
2616 preliminary study. *Arch. Balkan Med. Union* 52, 291–297.
- 2617 Petrou, K., Ralph, P.J., 2011. Photosynthesis and net primary productivity in three Antarctic  
2618 diatoms: possible significance for their distribution in the Antarctic marine ecosystem.  
2619 *Mar. Ecol. Prog. Ser.* 437, 27–40.

- 2620 Pfaffen, S., Abdulqadir, R., Le Brun, N.E., Murphy, M.E., 2013. Mechanism of ferrous iron  
2621 binding and oxidation by ferritin from a pennate diatom. *J. Biol. Chem.* 288, 14917–  
2622 14925.
- 2623 Pfaffen, S., Bradley, J.M., Abdulqadir, R., Firme, M.R., Moore, G.R., Le Brun, N.E., M.E.P.  
2624 Murphy., 2015. A diatom ferritin optimized for iron oxidation but not iron storage. *J. Biol*  
2625 *Chem.* 290, 28416–28427.
- 2626 Philips, E.J., Badylak, S., Christman, M., Wolny, J., Brame, J., Garland, J., Hall, L., Hart, J.,  
2627 Landsberg, J., Lasi, M., Lockwood, J., Paperno, R., Scheidt, D., Staples, A., Steidinger,  
2628 K., 2011. Scales of temporal and spatial variability in the distribution of harmful algae  
2629 species in the Indian River Lagoon, Florida, USA. *Harmful Algae* 10, 277–290.
- 2630 Pistocchi, R., Guerrini, F., Pezzolesi, L., Riccardi, M., Vanucci, S., Ciminiello, P.,  
2631 Dell'Aversano, C., Forino, M., Fattorusso, E., Tartaglione, L., Milandri, A., Pompei, M.,  
2632 Cangini, M., Pigozzi, S., Riccardi, E., 2012. Toxin levels and profiles in microalgae from  
2633 the North-Western Adriatic Sea – 15 years of studies on cultured species. *Mar. Drugs* 10,  
2634 140–162.
- 2635 Pitcher, G.C., Cembella, A.D., Krock, B., Macey, B., Mansfield, L., Probyn, T., 2014.  
2636 Identification of the marine diatom *Pseudo-nitzschia multiseries* (Bacillariophyceae) as a  
2637 source of the toxin domoic acid in Algoa Bay, South Africa. *African J. Mar. Sci.* 36, 523–  
2638 528.
- 2639 Pizarro, G., Frangópulos, M., Krock, B., Zamora, C., Pacheco, H., Alarcón, C., Toro1, C., Pinto,  
2640 M., Torres, R., Guzmán, L., 2017. Watch out for ASP in the Chilean Subantarctic region.  
2641 In: Proença, L.A.O., Hallegraeff, G.M. [Eds.], *Marine and Fresh-Water Harmful Algae*.  
2642 Proceedings of the 17<sup>th</sup> International Conference on Harmful Algae. International Society  
2643 for the Study of Harmful Algae, and Intergovernmental Oceanographic Commission of  
2644 UNESCO, pp. 30–33.
- 2645 Port, J.A., Parker, M.S., Kodner, R.B., Wallace, J.C., Armbrust, E.V., Faustman, E.M., 2013.  
2646 Identification of G protein-coupled receptor signaling pathway proteins in marine diatoms  
2647 using comparative genomics. *BMC Genomics* 14, 503.
- 2648 Prince, E.K., Irmer, F., Pohnert, G., 2013. Domoic acid improves the competitive ability of  
2649 *Pseudo-nitzschia delicatissima* against the diatom *Skeletonema marinoi*. *Mar. Drugs* 11,  
2650 2398–2412.
- 2651 Pugliese, L., Casabianca, S., Perini, F., Andreoni, F., Penna, A., 2017. A high resolution melting  
2652 method for the molecular identification of the potentially toxic diatom *Pseudo-nitzschia*  
2653 spp. in the Mediterranean Sea. *Sci. Rep.* 7, 4259.
- 2654 Pulido, O.M., 2014a. Domoic acid: biological effects and health implications. In: Rossini, G.P.  
2655 [Ed.], *Toxins and Biologically Active Compounds from Microalgae. Volume 2. Biological*  
2656 *Effects and Risk Management*. CRC Press, Taylor & Francis Group, Boca Raton, FL, pp.  
2657 219–252.
- 2658 Pulido, O.M., 2014b. Marine algal toxins: seafood safety, human health and beyond.  
2659 *Oceanography* 2, e111.
- 2660 Pulido, O.M., 2016. Phycotoxins by Harmful Algal Blooms (HABS) and human poisoning: an  
2661 overview. *Internat. Clin. Pathol.* J. 2, 00062.

- 2662 Quijano-Scheggia, S.I., Garcés, E., Lundholm, N., Moestrup, Ø., Andree, K., Camp, J., 2009a.  
2663 Morphology, physiology, molecular phylogeny and sexual compatibility of the cryptic  
2664 *Pseudo-nitzschia delicatissima* complex (Bacillariophyta), including the description of *P.*  
2665 *arenysensis* sp. nov. *Phycologia* 48, 492–509.
- 2666 Quijano-Scheggia, S., Garcés, E., Andree, K., Fortuño, J.M., Camp, J., 2009b. Homothallic  
2667 auxosporulation in *Pseudo-nitzschia brasiliiana* (Bacillariophyta). *J. Phycol.* 45, 100–107.
- 2668 Quijano-Scheggia, S., Garcés, E., Andree, K.B., de la Iglesia, P., Diogène, J., Fortuño, J.-M.,  
2669 Camp, J., 2010. *Pseudo-nitzschia* species on the Catalan coast: characterization and  
2670 contribution to the current knowledge of the distribution of this genus in the Mediterranean  
2671 Sea. *Sci. Mar.* 74, 395–410.
- 2672 Quijano-Scheggia, S., Olivos-Ortiz, A., Gaviño-Rodríguez, J.H., Castro-Ochoa, F., Rivera-  
2673 Vilarelle, M., Galicia-Pérez, M., Patiño-Barragan, M., 2011. First report of *Pseudo-*  
2674 *nitzschia brasiliiana* and *P. micropora* (Bacillariophyceae) found in Cuyutlan Lagoon,  
2675 Mexico. *Rev. Biol. Mar. Oceanogr.* 46, 189–197.
- 2676 Rachman, A., Thoha, H., 2014. Seasonal change in the diel pattern of the *Pseudo-nitzschia*  
2677 population in the Cisadane River estuary: response to the changes in the water's physical-  
2678 chemical parameters. *Mar. Res. Indonesia* 39, 39–50.
- 2679 Radan, R.L., Cochlan, W.P., 2018. Differential toxin response of *Pseudo-nitzschia multiseries* as  
2680 a function of nitrogen speciation in batch and continuous cultures, and during a natural  
2681 assemblage experiment. *Harmful Algae* 73, 12–29.
- 2682 Ramsdell, J.S., Gulland, F.M., 2014. Domoic acid epileptic disease. *Mar. Drugs* 12, 1185–1207.
- 2683 Reizopoulou, S., Strogyloudi, E., Giannakourou, A., Granéli, E., Pagou, K., 2012. Toxin  
2684 accumulation in benthic populations under blooms of *Dinophysis acuminata* and *Pseudo-*  
2685 *nitzschia multiseries*. In: Pagou, P., Hallegraeff, G. [Eds.], *Proceedings of the 14th*  
2686 *International Conference on Harmful Algae*. International Society for the Study of  
2687 Harmful Algae and Intergovernmental Oceanographic Commission of UNESCO, Paris,  
2688 pp. 178–180.
- 2689 Rengefors, K., Kremp, A., Reusch, T.B.H., Wood, M., 2017. Genetic diversity and evolution in  
2690 eukaryotic phytoplankton: revelations from population genetic studies. *J. Plank. Res.* 39,  
2691 165–179.
- 2692 Resetarits, E., 2011. Two toxic *Pseudo-nitzschia* species occupy different niches during a  
2693 Georges Bank summer transition. Senior Undergraduate Thesis, Columbia University.
- 2694 Rhodes, L.L., Scholin, C., Garthwaite, I., Haywood, A., Thomas, A., 1998. Domoic acid  
2695 producing *Pseudo-nitzschia* species educed by whole cell DNA probe-based and  
2696 immunochemical assays, In: Reguera, B., Blanco, J., Fernández, M.L., Wyatt, T. [Eds.],  
2697 *Harmful Algae*. Xunta de Galicia and the IOC of UNESCO, Paris, pp. 274–277.
- 2698 Rhodes, L., Adamson, J., Scholin, C., 2000. *Pseudo-nitzschia multistriata* (Bacillariophyceae) in  
2699 New Zealand. *New Zealand J. Mar. Freshwater Res.* 34, 463–467.
- 2700 Rhodes, L.L., Holland, P.T., Adamson, J.E., McNabb, P., Selwood, A.I., 2003. Production of a  
2701 new isomer of domoic acid by New Zealand isolates of the diatom *Pseudo-nitzschia*  
2702 *australis*. In: Villalba, A., Reguera, B., Romalde, J.L., Beiras, R. [Eds.], *Molluscan*

- 2703           Shellfish Safety. Xunta de Galicia and Intergovernmental Oceanographic Commission of  
2704           UNESCO, Paris, pp. 43–48.
- 2705           Rhodes, L., Jiang, W., Knight, B., Adamson, J., Smith, K., Langi, V., Edgar, M., 2013. The  
2706           genus *Pseudo-nitzschia* (Bacillariophyceae) in New Zealand: analysis of the last decade's  
2707           monitoring data. *New Zealand J. Mar. Freshwater Res.* 47, 490–503.
- 2708           Rijat Leblad, B., Lundholm, N., Goux, D., Veron, B., Sagou, R., Taleb, H., Nhhala, H., Er-  
2709           Raioui, H., 2013. *Pseudo-nitzschia* Peragallo (Bacillariophyceae) diversity and domoic  
2710           acid accumulation in tuberculate cockles and sweet clams in M'diq Bay, Morocco. *Acta*  
2711           *Bot. Croat.* 72, 35–47.
- 2712           Rivera-Vilarelle, M., Quijano-Scheggia, S., Olivos-Ortiz, A., Gaviño-Rodríguez, J.H., Castro-  
2713           Ochoa, F., Reyes-Herrera, A., 2013. The genus *Pseudo-nitzschia* (Bacillariophyceae) in  
2714           Manzanillo and Santiago Bays, Colima, Mexico. *Bot. Mar.* 56, 357–373.
- 2715           Rivera-Vilarelle, M., Valdez-Velázquez, L.L., Quijano-Scheggia, S.I., 2018. Description of  
2716           *Pseudo-nitzschia cuspidata* var. *manzanillensis* var. nov. (Bacillariophyceae): morphology  
2717           and molecular characterization of a variety from the central Mexican Pacific. *Diatom Res.*  
2718           (in press)  
2719           <http://www.tandfonline.com/doi/abs/10.1080/0269249X.2018.1426633?journalCode=tdia20>
- 2721           Romero, M.L.J., Lirdwitayaprasit, T., Kotaki, Y., Lundholm, N., Relox, Jr., R.J., Furio, E.F.,  
2722           Terada, R., Yokoyama, T., Kodama, M., Fukuyo, Y., 2008. Isolation of ASP toxin-  
2723           producing *Nitzschia* from Thailand. *Mar. Res. Indonesia* 33, 225–228.
- 2724           Romero, M.L.J., Kotaki, Y., Lundholm, N., Thoha, H., Ogawa, H., Relox, J.R., Terada, R.,  
2725           Takeda, S., Takata, Y., Haraguchi, K., Endo, T., Lim, P.T., Kodama, M., Fukuyo, Y.,  
2726           2011. Unique amnesic shellfish toxin composition found in the South East Asian diatom  
2727           *Nitzschia navis-varingica*. *Harmful Algae* 10, 456–462.
- 2728           Romero, M.L.J., Kotaki, Y., Relox, J.R., Lundholm, N., Takata, Y., Kodama, M., Fukuyo, Y.,  
2729           2012. Two new ASP toxin production types in strains of *Nitzschia navis-varingica* from  
2730           the Philippines. *Coast. Mar. Sci.* 35, 67–69.
- 2731           Rossini, G.P., Hess, P., 2010. Phycotoxins: chemistry, mechanisms of action and shellfish  
2732           poisoning. In: Luch, A. [Ed.], *Molecular, Clinical and Environmental Toxicology. Volume*  
2733           *2: Clinical Toxicology*. Birkhäuser, Basel, pp. 65–122.
- 2734           Ruggiero, M.V., Sarno, D., Barra, L., Kooistra, W.H.C.F., Montresor, M., Zingone, A., 2015.  
2735           Diversity and temporal pattern of *Pseudo-nitzschia* species (Bacillariophyceae) through  
2736           the molecular lens. *Harmful Algae* 42, 15–24.
- 2737           Ruggiero, M.V., D'Alelio, D., Ferrante, M.I., Santoro, M., Vitale, L., Procaccini, G., Montresor,  
2738           M., 2018. Clonal expansion behind a marine diatom bloom. *ISME J.* 12, 463–472
- 2739           Russell, A.D.P.G., de Souza, M.S., Mendes, C.R.B., Jesus, B., Tavano, V.M., Garcia, C.A.E.,  
2740           2015. Photophysiological effects of Fe concentration gradients on diatom-dominated  
2741           phytoplankton assemblages in the Antarctic Peninsula region. *J. Exp. Mar. Biol. Ecol.* 466,  
2742           49–58.

- 2743 Russell, T., 2016. *Pseudo-nitzschia* spp. and domoic acid in the waters of Haida Gwaii, British  
2744 Columbia – A summary of occurrences and details on anthropogenic and environmental  
2745 consideration. PICES 2016 Annual Meeting, Nov 2–13, 2016, San Diego, U.S.A.  
2746 <http://meetings.pices.int/Publications/Presentations/PICES-2016/W2-Russell.pdf>
- 2747 Rust, L., Gulland, F., Frame, E., Lefebvre, K., 2014. Domoic acid in milk of free living  
2748 California marine mammals indicates lactational exposure occurs. Mar. Mam. Sci. 30,  
2749 1272–1278.
- 2750 Ryabushko, L.I., Besiktepe, S., Ediger, D., Yilmaz, D., Zenginer, A., Ryabushko, V.I., Lee, R.I.,  
2751 2008. Toxic diatom of *Pseudo-nitzschia calliantha* Lundholm, Moestrup et Hasle from the  
2752 Black Sea: morphology, taxonomy, ecology. Mar. Ecol. J. ECOSI-Gidrofizika, Sevastopol  
2753 7, 51–60.
- 2754 Ryan, J.P., Kudela, R.M., Birch J.M., Blum, M., Bowers, H.A., Chavez, F.P., Doucette, G.J.,  
2755 Hayashi, K., Marin III, R., Mikulsk,i C.M., Pennington, J.T., Scholin, C.A., Smith, G.J.,  
2756 Woods, A., Zhang, Y., 2017. Causality of an extreme harmful algal bloom in Monterey  
2757 Bay, California, during the 2014–2016 northeast Pacific warm anomaly. Geophys. Res.  
2758 Lett. 44, 5571–5579.
- 2759 Sabatino, V., Russo, M.T., Patil, S., d’Ippolito, G., Fontana, A., Ferrante, M.I., 2015.  
2760 Establishment of genetic transformation in the sexually reproducing diatoms *Pseudo-*  
2761 *nitzschia multistriata* and *Pseudo-nitzschia arenysensis* and inheritance of the transgene.  
2762 Mar. Biotechnol. 17, 452–462.
- 2763 Sackett, O., Petrou, K., Reedy, B., De Grazia, A., Hill, R., Doblin, M., Beardall, J., Ralph, P.,  
2764 Heraud, P., 2013. Phenotypic plasticity of Southern Ocean diatoms: key to success in the  
2765 sea ice habitat? PLoS ONE 8, e81185.
- 2766 Saeed, A.F., Awan, S.A., Ling, S., Wang, R., Wang, S., 2017. Domoic acid: attributes, exposure  
2767 risks, innovative detection techniques and therapeutics. Algal Res. 24, 97–110.
- 2768 Sahraoui, I., Sakka Hlaili, A., Hadj Mabrouk, H., Léger, C., Bates, S.S., 2009. Blooms of the  
2769 diatom genus *Pseudo-nitzschia* H. Peragallo in Bizerte Lagoon (Tunisia, SW  
2770 Mediterranean). Diatom Res. 24, 175–190.
- 2771 Sahraoui, I., Bates, S.S., Bouchouicha, D., Hadj Mabrouk, H., Sakka Hlaili, A., 2011. Toxicity of  
2772 *Pseudo-nitzschia* populations from Bizerte Lagoon, Tunisia, southwest Mediterranean and  
2773 first report of domoic acid production by *P. brasiliiana*. Diatom Res. 26, 293–303.
- 2774 Sahraoui, I., Grami, B., Bates, S.S., Bouchouicha, D., Chikhaoui, M.A., Hadj Mabrouka, H.,  
2775 Sakka Hlaili, A., 2012. Response of potentially toxic *Pseudo-nitzschia* (Bacillariophyceae)  
2776 populations and domoic acid to environmental conditions in a eutrophied, SW  
2777 Mediterranean coastal lagoon (Tunisia). Estuar. Coast. Shelf Sci. 102–103, 95–104.
- 2778 Sakka Hlaili, A., Sahraoui Khalifa, I., Bouchouicha-Smida, D., Melliti Garali, S., Ksouri, J.,  
2779 Chalghaf, M., Bates, S.S., Lundholm, N., Kooistra, W.H.C.F., de la Iglesia, P., Diogène,  
2780 J., 2016. Toxic and potentially toxic diatom blooms in Tunisian (SW Mediterranean)  
2781 waters: review of ten years of investigations. Adv. Environ. Res. 48, 51–69.
- 2782 Santiago-Morales, I.S., García-Mendoza, E., 2011. Growth and domoic acid content of *Pseudo-*  
2783 *nitzschia australis* isolated from northwestern Baja California, Mexico, cultured under

- 2784           batch conditions at different temperatures and two Si:NO<sub>3</sub> ratios. *Harmful Algae* 12, 82–  
2785           94.
- 2786     Sarno, D., Zingone, A., Montresor, M., 2010. A massive and simultaneous sex event of two  
2787           *Pseudo-nitzschia* species. *Deep-Sea Res. II* 57, 248–255.
- 2788     Sato, S., Beakes, G., Idei, M., Nagumo, T., Mann, D.G., 2011. Novel sex cells and evidence for  
2789           sex pheromones in diatoms. *PLoS ONE* 6, e26923.
- 2790     Scalco, E., Stec, K., Iudicone, D., Ferrante, M.I., Montresor, M., 2014. The dynamics of sexual  
2791           phase in the marine diatom *Pseudo-nitzschia multiseries* (Bacillariophyceae). *J. Phycol.*  
2792           50, 817–828.
- 2793     Scalco, E., Amato, A., Ferrante, M.I., Montresor, M., 2016. The sexual phase of the diatom  
2794           *Pseudo-nitzschia multiseries*: cytological and time-lapse cinematography characterization.  
2795           *Protoplasma* 253, 1421–1431.
- 2796     Schiebel, R., Zeltner, A., Treppke, U.F., Waniek, J.J., Bollmann, J., Rixen, T., Hemleben, C.,  
2797           2004. Distribution of diatoms, coccolithophores and planktic foraminifers along a trophic  
2798           gradient during SW monsoon in the Arabian Sea. *Mar. Micropaleontol.* 51, 345–371.
- 2799     Schnetzer, A., Lampe, R.H., Benitez-Nelson, C.R., Marchetti, A., Osburn, C.L., Tatters, A.O.,  
2800           2017. Marine snow formation by the toxin-producing diatom, *Pseudo-nitzschia australis*.  
2801           *Harmful Algae* 61, 23–30.
- 2802     Selander, E., Thor, P., Toth, G., Pavia, H., 2006. Copepods induce paralytic shellfish toxin  
2803           production in marine dinoflagellates. *Proc. Biol. Sci. / Royal Soc.* 273, 1673–1680.
- 2804     Shanks, A.L., Morgan, S.G., MacMahan, J., Reniers, A.J.H.M., Kudela, R., Jarvis, M., Brown,  
2805           J., Fujimura, A., Zuccarelli, L., Griesemer, C., 2016. Variation in the abundance of *Pseudo-*  
2806           *nitzschia* and domoic acid with surf zone type. *Harmful Algae* 55, 172–178.
- 2807     Shaw, B.A., Andersen, R.J., Harrison, P.J., 1997. Feeding deterrent and toxicity effects of apo-  
2808           fucoxanthinoids and phycotoxins on a marine copepod (*Tigriopus californicus*). *Mar. Biol.*  
2809           128, 273–280.
- 2810     Shiganova, T., Öztürk, B., 2010. Trend on increasing Mediterranean species arrival into the  
2811           Black Sea. In: Briand, F. [Ed.], CIESM, 2010. Climate Forcing and its Impacts on the  
2812           Black Sea Marine Biota, N° 39 CIESM Workshop Monographs. Monaco, pp. 75–91.
- 2813     Shuler, A.J., Paternoster, J., Brim, M., Nowocin, K., Tisdale, T., Neller, K., Cahill, J.A.,  
2814           Leighfield, T.A., Fire, S., Wang, Z., Morton, S., 2012. Spatial and temporal trends of the  
2815           toxic diatom *Pseudo-nitzschia* in the southeastern Atlantic United States. *Harmful Algae*  
2816           17, 6–13.
- 2817     Sison-Mangus, M.P., Jiang, S., Tran, K.N., Kudela, R.M., 2014. Host-specific adaptation  
2818           governs the interaction of the marine diatom, *Pseudo-nitzschia* and their microbiota. *ISME J.* 8, 63–76.
- 2820     Sison-Mangus, M.P., Jiang, S., Kudela, R.M., Mehic, S., 2016. Phytoplankton-associated  
2821           bacterial community composition and succession during toxic diatom bloom and non-  
2822           bloom events. *Front. Microbiol.* 7, Article 1433.

- 2823 Sitprija, V., Sitprija, S., 2016. Renal injury induced by marine toxins: role of ion channels. *SRL*  
2824 *Nephrol. Therap.* 2, 001–006.
- 2825 Skov, J., Ton, T.P., Do, T.B.L., 2004. Bacillariophyceae. In: Larsen, J., Nguyen, N.L. [Eds.],  
2826 Potentially Toxic Microalgae of Vietnamese Waters. *Opera Botanica* Vol 140, Council for  
2827 Nordic Publications in Botany, Copenhagen, pp. 23–51.
- 2828 Smeti, E., Roelke, D.L., Gremion, G., Linhart, J.M., Danielidis, D.B., Spatharis, S., 2015.  
2829 Potential mechanisms of coexistence between two globally important *Pseudo-nitzschia*  
2830 (Bacillariophyta) species. *Hydrobiologia* 762, 89–101.
- 2831 Smith, M.W., Maier, M.A., Suci, D., Peterson, T.D., Bradstreet, T., Nakayama, J., Simon,  
2832 H.M., 2012. High resolution microarray assay for rapid taxonomic assessment of *Pseudo-*  
2833 *nitzschia* spp. (Bacillariophyceae) in the field. *Harmful Algae* 19, 169–180.
- 2834 Smith, G.J., Bowers, H.A., Epperson, Z., Woods, A., Sobrinho, B.F., San Miguel, R.A., 2015.  
2835 Application of ISSR PCR to assess intraspecific genetic diversity in local populations of  
2836 *Pseudo-nitzschia* and relevance to toxigenicity patterns. Eighth Symposium on Harmful  
2837 Algae in the U.S., Long Beach, CA, p. 85.  
2838 <http://www.whoi.edu/fileserver.do?id=235844&pt=2&p=241409>
- 2839 Smith, J., Gellene, A.G., Hubbard, K.A., Bowers, H.A., Kudela, R.M., Hayashi, K., Caron, D.A.,  
2840 2018. *Pseudo-nitzschia* species composition varies concurrently with domoic acid  
2841 concentrations during two different bloom events in the Southern California Bight. *J.*  
2842 *Plank. Res.* 40, 29–45.
- 2843 Sobrinho, B.F., Mocelin de Camargo, L., Sandrini-Neto, L., Kleemann, C.R., da Costa Machado  
2844 E., Mafra, Jr., L.L., 2017. Growth, toxin production and allelopathic effects of *Pseudo-*  
2845 *nitzschia multiseries* under iron-enriched conditions. *Mar. Drugs* 15, 331.
- 2846 Stauffer, B.A., Gellene, A.G., Schnetzer, A., Seubert, E.L., Oberg, C., Sukhatme, G.S., Caron  
2847 D.A., 2012. An oceanographic, meteorological and biological ‘perfect storm’ yields a  
2848 massive fish kill. *Mar. Ecol. Prog. Ser.* 468, 231–243.
- 2849 Stone, E.E., 2017. Obligate microbial communities associated with the toxic diatom *Pseudo-*  
2850 *nitzschia australis* genome? Implications for genome assemblies. Master’s Thesis, Clark  
2851 University, Worcester, MA, 58 p.  
2852 <https://search.proquest.com/openview/9adba9c6bc1f8659c5f70f80aeadd2fa/1?pq-origsite=gscholar&cbl=18750&diss=y>
- 2854 Stonik, I.V., Orlova, T.Yu., 2013. The species composition and quantitative distribution of the  
2855 diatom genus *Pseudo-nitzschia* H. Peragallo, 1990 in Russian waters of the Sea of Japan  
2856 and the Sea of Okhotsk. *Russ. J. Mar. Biol.* 39, 238–245.
- 2857 Stonik, I.V., Orlova, T.Yu., Propp, L.N., Demchenko, N.L., Skriptsova, A.V., 2012. An autumn  
2858 bloom of diatoms of the genus *Pseudo-nitzschia* H. Peragallo, 1900 in Amursky Bay, the  
2859 Sea of Japan. *Russ. J. Mar. Biol.* 38, 211–217.
- 2860 Stonik, I.V., Kapustina, I.I., Aizdaicher, N.A., Svetashev V.I., 2016. Sterols and fatty acids  
2861 isolated from laboratory culture of the toxic marine diatom *Pseudo-nitzschia pungens*.  
2862 *Chem. Nat. Compd.* 52, 296–298.

- 2863 Sugie, K., Yoshimura, T., 2013. Effects of  $p\text{CO}_2$  and iron on the elemental composition and cell  
2864 geometry of the marine diatom *Pseudo-nitzschia pseudodelicatissima* (Bacillariophyceae).  
2865 *J. Phycol.* 49, 475–488.
- 2866 Suriyanti, S.N.P., Usup, G., 2015. First report of the toxigenic *Nitzschia navis-varingica*  
2867 (Bacillariophyceae) isolated from Tebrau Straits, Johor, Malaysia. *Toxicon* 108, 257–263.
- 2868 Suriyanti, S.N.P., Dzulhelmi, M.N., Usup, G., 2017. Screening of toxic marine *Nitzschia* species  
2869 (Bacillariophyceae) in Malaysia. *Indonesian J. Mar. Sci. Technol.* 10, 97–102.
- 2870 Tammilehto, A., Nielsen, T.G., Krock, B., Møller, E.F., Lundholm, N., 2012. *Calanus* spp.–  
2871 Vectors for the biotoxin, domoic acid, in the Arctic marine ecosystem? *Harmful Algae* 20,  
2872 165–174.
- 2873 Tammilehto, A., Nielsen, T.G., Krock, B., Møller, E.F., Lundholm, N., 2015. Induction of  
2874 domoic acid production in the toxic diatom *Pseudo-nitzschia seriata* by calanoid  
2875 copepods. *Aquat. Toxicol.* 159, 52–61.
- 2876 Tan, S.N., Lim, H.C., Teng, S.T., Lim, P.T., Leaw, C.P., 2015. *Pseudo-nitzschia* species  
2877 (Bacillariophyceae) identification and delineation using mitochondrial *cox1* gene  
2878 sequences as compared to LSU rDNA. *Fish. Sci.* 81, 831–838.
- 2879 Tan, T.H., Leaw, C.P., Leong, S.C.Y., Lim, L.P., Chew, S.M., Teng, S.T., Lim, P.T., 2016a.  
2880 Marine micro-phytoplankton of Singapore, with a review of harmful microalgae in the  
2881 region. *Raff. Bull. Zool. Suppl.* No. 34, 78–96.
- 2882 Tan, S.N., Teng, S.T., Lim, H.C., Kotaki, Y., Bates, S.S., Leaw, C.P., Lim, P.T., 2016b. Diatom  
2883 *Nitzschia navis-varingica* (Bacillariophyceae) and its domoic acid production from the  
2884 mangrove environments of Malaysia. *Harmful Algae* 60, 139–149.
- 2885 Tas, S., Lundholm, N., 2017. Temporal and spatial variability of the potentially toxic *Pseudo-*  
2886 *nitzschia* spp. in a eutrophic estuary (Sea of Marmara). *J. Mar. Biol. Assoc. UK* 49, 951–  
2887 958.
- 2888 Tas, S., Dursun, F., Aksu, A., Balkis, N., 2016. Presence of the diatom genus *Pseudo-nitzschia*  
2889 and particulate domoic acid in the Golden Horn Estuary (Sea of Marmara, Turkey).  
2890 *Diatom Res.* 31, 339–349.
- 2891 Tasker, R.A., 2014. Chemistry and detection of domoic acid and isomers. In: Rossini, G.P. [Ed.],  
2892 Toxins and Biologically Active Compounds from Microalgae, Volume 1. Origin,  
2893 Detection and Chemistry. CRC Press, Taylor & Francis Group, Boca Raton, FL, pp. 232–  
2894 250.
- 2895 Tasker, R.A., 2016. Domoic acid and other amnesic toxins: toxicological profile. In:  
2896 Gopalakrishnakone, P., Haddad, V., Jr., Kem, W.R., Tubaro, A., Kim, E. [Eds.], Marine  
2897 and Freshwater Toxins. Springer Science+Business Media Dordrecht, Dordrecht  
2898 Netherlands, pp. 93–112.
- 2899 Tatters, A.O., Fu, F.-X., Hutchins, D.A., 2012. High  $\text{CO}_2$  and silicate limitation synergistically  
2900 increase the toxicity of *Pseudo-nitzschia fraudulenta*. *PLoS ONE* 7, e32116.
- 2901 Teng, S.T., Leaw, C.P., Lim, H.C., Lim, P.T., 2013. The genus *Pseudo-nitzschia*  
2902 (Bacillariophyceae) in Malaysia, including new records and a key to species inferred from  
2903 morphology-based phylogeny. *Bot. Mar.* 56, 375–398.

- 2904 Tatters, A.O., Schnetzer, A., Xu, K., Walworth, N.G., Fu, F., Spackeen, J.L., Sipler, R.E.,  
2905 Bertrand, E.M., McQuaid, J.B., Allan, A.E., Bronk, D.A., Gao, K., Sun, J., Caron, D.A.,  
2906 Hutchins, D.A., 2018. Interactive effects of temperature, CO<sub>2</sub> and nitrogen source on a  
2907 coastal California diatom assemblage. *J. Plank. Res.* 40, 151–164.
- 2908 Teng, S.T., Lim, H.C., Lim, P.T., Dao, V.H., Bates, S.S., Leaw, C.P., 2014a. *Pseudo-nitzschia*  
2909 *kodamae* sp. nov. (Bacillariophyceae), a toxicogenic species from the Strait of Malacca,  
2910 Malaysia. *Harmful Algae* 34, 17–28.
- 2911 Teng, S.-T., Lim, P.-T., Lim, H.-C., Leaw, C.-P., 2014b. *Pseudo-nitzschia* (Bacillariophyceae) in  
2912 Malaysia: a record of taxa from field investigations. In: Kim, H.G., Reguera, B.,  
2913 Hallegraeff, G.M., Lee, C.K., Han, M.S., Choi, J.K. [Eds.], *Harmful Algae 2012*,  
2914 Proceedings of the 15th International Conference on Harmful Algae. International Society  
2915 for the Study of Harmful Algae, Maple Design, Busan, Korea, pp. 145–148.
- 2916 Teng, S.T., Dmitriev, D.A., Bates, S.S., Lim, H.C., Lim, P.T., Leaw, C.P., 2014c. Introducing a  
2917 web-based interactive identification key to species of *Pseudo-nitzschia*  
2918 (Bacillariophyceae). *Harmful Algae News* No. 49, pp. 14.  
2919 <http://dmitriev.speciesfile.org/key.asp?key=Bacillariales&lng=En&i=1&keyN=2>
- 2920 Teng, S.T., Lim, H.C., Lim, P.T., Rivera-Vilarelle, M., Quijano-Scheggia, S., Takata, Y.,  
2921 Quilliam, M.A., Wolf, M., Bates, S.S., Leaw, C.P., 2015. A non-toxicogenic but  
2922 morphologically and phylogenetically distinct new species of *Pseudo-nitzschia*, *Pseudo-*  
2923 *nitzschia sabit* sp. nov. (Bacillariophyceae). *J. Phycol.* 51, 706–725.
- 2924 Teng, S.T., Tan, S.N., Lim, H.C., Dao, V.H., Bates, S.S., Leaw, C.P., 2016. High diversity of  
2925 *Pseudo-nitzschia* along the northern coast of Sarawak (Malaysian Borneo), with  
2926 descriptions of *P. bipertita* sp. nov. and *P. limii* (Bacillariophyceae). *J. Phycol.* 52, 973–  
2927 989.
- 2928 Tenorio, C., Uribe, E., Gil-Kodaka, P., Blanco, J., Álvarez, G., 2016. Morphological and  
2929 toxicological studies of *Pseudo-nitzschia* species from the central coast of Peru. *Diatom*  
2930 *Res.* 31, 331–338.
- 2931 Terenko, L., Terenko, G., 2012. Dominant *Pseudo-nitzschia* (Bacillariophyta) species in the  
2932 Black Sea (Ukraine). *Bot. Lith.* 18, 27–34.
- 2933 Terseleer, N., Gypens, N., Lancelot, C., 2013. Factors controlling the production of domoic acid  
2934 by *Pseudo-nitzschia* (Bacillariophyceae): a model study. *Harmful Algae* 24, 45–53.
- 2935 Thessen, A.E., Bowers, H.A., Stoecker, D.K., 2009. Intra- and interspecies differences in growth  
2936 and toxicity of *Pseudo-nitzschia* while using different nitrogen sources. *Harmful Algae* 8,  
2937 792–810.
- 2938 Tesson, S.V.N., Legrand, C., van Oosterhout, C., Montresor, M., Kooistra, W.H.C.F., Procaccini,  
2939 G., 2013. Mendelian inheritance pattern and high mutation rates of microsatellite alleles in  
2940 the diatom *Pseudo-nitzschia multistriata*. *Protist* 164, 89–100.
- 2941 Tesson, S.V.M., Montresor, M., Procaccini, G., Kooistra, W.H.C.F., 2014. Temporal changes in  
2942 population structure of a marine planktonic diatom. *PLoS ONE* 9, e114984.
- 2943 Tester, P.A., Pan, Y., Doucette, G.J., 2001. Accumulation of domoic acid activity in copepods.  
2944 In: Hallegraeff, G.M., Blackburn, S.I., Bolch, C.J., Lewis, R.J. [Eds.], *Harmful Algal*

- 2945 Blooms 2000. Intergovernmental Oceanographic Commission of UNESCO, Paris, pp.  
2946 418–420.
- 2947 Thoha, H., Kotaki, Y., Panggabean, L., Lundholm, N., Ogawa, H., Lim, P.T., Takata, Y.,  
2948 Kodama, M., Fukuyo, Y., 2012. Screening of diatoms that produce ASP toxins in  
2949 Southernmost Asian waters. *Coast. Mar. Sci.* 35, 34–38.
- 2950 Thorel, M., Fauchot, J., Morelle, J., Raimbault, V., Le Roy, B., Miossec, C., Kientz-Bouchart,  
2951 V., Claquin, P., 2014. Interactive effects of irradiance and temperature on growth and  
2952 domoic acid production of the toxic diatom *Pseudo-nitzschia australis*  
2953 (Bacillariophyceae). *Harmful Algae* 39, 232–241.
- 2954 Thorel, M., Claquin, P., Schapira, M., Le Gendre, R., Riou, P., Goux, D., Le Roy, B., Raimbault,  
2955 V., Deton-Cabanillas, A.-F., Bazin, P., 2017. Nutrient ratios influence variability in  
2956 *Pseudo-nitzschia* species diversity and particulate domoic acid production in the Bay of  
2957 Seine (France). *Harmful Algae* 68, 192–205.
- 2958 Thornhill, D.J., Lajeunesse, T.C., Santos, S.R., 2007. Measuring rDNA diversity in eukaryotic  
2959 microbial systems: how intragenomic variation, pseudogenes, and PCR artefacts confound  
2960 biodiversity estimates. *Molec. Ecol.* 16, 5326–5340.
- 2961 Tibiriçá, C.E.J.A., Fernandes L.F., Mafra, L.L., Jr., 2015. Seasonal and spatial patterns of  
2962 toxicogenic species of *Dinophysis* and *Pseudo-nitzschia* in a subtropical Brazilian estuary.  
2963 *Brazil. J. Oceanogr.* 63, 17–32.
- 2964 Timmerman, A.H.V., McManus, M.A., Cheriton, O.M., Cowen, R.K., Greer, A.T., Kudela,  
2965 R.M., Ruttenberg K., Sevadjian, J., 2014. Hidden thin layers of toxic diatoms in a coastal  
2966 bay. *Deep-Sea Res. II* 101, 129–140.
- 2967 Trainer, V.L., Hickey, B.M., Bates, S.S., 2008. Toxic diatoms. In: Walsh, P.J., Smith, S.L.,  
2968 Fleming, L.E., Solo-Gabriele, H.M., Gerwick, W.H. [Eds.], *Oceans and Human Health:*  
2969 Risks and Remedies from the Seas. Elsevier Science Publishers, New York, pp. 219–237.
- 2970 Trainer, V.L., Wells, M.L., Cochlan, W.P., Trick, C.G., Bill, B.D., Baugh, K.A., Beall, B.F.,  
2971 Herndon, J., Lundholm, N., 2009. An ecological study of a massive bloom of toxicogenic  
2972 *Pseudo-nitzschia cuspidata* off the Washington State coast. *Limnol. Oceanogr.* 54, 1461–  
2973 1474.
- 2974 Trainer, V.L., Bates, S.S., Lundholm, N., Thessen, A.E., Cochlan, W.P., Adams, N.G., Trick,  
2975 C.G., 2012. *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and  
2976 impacts on ecosystem health. *Harmful Algae* 14, 271–300.
- 2977 Twinner, M.J., Flewelling, L.J., Fire, S.E., Bowen-Stevens, S.R., Gaydos, J.K., Johnson, C.K.,  
2978 Landsberg, J.H., Leighfield, T.A., Mase-Guthrie, B., Schwacke, L., Van Dolah, F.M.,  
2979 Wang, Z., Rowles, T.K., 2012. Comparative analysis of three brevetoxin-associated  
2980 bottlenose dolphin (*Tursiops truncatus*) mortality events in the Florida panhandle region  
2981 (USA). *PLoS ONE* 7, e42974.
- 2982 Vale, C., 2014. Domoic acid: chemistry and pharmacology. In: Botana, L.M. [Ed.], *Seafood and*  
2983 *Freshwater Toxins: Pharmacology, Physiology and Detection*, 3rd Edition. CRC Press,  
2984 Boca Raton FL, pp. 875–894.

- 2985 Van Meerssche, E., Pinckney, J.L., 2017. The influence of salinity in the domoic acid effect on  
2986 estuarine phytoplankton communities. *Harmful Algae* 69, 65–74.
- 2987 van Tol, H.M., Amin, S.A., Armbrust, E.V., 2017. Ubiquitous marine bacterium inhibits diatom  
2988 cell division. *ISME J.* 11, 31–42.
- 2989 Veschaisit, O., Meksumpun, S., Thawonsode, N., Lirdwitayaprasit, T., 2017. Accumulation of  
2990 domoic acid in marine organisms from Sriracha Bay, Chonburi province, Thailand.  
2991 *ScienceAsia* 43, 207–216.
- 2992 Villac, M.C., Fryxell, G.A., 1998. *Pseudo-nitzschia pungens* var. *cinctula* var. nov.  
2993 (Bacillariophyceae) based on field and culture observations. *Phycologia* 37, 269–274.
- 2994 Villac, M.C., Kaczmarcza, I., 2011. Marine planktonic diatoms, including potentially toxic  
2995 species. In: Seckbach, J., P. Kociolek [Eds.], *The Diatom World. (Cellular Origin, Life in*  
2996 *Extreme Habitats and Astrobiology*, 19). Springer+Business Media B.V., Dordrecht,  
2997 Netherlands, pp. 465–490.
- 2998 Villac, M.C., Roelke, D.L., Chavez, F.P., Cifuentes, L.A., Fryxell, G.A., 1993. *Pseudonitzschia*  
2999 *australis* and related species from the west coast of the U.S.A.: occurrence and domoic  
3000 acid production. *J. Shellfish Res.* 12, 457–465.
- 3001 Villac, M.C., Doucette, G.J., Kaczmarcza, I., 2010. Toxic marine diatoms. In: Smol, J.P.,  
3002 Stoermer, E.F. [Eds.], *The Diatoms – Applications for the Environmental and Earth*  
3003 *Sciences*, 2nd Edition. Cambridge University Press, Cambridge, pp. 540–551.
- 3004 Villareal, T.A., Roelke, D.L., Fryxell, G.A., 1994. Occurrence of the toxic diatom *Nitzschia*  
3005 *pungens* f. *multiseries* in Massachusetts Bay, Massachusetts, U.S.A. *Mar. Environ. Res.*  
3006 37, 417–423.
- 3007 Wadt, P.R., Mafra, L.L., Jr., dos Santos Tavares, C.P., Fernandes, L.F., Proença, L.A.O., 2017.  
3008 Growth, chain formation, and toxin production by southern Brazilian *Pseudo-nitzschia*  
3009 isolates under laboratory conditions. *Environ. Monit. Assess.* 189, 621.
- 3010 Wang, P., Liang J., Lin, X., Chen, C., Huang, Y., Gao Y., Gao., Y., 2012. Morphology,  
3011 phylogeny and ITS-2 secondary structure of *Pseudo-nitzschia brasiliiana*  
3012 (Bacillariophyceae), including Chinese strains. *Phycologia* 51, 1–10.
- 3013 Wells, M.L., Trick, C.G., Cochlan, W.P., Hughes, M.P., Trainer, V.L., 2005. Domoic acid: the  
3014 synergy of iron, copper, and the toxicity of diatoms. *Limnol. Oceanogr.* 50, 1908–1917.
- 3015 Whyte, J.N.C., Townsend, L.D., Ginther, N.G., 1996. Fecundity, toxin and trophic levels of the  
3016 rotifer *Brachionus plicatilis* fed *Pseudo-nitzschia pungens* f. *multiseries*. In: Yasumoto, T.,  
3017 Oshima, Y., Fukuyo, Y. [Eds.], *Harmful and Toxic Algal Blooms*. Intergovernmental  
3018 Oceanographic Commission of UNESCO, Paris, pp. 401–404.
- 3019 Widiarti, R., Mulyani, Y., Kurnia, N., Razi, F., 2013. Phytoplankton species composition in  
3020 seawater and tissue of green mussels (*Perna viridis*), at Kali Baru-Cilincing, North Jakarta.  
3021 *Mar. Res. Indonesia* 38, 67–70.
- 3022 Wilson, C., Sastre, A.V., Hoffmeyer, M., Rowntree, V.J., Fire, S.E., Santinelli, N.H.,  
3023 Delegrange, S., Ovejero, D'Agostino, V., Marón, C.F., Doucette, G.J., Broadwater, M.H.,  
3024 Wang, Z., Montoya, N., Seger, J., Adler, F.R., Sironi M., Uhart, M.M., 2016. Southern

- 3025 right whale (*Eubalaena australis*) calf mortality at Península Valdés, Argentina: Are  
3026 harmful algal blooms to blame? *Mar. Mam. Sci.* 32, 423–451.
- 3027 Windust, A., 1992. The responses of bacteria, microalgae, and zooplankton to the diatom  
3028 *Nitzschia pungens* f. *multiseries* and its toxic metabolite domoic acid. M.S. Thesis,  
3029 Dalhousie University, Halifax, Canada, 107 pp. Accessed at <http://www.inter.dfo-mpo.gc.ca/folios/01017/docs/ref-343-77.pdf>
- 3031 Wingert, C.J., 2017. The effects of ocean acidification on growth, photosynthesis, and domoic  
3032 acid production by the toxicogenic diatom, *Pseudo-nitzschia australis*. Master's Thesis. San  
3033 Francisco State University, 134 pp.
- 3034 Woods, A., 2016. Domoic acid production in *Pseudo-nitzschia* (Bacillariophyceae) as a general  
3035 response to unbalanced growth: the role of photo-oxidative stress. Master's Thesis.  
3036 California State University, Monterey Bay. Capstones and Theses, Paper 575, 86 pp.
- 3037 Yap-Dejeto, L.G., Omura, T., Nagahama, Y., Nagahama, Y., Fukuyo, Y., 2010. Observations of  
3038 eleven *Pseudo-nitzschia* species in Tokyo Bay, Japan. *La Mer* 48, 1–16.
- 3039 Yap-Dejeto, L.G., Omura, T., Cinco, G.F., Cobacha, M.M., Fukuyo, Y., 2013. Species account  
3040 of marine diatoms of the genus *Pseudo-nitzschia* in San Pedro Bay, Philippines. *Philip. J.*  
3041 *Sci.* 142, 27–37.
- 3042 Yuan, X.-L., Cao, M., Bi, G.-Q., 2016. The complete mitochondrial genome of *Pseudo-nitzschia*  
3043 *multiseries* (Bacillariophyta). *Mitochondrial DNA* 27, 2777–2778.
- 3044 Xu, G.-s., Li, Y., 2015. Two new records of diatom genus *Pseudo-nitzschia* from Chinese waters  
3045 and analysis of their domoic acid production. *J. Trop. Subtrop. Bot.* 23, 614–624.
- 3046 Xu, B., Luo, C.-S., Liang, J.-R., Chen, D.-D., Zhuo, W.-H., Gao, Y.-H., Chen, C.-P., Song S.-S.,  
3047 2014. Cellular metabolic responses of the marine diatom *Pseudo-nitzschia multiseries*  
3048 associated with cell wall formation. *Mar. Genomics* 16, 29–38.
- 3049 Xu, N., Tang, Y.Z., Qin, J., Duan S., Gobler, C.J., 2015. Ability of the marine diatoms *Pseudo-*  
3050 *nitzschia multiseries* and *P. pungens* to inhibit the growth of co-occurring phytoplankton  
3051 via allelopathy. *Aquat. Microb. Ecol.* 74, 29–41.
- 3052 Zabaglo, K., Chrapusta, E., Bober, B., Kaminski, A., Adamski, M., Bialczyk, J., 2016.  
3053 Environmental roles and biological activity of domoic acid: a review. *Algal Res.* 13, 94–  
3054 101.
- 3055 Zamudio-Resendiz, E., González-Rivas, D., Meave del Castillo, M.E., 2014. Evaluation of  
3056 *Pseudo-nitzschia* spp. in a tropical bay of the Mexican Pacific. In: Kim, H.G., Reguera, B.,  
3057 Hallegraeff, G.M., Lee, C.K., Han, M.S., Choi, J.K. [Eds.], *Harmful Algae 2012*,  
3058 Proceedings of the 15th International Conference on Harmful Algae. International Society  
3059 for the Study of Harmful Algae. Maple Design, Busan, Korea, pp. 33–36.
- 3060 Zhu, Z., Xu, K., Fu, F., Spackeen, J.L., Bronk, D.A., Hutchins, D.A., 2016. A comparative study  
3061 of iron and temperature interactive effects on diatoms and *Phaeocystis antarctica* from the  
3062 Ross Sea, Antarctica. *Mar. Ecol. Prog. Ser.* 550, 39–51.
- 3063 Zhu, Z., Qu, P., Gale, J., Fu, F., Hutchins, D.A., 2017a. Individual and interactive effects of  
3064 warming and CO<sub>2</sub> on *Pseudo-nitzschia subcurvata* and *Phaeocystis antarctica*, two  
3065 dominant phytoplankton from the Ross Sea, Antarctica. *Biogeosciences* 14, 5281–5295.

- 3066 Zhu, Z., Qu P., Fu, F., Tennenbaum, N., Tatters, A.O. and Hutchins, D.A., 2017b. Understanding  
3067 the blob bloom: warming increases toxicity and abundance of the harmful bloom diatom  
3068 *Pseudo-nitzschia* in California coastal waters. Harmful Algae 67, 36–43.
- 3069 Zimmermann, J., Abarca, N., Enk, N., Skibbe, O., Kusber, W.-H., Jahn, R., 2014. Taxonomic  
3070 reference libraries for environmental barcoding: a best practice example from diatom  
3071 research. PLoS ONE 9, e108793.
- 3072 Zúñiga, D., Santos, C., Froján, M., Salgueiro, E., Rufino, M.M., De la Granda, F., Figueiras,  
3073 F.G., Castro, C.G., Abrantes, F., 2017. Diatoms as a paleoproductivity proxy in the NW  
3074 Iberian coastal upwelling system (NE Atlantic). Biogeosciences 14, 1165–1179.
- 3075
- 3076

3077 **Figure Legends**

3078

3079 Figure 1. Distribution of *Nitzschia navis-varingica* in the Western Pacific region, with their toxin  
3080 compositions as shown in the pie charts (modified from Tan et al. (2016b)).

3081

3082 Figure 2. World distribution of *Pseudo-nitzschia* spp. that have been reported since Lelong et al.  
3083 (2012a) and Trainer et al. (2012); see also Tables 3 and 4. Toxigenic species are in bold; note  
3084 that only certain strains of these species are toxigenic at some locations. A map showing the  
3085 world distribution of all reports of *Pseudo-nitzschia* spp. is shown in Bates et al. (2017), and a  
3086 list of all species shown here, plus in Trainer et al. (2012), is shown in Supplementary Table 1.

3087

3088 Figure 3. Locations where domoic was detected in stranded (s) and harvested (h) marine  
3089 mammals. Marine mammal species are listed as follows: (A) humpback whales, (B) bowhead  
3090 whales, (C) beluga whales, (D) harbor porpoises, (E) northern fur seals, (F) Steller sea lions, (G)  
3091 harbor seals, (H) ringed seals, (I) bearded seals, (J) spotted seals, (K) ribbon seals, (L) Pacific  
3092 walruses and (M) northern sea otters. Modified from Lefebvre et al. (2016).

3093

3094 Figure 4. Schematic drawing of the life cycle of *Pseudo-nitzschia multistriata*. See Basu et al.  
3095 (2017) for details.

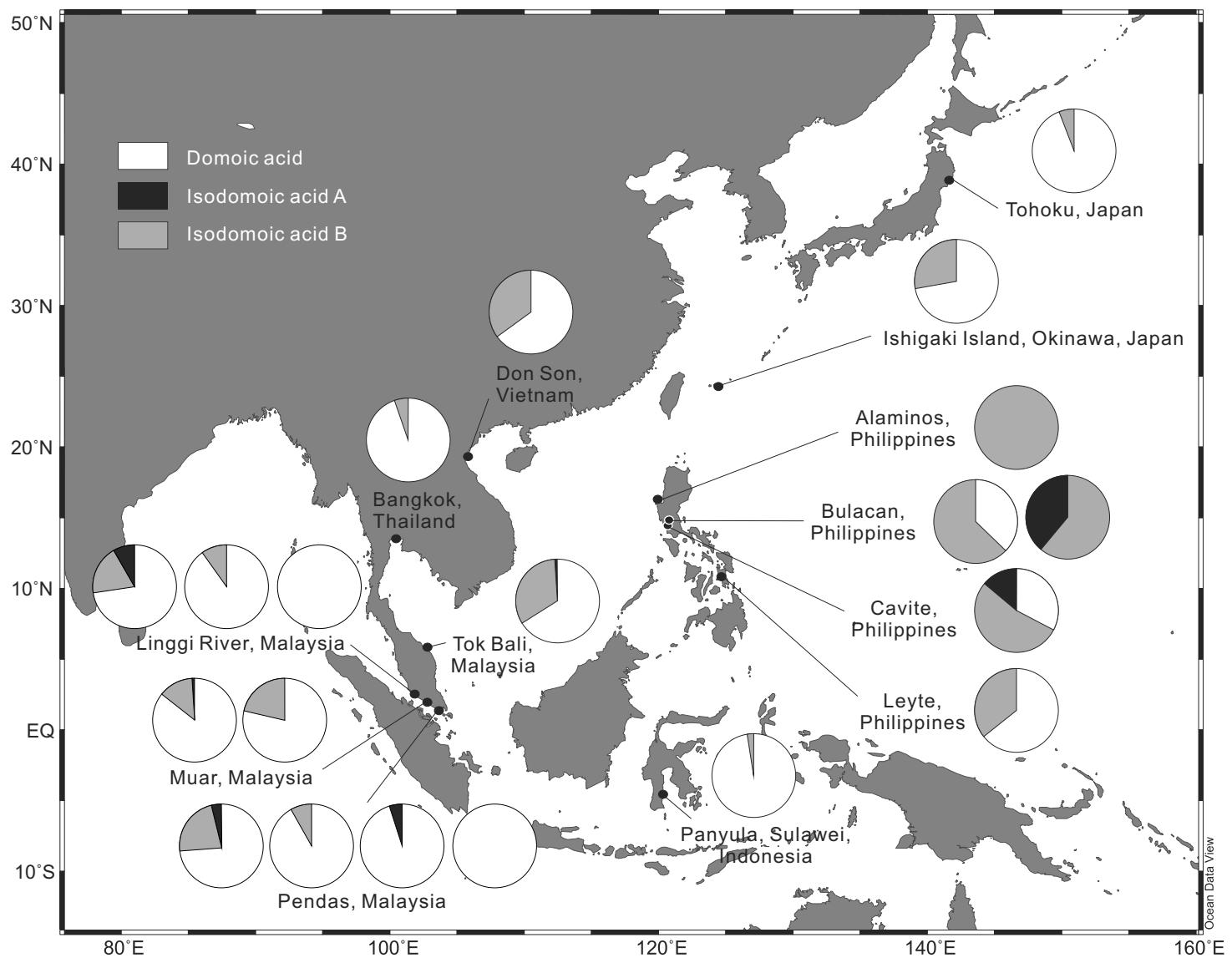
3096

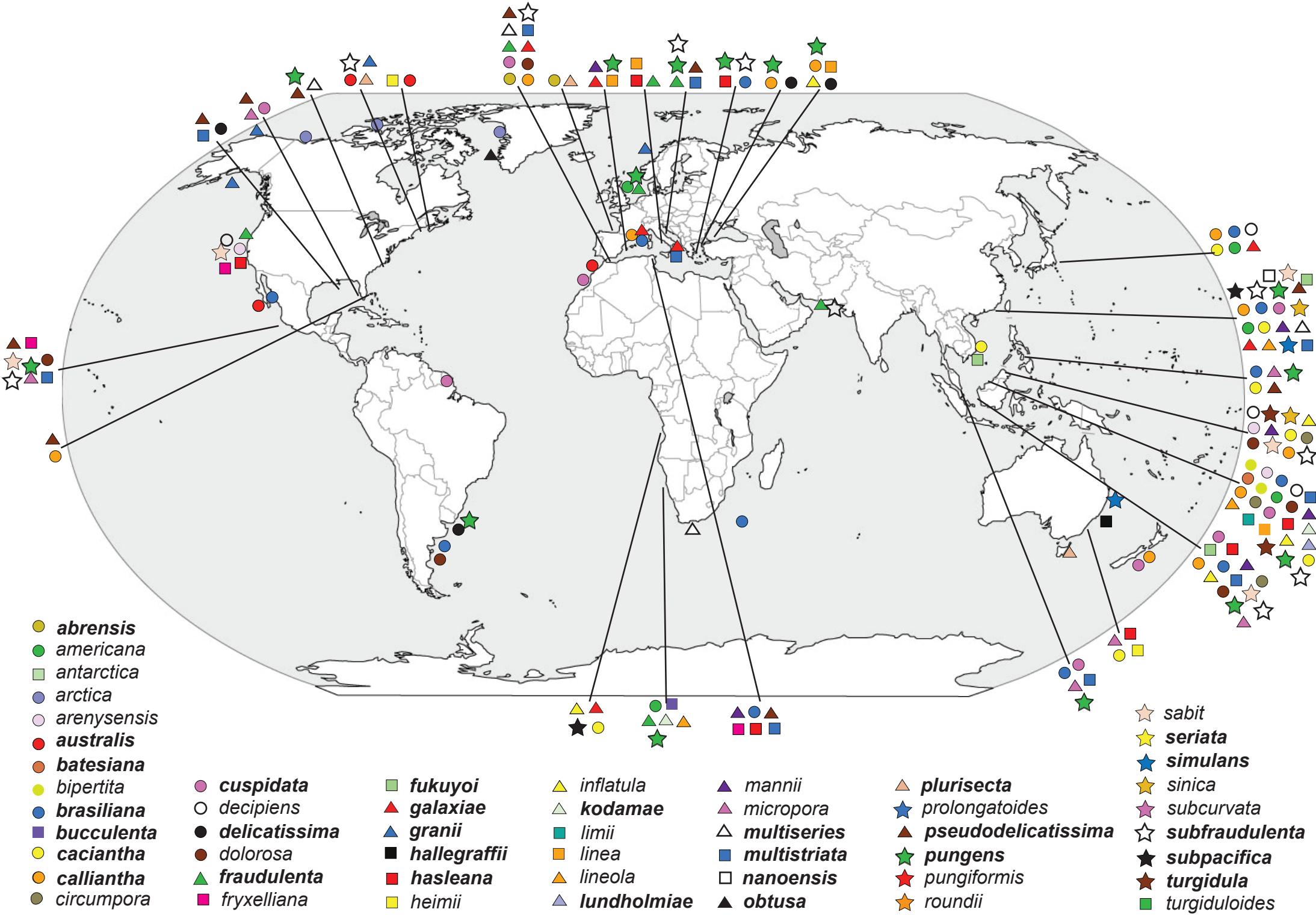
3097 Figure 5. Examples of *Pseudo-nitzschia* cells infected by an oomycete parasitoid. A) Early stage  
3098 of infection of *Pseudo-nitzschia australis* from the Gulf of Biscay (France). B) Later stage of  
3099 infection of *P. fraudulenta* from the Bay of Brest (France) showing the sporangium inside the  
3100 diatom cell. Note that the cell has become widened due to the infection, and the remnants of the  
3101 host chloroplasts have been pushed to the ends of the cells. An uninfected cell of normal width is  
3102 also shown. Light micrographs courtesy of Elisabeth Nézan (IFREMER, Station de Biologie  
3103 Marine de Concarneau, France). *Pseudo-nitzschia australis* was identified by SEM and *P.*  
3104 *fraudulenta* by light microscopy, using cells sampled from the same locations as the parasitized  
3105 diatoms.

3106

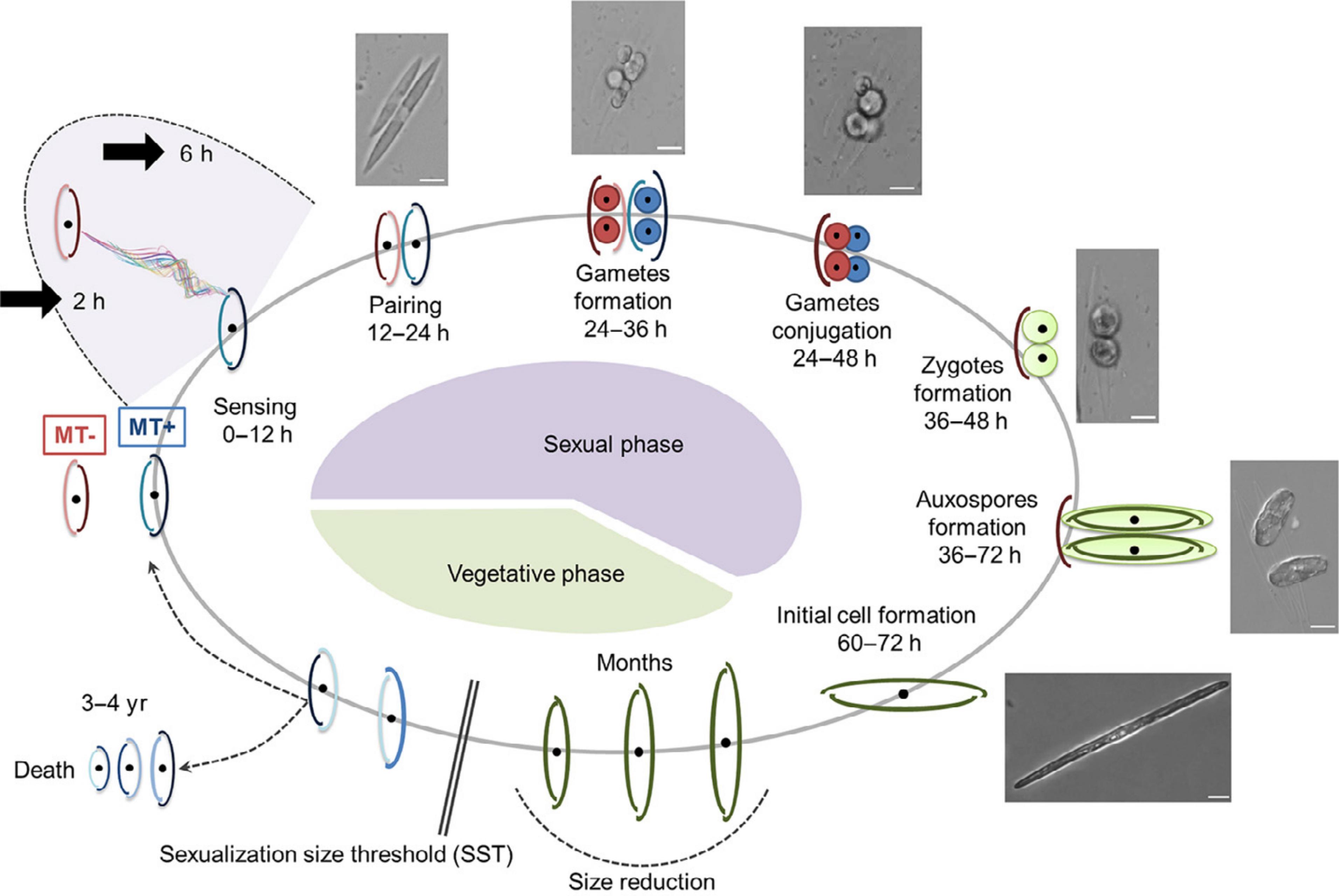
3107 Figure 6. Schematic drawing of poroid morphology of striae in *Pseudo-nitzschia* species,  
3108 showing hymen sectors (when present) within the poroids. (A) Striation with one row of poroids.  
3109 (B) Two rows of poroids with poroids divided into sectors. (C) Striation with rows of simple  
3110 poroids. Updated from Teng et al. (2013, 2016).

3111









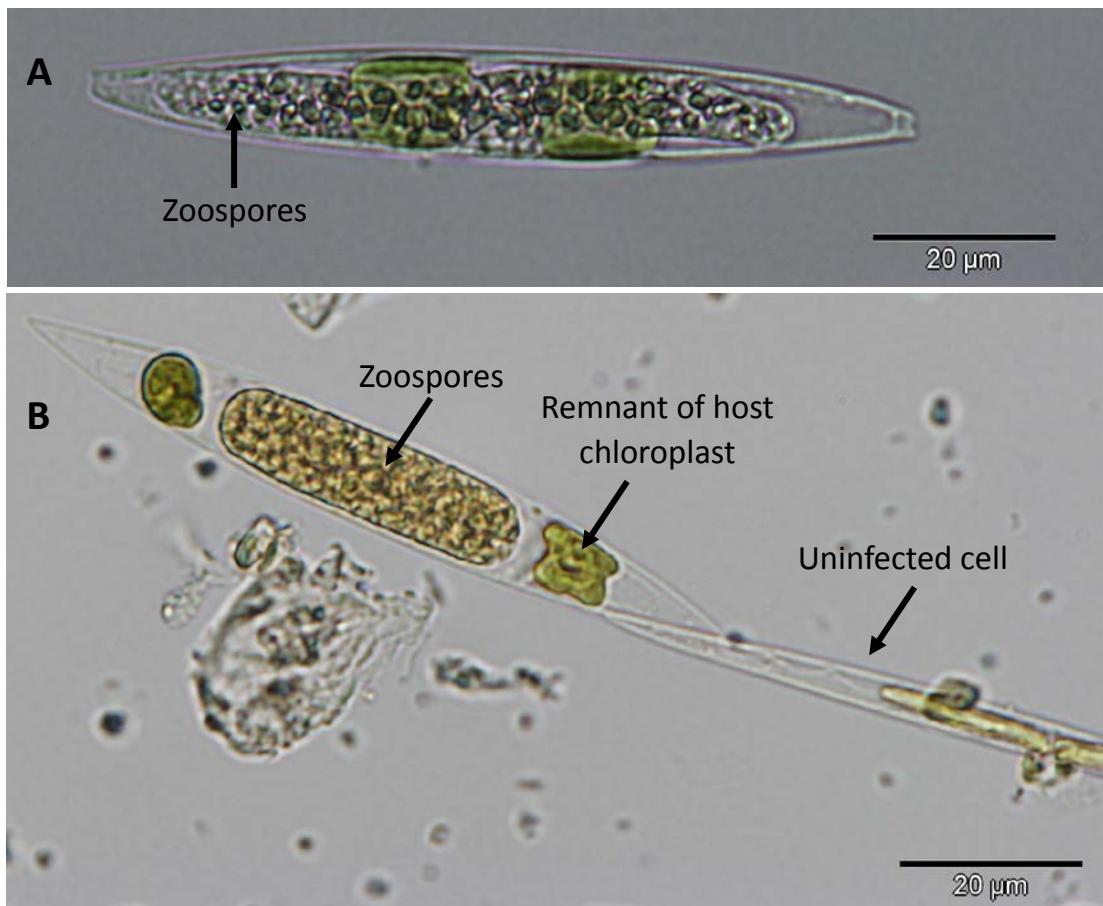
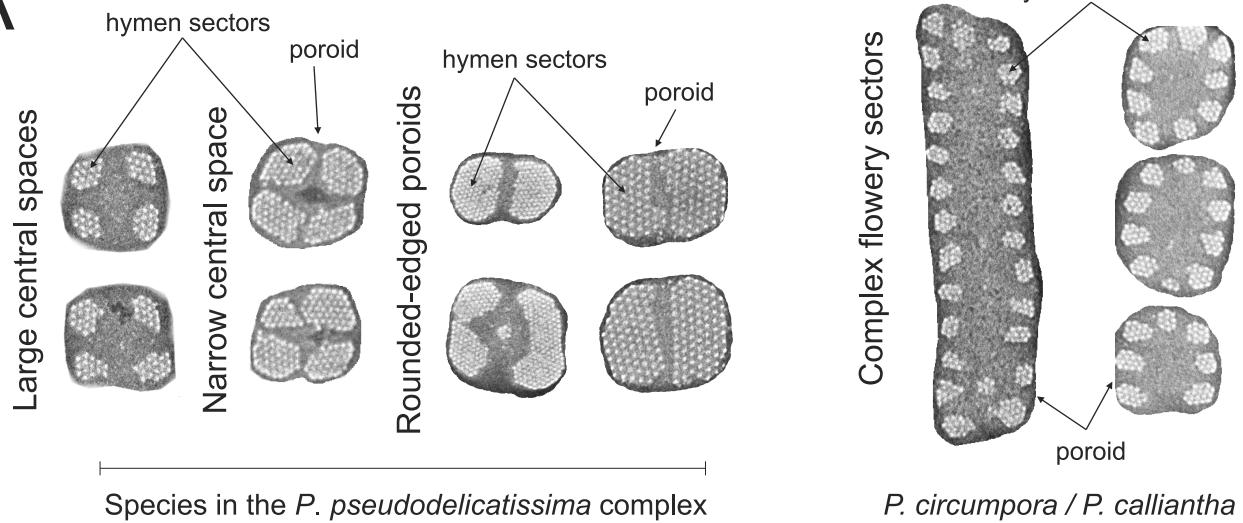
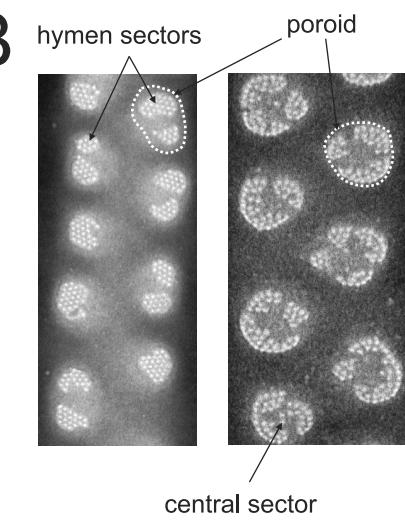


Fig. 5. Examples of *Pseudo-nitzschia* cells infected by an oomycete parasitoid. A) Early stage of infection of *Pseudo-nitzschia australis* from the Gulf of Biscay (France) showing zoospores inside of the cell. B) Later stage of infection of *P. fraudulenta* from the Bay of Brest (France) showing zoospores inside the cell, which has become widened due to the infection, and the remnants of the host chloroplasts that have been pushed to the ends of the cells. An uninfected cell of normal width is also shown. Light micrographs courtesy of Elisabeth Nézan (Ifremer, Station de Biologie Marine de Concarneau, France).

**A****B**

*P. fraudulenta* / *P. subfraudulenta* /  
*P. bipertita*

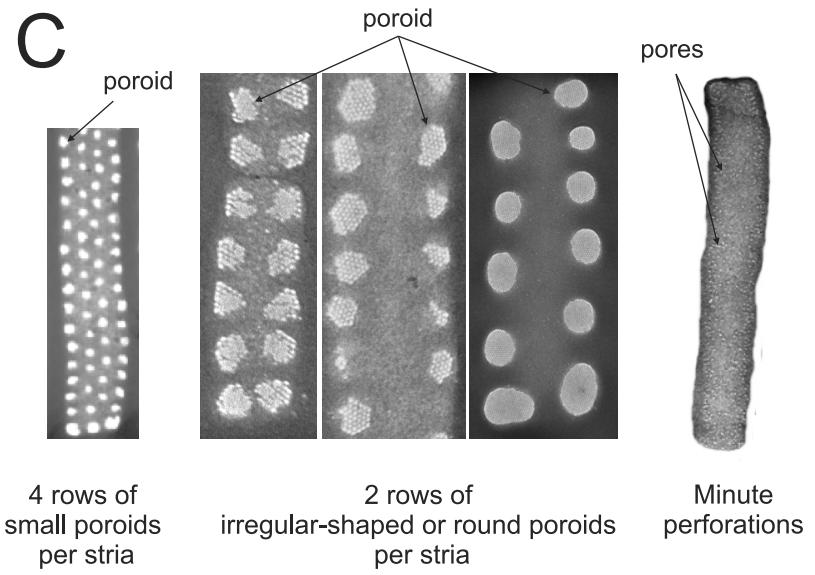
**C**

Table 1. Previous reviews of domoic acid and *Pseudo-nitzschia*.

Year	Title	Reference
1998	Ecophysiology and metabolism of ASP toxin production	Bates, 1998
1998	Bloom dynamics and physiology of domoic-acid-producing <i>Pseudo-nitzschia</i> species	Bates et al., 1998
2006	The ecology of harmful diatoms	Bates and Trainer, 2006
2008	Ecobiology of ASP producing diatoms	Kotaki, 2008
2008	Toxic diatoms	Trainer et al., 2008
2008	Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: a review	Bejarano et al., 2008
2010	Toxic marine diatoms	Villac et al., 2010
2011	Toxic diatom <i>Pseudo-nitzschia</i> and its primary consumers (vectors)	Bargu et al., 2011
2011	Marine planktonic diatoms, including potentially toxic species	Villac and Kaczmarzka, 2011
2012	<i>Pseudo-nitzschia</i> (Bacillariophyceae) species, domoic acid and amnesic shellfish poisoning: revisiting previous paradigms	Lelong et al., 2012a
2012	<i>Pseudo-nitzschia</i> physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health	Trainer et al., 2012
2014	Domoic acid as a neurotoxin	Pérez-Gómez and Tasker, 2014
2014	Domoic acid: biological effects and health implications	Pulido, 2014a
2014	Domoic acid	La Barre et al., 2014
2014	The mechanisms of action of domoic acid: from pathology to physiology	Du et al., 2016 et al., 2014
2014	Domoic acid epileptic disease	Ramsdell and Gulland, 2014
2014	Chemistry and detection of domoic acid and isomers	Tasker, 2014
2014	Domoic acid: chemistry and pharmacology	Vale, 2014
2016	Domoic acid and other amnesic toxins: toxicological profile	Tasker, 2016
2016	Environmental roles and biological activity of domoic acid: a review	Zabaglo et al., 2016
2017	Domoic acid: attributes, exposure risks, innovative detection techniques and therapeutics	Saeed et al., 2017
2018	Toxic and harmful diatoms	Bates et al., 2018

Table 2. Geographical distribution of toxic *Nitzschia navis-varingica* and levels of domoic acid (DA) and DA isomers (IA = isodomoic acid A; IB = isodomoic acid B) in cultures; nd = not detected; – = not tested. FMOC-LC-FLD = precolumn derivatization using 9-fluorenylmethylchloroformate and fluorescence detection; LC/ESI-MS = liquid chromatography coupled with electrospray ionization mass spectrometry; LC-MS/MS = liquid chromatography coupled with tandem mass spectrometry.

Geographical distribution	Reference for distribution	Domoic acid (pg DA cell <sup>-1</sup> )			Analytical method	Reference for toxicity
		DA	IA	IB		
Do Son (Vietnam)	Lundholm and Moestrup, 2000	0.7–1.7	–	–	FMOC-LC-FLD, LC/ESI-MS	Kotaki et al., 2000
Western Port Bay, Victoria (Australia)	Higgins et al., 2003	–	–	–	–	–
Nagura estuary, Ishigaki Island, Okinawa (Japan)	Kotaki et al., 2004	0.1–2.8	–	–	LC/ESI-MS	Kotaki et al., 2004
Natori estuary, Ishigaki Island, Okinawa (Japan)	Kotaki et al., 2004	2.1–3.9	–	–	LC/ESI-MS	Kotaki et al., 2004
Bacoor estuary, Manila Bay (Philippines)	Kotaki et al., 2004, 2005, 2006	1.4–15.3	–	–	LC/ESI-MS	Kotaki et al., 2004
Tanauan, San Pedro Bay (Philippines)	Kotaki et al., 2005, 2006	0.04–3.7	–	–	FMOC-LC-FLD	Kotaki et al., 2005
San Roué, San Pedro Bay (Philippines)	Kotaki et al., 2005, 2006	1.3–5.3	–	–	FMOC-LC-FLD	Kotaki et al., 2005
Bacoor estuary, Manila Bay (Philippines)	Kotaki et al., 2004, 2005, 2006	3.1–5.3	–	–	FMOC-LC-FLD	Kotaki et al., 2005
Tohoku district (Japan)	Kotaki et al., 2004, 2008	0.4–2.9	–	–	FMOC-LC-FLD	Kotaki et al., 2008
Okinawa (Japan)	Kotaki et al., 2008	0.1–9.8	–	–	FMOC-LC-FLD	Kotaki et al., 2008
Haiphong (Vietnam)	Kotaki et al., 2008	0.5–11.3	–	–	FMOC-LC-FLD	Kotaki et al., 2008

Geographical distribution	Reference for distribution	Domoic acid (pg DA cell <sup>-1</sup> )			Analytical method	Reference for toxicity
		DA	IA	IB		
Iba, Zambales estuary (Philippines)	Bajarias et al., 2006	nd	0.3–4.0	0.1–5.5	FMOC-Cl-FLD	Bajarias et al., 2006
Alaminos (Philippines)	Romero et al., 2011, 2012	nd	nd	2.4–3.05	FMOC-LC-FLD, LC-MS/MS	Romero et al., 2011, 2012
Bulacan, Manila Bay (Philippines)	Romero et al., 2011, 2012	0.68	1.18	nd	FMOC-LC-FLD, LC-MS/MS	Romero et al., 2011, 2012
Cavite, Manila Bay (Philippines)	Romero et al., 2011, 2012	0.58	0.20	0.92	FMOC-LC-FLD, LC-MS/MS	Romero et al., 2011, 2012
Panyula, Bone, South Sulawesi (Philippines)	Romero et al., 2011; Thoha et al., 2012	2.38	0.06	nd	FMOC-LC-FLD, LC-MS/MS	Romero et al., 2011; Thoha et al., 2012
Tok Bali, Kelantan (Malaysia)	Tan et al., 2016b	0.24–1.97	0–0.03	0.24–0.97	FMOC-LC-FLD, LC-MS/MS	Tan et al., 2016b
Lingga River, Negeri Sembilan (Malaysia)	Tan et al., 2016b	0.30–0.79	0–0.09	0–0.14	FMOC-LC-FLD, LC-MS/MS	Tan et al., 2016b
Muar, Johor (Malaysia)	Tan et al., 2016b	0.56–9.70	0–0.08	0.27–1.28	FMOC-LC-FLD, LC-MS/MS	Tan et al., 2016b
Pendas, Johor (Malaysia)	Suriyanti and Usup, 2015; Tan et al., 2016b	0.82–10.5	0–0.16	0–0.57	FMOC-LC-FLD, LC-MS/MS	Tan et al., 2016b

Table 3. List of described species of *Pseudo-nitzschia*. New species since Lelong et al. (2012a) are indicated by an \*, and new references for distributions are after 2011, except when not included in Lelong et al. (2012a). References for toxicity (domoic acid production) in cultures and for the distribution of species prior to 2012 are given in Lelong et al. (2012a). “Yes/No” indicates that some strains of a given species produce detectable levels of domoic acid, whereas others do not. Updates for new species and toxicity are found in Wikipedia (<https://en.wikipedia.org/wiki/Pseudo-nitzschia>) and Lundholm (2017). A compilation of species, their toxicity and distribution, from Lelong et al. (2012a) and this table, is given in Supplementary material Table S1.

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. abrensis</i> *	Yes	Teng et al., 2016	Malaysia, Spain (Atlantic)	Orive et al., 2013; Teng et al., 2016
<i>P. americana</i>	No	Villac et al., 1993; Churro et al., 2009; Ajani et al., 2013a,b; Rhodes et al., 2013	Australia, Bay of Fundy, China, France (Atlantic), Gulf of Maine, Japan, Malaysia, Mexico (Pacific), <b>Namibia</b> , North Sea (Helgoland), Washington State (USA)	Yap-Dejeto et al., 2010; Downes-Tettmar 2012; Lü et al., 2012; Ajani et al., 2013a,b; Rivera-Vilarelle et al., 2013; Teng et al., 2013, 2014b; Fernandes et al., 2014; Hubbard et al., 2014a; Mogensen, 2014; Bresnan et al., 2015; Carlson et al., 2016; Thorel et al., 2017; <b>Louw et al., 2018</b>
<i>P. antarctica</i>	Not tested	–	See Lelong et al., 2012a	See Lelong et al., 2012a
<i>P. arctica</i> *	No	Percopo et al., 2016	Arctic (Barrow Strait, Beaufort Sea, Baffin Bay, Greenland)	Percopo et al., 2016; Balzano et al., 2017
<i>P. arenysensis</i>	No	Orsini et al., 2002 <sup>1</sup> ; Quijano-Scheggia et al., 2009a, 2010; Ajani et al., 2013a,b	Australia, California (USA), Italy (Gulf of Naples), Malaysia, Spain (Mediterranean Sea)	Ajani et al., 2013a,b, 2016; Teng et al., 2013; Ruggiero et al., 2015; Bowers et al., 2016, 2017; Busch et al., 2016
<i>P. australis</i>	Yes/No	Sison-Mangus et al., 2014; Thorel et al., 2014; Woods, 2016; Lema et al., 2017; Zhu et al., 2017; <b>Gai et al., 2018</b> for “Yes”; Villac et al., 1993; Churro et al., 2009 for “No”; Lapworth et al., 2001; Rhodes et al.,	Argentina, Bay of Fundy, Brazil, British Columbia (Canada), California (USA), France (Atlantic), Gulf of Maine, Mexico (Baja California, Pacific), Morocco (Atlantic), <b>Namibia</b> , Scotland, Washington State (USA)	Fernandes and Brandini, 2010; Santiago-Morales et al., 2011; Ennaffah et al., 2012; Rivera-Vilarelle et al., 2013; Sison-Mangus et al., 2014, 2016; Thorel et al., 2014; Bowers et al., 2015; D’Agostino et al., 2015, 2017; Bresnan et al., 2015, 2017; Krock

Species	Toxicity	References for toxicity 1996, 2013 for “Yes/No”	Distribution	References for distribution
<i>P. batesiana</i> *	Yes/No	Teng et al., 2016 for “Yes”; Teng et al., 2014a for “No”	Malaysia	et al., 2015; Carlson et al., 2016; Du et al., 2016; Haigh et al., 2016; Johnson et al., 2016; McCabe et al., 2016; Woods, 2016; Bowers et al., 2017; Lema et al., 2017; Thorel et al., 2017; Ryan et al., 2017; Zhu et al., 2017; Hubbard et al., 2017; J. Martin, pers. commun.; Gai et al., 2018; Louw et al., 2018
<i>P. bipertita</i> *	No	Teng et al., 2016	Malaysia	Teng et al., 2016
<i>P. brasiliiana</i>	Yes/No	Sahraoui et al., 2011; Sakka Hlaili et al., 2016 for “Yes”; Lundholm et al., 2002a; Skov et al., 2004; Quijano-Scheggia et al., 2010; Lim et al., 2012a; Moschandreas et al., 2012; Wang et al., 2012; Dao et al., 2014 for “No”	Argentina, China, Florida (Gulf of Mexico), France (Mediterranean Sea), Greece, Japan, Malaysia, Mexico (Gulf of Mexico, Pacific), Philippines, Singapore, Tunisia, Vietnam	Yap-Dejeto et al., 2010, 2013; Quijano-Scheggia et al., 2011; Lim et al., 2012b,c,d, 2014c; Lü et al., 2012; Moschandreas et al., 2012; Parsons et al., 2012; Wang et al., 2012; Gárate-Lizárraga et al., 2013; Rivera-Vilarelle et al., 2013; Teng et al., 2013, 2014b, 2015; Dao et al., 2014; Lim et al., 2014c; Tan et al., 2015, 2016a; Sakka Hlaili et al., 2016; Almandoz et al., 2017; Grzebyk et al., 2017
<i>P. bucculenta</i> *	No	Gai et al., 2018	Namibia	Gai et al., 2018
<i>P. ciciantha</i>	Yes/No	Dao et al., 2014 <sup>2</sup> for “Yes”; Lundholm et al., 2003; Quijano-Scheggia et al., 2010; Lim et al., 2012a; Rhodes et al., 2013; Teng	Angola, Australia, China, Japan, Malaysia, Mexico (Gulf of Mexico) Morocco (Mediterranean), Philippines, Thailand	Lundholm et al., 2003; Yap-Dejeto et al., 2010, 2013; Lim et al., 2014a; Lü et al., 2012; Rijal Leblad et al., 2013 <sup>2</sup> ; Teng et al., 2013, 2014a,b; Guannel et al.,

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. calliantha</i>	Yes/No	et al., 2014a; Ajani et al., 2016 for “No” Besiktepe et al., 2008; Ryabushko et al., 2008; Sakka Hlaili et al., 2016 for “Yes”; Skov et al., 2004; Quijano-Scheggia et al., 2010; Lundholm et al., 2012; Moschandrou et al., 2012; Ajani et al., 2013a, b; Penna et al., 2013; Rhodes et al., 2013; Garali et al., 2016 for “No”; Lapworth et al., 2001; Álvarez et al., 2009; Sahraoui et al., 2009; Wadt et al., 2017 for “Yes/No”	Argentina, Australia, Brazil, <b>Bulgaria (Black Sea)</b> , China, Croatia (Adriatic Sea), Florida (Atlantic, Gulf of Mexico), France (Mediterranean Sea), Italy (Adriatic Sea, Gulf of Naples), Japan, Louisiana (Gulf of Mexico), Malaysia, Mexico (Gulf of Mexico), Morocco (Mediterranean Sea), Russia (NW Sea of Japan), Spain (Mediterranean Sea), Tunisia, Turkey (Black Sea, Sea of Marmara), Ukraine (Black Sea)	2015; Okolodkov et al., 2015; Ajani et al., 2016 Ryabushko et al., 2008; Fernandes and Brandini, 2010; Yap-Dejeto et al., 2010; Phlips et al., 2011; Lü et al., 2012; O’Dea, 2012; Parsons et al., 2012; Stonik et al., 2012; Terenko and Terenko, 2012; Ajani et al., 2013a,b, 2016; Baytut et al., 2013; Rijal Leblad et al., 2013; Penna et al., 2013; Rhodes et al., 2013; Teng et al., 2013, 2014b; Fernandes et al., 2013; Ruggiero et al., 2015; Tibiriçá et al., 2015; Arapov et al., 2016, 2017; Bargu et al., 2016; Busch et al., 2016; Garali et al., 2016; GRIIS; Sakka Hlaili et al., 2016; Tas et al., 2016; Tas and Lundholm, 2017; Chai et al., 2017; D’Agostino et al., 2017; <b>Dzhembekova et al., 2017a</b> ; Grzebyk et al., 2017; Wadt et al., 2017
<i>P. circumpora*</i>	No	Lim et al., 2012a	Malaysia	Lim et al., 2012a,d, 2013, 2014a; Teng et al., 2013, 2014b; Tan et al., 2015
<i>P. cuspidata</i>	Yes/No	Auro and Cochlan, 2013; Ajani et al., 2013a for “Yes”; Lim et al., 2012a; Rhodes et al., 2013 for “No”; Lundholm et al., 2012 for “Yes/No”	Angola, Australia, California (USA), China, Florida (Gulf of Mexico), Italy, Malaysia, Mexico (Gulf of Mexico), Morocco (Mediterranean Sea), Singapore, Washington State (USA)	Lim et al., 2012a,d; Lü et al., 2012; O’Dea, 2012; Parsons et al., 2012; Ajani et al., 2013a,b, 2016; Rijal Leblad et al., 2013; O’Dea et al., 2013; Rhodes et al., 2013; Teng et al., 2013, 2015; Hubbard et al., 2014a, 2015; Guannel et al.,

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. cuspidata</i> var. <i>manzanillensis</i> *	No	Rivera-Vilarelle et al. 2018	Mexico (Pacific)	2015; Tan et al., 2016a; Smith et al., 2018 Rivera-Vilarelle et al., 2018
<i>P. decipiens</i>	No	Lundholm et al., 2006	California (USA), China, France, Japan, Malaysia, <b>Namibia</b>	Teng et al., 2013, 2014b, 2015; Huang et al., 2017a; Nagai et al., 2017; Gai et al., 2018; Smith et al., 2018
<i>P. delicatissima</i> <sup>3</sup>	Yes/No	Sahraoui et al., 2011; Fuentes and Wikfors, 2013; Penna et al., 2013; Fernandes et al., 2014 for “Yes”; Villac et al., 1993; Rhodes et al., 1998; Lundholm et al., 2006; Quijano-Scheggia et al., 2010; Guannel et al., 2011; Lelong et al., 2012b, 2013, 2014; Moschandreas et al., 2012; Pistocchi et al., 2012; Prince et al., 2012; Woods, 2016 for “No”	Albania, Arabian Sea, Bay of Fundy, China, Croatia (Adriatic Sea), France (Atlantic, Mediterranean Sea), Gulf of Maine, Greece, Italy (Adriatic Sea, Gulf of Naples), Japan, Korea, Louisiana (Gulf of Mexico), Malaysia, Mexico (Gulf of Mexico, Pacific), Russia (Sea of Japan, Sea of Okhotsk), Scotland, Spain (Mediterranean Sea), Tunisia, Turkey (Aegean Sea), Ukraine (Black Sea), Uruguay, Washington State (USA)	GRIIS; Schiebel et al., 2004; Yap-Dejeto et al., 2010; Guannel et al., 2011; Bushati et al., 2012; Méndez et al., 2012; Parsons et al., 2012, 2013; Terenko and Terenko, 2012; Kesici et al., 2013; Penna et al., 2013; Rivera-Vilarelle et al., 2013; Stonik and Orlova, 2013; Teng et al., 2013; Mogensen, 2014; Fernandes et al., 2014; Hubbard et al., 2014a; Ruggiero et al., 2015; Smetti et al., 2015; Busch et al., 2016; Carlson et al., 2016; Dursun et al., 2016; Sakka Hlaili et al., 2016; Arapov et al., 2017; Bresnan et al., 2017; Geng et al., 2017; Grzebyk et al., 2017; Thorel et al., 2017
<i>P. dolorosa</i>	No	Lundholm et al., 2006; Marchetti et al., 2008; Lim et al., 2012a	Argentina, Australia, Italy (Gulf of Naples), Malaysia, Mexico (Pacific), Morocco (Mediterranean Sea), <b>Namibia</b>	Lim et al., 2012a; Rijal Leblad et al., 2013; Rivera-Vilarelle et al., 2013; Teng et al., 2013; see Zamudio-Resendiz et al., 2014; Ruggiero et al., 2015; Ajani et al., 2016; Almandoz et al., 2017; Gai et al., 2018

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. fraudulenta</i>	Yes/No	Tatters et al., 2012; Fernandes et al., 2014; Sison-Mangus et al., 2014; Lema et al., 2017 for “Yes”; Hargraves et al., 1993; Lapworth et al., 2001; Orsini et al., 2002; Rhodes et al., 2003; Churro et al., 2009; Quijano- Scheggia et al., 2010; Moschandreas et al., 2012; Pistocchi et al., 2012; Ajani et al., 2013a,b; Woods, 2016 for “No”; Thessen et al., 2009; Rhodes et al., 2013 for “Yes/No”	Argentina, Australia, Bay of Fundy, California (USA), Croatia (Adriatic Sea), France (Atlantic), Italy (Adriatic Sea, Gulf of Naples), Japan, Morocco (Mediterranean Sea), <b>Namibia</b> , North Sea (Helgoland), Pakistan, Scotland, Turkey (Sea of Marmara), Washington State (USA)	Yap-Dejeto et al., 2010; Naz et al., 2012; Pistocchi et al., 2012; Tatters et al., 2012; Ajani et al., 2013a,b, 2016; Rijal Leblad et al., 2013; Fernandes et al., 2014; Hubbard et al., 2014a; Sison- Mangus et al., 2014, 2016; Bresnan et al., 2015, 2017; D’Agostino et al., 2015, 2017; Ruggiero et al., 2015; Woods, 2016; Arapov et al., 2017; Bowers et al., 2017; Lema et al., 2017; Nagai et al., 2017; Thorel et al., 2017; <b>Gai et al., 2018; Louw et al.,</b> <b>2018</b>
<i>P. fryxelliana</i>	No	Lundholm et al., 2012; Sakka Hlaili et al., 2016	California (USA), Mexico (Pacific), Tunisia, Washington State (USA)	Lundholm et al., 2012; Auro and Cochlan, 2013; Rivera-Vilarelle et al., 2013 <sup>2</sup> ; Hubbard et al., 2014a; Sakka Hlaili et al., 2016; Smith et al., 2018
<i>P. fukuyoi*</i>	Yes/No	Dao et al., 2015 for “Yes”; Teng et al., 2014a for “No”	Malaysia, Vietnam	Lim et al., 2013; Dao et al., 2015; Tan et al., 2015; Teng et al., 2016
<i>P. galaxiae</i>	Yes/No	Cerino et al., 2005; Lundholm and Moestrup, 2002; Quijano-Scheggia et al., 2010; Xu and Li, 2015 for “No”; Moschandreas et al., 2012 for “Yes/No”	Angola, Australia, California (USA), China, France (Mediterranean Sea), Greece, Italy (Gulf of Naples, Ionian Sea), Japan, Morocco (Mediterranean Sea), Mozambique, Spain (Mediterranean Sea), South Africa	Yap-Dejeto et al., 2010; Rijal Leblad et al., 2013; Guannel et al., 2015; Ruggiero et al., 2015; Smeti et al., 2015; Teng et al., 2015; Xu and Li, 2015; Ajani et al., 2016; Busch et al., 2016; Caroppo et al., 2016 <sup>2</sup> ; Grzebyk et al., 2017; Nagai et al., 2017; Smith et al., 2018
<i>P. granii</i>	Yes/No	Fuentes and Wikfors, 2013	Arctic (Chukchi Sea, Gulf of	Skov et al., 2004; Guannel et al.,

Species	Toxicity	References for toxicity	Distribution	References for distribution
		for “Yes”; Marchetti et al., 2008; Guannel et al., 2011 for “No”	Alaska, Norwegian Sea), New Hampshire (USA), Subarctic Pacific (Ocean Station Papa), Vietnam Norwegian Sea <b>Australia</b>	2011; Marchetti et al., 2012; Fuentes and Wikfors, 2013; Balzano et al., 2017; Cohen et al., 2017 Hasle, 1974 <b>Ajani et al., 2018</b>
<i>P. granii</i> var. <i>curvata</i>	Not tested	–		
<i>P. hallegraeffii</i> *	No	<b>Ajani et al., 2018</b>		
<i>P. hasleana</i>	Yes/No	Sakka Hlaili et al., 2016 for “Yes”; Lundholm et al., 2012; Ajani et al., 2013a,b for “No”	Australia, California (USA), Greece, Italy, Malaysia, Tunisia, Washington State (USA)	Lundholm et al., 2012; Moschandrou et al., 2012; Teng et al., 2013, 2015; Ajani et al., 2013a,b, 2016; Ruggiero et al., 2015; Bowers et al., 2016; Carlson et al., 2016; Sakka Hlaili et al., 2016; <b>Tatters et al., 2018</b>
<i>P. heimii</i>	No	Rhodes et al., 1998; Marchetti et al., 2008; Woods, 2016	Australia, Bay of Fundy, California (USA), Gulf of Maine, Mexico (Pacific)	Fernandes et al., 2014; Mogensen, 2014; Ajani et al., 2013, 2016; see Zamudio-Resendiz et al., 2014; Woods, 2016; Bowers et al., 2017
<i>P. inflatula</i>	No	Marchetti, 2005 <sup>2</sup> ; Huang et al., 2017b	Angola, China, Malaysia, Mexico (Pacific), Ukraine (Black Sea)	Shiganova and Öztürk, 2010; Terenko and Terenko, 2012; Teng et al., 2013, 2014b; Zamudio-Resendiz et al., 2014; Guannel et al., 2015; Huang et al., 2017b
<i>P. kodamae</i> *	Yes/No	Teng et al., 2014a for “Yes”; Teng et al., 2016 for “No”	<b>Malaysia, Namibia</b>	Teng et al., 2014a, 2016; <b>Louw et al., 2018</b>
<i>P. limii</i> *	No	Teng et al., 2016	Malaysia	Teng et al., 2016
<i>P. linea</i>	No	Quijano-Scheggia et al., 2010	Brazil, <b>Bulgaria (Black Sea)</b> , Italy, Malaysia	Fernandes and Brandini, 2010; Teng et al., 2013, 2014b; Ruggiero et al., 2015; <b>Dzhembekova et al., 2017a</b>
<i>P. lineola</i>	No	Kang et al., 1993; Lundholm et al., 2012	Australia, China, Malaysia, <b>Namibia</b>	Lü et al., 2012; Ajani et al., 2013b; Teng et al., 2013, 2014b; <b>Louw et al., 2018</b>

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. lundholmiae</i> *	Yes/No	Teng et al., 2016 for “Yes”; Teng et al., 2014a for “No”	Malaysia	Lim et al., 2013; Tan et al., 2015; Teng et al., 2016
<i>P. manni</i>	No	Amato and Montresor, 2008; Quijano-Scheggia et al., 2010; Moschandreas et al., 2012; Sakka Hlaili et al., 2016	China, Croatia (Adriatic Sea), Italy (Adriatic Sea, Gulf of Naples), Malaysia, Tunisia	Lü et al., 2012; Penna et al., 2013; Teng et al., 2013; Ruggiero et al., 2015; Sakka Hlaili et al., 2016; Grbin et al., 2017
<i>P. micropora</i>	No	Skov et al., 2004; Lim et al., 2014b; Ajani et al., 2013a,b; Xu and Li, 2015	Australia, China, Florida (Gulf of Mexico), Malaysia, Mexico (Pacific), Philippines, Singapore	Quijano-Scheggia et al., 2011; Ajani et al., 2013a,b, 2016; Lim et al., 2012a,d, 2013; O’Dea et al., 2013; Rivera-Vilarelle et al., 2013; Teng et al., 2013; Yap-Dejeto et al., 2013; see Zamudio-Resendiz et al., 2014; Guannel et al., 2015; Xu and Li, 2015; Tan et al., 2016a
<i>P. multiseries</i>	Yes/No	Kotaki et al., 2008; Lelong et al., 2012b; Pitcher et al., 2014; Woods, 2016 for “Yes”; Villareal et al., 1994; Ajani et al., 2013a,b; for “No”; Kotaki et al., 1999 for “Yes/No”	Australia, Brazil, California (USA), China, Japan, Mexico, Morocco (Mediterranean Sea), <b>Namibia</b> , North Carolina (USA), North Sea (Helgoland), South Africa, Uruguay, Washington State (USA)	Fernandes and Brandini, 2010; Yap-Dejeto et al., 2010; Lü et al., 2012; Shuler et al., 2012; Méndez et al., 2012; Fernandes et al., 2013; Rijal Leblad et al., 2013; Ajani et al., 2013a,b, 2016; Hubbard et al., 2014a; Pitcher et al., 2014; Bresnan et al., 2015; GRIIS; Bowers et al., 2017; Nagai et al., 2017; Ryan et al., 2017; <b>Gai et al., 2018; Louw et al., 2018; Tatters et al., 2018</b>
<i>P. multistriata</i>	Yes/No	Orsini et al., 2002; Pistocchi et al., 2012; Ajani et al., 2013a,b for “Yes”; Rhodes et al., 2000; Lundholm et al., 2002b;	Australia, China, Italy (western Adriatic Sea, Gulf of Naples, Ionian Sea), India (Goa), Japan, Louisiana (Gulf of Mexico), Malaysia, Mexico (Pacific),	Yap-Dejeto et al., 2010; Lü et al., 2012; Méndez et al., 2012; Pistocchi et al., 2012; Sahraoui et al., 2012; Stonik et al., 2012; Ajani et al., 2013b; Rijal Leblad et al.,

Species	Toxicity	References for toxicity	Distribution	References for distribution
		Churro et al., 2009; Quijano-Scheggia et al., 2010; Moschandreas et al., 2012; Teng et al., 2016 for “No”; Amato et al., 2010; Rhodes et al., 2013 for “Yes/No”	Morocco (Mediterranean Sea), Russia (NW Sea of Japan), Singapore, Tunisia, Uruguay	2013; Parsons et al., 2013; Rivera-Vilarelle et al., 2013; Teng et al., 2013, 2014b, 2016; see Zamudio-Resendiz et al., 2014; Ruggiero et al., 2015; Sakka Hlaili et al., 2016; Caroppo et al., 2016 <sup>2</sup> ; Tan et al., 2016a; Chai et al., 2017; Geng et al., 2017; <b>Hernández-Becerril and Villagrán-Lorenzana, 2017</b> ; Nagai et al., 2017
<i>P. nanaoensis</i>	No	Li et al., 2018	China	Li et al., 2018
<i>P. obtusa</i>	Yes/No	Harðardóttir et al., 2015 for “Yes”; Hasle and Lundholm, 2005 for “No”	Arctic (Disco Bay [Greenland])	Harðardóttir et al., 2015
<i>P. plurisecta</i> *	Yes	Fernandes et al., 2014 <sup>4</sup> ; Teng et al., 2014a <sup>4</sup> ; <b>Gai et al., 2018</b>	Australia (Tasmania), Gulf of Maine, <b>Namibia</b> , Spain (Atlantic)	Orive et al., 2013; Fernandes et al., 2014 <sup>4</sup> ; <b>Gai et al., 2018</b>
<i>P. prolongatoides</i>	Not tested	–	See Lelong et al., 2012a	See Lelong et al., 2012a
<i>P. pseudodelicatissima</i>	Yes/No	Orsini et al., 2002; Lundholm et al., 2003; Sahraoui et al., 2011 for “No”; Lapworth et al., 2001; Moschandreas et al., 2012 for “Yes/No”	Alabama (Gulf of Mexico), Arabian Sea, Argentina, Brazil, <b>Bulgaria (Black Sea)</b> , China, Croatia (Adriatic Sea), Florida (Gulf of Mexico), Italy (western, northern Adriatic), Louisiana (Gulf of Mexico), Malaysia, Mexico (Gulf of Mexico, Pacific), Morocco (Mediterranean Sea), North Carolina (USA), Philippines, Scotland, Tunisia	Schiebel et al., 2004; Twinner et al., 2012; Lü et al., 2012; Parsons et al., 2012, 2013; Pistocchi et al., 2012; Sahraoui et al., 2012; Shuler et al., 2012; Fernandes et al., 2013; Gárate-Lizárraga et al., 2013 <sup>2</sup> ; Liefer et al., 2013; Rijal Leblad et al., 2013; Teng et al., 2013, 2014b; Yap-Dejeto et al., 2013; Facca et al., 2014; see Zamudio-Resendiz et al., 2014; Bresnan et al., 2015, 2017; Arapov et al., 2016, 2017; Bargu et al., 2016; Sakka Hlaili et al., 2016; D’Agostino et al., 2017; <b>Dzhembekova et al., 2017b</b>

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. pungens</i> <sup>5</sup>	Yes/No	Fernandes et al., 2014; Woods, 2016; Lema et al., 2017; Pednekar et al., 2018 for “Yes”; Villac et al., 1993; Lapworth et al., 2001; Lundholm et al., 2002b; Skov et al., 2004; Bajarias et al., 2006; Kotaki et al., 2008; Guannel et al., 2011; Ajani et al., 2013a,b; Penna et al., 2013; <b>García Blásquez, 2014</b> ; Lim et al., 2010; Sison-Mangus et al., 2014; Teng et al., 2016; Tenorio et al., 2016; Zu et al., 2015 for “No”; Rhodes et al., 2003, 2013; Lim et al., 2014b for “Yes/No”	Angola, Argentina, Australia, Bay of Fundy, Brazil, <b>Bulgaria (Black Sea)</b> , California (USA), China, Croatia (Adriatic Sea), Florida (Gulf of Mexico), France (Atlantic), Gulf of Maine, India (Goa), Italy (Adriatic Sea), Japan, Malaysia, Mexico (Gulf of Mexico, Pacific), <b>Namibia</b> , North Carolina (USA), North Sea (Helgoland), Peru, Philippines, Russia (Sea of Japan, Vostok Bay), Scotland, Singapore, Spain (Atlantic), Turkey (Sea of Marmara), Ukraine (Black Sea), Uruguay, Vietnam, Washington State (USA)	Fernandes and Brandini, 2010; Yap-Dejeto et al., 2010, 2013; Guannel et al., 2011; O’Dea, 2012; Lim et al., 2012b,2c,d; Lü et al., 2012; Méndez et al., 2012; Parsons et al., 2012; Terenko and Terenko, 2012; Shuler et al., 2012; Ajani et al., 2013a,b, 2016; Fernandes et al., 2013, 2014; Penna et al., 2013; Teng et al., 2013, 2014b, 2016; Fernandes et al., 2014; <b>García Blásquez, 2014</b> ; Lim et al., 2014c; Mogensen, 2014; Hubbard et al., 2014a; Sison-Mangus et al., 2014; Zamudio-Resendiz et al., 2014; Bresnan et al., 2015, 2017; D’Agostino et al., 2015, 2017; Krock et al., 2015; Tan et al., 2015, 2016; Tibiriçá et al., 2015; Xu et al., 2015; Markina and Aizdaicher, 2016; Arapov et al., 2016; Carlson et al., 2016; Dursun et al., 2016; Tas et al., 2016; Tenorio et al., 2016; Woods, 2016; Bowers et al., 2017; <b>Dzhembekova et al., 2017b</b> ; Lema et al., 2017; Geng et al., 2017; Licea et al., 2017; Nagai et al., 2017; Tas and Lundholm, 2017; Thorel et al., 2017; Zúñiga et al., 2017; Chen et al., 2018; <b>Louw et al., 2018</b> ; Pednekar et al., 2018; Smith et al.,

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. pungens</i> var. <i>pungens</i>	Yes/No	Lim et al., 2010, 2012a; Quijano-Scheggia et al., 2010 for “No”; Moschandreas et al., 2012 for “Yes/No”	Australia, Canada (Bay of Fundy, New Brunswick; Prince Edward Island), Greece, Irish Sea, Italy (Adriatic Sea), Japan, Korea, Malaysia, New Zealand, North Sea, Spain (Mediterranean Sea), Washington State	2018 Casteleyn et al., 2008, 2009, 2010; Churro et al., 2009; Quijano-Scheggia et al., 2010; Lim et al., 2012a,b; Moschandreas et al., 2012; Ajani et al., 2013b; Penna et al., 2013; Kim et al., 2015
<i>P. pungens</i> var. <i>cincta</i>	No	Villac and Fryxell, 1998	Brazil, California (Monterey Bay), <b>Namibia</b> , Washington State (USA)	Villac and Fryxell, 1998; Churro et al., 2009; Fernandes and Brandini, 2010; Kim et al., 2015; <b>Gai et al., 2018</b>
<i>P. pungens</i> var. <i>aveirensis</i>	No	Churro et al., 2009; Lim et al., 2010, 2014b	<b>Bulgaria (Black Sea)</b> , East China Sea, Korea, Malaysia, Mexico (Gulf of Mexico, Pacific) Morocco (Mediterranean Sea), Philippines, Portugal, Turkey (Black Sea), Vietnam, Washington State (USA)	Casteleyn et al., 2008; Churro et al., 2009; Moschandreas et al., 2012; Rijal Leblad et al., 2013; Baytut, 2013; Baytut et al., 2013; Rivera-Vilarelle et al., 2013; Lim et al., 2014b; Kim et al., 2015; <b>Dzhembekova et al., 2017a</b>
<i>P. pungiformis</i>	Not tested	–	See Lelong et al., 2012a	See Lelong et al., 2012a
<i>P. roundii</i>	Not tested	–	See Lelong et al., 2012a	See Lelong et al., 2012a
<i>P. sabit*</i>	No	Teng et al., 2015; Huang et al., 2017b	California (USA), China, Malaysia, Mexico (Pacific)	Rivera-Vilarelle et al., 2013 <sup>6</sup> ; Teng et al., 2015 <sup>6</sup> ; Huang et al., 2017b; Smith et al., 2018
<i>P. seriata</i>	Yes/No	Tammilehto et al., 2012, 2015; Fernandes et al., 2014; Harðardóttir et al., 2015 for “Yes”; Bates et al., 1989 for “No”	Arctic (Disco Bay [Greenland]), Bay of Fundy, Gulf of Maine, Scotland, Singapore, Ukraine (Black Sea)	Terenko and Terenko, 2012; Tammilehto et al., 2012, 2015; Fernandes et al., 2014; Mogensen, 2014; Bresnan et al., 2015, 2017; Harðardóttir et al., 2015; Tan et al., 2016
<i>P. simulans</i> *	Yes/no	Li et al., 2017 for “Yes/no”	Australia, China (Yellow Sea, East China Sea, South China Sea)	Li et al., 2017; P. Ajani, pers. comm. for Australia
<i>P. sinica</i>	Not tested	–	China, Malaysia, Thailand,	Lü et al., 2012; Teng et al., 2013

Species	Toxicity	References for toxicity	Distribution	References for distribution
<i>P. subcurvata</i>	No	Fryxell et al., 1991; Kang et al., 1993	Vietnam Antarctica (Ross Sea), Southern Ocean	Petrou and Ralph, 2011; Sackett et al., 2013; Teng et al., 2015; Zhu et al., 2016, 2017a
<i>P. subfraudulenta</i>	Yes/No	Teng et al., 2016 for “Yes”; Orsini et al., 2002; Álvarez et al., 2009; Moschandreu et al., 2012 for “No”	Alabama (Gulf of Mexico), China, Croatia (Adriatic Sea), Japan, Malaysia, Mexico (Pacific), Morocco (Mediterranean Sea), Pakistan	Lü et al., 2012; Naz et al., 2012; Liefer et al., 2013; Rijal Leblad et al., 2013; Rivera-Vilarelle et al., 2013; Teng et al., 2013, 2014b, 2016; Zamudio-Resendiz et al., 2014; Arapov et al., 2016, 2017; Nagai et al., 2017
<i>P. subpacifica</i>	Yes/No	Fernandes et al., 2014 for “Yes”; Lundholm et al., 2002b <sup>2</sup> ; Moschandreu et al., 2012; Rhodes et al., 2013; García Blásquez, 2014; Tenorio et al., 2016 for “No”	Angola, Australia, China, Greece, Hong Kong, Morocco (Mediterranean Sea), Peru, Scotland	Lü et al., 2012; Moschandreu et al., 2012; Ajani et al., 2013b; Rijal Leblad et al., 2013; Rhodes et al., 2013; García Blásquez, 2014; Bresnan et al., 2015; Guannel et al., 2015; Teng et al., 2015; Tenorio et al., 2016
<i>P. turgidula</i>	Yes/No	Fernandes et al., 2014 for “Yes”; Marchetti et al., 2008 for “No”	Antarctica (Weddell Sea), Bay of Fundy, Malaysia, Subarctic Pacific (Ocean Station Papa)	Bill et al., 2012; Teng et al., 2013, 2014b; Fernandes et al., 2014; Hoppe et al., 2013 <sup>2</sup>
<i>P. turgiduloides</i>	No	Kang et al., 1993	Antarctica (Weddell Sea)	Hoppe et al., 2013 <sup>2</sup>

<sup>1</sup> Strain SZN-B18 in Orsini et al. (2002) is *P. arenysensis*.

<sup>2</sup> The *Pseudo-nitzschia* species is indicated as “cf.”.

<sup>3</sup> Some species may be *P. arenysensis* (*sensu* Quijano-Scheggia et al., 2009).

<sup>4</sup> Toxigenic *P. sp.* GOM in Fernandes et al. (2014) shown to *P. plurisecta* in Teng et al. (2014a).

<sup>5</sup> Variety of *P. pungens* not specified.

<sup>6</sup> Teng et al. (2015) indicate that the *P. cf. delicatissima* reported by Rivera-Vilarelle et al. (2013) is *P. sabit*.

Table 4. New records for *Pseudo-nitzschia* spp. at different locations since reviewed by Lelong et al. (2012a), and the method by which the species were identified. \* = new species/strains since Lelong et al. (2012a); ARISA = Automated Ribosomal Intergenic Spacer Analysis; LM = light microscopy; LSU = large subunit of ribosomal DNA; ITS1 = first internal transcribed spacer region of rDNA; ITS2 = second internal transcribed spacer region of rDNA; SEM = Scanning Electron Microscopy; TEM = Transmission Electron Microscopy.

Species	Location	Method	Reference
<i>P. abreensis</i> *	Malaysia	TEM, LSU, ITS2	Teng et al., 2016
	Spain (Atlantic)	TEM, LSU, ITS2	Orive et al., 2013
<i>P. americana</i>	China	LM, TEM	Lü et al., 2012
	Japan (Tokyo Bay)	LM, TEM	Yap-Dejeto et al., 2010
	Malaysia	TEM	Teng et al., 2013, 2014b
	Namibia	TEM	Louw et al., 2018
<i>P. arctica</i> *	North Sea (Helgoland)	TEM	Bresnan et al., 2015
	Arctic (Barrow Strait, Beaufort Sea, Greenland, Baffin Bay)	LM, SEM, TEM, LSU	Percopo et al., 2016; Balzano et al., 2017
	Australia	TEM, ITS2	Ajani et al., 2016
	California (USA)	SEM	Bowers et al., 2016, 2017
<i>P. australis</i>	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	Bay of Fundy	SEM	J. Martin, pers. commun.
	Gulf of Maine	SEM, ITS1	Hubbard et al., 2017; Lewis et al., 2017
	Mexico (Baja California)	SEM	Santiago-Morales et al., 2011
<i>P. batesiana</i> *	Morocco (Atlantic)	SEM	Ennaffah et al., 2012
	Malaysia	TEM, LSU, ITS2	Lim et al., 2013; Tan et al., 2015; Teng et al., 2016
<i>P. bipertita</i> *	Malaysia	TEM, LSU, ITS2	Teng et al., 2016
<i>P. brasiliiana</i>	Argentina	SEM	Almandoz et al., 2017
	China	LM, TEM, LSU	Wang et al., 2012
	France (Mediterranean Sea)	ITS2	
	Greece	LM, TEM, ITS2	Wang et al., 2012
	Japan (Tokyo Bay)	LSU	Grzebyk et al., 2017
	Malaysia	TEM, ITS2	Moschandreou et al., 2012
	Mexico (Gulf of California)	LM, SEM, TEM, LSU, ITS2	Yap-Dejeto et al., 2010
	Philippines	SEM	Lim et al., 2012b,c; Teng et al., 2014b; Tan et al., 2015
	Singapore	TEM	Gárate-Lizárraga et al., 2013
		SEM, TEM	Yap-Dejeto et al., 2013
			Tan et al., 2016

Species	Location	Method	Reference
<i>P. bucculenta</i> *	Tunisia	SEM	Sakka Hlaili et al., 2016
<i>P. bucculenta</i> *	Namibia	TEM, ITS1, ITS2	Gai et al., 2018
<i>P. caciantha</i>	Angola	ARISA	Guannel et al., 2015
	Australia	TEM, ITS2	Ajani et al., 2016
	China	LM, TEM	Lü et al., 2012
	Japan (Tokyo Bay)	LM, TEM	Yap-Dejeto et al., 2010
	Malaysia	TEM, LSU, ITS2	Teng et al., 2014a,b
	Philippines	TEM	Yap-Dejeto et al., 2013
	Vietnam	TEM	Dao et al., 2014
<i>P. calliantha</i>	Bulgaria (Black Sea)	LSU	Dzhembekova et al., 2017a
	China	LM, TEM	Lü et al., 2012
	Florida (Atlantic)	LM, SEM	Phlips et al., 2011
	Japan (Tokyo Bay)	LM, TEM	Yap-Dejeto et al., 2010
	France (Mediterranean Sea)	LSU	Grzebyk et al., 2017
	Malaysia	TEM	Teng et al., 2013, 2014b
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	New Zealand	TEM, LSU	Rhodes et al., 2013
	Turkey (Sea of Marmara)	SEM	Tas et al., 2016; Tas and Lundholm, 2017
	Ukraine (Black Sea)	SEM, TEM	Ryabushko et al., 2008
<i>P. circumpora</i> *	Malaysia	TEM, LSU, ITS2	Lim et al., 2012a, 2013, 2014a; Teng et al., 2014b; Tan et al., 2015
<i>P. cuspidata</i>	China	LM, TEM	Lü et al., 2012
	Florida (Atlantic, Gulf of Mexico)	TEM, ITS1	O'Dea et al., 2013; Flewelling et al., 2015; Hubbard et al., 2015
	Malaysia	TEM, LSU, ITS2	Lim et al., 2012a, Teng et al., 2013
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	New Zealand	TEM, LSU	Rhodes et al., 2013
	Singapore	SEM, TEM	Tan et al., 2016
<i>P. cuspidata</i> var. <i>manzanillensis</i> *	Mexico (Pacific)	LM, SEM, TEM, ITS1, ITS2	Rivera-Vilarelle et al., 2018
<i>P. decipiens</i>	California (USA)	ITS1	Smith et al., 2017
	China	LM, TEM, ITS2	Huang et al., 2017a
	Japan	LSU	Nagai et al., 2017
	Malaysia	TEM, LSU, ITS2	Teng et al., 2014b, 2015
<i>P. delicatissima</i>	Louisiana (Gulf of Mexico)	LM, SEM	Parsons et al., 2013
	Tunisia	SEM	Sakka Hlaili et al., 2016
	Turkey (Aegean Sea)	SEM, ITS2	Kesici et al., 2013

Species	Location	Method	Reference
<i>P. dolorosa</i>	Turkey (Sea of Marmara)	SEM	Tas et al., 2016
	Ukraine (Black Sea)	LM, SEM	Terenko and Terenko, 2012
	Uruguay	SEM, TEM	Méndez et al., 2012
	Argentina	SEM	Almandoz et al., 2017
	Malaysia	TEM, LSU, ITS2	Lim et al., 2012a; Teng et al., 2013
<i>P. fraudulenta</i>	Mexico (Pacific)	SEM	Rivera-Vilarelle et al., 2013
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	California (USA)	SEM	Bowers et al., 2016
	Italy (Gulf of Naples, western Adriatic Sea)	SEM; LSU	Pistocchi et al., 2012; Ruggiero et al., 2015
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
<i>P. fryxelliana</i>	Namibia	TEM	Louw et al., 2018
	North Sea (Helgoland)	TEM	Bresnan et al., 2015
	Pakistan	LM, SEM	Naz et al., 2012
	California (USA)	ITS1	Smith et al., 2017
	Mexico (Pacific)	SEM	Rivera-Vilarelle et al., 2013 <sup>1</sup>
<i>P. fukuyoi</i> *	Tunisia	SEM	Sakka Hlaili et al., 2016
	China	LM, TEM, ITS2	Huang et al., 2017c
	Malaysia	TEM, LSU, ITS2	Lim et al., 2013; Tan et al., 2015; Teng et al., 2016
	Vietnam	TEM, LSU, ITS2	Dao et al., 2015
	Angola	ARISA	Guannel et al., 2015
<i>P. galaxiae</i>	China	TEM, ITS2	Xu and Li, 2015
	France (Mediterranean Sea)	LSU	Grzebyk et al., 2017
	Italy (Ionian Sea)	TEM	Caroppo et al., 2016 <sup>1</sup>
	Japan (Tokyo Bay)	LM, TEM	Yap-Dejeto et al., 2010
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
<i>P. granii</i>	Spain (Mediterranean Sea)	SEM, ITS1, ITS2	Quijano-Scheggia et al., 2010
	Arctic (Chukchi Sea, Gulf of Alaska, Norwegian Sea)	LM, SEM, TEM, LSU	Balzano et al., 2017
	New Hampshire (USA)	LM, ITS1	Fuentes and Wikfors, 2013
	Australia	LM, TEM, LSU, ITS2	Ajani et al., 2018
<i>P. hasleana</i>	Australia	TEM, LSU, ITS2	Ajani et al., 2013a,b, 2016
	California (USA)	SEM	Bowers et al., 2016, 2017
	Greece	TEM, ITS2	Moschandreas et al., 2012

Species	Location	Method	Reference
<i>P. heimii</i>	Italy (Gulf of Naples)	LSU	Ruggiero et al., 2015
	Malaysia	TEM	Teng et al., 2013, 2015
	Tunisia	SEM	Sakka Hlaili et al., 2016
	Australia	TEM, ITS2	Ajani et al., 2013, 2016
<i>P. inflatula</i>	Bay of Fundy	LM, SEM, TEM	Fernandes et al., 2014
	Angola	ARISA	Guannel et al., 2015
	China	LM, TEM, ITS2	Huang et al., 2017b
<i>P. kodamae*</i>	Malaysia	TEM	Teng et al., 2013, 2014b
	Ukraine (Black Sea)	LM, SEM	Terenko and Terenko, 2012
	Malaysia	TEM, LSU, ITS2	Teng et al., 2014a, 2016
	Namibia	TEM	Louw et al., 2018
<i>P. limii*</i>	Malaysia	TEM, LSU, ITS2	Teng et al., 2016
<i>P. linea</i>	Bulgaria (Black Sea)	LM, LSU	Dzhembekova et al., 2017a
<i>P. lineola</i>	Italy (Gulf of Naples)	LSU	Ruggiero et al., 2015
	Malaysia	TEM, LSU, ITS2	Teng et al., 2013, 2014b
	Spain (Mediterranean Sea)	SEM, ITS1, ITS2	Quijano-Scheggia et al., 2010
	China	LM, TEM	Lü et al., 2012
<i>P. lundholmiae*</i>	Malaysia	TEM	Teng et al., 2014b
	Namibia	TEM	Louw et al., 2018
	Malaysia	TEM, LSU, ITS2	Tan et al., 2015; Teng et al., 2016
<i>P. mannii</i>	China	LM, TEM	Lü et al., 2012
	Malaysia	TEM, LSU, ITS2	Teng et al., 2013
	Spain (Mediterranean Sea)	SEM, ITS1, ITS2	Quijano-Scheggia et al., 2010
	Tunisia	SEM	Sakka Hlaili et al., 2016
<i>P. micropora</i>	Australia	TEM, LSU, ITS2	Ajani et al., 2013a,b, 2016
	China	TEM, ITS2	Xu and Li, 2015
	Malaysia	TEM, LSU, ITS2	Lim et al., 2012a
	Florida (Gulf of Mexico)	TEM, SEM	O'Dea, 2012
	Mexico (Pacific)	SEM	Quijano-Scheggia et al., 2011; Rivera-Vilarelle et al., 2013
	Philippines	TEM	Yap-Dejeto et al., 2013
<i>P. multiseries</i>	Singapore	SEM, TEM	Tan et al., 2016
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	North Carolina (USA)	TEM	Shuler et al., 2012
<i>P. multistriata</i>	South Africa	LM, SEM, LSU	Pitcher et al., 2014
	Italy (Ionian Sea)	TEM	Caroppo et al., 2016 <sup>1</sup>
	Italy (western Adriatic Sea)	SEM; LSU	Pistocchi et al., 2012
	Louisiana (Gulf of	LM, SEM	Parsons et al., 2013

Species	Location	Method	Reference
	Mexico)		
	Malaysia	TEM, LSU, ITS2	Teng et al., 2013, 2014b, 2016
	Mexico (Pacific)	SEM	Rivera-Vilarelle et al., 2013
	Morocco	TEM	Rijat Leblad et al., 2013
	(Mediterranean Sea)		
	Singapore	SEM, TEM	Tan et al., 2016
	Tunisia	SEM	Sahraoui et al., 2012; Sakka Hlaili et al., 2016
<i>P. plurisecta</i> *	Australia (Tasmania)	TEM, LSU, ITS2	Orive et al., 2013
	Gulf of Maine	SEM, LSU, ITS2	Fernandes et al., 2014 <sup>2</sup>
	Spain (Atlantic)	TEM, LSU, ITS2	Orive et al., 2013
<i>P.</i>	Brazil	LM, TEM	Fernandes et al., 2013
<i>pseudodelicatissima</i>	Florida (Gulf of Mexico)	SEM	Twiner et al., 2012
	Italy (western Adriatic Sea)	SEM; LSU	Pistocchi et al., 2012
	Italy (northern Adriatic Sea)	LM, SEM	Facca et al., 2014
	Japan (Tokyo Bay)	LM, TEM	Yap-Dejeto et al., 2010
	Louisiana (Gulf of Mexico)	LM, SEM	Parsons et al., 2013
	Malaysia	TEM	Teng et al., 2014b
	Morocco	TEM	Rijat Leblad et al., 2013
	(Mediterranean Sea)		
	North Carolina (USA)	TEM	Shuler et al., 2012
	Philippines	TEM	Yap-Dejeto et al., 2013
	Tunisia	SEM	Sahraoui et al., 2012; Sakka Hlaili et al., 2016
<i>P. pungens</i> <sup>3</sup>	Namibia	TEM	Louw et al., 2018
	North Carolina (USA)	TEM	Shuler et al., 2012
	Philippines	TEM	Yap-Dejeto et al., 2013
	Singapore	SEM, TEM	Tan et al., 2016
	Turkey (Sea of Marmara)	SEM	Tas et al., 2016; Tas and Lundholm, 2017
	Uruguay	SEM, TEM	Méndez et al., 2012
<i>P. pungens</i> var.	Australia	TEM, LSU, ITS2	Ajani et al., 2013b
<i>pungens</i> (Clade I)	Brazil	LM, SEM, TEM	Cavalcante, 2011
	Greece	TEM, ITS2	Moschandrou et al., 2012
	Korea, East China Sea	LM, SEM, ITS1, ITS2	Kim et al., 2015, 2018
	Italy (Adriatic Sea)	ITS-5.8S	Penna et al., 2013

Species	Location	Method	Reference
<i>P. pungens</i> var. <i>cingulata</i> (Clade II)	Malaysia	LM, TEM, SEM, LSU, ITS2	Lim et al., 2012b,c; Teng et al., 2014b; Tan et al., 2015
	Spain (Mediterranean Sea)	SEM, ITS1, ITS2	Quijano-Scheggia et al., 2010
	Brazil	LM, TEM	Fernandes and Brandini, 2010
	Namibia	TEM, ITS1, ITS2	Gai et al., 2018
	Washington State	LM, SEM, ITS1, ITS2	Kim et al., 2015
<i>P. pungens</i> var. <i>aveirensis</i> (Clade III)	Bulgaria (Black Sea)	LSU	Dzhembekova et al., 2017a
<i>P. sabit</i> *	Korea, East China Sea	LM, SEM, ITS1, ITS2	Kim et al., 2015, 2018
	Malaysia	LM, TEM, SEM, LSU, ITS2	Lim et al., 2014b
	Mexico (Pacific)	SEM	Rivera-Vilarelle et al., 2013
	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	Philippines	LM, SEM, ITS1, ITS2	Kim et al., 2015, 2018
	Turkey (Black Sea)	TEM, SEM, LSU	Baytut, 2013; Baytut et al., 2013
	China	LM, TEM, ITS2	Huang et al., 2017b
<i>P. simulans</i> *	Malaysia	TEM, LSU, ITS2	Teng et al., 2015
	Mexico (Pacific)	SEM	Rivera-Vilarelle et al., 2013 <sup>4</sup> ; Teng et al., 2015 <sup>4</sup>
<i>P. sinica</i>	California (USA)	ITS1	Smith et al., 2017
	China (Yellow Sea, East China Sea, South China Sea)	TEM, LSU, ITS2	Li et al., 2017
	Australia (Brisbane)	TEM, ITS2	P. Ajani, pers. comm.
<i>P. seriata</i>	Malaysia	TEM, LSU, ITS2	Teng et al., 2013
<i>P. subraudulenta</i>	Singapore	SEM, TEM	Tan et al., 2016
<i>P. subpacifica</i>	Croatia (Adriatic Sea)	SEM	Arapov et al., 2016, 2017
	Greece	TEM, ITS2	Moschandrou et al., 2012
	Gulf of Maine	SEM, LSU, ITS2	Fernandes et al., 2014
	Malaysia	TEM, LSU, ITS2	Teng et al., 2013, 2014b, 2016
	Mexico (Pacific)	SEM	Rivera-Vilarelle et al., 2013
<i>P. subpacifica</i>	Morocco (Mediterranean Sea)	TEM	Rijat Leblad et al., 2013
	Pakistan	LM, SEM	Naz et al., 2012
	Angola	ARISA	Guannel et al., 2015
	Brazil	TEM	Calvancante, 2011

Species	Location	Method	Reference
<i>P. turgidula</i>	Hong Kong	TEM, LSU, ITS2	Teng et al., 2015
	New Zealand	TEM	Stonik et al., 2013
	Peru	SEM	Tenorio et al., 2016
	Scotland	TEM	Bresnan et al., 2015
	Malaysia	TEM, LSU, ITS2	Teng et al., 2013, 2014b

<sup>1</sup> The *Pseudo-nitzschia* species is indicated as “cf.”.

<sup>2</sup> The toxigenic *P. sp.* GOM in Fernandes et al. (2014) shown to be *P. plurisecta* in Teng et al. (2014a).

<sup>3</sup> Variety of *P. pungens* not specified.

<sup>4</sup> Teng et al. (2015) indicate that the *P. cf. delicatissima* reported by Rivera-Vilarelle et al. (2013) is *P. sabit*.

Table 5. Evidence of domoic acid (DA) in the food web, updated since Trainer et al. (2012). Action level for closure of shellfish harvesting = 20 µg g<sup>-1</sup>. dw = dry weight; ww = wet weight (not always indicated in the publications); nd = not detected

Common name	Organism Scientific name	Location	Level	Domoic acid		Reference
				Unit	Body part	
<b>Invertebrates</b>						
Mediterranean mussel	<i>Mytilus galloprovincialis</i>	North Black Sea (Bulgaria)	0.493–0.919	µg g <sup>-1</sup>	Digestive gland	Peteva et al., 2017
		Ria de Vigo (Spain)	3,884	ng g <sup>-1</sup>	Digestive gland	Pazos et al., 2017
		Ria de Vigo (Spain)	nd–186	ng g <sup>-1</sup> dw	Whole tissue	Reizopoulou et al., 2012
Sea cucumber	<i>Cucumaria</i> sp.		161–1,376	ng g <sup>-1</sup> dw	Whole tissue	
Brittle star	<i>Amphiura chiajei</i>		442–836	ng g <sup>-1</sup> dw	Whole tissue	
Serpent star	<i>Ophiura ophiura</i>		36–405	ng g <sup>-1</sup> dw	Whole tissue	
Pelican's foot (sea snail)	<i>Aporrhais pespelecani</i>		108–2173	ng g <sup>-1</sup> dw	Whole tissue	
Netted dog whelk (sea snail)	<i>Nassarius reticulatus</i>		308–4,1052	ng g <sup>-1</sup> dw	Whole tissue	
American horse mussel	<i>Modiolus americanus</i>	Florida (Gulf of Mexico, USA)	13.25	µg g <sup>-1</sup>	Whole tissue	O'Dea, 2013
Eastern oyster	<i>Crassostrea virginica</i>		5.15	µg g <sup>-1</sup>	Whole tissue	
Prickly cockle	<i>Trachycardium egmontianum</i>		0.27	µg g <sup>-1</sup>	Whole tissue	
Atlantic coquinas	<i>Donax variabilis</i>		1.39	µg g <sup>-1</sup>	Whole tissue	
Eastern oyster	<i>Crassostrea virginica</i>	Florida (Gulf of Mexico, USA)	76.0	µg g <sup>-1</sup>	Whole tissue	O'Dea et al., 2013
Bay scallop	<i>Argopecten irradians</i>		28.2	µg g <sup>-1</sup>	Whole tissue	
Pen shell	<i>Atrina rigida</i>		52.1	µg g <sup>-1</sup>	Whole tissue	
Abalone	Four species	Australia	0.14	µg g <sup>-1</sup>	Foot	Malhi et al., 2014
Eastern oyster	<i>Crassostrea virginica</i>		0.77	µg g <sup>-1</sup>	Viscera	
Sweet clam	<i>Challista chione</i>	Morroco	4.9	µg g <sup>-1</sup>	Whole tissue	Rijat Leblad et al., 2013
Tuberculate cockle	<i>Acanthocardia tuberculata</i>		2.1	µg g <sup>-1</sup>	Whole tissue	
Surf clam	<i>Mesodesma donacium</i>	Chile	10	µg g <sup>-1</sup>	Whole tissue	Álvarez et al., 2015
Brown mussel	<i>Perna perna</i>	Brazil	0.5–98.5	µg g <sup>-1</sup> ww	Whole tissue	Fernandes et al., 2013

Organism		Location	Level	Domoic acid			Reference
Common name	Scientific name			Unit	Body part		
Asian green mussel	<i>Perna viridis</i>	Thailand	214–454	ng g <sup>-1</sup> ww	Whole tissue	Veschasit et al., 2017	
Black-scar oyster	<i>Crassostrea lugubris</i>		171–371	ng g <sup>-1</sup> ww	Whole tissue		
Pearl oyster	<i>Pinctada fucata</i>		117–282	ng g <sup>-1</sup> ww	Whole tissue		
Ribbed mussel	<i>Aulacomya atra</i>	Chile	19.75	µg g <sup>-1</sup>	Whole tissue	Pizarro et al., 2017	
Sea scallops	<i>Chlamys vitrea</i>		5–14	µg g <sup>-1</sup>	Whole tissue		
Blue mussel	<i>Mytilus chilensis</i>		1.53	µg g <sup>-1</sup>	Whole tissue		
Ribbed mussel	<i>Aulacomya atra</i>		0.75	µg g <sup>-1</sup>	Whole tissue		
Sand crab	<i>Emerita analoga</i>	California (USA)	2.2–27.3	µg g <sup>-1</sup>	Whole tissue	Shanks et al., 2016	
Dungeness crab	<i>Metacarcinus magister</i>	Washington (USA)	65	µg g <sup>-1</sup>	Whole tissue	McCabe et al., 2016	
		California (USA)	270	µg g <sup>-1</sup>	Whole tissue		
		Oregon (USA)	70	µg g <sup>-1</sup>	Whole tissue		
Rock crab	<i>Cancer antennarius</i>	California (USA)	1,000	µg g <sup>-1</sup>	Whole tissue		
Razor clam	<i>Siliqua patula</i>	Oregon (USA)	170	µg g <sup>-1</sup>	Whole tissue		
Mussel	<i>Mytilus</i> sp.	California (USA)	75	µg g <sup>-1</sup>	Whole tissue		
Zooplankton	–	Thailand	0.04–0.21	ng l <sup>-1</sup>	Whole body	Veschasit et al., 2017	
<b>Cephalopods</b>							
Humboldt squid	<i>Dosidicus gigas</i>	Southern California Bight (USA)	0.2–0.3	µg g <sup>-1</sup> ww	Stomach	Mazzillo et al., 2011	
		British Columbia (Canada)	0.23	ng g <sup>-1</sup>	Digestive gland	Braid et al., 2012	
Common cuttlefish	<i>Sepia officinalis</i>	Morocco	50	µg g <sup>-1</sup>	Digestive gland	Ben Haddouch et al., 2016	
		Portugal	2.99–75.91 0.03–0.29 2.14	µg g <sup>-1</sup> ww µg g <sup>-1</sup> ww µg g <sup>-1</sup> ww	Digestive gland Brain Brain	Lopes et al., 2018	
Common octopus	<i>Octopus vulgaris</i>						
<b>Fish</b>							
Pacific sardine	<i>Sardinops sagax</i>	Redondo Beach, California (USA)	5.25–72.20	µg g <sup>-1</sup> ww	Stomach	Stauffer et al., 2012	
Striped mullet	<i>Mugil cephalus</i>	Florida (Gulf of Mexico, USA)	90	ng g <sup>-1</sup> ww	Stomach	Twinner et al., 2012	
Pinfish	<i>Lagodon rhomboides</i>		39	ng g <sup>-1</sup> ww	Stomach		
Pigfish	<i>Orthopristis</i>		76	ng g <sup>-1</sup> ww	Stomach		

Common name	Organism Scientific name	Location	Level	Domoic acid		Reference
				Unit	Body part	
Striped mojarra	<i>Eugerres plumieri</i>	Alabama (Gulf of Mexico, USA)	65	ng g <sup>-1</sup> ww	Stomach	Liefer et al., 2013
Scaled sardines	<i>Harengula jaguana</i>		440	ng g <sup>-1</sup> ww	Stomach	
Striped mullet	<i>Mugil cephalus</i>		54	ng g <sup>-1</sup> ww	Stomach	
Gulf kingfish	<i>Menticirrhus littoralis</i>		0.003–0.14	µg g <sup>-1</sup>	Whole tissue	
Striped anchovy	<i>Anchoa hepsetus</i>	Scotland	nd–0.72	µg g <sup>-1</sup>	Whole tissue	Jensen et al., 2015
White mullet	<i>Mugil curema</i>		0.027–0.056	µg g <sup>-1</sup>	Whole tissue	
Plaice	<i>Pleuronectes</i> sp.		177.4	µg g <sup>-1</sup>	Whole tissue	
Common dab	<i>Limanda limanda</i>		51.1	µg g <sup>-1</sup>	Whole tissue	
Cod	<i>Gadhus morhua</i>		2.7	µg g <sup>-1</sup>	Whole tissue	
Long rough dab	<i>Hippoglossoides platessoides</i>		2.8	µg g <sup>-1</sup>	Whole tissue	
Whiting	<i>Merlangius merlangus</i>	California to Washington (USA)	0.3	µg g <sup>-1</sup>	Whole tissue	McCabe et al., 2016
Northern anchovy	<i>Engraulis mordax</i>		1–505	µg g <sup>-1</sup>	Whole tissue	
			1–3,239	µg g <sup>-1</sup>	Viscera	
			2,076	µg g <sup>-1</sup>	Viscera	
			184	µg g <sup>-1</sup>	Head	
			35	µg g <sup>-1</sup>	Whole body	
Halibut	<i>Paralichthys californicus</i>	California (USA)	2.5	µg g <sup>-1</sup>	Red muscle	Kudela et al., 2015
Ling cod	<i>Ophiodon elongatus</i>		0.5	µg g <sup>-1</sup>	Red muscle	
Bigscale ponyfish	<i>Secutor megalolepis</i>	Thailand	nd–44.2	ng g <sup>-1</sup> ww	Whole tissue	Veschaisit et al., 2017
Pilchard	<i>Sardinops sagax</i>	Namibia	nd–0.12	ng g <sup>-1</sup>	Viscera	Louw et al., 2018
<b>Reptiles</b>						
Green sea turtle	<i>Chelonia mydas</i>	Florida (Atlantic, USA)	29	µg g <sup>-1</sup>	Intestines	Flewelling et al., 2015
			<1	ng ml <sup>-1</sup>	Plasma	
<b>Marine birds</b>						
Common murre	<i>Uria aalge</i>	California (USA)	nd–654.12	ng g <sup>-1</sup>	Cloaca	Gibble et al., 2018
			nd–361.99		Liver	
			nd–10.77		Stomach	
			nd–85.91		Kidney	

<b>Common name</b>	<b>Organism</b> <b>Scientific name</b>	<b>Location</b>	<b>Level</b>	<b>Domoic acid</b>			<b>Reference</b>
				<b>Unit</b>	<b>Body part</b>		
<b>Marine mammals</b>							
Bottlenose dolphin	<i>Tursiops truncatus</i>	Florida (Gulf of Mexico, USA)	4–45.1	ng g <sup>-1</sup> ww	Feces		Twiner et al., 2012
Pacific harbor seal	<i>Phoca vitulina richardii</i>	California (USA)	2.4–2,887	ng g <sup>-1</sup>	Feces		McHuron et al., 2013
			0.4–11.7	ng ml <sup>-1</sup>	Urine		
			1.4	ng g <sup>-1</sup>	Stomach		
			2.2	ng ml <sup>-1</sup>	Milk		
			9.7	ng ml <sup>-1</sup>	Amniotic fluid		
			14.6–39.8	ng g <sup>-1</sup>	Fetal meconium		
Harbour seal	<i>Phoca vitulina</i>	Scotland	63.17	ng ml <sup>-1</sup>	Urine		Jensen et al., 2015
			100.46	µg g <sup>-1</sup>	Feces		
Beluga whale	<i>Delphinapterus leucas</i>	Alaska (USA)	4.8	ng ml <sup>-1</sup>	Fetal tissue		Burek-Huntington et al., 2015
Southern right whale	<i>Eubalaena australis</i>	Argentina	7.0	ng ml <sup>-1</sup>	Juvenile tissue		
			3–7	ng ml <sup>-1</sup>	Blood		Wilson et al., 2016
California sea lion	<i>Zalophus californianus</i>	Washington (USA) California (USA)	0.30–710	µg g <sup>-1</sup> dw	Feces		D'Agostino et al., 2017
			1,045	µg g <sup>-1</sup>	Feces		McCabe et al., 2016
			1.0–142.6	ng ml <sup>-1</sup>	Milk		Rust et al., 2014
			0.004–134.2	µg g <sup>-1</sup>	Feces		
			2.3–19,340	ng ml <sup>-1</sup>	Urine		
			nd–344.7	ng ml <sup>-1</sup>	Bile		
Harbor porpoise	<i>Phocoena phocoena</i>		1.1–180.3	ng ml <sup>-1</sup>	Milk		
			9.5	ng g <sup>-1</sup>	Feces		
			1,352	ng g <sup>-1</sup>	Stomach		
Northern fur seal	<i>Callorhinus ursinus</i>		1.3–154.6	ng ml <sup>-1</sup>	Milk		
			5–6,730	ng g <sup>-1</sup>	Feces		
			64.9	ng g <sup>-1</sup>	Stomach		
Harbor seal	<i>Phoca vitulina</i>		2.2	ng ml <sup>-1</sup>	Milk		
			0.8	ng ml <sup>-1</sup>	Urine		
Peruvian fur seal	<i>Arctocephalus australis</i>	Peru	nd–533	ng g <sup>-1</sup>	Feces		Fire et al., 2017
South American sea lion	<i>Otaria byronia</i>		nd–525	ng g <sup>-1</sup>	Feces		
Humpback whale	<i>Megaptera</i>	Alaska (USA)	51	ng g <sup>-1</sup>	Feces		Lefebvre et al., 2016

May 9, 2018

Common name	Organism Scientific name	Location	Level	Domoic acid		Reference
				Unit	Body part	
Bowhead whale	<i>Balaena mysticetus</i> <i>novaehangliae</i>		359	ng g <sup>-1</sup>	Feces	
Beluga whale	<i>Delphinapterus leucas</i>		7	ng g <sup>-1</sup>	Stomach	
Harbor porpoise	<i>Phocoena phocoena</i>		15	ng g <sup>-1</sup>	Feces	
Northern fur seal	<i>Callorhinus ursinus</i>		14	ng ml <sup>-1</sup>	Serum	
Steller sea lion	<i>Eumetopias jubatus</i>		7	ng g <sup>-1</sup>	Stomach	
Harbor seal	<i>Phoca vitulina</i>		8	ng g <sup>-1</sup>	Feces	
Ringed seal	<i>Phoca hispida</i>		127	ng g <sup>-1</sup>	Feces	
Bearded seal	<i>Erignathus barbatus</i>		18	ng g <sup>-1</sup>	Intestine	
Spotted seal	<i>Phoca largha</i>		40	ng g <sup>-1</sup>	Stomach	
Ribbon seal	<i>Histriophoca fasciata</i>		7	ng g <sup>-1</sup>	Feces	
Pacific walrus	<i>Odobenus rosmarus</i>		6457	ng g <sup>-1</sup>	Stomach	
Northern sea otter	<i>Enhydra lutra</i>		162	ng ml <sup>-1</sup>	Urine	

Table 6. List of *Pseudo-nitzschia* species for which information on the occurrence of a sexual phase is available. Evidence for sexual events is provided by observations and experiments carried out in the laboratory with culture material (“Culture”), and/or from observations of sexual stages in natural populations at sea (“Nature”). Modified from Montresor et al. (2016).

Species	Evidence for sex	Reference
<i>P. arenysensis</i>	Culture	Quijano-Scheggia et al., 2009a; Adelfi et al., 2014
<i>P. arenysensis</i> as <i>P. delicatissima</i>	Culture	Amato et al., 2005, 2007; Levialdi Ghiron et al., 2008
<i>P. australis</i>	Nature	Holtermann et al., 2010; Du et al., 2016
<i>P. brasiliiana</i>	Culture	Quijano-Scheggia et al., 2009b
<i>P. calliantha</i>	Culture	Amato et al., 2007
<i>P. cf. calliantha</i>	Nature	Sarno et al., 2010
<i>P. cuspidata</i>	Culture	Amato et al., 2007; Lundholm et al., 2012
<i>P. delicatissima</i>	Culture	Kaczmarska et al., 2008
<i>P. cf. delicatissima</i>	Nature	Sarno et al., 2010
<i>P. dolorosa</i>	Culture	Amato et al., 2007
<i>P. fraudulenta</i>	Culture	Chepurnov et al., 2004
<i>P. mannii</i>	Culture	Amato and Montresor, 2008
<i>P. multiseries</i>	Culture	Davidovich and Bates, 1998; Hiltz et al., 2000; Kaczmarska et al., 2000
<i>P. multistriata</i>	Culture/Nature	D’Alelio et al., 2009, 2010; Adelfi et al., 2014; Scalco et al., 2014
<i>P. pseudodelicatissima</i>	Culture	Davidovich and Bates, 1998; Amato et al., 2007
<i>P. pungens</i>	Culture/Nature	Chepurnov et al., 2005; Casteleyn et al., 2009; Holtermann et al., 2010
<i>P. pungens</i> var. <i>aveirensis</i>	Culture	Churro et al., 2009
<i>P. subcurvata</i>	Culture	Fryxell et al., 1991

May 9, 2018

Table 7. Studies on interactions between grazers (zooplankton unless otherwise indicated) and *Pseudo-nitzschia* spp., with respect to domoic acid (DA). Zooplankton organisms are copepods (cop), unless otherwise indicated. – = no data; n.a. = not applicable; / = separates data referring to different species

Grazing species (zooplankton or dinoflagellate)	Toxic <i>Pseudo-nitzschia</i> species or DA	Effect on zooplankton	Time period for experiment	DA (pg cell <sup>-1</sup> )	Induction of DA (pg cell <sup>-1</sup> )	DA concentration in zooplankton <sup>1,2</sup>	Retained as % of ingested DA	Reference
<i>Temora longicornis</i> / <i>Calanus glacialis</i>	<i>P. multiseries</i>	No grazing deterrence, selective grazing or mortality	24 h; 9 d	2.7–4.8	–	After 5 h: 603 µg g <sup>-1</sup> DW / Up to 44 µg g <sup>-1</sup>	50%	Windust, 1992
<i>Temora longicornis</i>	Dissolved DA (0.05–50 µg ml <sup>-1</sup> )	Lethal: LC <sub>50</sub> = 135 µg ml <sup>-1</sup>	72 h	n.a.	n.a.	–	n.a.	Windust, 1992
<i>Pseudocalanus acuspes</i>	Dissolved DA (50 ng ml <sup>-1</sup> –50 µg mL <sup>-1</sup> )	Lethal: LC <sub>50</sub> = 37.5 µg ml <sup>-1</sup>	72 h	n.a.	n.a.	–	n.a.	Windust, 1992
<i>Calanus glacialis</i>	Dissolved DA (0.05–50 µg ml <sup>-1</sup> )	No lethal effect	72 h	n.a.	n.a.	–	n.a.	Windust, 1992
<i>Temora longicornis</i>	<i>P. multiseries</i>	No effect on egg production or hatching success	48 h	0.06 and 0.65	–	–	–	Windust, 1992
<i>Calanus glacialis</i>	<i>P. multiseries</i>	No effect on behavioral responses (appetitive)	–	–	–	–	–	Windust, 1992
Rotifer <i>Brachionus plicatilis</i>	<i>P. multiseries</i>	Reduction in egg production; possible feeding inhibition	96 h; 15 d	3.71–3.98	–	18.5–76.2 µg g <sup>-1</sup>	–	Whyte et al., 1996
<i>Tigriopus californicus</i>	Dissolved DA (no range given)	Toxic: LC <sub>50</sub> = 8.62 µM = 2.68 µg ml <sup>-1</sup>	24 h	n.a.	n.a.	–	n.a.	Shaw et al., 1997
<i>Acartia tonsa</i> / <i>Temora longicornis</i>	<i>P. multiseries</i>	No grazing deterrence, selective grazing or effect on egg production or egg hatching success	14–26 h	0.14–8.10	–	0.37–0.82 ng cop <sup>-1</sup> / –	–	Lincoln et al., 2001
<i>Acartia tonsa</i>	<i>P. multiseries</i>	No grazing deterrence or selective grazing	16.5–20.5 h	1.3–2.3	–	3–7.4 ng cop <sup>-1</sup>	–	Tester et al., 2001
Krill <i>Euphausia</i>	<i>P. multiseries</i>	Effect on grazing pattern	24 h	0.15–1.2	–	34 µg g <sup>-1</sup>	–	Bargu et al.,

May 9, 2018

Grazing species (zooplankton or dinoflagellate)	Toxic <i>Pseudo-nitzschia</i> species or DA	Effect on zooplankton	Time period for experiment	DA (pg cell <sup>-1</sup> )	Induction of DA (pg cell <sup>-1</sup> )	DA concentration in zooplankton <sup>1,2</sup>	Retained as % of ingested DA	Reference
<i>pacifica</i>								
<i>Acartia clausi</i>	<i>P. multiseries</i>	No grazing deterrence, selective grazing, or effect on egg production or egg hatching success	48 h, 4 d	~1,100 fmol cop <sup>-1</sup>	n.a.	1.1 pmol cop <sup>-1</sup> ; 41–98 µg g <sup>-1</sup> DW = 255–609 µg g <sup>-1</sup>	4.8%	2003 Maneiro et al., 2005
Krill <i>Euphausia pacifica</i>	Dissolved DA (0.4 and 1 µg ml <sup>-1</sup> )	Decreased grazing rates (uncertain)	24 h	n.a.	n.a.	–	n.a.	Bargu et al., 2006
Dinoflagellate <i>Protoperdinium</i> sp.	Dissolved DA (0–1000 nM)	No effect on grazing rate	9 d	n.a.	n.a.	–	–	Olson and Lessard, 2010
Dinoflagellate <i>Protoperdinium</i> sp.	<i>Pseudo-nitzschia</i> spp.	No effect on grazing rate	9 d	0–4	–	–	–	Olson and Lessard, 2010
<i>Calanus finmarchicus</i>	<i>P. multiseries</i>	No effect on grazing rate	12 h	1.05–1.27	Yes induction; 2.42–2.52	11–46 ng cop <sup>-1</sup> / 5–23 µg g <sup>-1</sup>	12–34%	Leandro et al., 2010a
<i>Calanus finmarchicus</i> / <i>C. hyperboreus</i> / <i>C. glacialis</i>	<i>P. seriata</i>	Affected grazing pattern of <i>C. finmarchicus</i> and <i>C. hyperboreus</i> ; no lethal effect	12 h	1.83 2.29	Yes induction in one species;	290 µg g <sup>-1</sup> DW = 55 ng cop <sup>-1</sup> = 1,812 µg g <sup>-1</sup> / 118 µg g <sup>-1</sup> DW = 257 ng cop <sup>-1</sup> = 737 µg g <sup>-1</sup> / 68 µg g <sup>-1</sup> DW = 35 ng cop <sup>-1</sup> = 425 µg g <sup>-1</sup>	48% / 37% / 47%	Tammilehto et al., 2012
<i>Calanus hyperboreus</i> / <i>C. finmarchicus</i>	<i>P. seriata</i>	No grazing deterrence	8 d	0.01–0.1	Yes induction: 0.51–13.1 (+ up to 17 ng ml <sup>-1</sup> in water) / 2.6–4.2	–	–	Tammilehto et al., 2015

May 9, 2018

Grazing species (zooplankton or dinoflagellate)	Toxic <i>Pseudo-nitzschia</i> species or DA	Effect on zooplankton	Time period for experiment	DA (pg cell <sup>-1</sup> )	Induction of DA (pg cell <sup>-1</sup> )	DA concentration in zooplankton <sup>1,2</sup>	Retained as % of ingested DA	Reference
<i>Calanus</i> copepodite stages C3 and C4	<i>P. seriata</i>	No grazing deterrence	39 h	0.1	Yes induction: 2.6	70 ng cop <sup>-1</sup> = 410 $\mu\text{g g}^{-1}$ DW = 2,562	13	Harðardóttir et al., 2015
<i>Calanus</i> copepodite stages C3 and C4	<i>P. obtusa</i>	No grazing deterrence	39 h	Below detection	Yes induction: 0.2±0.1	8±2 ng cop <sup>-1</sup> ; 48 $\mu\text{g g}^{-1}$ DW	6	Harðardóttir et al., 2015
<i>Calanus</i> copepodite stages C3 and C4	<i>P. seriata</i>	No grazing deterrence	8 d	0.3±0.1– 0.4±0.0	Yes induction: 13.3±4.9	8–70 ng cop <sup>-1</sup> = 48–410 $\mu\text{g g}^{-1}$ DW	–	Harðardóttir et al., 2015
<i>Calanus</i> <i>finmarchicus</i>	<i>P. seriata</i>	No effect on grazing rate, egg production or hatching success	10 d	0.46	Yes induction: 2.34	0.91 ng cop <sup>-1</sup> ; 0.01 ng DA $\mu\text{g carbon}^{-1}$ ~30 $\mu\text{g g}^{-1}$	Miesner et al., 2016	
<i>C. glacialis</i>	<i>P. seriata</i>	No effect on grazing rate, egg production or hatching success	10 d	0.36	Yes induction: 1.98	20.98 ng cop <sup>-1</sup> ; 0.08 ng DA $\mu\text{g}$ carbon <sup>-1</sup> ~255 $\mu\text{g}$ $\text{g}^{-1}$	Miesner et al., 2016	

<sup>1</sup>wet weight unless otherwise stated.

<sup>2</sup>recalculated to wet weight (WW) based on 1 mg WW = 0.16 mg dry weight (DW).

Table 8. Summary of factors affecting the production of domoic acid (DA) or cell physiology by *Pseudo-nitzschia* spp. in culture; studies updated since the reviews of Lelong et al. (2012a) and Trainer et al. (2012).

Factor	Species	Effect on DA production or physiology	Reference
<b>Physicochemical</b>			
Temperature	<i>P. subcurvata</i>	Warming increases cell volume, growth rate No change in C:N and N:P ratios, but C:P, C:Si, and C:Chl <i>a</i> ratios significantly affected	Zhu et al., 2016 Zhu et al., 2017a
Irradiance	<i>P. australis</i> , <i>P. turgidula</i>	Higher growth and carbon uptake for neritic <i>P. australis</i> than oceanic <i>P. turgidula</i> at the same saturating irradiance level	Bill et al., 2012
	<i>P. cuspidata</i>	Higher DA production for slow-growing cells at subsaturating irradiance than at saturating irradiance	Auro and Cochlan, 2013
	<i>P. australis</i>	Increase in DA production after shift up in irradiance, accompanied by increase in superoxide dismutase activity	Woods, 2016
Salinity	<i>P. circumpora</i>	Growth only at salinities of 25–35; optimum growth at 30	Lim et al., 2014a
	<i>P. pungens</i>	Cell number decreased after 1 day exposure to salinity of 16; high number of dead cells at salinities of 4 and 8; inter-strain differences	Markina and Aizdaicher, 2016
		Low growth at salinity of 5, highest at 15–30, lower at 35; low DA production at 5–15, highest at 35	Pednekar et al., 2018
pH / $p\text{CO}_2$	<i>P. australis</i>	Lower growth at pH 7.8 than at 7.9–8.1; no change in DA production with pH during exponential growth, but it increased with decreasing pH during stationary phase	Wingert, 2017
	<i>P. pseudodelicatissima</i>	Increase in $p\text{CO}_2$ leads to increase in C:P and decrease in Si:C and Si:N ratios	Sugie and Yoshimura, 2013
	<i>P. subcurvata</i>	Growth rate increase with $p\text{CO}_2$ ; increase in C:Si ratio at higher	Zhu et al., 2017a

Factor	Species	Effect on DA production or physiology	Reference
		$p\text{CO}_2$ ; no change in C:N, N:P, C:P ratios	
<b>Trace metals</b>			
Cu	<i>P. delicatissima</i>	Mortality increased after 24-h Cu exposure; addition of DA does not protect survival	Lelong et al., 2012
	<i>P. multiseries</i>	No change in DA with Cu exposure; survived after 28-h exposue to Cu, but bacteria decreased	
	<i>P. delicatissima</i>	No change in photosynthetic efficiency; does not induce Fe limitation; no DA production	Lelong et al., 2013
Fe	<i>P. delicatissima</i>	Fe limitation decreases chlorophyll content, esterase activity, photosynthetic efficiency; no DA production	Lelong et al., 2013
	<i>P. subcurvata</i>	Fe limitation decreases C:P ratio, cell volume, photosynthetic efficiency	Zhu et al., 2016
	<i>P. multiseries</i>	Higher DA production and cellular Chl <i>a</i> at high Fe than low Fe concentrations	Sobrinho et al., 2017
<b>Nutritional</b>			
Nitrogen	<i>P. cuspidata</i>	Higher DA production when grown on $\text{NO}_3^-$ or $\text{NH}_4^+$ than on urea at subsaturating irradiance during exponential growth	Auro and Cochlan, 2013
	<i>P. fryselliana</i>	N preference follows the order: $\text{NH}_4^+ > \text{NO}_3^- >$ urea at high N concentrations, but higher affinity for urea than for $\text{NO}_3^-$ or $\text{NH}_4^+$ at low concentrations	
Nitrogen	<i>P. multiseries</i>	Growth rate decreased in the following order: $\text{NH}_4^+ > \text{NO}_3^- > > \text{arginine} > \text{glutamine} > \text{urea} > \text{glutamate}$ ; highest DA production on urea and $\text{NO}_3^-$	Martin-Jézéquel et al., 2015
	<i>P. australis</i>	Growth rate decreased in the following order: glutamine $> \text{NH}_4^+ > \text{NO}_3^- > \text{arginine} > \text{urea} > \text{glutamate} > \text{taurine}$ ; highest DA production on glutamate and $\text{NH}_4^+$ ; none on glutamine,	

Factor	Species	Effect on DA production or physiology	Reference
Nitrogen	<i>P. calliantha</i>	arginine or taurine Growth rate decreased in the following order: urea > $\text{NO}_3^-$ > $\text{NH}_4^+$ ; DA production not detected	Garali et al., 2016
Nitrogen	<i>P. multiseries</i>	Equal growth on $\text{NO}_3^-$ , $\text{NH}_4^+$ , urea; DA production on urea > than on $\text{NO}_3^-$ , $\text{NH}_4^+$	Radan and Cochlan, 2018
Nitrogen, phosphorus	<i>P. delicatissima</i> , <i>P. galaxiae</i>	Both species had a high growth requirement for N and low requirement for P; <i>P. galaxiae</i> had a superior competitive ability under N- and P-limitation	Smeti et al., 2015
Silicon	<i>P. multiseries</i>	Si and P deficiency induce alkaline phosphataze enzyme activity	Fuentes et al., 2014
<b>Interactions</b>			
Irradiance-nitrogen	<i>P. cuspidata</i>	Faster growth at high irradiance at all N substrates; DA production, growth not favored by any of the N substrates at saturating irradiance	Auro and Cochlan, 2013
Temperature-irradiance	<i>P. australis</i>	DA production increases with increasing temperature and irradiance	Thorel et al., 2014
Temperature-UV radiation	<i>P. pungens</i>	Lower levels of photoinhibition and faster recovery rates at higher than at lower temperatures	Chen et al., 2018
Temperature- $p\text{CO}_2$ -Nitrogen	<i>P. multiseries</i>	Higher cellular DA at 23 than 19 °C, with 800 ppm $p\text{CO}_2$ and with nitrate	Tatters et al., 2018
Temperature-iron	<i>P. subcurvata</i>	Growth and carbon fixation rates significantly stimulated by the interactive effects of concurrent temperature and Fe increase	Zhu et al., 2016
Temperature- $p\text{CO}_2$		Temperature and $p\text{CO}_2$ interactively decreased C:Chl <i>a</i> ratio	Zhu et al., 2017a
$p\text{CO}_2$ -silicate	<i>P. fraudulenta</i>	Increased $p\text{CO}_2$ , in combination with silicate limitation, greatly increases (>250%) DA production	Tatters et al., 2012

Factor	Species	Effect on DA production or physiology	Reference
<i>pCO<sub>2</sub></i> -iron	<i>P. pseudodelicatissima</i>	Increase in Fe and <i>pCO<sub>2</sub></i> leads to decrease in Si:C and Si:N ratios	Sugie and Yoshimura, 2013
Copper-iron	<i>P. delicatissima</i>	Able to grow under severe Fe and Cu co-limitation; decreased lipid content, esterase activity	Lelong et al., 2013
Copper-silicate	<i>P. multiseries</i>	Si uptake increased when Cu and Si are co-limiting growth; highest DA production under high Si/high Cu and low Si/sufficient Cu	Fuentes and Wikfors, 2013
Nitrate-silicate-phosphorus	<i>P. australis</i> , <i>P. pungens</i> , <i>P. fraudulenta</i>	High DA production with low initial phosphate and high nitrate and silicate concentrations, or with low initial nitrate and silicate and high phosphate concentrations	Lema et al., 2017
<b>Biological</b>			
Grazing copepods	<i>P. seriata</i>	DA production enhanced by 3300% in presence of grazing <i>Calanus</i> copepodites	Harðardóttir et al., 2015
	<i>P. seriata</i>	DA production induced in the previously non-toxic <i>P. obtusa</i>	Tammilehto et al., 2015
Bacteria	<i>P. fraudulenta</i> , <i>P. multiseries</i>	DA production increased in the presence of copepods ( <i>Calanus hyperboreus</i> , <i>C. finmarchicus</i> )	Sison-Mangus et al., 2014
	<i>P. fraudulenta</i>	Only foreign bacteria induced an increase in DA production Axenic culture had a higher DA content than the original non-axenic culture	