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Conditions Promoting Extreme *Pseudo-nitzschia* Events in the Eastern Pacific but not the Western Pacific

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Conditions Promoting Extreme *Pseudo-nitzschia* Events in the Eastern Pacific but not the Western Pacific

Edited by
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September 2017

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Foreword

The use of numbered biogeographic regions (10–25) in the Overview section (section 2) follows the terminology for numbered areas named in accordance with Decision 2016/s/11(vii) adopted by the PICES Governing Council. A map showing these biogeographic regions is provided in Appendix 1. The content of the country reports (sections 3 to 7), including the use of different geographic names remains the responsibility of the contributing authors.

Robin Brown
Executive Secretary, PICES

1 Introduction

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National Scientific Center of Marine Biology of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia

This publication includes reports given at a workshop on “Conditions promoting *Pseudo-nitzschia* events in the eastern Pacific but not the western Pacific” co-convened by Drs. Vera L. Trainer (USA) and Polina Kameneva (Russia) on November 3, 2016 at the PICES 2016 Annual Meeting in San Diego, USA (see Appendix 2). The workshop was focused on the diatom, *Pseudo-nitzschia*, historically associated with dramatic negative impacts on the economy of shellfish harvests, the well-being of marine life and human health in the northeastern Pacific PICES member countries (Canada, USA), with little or no impact in the northwestern Pacific (China, Japan, Korea and Russia).

Diatoms of the genus *Pseudo-nitzschia* (H. Peragallo, 1900) are widely distributed in the plankton assemblages of both coastal and open ocean waters (Hasle *et al*., 1996; reviewed in Lelong *et al*., 2012 and Trainer *et al*., 2012). Nineteen of the 46 currently recognized *Pseudo-nitzschia* species are known to produce the potent marine neurotoxin, domoic acid (DA; Teng *et al*., 2016), which can accumulate in fish and shellfish and leads to Amnesic Shellfish Poisoning (ASP) in humans, seabirds and marine mammals when they consume contaminated fish and shellfish (*e.g*., Lefebvre *et al*., 2002). It has been suggested that repeated exposure to low levels of DA may cause neuropathic injury to vertebrates, including people (Grattan *et al*., 2007).

There is clear evidence of contrasting occurrence and impact of the toxin-producing diatom, *Pseudo-nitzschia,* between the northwestern and northeastern Pacific. In Canadian and American Pacific waters, numerous cases of toxic *Pseudo-nitzschia* bloom events and mortalities of sea birds, sea lions, whales, and other marine mammals have been registered from the time of the discovery of DA in 1987 (Bates *et al*., 1989) up to the present day. In 2015, a massive bloom spanning from California to Alaska, linked to anomalously warm ocean conditions associated with both El Niño and the Pacific Decadal Oscillation (PDO cycles), had major impacts on the economic viability of the shellfish industry and on marine life health (McCabe *et al*., 2016). In contrast, *Pseudo-nitzschia* did not cause significant economic losses in the northwestern Pacific in 2015. Gathered data provide a unique opportunity to make East–West Pacific comparisons, and to identify and rank those environmental factors which promote *Pseudo-nitzschia* success as a harmful algae species. Special attention should be paid to the climate-driven environmental changes such as ocean acidification, warming of the sea surface, stratification pattern changes, and the availability of nutrients to definitively characterize those factors promoting toxic blooms. The recent PICES-supported symposium on HABs and Climate Change (May 19–22, 2015 Göteburg, Sweden) and the related synthesis paper (Wells *et al*., 2015) emphasize the importance of studying such extreme events to further our understanding of climate impacts on toxic blooms.

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2 Overview of eastern *versus* western Pacific differences in *Pseudo-nitzschia* abundance, speciation, and domoic acid impacts

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In the American and Canadian Pacific, long-lasting and intensive toxigenic blooms of *Pseudo-nitzschia* occur most often in spring and fall within extensive strong upwelling zones near retentive sites. Recent U.S. coastwide toxic bloom events are directly related to warm sea surface temperature anomalies associated with the El Niño and Pacific Decadal Oscillation (PDO) cycles, leading to faster growth (and northward expansion of *P. australis* habitat as seen in 2015), followed by increased toxin accumulation in razor clams (McCabe *et al*., 2016). Although many (and likely all) *Pseudo-nitzschia* species have the capacity to produce the neurotoxin domoic acid (DA), the most toxic *Pseudo-nitzschia* species within the California Current system are *P. australis*, *P. multiseries* and *P.* cf. *pseudodelicatissima* (also specified as *P. cuspidata*; reviewed in Trainer *et al*., 2012), whereas the most problematic highly toxic species off California appears to be *P. australis* (Table 1).

Smaller, more localized blooms of *Pseudo-nitzschia* species occur within extensive coastal areas of the northwestern Pacific. These events typically occur during fall, winter, and summer in enclosed and semi-enclosed areas influenced by intensive riverine discharge and in outer coastal areas near localized upwelling. Although no serious cases of amnesic shellfish poisoning (ASP) have been recorded in the northwestern Pacific region, DA-producing plankton have been found repeatedly in Russia, Japan, and Korea, with traces of DA (up to 3 mg kg^{-1}) recorded in mussels, scallops and razor clams. Contrary to the northeastern Pacific, *P. multiseries* generally is the most common highly toxic *Pseudo-nitzschia* species in the northwestern Pacific region (Table 1).

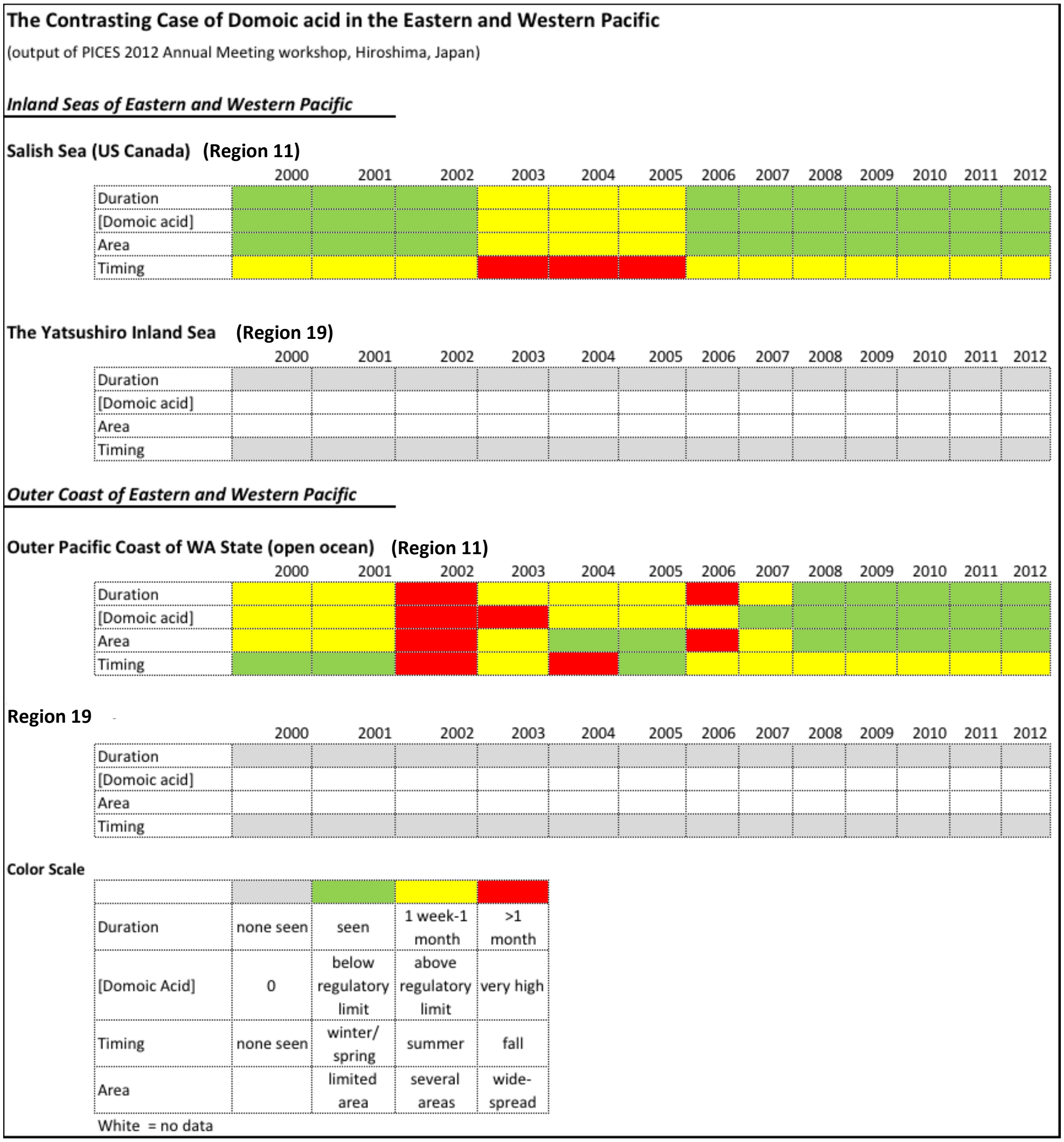
Table 1 Selected species of *Pseudo-nitzschia* isolated from the eastern and western Pacific that produce domoic acid (DA) in laboratory cultures.

	Maximum DA concentration (pg DA cell^{-1})	
Species	**Eastern Pacific**	**Western Pacific**
P. australis	21.8–37, Monterey Bay, USA[a]	1.1–2.0, New Zealand[b]
	1.74, Chile[c]	DA detected but concentration not reported, Australia[d]
P. multiseries	0.021–11.2, Monterey Bay, USA[e]	20.8, Peter the Great Bay, Russia[f]
		1.03–2.4, Jinhal and Chinhae bays, Korea[g]
		1.15–140, Okkiray Bay, Japan[h]
		5.7, Ofunato Bay, Japan[i]
P. cuspidata	0.019–0.031, Washington State USA[j]	25.4, Australia[k]

[a]Garrison *et al*., 1992; Guannel *et al*., 2011; [b]Rhodes *et al*., 1996, 2004; [c]Alvarez *et al*., 2009; [d]Lapworth *et al*., 2001; [e]Maldonaldo *et al*., 2002; Doucette *et al*., 2008; [f]Orlova *et al*., 2008; [g]Lee and Baik, 1997; Cho *et al*., 2001; [h]Lundholm *et al*., 2004; Trimborn *et al*., 2008; [i]Kotaki *et al*., 1999; [j]Auro, 2007 and Trainer *et al*., 2009b; [k]Ajani *et al*., 2013

The duration, toxin concentrations, abundance, area and timing of *Pseudo-nitzschia* blooms are drastically different between the eastern and western Pacific. The contrasting cases of harmful algal blooms (HABs) in these two regions, examined during the PICES 2012 Annual Meeting in Hiroshima, Japan, highlighted these differences. An example data set comparing the relative abundance of *Pseudo-nitzschia* and relative concentrations and impacts of DA from inland and coastal waterways of the U.S./Canada and Japan is shown in Figure 1.

Pseudo-nitzschia multiseries, isolated and cultured from Russian and Japanese waters, were found to contain a maximum of 5390 ng ml^{-1} and 317 ng ml^{-1} DA, respectively (Kotaki *et al.*, 1999; Orlova *et al.*, 2008). In contrast, estimates of maximum particulate DA in cultured isolates of *Pseudo-nitzschia* from Washington State are: *P. australis* (1.0 ng ml^{-1}), *P. multiseries* (24.0 ng ml^{-1}), *P.* cf. *pseudodelicatissima* (0.1 ng ml^{-1}; Baugh *et al.*, 2006) and from California: *P. australis* (up to 90 ng ml^{-1}; Wingert, 2017). However, natural monospecific blooms of *P. australis* have estimated maximum concentrations of ~20 mg ml^{-1}; McCabe *et al*., 2016) and of *P. cuspidata* (formerly identified as part of the *P.* cf. *pseudodelicatissima* complex) can reach concentrations of 13.3 ng ml^{-1}; Trainer *et al.*, 2009b, reported as 43 nmole L^{-1} particulate DA). However, it is likely that DA content can be much higher; in laboratory experiments a high proportion of DA is released from the cell (Wells *et al.*, 2005), and dissolved DA is measured within *Pseudo-nitzschia* blooms (*e.g.*, Trainer *et al.*, 2009a). It should be noted that in Russian western Pacific waters, and perhaps elsewhere in the northwestern Pacific, *P. multiseries* abundance has decreased drastically since 2002 (I. Stonik, unpubl. data), which may be considered as one of the probable causes of the low impact of *Pseudo-nitzschia* blooms in the northwestern Pacific over the past 15 years.



The Contrasting Case of Domoic acid in the Eastern and Western Pacific

(output of PICES 2012 Annual Meeting workshop, Hiroshima, Japan)

Inland Seas of Eastern and Western Pacific

Salish Sea (US Canada) (Region 11)

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Duration	🟩	🟩	🟩	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟩
[Domoic acid]	🟩	🟩	🟩	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Area	🟩	🟩	🟩	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩	🟩
Timing	🟨	🟨	🟨	🟥	🟥	🟥	🟨	🟨	🟨	🟨	🟨	🟨	🟨

The Yatsushiro Inland Sea (Region 19)

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Duration	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
[Domoic acid]													
Area													
Timing	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨

Outer Coast of Eastern and Western Pacific

Outer Pacific Coast of WA State (open ocean) (Region 11)

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Duration	🟨	🟨	🟥	🟨	🟨	🟨	🟥	🟨	🟩	🟩	🟩	🟩	🟩
[Domoic acid]	🟨	🟨	🟥	🟥	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	🟩
Area	🟨	🟨	🟥	🟨	🟩	🟩	🟥	🟨	🟩	🟩	🟩	🟩	🟩
Timing	🟩	🟩	🟥	🟨	🟥	🟩	🟨	🟨	🟨	🟨	🟨	🟨	🟨

Region 19

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Duration	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
[Domoic acid]													
Area													
Timing	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨

Color Scale

	▨	🟩	🟨	🟥
Duration	none seen	seen	1 week-1 month	>1 month
[Domoic Acid]	0	below regulatory limit	above regulatory limit	very high
Timing	none seen	winter/ spring	summer	fall
Area		limited area	several areas	wide-spread

White = no data

Fig. 1 Domoic acid concentrations and duration, timing and area of toxic events in U.S./Canadian and Japanese waters, including the inland seas (Salish Sea and Yatsushiro Sea) and outer waters (Region 19 and Pacific coast of Washington State – Region 11) from 2000–2012. The color scale describes intensity, extent or severity in each category.

Table 2 Evidence of the impact of *Pseudo-nitzschia* and domoic acid in the eastern and western Pacific.

Area	Damage
Coastal waters of the northeastern Pacific	Numerous cases of toxic *Pseudo-nitzschia* bloom events and related high DA concentrations in shellfish (up to 300 mg kg^{-1}) and events of sea bird, sea lion, whale, and other marine mammal mortalities (reviewed in Lelong *et al*., 2012 and Trainer *et al*., 2012).
Coastal waters of the northwestern Pacific	Cases of high DA content in mussels (up to 33 mg kg^{-1}) related to *P. multiseries* blooms were documented in the 1990s in Korea (Lee and Baik, 1997). No cases of ASP (animal mortality) were found. Traces of DA (up to 3 mg kg^{-1}) were found in shellfish from Japan and Russia.
South Asian waters	DA contamination in shellfish reported in the Philippines, Vietnam and some tropical Asian countries, with no records of human poisoning (reviewed in Fukuyo *et al*., 2011).

DA = domoic acid, ASP = amnesic shellfish poisoning

It is not surprising then, that the ecological and economic impacts of DA differ vastly between the northeastern and the northwestern Pacific Ocean (Table 2). There have been numerous highly toxic events along the U.S. and Canadian Pacific coasts over the past 15 years, in many cases leading to poisoning of marine mammals and birds, and high economic costs (Rowles *et al*., 2017). The most recent of these blooms (in 2015) lasted many consecutive months and caused prolonged closures of shellfish and crab fisheries (McCabe *et al*., 2016). Even so, blooms of toxigenic *Pseudo-nitzschia* spp. in northeastern Pacific coastal waters do not always generate toxic conditions. In contrast, *Pseudo-nitzschia* blooms in the northwestern Pacific are rarely highly toxic, and economic losses are minor in comparison (Table 2). While there is no definitive understanding of the reasons for these stark differences, some of the likely environmental factors potentially may include the following:

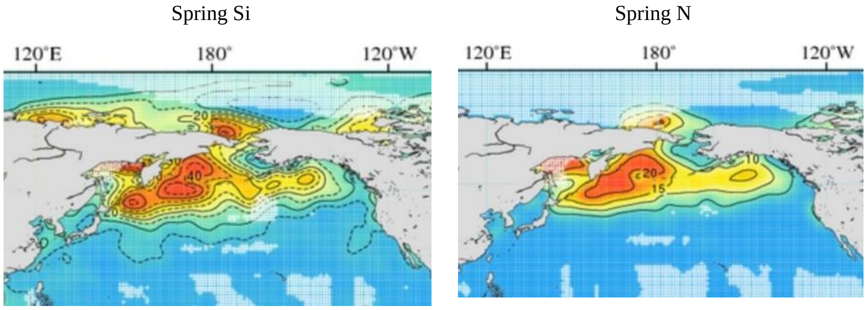
1) Intensive, broad upwelling zones along the continental shelves off the eastern margins of the Pacific appear to promote the competitive success of *Pseudo-nitzschia* species, and once firmly established as a seed population, the increased nutrient flux into surface waters facilitates the generation of high *Pseudo-nitzschia* biomass. By contrast, intensive upwelling zones are far more restricted in scale in western Pacific nearshore waters, where the coastal oceanography is influenced more by enclosed and semi-enclosed seas and periodic intensive riverine discharge (Table 3).

Table 3 Northeastern–northwestern Pacific general hydrological differences (modified from McKinnell and Dagg, 2010).

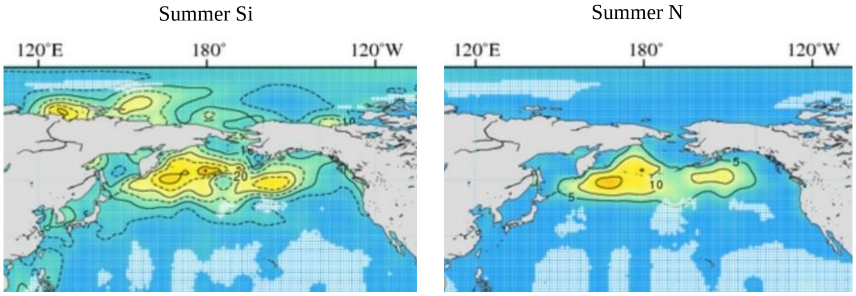
Northeastern Pacific	Northwestern Pacific
Intensive upwelling (California Current System)	Summer and winter monsoons, coastal upwelling during fall months (northwestern portion of Region 19)
Freshwater input and oceanic eddies (*e.g.*, in the Alaska Current region)	Large freshwater input *via* Amur River and local upwellings (Sea of Okhotsk). Inflows from the Kuroshio and Changjiang River (Yellow and East China seas (Regions 20 and 21)

The interannual variation of upwelling in the Russian waters of Region 19 is determined mainly by the varying pressure of the Siberian High, which is a major center of action in the East Asian region (Zhabin *et al.*, 2017). Upwelling intensity is also influenced by the magnitude of the atmospheric pressure gradient between the eastern part of the continent and the North Pacific Ocean. An increased upwelling intensity is observed in years with a developed Siberian High. Accordingly, during a weak Siberian High, the upwelling intensity in the northwestern portion of Region 19 declines. Intensive upwelling events appear to facilitate the competitive success of *Pseudo-nitzschia* species. For this reason, Zuenko and Rachkov (2015) hypothesize that the decrease in the abundance of *P. multiseries* in the 1990s in the northwestern portion of Region 19 can be related to the long-term tendency of the Siberian High and the summer monsoon to weaken during 1990–2015.

2) The supply of macronutrients (N, P, Si) often is cited as a contributing factor to increasing intracellular concentrations of DA in laboratory cultures (reviewed in Lelong *et al.*, 2012), although there is no consistent pattern for this relationship in coastal waters (*e.g.*, Trainer *et al.*, 2009a). Sea surface concentrations of dissolved nutrients (N, P, Si) are higher in the northwestern Pacific Ocean than in the northeastern region during winter, but are similar by mid-summer, reflecting a greater nutrient drawdown (Fig. 2). In addition, given the importance of Si in building diatom cell walls, it may be no coincidence that the Si/N drawdown ratio is greater in the northwestern Pacific than the northeastern Pacific and that diatoms have a greater predominance along the Asian coast (Yasunaka *et al.*, 2014). The success of toxic *Pseudo-nitzschia* and accumulation of cellular DA under nutrient stress in the northeastern Pacific have been demonstrated in recent field studies (McCabe *et al.*, 2016; McKibben *et al.*, 2017). *Pseudo-nitzschia* can grow and produce DA using various nitrogen sources (nitrate, ammonium and urea; Armstrong *et al.*, 2007). *Pseudo-nitzschia*'s resilience is demonstrated also in its ability to occupy the same ecological niche as flagellates (*e.g.*, the massive euglenoid bloom described in Trainer *et al.*, 2009b).


Spring Si

Spring N


Summer Si

Summer N

Fig. 2 World Ocean Atlas 2013 annual climatology (Garcia *et al.*, 2014). For silicate and nitrate concentrations (µmol L^{-1}) see https://www.nodc.noaa.gov/OC5/woa13f/index.html. Contour interval = 5.

3) Coastal waters of the northwestern Pacific obtain more dissolved micronutrients from riverine discharge (Amur, Yangtze and others) than in the northeastern Pacific, and there is greater iron enrichment in the west as a consequence the southward flow of the Oyashio Current enriched with iron from the Sea of Okhotsk (Nishioka *et al*., 2011; Whitney, 2011). Areas with limiting iron concentrations in the northeastern Pacific have been proposed to be sites where *Pseudo-nitzschia* outcompete other phytoplankton and become highly toxic (Trainer *et al*., 2009b). There is evidence for a direct linkage between the rate of DA production and limitation of cell growth by the micronutrient Fe (Maldonado *et al*., 2002; Wells *et al*., 2005). The more widely distributed riverine iron inputs, generally broader continental shelves (thus iron input from marginal shelf sediments), and higher rates of aerosol iron inputs (Fig. 3) in the northwestern Pacific region may all contribute to reducing the rates of DA production, and thus retained intracellular DA concentrations (*i.e*., reduced cell toxicity).

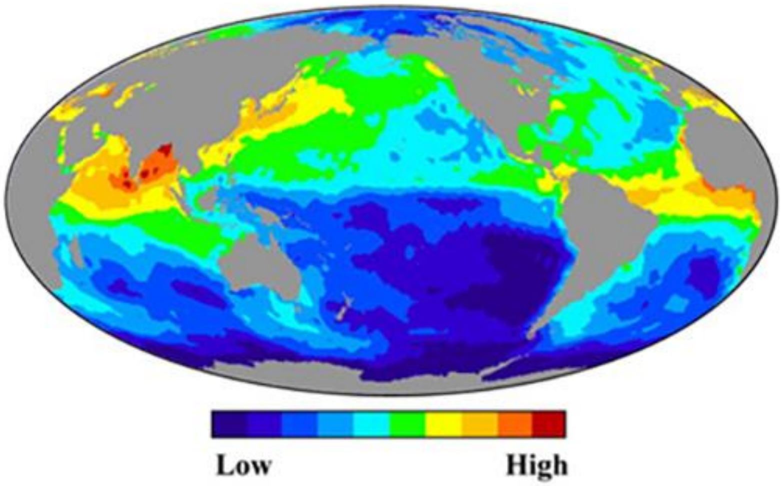


Fig. 3 Model of the distribution of the concentrations of iron dust in surface waters of the Pacific Ocean based on real-world observations (modified from https://www.nasa.gov/topics/earth/features/modis_fluorescence_briefing.html, Mike Behrenfeld, Oregon State University).

It is clear that frequent and intense *Pseudo-nitzschia* blooms cause high DA toxicity in shellfish and the mortality of marine birds and mammals in the northeastern Pacific, and that despite the presence of *Pseudo-nitzschia* species capable of DA production in the northwestern Pacific, sometimes reaching bloom concentrations of >1 million cells L^{-1}, no severe toxic episodes have been reported from the northwestern Pacific. Part of this disparity may be related to the apparent dominance of only one highly toxic species (*P. multiseries*) in the northwestern region *vs*. three primary species (*P. multiseries*, *P. australis*, and *P. cuspidata*) in the northeastern Pacific, but it is far more likely that differences in the frequencies and magnitude of these toxic bloom events are tied to underlying differences in oceanographic conditions in ways that are not yet understood. Two challenges guide the way forward. The first is to understand the fundamental limitations to the development of large blooms of toxigenic *Pseudo-nitzschia* spp., *vs*. other diatom species, and how these drivers differ between the northeastern and northwestern Pacific. The second is to constrain the environmental circumstances that, once a *Pseudo-nitzschia* bloom develops, leads to increased intracellular retention of DA. The North Pacific provides a unique platform that, through collaboration among PICES member countries, can serve as a resource for investigating the root mechanistic causes for toxic diatom blooms.

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3 Amnesic shellfish poisoning potential in Japan

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Monitoring domoic acid contamination in shellfish

Domoic acid (DA; Fig. 1) was first recognized as an amnesic shellfish poisoning (ASP) toxin in Canada (Wright *et al*., 1989) and the causative organism was identified as *Pseudo-nitzschia multiseries* (formerly *Nitzschia pungens* f. *multiseries*) (Bates *et al*., 1989). After this incident, monitoring systems were established in Canada, U.S., and in some European countries so as to prevent further ASP incidents. The Japanese government also took an interest in determining whether ASP occurs in Japan. The Fisheries Agency started to investigate the possibility of shellfish as a vector of ASP in Japan. This investigation was initiated by starting a series of projects coordinated through regional branches of the Fisheries Research Institute and a few university teams, including Kitasato University.

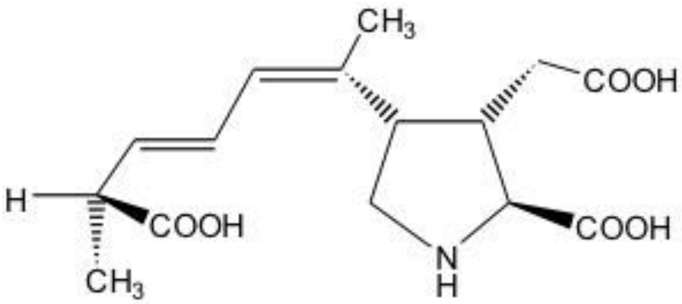

Fig. 1 Structure of domoic acid (DA).

In order to determine the possibility of ASP occurrences in eastern Japan, the monitoring of DA in shellfish began in Ofunato Bay in 1994. Five specimens of cultured scallops, *Mizuhopecten yessoensis*, and mussels, *Mytilus galloprovincialis*, were collected from the Shizu station (Ofunato Bay, station A, Fig. 2) monthly from April 1994 through February 1995. The digestive gland of scallops and edible part of mussels were extracted with 50% methanol, then analyzed by HPLC with UV detection (242 nm) after purification through a Sep-Pack C18 cartridge (Quilliam *et al*., 1989). DA was detected from November 1994 to

February 1995 (Table 1, Kotaki *et al.*, 1996). Maximum toxin levels were 0.8 µg g^{-1} (January 1995) in scallops and 2.8 µg g^{-1} (November 1994) in mussels. These values were well below the regulatory action level of 20 µg g^{-1}. This monitoring was performed monthly until 1997 and irregularly thereafter but since that time, no DA has been detected in shellfish.

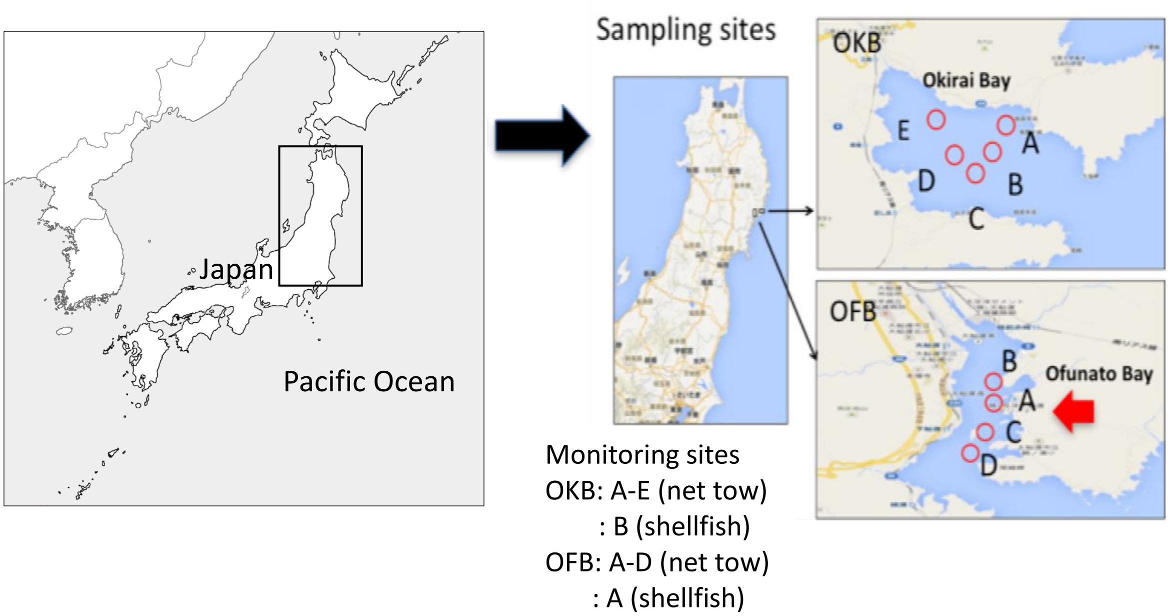

Fig. 2 Map of the monitoring sites. Monitoring of DA in shellfish and *Pseudo-nitzschia* were performed only at the Ofunato Bay station A (known for PSP toxins; Ogata *et al*., 1982). Monitoring of DA in shellfish after the 2011 tsunami was performed at Ofunato Bay station A and Okirai Bay station B. Monitoring sites OKB: A–E (net tow), B (shellfish), OFB: A–D (net tow), A (shellfish).

Table 1 DA concentrations in shellfish collected from Ofunato Bay (from Kotaki *et al*., 1996).

Date of collection[1]	**Mussel (µg g^{-1})**[2]	**Scallop (µg g^{-1})**[3]
Nov. 16, 1994	2.8	0.3
Dec. 14, 1994	0.5	ND
Jan. 11, 1995	0.5	0.8
Feb. 15, 1995	1.2	ND

ND = not detected

[1] Shellfish were collected from April, 1994 to February, 1995.

[2] Edible tissue was used for the analysis.

[3] Digestive gland was used for the analysis.

Screening of domoic acid-producing diatoms

Screening for DA-producing *Pseudo-nitzschia* was also performed in Ofunato Bay in 1994. Pennate diatoms were isolated from plankton net samples obtained at the same station (see Fig. 2) and cultured for the detection of DA by HPLC-fluorescence analysis (Pocklington *et al*., 1990). DA was analyzed two weeks after cells reached stationary growth, at about three to four weeks in culture, because it has been shown that DA content increases in the late stationary growth phase for some species of *Pseudo-nitzschia* (Bates *et al*., 1991). One out of 44 isolates showed a large peak identical to the known DA peak (Kotaki *et al*., 1999). The strain was mass cultured and confirmed to produce DA by electrospray ionization mass-spectrometry (ESI/MS) and proton nuclear magnetic resonance (NMR). This isolate was morphologically identified as *P. multiseries*, the same species as identified in the Canadian ASP incident. The distribution of *P. multiseries* was assessed in Ofunato Bay and nearby Okirai Bay. All of the *P. multiseries* isolated from these locations were confirmed to produce high levels of DA in culture experiments. The cells grew from 1,000 cells mL^{-1} to approximately 100,000 cells mL^{-1} in one week, reaching a maximum of approximately 120,000 cells mL^{-1} at day 12 and decreasing gradually thereafter until the last day of culture (day 27; Fig. 3). DA did not increase much during the exponential growth phase. However, DA content started to increase one week after reaching the stationary growth phase and suddenly increased after two weeks in culture until the end of the culture, with a maximum DA concentration of 6.7 pg cell^{-1}. High DA content was observed only in the late stationary growth phase.

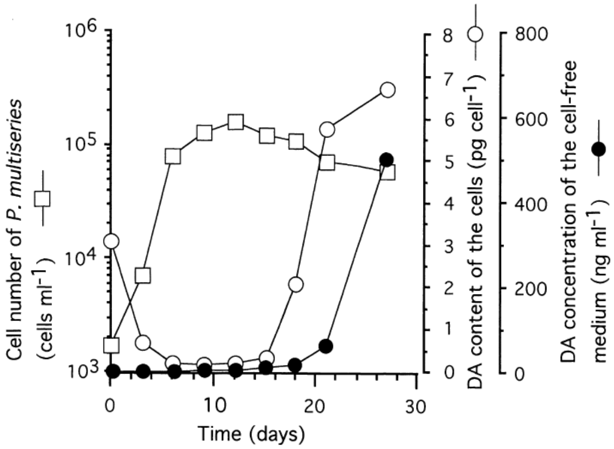

Fig. 3 Cell growth and DA production in *Pseudo-nitzschia multiseries* (Kotaki *et al*., 1999).

Domoic acid-producing diatoms other than *P. multiseries*

Ofunato Bay isolates of *Pseudo-nitzschia* were cultured for DA measurement using the same protocol as for *P. multiseries*, followed by morphological identification. Using HPLC-fluorescence analyses, the *Pseudo-nitzschia* species *P. pseudodelicatissima*, *P. delicatissima*, *P. pungens*, *P. turgidula*, *P. fraudulenta*, *P. cuspidata*, *P. subpacifica*, *P. subfraudulenta*, *P. heimii* and two unidentified *Pseudo-nitzschia*-like pennate diatoms were confirmed to produce low levels of DA (< 1 pg cell^{-1}) (Kotaki, 2008).

Monitoring after the 2011 Great East Japan Earthquake

A large tsunami that occurred following the 2011 Great East Japan Earthquake impacted Ofunato Bay and Okirai Bay. The habitat of *Pseudo-nitzschia* and their association with bacterial assemblages may have been altered in response to the tsunami. To estimate the ASP potential after this disaster, monitoring of DA in scallops and mussels was performed every three months at one station in both bays in 2013, 2014 and 2015. At the same time, DA analysis was performed by HPLC with fluorescence detection on plankton net samples towed twice from 20 m depth at five stations in Okirai Bay and 2 to 4 stations in Ofunato Bay (Fig. 2). DA was not detected in any of the shellfish samples during the monitoring period. However, DA was detected in the plankton net tow samples at concentrations (converted to 1 L of seawater equivalent) of 45–330 pg L^{-1} (Ofunato Bay, September and October, 2013), 16–205 pg L^{-1} (Okirai Bay, June and October, 2013), 7–163 pg L^{-1} (Ofunato Bay, Jun 2014 and January 2015), 17–154 pg L^{-1} (Okirai Bay, May, June and August 2014), 7–720 pg L^{-1} (Ofunato Bay, September and December 2015) and 33–56 pg L^{-1} (Okirai Bay, May 2015).

Representative positive samples collected in 2014 and 2015 were further purified by a Sep-Pak C18 cartridge and DA was confirmed by liquid chromatography/time-of-flight/tandem mass spectrometry (LC-TOF MS/MS). An attempt was made to isolate *Pseudo-nitzschia* from each net sample. In 2013, three *P. multiseries* isolates showed low DA content (a mean DA concentration of 0.64 ± 0.38 pg $cell^{-1}$) in culture experiments. In 2014, one isolate of *P. multiseries* was established and showed high DA content after five weeks in culture (314 ng mL^{-1}, 9.0 pg $cell^{-1}$). These results suggest that the origin of at least some of the DA in plankton net samples was *P. multiseries*. These results showed that although *P. multiseries* was present in both bays after the 2011 tsunami, this species did not bloom to high enough concentrations for significant levels of DA to be accumulated by shellfish. The ASP potential in both bays before and after the disaster appeared to be low.

Factors affecting DA production

The effects of nitrogen ($NO_3 + NO_2$), phosphate (PO_4), silicate (SiO_2), and dissolved iron on DA production in batch culture experiments were investigated under axenic and non-axenic conditions. As shown in Fig. 4A, the growth of both axenic and non-axenic cultures was almost the same, reaching stationary growth phase after ~10 days and maintaining the same cell concentrations until the end of the culture experiment (37 days). However, DA production was very different between axenic and non-axenic cultures. In non-axenic cultures, moderate DA production was seen immediately after reaching stationary phase and significant DA production was observed one week after reaching stationary growth (Fig. 5B and C). Changes in the four nutrients described above were measured and their relationship with DA production was assessed.

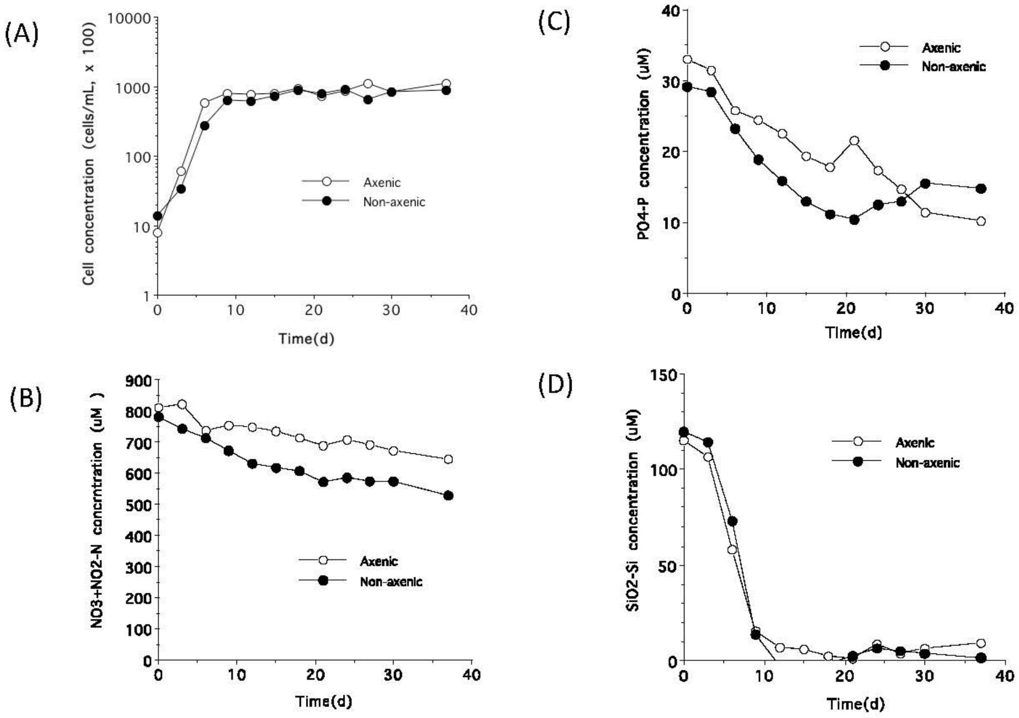

Fig. 4 Batch culture experiments on DA production by *P. multiseries* under axenic and non-axenic conditions. (A) Control growth curves, (B) N (NO_3 + NO_2) treatments, (C) PO_4 treatments (D) SiO_2 treatments.

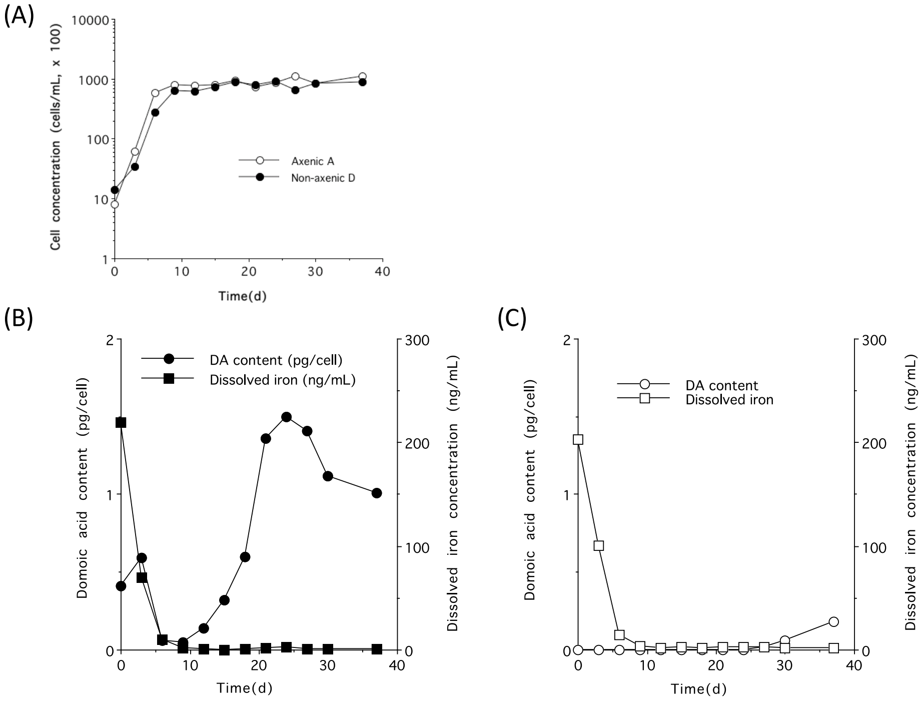

Fig. 5 Batch culture experiments on DA production by *P. multiseries* under axenic and non-axenic conditions. (A) Control growth curves, (B) Concentrations of dissolved iron and DA under non-axenic conditions, (C) Concentrations of dissolved iron and DA under axenic conditions.

The changes in N and P did not appear to explain the difference in cellular DA content under axenic and non-axenic conditions. Significant decreases of Si and dissolved iron were observed and were almost depleted at 10 days both in axenic and non-axenic cultures. DA increased remarkably just after stationary growth was reached in non-axenic culture, while DA increased slightly three weeks after reaching stationary growth in axenic culture. This result shows the potential role of bacteria on DA production of *P. multiseries*. The remaining question was whether depletion of silicate or iron was correlated with DA production and associated with bacterial effects. The iron concentration in cells increased continuously during the exponential growth phase in axenic and non-axenic cultures. After reaching the stationary growth phase, iron concentrations did not increase but were maintained at the same level in the axenic culture while iron concentrations increased continuously in the non-axenic culture (insoluble iron became soluble and was taken into cells in non-axenic culture but not in axenic culture), indicating the possibility that bacteria might help *P. multiseries* cells acquire insoluble iron, resulting in DA production (or accumulation in the stationary phase when growth ceases).

More supporting data were obtained by a 3-fold decrease in DA production after the addition of a 1× concentration of iron at 14 days. Likewise, a 5- and 10-fold iron addition corresponded to the decrease in DA but this decrease was not correlated with added EDTA-Fe (Kotaki *et al*., 2004).

As *P. multiseries* produced low levels of DA even under axenic conditions, intracellular bacteria were confirmed by measurement of bacterial rRNA genes in *P. multiseries* cells (Kobayashi *et al*., 2003). Amounts and species of bacteria appear to be important factors for the production of DA by *P. multiseries*.

Discussion

Monitoring of DA in shellfish and the screening of DA-producing diatoms were performed in Ofunato Bay after the Canadian ASP incident, resulting in detection of a small amount of DA in scallops and blue mussels. The pennate diatom *P. multiseries* was isolated and identified as the source of DA in shellfish from Ofunato Bay and Okirai Bay. DA production was investigated in batch culture experiments, showing that *P. multiseries* produces DA with a significant accumulation only in late stationary growth.

Conditions necessary for ASP occurrence are: 1) presence of highly toxic *Pseudo-nitzschia* such as *P. multiseries*, 2) high abundance of toxic *Pseudo-nitzschia*, 3) sustenance of the bloom for more than two weeks with low nutrient supply (stress), 4) uptake of toxic *Pseudo-nitzschia* by shellfish, and 5) consumption of the toxic shellfish by humans.

Very few bays in Japan satisfy the above conditions, which apparently is the reason why ASP has not occurred, and the potential for ASP in Japan is low. Nine species of *Pseudo-nitzschia* isolated from Ofunato Bay and confirmed to be DA producers do not seem to contribute to ASP occurrences because of their very low toxin content. The National Government decided against continuing DA monitoring in shellfish in Japan after several years of monitoring concluded without detection of DA in excess of the regulatory limit (20 µg g^{-1}). Environmental parameters related to ASP have not been measured in Japan for the same reason. Monitoring of DA in shellfish and *Pseudo-nitzschia* were performed for a number of years in Ofunato Bay and Okirai Bay after the East Japan tsunami in 2011. Monitoring of DA after this tsunami gave the results as before: the ASP potential seems to be low in eastern Japan coastal areas.

DA production characteristics were further investigated in batch culture experiments using *P. multiseries*. The results of these experiments are: 1) cellular DA increases significantly only in late stationary growth after nutrient depletion, 2) co-existing bacteria help facilitate DA production, 3) DA production appears to correlate with the uptake of insoluble iron into the *P. multiseries* cells, 4) bacteria may help with insoluble iron acquisition by *P. multiseries* cells.

How bacteria help *P. multiseries* cells acquire insoluble iron is a remaining problem to be solved in the future. One possible idea is that bacteria make the siderophore-like DA or DA derivatives (Wells *et al.*, 2005) to help *P. multiseries* to assimilate insoluble iron in iron-deficient waters. The comparison of environmental parameters, including iron concentrations, will help to explain the immense differences in DA problems faced by countries in the eastern *versus* western Pacific coastal areas.

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4 *Pseudo-nitzschia* blooms in China

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Sixteen blooms of *Pseudo-nitzschia* (including co-occurrence with other species) have been recorded since this species was first observed in 1987 along the Chinese coastline. The cumulative area in which these past blooms have been observed is 1,280 km^2. Blooms dominated by *Pseudo-nitzschia* were observed nine times and have not caused the death of marine life, or large economic losses.

Pseudo-nitzschia species

Pseudo-nitzschia delicatissima

There are four records of *Pseudo-nitzschia delicatissima* blooms along China's coastline, with three of them occurring in the East China Sea, in May, August, and September 2009 (Table 1). The recorded area of these three blooms was relatively small, with the largest area of only 25 km^2. However, in May 2009, the bloom occurred together with *Noctiluca scintillans*, with a density of 2.0×10^5 cells L^{-1} and in August 2007, a *Pseudo-nitzschia delicatissima* bloom coincided with *Gymnodinium catenatum* in an area of about 400 km^2 in Liaodong Bay in the Bohai Sea coastal area. The *Pseudo-nitzschia delicatissima* bloom did not cause the death of marine life, or any other major disasters.

Table 1 *Pseudo-nitzschia delicatissima* blooms in China's coastal areas.

Date	Location	Region code*	Area (km^2)	Major components
Aug. 21–24, 2007	Liaodong Bay Huludao Bohai Sea	CN-02	400	*Gymnodinium catenatum, Pseudo-nitzschia delicatissima*
May 30, 2009	Ningde Beishuang Island East China Sea	CN-08	10	*Noctiluca scintillans, Pseudo-nitzschia delicatissima*
Aug. 4–6, 2009	Fujian Huangqi Peninsula East China Sea	CN-08	25	*Pseudo-nitzschia delicatissima*
Sep. 1–7, 2009	Fujian Huangqi Peninsula East China Sea	CN-08	3	*Pseudo-nitzschia delicatissima*

*Region code locations are shown in the Harmful Algae Event Database (HAEDAT) website, http://haedat.iode.org/browseGrids.php?countryID=7&mapOnly=1.

Pseudo-nitzschia pungens

There have been nine blooms of *Pseudo-nitzschia pungens* in China's coastal areas since 1987. Among them, five events were observed from May to September 2008 (Table 2). Six cases of *P. pungens* blooms occurred in the East China Sea, with a cumulative area greater than 830 km^2. All these blooms occurred together with other species, such as the diatoms *Eucampia cornuta*, *Thalassiosira rotula*, *Skeletonema costatum*, and the dinoflagellates *Prorocentrum donghaiense* and *Ceratium furca*.

From May 23–24, 2008, *Prorocentrum donghaiense* dominated but co-occurred with *P. pungens*, resulting in a bloom of 500 km^2 near Ningbo Youcai Island in the East China Sea. From September 10–13, 2008, *P. pungens* dominated with *Skeletonema costatum* causing a bloom of 200 km^2 with a density of 6.37×10^7 cells L^{-1} in the Shengshan Island area. The density of *Prorocentrum donghaiense* was 2.45×10^6 cells L^{-1}. Two cases of small-scale *P. pungens* blooms, with a total area of 1 and 6 km^2, occurred in the Bohai Sea in September 2008 and 2010, coinciding once with a *Skeletonema costatum* bloom. In May 2011, a *P. pungens* bloom of 20 km^2 was recorded in the Yellow Sea .

These blooms of *Pseudo-nitzschia pungens* were nontoxic and did not cause serious ecological and economic losses.

Table 2 Statistics of *Pseudo-nitzschia pungens* blooms in China's coastal areas.

Date	Position	Region code*	Area (km^2)	Major components
May 1987	Xiamen Baozhu Island East China Sea	CN-08	14	*Eucampia cornuta, Pseudo-nitzschia pungens*
Late Aug., 2003	Xiangshan Damutu East China Sea	CN-07	1	*Pseudo-nitzschia pungens,* *Prorocentrum donghaiense*
May 23–24, 2008	Ningbo Youcai Island East China Sea	CN-07	500	*Prorocentrum donghaiense,* *Pseudo-nitzschia pungens*
June 10–14, 2008	Xiapu Sansha Bay East China Sea	CN-08	15	*Pseudo-nitzschia pungens,* *Thalassiosira rotula*
July 16–18, 2008	Daishan Dachangtu East China Sea	CN-07	100	*Ceratium furca,* *Pseudo-nitzschia pungens*
Sep. 5, 2008	Yantai Sishili Bay Bohai Sea	CN-05	1	*Pseudo-nitzschia pungens*
Sep. 10–13, 2008	Shengshan Island East China Sea	CN-07	200	*Pseudo-nitzschia pungens,* *Skeletonema costatum*
Sep. 6–10, 2010	Yantai Mashanzhai Bohai Sea	CN-04	6	*Pseudo-nitzschia pungens* *Skeletonema costatum*
May 23, 2011	Liaoning Donggang Yellow Sea	CN-01	20	*Pseudo-nitzschia pungens*

* Region code locations are shown in the Harmful Algae Event Database (HAEDAT) website, http://haedat.iode.org/browseGrids.php?countryID=7&mapOnly=1

Newly recorded species of *Pseudo-nitzschia* in China

New species of *Pseudo-nitzschia* were recorded in June 2000, September 2001, and July 2008. All these events were found near Shenzhen Bay, South China Sea. The scale of these blooms was small and did not cause obvious harm (Table 3). The blooms were caused by a mixture of species. Together with *Pseudo-nitzschia* spp., the dominant species included *Chaetoceros* sp., *Ceratium* sp., *Leptocylindrus danicus*.

Table 3 *Pseudo-nitzschia* blooms of undetermined species in China's coastal areas.

Date	Location	Region code*	Area (km^2)	Major components
June 12, 2000	Shenzhen Bay South China Sea	CN-09	1.4	*Chaetoceros* sp., *Pseudo-nitzschia* sp., *Ceratium* sp.
Sep. 16–20, 2001	Shenzhen Baguang South China Sea	CN-09	2	*Leptocylindrus danicus*, *Rhizosolenia fragilissima*, *Pseudo-nitzschia* sp.
July 30–31, 2008	Shenzhen Baguang South China Sea	CN-09	5	*Pseudo-nitzschia* sp., *Chaetoceros* sp.

* Region code locations are shown in the Harmful Algae Event Database (HAEDAT) website, http://haedat.iode.org/browseGrids.php?countryID=7&mapOnly=1

Research on *Pseudo-nitzschia* in China

Morphology and taxonomy

The earliest research on the genus *Pseudo-nitzschia* in China was in 1965, when two species, *P. pungens* and *P. delicatissima*, were observed by microscope, but classified as the genus *Nitzschia* (Chin *et al*., 1965). Since then, 19 species of *Pseudo-nitzschia* have been identified and their distribution has been monitored throughout Chinese coastal waters as shown in Table 4.

Ten *Pseudo-nitzschia* species were recently recorded in China and two *P. sinica* and *P. micropora* are new species that previously have not been observed anywhere in the world. In addition, two new records were described using morphological characteristics as well as a molecular phylogenetic tree based on sequences of the internal transcribed spacer region (ITS1-5.8S-ITS2) of *P. galaxiae* Lundholm & Moestrup and *P. micropora* Priisholm, Moestrup & Lundholm (Xu and Li, 2015). All strains of these two new species showed no DA content using high performance liquid chromatography (HPLC).

Table 4 *Pseudo-nitzschia* species recently identified in China's coastal areas.

P. americana (Hasle) Fryxell
P. australis Frenguelli
P. brasiliana Lundholm Hasle & Frywell (new record)
P. caciantha Lundholm, Moestrup & Hasle (new record)
P. calliantha Lundholm, Moestrup & Hasle
P. cuspidata (Hasle) Lundholm, Moestrup & Hasle
P. delicatissima (Cleve) Heiden
P. galaxiae Lundholm & Moestrup (new record)
P. cf. *lineola* (Cleve) Hasle (new record)
P. mannii Amato & Montresor (new record)
P. micropora Priisholm, Moestrup & Lundholm (new record, new species)
P. multiseries (Hasle) Hasle
P. multistriata (Takano) Takano (new record)
P. pseudodelicatissima (Hasle) Lundholm, Hasle & Moestrup
P. pungens (Grunow & Cleve) Hasle
P. sinica Qi & Wang (new record, new species)
P. subfraudulenta (Hasle) Hasle (new record)
P. subpacifica (Hasle) Hasle (new record)
P. turgidula (Hustedt) Hasle

Pseudo-nitzschia pungens is a common, cosmopolitan species confined to coastal waters and has been sometimes reported to produce DA, the causative agent of amnesic shellfish poisoning (ASP). Zhang *et al.* (1994) studied the subspecific taxonomy of *Nitzschia pungens* (Grunow) from the Yellow Sea. The samples were identified as *Nitzschia pungens* (Grunow f. *pungens*) but not *N. pungens* (Grunow f. *multiseries*). *Pseudo-nitzschia pungens* is ubiquitous in China, and forms blooms along the Chinese coast from north to south, in Dalian Bay, Jiaozhou Bay, Changjiang River Estuary, Xiamen Bay and Daya Bay. It commonly occurs in all seasons, but especially in the summer and fall (Lü and Qi, 1993; Zou *et al.*, 1993; Qi *et al.*, 1994). Li (2010) did more research on the morphology and taxonomy of the *Pseudo-nitzschia* genus in Chinese coastal waters and found that it formed two additional complexes: the *P. pseudodelicatissima* and *P. americana* complexes.

Physiology and growth kinetics

Chen *et al.* (2002) studied the dynamics of *Pseudo-nitzschia* spp. and associated environmental factors in Daya Bay which is a semi-closed gulf in the South China Sea that has been developed as a mariculture base in Guangdong Province. *Pseudo-nitzschia* spp. are a common dominant species in this area. Seasonal changes of *Pseudo-nitzschia* spp. as well as the influences of environmental factors (especially nutrients) on the population dynamics were analyzed from July 1997 to June 1998. Chen *et al.* (2002) observed the genus in the bay throughout the year, and noted that cell concentrations were highest in spring and autumn.

Peak densities of *Pseudo-nitzschia* spp. were observed at temperatures ranging from 25.0–30.0℃ and salinity from 28.4–31.3‰. Dissolved inorganic nitrogen (DIN) and silicate were abundant in the bay while dissolved inorganic phosphate (DIP) appeared to be the limiting factor. The ratios of DIN, DIP, and Si are important for the growth of *Pseudo-nitzschia* and the optimal ranges of N:P, Si:P, Si:N are 6.21–32.98, 59.67–119.71, 3.36–17.89, respectively.

Lü *et al*. (2006) analyzed the effects of nitrogen, phosphorus and N:P ratios on the growth of *P. pungens* collected and isolated from Daya Bay. Results showed that *P. pungens* is a nutrient-dependent species, and its growth is stimulated by high levels of nitrogen and phosphorus. Therefore, the N:P ratio is another important factor to affect the population growth. The optimal N:P ratio for the growth of *P. pungens* ranges from 10–3.

Qin *et al*. (2014) compared physiological characteristics of alkaline phosphatase (AP) in *P. pungens* and *Aureococcus anophagefferens* and showed that AP of *P. pungens* and *A. anophagefferens* was inducible and that the expression of AP activity was co-regulated by DIP and particulate phosphorus. The response of *P. pungens* to AP activity was more sensitive than that of *A. anophagefferens*.

Ecology and *Pseudo-nitzschia* blooms

Pseudo-nitzschia spp. can succeed at low temperatures due to their ability to obtain enough nutrients for growth, compared to dinoflagellates. Population densities and relationships between *Pseudo-nitzschia*, phytoplankton and zooplankton were investigated daily from April to May, 2000, in Daya Bay (Chen *et al*., 2005). At the beginning of survey, species composition and densities of the planktonic community were low followed by the rapidly increasing growth of *Pseudo-nitzschia* with a maximum density of 2.93×10^6 cells L^{-1}. Both *P. pseudodelicatissima* and *P. pungens* bloomed, and other diatoms and dinoflagellates were also enriched during this bloom. The authors determined that *Pseudo-nitzschia* was the most important component of the spring diatom bloom due to its competitive advantage over other species. The effect of zooplankton (especially copepods) top-down control on *Pseudo-nitzschia* was not significant because of reduced grazing pressure. This lack of grazing on *Pseudo-nitzschia* contributed to its success in the study area.

Toxins and toxicology

Pseudo-nitzschia species, including *P. pungens*, *P. cuspidata*, *P. multistriata*, *P. brasiliana*, *P. galaxiae* and *P. micropora*, isolated from the coast of China, showed either no measurable or only a trace of DA using HPLC; Li *et al*., 2002). Recently, traces of DA have been measured in some shellfish samples but not in seawater or freshwater (Table 5; Chen *et al*., 2001). In May 2001, five species of shellfish samples normally collected and consumed in the Dalian area (the Yellow Sea) were analyzed by capillary electrophoresis with UV detection and only one species (*Chlamys farreri*) was found to contain DA (Table 6).

Table 5 Analytical results of DA in shellfish using HPLC analysis (Chen *et al*., 2001).

Sample	**Sample weight (g)**	**DA (µg/g)**
Shrimp	10.5	0.50
Scapharca subcrenata #1 (blood clam)	9.0	Not detected
Scapharca subcrenata #2	8.0	Not detected
Scapharca subcrenata #3	10.0	0.57
Conch Shell #1	11.5	0.80
Conch Shell #2	9.5	Not detected
Scapharca subcrenata #4	16.0	Not detected
Panopea abrupta (geoduck)	11.6	8.14
Pteria margaritifera (clam)	14.2	4.04
Paphia undulate (clam)	9.7	Not detected
Meretrix meretrix L. (clam)	14.4	4.16
Ruditapes philippinarum (clam)	12.6	0.43
Cyclina sinensis (clam)	9.7	Not detected
Conch (n = 3)	10.3	Not detected

Table 6 Analysis of DA in shellfish using capillary electrophoresis with UV detection (Li *et al*., 2002).

Sample	**DA (µg/g)**
Ruditapes philippinarum (clam)	Not detected
Mytilus edulis (mussel)	Not detected
Oyster	Not detected
Callista chione (clam)	Not detected
Chlamys farreri (scallop)	5.2
MUS-1 reference material	32.6

Summary

1) Sixteen cases of *Pseudo-nitzschia* blooms in China have been recorded from 1987 to the present, often co-occurring with other species.
2) The cumulative area in which these past blooms have been observed is 1,280 km^2, and no death of marine life, or large economic loss, due to *Pseudo-nitzschia* has occurred.
3) Two or more *Pseudo-nitzschia* sp. may cause blooms in China, including *P. delicatissima*, *P. pungens*, as well as other newly recorded *Pseudo-nitzschia* species.
4) Some species of *Pseudo-nitzschia* in China may produce trace DA (but less than the regulatory limit of 20 µg g^{-1}).
5) Due to the potential threat of DA production among *Pseudo-nitzschia* species, it is suggested that routine monitoring of these blooms should be intensified to protect seafood safety.

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5 Temporal changes and toxicity of *Pseudo-nitzschia* species

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Since the first occurrence of *Pseudo-nitzschia* blooms in Masan Bay, Korea, in 1975, these blooms have been observed mostly in semi-closed bays (such as Jinhae, Deukryang). Since 1990, these blooms have been observed in coastal waters (*e.g.*, near Jinhae, coastal ports, mouth of Nak-dong River; Lee, 1994). Despite observations of recurrent *Pseudo-nitzschia* blooms, amnesic shellfish poisoning (ASP) has not yet been reported in Korea. Koh and Kwon (2002) reported no detectable domoic acid (DA) in one gastropod species and 11 bivalve species which were sampled from May to December in 1999. Nationwide shellfish poisoning monitoring performed by the National Institute of Fisheries Science (NIFS) also showed that DA was rarely detected in Korean waters. DA has not been detected in any shellfish sample since 2009 (Table 1). In Korea, *Cochlodinium polykrikoides* and some other dinoflagellates are major research subjects as these harmful dinoflagellates are the primary causes of massive fish mortality. Because *Pseudo-nitzschia* bloom events and bloom periods have gradually decreased since the 1980s (Figs. 1 and 2), few studies have been conducted about boom dynamics and DA production by *Pseudo-nitzschia* species in Korea.

Table 1 Concentrations of domoic acid (DA; µg g^{-1}) according to a nationwide shellfish toxin monitoring program conducted by the National Institute of Fisheries Science (NIFS) from 2003–2015 in Korean waters.

	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
Month	Jan-May	Feb-Apr	Jan	Aug	Mar	Mar	ND	ND	ND	ND	ND	ND	ND
	Jan-May	Jun	Feb	Aug	Apr	Mar							
	Jan-May	Jun	Feb	Aug	Aug	Mar							
DA	0.3–0.5	0.2–0.9	1.46–2.85	0.73–0.86	0.3–0.44	1.07–2.17	–	–	–	–	–	–	–

ND, not detected by high performance liquid chromatography (HPLC)

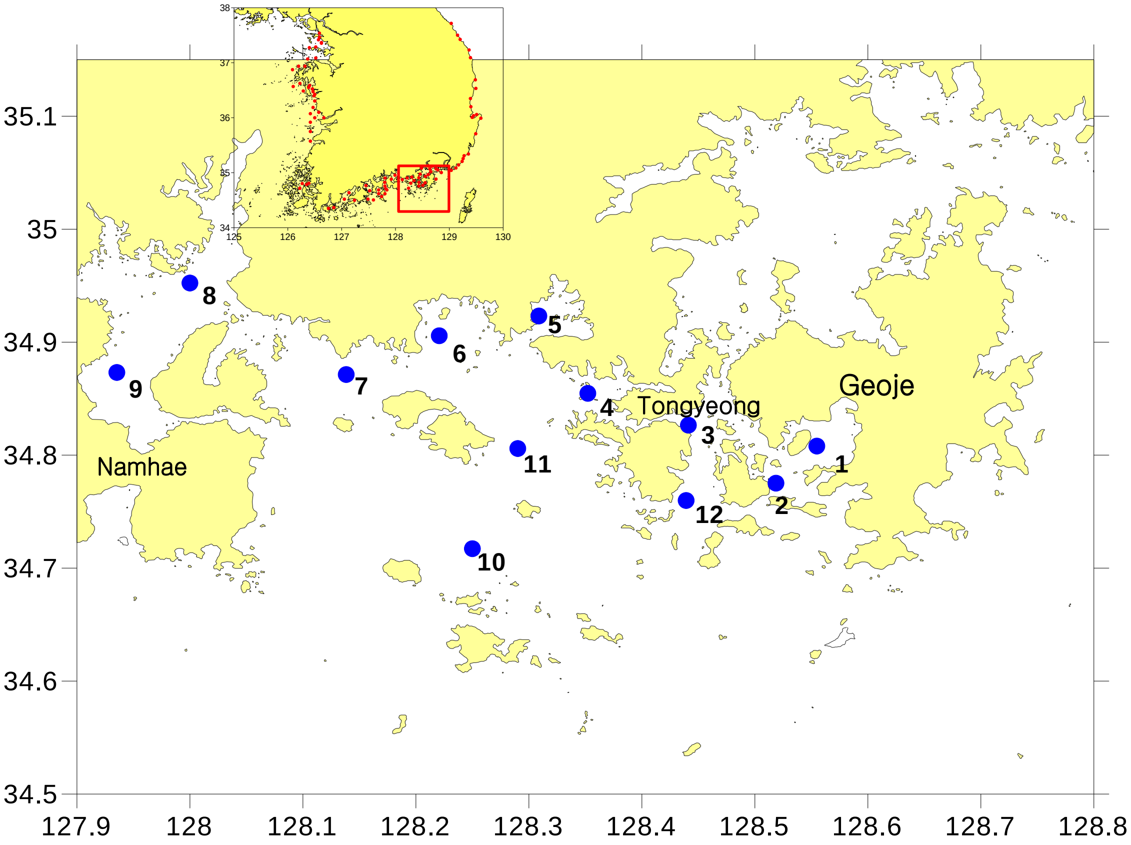

Fig. 1 Map showing the sampling stations in the study area.

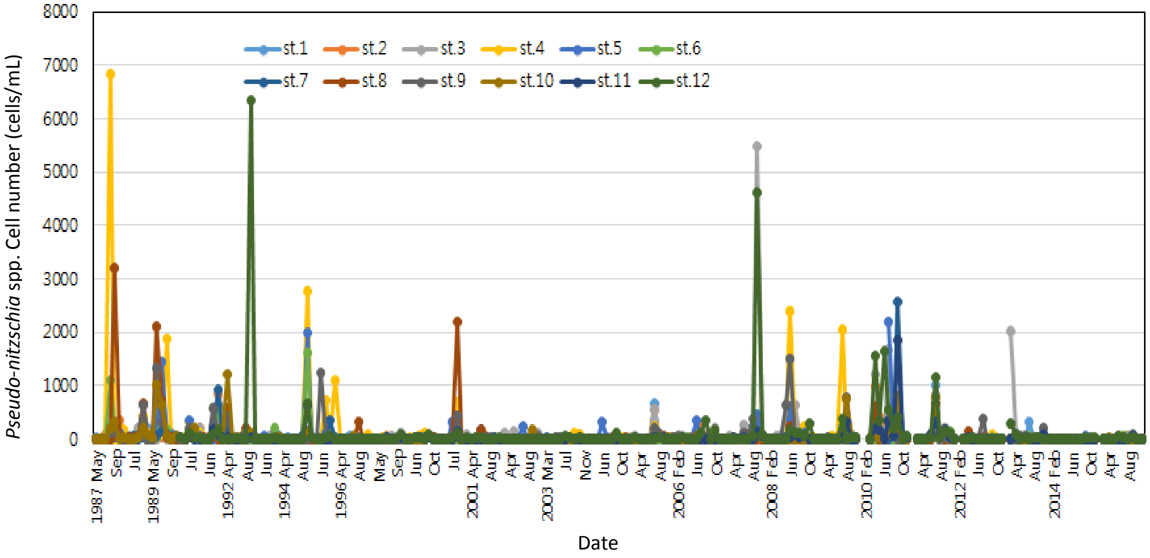

Fig. 2 Cell densities of *Pseudo-nitzschia* spp. in southeast coastal waters, Korea, from 1987–2014.

Lee (1994) compared the morphological characteristics between *Pseudo-nitzschia pungens* f. *multiseries* and *P. pungens* f. *pungens* sampled from 1974 to 1994 in the waters of southern Korea. Lee and Baik (1997) isolated and cultured *P. multiseries* from the waters of southern Korea, and detected 1.03 pg cell^{-1} DA in stationary phase cultures. *Pseudo-nitzschia multiseries* was dominant from April to May at a temperature range of 14.8°–19°C.

Park *et al*. (2009) reported that 13 *Pseudo-nitzschia* species (*P. americana*, *P. brasiliana*, *P. caciantha*, *P. calliantha*, *P. cuspidata*, *P. delicatissima*, *P. micropora*, *P. multiseries*, *P. multistriata*, *P. pseudodelicatissima*, *P. pungens*, *P. subfraudulenta* and *P. subpacifica*) appeared in southeastern coastal waters in 2008 (Fig. 3). Only four species appeared in May, 12 species in September, and 5 species in November (Table 2).

Lim (2010) found that *Pseudo-nitzschia* cell density has fluctuated since 1987, with a possible decrease (Fig. 2). *Pseudo-nitzschia* blooms were highly correlated with rainfall. Cell density increased when silicate concentrations were high rather than when nitrogen and phosphorus concentrations were high. High cell densities were frequently found in inner sea areas where an elevated inflow of fresh water from land occurred.

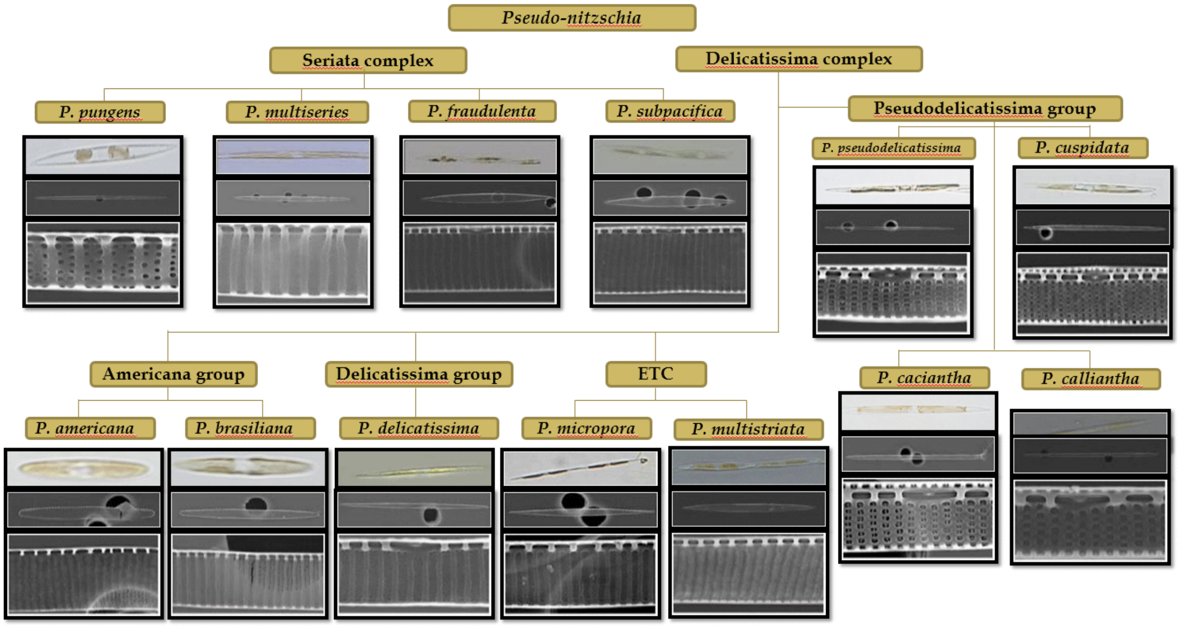

Fig. 3 *Pseudo-nitzschia* species in southeastern coastal waters, Korea, in 2008. ETC indicates the species that do not belong to any group.

Table 2 *Pseudo-nitzschia* species detected in the coastal waters of Tongyeong, Korea.

	2008						
Taxon	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
Pseudo-nitzschia americana	+	+	+	+	+	+	+
P. brasiliana				+	+		
P. caciantha			+	+	+	+	+
P. calliantha		+	+		+		
P. cuspidata	+	+	+	+	+		
P. delicatissima	+	+	+	+	+	+	
P. micropora		+	+	+	+		
P. multiseris				+	+	+	+
P. multstriata			+	+	+	+	+
P. pseudodelicatissima			+		+		
P. pungens	+	+		+	+	+	+
P. subpacifica				+	+		
P. subfraudulenta					+	+	
Total number of species	4	6	8	10	13	7	5

Table 3 Field observation and cultures of *Pseudo-nitzschia* species in 2008 and 2009 (Lim, 2010).

Field strain	**Culture strain**
Pseudo-nitzschia americana	–
P. brasiliana	*P. brasiliana*
P. caciantha	*P. caciantha*
P. calliantha	–
P. cuspidata	–
P. delicatissima	*P. delicatissima*
P. fraudulenta	–
P. micropora	–
P. multiseris	*P. multiseris*
P. multstriata	*P. multstriata*
P. pseudodelicatissima	–
P. pungens	*P. pungens*
P. subpacifica	–
P. subfraudulenta	–
–	*P. mannii*
–	*P. americana*
14 species	8 species

Lim (2010) reported that 14 species of *Pseudo-nitzschia* were observed in the southern East Sea and 8 species were cultured in 2008 and 2009 (Table 3). The analysis of DA in seawater samples and cultures showed that 3 species of *Pseudo-nitzschia*, *P. calliantha*, *P. multiseries* and *P. multistriata*, produced DA. The concentrations of DA varied significantly throughout the year. The concentrations of DA were higher in eutrophic inner sea areas than in shellfish farm areas, and varied among *Pseudo-nitzschia* species.

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6 *Pseudo-nitzschia* bloom events in the Russian waters of the Japan/East Sea

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Introduction

Pseudo-nitzschia monitoring in the northwestern Japan/East Sea has been conducted for over two decades by the Center for Monitoring of Harmful Algal Blooms and Biotoxins, the National Scientific Center of Marine Biology. Phytoplankton species diversity and abundance have been studied since 1992 and domoic acid (DA) concentrations in diatoms and bivalve samples have been quantified since 2007.

Pseudo-nitzschia is one of the most widespread and abundant microalgae groups capable of toxin production in the Russian waters of the Japan/East Sea where nine *Pseudo-nitzschia* species have been identified by light and electron microscopy (Stonik and Orlova, 2012). At least seven of these species (*P. calliantha*, *P. delicatissima*, *P. fraudulenta*, *P. multiseries*, *P. multistriata*, *P. pungens*, *P. seriata*) are known to produce DA in other parts of the world.

Pseudo-nitzschia bloom events

Blooms of *P. calliantha*, *P. multiseries*, *P. multistriata* and *P. pungens* have been observed in the Russian coastal waters of the western North Pacific in the summer and autumn with abundances exceeding 1 million cells L^{-1} and constituting 75–98% of the total phytoplankton density (Stonik *et al.*, 2011a). The most intensive *Pseudo-nitzschia* bloom events in Russian waters of the Japan/East Sea were registered from 1992–1997 (Stonik *et al.*, 2001). On a decadal timescale, there has been a sharp decrease in *P. multiseries* abundance (from 11.0 to 1.4 million cells L^{-1}) from 1992–2002, and a shift from *P. multiseries* to *P. calliantha* and *P. multistriata* from 2002–2012 (Table 1). For example, one of the potentially toxic diatom blooms of the genus *Pseudo-nitzschia* was observed in October–November 2005 in the northeastern part of Amur Bay in the coastal waters of Vladivostok with salinity ranging from 31–33.5‰ and water temperature from 6–12°C. The peak of *Pseudo-nitzschia multistriata/calliantha* cell density ($0.8 \cdot 10^6$ cells L^{-1}), recorded after heavy rains, was mainly caused by a massive bloom of *P. multistriata* (67% of total density) and *P. calliantha* (9%). A negative correlation was found between *Pseudo-nitzschia* spp. cell density and water salinity and NH_4 concentration; a positive correlation was observed between diatom population density and water temperature (Stonik *et al.*, 2012).

Table 1 The most intensive *Pseudo-nitzschia* bloom events in the northwestern Japan/East Sea from 1992–2012.

Species	Date	Maximum concentration (cells L^{-1})	Condition
P. multiseries	June 1992 June 1993 September 2002	$11 \cdot 10^6$ $1 \cdot 10^6$ $1.4 \cdot 10^6$	After heavy rainfalls at SST of 14.2–16.1°C
P. calliantha	November 1997	$2.7 \cdot 10^6$	SST of 5–6°C and salinity of 34.5‰
P. calliantha/fraudulenta	October–November 2002	$3.6 \cdot 10^5$	SST of 6–16°C and salinity of 28.8–33.5‰
P. multistriata/calliantha	October–November 2005	$0.8 \cdot 10^6$	SST temperature of 6–12°C and salinity of 31–33.5‰. The bloom event was found after heavy rains. There was a negative correlation between the diatom abundance, water salinity, and ammonium concentrations. There was a positive correlation between diatom abundance and water temperature.
P. multistriata	September 2012	$2.5 \cdot 10^5$	SST of 17.8–18°C and salinity of 26.8–28 ‰.
P. calliantha	November 2012	$1.4 \cdot 10^6$	SST of 5–6°C

After this bloom, samples of phytoplankton were collected on a routine basis from 2012–2015 in the Russian waters of the Japan/East Sea. The total abundance of *Pseudo-nitzschia* species varied from $3 \cdot 10^2$ to $1.4 \cdot 10^6$ cells L^{-1}. The highest concentrations of toxigenic *Pseudo-nitzschia* species in Amur and Ussuri bays reached 0.2–$1.4 \cdot 10^6$ cells L^{-1}, well above the European Union and Canadian threshold guidance level (10^4–10^5 cells L^{-1} in Andersen, 1996). The density of toxic species exceeded the limit of $1 \cdot 10^5$ cells L^{-1} on four occasions ($2.5 \cdot 10^5$ cells L^{-1} in September 2012 in northern Amur Bay, dominated by *P. multistriata*; 1.2–$1.4 \cdot 10^6$ cells L^{-1} in November 2012 in Ussuri Bay, dominated by *P. calliantha*; $3 \cdot 10^5$ cells L^{-1} in November 2013 in Ussuri Bay, dominated by *P. calliantha*; $1.7 \cdot 10^5$ cells L^{-1} in October 2015 in Usauri Bay, dominated by *P. multistriata*, Stonik, unpubl. data).

Regular fall blooms of toxigenic *P. calliantha* and *P. multistriata* were recorded in Ussuri Bay, coinciding with a period when local wind-driven upwelling occurs (V.B. Lobanov, V.I. Il'ichev Pacific Oceanological Institute, Far Eastern Branch of the Russian Academy of Sciences, pers. comm.).

DA measurements in *Pseudo-nitzschia* laboratory cultures and bivalve samples

In the summer and fall prior to 2002, *Pseudo-nitzschia multiseries* bloomed in Peter the Great Bay, with cell abundances exceeding 10^6 cells L^{-1}. Total DA was measured using high performance liquid chromatography (HPLC) with a fluorenylmethoxycarbonyl derivative (Pocklington *et al*., 1990) in "whole-culture" samples (cells plus medium) isolated during a *P. multiseries* bloom in 2002. Domoic acid was found in stationary-phase (days 20–35) cultures of *P. multiseries* isolated from Peter the Great Bay during fall 2002 when concentrations varied from 180–5390 ng ml^{-1}, equivalent to 2 to 21 pg $cell^{-1}$, which is in the range reported for other isolates of *P. multiseries* (Orlova *et al*., 2008). The Russian isolate showed greater ability to produce DA over time in culture. The change in toxicity over time could be related to changes in the bacterial composition in the diatom culture. A gamma proteobacterium (tentatively identified as *Alteromonas macleodii*) was isolated from *P. multiseries* strain PM-02, and has been shown to significantly enhance the DA concentration of an axenic culture of another strain of *P. multiseries* (S.S. Bates, I. Kaczmarska, C. Leger, J. Ehrman and D.H. Green, unpublished data).

Concentrations of DA (up to 0.5 pg $cell^{-1}$), determined using the "ASP direct ELISA" kit (Biosense Laboratories AS, Norway, AOAC official method 2006.02) were measured in cultures of *P. calliantha*, *P. delicatissima*, *P. multistriata* and *P. pungens* (Stonik *et al*., 2011b; Stonik, unpublished data). The monitoring of DA in shellfish collected from Peter the Great Bay during 2009–2013 showed that concentrations in tissues of the bivalves *Mytilus trossulus*, *Crenomytilus grayanus* and *Mizuhopecten yessoensis* ranged from 0.01–0.3 mg kg^{-1}, well below the permissible regulatory limit of 20 mg kg^{-1} (Stonik and Orlova, 2012).

In summary, to date there have been no economic or social impacts connected with toxigenic *Pseudo-nitzschia* blooms in Russian waters. However, the high concentrations of toxigenic *Pseudo-nitzschia* species able to produce DA suggest a potential threat of future DA contamination in shellfish in the northwestern Japan/East Sea.

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7 *Pseudo-nitzschia* blooms in the northeastern Pacific Ocean

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Coastal managers became keenly aware of the possibility of amnesic shellfish poisoning (ASP) in the northeastern Pacific region after sea lions and sea birds in Monterey Bay, California, showed symptoms of this poisoning (also called domoic acid poisoning) after ingesting sardines and anchovies filled with cells of *Pseudo-nitzschia australis* in 1991 (Scholin *et al.*, 2000). Soon after this event, toxigenic *Pseudo-nitzschia* blooms were identified as a problem in Washington State after the first closure of the razor clam fishery was announced in the same year. Harmful DA was identified after a shellfish manager from the Washington State Department of Health (WDOH), collected razor clams at Long Beach, Washington, for routine paralytic shellfish toxin testing. When the shellfish homogenate was injected into a mouse at the lab, unusual behavior not characteristic of paralytic shellfish poisoning was noticed, including scratching behind the ear, a symptom of domoic acid (DA) poisoning. This observation was communicated to Canadian scientists, who first described DA in 1987, as a new toxin produced by *Pseudo-nitzschia*. The shellfish sample was shipped to them for analysis, resulting in the confirmation of harmful levels of DA in Washington State shellfish for the first time in 1991.

Since that year, DA closures have affected the commercial, recreational and tribal subsistence harvest of shellfish, including blue mussels, razor clams and Dungeness crabs in Oregon, Washington State, and British Columbia (BC), Canada, although the intensity and frequency of these closures vary spatially. Shellfish monitoring for public health safety is conducted on a regular basis by the WDOH, the Oregon Department of Agriculture, and the Canadian Shellfish Sanitation Program. The analysis of razor clam tissue for DA content (Fig. 1) at two representative Washington State coastal beaches (Kalaloch and Long Beach; Fig. 2) from 1991–2017 illustrates the frequency of razor clam closure events. Although toxic razor clams are frequently present at the central (Kalaloch Beach) and southern (Long Beach) sites, the level of toxicity varies, suggesting that physical processes impact bloom access to the coast from different source regions (described below). It is interesting to note that during 2007–2014, no DA closures occurred on the Washington coast, but this non-bloom period was followed by the west coastwide *Pseudo-nitzschia* bloom in 2015 that had the highest economic impact (~$100 million economic loss to the Dungeness crab harvest alone; Lowther and Liddel, 2016) compared to previous toxic *Pseudo-nitzschia* events (McCabe *et al.*, 2016).

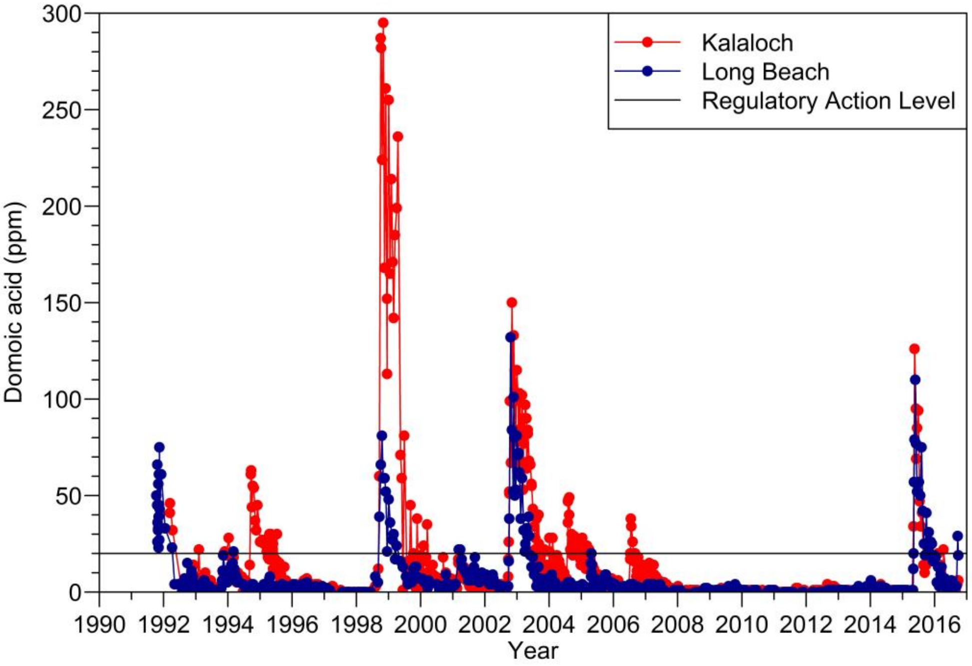

Fig. 1 Historical DA in razor clams at Kalaloch and Long Beach, WA (locations shown in Fig. 2) from 1991–2017. The regulatory action level of 20 ppm (or µg g^{-1}) is shown as a black horizontal line.

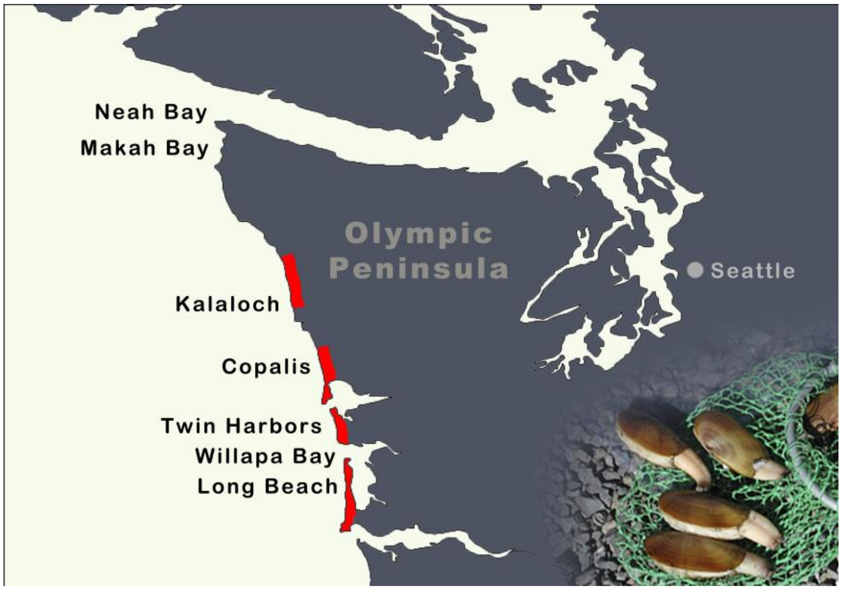

Fig. 2 Core phytoplankton sampling sites on the Washington coast monitored by the Olympic Region Harmful Algal Bloom (ORHAB) partnership.

In British Columbia, DA levels have generally been lower than in the U.S. northeastern Pacific Ocean, in part due to the general lack of razor clams on the outer coast of BC. The first significant amount of DA detected in shellfish was in razor clams from Haida Gwaii in April 1992, and Dungeness crab viscera in western Vancouver Island inlets in August of the same year. Figure 3 shows the maximum monthly DA (Canadian Food Inspection Agency data) in samples from four separate areas on the BC coast: Haida Gwaii, West Coast Vancouver Island (WCVI), East Coast Vancouver Island (ECVI), and the Central Coast (Fig. 4). DA levels in 1992 were the highest seen in B.C. to date; since 1995 shellfish from Haida Gwaii sites, primarily razor clams, are most commonly found to be contaminated although there have been distinct periods (spring 2002–winter 2004, spring 2008–spring 2012) where no DA was detected. In spring 2015, unusually high levels of DA were seen in samples from WCVI, and in November of that year, DA in mussel samples from ECVI were measured for the first time above the regulatory guidance level (50.4 µg g^{-1}, at Patricia Bay in Saanich Inlet).

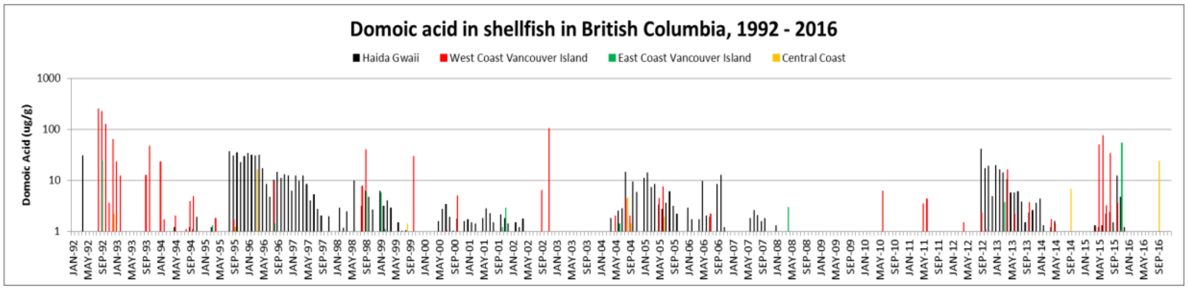

Fig. 3 Domoic acid (DA) in shellfish samples in British Columbia, Canada, from 1992–2016; maximum values, pooled by area and month. Data from Canadian Food Inspection Agency.

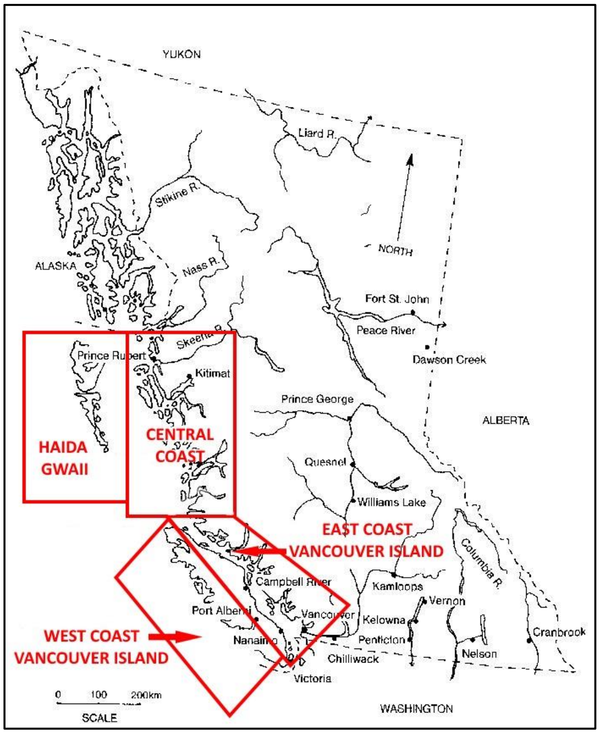

Fig. 4 DA monitoring areas in British Columbia, Canada.

Phytoplankton monitoring has been established to provide an early warning of harmful algal blooms (HABs) in the northeastern Pacific Ocean including the Monitoring of Oregon's Coast for Harmful Algae (MOCHA), the Olympic Region HAB program (ORHAB, Washington State – for details on the ORHAB program methods, see Appendix 3 and www.orhab.org), the Harmful Algae Monitoring Program (HAMP, British Columbia, Canada), and the Southeast Alaska Tribal Toxins network (SEATT; www.seator.org). Phytoplankton monitoring is not required as part of U.S. regulatory testing, but has been established through funding programs such as NOAA's Monitoring and Event Response to HABs (MERHAB), through a small fee paid by shellfish farmers, and through a surcharge for shellfish license fees. However, it has been difficult to maintain these important phytoplankton monitoring programs without consistent funding.

Phytoplankton monitoring has helped scientists to establish the annual seasonality of *Pseudo-nitzschia* blooms, the species and their abundance associated with the most toxic events, and the potential source regions for these blooms. In particular, the ORHAB program has a dataset of phytoplankton abundance established in 2000, allowing for the analysis of annual patterns in *Pseudo-nitzschia*. The beaches at which phytoplankton samples are collected at least weekly by the Washington Department of Fish and Wildlife, the University of Washington's Olympic Natural Resource Center, the Quinault Indian Nation, the Quileute Tribe and the Makah Tribe are shown in Figure 2. In general, *Pseudo-nitzschia* blooms occur in the spring and late summer, and are now known to originate from the source regions, Heceta Bank and the Juan de Fuca Eddy. The *Pseudo-nitzschia* species that have been present during the major toxic events on the Washington coast since 1998, their total maximum abundance, and the clamming days lost due to these events, are shown in Table 1. Although several species have been observed, including *P. heimii*, *P. pungens*, *P. delicatissima*, *P. fraudulenta*, *P. seriata*, *P. lineola*, and *P. multiseries*, the two *Pseudo-nitzschia* species responsible for the major razor clam closures in Washington and Oregon are *P.* cf. *pseudodelicatissima* (recently identified as *P. cuspidata* by scanning (SEM) or transmission (TEM) electron microscopy; Lundholm *et al*., 2003) and *P. australis*. Fewer *Pseudo-nitzschia* species have been identified in BC waters. Forbes and Denman (1991) reviewed the distribution of *P. pungens* and *P. multiseries* (as *Nitzschia pungens* f. *pungens*, and *N. pungens* f. *multiseries*) in samples taken from 1980–1988. Samples were analyzed with a light microscope, so differentiation between the two forms was not possible; only one sample was examined using SEM, and in this sample *P.* f. *pungens* outnumbered f. *multiseries* by 25-fold. These researchers reported *P. seriata* from WCVI sites. *P. australis* and *P. delicatissima* have also been reported from Barkley Sound (WCVI; Taylor and Haigh, 1996), with *P. australis* more common in early summer, and *P. delicatissima*, with *P. pungens*, in late summer and autumn. In 2015, *Pseudo-nitzschia* species seen in WCVI sites taking part in the Harmful Algae Monitoring Program (HAMP, see http://www.microthalassia.ca/hamp/) were *P. australis*, *P. fraudulenta*, *P. pungens* and *P.* cf. *delicatissima*.

Table 1 Description of historical DA closure events in Washington State (1998–2017).

Event[1]	Closure	Max. PN[2] (cells L^{-1})	Dominant PN species	Other PN species	Max DA in clams (ppm)	Action (closure period)	Clamming days lost	% days lost
Summer 1998	coastwide	17×10^6	cf. psdeli[3]	heimii, pun	300	pre-season cancellation	116	64
Spring 2001	southern beaches	0.7×10^5	aus	pun	17	pre-season cancellation	1	7
Summer 2002	coastwide	4.3×10^6	aus	heimi, pun, deli	99	pre-season cancellation	268	85
Summer 2003	Kalaloch Beach	2.4×10^6	cf. psdeli	cusp, fraud, pun, aus	25	season postponement, cancellation	23	16
Summer 2004	Kalaloch Beach	4.8×10^6	cf. psdeli	fraud, heimii, cusp, deli, pun	49	season postponement, cancellation	147	28
Spring 2005	Long Beach	2.0×10^5	aus	fraud	20	season postponement, cancellation	5	8
Summer 2006	Kalaloch Beach	9.3×10^6	cf. psdeli	–	38	no action, outside razor clam season	0	0
2007–2014	*No coastal razor clam toxicity*							
Spring 2015	coastwide	2.2×10^6	aus	psdeli, heimii, multi, lineola, fraud, pun	169	in-season closure	not scheduled	100
Fall 2016	southern beaches	2.7×10^6	cusp (Sep), aus (Oct)	pun, multi, deli	29	season postponement, in-season closure	156	69
Spring 2017	southern beaches	7.9×10^4	aus	pun	44	season postponement, in-season closure	46	41

[1] Season when event began.

[2] PN = *Pseudo-nitzschia*. Indicates maximum number of all *Pseudo-nitzschia* species.

[3] Species abbreviations: psdeli = *P.* cf. *pseudodelicassima*; aus = *P. australis*; pun = *P. pungens*; heimii = *P. heimii*; deli = *P. delicatissima*; lineola = *P. lineola*; cusp = *P. cuspidata*; fraud = *P. fraudulenta*; seriata = *P. seriata*

A series of research cruises since 1997 has shown that toxic *Pseudo-nitzschia* blooms affecting the U.S. Pacific Northwest originate from source regions in the northeastern Pacific, including Point Conception/Santa Barbara Channel (California), Monterey Bay (California), Heceta Bank (Oregon), and the Juan de Fuca Eddy (Washington/BC) (Fig. 5). These "hotspot" sites form during late spring through late summer and provide nutrients from upwelling and physical retention that enables phytoplankton growth and maintenance. Often, these features are visible by satellite as regions of high chlorophyll and cooler upwelled water. It has been shown that average cumulative upwelling (*i.e.*, periods of moderate upwelling followed by moderate storms) was observed in summers when the Juan de Fuca Eddy was most retentive, and DA closures in coastal Washington occurred (compare Fig. 6 to Table 1). In 1991, 1998, 2002, 2003, 2004, 2006 (through late June) and 2016 the cumulative upwelling index was "average" compared to past years, shown in light gray (Fig. 6). In contrast, the 2015 springtime bloom was delivered to the coast by the anomalously warm nutrient-depleted waters (nicknamed "the Blob"; see description below), and a higher than average cumulative upwelling index was observed (Fig. 6, dotted purple line).

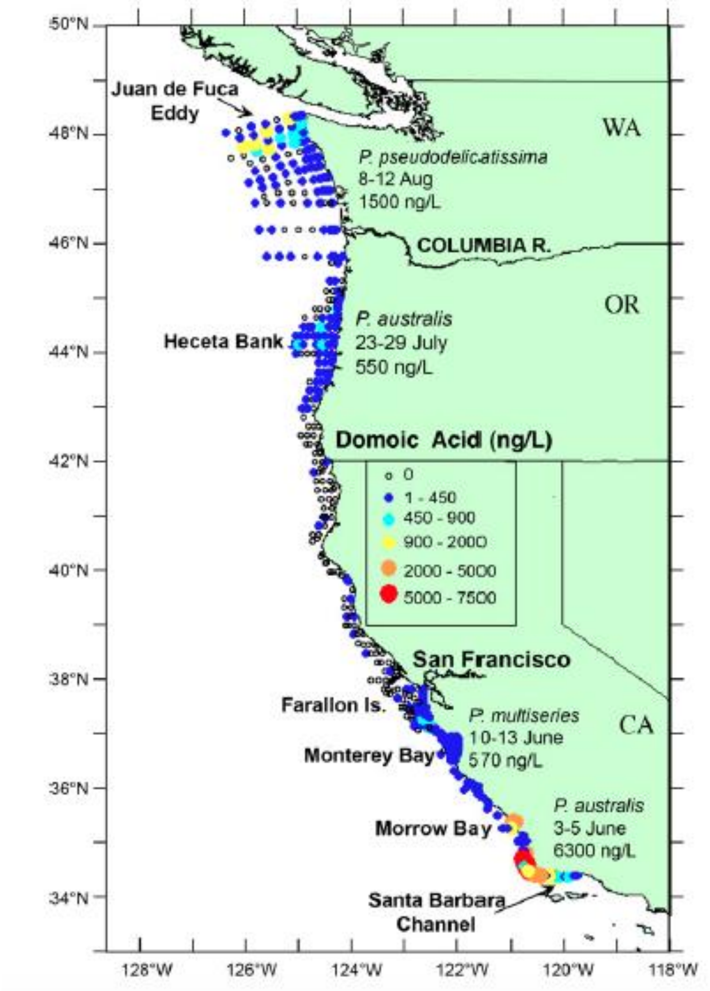

Fig. 5 Particulate DA in the northeastern Pacific Ocean in the summer 1998. Maximum concentrations of DA and toxic species are indicated to the right of each toxic hotspot (redrawn from Trainer *et al*., 2001; Hickey and Banas, 2003).

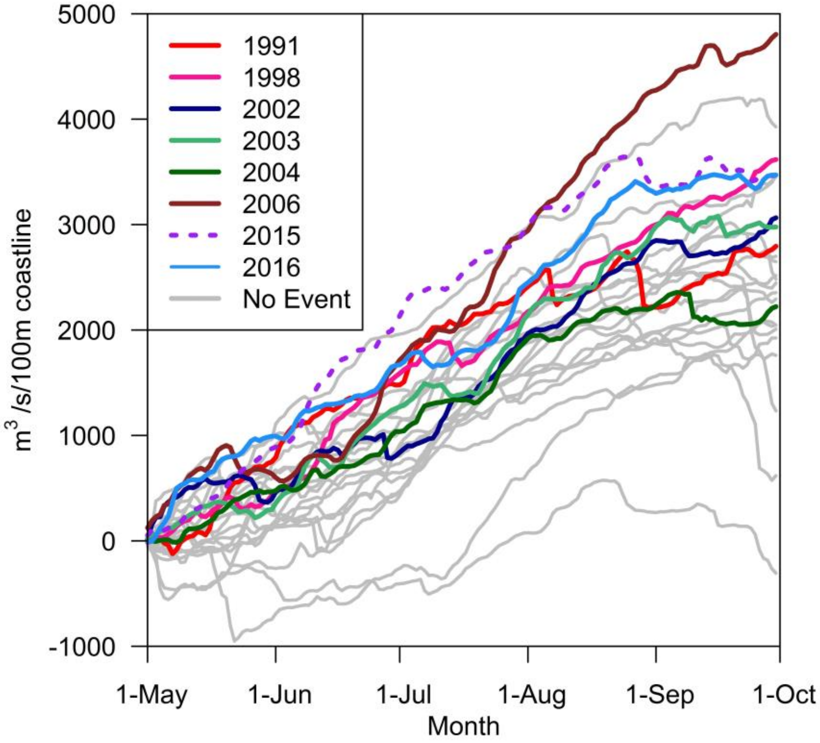

Fig. 6 Daily upwelling indices at 48°N, 125°W for 1991–2016 from May–September obtained from NOAA Pacific Fisheries Environmental Lab (https://www.pfeg.noaa.gov/products/PFEL/modeled/indices/upwelling/upwelling.html). Cumulative upwelling indices were derived by calculating a running total of the data for each year starting on May 1 and ending on September 30. Colored lines represent years where DA events occurred on the coast of Washington State and gray lines represent years where there were no DA events.

The successful transport of *Pseudo-nitzschia* from the hotspot sites to beaches is influenced by the position and intensity of the Columbia River, the geographical boundary between Washington and Oregon, with the greatest discharge of any North American river entering the Pacific. During the summer, or good-weather periods, the predominant direction of the Columbia River freshwater plume is southward and during the winter, or storm periods, the primary direction is northward (Fig. 7). However, it can take days or even weeks for the Columbia River to respond to changing weather and at times the plume is bi-directional. This freshwater gradient often acts as a barrier to *Pseudo-nitzschia* transport to the coast, sometimes resulting in higher concentrations of DA in shellfish at northern Washington State beaches (see razor clam DA in 1998 at Kalaloch Beach compared to Long Beach, Fig. 1). However, at times, the plume can act as a conduit for *Pseudo-nitzschia* cell transport from Heceta Bank to the Washington coast (Hickey *et al*., 2013), resulting in toxic clams at Long Beach, but not Kalaloch Beach (Table 1; see spring 2017 closure at southern beaches only).

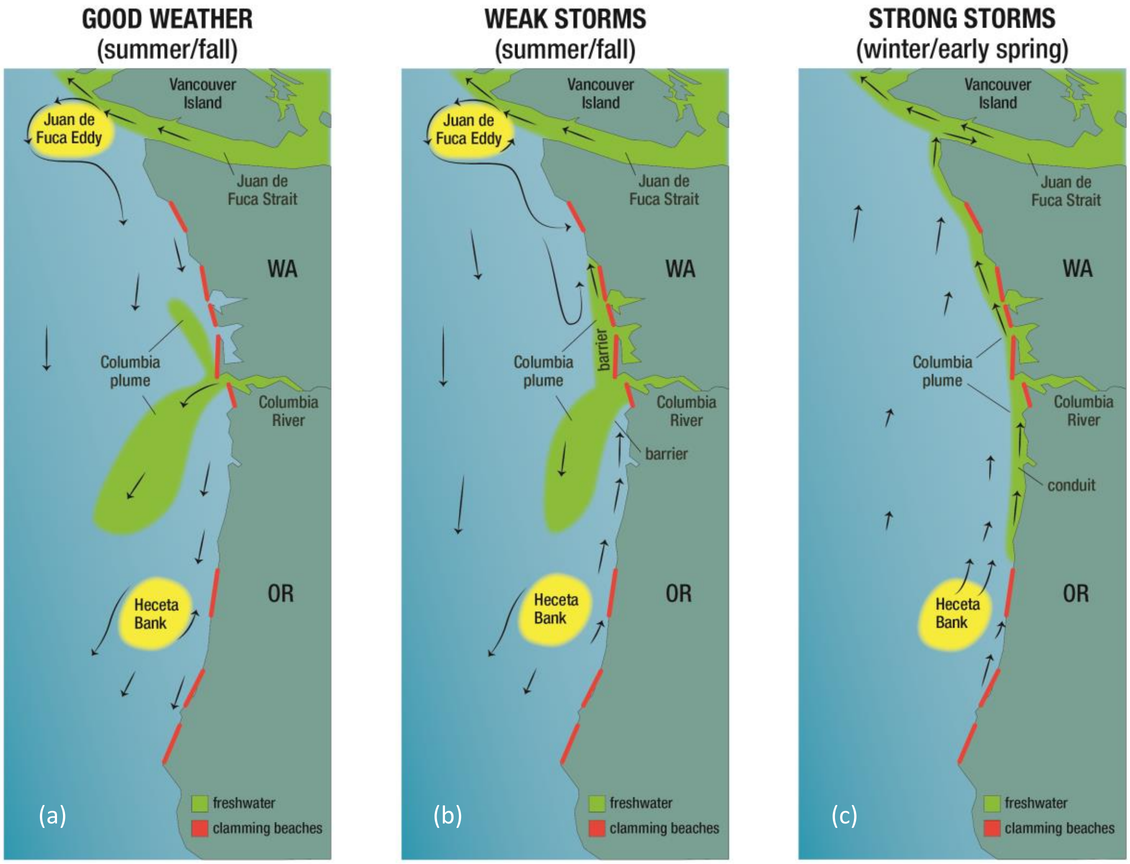

Fig. 7 Environmental conditions in the U.S. Pacific Northwest that transport *Pseudo-nitzschia* cells (a) southward from the Juan de Fuca Eddy in summer/fall, or during good weather conditions, (b) during weak storms, or downwelling-favorable wind reversals, and (c) in the late winter/spring during strong storms. Surface currents are shown with arrows, clamming beaches in red, and green shading indicates freshwater from the Columbia River and Juan de Fuca Strait. The Columbia River plume can act as a conduit or barrier. The offshore retentive sites, Juan de Fuca Eddy and Heceta Bank, where harmful algal blooms typically initiate, are shown as yellow ovals (redrawn from Fig. 9 in Hickey *et al*., 2013).

Data collected during five Ecology and Oceanography of Harmful Algal Blooms–Pacific Northwest (ECOHAB PNW) cruises have allowed scientists to determine the environmental factors correlated with toxic *Pseudo-nitzschia* blooms. These cruises confirmed that *P*. cf. *pseudodelicatissima/cuspidata* and *P*. *australis* are the primary species responsible for toxic events in Washington State while *P*. *multiseries* is also present at lower abundance (Table 2). Environmental parameters consistently correlated with *Pseudo-nitzschia* abundance and particulate DA (pDA) in both the whole cruise grid and the Juan de Fuca Eddy region were Chl-*a*, and Si:P ratios (Table 3). However, it is important to remember that these factors were coincident with *Pseudo-nitzschia* and pDA measurements and were not necessarily factors that promoted the toxic blooms. In addition, the presence of pDA in the Juan de Fuca Eddy region appears to be elevated in regions of reduced iron (Trainer *et al*., 2009); this finding is supported by culture studies demonstrating linkage of DA with trace metal availability (Wells *et al*., 2005).

Table 2 *Pseudo-nitzschia* species, maximum total abundance, and toxicity observed in selected surface samples collected during Ecology and Oceanography of Harmful Algal Blooms–Pacific Northwest (ECOHAB PNW) cruises.

Date	**Eddy or coast**	***Pseudo-nitzschia*** **(cells L^{-1})**	***Pseudo-nitzschia*** **species (%)**	**pDA (nM)**
June 4, 2003	coast	21,000	fraud (65)[1], pun (25), psdeli (5), aus (5); n = 20	1.26
June 4, 2003	coast	24,000	pun (48), fraud (28), deli (8), aus (16); n =25	1.48
June 14, 2003	eddy	8,000	pun (60), psdeli/deli (20), aus (17), multi (3); n = 121	0.33
June 15, 2003	eddy	5,000	pun (58), aus (26), multi (10), psdeli/deli (5); n = 165	0.05
Sep. 1, 2003	coast	324,000	heimii/fraud (96), aus (1), psdeli/deli (3); n = 290	0.01
Sep. 4, 2003	eddy	278,000	aus (97), psdeli/deli (2), heimii (1); n = 342	2.54
Sep. 8, 2003	eddy	69,000	psdeli/deli (57), aus (43); n = 21	0.41
Sep. 11, 2004	coast	250,000	psdeli/deli (99), fraud (1), multi; n = 269	0.21
Sep. 10, 2004	tendril[2]	67,000	psdeli/deli (98), fraud (2); n = 298	1.02
Sep. 13, 2004	eddy	1,467,000	cusp (96)[3], fraud (2), pun (1), aus (1); n = 344	12.65
Sep. 14, 2004	eddy	1,900,000	cusp (99), aus, fraud; n = 402	16.42
Sep. 14, 2004	eddy	1,040,000	cusp (99), multi, aus, fraud; n = 233	7.65
Sep. 15, 2004	eddy	3,280,000	cusp (98), deli (1), pun (1); n = 177	20.25
July 19, 2005	coast	12,000	deli (58), pun (24), heimii (12), fraud (6); n = 17	0
July 22, 2005	eddy	5,333	multi (47), aus/fraud (27), psdeli/deli (11), pun (1); n = 73	0.09
Sep. 14, 2005	coast	299,231	cusp (99), heimii (1); n = 300	1.08
Sep. 15, 2005	coast	1,126,923	psdeli (92), heimii (7), fraud (1); n = 132	1.21
Sep. 15, 2005	coast	43,846	cusp (90), heimii (10); n = 78	1.17
Sep. 20, 2005	eddy	36,000	psdeli (98), heimii (2); n = 212	2.21
Sep. 19, 2005	eddy	1,429	pun (34), heimii (28), psdeli (36), multi (2); n = 61	0.19
Sep. 14, 2006	coast	73,846	psdeli/deli (49), heimii (43), fraud (5), multi (3); n = 139	0.04
Sep. 15, 2006	coast	388,462	psdeli/deli (83), heimii (15), fraud (3), pun (1); n = 238	0.02
Sep. 26, 2006	eddy	80,714	psdeli/deli (68), heimii (30), multi (1), lineola; n = 79	0
Sep. 27, 2006	eddy	50,769	psdeli/deli (65), heimii (35), lineola; n = 47	0.01

[1] Species abbrevations: *P. fraudulenta* = fraud, P. pungens = pun, *P.* cf. *pseudodelicatissima* = psdeli, *P. australis* = aus, *P. multiseries* = multi, *P. cuspidata* = cusp, *P. heimii* = heimii, *P. delicatissima* = deli. Percentage of a species in each sample is shown in parenthesis. When no percentage is noted, the species is present at less than 1%.

[2] A tendril is a patch of *Pseudo-nitzschia* ejected from the Juan de Fuca Eddy.

[3] *P. cuspidata* was identified using transmission (TEM) and scanning (SEM) electron microscopy of samples collected during the September 2004 ECOHAB PNW cruise, and subsequent cruises. This is a new species resulting from the recent separation of *P. pseudodelicatissima* into three species (Lundholm *et al.*, 2003). Therefore, *P. cuspidata* is not positively identified in samples prior to 2004 and the nomenclature psdeli is used. The species psdeli/deli are grouped together when they cannot be distinguished by SEM (modified from Table 2 in Trainer *et al.*, 2009).

Table 3 Spearman's rho (rs) non-parametric correlation analyses for *Pseudo-nitzschia* (PN) and particulate domoic acid (pDA) in Washington State and the Juan de Fuca Eddy region.

	Offshore Washington State		**Eddy region**	
	***Pseudo-nitzschia* (PN)**	**pDA**	***Pseudo-nitzschia* (PN)**	**pDA**
PN	1.00	0.55_{323}^{**}	1.00	0.59_{157}^{**}
pDA	0.49_{564}^{**}	1.00	0.49_{274}^{**}	1.00
Chl-*a*	0.52_{531}^{**}	0.19_{307}^{**}	0.46_{272}^{**}	0.20_{143}^{*}
Temp.	-0.06_{579}	$<0.01_{323}$	0.03_{286}	-0.16_{157}^{*}
Salinity	0.11_{579}^{*}	0.13_{323}^{*}	-0.10_{286}	0.21_{157}^{**}
N	-0.08_{331}	0.08_{188}	-0.21_{157}^{**}	0.25_{88}^{*}
P	-0.10_{286}	0.03_{165}	-0.21_{127}^{*}	0.14_{72}
Si	-0.03_{331}	0.09_{188}	-0.14_{157}	0.26_{88}^{*}
N:P	0.05_{283}	0.19_{163}^{*}	-0.08_{124}	0.37_{70}^{**}
N:Si	-0.08_{331}	0.10_{188}	-0.25_{157}^{**}	0.25_{88}^{*}
Si:P	0.34_{282}^{**}	0.27_{162}^{**}	-0.39_{124}^{**}	0.42_{70}^{**}

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level. Correlations with pDA (shaded) were calculated only using data at sites where *Pseudo-nitzschia* > 0.

Although toxigenic blooms of *Pseudo-nitzschia* have been demonstrated to initiate at offshore retentive sites, the large coastwide bloom that impacted the entire northeastern Pacific region in spring 2015 challenged that paradigm. During this event, the Blob, which approached the west coast of North America, contained low abundances of *Pseudo-nitzschia* cells. Brought to the coast by a series of storms, these cells were met by a fresh supply of nutrients from upwelling. The nutrient-starved *Pseudo-nitzschia* from the Blob were able to quickly assimilate these upwelled nutrients and develop into a massive bloom (McCabe *et al*., 2016). In fact, attempts to collect zooplankton samples during cruises in April and May 2015 failed because large, healthy chains of *Pseudo-nitzschia*, up to 165 cells per chain, clogged zooplankton nets (~200 µm mesh size). The geographical extent of this *Pseudo-nitzschia* bloom and its impacts on DA concentrations in rock crab, Dungeness crab, mussels, razor clams, sardines and anchovies were unprecedented. DA was measured even in the flesh of finfish, although at levels below the regulatory limit. Even more disturbing is the documented relationship of DA closure events with warm water anomalies, including the Blob and El Niño events (McCabe *et al*., 2016; McKibben *et al*., 2017), suggesting that DA events may become more prevalent as surface waters continue to warm.

The economic impacts of DA events can be significant. A study in coastal Washington by Dyson and Huppert (2010) showed that a year-long closure of the recreational razor clam harvest resulted in almost $25 million US in lost income, including over 400 jobs, lost wages and lost income for local businesses, tourism and recreation. In contrast, the 2015 coastwide bloom event resulted in a direct economic impact to California alone of $48.3 million for Dungeness crab (representing 70% of the total estimated commercial value) and $376,000 for rock crab (representing 37% of the total estimated value) from

November 2015 through June 2016[1]. The 2015 closure of the recreational razor clam fishery resulted in at least a $40.3 million loss in tourist-related spending in Washington[2]. Oregon reported an estimated $5.8 million loss. The total loss to Dungeness crab harvest alone was estimated at $100 million (Lowther and Liddel, 2016).

However, these estimates do not include economic losses due to public perceptions of unsafe seafood resulting from incomplete or insufficient information during this large bloom. Education of coastal communities about HABs, their toxins, the retention and release of toxins from seafood, the nutritional value relative to the risk of low-level toxins in this seafood and the impacts of climate change on blooms, is more important now than ever. Efforts to study the large 2015 west coastwide bloom and its impact on different communities will provide a resource to shellfish consumers and enable them to adapt and become more resilient during future blooms.

Acknowledgements

We thank Dr. Stephanie Moore for assistance with the statistics in Table 3.

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[1] Letter from the governor of California, Mr. Edmund Brown, to Ms. Penny Pritzker, former U.S. Department of Commerce Secretary, requesting a fishery resource disaster relief, dated February 9, 2016.

[2] Congressional briefing by Dr. Mary Erickson, Former Director of the NOAA National Centers for Coastal Ocean Science, November 2015 in Washington, DC.

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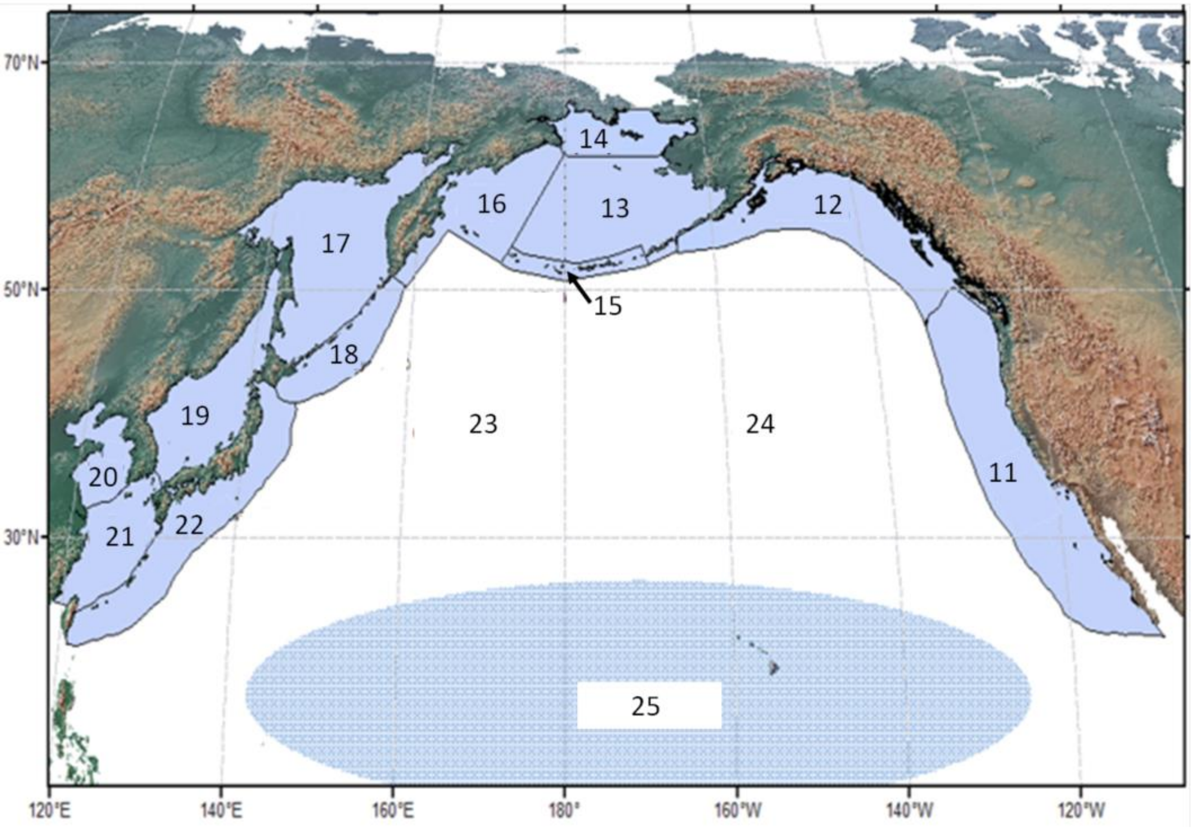
Appendix 1

Biogeographic regions in the PICES Convention Area



Appendix 2

MEQ Workshop (W2) on "Conditions promoting extreme *Pseudo-nitzschia* events in the eastern Pacific but not the western Pacific" at PICES-2016

Co-Convenors**:** Vera L. Trainer (U.S.A.) and Polina Kameneva (Russia)

Abstract

There is clear evidence of contrasting occurrence and impacts of the toxin-producing diatom, *Pseudo-nitzschia*, between the western and eastern Pacific. In 2015, a massive bloom spanning from California to Alaska, had major impacts on the economic viability shellfish industry and on wildlife health. In contrast, *Pseudo-nitzschia* are not highly toxic and do not cause economic losses in the western Pacific. These data provide a unique opportunity for east–west Pacific comparisons to identify and rank those environmental factors that promote harmful algal bloom (HAB) success at different times. The recent PICES-funded workshop on HABs and Climate Change emphasized the importance of studying such extreme events to further our understanding of climate impacts. This workshop will focus on *Pseudo-nitzschia*—a diatom that historically had massive economic impacts in the eastern PICES member countries, with low or no impacts in the western Pacific. The workshop foundation will be an extension of the current dataset to the 1990s and earlier where available, with PICES participants pre-submitting available data on: HAB species presence, maximum abundance, toxicity, optimal conditions for growth, time of year, temperature range, salinity range, water clarity, nutrients, wind, river flow (flooding), and upwelling indices.

Speakers:[3]

Inna V. Stonik (Invited)

Pseudo-nitzschia diversity, bloom events and their impacts in the North Pacific: An East-West comparison

Nicola Haigh

Pseudo-nitzschia species and domoic acid on the west coast of Vancouver Island, British Columbia, in 2015

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[3] See http://meetings.pices.int/publications/presentations/PICES-2016#workshop2 for individual presentations.

Yuichi Kotaki

Amnesic shellfish poisoning (ASP) potential in Japan

Vera L. Trainer

Pseudo-nitzschia and domoic acid on the US west coast: State of our knowledge and implications for the future

Meredith L. Elliott

Pseudo-nitzschia occurrence in the central California Current

William P. Cochlan

The effects of temperature and ocean acidification on the growth and toxicity of *Pseudo-nitzschia australis* from the California Current upwelling system

Lin Yang

Pseudo-nitzschia harmful algal blooms (HAB) in the coast of China

Weol-Ae Lim

Temporal changes and toxicity of *Pseudo-nitzschia* species in Korean coastal waters

Tamara Russell

Pseudo-nitzschia spp. and domoic acid in the waters of Haida Gwaii, British Columbia: A summary of occurrences and details on anthropogenic and environmental considerations

Devan Johnson

Pseudo-nitzschia species and domoic acid in southeast Vancouver Island, November 2015 to July 2016

Anthony Odell

Washington State Pacific coast *Pseudo-nitzschia* bloom of 2016

Appendix 3

Olympic Region Harmful Algal Bloom (ORHAB) partnership sampling methods

1. Collect whole water and net tow samples twice weekly (March 1–October 31) and once weekly (November 1–February 28). The number of sample sites as well as sample frequency may be increased when *Pseudo-nitzschia* (PN) and domoic acid (DA) are present. One L of whole water is filtered onto a Nucleopore HA filter (0.45 µm pore size) and frozen at –80°C until analyzed for particulate DA (pDA).
2. Count settled whole water samples for total PN. When >50,000 large PN cells L^{-1} or >1 million small PN cells L^{-1} are observed, State managers are notified that PN abundance is over the "action level" and a test for particulate DA is performed as soon as possible using the Mercury Science DAK-36 enzyme linked immunosorbent assay (ELISA). If it is apparent from net tow samples that PN abundance may be above the action level, samplers may run an ELISA for pDA prior to counting PN from whole water which often need to be settled overnight.
3. If ELISA results for pDA are >200 ng L^{-1}, state managers are notified and an ELISA is run on shellfish samples (in Washington State, razor clam samples are the primary shellfish species analyzed because they can retain DA for many months). Composite razor clam samples (a composite of 12 individual clams when possible) are analyzed for DA using ELISA. If ELISA results show elevated levels of DA in shellfish, additional tissue samples are also sent to the Public Health Lab for analysis of DA by high-performance liquid chromatography (HPLC; the standard regulatory method).
4. Mercury Science[4] DA ELISAs allow individual particulate (cellular) DA filters to be tested in duplicate in strips – up to ~12 individual samples per ELISA plate because a full standard curve is analyzed with each sample at a total cost of ~$250. Biosense[5] ELISA can also be used, but these cost ~$500 per plate for fewer samples. The Mercury Science ELISA is not Association of Analytical Communities (AOAC) or Interstate Shellfish Sanitation Conference (ISSC) approved. This method is used to screen for DA; positive tests must be confirmed by approved regulatory methods for detection of DA in shellfish. Emergency closures can be enacted by using screening methods, while reopening of shellfish beds after closure requires testing using approved methods.
5. Depending on the timing of the scheduled razor clam openers, the timing of the observed PN bloom and the ability of the coastal ORHAB samplers to run immediate ELISA, pre-emptive closures may be decided upon by the Washington State Department of Health and the Washington Department of Fish and Wildlife. However, this is rare. Closures are enacted when a value of 20 ppm or greater in razor clam is measured by HPLC.

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[4] http://www.mercuryscience.com/DA.html

[5] https://www.abraxiskits.com/products/algal-toxins/

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BOOKS – are peer-reviewed, journal-quality publications of broad interest.

PICES PRESS – is a semi-annual newsletter providing timely updates on the state of the ocean/climate in the North Pacific, with highlights of current research and associated activities of PICES.

ABSTRACT BOOKS – are prepared for PICES Annual Meetings and symposia (co-)organized by PICES.

For further information on our publications, visit PICES at www.pices.int.

Front cover figure

Clockwise from left: Sign on a Washington State beach warning razor clam diggers of beach closures due to dangerous levels of domoic acid in shellfish, a recreational razor clam harvester digging clams with a “tube”, chains of *Pseudo-nitzschia* sampled off central California during a massive coastwide 2015 harmful algal bloom, and a net filled with razor clams.