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ENVIRONMENTAL CONDITIONS ASSOCIATED WITH DOMOIC ACID IN RAZOR CLAMS ON THE WASHINGTON COAST

NICOLAUS G. ADAMS,*[1] MITCH LESOING,[2] AND VERA L. TRAINER[1]

[1]*National Marine Fisheries Service*
Northwest Fisheries Science Center
Environmental Conservation Division
2725 Montlake Blvd. E.
Seattle, Washington 98112
[2]*Quileute Natural Resources*
Quileute Indian Tribe
234 Front St.
La Push, Washington 98350

ABSTRACT In October 1998, record levels of the neurotoxin domoic acid (DA) were detected in razor clams (*Siliqua patula*, Dixon) resulting in the closure of shellfish harvesting areas along the Washington coast. This toxin was detected in seawater samples collected at Kalaloch Beach and Second Beach on the central Washington coast using a receptor binding assay and liquid chromatography-tandem mass spectroscopy. Domoic acid levels ranging from 0–2700 ng/L were measured in seawater samples containing from 70–100% *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle at concentrations of 1.0–15 × 10^6 cells/L, resulting in maximum levels of cellular toxin of approximately 500 fg/cell. A cultured isolate of this species collected from Kalaloch Beach also produced DA, as determined by the receptor binding assay, during late exponential and stationary stages of growth. The toxic *P. pseudodelicatissima* bloom in the late summer and autumn of 1998 occurred 2–3 weeks after strong coastal upwelling during a period of anomalously low rainfall, typical in post-El Niño years. Higher toxin levels in seawater at Kalaloch Beach compared to Second Beach were attributed to the periodic nature of upwelling at Kalaloch Beach, demonstrated by a 175-fold increase in nitrate in seawater coincident with a 5 °C decrease in sea surface temperature on September 1. The upwelling event in September was followed by wind relaxation and reversal at the end of that month, resulting in the transport of toxic cells toward the coast where nutrients were already present to fuel the algal bloom. A pulse of nutrients, either from rainfall or upwelling, to coastal regions that have experienced several weeks of low nutrients, followed by wind relaxation or reversal events that transport cells to inshore regions, are suggested to be important factors in the initiation of the most toxic *Pseudo-nitzschia* species blooms on the Washington coast.

KEY WORDS: *Pseudo-nitzschia*, domoic acid, razor clams, upwelling

INTRODUCTION

The first domoic acid (DA) poisoning of humans was reported in 1987 in eastern Canada (Todd 1993). A series of collaborative studies demonstrated that the toxin was concentrated in mussels that had fed on a bloom of *Pseudo-nitzschia multiseries*, the first pennate diatom from which DA was isolated (Bates et al. 1989). In 1991, DA was implicated in the illness and death of brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in Monterey Bay, California (Work et al. 1993). Using laboratory isolates, Garrison et al. (1992) determined that *P. australis* was the DA-producing diatom responsible for the mortalities. Unlike the outbreak in eastern Canada, where mussels were the toxin vector (Wright et al. 1989), anchovies became toxic to seabirds after feeding on the diatom, *P. australis*, in Monterey Bay (Buck et al. 1992, Fritz et al. 1992), illustrating that DA has at least two means of entering the higher food web, through filter-feeding molluscan shellfish and suspension-feeding finfish.

The poisoning event in Monterey Bay resulted in the establishment of a DA monitoring program in the states of Washington and Oregon. In October 1991, about 1 month following the toxic bloom event in California, levels of DA above the regulatory limit of 20 μg/g shellfish tissue were found in the edible parts of razor clams (*Siliqua patula*) and Dungeness crabs (*Cancer magister*) collected on the Washington coast (Wekell et al. 1994). Consequently, beaches were temporarily closed to recreational and commercial shellfish harvesting (Horner and Postel 1993), resulting in a substantial loss of revenue ($15–20 million) to local fishing communities (Anderson 1995). The *Pseudo-nitzschia* species responsible for the DA poisoning event was not determined, because there were no phytoplankton samples collected on the Washington coast immediately before the measurement of toxin in razor clams (Horner et al. 1993). Several species that had been determined to be toxic in some, but not all, geographical regions (i.e., *P. multiseries, P. australis, P. pungens*, and *P. pseudodelicatissima*) were found in Washington coastal waters (Horner et al. 1997). Among those species, *P. australis* was thought to be responsible for the presence of DA, because it was identified in samples collected off Grays Harbor, Washington, several months after the initial event (Horner and Postel 1993). Taylor and Horner (1994) suggested that the 1991 toxic bloom event off the Washington coast might have been part of a widespread bloom of *P. australis*. It was estimated that a bloom starting in California in September 1991 and carried up the coast by currents at speeds of nearly 20–40 cm/sec could have reached the Washington coast by late October to November (Horner et al. 1997).

More recently, in May and June of 1998, the first confirmed deaths of a marine mammal species attributable to DA poisoning were documented in sea lions along the central California coast. In this mortality event, sea lions fed on anchovies and sardines that

*Corresponding author. E-mail: nicolaus.adams@noaa.gov

had ingested toxic *P. australis* cells (Gulland et al. 1999, Lefebvre et al. 1999, Scholin et al. 2000). Because razor clam toxification on the Washington and Oregon coasts in 1991 was preceded by the death of seabirds in Monterey Bay earlier that summer, we suspected that the poisoning of sea lions by DA in 1998 was an early warning of impending toxicity on the Washington and Oregon coasts.

Because concentrations of *Pseudo-nitzschia* spp. increase and subside rapidly in Washington coastal waters (R. Horner, pers. comm.), a more rigorous and frequent monitoring effort was implemented by sampling at two beaches in order to investigate the relationships among *Pseudo-nitzschia* spp. cells, DA levels in seawater and shellfish, nutrients, and a variety of environmental variables. This paper describes weekly sampling of the surf zone, which enables us to describe the chemical, biological, and physical processes preceding and during the razor clam toxification event that occurred in the late summer and early autumn of 1998.

METHODS

Sample Collection

Seawater was sampled on a weekly basis from the surf zone of two accessible beaches on the Pacific coast of Washington State: Second Beach, near La Push, and Kalaloch Beach, approximately 65 km south of La Push (Fig. 1). Seawater samples were collected using a bucket and preserved for phytoplankton species identification and enumeration, as well as DA, chlorophyll *a*, and nutrient analyses as described below.

Nutrient and Chlorophyll a Analyses

Seawater was filtered through a 25-mm Whatman #1 filter and collected in a 60-mL polyethylene bottle, frozen, and later analyzed for nutrient concentrations using standard autoanalyzer methods (Whitledge et al. 1981). Aliquots (50 mL) of seawater samples were filtered through Whatman GF/F filters for chlorophyll *a* detection. Filters were stored at −20 °C until analysis by extraction with 10 mL 90% acetone overnight at 4 °C in the dark. Extracts were analyzed using the standard fluorometric method (Welschmeyer 1994) using a Turner Designs (TD-700) fluorometer with narrow bandpass filters.

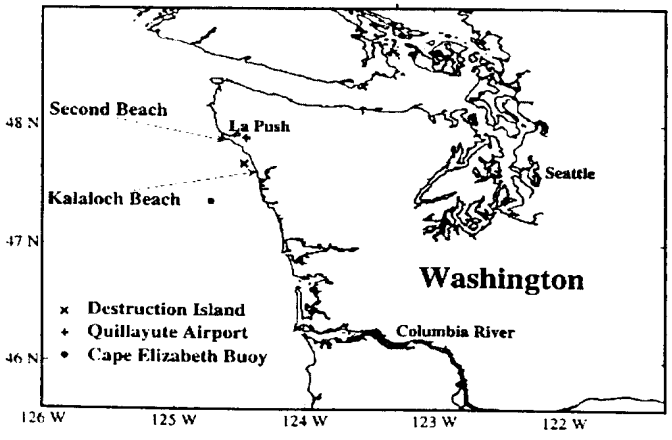

Figure. 1. Sampling sites on the central Washington coast and locations of coastal environmental monitoring stations.

DA Analysis in Phytoplankton

Cellular DA was measured by filtering 2 liters of seawater through a 47-mm 0.45-μm (Millipore HA) filter. Depending on the density of the material in the sample, up to three filters were used. Filters were folded in half with forceps, wrapped in aluminum foil, and frozen until analysis using the method described by Van Dolah et al. (1997). A glutamate decarboxylase digestion step was used before analysis to remove endogenous glutamate in all samples. Binding experiments were initiated by incubation of 100 μL of cloned GluR6 membrane preparation (Taverna and Hampson 1994) with 50 μL of a 5 nM solution of [^{3}H] kainic acid and 50 μL of standard or sample in a 13 × 10 mm glass test tube. Samples were vortexed briefly, incubated at 4 °C for 1 h, poured over Whatman GF/C filters (25-mm diameter), and rinsed twice with 5 mL of 50 mM Tris-citrate buffer (pH 7.4). Filters were placed in scintillation vials, soaked overnight in 10-mL scintillation fluid, and radioactivity was measured using scintillation spectroscopy. For quantification of DA in selected seawater samples, liquid chromatography-tandem mass spectroscopy (LC-MS/MS) was performed according to standard protocols (Scholin et al. 2000).

Cell Counts

Phytoplankton cells were counted by first pouring 100 mL of seawater into a graduated cylinder, then fixing with formaldehyde to a final concentration of approximately 1 % and settling for 24–72 hours. Ninety mL of water were carefully drawn off with a pipette and the settled material was resuspended. A 0.1 mL subsample was loaded into a Palmer–Maloney slide, and a minimum of 100 individual algal cells were counted at 100X magnification using light microscopy. Phytoplankton cells were identified to the lowest possible taxon and to species when possible. The percentage contribution of the *Pseudo-nitzschia* species for the whole phytoplankton assemblage, viewed at 100X, was calculated.

Scanning Electron Microscopy

In samples where *Pseudo-nitzschia* spp. were numerous, aliquots were prepared using a modified $KMnO_4$/HCl oxidation method (Miller and Scholin 1998). Filter membranes with processed samples were bonded to aluminum stubs, air-dried, coated with gold-palladium, and examined with an AMRAY 1000 SEM.

Growth Study

Pseudo-nitzschia pseudodelicatissima, clone NWFSC-047, isolated from a plankton sample collected at Kalaloch Beach, Washington on July 31, 1999, was grown in batch culture. The isolate used in the growth study was not axenic, but sterile technique was used throughout the experiment. Cells were maintained in f/2 medium (Guillard and Ryther 1962) made with filtered natural seawater. An inoculum of exponentially growing cells was used. Duplicate cultures (200 mL each) were grown in 1-L borosilicate culture flasks at 13 °C. Irradiance was provided by a bank of Cool-White fluorescent lamps (15 W). Cells were exposed to a 12:12 h light:dark cycle at a light intensity of 80 μE m^{-2} s^{-1}. Every 3–4 days during the study, 20-mL aliquots from each flask were filtered onto Millipore HA, 0.45-μm, 25-mm diameter filters using vacuum (< 15 psi). Filters were stored and analyzed for DA by receptor binding assay as described above, and a 0.1-mL aliquot of culture was collected for cell enumeration.

Environmental Data

Wind speed and direction were obtained from the National Data Buoy Center's weather station on Destruction Island, Washington (NDBC #DESW1, 47.68 °N, 124.49 °W), which is about 7 km northwest of Kalaloch Beach. Rainfall data were obtained from the Quillayute airport, near La Push, Washington and from the Kalaloch Ranger Station in Kalaloch, Washington. Water temperature at beach sampling sites was measured with a thermometer. Sea surface temperatures were obtained from the Cape Elizabeth buoy (NDBC #46041) at 47.4 °N, 124.5 °W. The locations of coastal monitoring stations are shown in Figure 1.

Domoic Acid Analysis in Shellfish

Razor clams were collected at Kalaloch Beach for DA analysis. Concentrations of DA were determined utilizing a methanol/water extraction and analysis by high-performance liquid chromatography (HPLC) (Hatfield et al. 1994). Additional razor clam toxin concentration data were obtained from samples collected by Quileute Natural Resources staff and analyzed by the Washington Department of Health (WDOH). Only the edible parts of the clams were analyzed for DA (i.e., viscera were not analyzed).

RESULTS

Pseudo-nitzschia Cell Numbers and Domoic Acid Levels

As *Pseudo-nitzschia* spp. cell counts increased, there was a corresponding increase in the level of DA activity detected in seawater at Kalaloch Beach (Fig. 2a). Both cell counts and DA activity in seawater reached a maximum on September 22 at 17.1 $\times 10^6$ total *Pseudo-nitzschia* spp. cells/L and 2,700 ng DA/L seawater. A smaller peak of DA activity on August 25 also corresponded with elevated *Pseudo-nitzschia* spp. cell counts. The highest recorded level of DA detected in razor clams (295 μg/g) occurred within 18 days after the maximum *Pseudo-nitzschia* spp. cell number and level of seawater toxicity were measured.

At Second Beach (Fig. 2b), the highest number of *Pseudo-nitzschia* spp. cells was about three times lower than that at Kalaloch Beach, reaching a maximum of 5.9×10^6 cells/L on September 23; whereas, the level of DA activity detected in seawater reached a maximum of 350 ng/L on October 6. There were also smaller peaks in *Pseudo-nitzschia* spp. cell counts on August 12 and 26 associated with increased DA activity in seawater. An increase in *Pseudo-nitzschia* spp. cells on September 9 did not correspond to elevated DA in seawater; however, toxin levels increased by September 13, corresponding to levels of 2.2×10^6 total *Pseudo-nitzschia* cells/L. Razor clams were not collected from Second Beach for measurement of DA.

Phytoplankton Assemblage Observations

Pseudo-nitzschia pseudodelicatissima (Fig. 3) was the only known DA-producer present in significant numbers within the phytoplankton assemblage (Table 1). Most of the other *Pseudo-nitzschia* were *P.* cf. *heimii* (up to 90% of the total *Pseudo-nitzschia* on August 25 at Kalaloch Beach) plus relatively small amounts of *P. pungens* (less than 3% on dates when SEM was done) and *P. delicatissima* (8% on September 1 at Kalaloch Beach, the only sample in which this species was observed).

Table 1 shows *Pseudo-nitzschia* spp. as a percentage of the whole phytoplankton assemblage and *P. pseudodelicatissima* as a percentage of total *Pseudo-nitzschia* spp. at Kalaloch and Second Beaches. At both sampling sites, *Pseudo-nitzschia* spp., as a percent of total phytoplankton species, increased until the end of

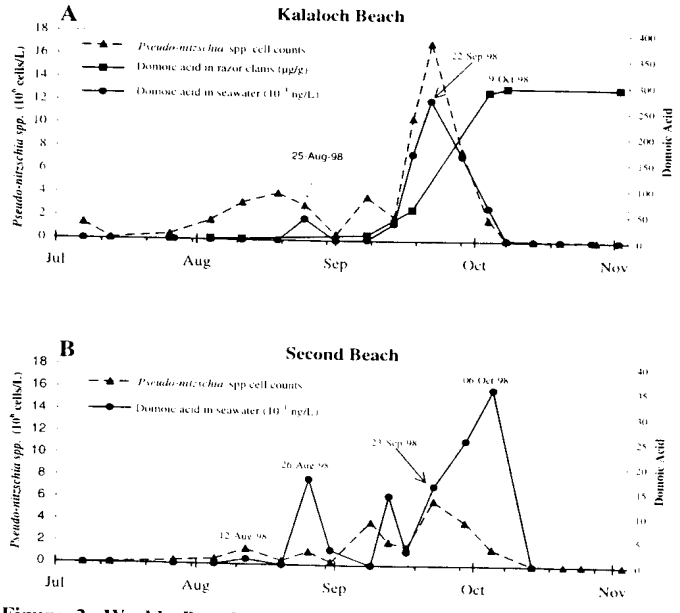

Figure. 2. Weekly *Pseudo-nitzschia* spp. cell counts and domoic acid in seawater from July through October 1998, at Kalaloch Beach (A) and Second Beach (B). Domoic acid levels in razor clams are shown for Kalaloch Beach.

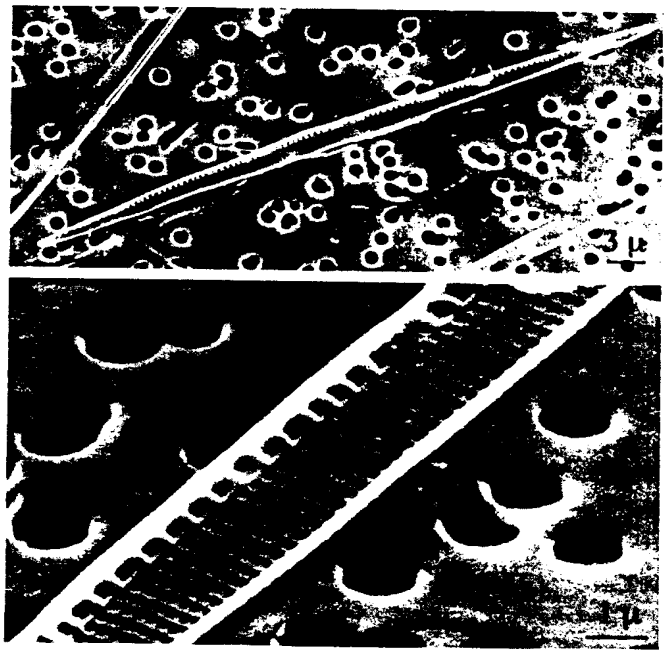

Figure. 3. Scanning electron micrographs of a field isolate of *P. pseudodelicatissima* from Kalaloch Beach. (top) a whole valve. (bottom): higher magnification showing 1 row of square poroids between interstriae. Scale bars indicate size.

TABLE 1.

***Pseudo-nitzschia* spp. abundance and domoic acid levels on the central Washington coast in 1998.**

Kalaloch						
Date	*Pseudo-nitzchia* spp. (10^6 cells/L)	*Pseudo-nitzchia* spp. abundance[1]	Domoic Acid (ng/L)	Domoic Acid (fg/cell)[2]	Domoic Acid by LC-MS/MS	*P. pseudodelicatissima* abundance[3]
7 July	1.4	37	0			
13 July	0.1	30	0			
26 July	0.5	34	0		–	
4 Aug	1.7	48	0			
11 Aug	3.2	30	0			
19 Aug	4.1	59	0			40
25 Aug	3.0	64	410			
1 Sept	0.6	41	0			70
8 Sept	3.8	83	10			90
14 Sept	2.0	93	330			
18 Sept	10.6	97	1700	170	+	90
22 Sept	17.1	99	2700	180	+	90
29 Sept	7.8	99	1600	210	+	100
5 Oct	1.8	92	640	500	+	70
9 Oct	0.1	41	10			20
15 Oct	0.0	15	0			
21 Oct	0.0	16	0			
29 Oct	0.0	4	no data			
3 Nov	0.0	3	0			

Second Beach						
Date	*Pseudo-nitzchia* spp. (10^6 cells/L)	*Pseudo-nitzchia* spp. abundance[1]	Domoic Acid (ng/L)	Domoic Acid (fg/cell)[2]	Domoic Acid by LC-MS/MS	*P. pseudodelicatissima* abundance[3]
7 July	0.0	18	0			
13 July	0.1	38	0			
27 July	0.3	54	0			
5 Aug	0.5	56	0			60
12 Aug	1.4	78	10			60
20 Aug	0.4	71	0			
26 Aug	1.2	88	170	160		90
31 Aug	0.3	84	30			60
9 Sept	3.9	95	0			
13 Sept	2.2	97	140	109		90
17 Sept	1.7	99	30			
23 Sept	5.9	99	160	40		70
30 Sept	3.9	100	250			
6 Oct	1.6	98	360	230	+	100
15 Oct	0.0	50	0			
22 Oct	0.0	3	no data			
26 Oct	0.0	26	no data			
4 Nov	0.0	6	no data			

[1] as a percentage of the phytoplankton assemblage viewable at 100X; [2]domoic acid per cell was only calculated where DA was > 100 ng/L, and *P. pseudodelicatissima* abundance was >70%; [3]estimated values as a percentage of total *Pseudo-nitzschia* spp.

September and early October, at which time the percentage declined. The increase was due to a bloom of *P. pseudodelicatissima*, which comprised the majority of all *Pseudo-nitzschia* observed. *Pseudo-nitzschia pseudodelicatissima* reached maximum numbers on September 22–23, corresponding to measurable levels of DA at both sites and the highest measured DA levels at Kalaloch Beach. After October 6, numbers of *Pseudo-nitzschia* spp. declined dramatically, as did toxin levels at both sites. The LC-MS/MS results confirm that DA was present on September 18, 22, 29, and October 5 at Kalaloch Beach and on October 6 at Second Beach.

Nutrients and Chlorophyll a

Figure 4 shows the concentrations of phosphate, silicate, and nitrate relative to *Pseudo-nitzschia* spp. cell counts at Kalaloch Beach and Second Beach (*Pseudo-nitzschia* spp. cell counts are duplicated from Fig. 2). On July 26, there was an increase in the levels of all three nutrients at Kalaloch Beach (Fig. 4a). Phosphate and silicate increased by a factor of approximately 4 compared to the previous sampling date, and nitrate increased by a factor of about 40. Approximately 3 weeks after the influx of nutrients, there was a subsequent rise in the number of *Pseudo-nitzschia* spp.

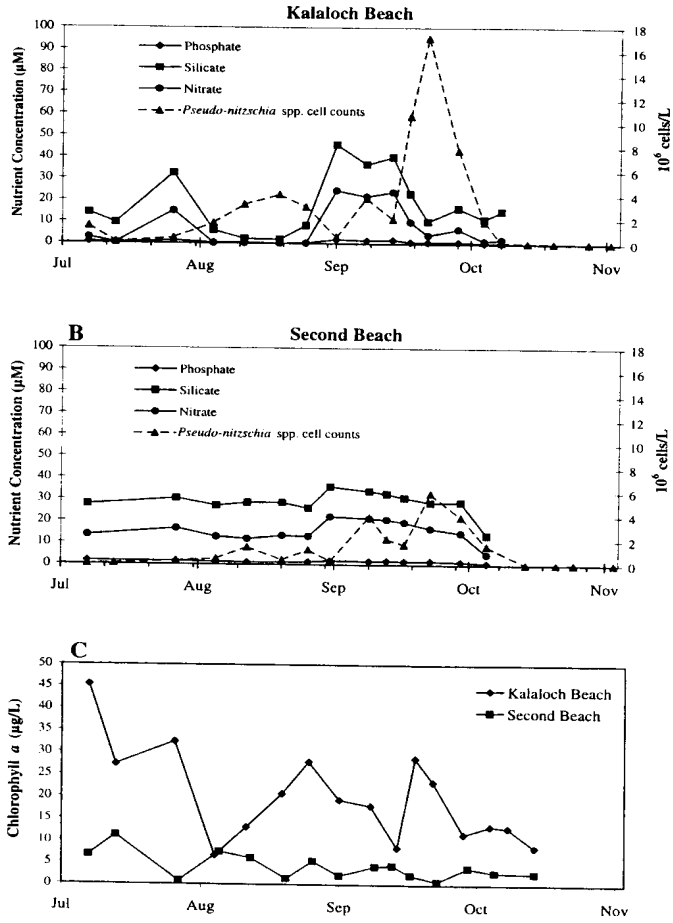

Figure. 4. Weekly concentrations of nitrate, silicate, phosphate from July through October 1998, at Kalaloch Beach (A) and Second Beach (B). *Pseudo-nitzschia* spp. cell counts are duplicated from Figure 2 for reference. Weekly chlorophyll *a* concentrations at Kalaloch Beach and Second Beach are shown (C).

cells that reached a maximum of 4 × 10^6 cells/L in August. There was another increase in nutrient levels on September 1; phosphate and silicate increased by a factor of 5 from the previous sampling date, and nitrate increased by a factor of 175. Until about September 14, nutrient levels remained relatively constant at Kalaloch Beach, with phosphate ranging from 1–2 μM, silicate 37–46 μM, and nitrate 22–24 μM, with the highest levels occurring on September 1. About 2–3 weeks following this second increase in nutrients, *Pseudo-nitzschia* spp. cell numbers increased from 0.5 × 10^6 cells/L on September 1 to 2.0 × 10^6 cells/L on September 13. Nutrient levels decreased substantially on September 18, corresponding to an increase in the number of *Pseudo-nitzschia* spp. to 10.6 × 10^6 cells/L. *Pseudo-nitzschia* spp. cell numbers reached their maximum of over 17 × 10^6 cells/L several days later on September 22, while nutrients continued to decline. After reaching this peak, *Pseudo-nitzschia* spp. cell counts rapidly decreased and remained low throughout the remainder of 1998. Chlorophyll *a* levels at Kalaloch Beach were routinely higher than those at Second Beach, except for the August 5 sampling date, when they were similar (Fig. 4c).

Second Beach showed more sustained high levels of nutrients compared to Kalaloch Beach (Fig. 4b). At Second Beach from July 7 to September 30, phosphate ranged from 1–2 μM, silicate from 26–36 μM, and nitrate from 12–22 μM. The concentrations of these nutrients peaked on August 31. After nutrient concentrations reached their maximum, there were two distinct peaks in *Pseudo-nitzschia* spp. cell numbers (4 × 10^6 cells/L on 20 August and 6 × 10^6 cells/L on September 23), which coincided with slightly decreased nutrient concentrations. On September 30, the number of *Pseudo-nitzschia* spp. cells began to decrease and on October 6 the levels of nutrients also began to decline. Chlorophyll *a* levels at Second Beach were less than 10 μg/L on all sampling dates (Fig. 4c).

Meteorological and Oceanographic Conditions

Monthly precipitation and sea surface temperatures (SST) are shown in Table 2 (see Fig. 1 and Methods for the locations of these monitoring sites). Average values for June through October for the period of record are compared to data for those months in 1998. Water temperatures for 1998 were similar to levels documented during the period of record (1987–1993). However, levels of precipitation for the summer of 1998 were lower than the average for the period of record (1948–1997), with September 1998 values almost an order of magnitude less than the average. There were also comparatively low amounts of rainfall at Kalaloch Beach during August and September of 1998 (B. Rhode pers. comm.).

Wind vectors are shown in Figure 5a. Data from the end of July through September showed periods of upwelling favorable winds (vectors pointing toward the southeast), with the most sustained period of strong upwelling in late August through early September. Periods of wind relaxation and mild reversal were common

TABLE 2.

Monthly mean sea surface temperature and precipitation on the Washington coast.

	Period of Record				
	June	**July**	**August**	**September**	**October**
Precipitation (cm) 1948–1998	8.0	6.6	5.7	12.2	26.4
Sea surface temperature (°C) 1987–1993	12.9	13.5	13.8	13.2	12.3
	1998				
	June	**July**	**August**	**September**	**October**
Precipitation (cm)	3.2	5.3	0.4	1.3	20.8
Sea surface temperature (°C)	12.6	13.8	14.0	12.4	12.6

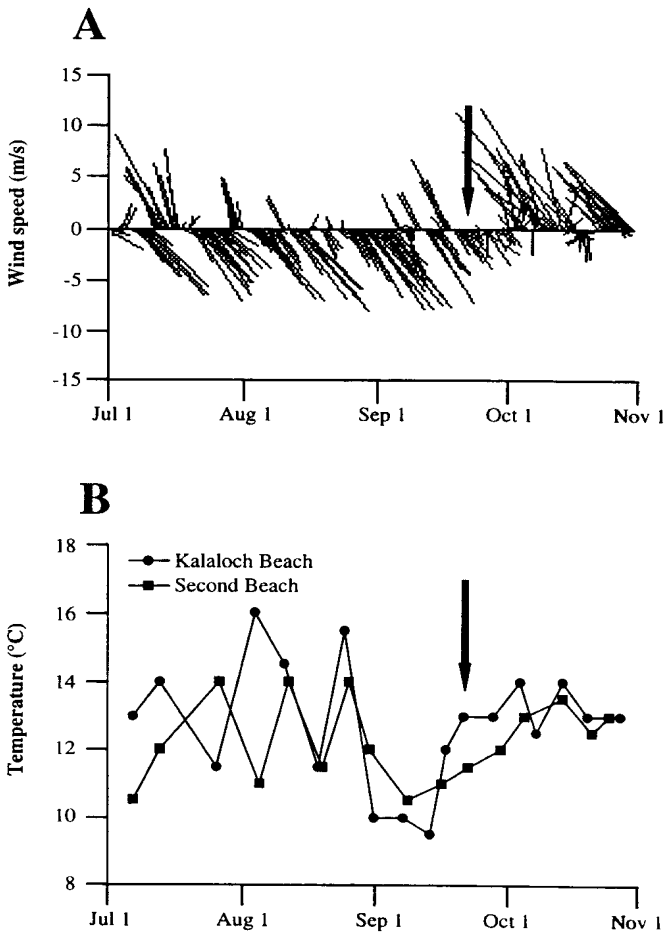

Figure. 5. A. Vector time series of daily average winds from June through October 1998. Length of lines refer to speed, angle of vector refers to direction. B. Weekly sea surface temperature at Kalaloch Beach and Second Beach from July through October 1998. Solid arrows indicate the date when the maximum numbers of *Pseudo-nitzschia* spp. cells were seen at both beaches (September 22–23). Wind reversal immediately before arrow is on September 16–19.

through the summer months. Upwelling favorable winds were sustained from late August through early September, and then began to weaken in strength toward the end of September. In the beginning of October, a strong transition to northward (non-upwelling) winds was observed.

A decrease in water temperature was recorded at both beaches in mid-August, followed 1 week later by a warming event, which corresponded to the first observation of toxic *Pseudo-nitzschia* spp. (Fig. 5b). Water temperature decreased by approximately 6.0 °C at Kalaloch Beach and 4.5 °C at Second Beach from late August to mid-September, coincident with strong northwesterly winds. Water temperature increased at both sites during the last 2 weeks in September and into early to mid-October. At both beaches, the maximum numbers of *Pseudo-nitzschia* spp. cells were seen during this temperature increase (solid arrow).

Pseudo-nitzschia pseudodelicatissima Growth Study

Pseudo-nitzschia pseudodelicatissima in culture had apical axes of 38–60 μm, transapical axes of 2–2.5 μm, 33–40 striae in 10 μm, 18–21 fibulae in 10 μm, and 5–6 poroids per 1 μm. Except for the apical axes, which were approximately half of the reported values, these measurements are similar to those describing *P. pseudodelicatissima* in Hasle et al. (1996). Figure 6 shows average growth rate and DA production for duplicate cultures of *P. pseudodelicatissima*. DA activity increased steadily through exponential growth phase, and as the cells reached the late stationary growth phase, DA production dramatically increased. This experiment illustrates that an isolate of *P. pseudodelicatissima* from Washington coastal waters does produce DA as measured by a receptor binding assay, and toxin production increases as the cells reach stationary phase.

DISCUSSION

Domoic Acid Observations on the U.S. West Coast in 1998

The same species of *Pseudo-nitzschia* was not responsible for all toxic events along the U.S. west coast during 1998. DA was first detected in sardines and anchovies in California coastal waters in May 1998, then subsequently measured in razor clams on the Oregon coast beginning in late July and in Washington State razor clams later that summer. *Pseudo-nitzschia australis* was responsible for the death of over 50 sea lions in May and June 1998 along the central California coast (Gulland et al. 1999, Lefebvre et al. 1999, Scholin et al. 2000). Levels of DA in razor clams in Oregon, above the 20 μg/g regulatory limit, coincided with the presence of *P. australis* in coastal waters beginning in late July (D. Cannon pers. comm., Trainer et al. in press.). In contrast, we report here that the toxic event on the Washington coast that resulted in record levels of DA in razor clams in October 1998 was attributable to a bloom of *P. pseudodelicatissima*.

The association of different *Pseudo-nitzschia* species with distinct toxification events indicates that the hydrographic factors influencing toxic bloom occurrence and toxic cell transport may be unique to a given U.S. West Coast region. Given the southward surface flow of the California current system and maximum Columbia River discharge as a low salinity, low density, offshore plume lying to the southwest during summer months, it is unlikely that a northward transport of phytoplankton cells is a primary means by which toxic cells are spread northward along the coast from California to Washington. We hypothesize that onshore advection of localized offshore populations of *Pseudo-nitzschia* spp. cells that are present in U.S. West Coast waters during summer months (Horner et al. in press) is the likely means of toxic bloom initiation in Washington State waters.

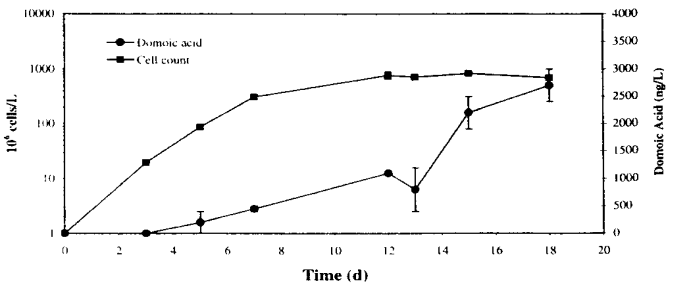

Figure. 6. Cell counts and domoic acid levels in a cultured isolate of *P. pseudodelicatissima*. The error bars indicate the range of values from duplicate measurements at each time point.

Environmental Conditions

What were the environmental conditions that allowed a toxic bloom to occur? Observations of toxic blooms in eastern Canada, central California, and Puget Sound, Washington, have indicated that rainfall may provide a significant source of nutrients that are important in bloom initiation. High precipitation following an unusually dry summer was suggested as a causative factor in 1987 DA incident in eastern Canada (Bird and Wright 1989, Smith et al. 1990), with runoff supplying the nitrogen source. Similar conditions were documented in the 1991 DA episode off the Washington coast, which occurred after a hot, dry period followed by rain (Horner and Postel 1993). Lack of runoff in central California during spring through autumn is the norm (Buck et al. 1992); however, record levels of rainfall were experienced in central California during the early months of 1998 before the outbreak of *P. australis* (Trainer et al. 2000, Scholin et al. 2000). Rainfall and corresponding river runoff, followed by calm weather, were important factors in the development of a *Pseudo-nitzschia* spp. bloom in Penn Cove, Puget Sound in the summer of 1997 (Trainer et al. 1998).

Rainfall, however, is not the only source of nutrients to coastal environments. The small upwelling events that punctuate the oceanic autumn/winter transition in the northeastern Pacific (Bolin and Abbott 1963) may substitute for freshwater runoff as a nitrogen source. The toxic *P. australis* blooms observed off the California coast in 1998 were positioned in coastal upwelling zones (Trainer et al. 2000). The present study shows that nutrient inputs from upwelling in the absence of significant rainfall fueled the *Pseudo-nitzschia* spp. bloom off the central Washington coast in late September 1998. These case histories make it clear that a pulse of nutrients, whether from rainfall and subsequent river runoff or from coastal upwelling events, is required to fuel toxic *Pseudo-nitzschia* spp. blooms.

The transition to wind relaxation and reversal events in mid- to late September and a strong reversal in early October, which marked the beginning of the autumn transition, resulted in the Ekman transport of surface water toward the coast beginning in mid-September. When upwelling winds reverse direction or relax, surface waters can be transported rapidly into the nearshore region where algal cells can mix vertically to the bottom (Donaghay and Osborn 1997). The beginning of the autumn transition in 1998 corresponded with a dramatic increase in water temperature at Kalaloch Beach (+ 4 °C) and at Second Beach (+ 2 °C; Fig. 5b). The subsequent relaxation of upwelling winds (Fig. 5a) corresponded to an increase in the number of cells observed at both beach sites, reaching a maximum on September 22–23 (Fig. 4a,b).

Difference between Kalaloch Beach and Second Beach

Although species composition and cellular levels of DA were similar at both beaches during the course of this study, the maximum number of *Pseudo-nitzschia* spp. cells, specifically *P. pseudodelicatissima*, measured at Kalaloch Beach was at least three times higher than the levels measured at Second Beach. Our results suggest that a pulse of nutrients, especially nitrate, rather than a sustained nutrient supply could account for the difference in the intensity of the bloom and, thus, the total levels of DA in the seawater. Pulses of nitrate, possibly from resuspended sediments after wind events or from river runoff have been previously associated with blooms of *P. multiseries* in eastern Canada (Smith et al. 1990). The highest concentration of nitrate at Kalaloch Beach was measured on September 1, a significant increase (175-fold) over levels recorded on August 25. Although nitrate levels increased slightly on September 1 at Second Beach, the change in concentration of this nutrient was not significant through September. Chlorophyll *a* levels were also routinely higher at Kalaloch Beach than at Second Beach (Fig. 4c). This clearly indicates that hydrographic factors at Kalaloch Beach were more supportive of phytoplankton blooms in the summer and early autumn months. The physical oceanography that explains the different productivity at Kalaloch Beach compared with Second Beach will be detailed in future studies.

Toxigenic Pseudo-nitzschia pseudodelicatissima

Our measurement of increasing DA levels in a cultured isolate of *P. pseudodelicatissima*, and confirmation of DA by mass spectroscopy of field samples consisting of 90–100% *P. pseudodelicatissima*, show that this species is a DA producer in Washington coastal waters. Significant numbers of other known toxigenic *Pseudo-nitzschia* species were not found in field samples collected before and during the razor clam toxification event in 1998. The 1998 coastal event was not the only time that *P. pseudodelicatissima* has been linked to toxin production in Washington waters. This organism was the primary *Pseudo-nitzschia* species seen in offshore areas of DA production during cruises aboard the R/V McArthur in the summers of 1997 and 1998 (Horner et al. in press). However, *P. pseudodelicatissima* does not produce toxin in all areas of the world. For example, DA has not been measured in seawater at times when *P. pseudodelicatissima* formed dense blooms in Monterey Bay (Walz et al. 1994, Scholin et al. 2000) but it is known to produce toxin in the Bay of Fundy (Martin et al. 1990), in the Gulf of Mexico (M. Parsons pers. comm.), and in certain clones isolated from Danish waters (Lundholm et al. 1997). Genetic variability or differences in gene expression in various strains of *P. pseudodelicatissima* may explain the variation in toxin production by this organism in different regions.

Indeed, *P. pseudodelicatissima* did not produce measurable amounts of toxin at all times in both cultured and field samples. For example, it was observed at Kalaloch Beach on August 19 (Table 1) when no toxin was measured in seawater. Laboratory cultures showed that this alga produces maximum amounts of toxin in late exponential and stationary phases of growth. The highest cellular levels of DA in field samples were measured in early October as the bloom was declining. Therefore, to predict accurately the levels of toxin that will be produced by a bloom, some knowledge of the growth stage of the bloom population must be obtained. In the field, maximum numbers of *P. pseudodelicatissima* were observed at Kalaloch Beach about 2–3 weeks after a pulse of nutrients was recorded. Similarly, the cultured isolate reached stationary phase of growth in approximately 12 days. Perhaps similar growth rates and dependence of toxin production on growth stage can be expected in field samples compared to laboratory cultures, a possibility that needs to be determined empirically in future studies. This information will assist in the complete characterization of natural blooms of toxic *Pseudo-nitzschia*.

Pseudo-nitzschia spp. cells were present in nearshore waters through most of the summer at both beaches, indicating that they may be able to bloom during much of the year, given the appropriate environmental conditions. During most of the summer of 1998, nontoxic or weakly toxic species such as *P.* cf. *heimii* and *P.*

pungens, respectively, were present at times when DA was not detectable in the seawater. When a rich supply of nutrients became available, toxic *P. pseudodelicatissima* cells increased in number and reached bloom proportions (over 10^6 cells/L) over a 2–3 week period. It is not clear which specific environmental conditions caused *P. pseudodelicatissima* to become dominant and not any of the other species of *Pseudo-nitzschia* that were present in the assemblage during the summer months.

Prediction

The influx of nutrients in early September 1998 was a result of one of the strongest coastal upwelling events on the Washington coast that season as evidenced by persistent northwest winds (Fig 4a). Although toxic blooms in Monterey Bay occur in the spring and autumn, the Washington coast has recorded DA events primarily in autumn months. This observation makes toxic blooms in Washington potentially more predictable and perhaps more strongly linked to nutrient pulses during coastal upwelling events, especially in years of low rainfall. If nutrient concentrations and environmental conditions are to be used as predictive factors, more in-depth studies of these parameters as they relate to the composition of the phytoplankton assemblage must be undertaken.

Close observation of the developing *Pseudo-nitzschia* population during late summer and early autumn months and daily monitoring of environmental factors such as upwelling indices could provide an early warning of toxic blooms that affect coastal razor clam populations. Molecular probes able to detect toxic *Pseudo-nitzschia* species found seasonally in Washington coastal regions, possibly in the form of automated sensors placed on buoys, could warn recreational and subsistence shellfishers of an impending bloom event (Scholin et al. 1999). Although we know that razor clams can become toxic within 18 days of the appearance of a toxic bloom, the precise timing between the appearance of high levels of DA in seawater, and the accumulation of toxin in clams must still be determined in order to fine tune our predictive ca bilities.

CONCLUSIONS

The late summer/early autumn outbreak of toxic *Pseu nitzschia* was strongly linked to the relaxation of seasonal welling in a year of unusually low rainfall. The bloom occurre a time when winds reversed direction, allowing cells to be brou to the coast. This scenario presumes an established, offshore po lation of *P. pseudodelicatissima*, of which recent research crui provide evidence (Horner et al. in press, Trainer et al. in press). conclude that the pennate diatom *P. pseudodelicatissima* was major source of DA on the central Washington coast in the summer and autumn of 1998, with record levels of toxin in ra clams detected in October of that year. Our study illustrates t toxic *Pseudo-nitzschia* can, indeed, contribute to the surf-z diatom community on which razor clams feed. Natural depurat of razor clams was still not complete as of October 1999. This sl depuration of toxin from razor clams has made a further impact the already depressed coastal economies of Washington State necessitating a second season of beach closures likely resulti from one toxic bloom.

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