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Harmful Algae xxx (2007) xxx–xxx
HARMFUL ALGAE
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Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways

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Received 24 September 2006; received in revised form 24 November 2006; accepted 20 December 2006

Abstract

Several species of the toxigenic diatom *Pseudo-nitzschia*, together with low concentrations of domoic acid (DA) in shellfish have been observed in Puget Sound, Washington State, since 1991. However, for the first time in September 2003, high-density blooms of *Pseudo-nitzschia* forced the closure of recreational, commercial, and tribal subsistence shellfish harvesting in Puget Sound. Here we report on the environmental conditions associated with shellfish closures in two inland waterways of Washington State during the Fall 2005. In Sequim Bay, shellfish harvest losses occurred on September 12 following the measurement of elevated macronutrient levels on September 2, and a bloom of *P. pseudodelicatissima* (up to 13 million cells/L) on September 9. Ambient NH_4 concentrations >12 μM (measured on September 2) were likely due to anthropogenic sources, ostensibly from sewage inputs to Sequim Bay. The closure of a Penn Cove commercial shellfish farm on October 16 was caused by a bloom of *P. australis* that followed a period of sustained precipitation, elevated Skagit River flow, and persistent southeasterly winds. The relative importance of a number of environmental factors, including temperature, stratification caused by rivers, and nutrient inputs, whether natural or anthropogenic, must be carefully studied in order to better understand the recent appearance of massive blooms of toxigenic *Pseudo-nitzschia* in the inland waterways of Washington State.
Published by Elsevier B.V.

Keywords: Domoic acid; *Pseudo-nitzschia*; Puget Sound; Shellfish closure; Harmful algal bloom

1. Introduction

The razor clam and Dungeness crab fisheries on the outer coast of Washington State have been plagued by domoic acid (DA) closures since 1991 (Horner et al., 1993; Horner and Postel, 1993; Wekell et al., 1994). Commercial, recreational and subsistence razor clam fisheries suffered total coast wide closures in 1991, 1998 and 2002, however, due to the monitoring efforts of the Olympic Region Harmful Algal Bloom (ORHAB) partnership formed in 2000, selective closures were possible in 2001 and 2003–2005. Because razor clams can retain DA for periods of up to a year due

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1568-9883/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.hal.2006.12.001

to the presence of a high affinity glutamate binding protein (Trainer and Bill, 2004), closures on the outer coast lasting for up to a year caused serious economic hardship to the tribal communities which rely on this subsistence fishery. The importance of this fishery to the indigenous tribes of the Pacific Northwest is evidenced by a phrase in the Quinault tribal language ''ta-aWshi xa-iits'os'' that translates as ''clam hungry'' and refers to times when clam harvests have been poor.

Low levels of DA and several species of *Pseudo-nitzschia* have been observed in Puget Sound since 1991 (Bill et al., 2006; Trainer et al., 1998; Stehr et al., 2002; Horner, 2003), but the Sound was presumed less susceptible to DA closures due to the absence of DA-retaining razor clams in this region. Certain shellfish species that are harvested in Puget Sound, such as mussels, littleneck clams, and oysters, are able to depurate DA over a period of hours or days (e.g., Novaczek et al., 1992; F. Cox, pers. comm.), whereas the ability of other species to retain or release DA (e.g., geoduck) has not yet been characterized. However, in early July 1997, the Washington State Department of Health (WDOH) officially reported the presence of DA in commercial mussels cultivated at a shellfish farm (Penn Cove Shellfish, LLC) located on the eastern side of Whidbey Island in Puget Sound. This was the first report of DA in shellfish in Whidbey basin which caused the concern that these shellfish closures might spread to central and south Puget Sound.

Recently, DA closures have occurred in three Washington State inland waterways, causing great concern to State shellfish managers. To date, concentrations of DA below the regulatory limit of 20 ppm have been detected in Puget Sound blue mussel (*Mytilus edulis*), littleneck clam (*Protothaca staminea*), geoduck clam (*Panopea abrupta*), manila clam (*Tapes philippinarum*), Pacific oyster (*Crassostrea gigas*), and Dungeness crab (*Cancer magister*) (Bill et al., 2006; F. Cox, pers. comm.). If future DA concentrations are found at levels in excess of the regulatory limit ($\geq$20 ppm) in more areas of Puget Sound, the resulting economic losses could be large. Washington State is the U.S. west coast's largest producer of shellfish, with an estimated annual production of 86 million lb that generates over $77 million in sales (Puget Sound Action Team, 2003). Puget Sound accounts for most of the clam and mussel sales (13.5 million lb at a value of approximately $19 million) while Willapa Bay on the outer Washington coast leads in oyster sales. Shellfish harvesting provides a strong and steady employment base and fulfills recreational and subsistence needs in Washington State. Because the first paralytic shellfish poisoning (PSP) outbreaks occurred in northern Puget Sound and the Strait of Juan de Fuca (JDF) in the 1930–1950s, then spread into southern Puget Sound regions during the 1970–1990s (Nishitani and Chew, 1988; Rensel, 1993; Trainer et al., 2003), there is great concern that these DA closures that have recently been documented in northern Puget Sound will parallel the PSP events by spreading gradually into most regions of Puget Sound.

2. Methods

2.1. Sample collection

Surface seawater samples were collected from several sites off Whidbey Island using a bucket, either directly from the beach or from piers or wharfs at the sampling sites. A Niskin bottle, deployed to 2 m depth, was used to collect seawater from the Sequim Bay State Park pier. Discrete samples were then processed and analyzed for concentrations of particulate DA (pDA), dissolved DA (dDA), *Pseudo-nitzschia* cells and inorganic nutrients. Samples for species identification at all sites were collected using a 20-μm mesh phytoplankton net and preserved in 1% formalin.

2.2. DA analysis

Particulate DA was measured by filtering 1 L of seawater onto a nitrocellulose filter and freezing until analysis following the procedure outlined by Bill et al. (2006). Dissolved DA was measured by filtering seawater through a 25-mm diameter (0.45 μm pore size) mixed-cellulose ester filter (Millipore Corp.) and analyzed for DA using the direct competitive enzyme linked immunoassay (cELISA) Biosense kits (Biosense Laboratories, Bergen, Norway). Analysis of DA in shellfish was performed by the WDOH using high performance liquid chromatography (HPLC) (Hatfield et al., 1994).

2.3. *Pseudo-nitzschia* cell counts and species identification

Pseudo-nitzschia cells were counted in a Palmer-Maloney counting chamber using a Zeiss Axiovert 135 inverted light microscope. Because of the diverse number of species of *Pseudo-nitzschia* found in Puget Sound, cells were characterized by relative size as described in Trainer and Suddleson (2005), including the short and narrow *P. pseudodelicatissima/delicatissima* (pd/d) group, the long and wide *P. australis/fraudulenta/heimii* (a/f/h) group, and the long and narrow *P. pungens/multiseries* (p/m) group. *Pseudo-*

nitzschia cells were positively identified to the species level in selected preserved net-tow samples using scanning and transmission electron microscopy (SEM and TEM, respectively) following published morphological characteristics (Hasle and Syvertsen, 1997). The SEM and TEM methods followed those described in Bill et al. (2006) and Lundholm et al. (2003), respectively. Calculations of cell volumes were estimated using the procedures described in Hillebrand et al. (1999).

2.4. Nutrient analysis

Seawater for inorganic nutrient determinations was filtered through a Whatman No. 1 filter (11 μm particle retention), frozen, and later analyzed for nitrate plus nitrite ($NO_3 + NO_2$), hereafter referred to as nitrate (NO_3), ammonium (NH_4), phosphate (PO_4) and silicic acid ($Si[OH]_4$) according to standard colorimetric methods (UNESCO, 1994) using a AlpkemRFP/2 autoanalyzer.

3. Results

3.1. Sequim Bay

The WDOH Office of Food Safety and Shellfish program conducts a seasonal shellfish monitoring program that includes both PSP toxins and DA. Elevated levels of DA in shellfish collected from Sequim Bay on September 12 (Fig. 1) resulted in the closure of this bay to commercial, recreational, and subsistence shellfish harvest. A weekly sampling program initiated in Sequim Bay in early June by the Jamestown S'Klallam Tribe showed sustained, high levels of pDA in seawater samples beginning on September 9 (14,264 ng/L, Table 1). Three locations in central and southern Sequim Bay showed elevated DA in shellfish on September 12, including blue mussels at Sequim Bay State Park (26 ppm), manila clams at Blyn (36 ppm) and littleneck clams at Hardwick Point (27 ppm), illustrating the range of species affected and the extent of the bloom (Fig. 2). Although blue

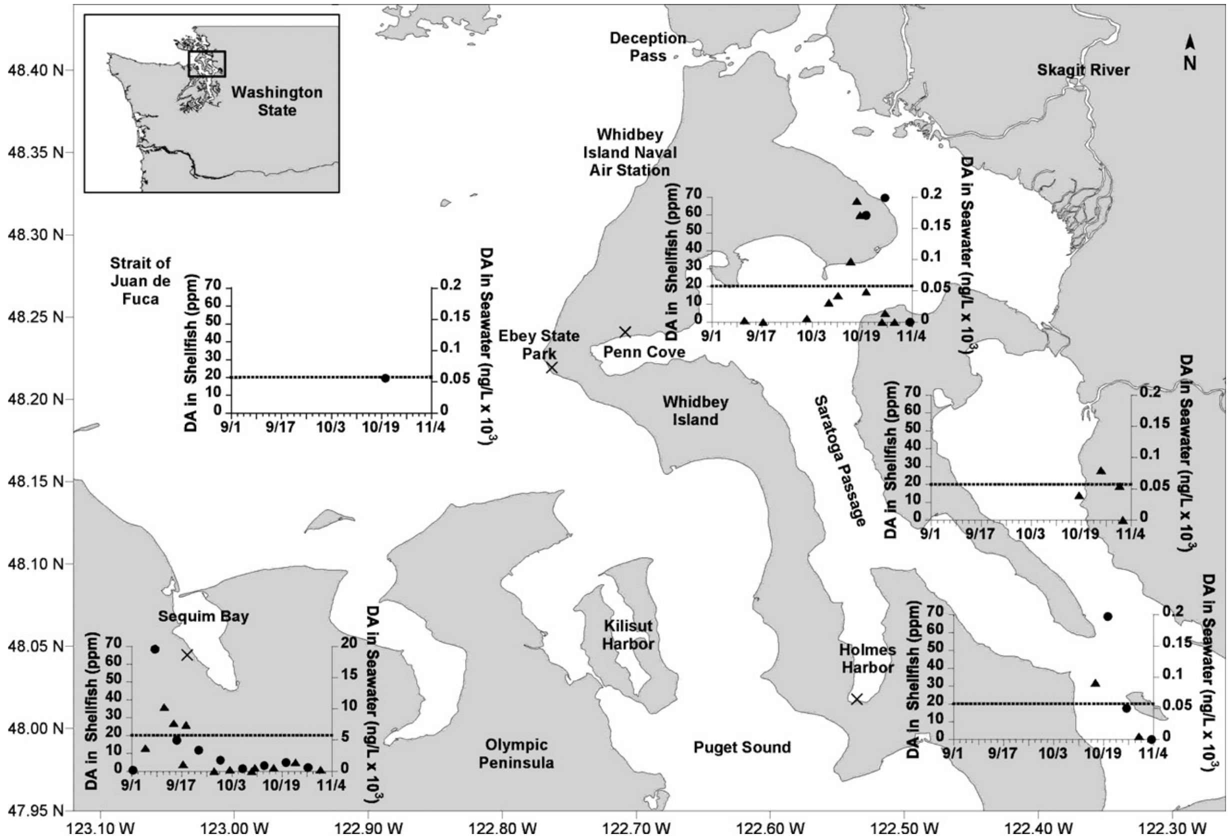

Fig. 1. DA in shellfish and seawater during Puget Sound closure events in September–October, 2005. Both DA in shellfish (circles) and in seawater (triangles) are shown from September 1 through November 4, 2005. The highest DA in shellfish on any given date is shown. Shellfish data from Saratoga Passage are from Dungeness crab and from all other sites (marked by X) are from bivalve shellfish including manila clams, Pacific oysters, littleneck clams and blue mussels. The regulatory limit of 20 ppm in bivalve shellfish is shown by a dotted line.

Table 1
Physical, chemical and biological characteristics of seawater collected off the Sequim Bay State Park pier

Date	Temperature (°C)	Pn counts (cells/L)	pd/d (%)	a/f/h (%)	p/m (%)	pDA (ng/L)	dDA (ng/L)	$Si(OH)_4$ (μM)	NO_3 (μM)	NH_4 (μM)	PO_4 (μM)
6/3/2005	14.0	501,000	25	75	25	280	3308	13.57	1.68	1.29	0.82
6/10/2005	14.5	6,400	0	100	0	0	1275	28.21	6.56	3.71	1.28
6/17/2005	15.0	0	0	0	0	38	299	29.69	2.98	1.21	0.98
6/24/2005	16.0	21,000	100	0	0	0	102	25.94	3.14	1.62	0.97
7/1/2005	15.0	0	0	0	0	0	78	13.56	0.74	6.48	0.59
7/8/2005	15.5	3,000	75	25	0	0	60	18.87	1.40	1.62	0.77
7/15/2005	15.5	58,000	0	50	50	0	88	21.31	4.69	0.83	0.91
7/22/2005	16.0	23,000	0	50	50	42	389	11.36	0.77	2.68	1.19
7/29/2005[a]	19.0	21,000	85	15	0	0	315	9.53	0.44	0.36	0.67
8/5/2005	18.0	145,000	99	1	0	67	1366	12.49	0.72	6.98	0.86
8/12/2005	17.0	102,000	90	9	1	102	2999	20.95	1.36	1.04	1.06
8/19/2005	17.0	57,000	95	5	0	0	676	18.33	0.75	1.27	0.95
8/26/2005	15.0	20,000	100	0	0	38	171	26.54	7.32	3.28	1.57
9/2/2005	14.5	46,000	100	0	0	44	764	37.64	12.88	12.67	2.10
9/9/2005[b]	15.0	23,000,000	100	0	0	14264	135600	14.39	0.78	0.29	0.74
9/16/2005	13.0	6,500,000	100	0	0	4943	31550	34.41	10.99	3.14	1.61
9/23/2005	15.0	15,290,000	100	0	0	3347	115600	25.92	7.54	3.43	1.77
9/30/2005	14.0	10,620,000	100	0	0	1774	32500	17.88	3.13	0.69	0.91
10/7/2005	13.0	1,670,000	70	30	0	396	7180	25.13	0.67	0.72	0.71
10/14/2005	13.0	677,000	7	93	0	897	1271	39.57	14.91	2.51	1.66
10/21/2005	12.0	593,000	0	90	10	1404	2115	37.82	15.99	1.25	1.58
10/28/2005	11.0	471,000	0	94	6	609	1788	43.08	14.44	5.80	1.83

[a] Shift in *Pseudo-nitzschia* species.
[b] *P. pseudodelicatissima* bloom begins.

mussels at Pitship Point, closer to the opening of Sequim Bay to the Strait of Juan de Fuca contained 10 ppm DA on September 6, toxin levels never reached the regulatory limit at this site.

Prior to September 9, total cell abundance of all *Pseudo-nitzschia* species was low and averaged <100,000 cells/L (Fig. 3A). During most of this period the community was composed of either the *P. pseudodelicatissima/delicatissima* (pd/d) group or the longer and wider *P. australis/fraudulenta/heimii* (a/f/h) group; not until July 29 did the assemblage become consistently dominated by the pd/d cell type (Table 1 and Fig. 3B, dotted vertical line 1). This corresponded to an increase in surface temperatures measured at Sequim Bay State Park from approximately 16 °C on July 22 to 19 °C on July 29 (Table 1). It also followed reduced ambient concentrations of $Si(OH)_4$ (approximately 10 μM) and NO_3 (only 0.4 μM) on July 29 (Table 1 and Fig. 3C). *Pseudo-nitzschia* cell abundance as well as pDA and dDA concentrations were variable over the next 6 weeks until September 9 when *Pseudo-nitzschia* cell numbers exceeded 20×10^6 cells/L, pDA and dDA reached 14.3 μg/L and 136 μg/L, respectively (Table 1 and Fig. 3B, dotted vertical line 2). The increased cell density to bloom proportions on September 9 followed the build up of ambient concentrations of $Si(OH)_4$ (37.6 μM), NO_3 (12.9 μM), and NH_4 (12.7 μM) measured on September 2 (Table 1), 2 weeks after substantial rainfall events on August 17 (1.1 cm) and August 28–29 (0.9 cm), and elevated stream flow on August 28–31 (Fig. 3D).

Species identification of *Pseudo-nitzschia* using SEM (Fig. 4) and TEM indicated that the species responsible for the bloom in Sequim Bay was *P. pseudodelicatissima* (N. Lundholm, pers. comm.). Because of the recent splitting of *P. pseudodelicatissima* into 3 species, *P. pseudodelicatissima*, *P. caciantha*, and *P. calliantha* (Lundholm et al., 2003), this appears to be one of the first confirmed toxic blooms caused by *P. pseudodelicatissima*. However, because other *Pseudo-nitzschia* species were present (although in low abundance) before and during the shellfish closure event, production of DA by *P. pseudodelicatissima* must be confirmed in future culture studies with isolates from the region.

3.2. Penn Cove

Following the short closure event in Sequim Bay (DA in shellfish dropped below the regulatory limit by

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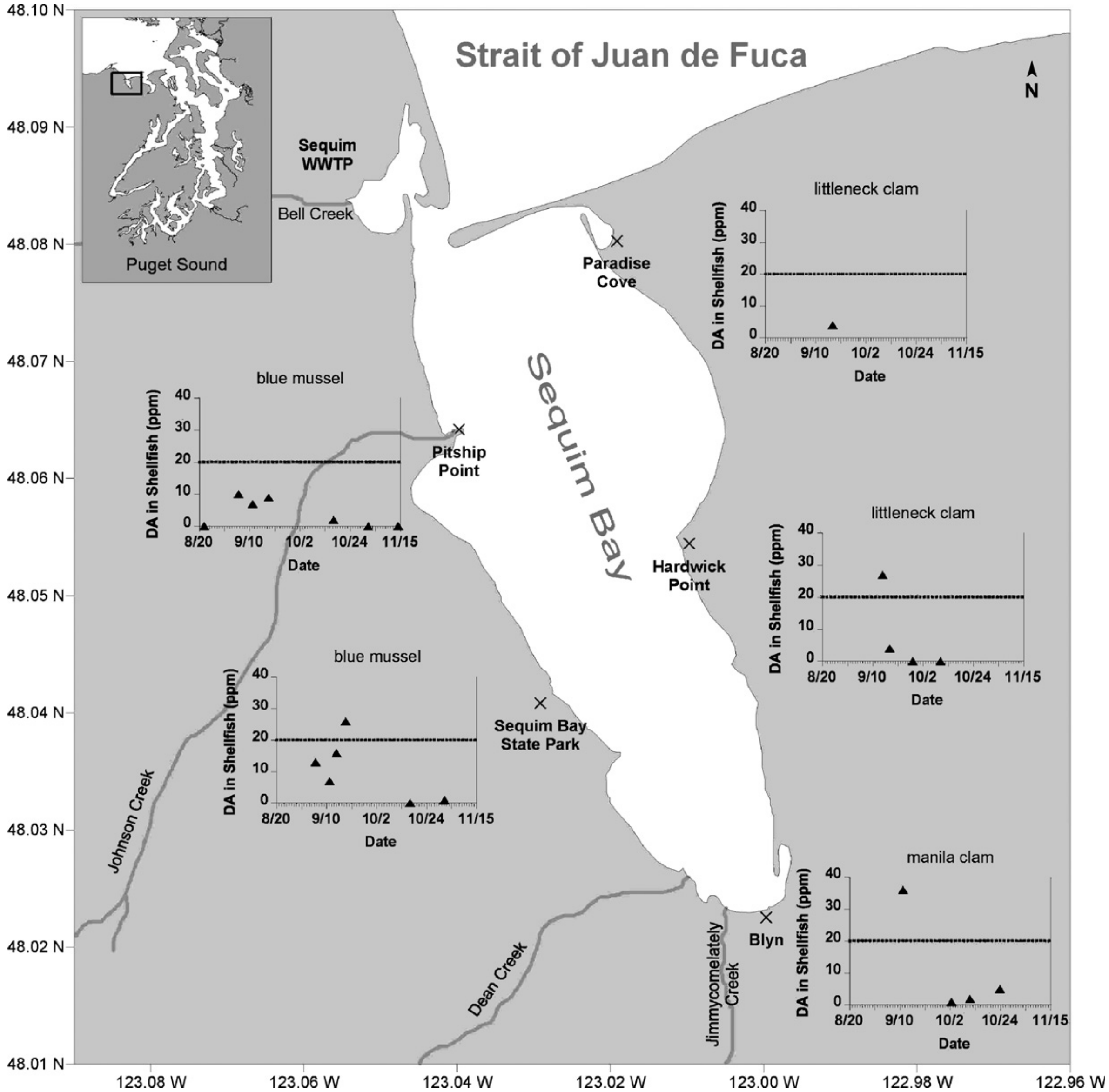

Fig. 2. DA in shellfish from Sequim Bay. Shellfish species monitored at each site are shown above each plot. The regulatory limit of 20 ppm in bivalve shellfish is shown by a dotted line. The locations of several creeks flowing into Sequim Bay and the Sequim Bay Waste Water Treatment Plant (WWTP) are shown.

September 18), increasing concentrations of DA in shellfish were measured during October in Penn Cove, the site of a large commercial mussel farm. Domoic acid concentrations in blue mussels rose from 2 ppm on October 2 to 11 ppm on October 11 and to 15 ppm on October 15, finally leading to closures on October 16 at concentrations of 34 and 60 ppm in blue mussels and manila clams, respectively (Fig. 1). The WDOH announced the official closures of Penn Cove, Saratoga Passage, and Holmes Harbor to shellfish harvest on October 18 due to record levels of DA in inland waters. Measurements of DA in shellfish and seawater throughout the Whidbey Basin indicated that the bloom responsible for this event was extensive. DA levels of 28 ppm were measured in Dungeness crab from Saratoga Passage (Fig. 1), just below the 30 ppm closure level for this important commercial shellfish species. Samples for pDA were collected on October 21, 27 and November 4. Measurable pDA was found in Penn Cove, Holmes Harbor on the first 2 sampling dates as well as on the west side of Whidbey Island at Ebey State Park and at Deception Pass (not shown) on

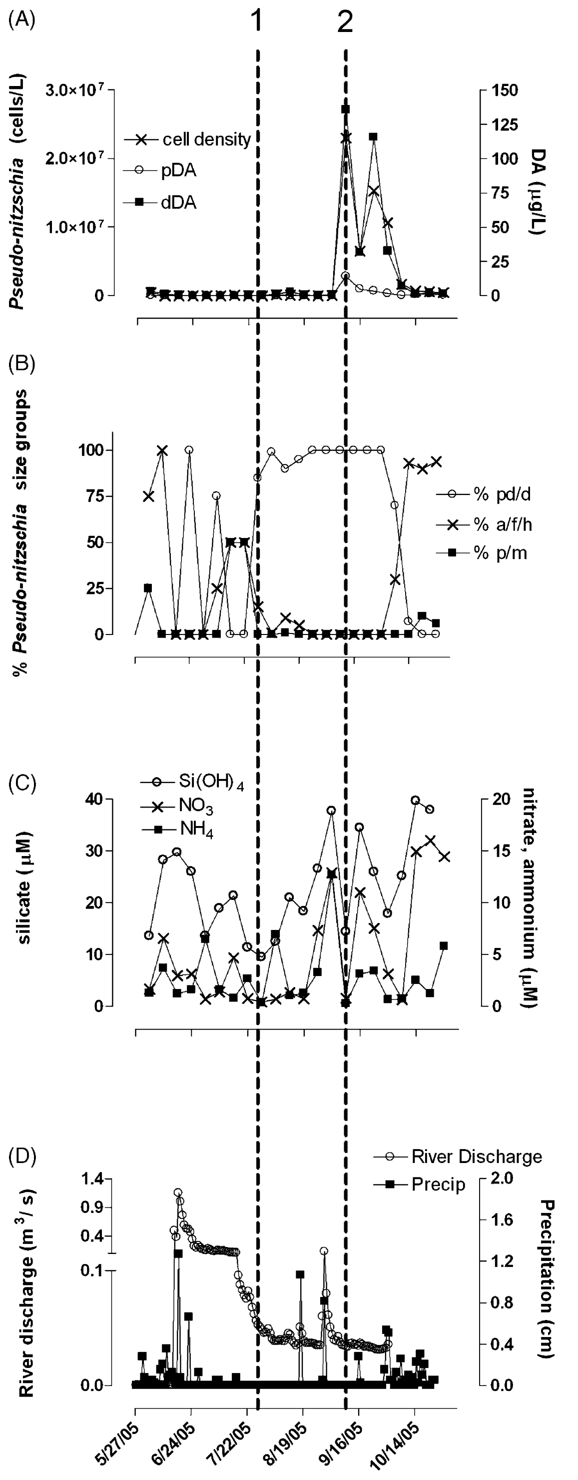

Fig. 3. Weekly biological, chemical and physical parameters in Sequim Bay during Spring–Fall 2005. (A) Cell abundance of *Pseudo-nitzschia*, and concentrations of pDA and dDA; (B) relative abundance (%) of the three *Pseudo-nitzschia* size groups; (C) ambient concentrations of NO_3, NH_4, and $Si(OH)_4$; and (D) river discharge and precipitation. Cell abundance and relative percentages of *Pseudo-nitzschia* (A and B); toxin concentrations (A); and nutrient data (C) were calculated in samples collected off the Sequim Bay State Park dock. Precipitation data were obtained from the National Climatic Data Center archives from Sequim Weather station 2E, approximately 4 km west of Sequim Bay; stream flow data were from the Washington State Department of Ecology gauge at Jimmycomelately Creek that enters into southern Sequim Bay. Dotted vertical line 1 corresponds to the shift in *Pseudo-nitzschia* species on July 29. Dotted vertical line 2 corresponds to the shellfish closures on September 12.

October 21. By November 4, pDA was not detectable at any of the sites sampled. SEM of a net tow sample collected from Penn Cove on October 18 showed a dense bloom of *P. australis* (Fig. 4C and D). Whole water samples collected at several sites in Whidbey basin (see Fig. 1) indicated that the maximum numbers of *P. australis* were found in Penn Cove (21,000 cells/L on October 21 and 41,000 cells/L on October 27, not shown), although these samples were collected following the shellfish closure and may represent cell numbers in the receding bloom.

Sustained precipitation and periods of above average Skagit River flow (Fig. 5B and C) were observed in October. An increase in mean air temperature from the end of September to early October was also observed (Fig. 5C). Strong and persistent southeasterly winds were observed in early through mid-October (Fig. 5A) compared to previous months. Nutrient data were not collected at this site.

4. Discussion

4.1. Sequim Bay

A WDOH news release was issued on September 16, 2005 to announce the temporary closure of Sequim Bay to commercial and recreational shellfish harvest due to elevated levels of DA in oysters and clams. This was only the second closure of Puget Sound benthic fisheries due to DA since routine monitoring of Washington State inland and coastal waters began in 1991.

Sequim Bay has a narrow opening into the Strait of JDF where waters from multiple sources including Puget Sound, the Pacific Ocean and the Strait of Georgia, with its associated strong freshwater inputs from the Fraser River, all mix and enter the Bay during flood tide. In addition to a direct source of nutrients from JDF, the waters of the northern and deeper portion of the Bay receive nutrients from two creeks and the Sequim Bay Waste Water Treatment Plant (Fig. 2), whereas the shallow and more stagnant waters of the southern portion of the Bay have nutrient inputs from two creeks on the most southern and southwestern sides

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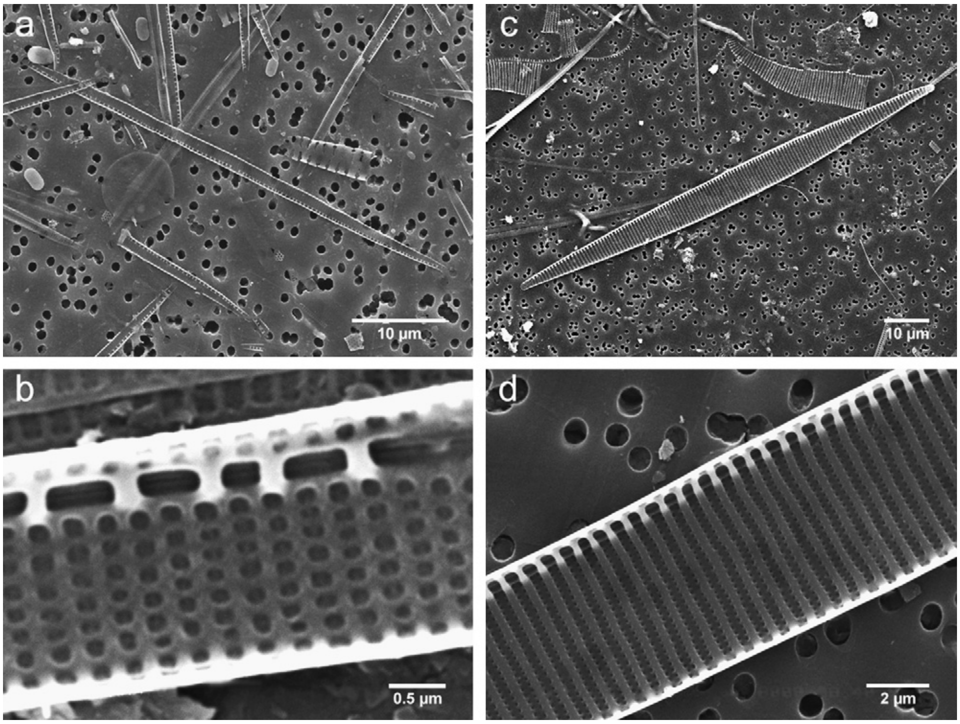

Fig. 4. Scanning electron micrographs of *Pseudo-nitzschia*. (A) *P. pseudodelicatissima*, whole value; (B) *P. pseudodelicatissima*, higher magnification. *Pseudo-nitzschia pseudodelicatissima* are characterized by a whole valve 55 μm long and 1.0–1.3 μm wide, 20–23 fibulae in 10 μm, 37–43 interstriae in 10 μm, 1 row of pores, 6 pores in 1 μm, pores divided into two parts, and valve view linear with central interspace and nodule present. Cell volume of *P. pseudodelicatissima* is 40 μm^3; area is 87 μm^3. (C) *P. australis*, whole valve; (D) *P. australis*, higher magnification. *Pseudo-nitzschia australis* are characterized by a whole valve of 90–110 μm long and 7.4–7.9 μm wide, 15 fibulae in 10 μm, 15 interstriae in 10 μm, 2 rows of pores, 5 pores in 1 μm, pores not divided into parts, and valve view asymmetrical with no central interspace and nodule present. Cell volume of *P. australis* is 1530 μm^3; area is 865 μm^3. Scale bars on the bottom of each micrograph indicate size.

(Elwha-Dungeness Watershed Planning Unit, 2005). The very weak circulation in the southern portions of the Bay can lead to low dissolved oxygen conditions, especially in the summer (Elwha-Dungeness Planning Unit, 2005), a direct result of decaying blooms of algae. Although the southern Bay is conditionally approved for shellfish harvest, shellfish toxin data for 2005 (Fig. 2) indicate that southern Sequim Bay is more susceptible to toxic blooms; whereas the northern portion is closed to shellfish harvest due to poor water quality (Parametrix, 2000).

The summer maximal concentrations of NO_3, NH_4, and $Si(OH)_4$ measured in Sequim Bay on September 2 (Table 1), were sufficient to fuel the high-density *Pseudo-nitzschia* bloom that appeared the following week on September 9. We suspect that the elevated NH_4 concentrations measured on September 2 were at least partially due to anthropogenic inputs, likely from the outdated sewage system at Sequim Bay State Park that currently discharges to an above-ground spray field (Puget Sound Initiative, 2006) and is known to be overloaded and at risk of failure during the recreational season (Elwha-Dungeness Planning Unit, 2005). The measured NH_4 concentrations are approximately 1–2 fold greater than ambient NH_4 concentrations found in the adjacent, surface waters of the Strait of JDF (∼1–2 μM, Cochlan unpublished results), the southeastern Strait of Georgia (<0.2 μM; Mackas and Harrison, 1997) and the coastal Pacific Ocean waters (0.1–0.2 μM; Cochlan, unpublished results), whereas the elevated ambient NO_3 concentrations are similar to the concentration range normally found in the adjacent inland waters of Washington State and southwestern British Columbia (e.g., see review by Mackas and Harrison, 1997). Although high levels of discharge from Jimmycomelately Creek (Fig. 3D) were observed in June and July, it is likely that there was dilution of any NH_4 inputs from land at that time due to sustained precipitation. The strong density gradient (observed during CTD casts) near Sequim Bay State Park in September of previous years (data not shown), suggests that benthic-derived NH_4 from bacterial remineralization in the sediment layer was isolated from the surface layer, and thus did not contribute substantially to the

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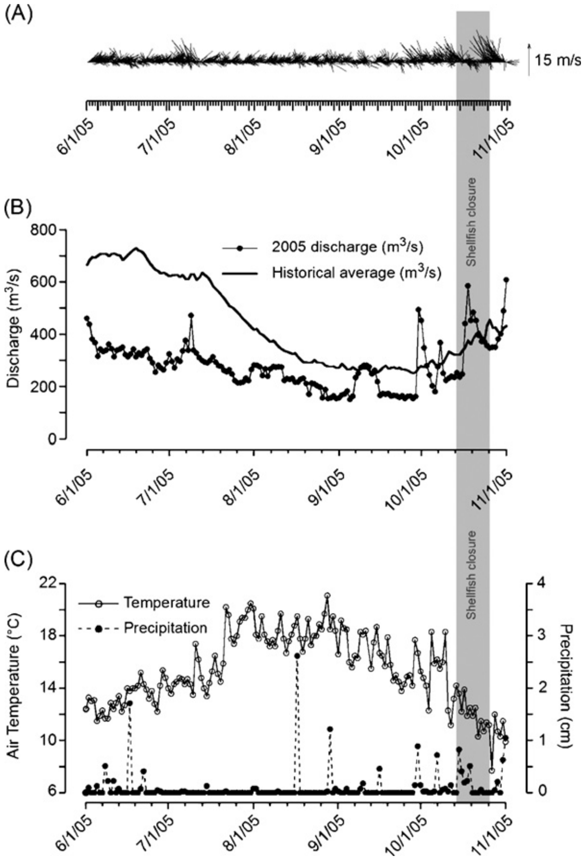

Fig. 5. Hourly winds, mean daily averaged air temperature, and total daily precipitation and Skagit River flow data. Skagit River discharge data were measured at Mount Vernon, a USGS Water Information Station about 30 km northeast of Penn Cove, and approximately 11 km from the point of discharge into Skagit Bay. The historical average Skagit River discharge is shown as a solid line. Weather data from the Whidbey Naval Air Station (WMO ID# 72797) were collected using an automated surface observing system that is located about 10 km north of Penn Cove. The shellfish closure period is shown as a shaded bar.

elevated, surface NH_4 concentrations reported here on September 2.

Macronutrient inputs have been hypothesized to be a possible cause of *Pseudo-nitzschia* blooms in eastern Canada in 1987 (Smith et al., 1990; Smith et al., 1993; Bates et al., 1998), in the northern Gulf of Mexico in the early 1990s (Dortch et al., 1997), on the Washington State coast in 1991 (Horner and Postel, 1993), and in Monterey Bay in 1991 (Bird and Wright, 1989). However, macronutrients from anthropogenic sources are likely not a causative factor for many toxigenic *Pseudo-nitzschia* blooms, for example those originating in the Juan de Fuca Eddy off the British Columbia and Washington State coasts (Trainer et al., 2002). These blooms are found in waters with low ambient concentrations of both NH_4 and urea (Cochlan, unpublished results), but replete in NO_3 at concentrations normally considered sufficient to saturate the N uptake capabilities of most coastal diatoms (e.g., see review by Smayda, 1997), including *Pseudo-nitzschia* (Cochlan et al., 2005).

Recently it has been suggested that silicate limitation may play a role in DA production in blooms of *Pseudo-nitzshia australis* in southern California (Anderson et al., 2006). However in Sequim Bay, the surface waters were consistently Si-replete, ranging from 9.5 to 43 μM (mean = 23.9 μM), and $Si(OH)_4$ only fell to 14.39 μM at the height of the toxic bloom on Sept. 9, whereas inorganic N concentrations were depleted to below 1 μM. We suggest that the availability of N, not Si, may be more important for toxigenic diatom bloom development in the Pacific Northwest, and that the dramatic shift to the smaller *Pseudo-nitzschia* cell type (*P. pseudodelicatissima*) that occurred on July 29 was a consequence of the greater ability of these smaller cells to acquire nitrogen substrates found at low (sub-saturating) concentrations. The low NO_3 and NH_4 concentrations (<0.5 μM) measured during that time are below the half-saturation constants for N uptake of most coastal diatoms and dinoflagellates (e.g., see review by Smayda, 1997), and will limit the ability of most coastal phytoplankton to acquire sufficient N for growth except at reduced rates. Therefore one would expect cell types with higher nutrient uptake efficiencies to dominate in these areas during periods of low ambient N concentrations. As cells decrease in size, their relative surface to volume ratio (S/V) increases so that small cells (for example, the S/V of *P. pseudodelicatissima* is 2.17, whereas the S/V of *P. australis* is 0.56, see calculations in Fig. 4 legend) are generally more efficient at acquiring macro- or micro-nutrients (like Fe or Cu) when available at low or sub-saturating concentrations. Recent work has demonstrated the role of DA in Fe and Cu acquisition, providing *Pseudo-nitzschia* spp. with a competitive advantage over other phytoplankton when concentrations of micronutrients are limiting (Wells et al., 2005). Recent culture studies also have demonstrated that N-deficient, small-sized species of *Pseudo-nitzschia* such as *P.* cf. *pseudodelicatissima* have the ability to take up pulses of high concentrations of NH_4 at initially elevated (surge) uptake rates that greatly exceed their growth requirements (Auro and Cochlan, unpublished results). Such enhanced, transient uptake rates in cultures (e.g., Conway et al., 1976; Cochlan and Harrison, 1991) and natural assemblages (e.g., Glibert and Goldman, 1981) have been suggested as an adaptive response to

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patterns of N availability in the sea, where those species with the ability to assimilate N rapidly during N starvation have a selective advantage over others to dominate in areas where N limitation can be a major environmental stress.

The availability of nitrogen is a major factor controlling the growth of diatoms, and NH_4 appears to be of equal or greater importance than NO_3 in supporting the growth of toxigenic *Pseudo-nitzschia* species isolated from the U.S. Pacific Northwest (Auro et al., 2006) and other West coast regions of the U.S. (Armstrong et al., in press). In Sequim Bay, limiting concentrations of dissolved inorganic nitrogen were observed throughout late July and most of August, followed by high inorganic nitrogen concentrations on August 26 and September 2 (up to 13 μM NO_3 and 13 μM NH_4, see Table 1) which fueled the September *Pseudo-nitzschia* bloom of over 23 million cells/L. Such an elevated concentration of NH_4 normally would only be found in open ocean or coastal regions receiving anthropogenic inputs (e.g., see review by McCarthy, 1981). This elevated concentration would effectively saturate the NH_4 uptake capability of *P. australis* ($K_s = 5.4$ μM NH_4; Cochlan et al., 2005) and most other neritic diatoms and assemblages, although there are exceptions (e.g., Fan et al., 2003). In addition, unialgal cultures of *P. australis* have been shown to take up saturating concentrations of NH_4 and NO_3 at equitable rates, and both exponential growth rate and toxin concentration per cell are comparable on both N sources (Cochlan et al., 2005; Armstrong et al., in press). Studies with *P. pseudodelicatissima* and other small *Pseudo-nitzschia* cells are currently underway to understand the role of different N sources in their growth and DA production capabilities. This will help to clarify the relative importance of natural and anthropogenic nitrogen sources in the success of toxigenic *Pseudo-nitzschia* species.

4.2. Penn Cove

Stratification appears to play a strong role in the appearance of toxigenic *Pseudo-nitzschia* in Penn Cove. It has been suggested that because *Pseudo-nitzschia* cells have an efficient nutrient uptake capability, they can survive in more macro- and micro-nutrient depleted environments, such as stratified systems with no advected inputs of nutrients, than many other diatoms (Cochlan et al., 2005; Wells et al., 2005). A previous study in Penn Cove (Trainer et al., 1998) documented a bloom of *Pseudo-nitzschia* after a period of strong discharge from the Skagit River and rain accompanied by elevated south and southeasterly winds. Stratification was facilitated by weak winds, sunshine and a freshwater lens at the mouth of the cove. The environmental conditions preceding and during the *Pseudo-nitzschia* bloom in July 1997 were replicated almost exactly by the toxigenic bloom in Penn Cove in October 2005.

Toxin-producing *Pseudo-nitzschia* blooms have been known to cause marine mammal mortalities on the U.S. west coast (Scholin et al., 2000). During June through August 2005, increased numbers of dead or moribund neonatal and juvenile harbor seal pups, particularly around the Smith and Minor Islands, 8 km west of Whidbey Island. In one animal from which a non-decayed brain sample was obtained, acute neuronal degeneration and necrosis with rarefaction of the brain parenchyma, similar to that observed in sea lions with DA poisoning in Monterey Bay (Scholin et al., 2000), was suggestive of DA exposure (Steven Raverty, veterinary pathologist, Ministry of Agriculture, Food and Fisheries, British Columbia, Canada, pers. comm.). In addition, during the Ecology and Oceanography of Harmful Algal Blooms in the Pacific Northwest (ECOHAB PNW) cruise in mid to late July 2005, pDA (approximately 250 ng/L) was measured in the Strait of JDF just off the WA coast between Port Angeles and Sequim Bay. SEM analysis indicated that the major toxin producer was *P. australis* (Fig. 4C and D) at concentrations up to about 150,000 cells/L.

As noted by Frank Cox, WDOH, "If DA should continue to move into more areas inside Puget Sound, the economic and public health implications could be significant. Closures will be more difficult to enforce inside the widely inhabited Puget Sound metropolis than on the coast." DA closures could impact the three categories of tidelands; commercial, public and private. Commercial closures would be relatively straightforward to enforce. However, private ownership of tidelands represents a major portion of the Puget Sound shellfishery and is problematic because these private owners of tidelands may not pay attention to warnings (public warning signs are not posted on their lands, so private landowners need to inform themselves regularly by visiting websites, etc.). In addition, DA retention rates of certain shellfish species such as geoduck clam, a major Puget Sound commercial shellfish species that can live for up to 150 years, are poorly defined, and therefore must be characterized in order to effectively protect public health from the effects of harmful algal blooms (HABs).

An early warning of Puget Sound HAB events would help to sustain the economic and social benefits of fisheries in this region by preventing recalls and allowing

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for selective beach openings or early harvest of shellfish prior to HAB events. A tiered sampling program consisting of twice-weekly counts of *Pseudo-nitzschia* cells, followed by DA determination in seawater if a critical cell number is reached, would give about a 1 week warning of shellfish toxification. Such a simple monitoring protocol is the basis of the ORHAB partnership, the Washington State outer coast program that acquired Washington State funding in 2005, thereby ensuring its longevity (Trainer and Suddleson, 2005). Environmental triggers, such as late summer precipitation or river flow following several weeks of dry weather, might also provide an early warning of DA events in Washington State inland waterways.

The collection of shellfish toxin data in Puget Sound over the past 40 years has provided a valuable sentinel tool for tracking the incidence and spread of both common and new toxins. *Pseudo-nitzschia* has been present in Puget Sound for decades (Bill et al., 2006; Trainer et al., 1998; Stehr et al., 2002; Horner, 2003), so why have they caused shellfish closures in Puget Sound only in recent years? One possibility is that more toxigenic strains have entered Puget Sound from offshore HAB initiation sites such as the Juan de Fuca Eddy (Trainer et al., 2002). A series of factors, including genetic relatedness of species and environmental triggers of these HABs must be understood in order to effectively mitigate these events. Because low levels of pDA have been detected in both Sequim Bay (Trainer et al., unpublished data) and Penn Cove (Trainer et al., 1998) within the last decade, it is tempting also to speculate on environmental conditions that may have exacerbated these recent highly toxigenic blooms. Warming ocean temperatures for example can certainly impact cell growth rates, duration of optimal growth period, and stratification intensity of nearshore waters due to increased glacial melt, but to date the relationship between ocean warming and its potential effects on the spatial and temporal magnitude, and toxicity of natural blooms of *Pseudo-nitzschia* is unknown both here and elsewhere in the world.

Acknowledgements

This publication was supported by the West Coast Center for Oceans and Human Health (WCCOHH) as part of the NOAA Oceans and Human Health Initiative, and is WCCOHH publication no. 14. The WCCOHH is part of the National Marine Fisheries Service's Northwest Fisheries Science Center, Seattle, WA. We thank Nina Lundholm for TEM identification of *P. pseudodelicatissima* in Sequim Bay. We thank Lyn Muench and Lohna O'Rourke of the Jamestown S'Kallam Tribe for coordination of sample collection in Sequim Bay; Keri Baugh, Shelly Nance and Bich-Thuy Eberhart for toxin analysis of seawater samples; Jessica Hendrickson for assistance with sample collection in Whidbey Basin; and Nick Adams and Sheryl Day for creating the figures. We thank Jack Rensel and one anonymous reviewer for helpful comments that have improved this manuscript. [SE]

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