

## Biological and physical dynamics of domoic acid production off the Washington coast

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### Abstract

The relationship among *Pseudo-nitzschia* distributions, particulate toxin levels in seawater, and the energetic and highly variable water masses of an upwelling-dominated region are explored using data collected during summer cruises off the Washington coast in 1997 and 1998. In the early summer of both years, an area of maximum domoic acid (DA) accumulation was located approximately 50 km off the coast within a counterclockwise, cold feature known as the Juan de Fuca eddy. The stations in the eddy with the highest domoic acid concentrations coincided with high numbers of *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle ( $2.7 \mu\text{g DA equivalents L}^{-1}$ , up to  $0.6 \times 10^6$  cells  $\text{L}^{-1}$  in 1997 and  $0.2 \mu\text{g DA equivalents L}^{-1}$ , up to  $0.2 \times 10^6$  cells  $\text{L}^{-1}$  in 1998), a species known to produce toxin in this region. Other known toxin-producing species were sometimes present, but at <5% of the total *Pseudo-nitzschia* population when  $>0.1 \mu\text{g DA equivalents L}^{-1}$  were measured. In 1998, large-scale surveys indicated that high levels of particulate DA in seawater persisted at least until 1 October, covering a maximum area of  $100 \text{ km}^2$  off the Washington coast in midsummer. The appearance of high levels of DA (up to  $2.7 \mu\text{g DA equivalents L}^{-1}$ ), coincident with high numbers of *P. pseudodelicatissima* (up to  $15.4 \times 10^6$  cells  $\text{L}^{-1}$ ) at Kalaloch beach on the central coast in late September, was followed by the accumulation of record levels of toxin in razor clams. This toxic episode was preceded by a downwelling-favorable wind event, with associated onshore transport in near-surface layers. We suggest that the Juan de Fuca eddy may be one source of DA to the Washington coast. The duration of upwelling and the exact timing of fall storms likely play an influential role in the intensity of the bloom and the movement of toxic cells from the eddy to the coast.

The neurotoxin domoic acid (DA) was first measured in razor clams and Dungeness crabs on the Washington coast in 1991 (Horner and Postel 1993; Horner et al. 1993; Wekell et al. 1994). Razor clam toxification on the Washington coast in 1991 was preceded by the death of seabirds in Monterey Bay due to ingestion of toxic planktivorous fish earlier that summer (Buck et al. 1992; Fritz et al. 1992; Work et al. 1993). More recently, in May and June 1998, the first confirmed death of a marine mammal species due to DA poisoning was documented in sea lions along the central California coast (Gulland et al. 1999; Scholin et al. 2000). By late September, high levels of DA were again measured in

razor clams on the Washington coast (Adams et al. 2000). These examples of toxin bioaccumulation result from food web transfer of DA, produced by several species of the pennate diatom *Pseudo-nitzschia*, to filter-feeding fish and shellfish and, at times, to higher level vertebrates, including birds and mammals.

The species of *Pseudo-nitzschia* responsible for toxin production along the West Coast vary in place and time. For example, in Monterey Bay, anchovies became toxic to seabirds after feeding on *Pseudo-nitzschia australis* Frenguelli (Buck et al. 1992; Fritz et al. 1992). Both *Pseudo-nitzschia multiseriata* (Hasle) Hasle and *P. australis* were associated with algal blooms near locations where sea lions with neurological symptoms stranded in 1998 (Scholin et al. 2000; Trainer et al. 2000). *P. australis* was the toxic species in nearshore waters off the central Oregon coast in the summer of 1998 when DA in razor clams was reported on the beaches (Trainer et al. 2001). *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle was present in Washington coastal waters immediately prior to the razor clam toxification in 1998 (Adams et al. 2000; Horner et al. 2000). Off the Washington coast, *P. pseudodelicatissima* has been the only species present in significant numbers in seawater at times when particulate DA was measured. However, the potentially toxic species *P. multiseriata*, *P. australis*, *Pseudo-nitzschia pungens* (Grunow) Hasle, and *Pseudo-nitzschia fraudulenta* (Cleve) Heiden have also been observed in Washington coastal waters (Horner et al. 1997).

In an effort to characterize the *Pseudo-nitzschia* species responsible for DA production and also to understand the

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Table 1. Sampling events.

Date	Location	Figure*
7–19 Jul 1997	Offshore	2
27 Jun–4 Jul 1998	Offshore	3
31 Jul–14 Aug 1998	Offshore	4a
25 Aug 1998	Nearshore	4b
15 Sep 1998	Nearshore	4c
1 Oct 1998	Nearshore	4d
1 Jul–31 Oct 1998	Kalaloch beach	5

\* Figure numbers indicate where data from each sampling event is shown in detail.

origins of *Pseudo-nitzschia* blooms off the Washington coast, we participated in ship-of-opportunity cruises in 1997 and 1998 and in more extensive offshore and nearshore sampling in 1998. In addition, seawater was analyzed for particulate DA, and *Pseudo-nitzschia* spp. were enumerated on a weekly basis during the summer of 1998 at Kalaloch beach on the central Washington coast. Levels of DA in razor clams on the coast were also measured in 1997 and 1998. The primary goals of our beach sampling and offshore surveys were to determine (1) potential sites of offshore bloom initiation, (2) the species of *Pseudo-nitzschia* responsible for toxic events, and (3) the atmospheric and physical oceanographic processes associated with the onshore movement of high concentrations of toxic cells, resulting in toxification of coastal razor clams.

## Methods

**Sample collection**—Seawater was sampled on offshore and nearshore cruises using Niskin bottles. Samples were collected at depth only on some cruises; therefore, only surface data are reported here. Samples from the surf zone at Kalaloch beach were collected using a bucket. Seawater samples were preserved for DA analysis as well as phytoplankton identification and enumeration as described below. A summary of all sampling events is shown in Table 1.

**Analysis of particulate DA**—Particulate DA was measured by filtering 1 liter (offshore sampling) or 2 liters (for Kalaloch beach sampling) of seawater through a 47-mm-diameter (0.45- $\mu$ m pore size) nitrocellulose filter (Millipore Corp.). The filter was frozen for domoic acid analysis using the receptor binding assay described by Van Dolah et al. (1997). Glutamate decarboxylase was used prior to analysis to remove endogenous glutamate in all samples.

**DA analysis of shellfish**—Razor clams were collected from Kalaloch beach on the Washington coast in the summer and fall of 1997 and 1998. Concentrations of DA in razor clams on a given date represent analysis of a composite of six clams (viscera discarded) using HPLC (Hatfield et al. 1994) by the Washington State Department of Health.

**Phytoplankton cell counts and *Pseudo-nitzschia* species identification**—Total *Pseudo-nitzschia* cells were quantified for contour plots using the light microscope methods described below.

Early summer cruises, June–July 1997 and 1998: Whole water was preserved with buffered formalin (<1% final concentration) and analyzed for phytoplankton (including total *Pseudo-nitzschia*) presence and abundance using the Utermöhl inverted microscope method (Hasle 1978). Samples (50 ml) were settled for 12–24 h. All cells were identified and enumerated in settled samples at  $\times 400$  magnification; however, some (e.g., small flagellates) were classified only by size. *Pseudo-nitzschia* cell numbers, as a percentage of the total phytoplankton population contained within whole water samples, were also estimated. *Pseudo-nitzschia* species identifications, but not cell numbers, were confirmed with scanning electron microscopy (SEM) for some samples using cells collected with a 0.25-m-diameter, 20- $\mu$ m mesh net and preserved in buffered formalin (<5% final concentration). Cells were acid cleaned (Hasle and Fryxell 1970), dried directly on aluminum SEM stubs, coated with Au-Pd, and examined in a JEOL JSM-840A SEM at an operating voltage of 15 kV. Some net samples were examined to determine *Pseudo-nitzschia* spp. cell numbers using other SEM methods (Trainer et al. 2000), but cells were too fragmented for accurate counts because they were processed for SEM several months after collection.

Late summer samples, August–October 1998: Whole water was preserved with buffered formalin (<1% final concentration) and analyzed for total *Pseudo-nitzschia* abundance using a Palmer–Maloney slide at  $\times 200$  magnification (Guillard 1978). Net tow samples (20- $\mu$ m mesh) were preserved with buffered formalin (<5% final concentration) for determination of other dominant phytoplankton genera and total *Pseudo-nitzschia* (as a percentage of the phytoplankton population) using a Palmer–Maloney slide. Net tow samples were also used for *Pseudo-nitzschia* species identification and enumeration using SEM by determining the percentage of each species in a total count of at least 200 *Pseudo-nitzschia* cells.

**Nutrients**—Seawater for nutrient determination was filtered through a Whatman No. 1 filter (11  $\mu$ m particle retention), frozen, and analyzed using autoanalyzer methods (Whitledge et al. 1981).

**Advanced very high resolution radiometry (AVHRR)**—Satellite imagery from 1997 and 1998 was purchased from Ocean Imaging and was derived from 1-km high-resolution picture transmission (HRPT) AVHRR data collected by NOAA-12 and -14 satellites.

**Wind and current data**—Wind velocity vectors were constructed using wind speed and direction data obtained from the National Data Buoy Center's weather station on Destruction Island, Washington (NDBC #DESW1, 47.68°N, 124.49°W), located about 7 km northwest of Kalaloch beach (Fig. 2a). Current speed and direction were measured with an electromagnetic current meter (in 1997) or an acoustic doppler profiling system (in 1998) suspended from a taut wire mooring at a location off the Washington coast, 46.52°N 124.14°W (Fig. 2a), at a bottom depth of about 55 m. Both

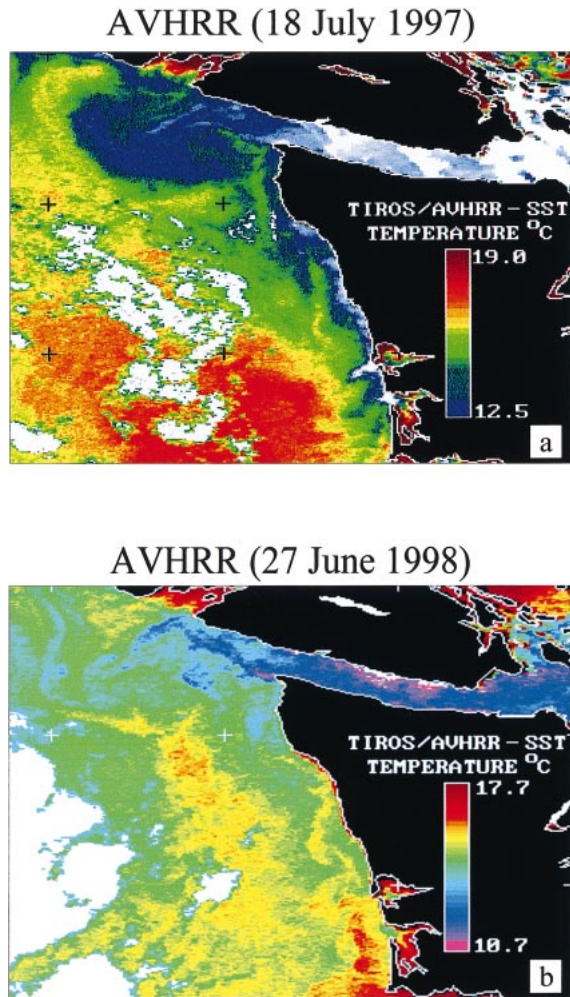


Fig. 1. AVHRR imagery off the Washington coast shows ocean surface temperature, indicated by the accompanying color legend (a) on 18 July 1997 at 2215 h GMT and (b) on 27 June 1998 at 2250 h GMT. The most cloud-free image during the 1998 McArthur cruise was obtained on 27 June during a downwelling period (Horner et al. 2000); therefore, the eddy feature is not as well formed as in (a).

wind and current hourly data were edited and then filtered to remove tidal frequencies.

## Results

A cold water gyre (shown in AVHRR imagery, Fig. 1) about 50 km off the northern Washington coast was the location of the highest DA levels and total *Pseudo-nitzschia* cell numbers during surveys in the early summers of 1997 and 1998 (Figs. 2, 3). The stations with the highest particulate DA concentrations in seawater coincided with high numbers of *P. pseudodelicatissima* ( $2.7 \mu\text{g}$  DA equivalents  $\text{L}^{-1}$ , up to  $0.6 \times 10^6$  cells  $\text{L}^{-1}$  in 1997 and  $0.2 \mu\text{g}$  DA equivalents  $\text{L}^{-1}$ , up to  $0.2 \times 10^6$  cells  $\text{L}^{-1}$  in 1998). *P. pseudodelicatissima* was the dominant toxigenic species of *Pseudo-nitzschia* in this area; however, *Pseudo-nitzschia* was not the dominant diatom and was usually  $\leq 10\%$  of the total

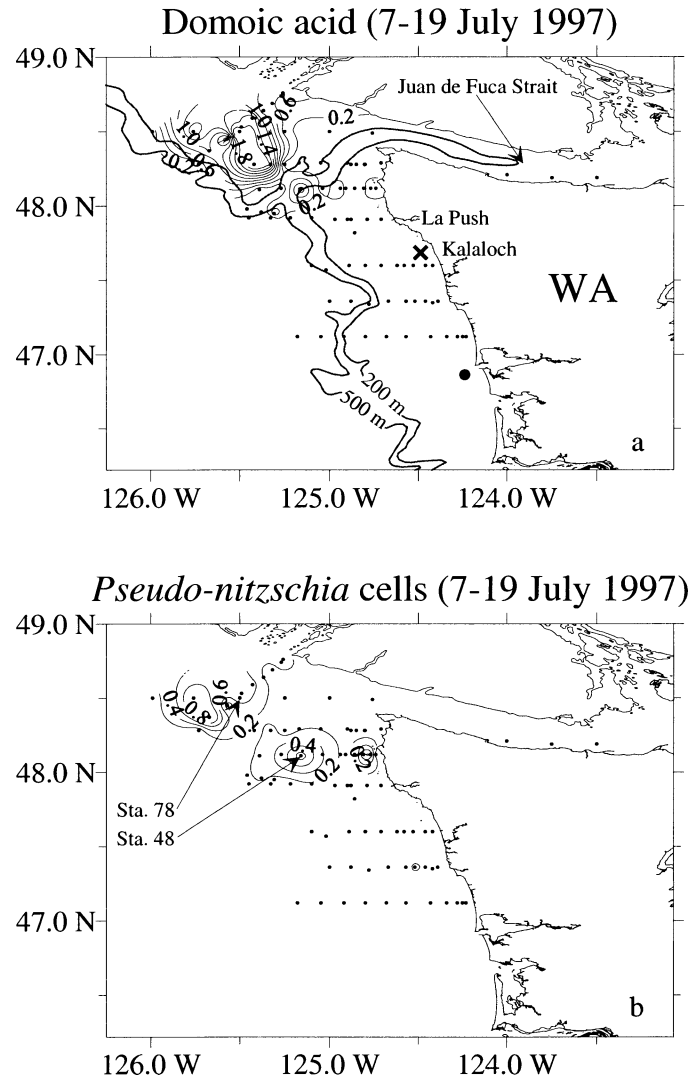


Fig. 2. DA and *Pseudo-nitzschia* concentration, 7–19 July 1997, thin contours show (a) DA concentrations ( $\mu\text{g}$  DA equivalents  $\text{L}^{-1}$ ) and (b) *Pseudo-nitzschia* cell numbers ( $\times 10^6$  cells  $\text{L}^{-1}$ ). Cruise transects (stations are shown as solid dots) were occupied in a south to north direction. Northern stations, where the highest DA and *Pseudo-nitzschia* cell numbers were detected, were occupied on 16–18 July. Thick contour lines (a) show the 200- and 500-m isobaths. Wind data were collected at the NDBC weather station on Destruction Island, shown by an X, and currents were collected at the University of Washington mooring shown as a solid circle (a). Arrows pointing to station locations (b) refer to data presented in Table 2.

phytoplankton population. Other *Pseudo-nitzschia* species were present, including *P. pungens*, *Pseudo-nitzschia delicatissima* (Cleve) Heiden, *Pseudo-nitzschia* cf. *heimii* Mangin, *P. australis*, *P. fraudulenta*, and *P. multiseriata*, but were never abundant. During a midsummer cruise in 1998, DA concentrations increased (Fig. 4a) relative to those measured in late June (Fig. 3a), with maximum total *Pseudo-nitzschia* cell numbers totaling  $4.7 \times 10^6$  cells  $\text{L}^{-1}$  (Table 2). Again, *P. pseudodelicatissima* was the dominant *Pseudo-nitzschia* species. The highest toxin levels measured during the August 1998 cruise were observed about 50 km off the

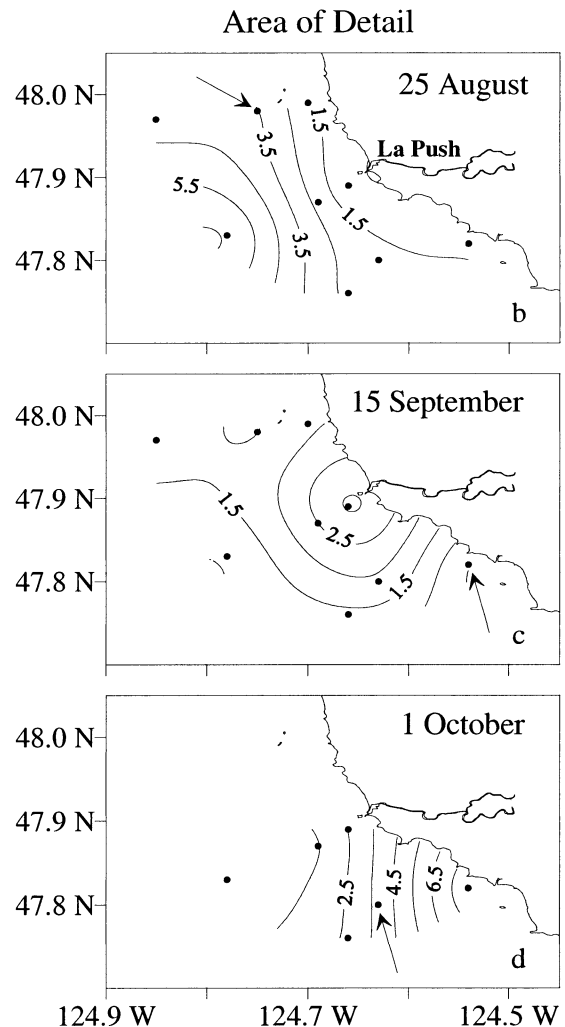
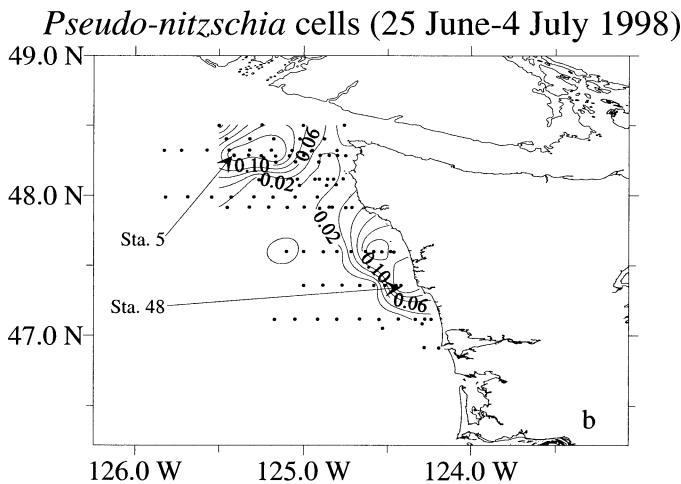
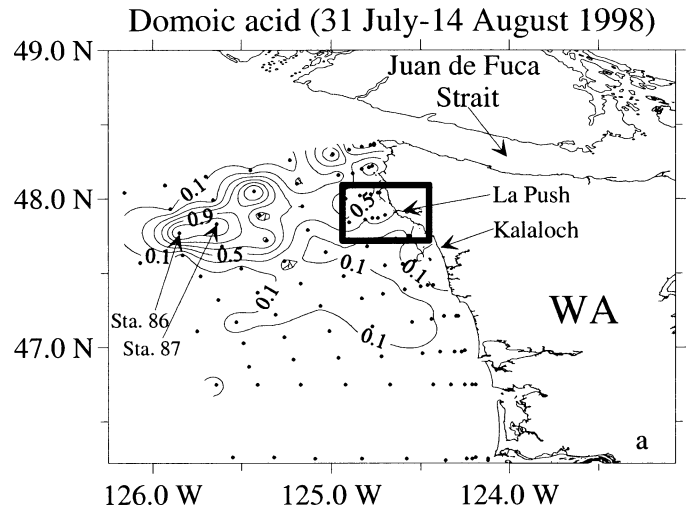
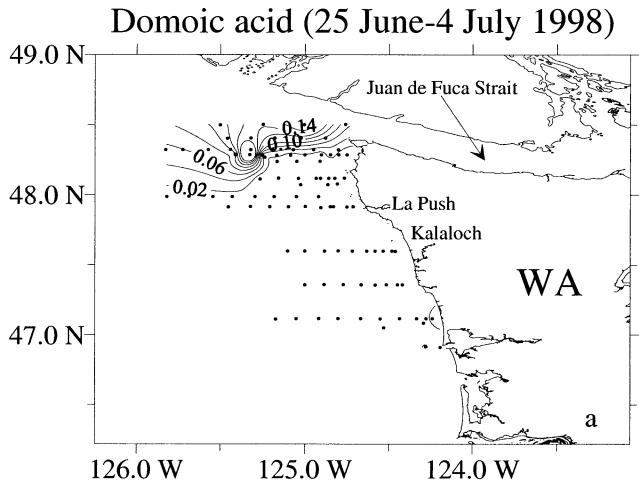


Fig. 3. DA and *Pseudo-nitzschia* concentration, 25 June–4 July 1998, contours show (a) DA concentrations ( $\mu\text{g DA equivalents L}^{-1}$ ) and (b) *Pseudo-nitzschia* cell numbers ( $\times 10^6$  cells  $\text{L}^{-1}$ ). Cruise transects (stations are shown as solid dots) were occupied in a north to south direction. Northern stations, where the highest DA and *Pseudo-nitzschia* cell numbers were detected, were occupied on 25–27 June. Arrows pointing to station locations (b) refer to data presented in Table 2.

coast in an area encompassing about 100 km<sup>2</sup>, beginning at the Juan de Fuca eddy area in the north and extending to 47.5°N, 126.0°W (west of Kalaloch beach) in the south.

DA patterns obtained from nearshore sampling off La Push on 25 August, 15 September, and 1 October are shown in Fig. 4b–d, respectively. On all three dates, *Pseudo-nitzschia* spp. were the dominant phytoplankton >20  $\mu\text{m}$  in size at all sites sampled, and *P. pseudodelicatissima* was the dominant *Pseudo-nitzschia* species. Arrows point to sites where detailed data are shown in Table 2. Estimates of cellular DA (Table 2) decreased from 0.7 to 0.1 pg cell<sup>-1</sup> from 25 August to 1 October. Silica and nitrate concentrations were high on all three dates. The highest DA levels and total *Pseudo-nitzschia* spp. cell numbers, respectively, measured at all stations shown in Fig. 4b–d were: 25 August (7.3  $\mu\text{g DA equivalents L}^{-1}$ , 13.3  $\times 10^6$  cells  $\text{L}^{-1}$ ), 15 September

Fig. 4. DA concentrations ( $\mu\text{g DA equivalents L}^{-1}$ ) in 1998. (a) DA concentrations, 31 July–14 August 1998. Cruise transects (stations are shown as solid dots) were occupied in a south to north direction. Northern stations, where the highest DA was detected, were occupied on 11–12 August. Three smaller panels (b–d) show results for the nearshore sampling grid on selected sampling dates. Arrows pointing to station locations (panels a–d) refer to data presented in Table 2. Beach samples were collected at Kalaloch beach indicated by an arrow (a).

Table 2. Domoic acid content, *Pseudo-nitzschia* species, other abundant phytoplankton, silicate, and nitrate in surface samples collected off the Washington coast in 1997 and 1998. ND, not done; equiv., equivalents.

Location*	Date	Cellular domoic acid (pg DA equiv. cell <sup>-1</sup> )†	Particulate domoic acid (µg DA equiv. L <sup>-1</sup> )	<i>Pseudo-nitzschia</i> abundance <sup>e</sup> (10 <sup>6</sup> cells L <sup>-1</sup> ) (% total)‡	Ppd and/or Pd abundances§ (10 <sup>6</sup> cells L <sup>-1</sup> )	Other most abundant general	Si, NO <sub>3</sub> (µM)
1. Sta. 78, Fig. 2	14 Jul 1997	4.6	2.66	0.6(4)	0.6	Ch(++), Em(+++), Th(+)	12.6, 0.4
2. Sta. 48, Fig. 2	15 Jul 1997	1.0	0.95	0.9(10)	0.9	Ch(++), Em(+++), Ph(+), Th(+)	8.5, 1.4
3. Sta. 5, Fig. 3	27 Jun 1998	0.6	0.09	0.1(4)	0.1	Ch(+++), Th(+++)	2.2, 7.5
4. Sta. 48, Fig. 3	1 Jul 1998	0	0	0.2(9)	0.1	Ch(+++), Ph(+), Sk(+++)	0.2, 0
5. Sta. 87, Fig. 4a	11 Aug 1998	0.3	1.36	4.7(95)	4.5	Ch(+), Th(+)	ND
6. Sta. 86, Fig. 4a	11 Aug 1998	0.6	1.47	2.5(ND)	2.1	Ph(++)	1.0, 0.2
7. Fig. 4b	25 Aug 1998	0.7	4.14	8.9(80)	5.7	As(+), Ch(+), Sk(+), Ph(++)	10.3, 4.2
8. Fig. 4c	15 Sep 1998	0.6	0.18	0.3(95)	2.8	Ch(+), Di(+), Sk(+), Ph(+), Pp(+)	33.0, 21.4
9. Fig. 4d	1 Oct 1998	0.1	3.69	3.9(95)	3.7	Ch(+), Rh(+), Th(+), Ph(+)	15.9, 9.2
10. Fig. 5	22 Sep 1998	0.2	2.70	17.1(99)	15.4	At(+), Ph(+)	10.4, 4.1

\* Sites were selected to focus in areas of high numbers of *Pseudo-nitzschia* (>100,000 cells L<sup>-1</sup>) during each survey and to give a range of cellular DA levels. See the Methods section for details of all measurements and calculations described below. Station locations are shown with arrows on each corresponding figure.

† Cellular toxin levels were estimated by dividing the concentration of particulate DA in 1 liter of seawater by the estimate of total *P. pseudodelicatissima* per liter using SEM (5–10) or the total of both *P. pseudodelicatissima* and *P. delicatissima* per liter using light microscopy (1–4).

‡ Numbers of *Pseudo-nitzschia* are estimated in whole water samples using light microscopy within ~1 month of collection. *Pseudo-nitzschia* and other genera are estimated as a percentage of the total phytoplankton using light microscopy of whole water (1–4) or net tow (5–10) samples.

§ Ppd, *Pseudo-nitzschia pseudodelicatissima*; Pd, *Pseudo-nitzschia delicatissima*.

|| The percentages of other genera and/or species are estimated as—+ (1–9%), ++ (10–39%), +++ (40–69%), ++++ (70–100%)—and are in alphabetical order as follows: As, *Asterionellopsis*; At, *Attheya*; Ch, *Chaetoceros*; Di, *Dinophysis*; Em, *Emiliania huxleyi*; Ph, *Pseudo-nitzschia cf. heimii*; Pp, *Pseudo-nitzschia pungens*; Rh, *Rhizosolenia*; Sk, *Skeletonema*; Th, *Thalassiosira*.

(3.6  $\mu\text{g}$  DA equivalents  $\text{L}^{-1}$ ,  $6.3 \times 10^6$  cells  $\text{L}^{-1}$ ), and 1 October (8.7  $\mu\text{g}$  DA equivalents  $\text{L}^{-1}$ , no determination of total *Pseudo-nitzschia* spp. on this date). The northern transect (northern three stations) could not be sampled on 1 October because of storm conditions and high winds.

Seawater sampling at Kalaloch beach included the time period of the nearshore sampling. In 1997, DA levels in razor clams were below the regulatory limit of 15  $\mu\text{g}$   $\text{g}^{-1}$  throughout the summer and fall (Washington Department of Health, data not shown). However, in 1998, DA levels in clams rose sharply in early fall at the same time as the particulate DA level detected in seawater and the *Pseudo-nitzschia* counts at Kalaloch beach increased (Fig. 5c). Both cell counts and particulate DA in seawater reached a maximum on 22 September at  $17.1 \times 10^6$  cells  $\text{L}^{-1}$  of total *Pseudo-nitzschia* spp. and 2.7  $\mu\text{g}$  DA equivalents  $\text{L}^{-1}$  seawater. The presence of particulate DA in Kalaloch beach seawater samples collected on 18, 22, 29 September and 5 October was confirmed by liquid chromatography–tandem mass spectrometry (LC-MS)/MS (Adams et al. 2000). The highest level of DA in razor clams (295  $\mu\text{g}$   $\text{g}^{-1}$  on 9 October) was recorded 18 d after the maximum *Pseudo-nitzschia* spp. cell number and particulate toxin in seawater at Kalaloch beach were measured (Adams et al. 2000). *P. pseudodelicatissima* was the only known DA producer present in significant numbers within the phytoplankton assemblage.

Selected biological and nutrient data from the sampling events in Figs. 2–5 are shown in Table 2. At each location where high numbers of *Pseudo-nitzschia* were measured, cellular DA, total particulate DA, silicate, and nitrate concentrations were variable. Cellular DA ranged from 0 to 4.6 pg cell $^{-1}$  in areas where total *Pseudo-nitzschia* abundance ranged from 0.1 to  $17 \times 10^6$  cells  $\text{L}^{-1}$  and particulate DA varied from 0 to 2.7  $\mu\text{g}$   $\text{L}^{-1}$ . Other abundant phytoplankton species included *Chaetoceros* spp., *Skeletonema costatum*, *Thalassiosira* spp., and *P. cf. heimii*, found in samples collected both from the eddy and nearshore regions. Silicate values ranged from 0.2 to 33.0  $\mu\text{M}$  and nitrate ranged from 0 to 21.4  $\mu\text{M}$ , but the lowest levels of these nutrients were found at station 48 in 1998 (Fig. 3), where particulate DA was not detected.

On the Washington coast, much of the current and water property variability is related to the strength and direction of local winds (Hickey 1989). Winds toward the south are associated with offshore and southward surface currents and upwelling of colder, nutrient-rich water. Winds toward the north (storm conditions) are associated with onshore and northward surface currents and downwelling of warmer, often nutrient-poor water. The close relationship between local winds and currents in summer 1997 and summer 1998 can be seen in Fig. 5a,b. In addition, under northward wind conditions, fresher water from the Columbia River to the south is generally moved northward up the Washington coast. Vertical lines through the vectors mark the dates on which the highest DA levels were measured in surface seawater samples collected during offshore surveys (Figs. 2–4). In 1997, maximum levels of toxin were measured in the Juan de Fuca region on 16–18 July during a period of relatively persistent southward (upwelling-favorable) winds from 10 July through early August. In 1998, southward winds were rela-

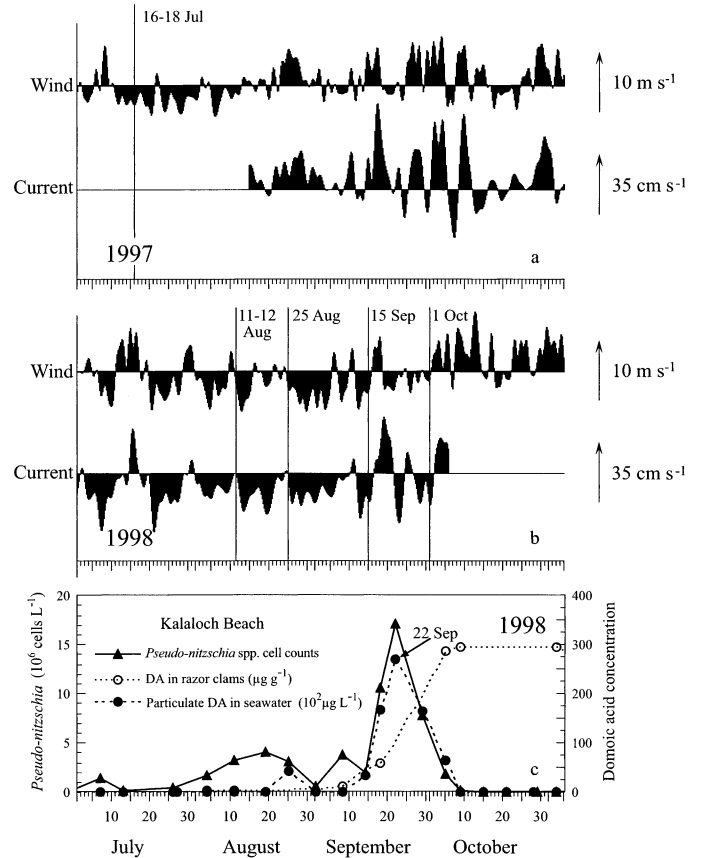


Fig. 5. North–south component of winds and currents off the Washington coast in (a) 1997 and (b) 1998 (see station locations in Fig. 1). Components above the axis are directed northward and below the axis are directed southward. Wind and current magnitude are indicated on the scales to the right of each time series. Dates when maximum DA was observed on surveys shown in Figs. 1–3 are indicated with vertical lines. *Pseudo-nitzschia* cell numbers, DA in razor clams, and particulate DA in seawater ( $\mu\text{g}$  DA equivalents  $\text{L}^{-1}$ ) at Kalaloch beach in 1998 are shown in the lower panel (c, adapted from Adams et al. 2000). The line marking 22 September 1998 indicates the date on which elevated levels of DA were measured in razor clams and seawater (particulate fraction) at Kalaloch Beach, immediately following the wind and current reversal on 16–18 September. Note that northward-tending winds and currents are generally associated with onshore-tending currents in the near-surface layers.

tively persistent beginning on about 20 July, with only weak reversals (northward winds) through the end of July to mid-August. Following this sustained period of southward winds, a DA maximum was measured on 11–12 August about 50 km offshore from La Push (Fig. 4a; Table 1). Mid-August through mid-September marked a period of prolonged, strong southward winds (Fig. 5b). The period of high cell numbers coincided with high particulate DA at stations near La Push on 25 August (Fig. 4b). This persistent upwelling was followed by storms (wind reversal to northward) recorded on 5, 10–12, and 15–18 September (Fig. 5b). The highest levels of particulate DA in seawater were measured on 15 September and 1 October at stations closer to shore than on the previous sampling date (Fig. 4c,d). Maximum

levels of particulate DA in seawater at Kalaloch beach on 22 September immediately followed the large storm event on 15–18 September (Fig. 5b,c). At the end of September, southward winds weakened and the autumn transition (wind reversal to primarily northward) began.

## Discussion

A zone of high phytoplankton biomass was found in a cyclonic gyre (the Juan de Fuca eddy) that persists throughout the summer over the continental shelf near the mouth of the Juan de Fuca Strait (Denman et al. 1981; Mackas and Sefton 1982). The eddy is a result of the interaction between the outflow from the Strait, southward wind-driven currents along the continental slope, and the underlying topography, a spur of the Juan de Fuca submarine canyon. Drifter studies indicate that particles are retained in the eddy for about 7 d and that the preferential direction taken when they leave the eddy is through the southeast quadrant toward the Washington coast (Freeland and McIntosh 1989). This was also suggested when a tanker sank in the eddy in July 1991, resulting in oil beached on the Washington coast 6 d later (Venkatesh and Crawford 1993). Thus, it seems likely that marine organisms residing in the Juan de Fuca eddy can, under certain ocean conditions, also be transported to the Washington coast.

Despite its potential importance as an area of high phytoplankton biomass, the eddy has not been studied intensively. The photic zone in this area is characterized by high nutrients supplied by wind mixing; episodic, wind-driven upwelling; topographically controlled upwelling (Freeland and Denman 1982); and the outflow from the Juan de Fuca Strait, where deep, nutrient-rich water is advected to the surface by estuarine circulation and tidal mixing (Mackas et al. 1989). High numbers of *Pseudo-nitzschia* cells (initially identified as *Nitzschia* spp.) are associated with these productive waters (Mackas and Sefton 1982; Forbes and Denman 1991; Horner et al. 2000). Measurements of DA, however, have not been made prior to our study. We show that toxigenic *P. pseudodelicatissima* cells were associated with the presence of particulate DA in this Juan de Fuca eddy in the early summer of two consecutive years, suggesting that this may be an area of *Pseudo-nitzschia* bloom initiation. Observations to date show that *Pseudo-nitzschia* spp. are frequently seen over the Washington shelf and near the Washington coast, as well as in the Juan de Fuca eddy (Horner and Postel 1993; Horner et al. 2000). With the limited and nonsynoptic data collected to date, we cannot rule out the possibility that DA in coastal shellfish can originate from nearshore phytoplankton blooms fueled by upwelling near the coast. Nevertheless, DA has not been observed near the coast in our summer surveys. Thus, we conclude that the Juan de Fuca eddy is a productive area where toxic cells are frequently observed and also that transport from the eddy to the Washington coast can occur under some environmental conditions. Although there may be other initiation sites of DA-producing blooms along the Washington coast, it is our hypothesis that the Juan de Fuca eddy is a productive area where toxic cells may be found in the summer of every year.

*P. pseudodelicatissima* comprised 75–95% of the *Pseudo-nitzschia* population observed in the surf zone at Kalaloch beach and in nearshore samples from August–October 1998 when particulate DA was measured. The presence of DA activity in field samples containing up to  $15 \times 10^6$  cells L<sup>-1</sup> *P. pseudodelicatissima* (this study) and in a cultured isolate of *P. pseudodelicatissima* collected from Kalaloch beach (Adams et al. 2000) gives strong evidence that this species was the primary toxin producer in offshore Washington waters in 1997 and 1998.

Cellular levels of DA ranged from 0 to 4.6 pg cell<sup>-1</sup> when *P. pseudodelicatissima* was present. The maximum level estimated in these field samples is two to three orders of magnitude higher than the level determined in cultured isolates (~5 fg cell<sup>-1</sup>, Adams et al. 2000; 38 fg cell<sup>-1</sup>, Pan et al. 2001), suggesting that lab measurements of specific cellular toxicity do not necessarily reflect actual levels in the field. The maximum cellular levels of DA that have been determined for field populations of other toxic species, namely *P. australis* and *P. multiseriis*, are greater than the highest value for cellular toxicity of *P. pseudodelicatissima* estimated here. Levels of up to 6 pg cell<sup>-1</sup> have been calculated for *P. multiseriis* from California (Trainer et al. 2000) and up to 9 pg cell<sup>-1</sup> for *P. multiseriis* from eastern Canada (Smith et al. 1990). The highest cellular levels of DA (75–78 pg cell<sup>-1</sup>) have been measured in field populations of *P. australis* from the central California coast (Scholin et al. 2000; Trainer et al. 2000). These data suggest that lower cell densities of *P. australis* or *P. multiseriis* than *P. pseudodelicatissima* may be required to produce similar amounts of DA. However in one example, where *P. australis* numbers off California reached  $1 \times 10^7$  cells L<sup>-1</sup>, cellular levels of DA ranged from 0.1 to 0.9 pg cell<sup>-1</sup> (Trainer et al. 2000), comparable to the cellular level of DA (0.2 pg cell<sup>-1</sup>), measured in similar numbers of *P. pseudodelicatissima* at Kalaloch beach on 22 September (Fig. 5). This range of cellular DA measured in field populations of *Pseudo-nitzschia* illustrates the difficulty in predicting the magnitude of toxicity attributable to any given species. Indeed, this study makes clear that *P. pseudodelicatissima* can present a significant threat to coastal fisheries and that the positive identification of all potentially toxigenic species should be part of routine monitoring programs.

The Juan de Fuca eddy is probably not the only initiation site for *Pseudo-nitzschia* blooms off the Washington coast. It is interesting to note that no DA was measured in the nearshore area at station 48 in 1998, but DA levels of ~0.6 pg cell<sup>-1</sup> were measured in the eddy region during the same cruise (Sta. 5, Table 2), suggesting that the offshore eddy might provide conditions more favorable to toxin production. The nearshore bloom of *Pseudo-nitzschia* was likely associated with a nearshore upwelling event that occurred from 27 June to 3 July (Horner et al. 2000; Fig. 1). Although our nutrient data cannot be used to establish a linkage between cellular DA levels and nutrient availability in situ (Table 2), they do suggest the importance of making field measurements of macro- and micronutrients, including trace metals such as iron, in conjunction with measurements of DA and *Pseudo-nitzschia*, to help validate theories developed from lab studies.

An understanding of physical oceanographic processes off the Washington coast is necessary to the detailed characterization of harmful algal bloom transport. In 1998, upwelling-favorable winds (southward) and associated southward currents in July and August are believed to have caused movement of toxin and cells southward from the Juan de Fuca eddy area to the Washington coast (Fig. 5b,c). In general, our data showed an increase in particulate DA levels in seawater from summer to fall. Although we do not know the extent of the bloom in August and September, high levels of particulate DA were detected within 20 km of the coast in late August (Fig. 4b). Southward winds were strong and persistent in late August (Fig. 5b) so that toxic cell growth was likely sustained by nutrients from upwelling. A substantial increase in numbers of toxic *P. pseudodelicatissima* and DA in razor clams above the 15- $\mu\text{g g}^{-1}$  regulatory limit was measured after the large wind/current reversal event on 16–18 September. We hypothesize that the toxic bloom that was sustained offshore, possibly throughout much of the summer, was brought to the coast during this major wind reversal event and the associated onshore surface currents in late summer. The observation of similar phytoplankton assemblages in the eddy and nearshore regions could lend credence to the idea that eddy and nearshore phytoplankton populations are sometimes mixed. Although we did observe several of the same phytoplankton genera in both the eddy and on the coast during the summer (Table 2), it is difficult to make any firm conclusions regarding transport processes from these data.

Why were high levels of DA present in razor clams in 1998 but not in 1997? The duration of upwelling, the timing of the fall transition, and the availability of macro- and micronutrients are believed to be key factors in the advection of high concentrations of toxic *Pseudo-nitzschia* to the coast, resulting in toxification of razor clams. The summer of 1998 followed one of the largest El Niño events in decades. Upwelling-favorable winds were anomalously weak and intermittent in summer 1997 and anomalously strong and persistent in summer 1998 (Huyer et al. in press; see also Fig. 5a,b). The exact timing of changes in the ocean currents in any given year likely plays an influential role in the movement of toxic cells to the Washington coast. For example, if upwelling, hence southward currents, are not sufficiently persistent, a bloom originating in the Juan de Fuca eddy might never reach the coast. If the transition to major fall storms occurs early in the growing season, as was observed in 1997, the population of toxic *Pseudo-nitzschia* that reaches the coast from the eddy could be sparse.

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