An ecological study of a massive bloom of toxigenic *Pseudo-nitzschia cuspidata* off the Washington State coast

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**Abstract**

In September 2004 a large, nearly monospecific diatom bloom of *Pseudo-nitzschia cuspidata* off the coast of the state of Washington reached cell concentrations of $6.1 \times 10^6$ cells L$^{-1}$ and produced maximum particulate domoic acid (pDA), dissolved domoic acid (dDA), and cellular domoic acid concentrations of 43 nmol L$^{-1}$, 4 nmol L$^{-1}$, and 63 pg cell$^{-1}$, respectively. This bloom co-dominated the phytoplankton assemblage with the euglenoid *Eutreptiella* sp. in the Juan de Fuca eddy region, a known initiation site for toxigenic *Pseudo-nitzschia* blooms. Two isolates of *P. cuspidata* collected during separate cruises produced domoic acid (DA) in culture. During the September 2004 survey, 84% of the stations ($n = 98$) had detectable *Pseudo-nitzschia* and 78% had detectable pDA. There were no significant correlations between either pDA or cellular DA and ambient concentrations of macronutrients; however, when considering only those stations where *Pseudo-nitzschia* was present, pDA was positively correlated with chlorophyll $a$ and negatively correlated with temperature ($p < 0.01$) at both 1- and 5-m depths. Correlations between cellular DA concentrations and total bacteria or cyanobacteria abundances were not significant. Variable ratios of pDA : dDA in the eddy region suggest that DA release was under cellular regulation by *Pseudo-nitzschia*. Stations where dissolved Fe concentrations were limiting (<0.5 nmol L$^{-1}$) had the highest *Pseudo-nitzschia* abundances and pDA and cellular DA values. These results provide enticing field evidence of the role of Fe limitation in controlling cellular DA levels.

The Juan de Fuca eddy is a nutrient-rich, physically retentive region off the U.S. and Canadian coasts that persistently supports the initiation and growth of toxigenic *Pseudo-nitzschia* spp. (Trainer et al. 2009) and thus serves as a natural laboratory for studying toxigenic diatom blooms. Recent evidence has demonstrated that this eddy is an advective source of toxic *Pseudo-nitzschia* to the Washington State coast (MacFadyen et al. 2005, 2008). Over 10 species of *Pseudo-nitzschia* have been documented in this region (Stehr et al. 2002), and blooms of *Pseudo-nitzschia* here are rarely dominated by a single species (Trainer et al. 2009). However, in September 2004, a nearly monospecific diatom bloom of *P. cuspidata*, which co-dominated the phytoplankton assemblage together with the euglenoid *Eutreptiella* sp. (Trainer et al. 2009), provided a unique opportunity to study the oceanography and ecology of a single-species bloom of *Pseudo-nitzschia*.

The environmental controls on in situ domoic acid (DA) production by *Pseudo-nitzschia* have been difficult to assess. Although toxin content of various *Pseudo-nitzschia* species in culture appears to change under macronutrient stress (Bates et al. 1991; Pan et al. 1996a,b), laboratory conditions do not always accurately replicate field scenarios. A recent analysis of >550 samples from the shelf region off Washington State over 4 yr showed that no simple predictive relationships exist between *Pseudo-nitzschia* abundance or particulate DA (pDA) concentrations and ambient concentrations of macronutrients (Trainer et al. 2009). The environmental controls on cellular DA content have been especially difficult to pinpoint because *Pseudo-nitzschia* blooms in coastal waters are most often composed of multiple species (Trainer et al. 2000, 2009) with widely varying cellular toxin quotas (Trainer et al. 2007).

In this study, we confirm that *P. cuspidata* was the bloom-forming species that produced DA in situ during the September 2004 cruise and under laboratory culture conditions. We compare the distributional patterns of *Pseudo-nitzschia* abundance and species as well as DA (pDA, dissolved DA [dDA], and cellular DA) concentrations from the September 2004 cruise with the concentrations of heterotrophic bacteria, cyanobacteria, dissolved macronutrients, and Fe. These findings are used to understand the potential environmental regulators of cellular toxin production during the largest *P. cuspidata* bloom recorded on the west coast of America to date.

**Methods**

This study was part of a 5-yr Ecology and Oceanography of Harmful Algal Blooms in the Pacific Northwest (ECOHAB-PNW) program to study the ecophysiology of
toxigenic *Pseudo-nitzschia* in coastal waters. The ECO-HAB-PNW survey grid encompassed waters off southern Vancouver Island, British Columbia, the Strait of Juan de Fuca, the Juan de Fuca eddy, and the Washington coast as far south as Grays Harbor (Fig. 1). In 2004, the complete survey grid was sampled from 09 to 16 September during a period of reasonably steady downwelling-favorable winds (Trainer et al. 2009). Drifter studies were performed on the same cruise (C. Trick unpubl.).

**Hydrographic data and sample collection**—Hydrographic conditions were characterized at each grid station using conductivity, temperature, and depth (CTD) profiles (SBE-911, Sea-Bird Electronics) and discrete seawater samples collected at the surface (1-m), 5-m, and 10-m depths using rosette-mounted 10-liter Niskin bottles for analysis of phytoplankton biomass as chlorophyll *a* (Chl *a*), macronutrients (nitrate + nitrite, phosphate, and silicic acid), phytoplankton species composition, dDA, and pDA. Samples for trace metal analysis were collected separately using an all-Teflon underway sampling system comprising a towed fish placed outboard of the ship’s wash. This water (3–4 m) was pumped onboard using a Teflon double-diaphragm pump to a sampling station within a Class 100 HEPA fan bench housed inside a custom-fabricated clean room. Seawater was filtered using Teflon capsule filters (Sterlitech) at low pressure (<70 kPa), and collected in rigorously cleaned fluorinated polyethylene bottles. To avoid ship contamination, sample collections were performed immediately before the vessel slowed for each station.

**Chlorophyll determinations**—Samples were analyzed at each station for phytoplankton biomass as Chl *a* using the non-acidification in vitro fluorometric analysis (Welschmeyer 1994). Seawater was filtered onto Whatman GF/F filters (25-mm diameter, 0.7-μm nominal pore size) at low pressure (<70 kPa) and immediately extracted in 90% acetone for ca. 24 h at −20°C to −80°C. Chl *a* concentrations were determined on a Turner Designs 10AU field fluorometer that was calibrated at the beginning of the cruise with pure Chl *a* in 90% acetone (Turner Designs) and monitored for instrument drift during the cruise using a solid secondary standard (Turner Designs).

**Pseudo-nitzschia species enumeration**—Samples for cell quantification were incubated with Triton X-100 (0.1% final concentration) to ensure complete recovery from 50-mL polypropylene tubes in which they were stored. *Pseudo-nitzschia* cell numbers were quantified microscopically (Zeiss Axiovert 135 inverted light microscope) from whole water samples preserved with buffered formalin (1–2% final concentration) using a Palmer-Maloney counting chamber, and when necessary, after 12–24 h of settling. All cells were enumerated at 100–200× magnification with a limit of detection of 700 cells L⁻¹. *Pseudo-nitzschia* species were grouped into the three size categories (in order of increasing size) of *pseudodelicatissima*, *delicatissima*, *cuspidata* (pd/d/c); *australis*, *fraudulenta*, *heimii* (a/f/h); and *pungens*, *multiseries* (p/m) (Trainer and Suddleson 2005). The efficacy of these categorizations in relation to species identification described by Lundholm et al. (2006) was established in Trainer and Suddleson (2005) and confirmed using transmission electron microscopy (TEM) analyses on ~1% of the survey grid surface samples (Lundholm et al. 2003). Because of sample loss on the September 2004 cruise, total cell abundances for 1-m samples at stations CB05, CB07, GH04, GH05, and GH06 were estimated from a
linear regression of pDA vs. *Pseudo-nitzschia* abundance from the filament region during the survey.

**DA analyses**—pDA samples were obtained from 1-, 5-, and 10-m depths by filtering 1 liter of seawater onto 47-mm-diameter nitrocellulose filters (HAWP04700 Millipore MF<sup>TM</sup> Membrane filters; 0.45-µm pore size). The filters were macerated and sonicated to ensure the release of intracellular DA, and analyzed in triplicate using the receptor binding assay method as outlined by Baugh et al. (2004). Standards were prepared with a certified DA standard (DACs-1D, Certified Reference Materials Program, National Research Council, Canada). The limit of detection was 0.1 nmol L<sup>−1</sup> with an overall precision of ∼5% coefficient of variation. Depth-integrated pDA was determined by trapezoidal integration for the upper 10 m of the water column by summing the areas of two four-sided polygons as follows:

$$pDA_{\text{depth int}} = \frac{(b_1 + b_2) \times h}{2} + \frac{(b_2 + b_1) \times h}{2}$$  

where \(b_1, b_2, b_3\) are pDA values at 1-, 5-, and 10-m depths, respectively, and \(h\) is the vertical distance between observations of pDA in the water column (i.e., 5 m).

dDA concentrations were measured separately on sample filtrates (0.45 µm, mixed cellulose ester filters; Millipore) using the direct competitive enzyme-linked immunoassay (cELISA) kits (Biosense Laboratories), a modified version of the indirect cELISA method described in Garthwaite et al. (1998). Samples were analyzed in duplicate, and the occasional poor replicates reanalyzed. The limit of detection for seawater samples was 22 pmol L<sup>−1</sup>, and the limit of quantification was 45 pmol L<sup>−1</sup>.

Cellular DA concentrations were estimated by dividing pDA concentrations by the corresponding *Pseudo-nitzschia* cell numbers. These estimates were restricted to samples containing a minimum of 50 cells, giving a 95% confidence interval of ±30% of the mean cell density (Lund et al. 1958). The central assumption in these cellular DA estimates is that there was low variability in toxin production among individual *P. cuspidata* cells in these natural samples.

**Flow cytometry (bacterioplankton enumeration)**—Flow cytometry samples were analyzed within 2 h of collection without fixative on a FACSCalibur flow cytometer (Becton-Dickinson). Samples not analyzed immediately were stored at ∼4°C in the dark until analysis. All data acquisitions were done with logarithmic signal amplification. Cytometer sample flow rates were calibrated using bead stocks of known concentration (Calibrite beads, Becton-Dickinson) and particle size was calibrated using beads of known size (Flow Cytometry Size Bead Kit, Invitrogen). Cyanobacteria were identified by size and by red and orange fluorescence (instrument settings: forward scatter = E00, FL2 = 280, and FL3 = 350). *Prochlorococcus* were identified by size and red fluorescence, but were present in low number and not considered in these analyses. Heterotrophic bacterioplankton were stained with the nucleic acid stain SYBR Green I (Invitrogen) (Marie et al. 1997) and cell abundances were calculated from acquisition duration, the number of events, and instrument flow rate.

**Iron determinations**—Dissolved Fe concentrations were determined in triplicate using flow injection analysis with luminol-induced chemiluminescence detection (Roy et al. 2008). Briefly, 100-µL samples were chemically reduced to Fe(II) with sulfite before analysis and results were quantified using standard curves obtained from standard additions to a subset of samples. Total Fe samples and standards were acidified to pH 2.0 for a minimum of 12 h with Optima HCl (Fisher Scientific). After acidification, samples were buffered to pH 4.5 with the minimum addition of acetate buffer and 200 µmol L<sup>−1</sup> sodium sulfite, then reacted for >12 h before analysis by flow injection. The reagent blank was determined as 40 pmol L<sup>−1</sup> with a detection limit of <50 pmol L<sup>−1</sup> (3 × SD of blank analyses). Analytical accuracy (>95%) was assessed by analysis of NASS-5 certified coastal seawater, and Sampling and Analysis of Iron (SAFe) cruise standards (Johnson et al. 2007).

**Nutrients**—Water samples for dissolved inorganic macronutrient analyses were collected at multiple depths, including the two inshore stations of each survey line and then at every other station continuing offshore. Unfiltered samples were collected in pre-cleaned polypropylene tubes and analyzed for nitrate plus nitrite (NO<sub>3</sub>− + NO<sub>2</sub>−; hereafter referred to as nitrate), orthophosphate (PO<sub>4</sub>−<sub>3</sub>), and silicic acid [Si(OH)<sub>4</sub>] with a Lachat QuikChem 8000 Flow Injection Analysis system using standard colorimetric techniques (Smith and Bogren 2001; Knapel and Bogren 2002; Wolters 2002, respectively).

**Laboratory culture studies establishing cell toxicity**—Single-cell isolates were obtained by placing a drop of seawater concentrated by a 20-µm mesh size plankton net into well plates containing filtered seawater medium amended with f/2 nutrients (Sigma). Single cells or chains of target *Pseudo-nitzschia* spp. were isolated with a capillary pipette and transferred to wells with fresh medium. Cell isolates were maintained onboard in a 12°C incubator until transfer to the shore laboratory. There, cells were transferred using sterile technique to 12-mL borosilicate tubes and maintained at 12°C in f/2 medium at a photosynthetic photon flux density of ~80 µmol photons m<sup>−2</sup> s<sup>−1</sup> (12:12 h light : dark cycle). Isolates were identified to the species level using TEM as described above.

Two clonal isolates of *P. cuspidata* were used in this study to determine the potential for DA production in this species. Clone NWFSC 191 was collected from the Juan de Fuca eddy region on the September 2004 cruise, and clone NWFSC 221 was collected at Sta. KB05 (Fig. 1B) during the September 2005 ECOHAB-PNW cruise. The growth experiments using these cultures were initiated 5 months after isolation (NWFSC 191) and 1 month after isolation (NWFSC 221). Approximately 1 mL of culture (or an
appropriate volume to yield an initial cell density of ca. 20–200 cells mL\(^{-1}\)) was transferred to 100 mL of medium in a 250-mL borosilicate flask. Once each culture entered mid-exponential phase, ca. 5 mL (or the appropriate volume to attain an initial cell density of 20–200 cells mL\(^{-1}\)) of culture was transferred to 1.5 liters of medium in a 2-liter borosilicate flask. The culture was sampled multiple times at each growth stage to obtain total cell counts and pDA measurements. For determination of pDA on selected days in culture, 100 mL of culture was filtered and analyzed in duplicate as described above.

**Sea surface temperature imagery**—Advanced very high resolution radiometer sea surface temperature (SST) imagery originated from the NOAA CoastWatch West Coast regional node in La Jolla, California.

**Statistical relationships**—Principal components (PC) analyses were conducted to explore the relationships between the environmental parameters (temperature, salinity, oxygen, nutrient concentrations, and nutrient ratios) and the biological parameters (in vitro Chl a concentration, in situ Chl a fluorescence, *Pseudo-nitzschia* abundance, pDA, and cellular DA). PC analysis summarizes the variability in the original observations by a series of orthogonal linear transformations of the data into PC (Everitt and Hothorn 2006). Analyses were conducted separately on the survey samples at 1- and 5-m depths using the R statistical package (version 2.6.2, R Foundation for Statistical Computing). Observations were centered around zero and rescaled to unit variance. Relationships between parameters are described by the similarity between the loading coefficients for each parameter for each PC axis. If the eigenvalue of PC was greater than one, they were deemed to be meaningful. Parameters loading coefficients greater than 0.3 were considered significant based on the number of parameters and the magnitudes of the loading coefficients.

Spearman's rank correlation analyses were conducted to further explore the relationships between ambient concentrations of pDA and cellular DA with environmental properties observed at 1- and 5-m depths during the September 2004 cruise. Relationships determined for DA used data only from sites where *Pseudo-nitzschia* spp. were detected. Relationships were determined using nonparametric analyses because the distributions of pDA and cellular DA concentrations could not be transformed to meet the assumption of normality. The Spearman's rank difference correlation coefficient (\(r_s\)) indicates the strength of general monotonic relationships by measuring the association between ranks of variables. All statistical analyses were conducted using the software package SPSS 14.0 (SPSS).

**Results**

This grid was sampled between 09 and 16 September 2004 over an 8-d period of variable but predominantly downwelling favorable winds (Trainer et al. 2009). The Juan de Fuca eddy was clearly seen as an area of high salinity (>33.3 at 50-m depth during the September 2004 cruise (Fig. 1A). The filament zone downstream of the eddy where high chlorophyll filaments have been observed (Trainer et al. 2009) is delineated in the offshore region south of the defined eddy (Fig. 1A). The resultant well-defined eddy was cohesive, with surface-water circulation around the eddy being successfully tracked by drifters (MacFadyen et al. 2008).

*Pseudo-nitzschia spatial distributions*—The broad spatial distribution of total *Pseudo-nitzschia* cell abundance in surface waters during September 2004, along with that of the three size groupings, is shown in Fig. 2. The highest levels of toxicity and *Pseudo-nitzschia* abundance were generally found in surface (1-m) samples (51\% of the stations), whereas a smaller percentage occurred at 5-m depth (22\% of the stations, \(n = 98\)). Cell densities were typically substantially lower in deeper waters. Overall, 65\% of the surface stations across the entire grid region had detectable *Pseudo-nitzschia* abundances (84\% of the stations when 5- and 10-m depths were also included; Trainer et al. 2009), and 65\% had detectable pDA (78\% of stations when 5- and 10-m depths were also included). Maximum pDA concentrations were observed at 1 m in 38\% of the stations, at 5 m in 34\% of the stations, and at 10 m in 28\% of the stations (\(n = 98\)).

The greatest surface densities of *Pseudo-nitzschia* cells during the grid survey were found at station LBC02 off Barkley Sound, Canada (3.3 \(\times\) 10\(^6\) cells L\(^{-1}\); Fig. 2A) and at 5 m at station LAB03 in the center of the eddy (6.1 \(\times\) 10\(^6\) cells L\(^{-1}\); see Fig. 1B for station locations). Light microscope observation of size groups (Trainer and Suddleson 2005), at each station where *Pseudo-nitzschia* were observed, indicated that the small, narrow cells of the pdil/dc group dominated at 97\% of the survey stations, whereas the large, broad cells (a/f/h) and large, narrow cells (p/im) each dominated less than 2\% of the stations sampled (Fig. 2).

**pDA and dDA**—The highest surface-water pDA concentrations (Fig. 3A) and the greatest depth-integrated pDA concentrations (Fig. 3B) were observed in the Juan de Fuca eddy and its southward-advecting filament (a tongue of cool water in the SST image; see Fig. 4). Nearshore stations also had lower, but measurable, pDA (with the exception of Sta. GH02). Stations having maximum pDA concentrations below the surface were situated in the eddy on lines B (LB), AB (LAB), and A (LA), and offshore on the Cape Flattery (CF), Ozette (OZ), and La Push (LP) lines (Fig. 3B). pDA was measurable at 68\% of eddy (\(n = 50\)) and 61\% of filament stations (\(n = 38\); Fig. 3B). The highest pDA concentration measured during the survey was 43 nmol L\(^{-1}\) in surface (1 m) waters on the CF line at the southern edge of the eddy (CF05). During the survey, 37\% of the stations had maximum pDA at the surface; in contrast, a majority of stations (51\%) had maximum *Pseudo-nitzschia* numbers at the surface.

Representative vertical profiles of *Pseudo-nitzschia* abundances and pDA and dDA concentrations in the eddy region are shown in Fig. 3C,D. In Fig. 3C, *Pseudo-
nitzschia abundance was greatest at 5 m, maximum pDA values were observed at 10 m, and maximum dDA at 15 m, whereas in Fig. 3D, Pseudo-nitzschia, pDA, and dDA concentrations were all highest at the surface. Practical limitations restricted dDA analyses to waters in the eddy region. dDA was detected at all but one of the stations sampled, with most values being in the range of 1–2 nmol L$^{-1}$. The maximum concentration of dDA in surface (1-m) waters was 4 nmol L$^{-1}$ at Sta. LAB03 (Fig. 5A). Of particular note is that high concentrations of dDA were observed at some stations (e.g., LA line) even though pDA was below the level of detection by receptor binding assay. pDA and dDA concentrations differed greatly among stations, and ratios of pDA to dDA ranged from 0 to almost 20. To date, the only mechanism for degradation of dDA reported is photochemical (Bouillon et
al. 2008). In the present study, the lack of any consistent pattern in the degradation of dDA with depth suggests that DA release is under cellular regulation.

**Cellular toxicity**—Unlike most recorded blooms of *Pseudo-nitzschia* spp., it was possible to estimate cellular toxin quotas with reasonable confidence during this bloom because *P. cuspidata* was the dominant *Pseudo-nitzschia* species at the majority of stations sampled. For surface samples having measurable *Pseudo-nitzschia* abundances and also showing detectable pDA, *P. cuspidata* represented 100% of the *Pseudo-nitzschia* assemblage in 40 samples, 90–99% in 14 samples, and less than 90% in 3 samples (CF06: 70% pdld/c, 30% plnr; LP01: 88% pdld/c, 12% alfth; LBC08: 100% plnr; Fig. 2). Cellular toxin quotas in surface samples were highest on the western edge of the eddy region and its filament (Fig. 4). Maximum surface values of cellular DA were observed at Sta. CF06 (20 pg cell⁻¹) and Sta. CF05 (13 pg cell⁻¹), both in the eddy filament (Fig. 4). At depth, the highest cellular DA values were measured at Sta. CF06 at 5 and 10 m (27 and 63 pg cell⁻¹, respectively) and Sta. LB09 at 5 m (35 pg cell⁻¹, not shown). *Pseudo-nitzschia* species belonging to size groups other than pdld/c were present at Sta. CF06; therefore, the high cellular DA at this station may be attributed, in part, to other species.

**Bacterial abundances**—Heterotrophic bacterial cell abundances varied slightly across the survey grid; however, the highest densities (up to $2.1 \times 10^6$ cells mL⁻¹) generally were found in the nearshore and eddy regions (Fig. 6A,
associated with higher-salinity waters (Fig. 1A). The highest cyanobacteria cell densities were found in the nearshore region (up to $1.1 \times 10^5$ cells mL$^{-1}$; Fig. 6B) and along the LB line near the center of the eddy.

**Dissolved iron**—We compare here both pDA and cellular DA concentrations to dissolved Fe concentrations across the region, though different methods were used to collect DA and Fe samples. The pDA and cellular DA data are from whole water samples collected at 1-m depth using Niskin bottles, whereas dissolved Fe samples were collected when arriving on station from 3- to 4-m depth using the trace metal–clean towed fish. Given that the average wind-mixed layer in the study region was considerably deeper (9 m), it is reasonable to expect that the samples collected using the two methods provide an equivalent assessment of these parameters.

Concentrations of dissolved Fe generally ranged from 1 to 5 nmol L$^{-1}$ in nearshore waters, and <1 nmol L$^{-1}$ in the outer regions of the sampling area well removed from inputs of wind-driven coastal upwelling. Values in the eddy were among the lowest in the region (generally <0.7 nmol L$^{-1}$). A strong trend was found towards increasing pDA concentrations with decreasing dissolved Fe concentrations, with the highest pDA occurring in waters containing <0.5 nmol L$^{-1}$ Fe (Fig. 7A). The highest Fe concentrations were measured in the nearshore zone at KB02 and KB03, and at Sta. LBC01 and LBC02, where eddy waters were entrained in the northwest-advecting Fe-rich Vancouver Island Coastal Current (VICC). Grouping data from several additional ECO-HAB-PNW cruises conducted in this region (June 2003, ECOHAB-PNW 1 cruise [EH1]; September 2004 [EH3]; July 2005 [EH4]; September 2005 [EH5]) provides a similar finding: pDA concentrations generally were highest in eddy waters containing <0.5 nmol L$^{-1}$ Fe (e.g., Sta. LAB03, LA07; Fig. 7C), pDA concentrations were <2 nmol L$^{-1}$ where Fe concentrations were >3 nmol L$^{-1}$ (Fig. 7C). The exception was the coastal Sta. GH02 during the September 2005 cruise (EH5), which had a pDA concentration of ~7 nmol L$^{-1}$, but this water was in a filament originating from the eddy region (Trainer et al. 2009). Where comparisons were possible (see Methods for estimation of cellular DA), there was a generally inverse relationship between cellular DA and dissolved Fe, with the majority of high cellular DA (>2 pg cell$^{-1}$) occurring at <0.5 nmol L$^{-1}$ Fe (Fig. 7B). The highest cellular DA was observed in eddy Sta. LA07 and LAB05, where Fe measured <0.3 nmol L$^{-1}$ (Fig. 7B). Finally, the highest numbers of *Pseudo-nitzschia* (>0.7 $\times$ 10$^6$ cells L$^{-1}$) were associated with Fe concentrations <1 nmol L$^{-1}$, with the exception of Sta. LBC02 in the VICC (Fig. 7D).

**Statistical analysis**—The first two PC described >60% of the variation in the observations (Table 1). The first PC described the inverse relationship between nutrient concen-
trations and temperature, which was consistent with nutrient input being driven by the advection and upwelling of cold, nutrient-rich water (MacFadyen et al. 2008). The second PC captured the positive relationship between Chl $a$ concentration, fluorescence, Pseudo-nitzschia abundance, and DA concentration. The orthogonal relationship between the first and second PC indicated that the biological parameters of phytoplankton biomass, Pseudo-

![Fig. 6. (A) Bacterial and (B) cyanobacterial abundances in the September 2004 grid survey region.](image)

![Fig. 7. Relationship of Fe concentrations with pDA, cellular DA, and Pseudo-nitzschia abundance. Iron vs. (A) pDA and (B) cellular DA during the September 2004 grid survey (EH3); and (C) pDA and (D) Pseudo-nitzschia abundance on the grid surveys using the combined data from June 2003 (EH1), September 2004 (EH3), July 2005 (EH4), and September 2005 (EH5) cruises.](image)
Pseudo-nitzschia abundance, and DA concentrations were not strongly connected to the changes in nutrient concentrations and ratios throughout the survey grid. Furthermore, changes in *Pseudo-nitzschia* abundance and pDA were linked to the general parameters describing phytoplankton biomass and oxygen concentrations in the second PC axis. The separate PC analysis for 1 and 5 m showed no substantial differences in the relationships between the environmental and biological parameters between these samples (Table 1). Virtually all of the significant (>0.3) coefficients were similar between the two analyses, and the diagnostic parameters for the PC (eigenvalues and cumulative sum of variance) were similar.

Spearman’s rank correlation analysis was used to further explore the potential associations of DA (both particular and cellular) with the suite of physical, chemical, and biological variables (Table 2). pDA concentrations showed positive correlations with concentrations of *Pseudo-nitzschia* (1 m), cellular DA, Chl *a*, heterotrophic bacteria, and cyanobacteria, but a negative correlation with water temperature (*p* ≤ 0.01). No significant relationship was observed between pDA and any ambient nutrient concentration measured or any of the nutrient concentration ratios. Cellular DA was positively correlated with pDA and negatively correlated with *Pseudo-nitzschia*, Chl *a*, and Si : P ratios at 5 m only.

**Table 1.** Parameter loading coefficients for principal components analysis of environmental and biological parameters during September 2004 grid survey at 1- and 5-m depths. Significant coefficients (>0.3) are bold.

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<tr>
<td></td>
<td>1 m</td>
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<tr>
<td>Chl <em>a</em></td>
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<td>Proportion of variation</td>
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DA, domoic acid.

**Table 2.** Spearman’s rank correlation coefficients (r) for *Pseudo-nitzschia* (*P-n*), particulate domoic acid (pDA) and pDA concentrations per cell (cell DA) with the physicochemical variables chlorophyll *a* (Chl *a*), temperature (temp), salinity (sal), nitrate (N), phosphate (P), silicic acid (Si), and the nutrient ratios nitrate : phosphate (N : P), nitrate : silicic acid (N : Si), silicic acid : phosphate (Si : P), and bacterial and cyanobacterial (cyano) abundance. Analyses used 1- and 5-m observations, except for bacterial abundance observations, which were only available for 5 m. For each entry of *r*, the subscript df indicates the number of observations used in the correlation. Significance at the 95% and 99% confidence level (i.e., *r* ≤ 0.05 and 0.01) is indicated by * and **, respectively. NA, not analyzed.

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</tr>
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<tr>
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<tr>
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Discussion

**Confirmation of *P. cuspidata* as the DA–producing species**—To our knowledge, this is the first definitive
report of DA production by *P. cuspidata*. *Pseudo-nitzschia cf. cuspidata* was suspected of having produced DA in the San Pedro Channel, California, but it “remains unclear if *P. cf. cuspidata* was the main source of DA” (Schnetzer et al. 2007). Similarly, the identity of the DA-producing cultures isolated from the Gulf of Mexico in Pan et al. (2001) could have been either *P. pseudodelicatissima* or *P. cuspidata*, as the valve shape was uncertain.

The delineation between *P. cuspidata* and *P. pseudodelicatissima* has historically not been clear. The initial description of *P. cuspidata* was from an isolate collected near Las Palmas, Canary Islands, as well as in a field sample obtained off northwest Africa (Hasle 1965). At roughly the same time, a morphologically similar species, *P. pseudodelicatissima*, was described (as *Nitzschia delicatula*) at multiple sites around the world (Hasle 1965). The apparent cosmopolitan distribution of *P. pseudodelicatissima* has attracted recent interest with the discovery that *P. cf. pseudodelicatissima* produces DA in culture (Martin et al. 1990; Lundholm et al. 1997; Adams et al. 2000).

The valve shapes of *P. cuspidata* and *P. pseudodelicatissima* have been described as slightly different, because *P. cuspidata* is lanceolate and *P. pseudodelicatissima* is linear (Lundholm et al. 2003). Phylogenetically, the two species are closely related, making up a clade well separated from other *Pseudo-nitzschia* species (Lundholm et al. 2003).
of PO$_{4}^{3-}$, Si(OH)$_4$, and NO$_3^-$ were rarely reduced to levels considered limiting for the growth of diatoms in the eddy region. These results conflict with reported laboratory culture experiments showing a putative relationship between macronutrient limitation and DA production by _Pseudo-nitzschia_. Consistent with these past studies using cultured _Pseudo-nitzschia_ species where toxin production may increase under phosphate or silicic acid limitation (Bates et al. 1991; Pan et al. 1996a,b), field studies of recent blooms of _P. australis_ in southern California have reported significant negative correlations between both pDA and _Pseudo-nitzschia_ concentrations and the ambient concentrations of Si(OH)$_4$, NO$_3^-$ (Anderson et al. 2006; Schnetzer et al. 2007), or PO$_{4}^{3-}$ (Schnetzer et al. 2007). As these authors suggest, such negative correlations with macronutrients are likely a result of their drawdown during the development of the toxic diatom blooms. Although the availability of nutrients is certainly a determinant for growth of _Pseudo-nitzschia_, a lack of a strong relationship of pDA and cellular DA with macronutrients or their ratios in the Pacific Northwest and elsewhere (Fehling et al. 2006) suggests that neither macronutrient abundance nor macronutrient stress are strong determinants for toxicity of natural assemblages of _Pseudo-nitzschia_. However, here we have assessed the consequence of past nutrient assimilation on DA concentrations at a single point in time. Future fieldwork is needed to elucidate the effects of nutrient uptake rates and availability on _Pseudo-nitzschia_ growth and DA production; however, given that _Pseudo-nitzschia_ cells are rarely the dominant species in natural assemblages, this will be challenging.

_Pseudo-nitzschia_ abundance and pDA concentrations were closely associated with phytoplankton biomass (Chl $a$), as would be expected given the dominance of _Pseudo-nitzschia_ in the community (Table 1). The only other significant PC with substantial _Pseudo-nitzschia_ abundance or DA loadings did not describe any relationships with the included environmental parameters. A significant ($p \leq 0.05$) negative relationship between cellular DA and the elemental ratio of Si(OH)$_4$:PO$_{4}^{3-}$ (Table 2) is suggestive of a potential role of PO$_{4}^{3-}$ stress in pDA production; however, concentrations of PO$_{4}^{3-}$ were rarely reduced to levels considered limiting for the growth of diatoms in this region, and this relationship was not seen consistently at 1- and 5-m depth.

**Association of _Pseudo-nitzschia_ with bacteria**—Laboratory studies have indicated that _Pseudo-nitzschia_ produce less DA in the absence of bacteria (Douglas et al. 1993; Bates et al. 1995) and that the association of _Pseudo-nitzschia_ with bacteria enhances DA production capability. The mechanism underlying this association is unclear, but it has been suggested that incorporation of bacterially derived precursor by _Pseudo-nitzschia_ fuels DA production (Bates et al. 2004). Alternatively, an apparent linkage between DA and Fe limitation in culture (Maldonado et al. 2002; Wells et al. 2005) may mean that siderophore production associated with bacterial growth drives DA production as a competitive response to Fe stress in culture. Data from the September 2004 cruise did not offer strong

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**Fig. 9.** Cell numbers and DA in _P. cuspidata_ cultured isolates (A) NWFSC 191 and (B) NWFSC 221. _P. cuspidata_ abundance (solid triangles) and pDA concentrations (solid squares) are shown over the 17- or 20-d period of growth in culture. The range is shown for DA values analyzed in duplicate. The average standard deviation for _Pseudo-nitzschia_ counts was <15%.

Considering that vegetative division results in decreasing length and therefore also the size of the frustules in diatoms, one could imagine how the linear valve shape of _P. pseudodelicatissima_ might gain the more lanceolate shape of _P. cuspidata_ after repetitive divisions. One may therefore regard the two species as cryptic (morphologically indistinguishable). Further sequencing and mating studies with additional isolates are needed to confirm whether they should be merged into a single species. If the two species are to be merged, we suggest the name _P. cuspidata_ be used, because past reports of _P. pseudodelicatissima_ have often mistakenly included other species of _Pseudo-nitzschia_.

**Lack of correlation of cellular toxin quotas with macronutrient concentrations**—Utilizing the natural laboratory of the Juan de Fuca eddy, the present study has demonstrated that there are no simple predictive relationships between the concentrations of pDA, _Pseudo-nitzschia_ cells, or cellular DA and the ambient concentrations of Si(OH)$_4$, NO$_3^-$, or PO$_{4}^{3-}$; in particular, low concentrations of these nutrients did not co-vary with elevated concentrations of these toxigenic cells or their toxin content (Trainer et al. 2009; this study). Marchetti et al. (2004) also found that there were adequate concentrations of Si(OH)$_4$ and NO$_3^-$ in the eddy region when DA was detected. Concentrations
Iron limitation and DA production—Pseudo-nitzschia are often the sole diatoms co-occurring with autotrophic dinoflagellates and other taxa that thrive on recycled nutrients (Olson et al. 2006). Their cosmopolitan nature and long residence in the euphotic zone suggest that either reduced mortality through grazing or unique nutrient acquisition capabilities (Maldonado et al. 2002; Wells et al. 2005) provide this genus a competitive advantage over other diatoms. Because Pseudo-nitzschia have been shown to benefit from low grazing mortality (Olson et al. 2006, 2008) and macronutrients from the eddy were never limiting during the September 2004 cruise at sites where P. cuspidata were present, it is plausible that unique micronutrient uptake capabilities may have played a role in population ecology of these cells during this event.

The success of Pseudo-nitzschia may in part be explained by the unique trace metal-binding capability afforded to this genus through its production of DA, a putative cuprophore that may assist with Fe acquisition (Wells et al. 2005). Phytoplankton in the eddy region are more Fe-stressed than those in the surrounding region, as determined initially by a laboratory addition experiment using seawater collected at a location on the outer edge of the eddy (Wells et al. 2005). Whether the eddy region becomes Fe-depleted through biological (frequent phytoplankton blooms at this macronutrient-rich site) or chemical means (distance from shore, thereby limiting Fe input from sediments) remains to be determined. Our ECOHAB-PNW cruises showed the highest pDA, cellular DA (September 2004 data only), and Pseudo-nitzschia abundances in regions of Fe limitation (Fig. 7). In general, these Fe-limited stations were in the eddy region (see selected stations labeled in Fig. 7). The exception was at Sta. LBC02, found in the VICC; toxigenic cells at this station likely originated from the eddy. Storms that occurred during the September 2004 grid survey (MacFadyen et al. 2008; Trainer et al. 2009) may have resulted in leakage of cells from the Fe-limited eddy region to the north where they were entrained into the VICC.

Diatoms of the Pseudo-nitzschia genus have been shown to possess an inducible high-affinity Fe uptake capability that makes them particularly well adapted to grow on chelated Fe forms relative to other coastal diatoms (Wells et al. 2005). The dominance of P. cuspidata in the eddy region and its filament during the September 2004 cruise may have been the result of the combination of this Fe uptake capability with high surface area to volume ratios and higher diffusivity in the cellular boundary layers associated with smaller cell size (Wells 2003; Marchetti and Harrison 2007). Another small Pseudo-nitzschia species, P. cf. calliantha, isolated from the Juan de Fuca eddy and subsequently subjected to Fe stress in culture, showed the highest increase in surface to volume ratio of six other Pseudo-nitzschia isolates tested (Marchetti and Harrison 2007). Recent work has demonstrated the ability of cultured Pseudo-nitzschia to sequester large amounts of Fe for later growth under conditions of Fe limitation (Marchetti et al. 2009). This combination of morphological advantage and Fe uptake and sequestration mechanisms suggests that Pseudo-nitzschia may have unique adaptability to low micronutrient concentrations. The variable ratios of pDA to dDA in the Juan de Fuca eddy also suggest a role of DA as an organic metal-complexing ligand under cellular regulation by Pseudo-nitzschia exposed to metal stress in this region. Such active release of DA by cells in response to metal stress, including conditions of Fe deficiency, has been observed in laboratory studies (Maldonado et al. 2002; Wells et al. 2005). However, we acknowledge that although Fe limitation appeared to play a role in the toxic Pseudo-nitzschia bloom that developed in the Juan de Fuca eddy in September 2004, Fe may not be a key factor in the development of similar blooms in all regions (Kudela et al. 2004).

The Juan de Fuca eddy retentiveness in September 2004—The eddy region was highly retentive in September 2004. Drifters deployed during the cruise were retained in this cyclonic feature for ~32 d (MacFadyen et al. 2008). This high degree of retention was because of the moderate seasonal upwelling intensity observed together with frequent northward wind reversals (MacFadyen et al. 2008). The average cumulative upwelling index (CUI) in 2004 was similar to the CUI observed in 1998 (MacFadyen et al. 2008), a year when Pseudo-nitzschia blooms led to coastwide closures of the coastal razor clam fishery occurring in early October (Adams et al. 2000; Trainer et al. 2002).

Fate of the highly toxic September 2004 bloom—In September 2004, cellular DA was highest in the eddy region (Sta. LB09), and the area just south of the eddy (Sta. CF05), ranging from 13 to 35 pg cell−1 (Fig. 4). These values are greater than those measured during a coastal bloom of P. cf. pseudodelicatissima (maximum abundance of 15 × 10⁶ cells L−1) in 1998, when maximum cellular DA was estimated at 0.5 pg cell−1 (Adams et al. 2000). As a result of the 1998 bloom, a coastwide closure of the razor clam fishery was in place for over 1 yr (Adams et al. 2000; Trainer et al. 2002). In contrast, DA in coastal shellfish populations did not exceed the regulatory limit of 20 ppm during the late summer and early fall of 2004 (Trainer and Suddleson 2005). This lack of shellfish toxicity was consistent with the southward advection of cells from the eddy during the upwelling period prior to the start of the cruise (Trainer et al. 2009) and the maintenance of toxigenic Pseudo-nitzschia offshore during and after the cruise (MacFadyen et al. 2008). Although drifters escaped
from the eddy to the south in late September, and remained offshore during a ~10-d period of steady southward winds (MacFadyen et al. 2008), a fall storm of a magnitude similar to one observed in 1998 (Adams et al. 2000; Trainer et al. 2002) did not bring those drifters onshore. Therefore, the highly toxic P. cuspidata bloom observed in the eddy and its filament during the September 2004 cruise fortunately was not transported to the Washington State coast.

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**References**


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