Vol. 281: 1–12, 2004 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published November 1

Environmental conditions and phytoplankton dynamics associated with *Pseudo-nitzschia* abundance and domoic acid in the Juan de Fuca eddy

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ABSTRACT: The Juan de Fuca eddy, located off the coasts of Washington (USA) and British Columbia (Canada), has been identified as a region that frequently contains relatively high levels of domoic acid (DA), a toxin produced by some members of the marine diatom genus, *Pseudo-nitzschia*. This seasonal eddy provides a unique environment to study the influence of nutrients on *Pseudo-nitzschia* abundance and DA accumulation in the field. Vertical sampling in the Juan de Fuca region was conducted in May, July and September of 2001 in an effort to determine environmental conditions and phytoplankton dynamics within the eddy compared to surrounding waters. The eddy was characterized by high primary productivity and high biomass in May and September relative to surrounding waters and was dominated by diatoms in the >5 µm size-fraction. In May, nitrate (NO_3^-) concentrations and the corresponding NO_3^- assimilation rates by phytoplankton within the eddy surface waters were relatively low. In contrast, in September, NO_3^- was high and NO_3^- assimilation rates increased by 7 times relative to those in May. DA was below detection levels at all stations in May and July. In September, *Pseudo-nitzschia* reached highest cell densities (~2×10^4 cells l^{-1}) and particulate DA (~30 ng DA equivalents l^{-1}) was detected in surface waters of the eddy. The presence of DA in healthy growing phytoplankton communities indicates a need to examine other environmental conditions that induce DA production in natural *Pseudo-nitzschia* populations than have previously been reported in nutrient-stressed laboratory studies.

KEY WORDS: Juan de Fuca eddy · *Pseudo-nitzschia* · Domoic acid · Nitrate uptake · Carbon uptake · Chlorophyll · Phytoplankton · Biological processes

INTRODUCTION

Toxin-producing harmful algae are frequently a natural occurrence in coastal waters worldwide. However, over the last several decades, heightened awareness and elevated monitoring have documented an increase in harmful algal bloom frequency and intensity (Hallegraeff 1993, Anderson 1995, Smayda 1997). The domoic acid-producing diatom *Pseudo-nitzschia* is present on the west coast of North America (Horner et al. 1997). Its blooms have been detrimental to marine mammal and bird populations, and have caused significant economic losses due to their impact on the shellfish and planktivorous fish industries (Fritz et al. 1992, Horner et al. 1993, Beltran et al. 1997, Scholin et al. 2000, Wekell et al. 2002).

The ubiquitous pennate diatom genus *Pseudo-nitzschia* is comprised of roughly 20 species (Hasle 1994, Lundholm et al. 2002), a number of which are responsible for the production of the neurotoxin, domoic acid (Subba Rao et al. 1988, Bates et al. 1998). Recent evidence suggests members of the genus *Nitzschia* may also be DA producers (Bates 2000). Since 1987, when the toxin was first identified, the conditions associated with its production in laboratory cultures have been studied extensively. In initial studies,

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cultures of *P. multiseries* were found to produce DA during stationary phase when N was in excess and silicon (Si) or phosphorus (P) was limiting to growth (Bates et al. 1991, 1993, Pan et al. 1996a, 1996b). However, recent findings suggest that DA may also be produced in early exponential phase (Pan et al. 2001) or mid-exponential phase when cells are iron-limited (Maldonado et al. 2002). *Pseudo-nitzschia* cultures exhibit a wide range of physiological tolerances demonstrating their ability to dominate a variety of marine habitats (for review, see Bates et al. 1998).

Verifying laboratory criteria for DA production within a natural setting is complicated by the unpredictability associated with *Pseudo-nitzschia* blooms and DA poisoning outbreaks. To date, there are few field studies that elucidate the environmental conditions associated with high concentrations of *Pseudo-nitzschia* cells and DA production. Monitoring programs along the west coast of Washington State have identified one area where there are frequently elevated levels of DA relative to the surrounding waters (Trainer et al. 2002). This region, located at the mouth of Juan de Fuca Strait, along the USA-Canadian border, is seasonally dominated by a cold-water gyre known as the Juan de Fuca eddy. *In situ*, particulate DA concentrations in the eddy have been observed to be some of the highest recorded (Trainer et al. 2002). Therefore, this eddy provides a unique natural setting to study factors contributing to high *Pseudo-nitzschia* abundance and the production of DA.

The Juan de Fuca eddy is formed through a combination of forcing mechanisms. These include tidal rectification, seasonal upwelling and buoyancy flux (Freeland & Denman 1982, Crawford & Thomson 1991, Hickey et al. 1991). The influence of each of these processes on eddy formation may vary depending on current flow, wind speed and direction, and estuarine outflow from Juan de Fuca Strait. By tracing local wind conditions and current flow, Trainer et al. (2002) suggested that during storm events, surface waters from the Juan de Fuca eddy may be advected towards the Washington coast, transporting the residential toxic phytoplankton populations within them. In 1998, *Pseudo-nitzschia* blooms that originated in the eddy were suggested to have caused the closure of razor clam beds on Kalaloch Beach, Washington (Trainer et al. 2002). The eddy could potentially provide seed populations of toxic *Pseudo-nitzschia* cells to coastal regions through wind-mediated advection of toxic cells onshore with subsequent toxin accumulation in shellfish.

Dense *Pseudo-nitzschia* blooms may not always coincide with high levels of DA. For example, high concentrations ($>10^6$ cells l^{-1}) of *P. pseudodelicatissima*, a known toxin producer (Pan et al. 2001), are frequently observed along the Louisiana coast in the Gulf of Mexico. However, there have been no documented DA poisoning events in this region (Dortch et al. 1997, Parsons et al. 2002). In contrast, equivalent concentrations of *P. pseudodelicatissima* observed on the northwest coast of North America are usually associated with high levels of DA. This discrepancy could be a result of differences in toxin-producing strains or specific physiological conditions conducive to DA production. Thus, the promotion of *Pseudo-nitzschia* blooms and the subsequent toxin production may require a distinct combination of environmental conditions.

The Juan de Fuca eddy is an area where *Pseudo-nitzschia* cells persist in variable concentrations throughout the summer months. Studying this region provided a unique opportunity to relate patterns of environmental conditions associated with high *Pseudo-nitzschia* abundance and DA production. In an effort to determine phytoplankton dynamics within the eddy region, sampling was conducted in May, July and September of 2001. The aims of this study were to determine: (1) physical and chemical properties of source waters supplying the eddy, (2) spatial and seasonal changes in phytoplankton abundance and composition as well as biological rate processes in the Juan de Fuca region, and (3) chemical and biological parameters associated with the production of DA by *Pseudo-nitzschia* in the eddy.

MATERIALS AND METHODS

Sampling sites and collection. Satellite imagery of sea surface temperatures obtained from Ocean Imaging were used to confirm the locations of the stations with respect to the eddy. Data were collected on 3 cruises to the Juan de Fuca eddy region in May, July and September of 2001 (Fig. 1). Sampling stations were chosen to specifically study the phytoplankton dynamics in the Juan de Fuca eddy (JFE) (48.52° N, 125.58° W) and representative source waters to the eddy. Two stations were selected as possible source waters based on the direction of currents. These stations were located in Juan de Fuca Strait (JFS) (48.38° N, 124.33° W), 92 km east of the eddy and an offshore station (OFS) (48.52° N, 126.20° W), 45 km west of the eddy. In the summer, because the upper layers of Juan de Fuca Strait are characterized by an outflow of fresher water predominantly on the north side (Hickey et al. 1991), Stn JFS was positioned to ensure outflow waters from Juan de Fuca Strait were being sampled. Stn OFS was positioned on the continental slope to sample the California Current and Undercurrent systems that supply upwelled water to the eddy during certain periods in the summer.

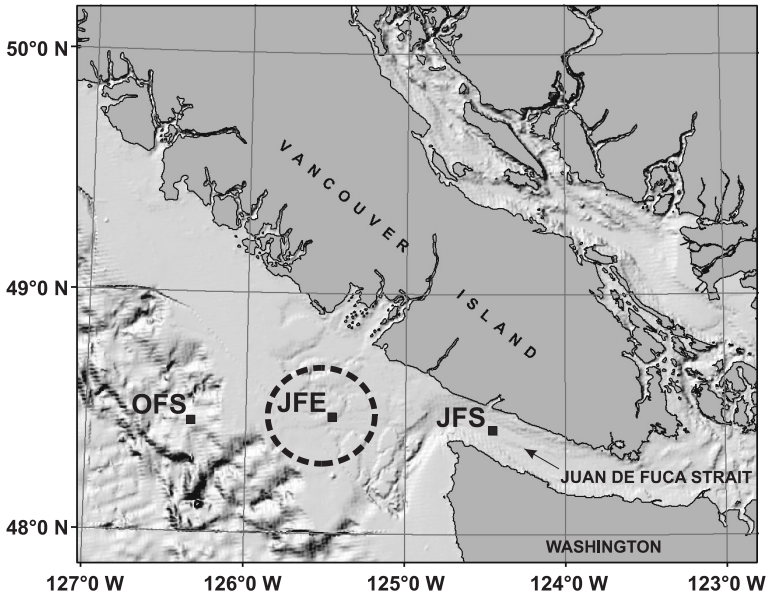

Fig. 1. Location of transect off the coast of Vancouver Island in the Juan de Fuca eddy region. The eddy sampling station was determined through AVHRR imagery of ocean surface temperature and is indicated by JFE (Juan de Fuca eddy). Sampling stations in Juan de Fuca Strait (JFS) and offshore (OFS) are also shown. The dashed circle indicates the general locality of the Juan de Fuca eddy

Seawater samples were collected using 10 l Niskin bottles mounted on a rosette. The photosynthetic active radiation (PAR) of the sampled waters was determined using a Li-Cor 2-pi sensor before water samples were obtained. Samples for phytoplankton biomass and biological rate processes were collected at 6 depths corresponding to 100, 50, 33, 10, 3 and 1% of incident irradiance (I_0).

Water properties. Temperature and salinity profiles were collected throughout the water column using a Seabird 911+ CTD. Surface mixed layer depths were determined subjectively as the depth above which there was no visible consistent change in temperature or salinity, thus the top of the pycnocline. For nutrient analysis, seawater was collected in triplicate, filtered through GF/F (0.7 µm nominal pore size) filters and frozen at −20°C until onshore analysis. The dissolved macronutrients silicic acid ($Si(OH)_4$), phosphate (PO_4^{3-}) and nitrate (NO_3^-) + nitrite (NO_2^-) were determined using a Bran + Luebbe Autoanalyzer 3.

Phytoplankton biomass and *Pseudo-nitzschia* species counts. To estimate size-fractionated chlorophyll *a*, 300 ml of seawater were filtered in triplicate by gravity (20 µm and 5 µm pore size) and gentle vacuum (0.2 µm pore size) onto polycarbonate filters in a filter cascade. Filters were frozen at −20°C until onshore analysis. The chlorophyll *a* content of the frozen samples was determined by extraction in 90% acetone and measured by *in vitro* fluorometry (Parsons et al. 1984). For data analysis, phytoplankton community structure was separated into <5 µm and >5 µm size-fractions. The >5 µm size-fraction was estimated by combining measurements from the 5 and 20 µm filters. Water-column, integrated chlorophyll *a* was calculated down to the 1% I_0 depth through trapezoidal integration.

For phytoplankton taxonomy, whole water samples were collected in duplicate in 100 ml dark glass bottles and preserved with 5% buffered formalin or acidic Lugols fixative. Samples were settled for 24 h and enumerated with an inverted microscope using 25, 50 or 100 ml Utermöhl settling chambers depending on phytoplankton density. Identification of *Pseudo-nitzschia* to the species level, along with other dominant phytoplankton genera, was confirmed by scanning electron microscopy (SEM). For SEM preparation, an aliquot of preserved cells was acid-cleaned according to Hasle & Fryxell (1970) and filtered onto a polycarbonate filter (1 µm pore size). Filters were dried, mounted onto aluminum stubs, coated with Au-Pd and examined using a Hitachi S-4700 field emission SEM.

Biological rate processes. Size-fractionated DIC uptake rates were estimated as outlined in Parsons et al. (1984). Water samples (300 ml) were collected in triplicate in polycarbonate bottles and inoculated with 10 µCi of $H^{14}CO_3^-$. Bottles were wrapped in layers of neutral density screening to achieve light intensities corresponding to the depth at which the samples were collected. Inoculated samples were incubated on-deck in Plexiglas® incubators maintained at near ambient sea surface temperatures using a seawater flow-through system. After 24 h, samples were filtered using the same size-fractions as outlined for chlorophyll *a*. When possible, incubations commenced at dawn, although for some stations time constraints did not permit this arrangement. Water-column, integrated DIC uptake rates were calculated down to the 1% I_0 depth through trapezoidal integration. DIC assimilation rates (rate of DIC uptake normalized to biomass) were estimated from DIC uptake rates and chlorophyll *a* data.

NO_3^- uptake rates were estimated using the short term (6 to 8 h), stable isotope technique as outlined in Dugdale & Goering (1967) with modifications according to Dugdale & Wilkerson (1986). $Na^{15}NO_3$ was added to duplicate 1 l samples at approximately 1 to 10% of the ambient concentrations of NO_3^-. Bottles were wrapped in layers of neutral density screening to achieve light intensities corresponding to the depth at which the samples were collected. Inoculated samples

were incubated on-deck in Plexiglas® incubators kept at near ambient sea surface temperatures using a seawater flow-through system. After 6 to 8 h, samples were filtered onto pre-combusted GF/F filters and frozen at –20°C until on-shore analysis. Frozen filters were dried at 50°C for 24 h and analyzed using a Europa Integra isotope mass spectrometer. Daily NO_3^- uptake rates were estimated by extrapolation assuming a constant hourly rate. Daily rates did not account for possible diel variations in NO_3^- uptake and are therefore considered to be slight overestimates (Cochlan et al. 1991). Water-column, integrated NO_3^- uptake rates were calculated down to the 1% I_0 depth through trapezoidal integration. NO_3^- assimilation rates (rate of NO_3^- uptake normalized to biomass) were estimated from NO_3^- uptake rates and chlorophyll *a* data.

Particulate DA. Duplicate 1 l seawater samples were filtered onto Millipore HA filters (0.45 µm pore size). The filters were frozen at –20°C and kept in the dark until onshore analysis. The filters were later analyzed for particulate DA using the receptor binding assay outlined in VanDolah et al. (1997). The sensitivity level of this particular assay for DA is approximately 5 ng ml^{-1}. A glutamate decarboxylase step was implemented to remove endogenous glutamate that interferes with DA quantification in the samples. Particulate DA concentrations are reported as ng DA equivalents l^{-1}. Cellular DA concentrations are estimated by dividing particulate DA concentrations by total *Pseudo-nitzschia* cell density. This estimate does not take into account possible variations in toxin production by different species of *Pseudo-nitzschia*.

RESULTS

Water properties

During this study, the presence of the Juan de Fuca eddy was verified using remote sensing sea surface temperatures (AVHRR). The eddy was characterized by cooler, cyclonic waters at the mouth of Juan de Fuca Strait and, though visible in May, appeared to strengthen throughout the summer (data not shown).

Stn JFS was characterized by cooler, lower salinity surface waters (Fig. 2). In May and July, surface waters at Stn JFE were also lower in salinity when compared to OFS. In September, Stn JFE surface waters were more saline than Stns JFS and OFS. Surface temperatures increased from the east to west stations throughout the sampling period. When surface water heating is considered, the water masses of Stns JFS and JFE were similar. At JFE, mixed layer depths ranged from 10 to 15 m and did not vary considerably between the 3 sampling periods (data not shown).

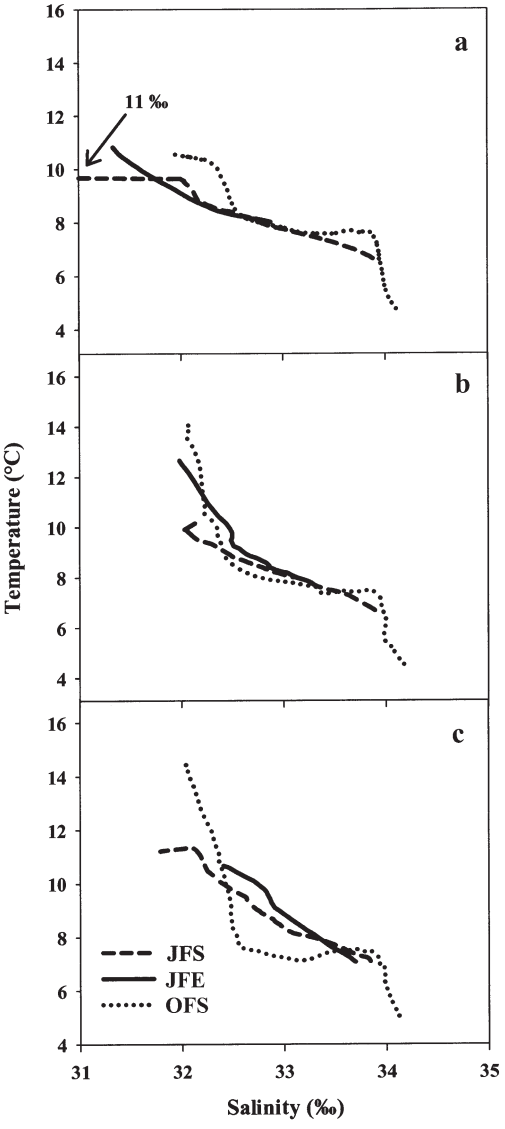

Fig. 2. Temperature versus salinity plots for the Juan de Fuca eddy (JFE), Juan de Fuca Strait (JFS) and offshore (OFS) stations in (a) May, (b) July and (c) September 2001. Lines start at the surface and end at the bottom depth

Nutrient concentrations in the Juan de Fuca region varied markedly both spatially and seasonally. In May, nutrients were high at Stn JFS with NO_3^-, $Si(OH)_4$ and PO_4^{3-} exceeding 20, 40 and 1.5 µM respectively (Fig. 3). At Stns JFE and OFS in May, surface waters were NO_3^--depleted and low in $Si(OH)_4$ (<10 µM) and PO_4^{3-} (<0.5 µM). For Stns JFS and OFS, these trends continued in July and September with JFS having consistently high nutrient concentrations throughout the water column and OFS having surface-depleted nutrients (NO_3^- being below detectable limits). In July, NO_3^- concentrations at JFE were low but not below levels

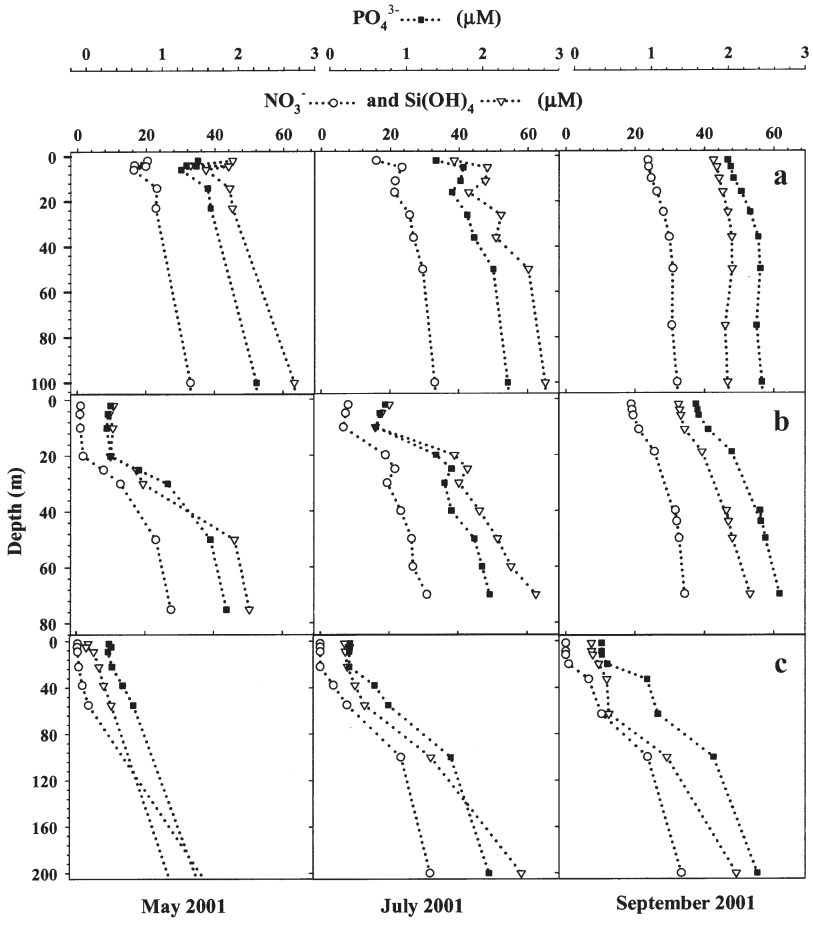

Fig. 3. Nitrate (NO_3^-), silicic acid ($Si(OH)_4$) and phosphate (PO_4^{3-}) concentrations in (a) Juan de Fuca Strait, (b) Juan de Fuca eddy and (c) offshore stations throughout the 2001 sampling period. Macronutrients were collected at specified PAR depths within the euphotic zone as well as other deeper depths. Lower depths at the offshore station are not shown. Error bars are not shown but were less than 5% of the mean (n = 3)

considered limiting to phytoplankton growth for this region ($K_s \approx 1$ to 2 μM, MacIsaac & Dugdale 1969). In September, mixed layer nutrient concentrations at JFE were high, with surface water concentrations of NO_3^-, $Si(OH)_4$ and PO_4^{3-} exceeding 18, 32 and 1.5 μM, respectively. The $Si(OH)_4$:NO_3^- ratio for surface waters at JFS and JFE averaged approximately 2, with the exception of May at Stn JFE, when the surface waters were depleted of NO_3^-, resulting in an elevated $Si(OH)_4$:NO_3^- ratio (Table 1). At Stn OFS, the mixed layer $Si(OH)_4$:NO_3^- ratio was consistently high due to a preferential removal of NO_3^- over $Si(OH)_4$ by dominant, non-diatom phytoplankton (see paragraph below). Deep waters of all 3 stations were high in nutrients throughout the sampling period. The $Si(OH)_4$:NO_3^- ratio for bottom waters at JFS and JFE were typically below 2, whereas for deep waters at Stn OFS, this ratio was usually greater than 2 (data not shown).

Phytoplankton dynamics and *Pseudo-nitzschia* abundance

Depth-integrated chlorophyll *a* within the sampled stations was highest at Stn JFE throughout the sampling period (Fig. 4a). At JFE, integrated chlorophyll

Table 1. $Si(OH)_4$:NO_3^- ratios, chlorophyll *a* distributions, DIC and NO_3^- assimilation rates and most abundant phytoplankton genera in surface waters of the Juan de Fuca region in 2001. NBDL: NO_3^- below detection limits; na: data not available. Phytoplankton genera listed were estimated by light microscopy and appear in order of relative abundance. Al: *Alexandrium*; Ch: *Chaetoceros*; Cr: *Ceratium*; Dc: *Dictyocha*; Di: *Ditylum*; Go: *Gonyaulax*; lc: large centrics; Pn: *Pseudo-nitzschia*; sc: small coccoids; sp: small pennates; Rh: *Rhizosolenia*; Sk: *Skeletonema*; Th: *Thalassiosira*; Tm: *Thalassionema*

Stn	Month	$Si(OH)_4$:NO_3^- ratio	Chl *a* (mg chl *a* m^{-3})	<5 μm (% total chl *a*)	>5 μm (% total chl *a*)	DIC assimilation (mg C mg chl a^{-1} h^{-1})	NO_3^- assimilation (mg N mg chl a^{-1} h^{-1})	Most abundant phytoplankton
JFS	May	2.2	3.18	69	31	2.2	0.55	sc, Tm, Pn
	July	2.4	0.91	42	58	2.3	na	na
	September	1.8	1.86	55	45	na	0.45	sc, Ch, Tm, Th, Di, lc, Cr
JFE	May	10.6	4.50	11	89	3.2	0.09	Tm, Sk, Ch, Pn, Th, Di
	July	2.5	1.29	36	64	3.4	0.05	na
	September	1.7	3.46	36	64	3.8	0.62	Ch, Pn, Di, Al, Th
OFS	May	8.8	0.67	72	28	na	0.04	sc, Rh, Pn
	July	NBDL	0.30	61	39	1.8	0.16	na
	September	NBDL	1.12	46	54	1.3	na	sp, Cr, Go, Dc, Pn, lc

a was 108.6 mg chl *a* m^3 in May, decreased to 35.8 mg chl *a* m^{-2} in July and then increased to 75.0 mg chl m^3 in September. Surface chlorophyll *a* concentrations exceeded 4 mg chl *a* m^{-3} in May and September, but were only 1.3 mg chl *a* m^3 in July (Fig. 4b). In May and September, size-fractionated chlorophyll *a* indicated that the larger phytoplankton (>5 µm size-fraction) made up the majority (64 to 89%) of biomass at Stn JFE, whereas Stns JFS and OFS were dominated by smaller phytoplankton (<5 µm size-fraction) (Table 1). The larger phytoplankton size-fraction was primarily composed of large and/or chain-forming diatoms, whereas the smaller phytoplankton size-fractions were primarily composed of flagellate species (data not shown).

In May, the pennate diatom *Thalassionema* spp., and in September, the centric diatom *Chaetoceros* spp. numerically dominated the diatom assemblage at JFE (Table 1). The highest abundance of *Pseudo-nitzschia* cells was observed at JFE in September (1.89×10^4 cells l^{-1}) (Table 2). *P.* cf. *pseudodelicatissima* appeared to be one of the dominant species (80 to 90% of total *Pseudo-nitzschia* spp.) present at this time and it was distributed throughout the mixed layer. In May, *Pseudo-nitzschia* cells were also present in the eddy at lower concentrations (0.3×10^4 cells l^{-1}); however, *P.* cf. *australis* appeared to be the dominant *Pseudo-nitzschia* species present at this time. At Stns JFS and OFS, *Pseudo-nitzschia* cells were rare or completely absent. Particulate DA was detected in low concentrations (27.2 ng DA equivalents l^{-1}) at Stn JFE in September and coincided with the highest *Pseudo-nitzschia* cell abundance, whereas in May and July, particulate DA levels were below detection (Table 2).

Depth-integrated rates of DIC uptake at Stns JFS and OFS were lower than rates measured at Stn JFE (Fig. 5a). Volumetric DIC uptake rates at JFE were highest in May and September, similar to trends in chlorophyll *a* (Fig. 5b). In May and September, the larger phytoplankton (>5 µm size-fraction) accounted for 70 to 80% of the DIC uptake at JFE (Fig. 5b), whereas at JFS and OFS, the smaller phytoplankton (<5 µm size-fraction) accounted for the highest proportions of DIC uptake (data not shown). DIC assimilation rates were highest and relatively consistent at JFE throughout the sampling period (3.2 to 3.8 mg C mg chl a^{-1} h^{-1}) (Table 1). At JFS and OFS, average DIC assimilation rates were lower (~2.2 and ~1.5 mg C mg chl a^{-1} h^{-1}, respectively) than at JFE.

Depth-integrated NO_3^- uptake rates varied considerably between stations (Fig. 6a). At JFS, integrated NO_3^- uptake rates were high (>400 mg NO_3^- m^{-2} d^{-1}) and at OFS, rates were low (<50 mg NO_3^- m^{-2} d^{-1}). At Stn JFE, integrated NO_3^- uptake rates were 3 to 6 times lower in May and July (~200 and 100 mg NO_3^- m^{-2} d^{-1}, respec-

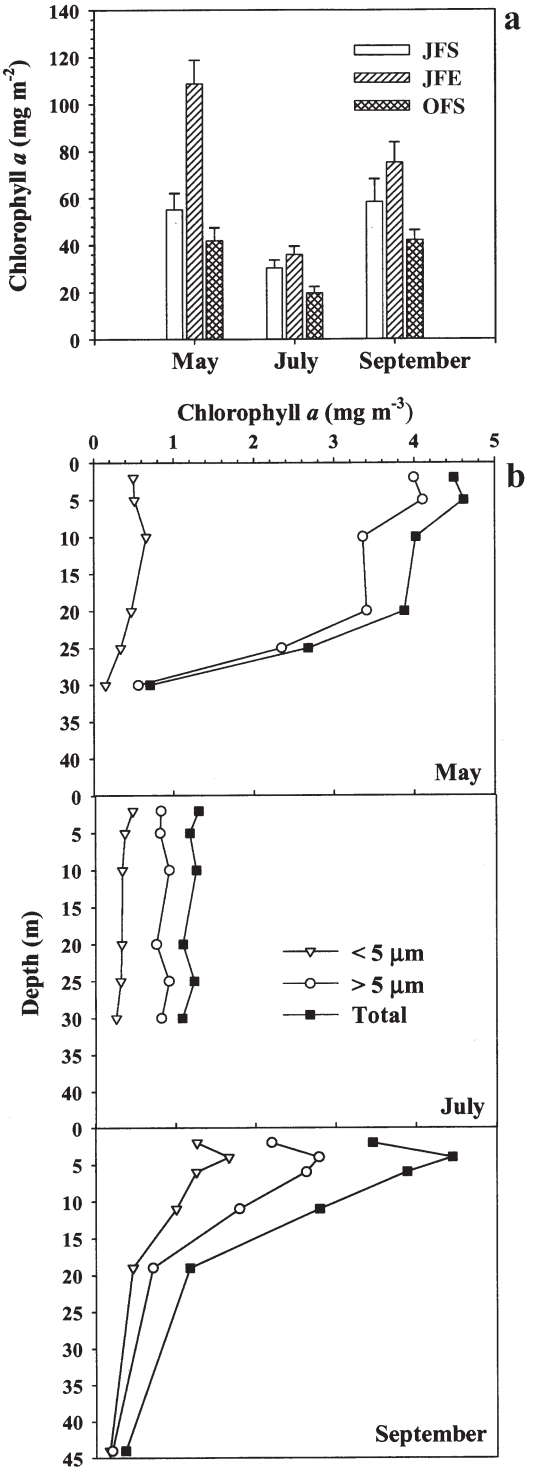

Fig. 4. (a) Depth-integrated chlorophyll *a* for Juan de Fuca Strait (JFS), Juan de Fuca eddy (JFE) and offshore (OFS) stations throughout the 2001 sampling period. Error bars represent one standard deviation associated with the mean concentration (n = 3). (b) Vertical profiles of size-fractionated and total phytoplankton volumetric chlorophyll *a* concentrations at JFE during May, July and September 2001. Error bars are not shown but were less than 10% of the mean (n = 3)

Table 2. Particulate domoic acid (DA) concentrations, *Pseudo-nitzschia* spp. abundance, total chlorophyll *a*, $Si(OH)_4$ and NO_3^- concentrations, biomass specific DIC and NO_3^- uptake rates and DIC:NO_3^- uptake ratios for samples collected in the Juan de Fuca eddy in 2001. BDL, below detection limit, DCM, depth of maximum chlorophyll *a* concentration and DDM, depth of maximum DA concentration. Numbers of *Pseudo-nitzschia* are estimated from duplicate, whole water samples using light microscopy

Month	Depth (m)	Particulate DA (ng DA equiv. l^{-1} ±SD)	*Pseudo-nitzschia* spp. abundance (10^4 cells l^{-1} ±SD)	Chl *a* (mg chl *a* m^{-3})	$Si(OH)^4$, NO_3^- (µM)	Chl *a*-specific DIC uptake (µmol C mg chl a^{-1} h^{-1})	Chl *a*-specific NO_3^- uptake (µmol NO_3^- mg chl a^{-1} h^{-1})	DIC:NO_3^- uptake ratio (mol:mol)
May	Surface (2)	BDL	0.34	4.50	10.6, 1.0	270	6.4	42.2
	DCM (5)	BDL	0.63 ± 0.05	4.62	9.3, 0.8	400		
July	Surface (2)[a]	BDL	0	1.29	19.8, 7.9	280	3.6	77.8
September	Surface (2)	14.0 ± 2.7	1.89 ± 0.80	3.46	32.6, 18.9	320	44	7.3
	DCM (4)	21.1	1.46 ± 0.23	4.45	32.9, 19.1	240	31	7.7
	DDM (11)	27.2 ± 3.9	0.87 ± 0.26	2.80	34.3, 21.1	110	25	4.4

[a]In July, the maximum chlorophyll *a* depth occurred at the surface

tively) than in September (664 mg NO_3^- m^{-2} d^{-1}). Maximum volumetric rates of NO_3^- uptake (51 mg NO_3^- m^{-3} d^{-1}) were also measured in JFE surface waters in September (Fig. 6b). At JFS, NO_3^- assimilation rates were consistently high, whereas at OFS, rates were consistently low (Table 1). At Stn JFE, NO_3^- assimilation rates were low in May and July (<0.1 mg N mg chl a^{-1} h^{-1}) but were high in September (0.6 mg N mg chl a^{-1} h^{-1}). In May and July, DIC:NO_3^- uptake ratios for surface waters at Stn JFE were much higher than the Redfield ratio (C:N = 6.6 by atom) due to low NO_3^- uptake rates. In September, DIC:NO_3^- uptake ratios at Stn JFE exhibited Redfield-like stoichiometry, indicative of nutrient-replete, healthy photosynthetic cells (Redfield 1958, Goldman et al. 1979) (Table 2).

DISCUSSION

Physical and chemical water properties in the Juan de Fuca region

The source waters that supply nutrients to the Juan de Fuca eddy are influenced by a number of physical processes. Seasonal upwelling in this region begins when shelf-break currents that flow north in the winter, reverse and flow to the south in the spring and summer (Freeland & Denman 1982). The appearance of the Juan de Fuca eddy coincides with this transition suggesting that current flow is a strong contributing factor to eddy formation. The salinity in Juan de Fuca Strait is lower than upwelled offshore waters due to mixing with the freshwater discharge from the Fraser River (Thomson et al. 1989, Hickey et al. 1991). The outflow from the Strait reaches its maximum in the summer and minimum in the winter (Thomson et al. 1989). During our study period, in May and July, this lower salinity signature was present within the Juan de Fuca eddy, suggesting that Juan de Fuca Strait was a dominant contributing source to eddy surface waters at this time. In September, the Juan de Fuca eddy was more saline than the surface waters of Juan de Fuca Strait and the offshore station, suggesting eddy waters were predominantly from deeper, upwelled sources. Previous studies have identified the upwelled, oxygen-poor, nutrient-rich waters from the California Undercurrent system as a main source of water to the Juan de Fuca eddy (Freeland & Denman 1982).

Juan de Fuca Strait contains high nutrient concentrations due to a deep inflow of nutrient-rich offshore seawater that enters the lower Juan de Fuca Strait and the subsequent entrainment of these waters due to estuarine circulation and tidal mixing (Thomson 1994, Mackas & Harrison 1997). This abundant nutrient supply should facilitate significant phytoplankton accumulation. Indeed, moderate C assimilation rates measured within Juan de Fuca Strait during this study suggests the potential for rapid growth of phytoplankton. However, in Juan de Fuca Strait, spring blooms have rarely been observed and phytoplankton biomass is consistently low throughout the year (Li et al. 2000). Furthermore, phytoplankton communities in Juan de Fuca Strait are typically dominated by smaller, non-diatom species. Since phytoplankton biomass is low and vertical mixing is high in the Strait, the outflow is normally high in nutrients that may potentially fuel high phytoplankton production in the Juan de Fuca eddy where current speeds are reduced.

The sources of nutrients to the Juan de Fuca eddy are important for our understanding of phytoplankton

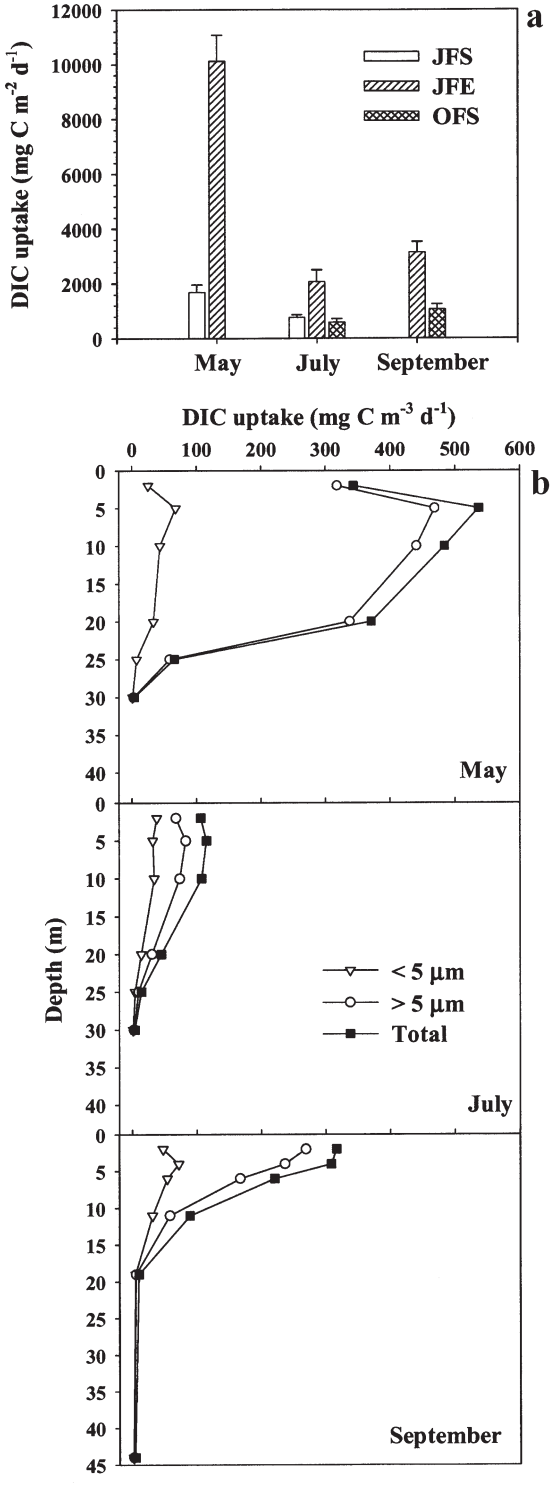

Fig. 5. (a) Depth-integrated DIC uptake rates for Juan de Fuca Strait (JFS), Juan de Fuca eddy (JFE) and offshore (OFS) stations throughout the 2001 sampling period. Error bars represent 1 SD associated with the mean DIC uptake rate (n = 3). Note: DIC uptake rate measurements were not performed at Stn OFS in May and JFS in September. (b) Vertical profiles of size-fractionated and total phytoplankton volumetric DIC uptake rates at JFE during May, July and September 2001. Error bars are not shown but were less than 10% of the mean (n = 3)

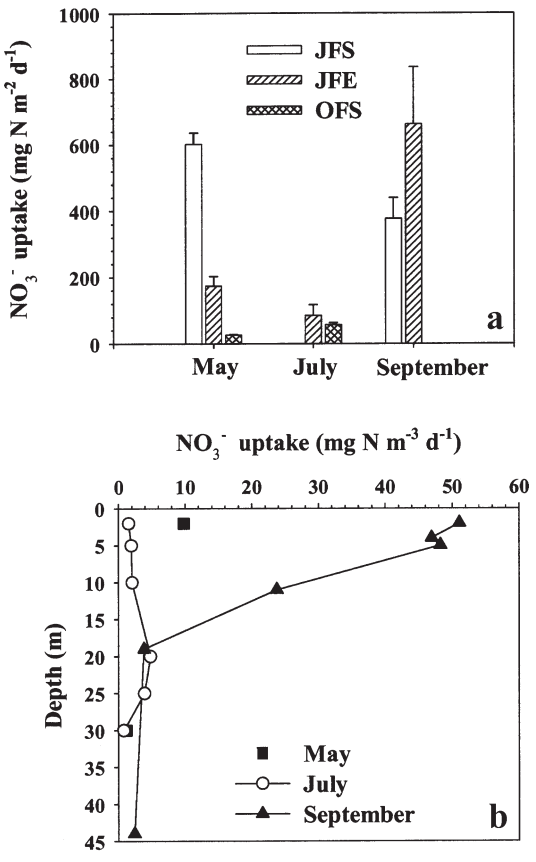

Fig. 6. (a) Integrated NO_3^- uptake rates for Juan de Fuca Strait (JFS), Juan de Fuca eddy (JFE) and offshore (OFS) stations throughout the 2001 sampling period. Error bars represent 1 SD associated with the mean NO_3^- uptake rate (n = 2). For May, only the 100 and 1% I_0 depth NO_3^- uptake rates were measured. NO_3^- uptake rate measurements were not performed at Stn JFS in May and Stn OFS in September. (b) Vertical profiles of volumetric NO_3^- uptake rates at JFE during May, July and September 2001. Error bars are not shown but were less than 10% of the mean (n = 2)

dynamics. These nutrients will fuel growth and sustain phytoplankton standing stocks in the eddy. On the occasion that these nutrients are drawn down to low or limiting concentrations, the ratio of incoming nutrients may further determine whether *Pseudo-nitzschia* will out-compete other diatoms. *Pseudo-nitzschia* cells are lightly silicified compared to other coastal diatoms (<2 pmol Si cell^{-1}, A. Marchetti & P. J. Harrison unpubl. data) and may have an advantage at low Si concentrations. In laboratory competition experiments, the lowest Si:N nutrient supply ratio favoured the growth of *P. multiseries* cells over other diatoms (Sommer 1994). In Louisiana/Texas shelf waters, where *Pseudo-nitzschia* cell densities have reached >10^8 cells l^{-1}, the increase in N and P inputs since the 1950s has resulted in a 4-fold decrease in the Si:N ratio (Turner & Rabalais

1991). This change in nutrient ratio is speculated to be the cause of the observed increase in *Pseudo-nitzschia* abundance in the northern Gulf of Mexico (Dortch et al. 1997). During our study, maximum *Pseudo-nitzschia* cell densities were observed when both $Si(OH)_4$ and NO_3^- concentrations were high. However, when compared to previous assessments of *Pseudo-nitzschia* abundances in the Juan de Fuca eddy, these cell densities are 2 orders of magnitude lower (Trainer et al. 2002). Therefore, the nutrient ratios and other environmental conditions present during our study may not have been conducive to maximum growth of *Pseudo-nitzschia* cells.

In the Juan de Fuca eddy, we observed conditions of adequate $Si(OH)_4$ and NO_3^- concentrations in the eddy when DA was detected. However, provided that these nutrients are drawn down to low or limiting concentrations, the $Si(OH)_4$:NO_3 ratio of source waters that contribute to the Juan de Fuca eddy will influence which nutrient may limit phytoplankton growth. The average water column $Si(OH)_4$:NO_3^- ratio in Juan de Fuca Strait was 2.3 in May and July, decreasing to 1.8 in September. Offshore, at mid-depth waters between 100 and 300 m, the ratio decreased below 2 and then increased to above 2 at depths below 300 m. This mid-depth minimum trend of a low $Si(OH)_4$:NO_3^- ratio is likely a result of greater regeneration of N than Si in shallower waters (Dugdale et al. 1995). If source waters to the Juan de Fuca eddy originated from Juan de Fuca Strait or from offshore mid-depth waters, they may contain a low Si:N (due to low Si and high N) and a high N:P ratio. In the presence of DA-producing *Pseudo-nitzschia* cells, these waters may contribute to conditions favorable for toxin production by reducing the likelihood of N limitation.

The highest DA concentrations were detected in the Juan de Fuca eddy within the upper euphotic zone. Other studies have also observed the highest abundance of *Pseudo-nitzschia* cells and DA in surface waters off the Washington coast (Adams et al. 2000, Trainer et al. 2000, 2002). In laboratory studies, light was verified as a requirement for the production of DA (Bates et al. 1991). In the Juan de Fuca eddy, the length of time which *Pseudo-nitzschia* cells are exposed to a well-lit region is a function of the eddy surface water residence time. From drifter studies, the average residence time in the upper 40 m of the Juan de Fuca eddy core was observed to be 4 d but this varied from as short as 2 d to as long as 17 d (Freeland 1988). This residence time may determine the extent of *Pseudo-nitzschia* bloom formation and subsequent DA production and accumulation. A better understanding of factors contributing to the increased residence time of waters in the Juan de Fuca eddy is necessary.

Phytoplankton dynamics in the Juan de Fuca eddy

Phytoplankton biomass was consistently high in the Juan de Fuca eddy relative to the surrounding waters. The phytoplankton assemblage was dominated by diatoms, whereas small flagellates and dinoflagellates were dominant in Juan de Fuca Strait and the offshore region. We observed the eddy to be an area of high primary productivity and accumulated phytoplankton biomass, possibly due to pulses of nutrient-rich waters decreasing in velocity from Juan de Fuca Strait, or reaching the euphotic zone from upwelling. In May and July, NO_3^- concentrations were low and NO_3^- assimilation rates in the Juan de Fuca eddy were reduced relative to DIC assimilation rates resulting in non-balanced growth. In September, abundant NO_3^- supply coincided with high rates of NO_3^- assimilation that resulted in DIC:NO_3^- uptake ratios closer to expected Redfield stoichiometric proportions. DIC:NO_3^- consumption ratios for healthy phytoplankton under nutrient replete conditions may range from 6 to 13, whereas higher ratios may be indicative of N stress or N limitation (Goldman et al. 1979, Geider & La Roche 2002). *In situ*, the use of regenerated forms of N, such as ammonium and urea, should also be considered and may have contributed to the highly elevated DIC:NO_3^- uptake ratios observed in the eddy during May and July. Other studies, however, have observed non-balanced uptake of DIC relative to NO_3^- in N-limited waters in the coastal upwelling region west of Vancouver Island (Ianson et al. 2003). This may be a result of the continued processing of DIC by phytoplankton in response to light, even in the absence of N.

Conditions for domoic acid production

The dominant *Pseudo-nitzschia* species in the Juan de Fuca eddy appeared to shift between May and September. Although both species were observed during the 2 sampling periods, in May, larger and broader *P.* cf. *australis* cells were most abundant, whereas in September the smaller and narrower *P.* cf. *pseudodelicatissima* cells were dominant. In laboratory studies, the conditions required for both of these species to produce DA were found to be similar (Garrison et al. 1992, Pan et al. 2001). Therefore, we suspect the environmental conditions and physiological status associated with DA production by these 2 species *in situ* may also be analogous.

Previous attempts to identify correlations between environmental conditions associated with natural *Pseudo-nitzschia* abundance and DA production have resulted in minimal success. This is largely due to the inherent complexity in factors affecting phytoplankton

growth and composition. In 1997 and 1998, several research cruises focused on measurements of DA and *Pseudo-nitzschia* abundance in the Juan de Fuca eddy region and the coastal waters of Washington State (Trainer et al. 2002). Traditional hydrographic properties (nutrients, chlorophyll *a*, salinity and temperature) were also collected at each sampling station. During these cruises, DA concentrations ranged from 0 to 2000 ng l^{-1}, whereas *Pseudo-nitzschia* abundance varied from complete absence to 10^6 cells l^{-1}. To assess the correlation of these environmental variables with DA and *Pseudo-nitzschia* cell abundance, we performed scatterplot regression analyses on this data set (data not shown). No clear correlations between DA concentrations, *Pseudo-nitzschia* abundance and nutrient concentrations were observed. A similar attempt to predict the presence of *Pseudo-nitzschia* cells from discriminant analysis of existing environmental conditions in the coastal waters of British Columbia produced potential, but inconclusive results (Forbes & Denman 1991). Thus, the traditional parameters measured during most field studies are unable to discern factors contributing to high *Pseudo-nitzschia* abundance and subsequent toxin production. This points to the importance of measuring biological rate processes in assessing possible linkages between *Pseudo-nitzschia* abundance and DA production to the physiological status of the phytoplankton community.

In the present study, DA was detectable only at the highest concentrations of *Pseudo-nitzschia* cells. The concentrations of measured DA at our eddy sampling station were low relative to the study performed in 1997 and 1998 (Trainer et al. 2002). Likewise, *Pseudo-nitzschia* cell abundances were not as high as previously observed in the eddy. However, the concentrations of DA produced are not always directly related to *Pseudo-nitzschia* abundance. Cellular production rates of DA by *Pseudo-nitzschia* spp. may differ between species and between physiological conditions (Bates et al. 1998). In our study, *Pseudo-nitzschia* cellular DA content (1 to 3 pg DA equivalents $cell^{-1}$) was in agreement with those estimated in the Juan de Fuca eddy during 1997 and 1998 (0.1 to 4.6 pg DA equivalents $cell^{-1}$) (Trainer et al. 2002). Therefore, low total DA concentrations in the eddy during our study appear to be due to low *Pseudo-nitzschia* density rather than a lower production of cellular DA.

Our study verifies that the production of particulate DA coincides with sufficient rates of NO_3^- assimilation, resulting in DIC:NO_3^- uptake ratios indicative of exponentially growing phytoplankton. When NO_3^- assimilation rates were low, and possibly limiting phytoplankton growth, *Pseudo-nitzschia* abundance was low and levels of particulate DA were below our detection limits. It must be noted, however, that we cannot distinguish whether the inability to detect DA during the May and July sampling periods was due to low cellular DA production or low *Pseudo-nitzschia* abundance. Additionally, it is not known whether the *Pseudo-nitzschia* spp./clones present at these times have the potential to produce DA, even under the appropriate environmental conditions. Nonetheless, these observations are consistent with culture experiments that showed a N source is required for the production of DA (Bates et al. 1991). In contrast, the finding of healthy growing *Pseudo-nitzschia* cells producing DA does not corroborate most laboratory findings. In these studies, numerous *Pseudo-nitzschia* spp. produced the toxin primarily during slow growth or stationary phase as a result of a physiological stress (Bates et al. 1998). Thus, although the production of DA by cells that are sufficient in N is consistent in all laboratory cultures and our findings in natural populations, further examination into the cellular status determining when *Pseudo-nitzschia* spp. begin to produce DA *in situ* is required.

How did environmental conditions in the Juan de Fuca eddy differ between this study in 2001 and cruises in 1997 and 1998 (Trainer et al. 2002), when bloom concentrations (>10^6 cells l^{-1}) of *Pseudo-nitzschia* were repeatedly observed? The 1997–1998 El Niño event resulted in significant changes in hydrographic conditions along the west coasts of Canada and the Northern USA, such as increased water temperatures and stratification. In particular, along the coast of British Columbia, increased stratification resulted in weak upwelling in the summer of 1998. These shallower upwelled waters had a $Si(OH)_4$:NO_3^- ratio of 1.4 or less (Whitney & Welch 2002) that may have decreased the likelihood of NO_3^- depletion in euphotic zone waters. In fact, at several locations in southern and northern British Columbia coastal waters, residual $Si(OH)_4$ was <1 µM and was likely limiting to the growth of some diatoms (Whitney & Welch 2002). Thus, in 1997 and 1998 if nutrients in low $Si(OH)_4$:NO_3^- input waters to the Juan de Fuca eddy were depleted to low concentrations, *Pseudo-nitzschia* growth may have been favoured over other diatoms. Furthermore, high cell densities combined with long residence times in these N-rich surface waters may have provided conducive conditions for toxin production by *Pseudo-nitzschia*. This hypothesized association between *Pseudo-nitzschia* occurrence and DA production and El Niño events warrants further investigation.

CONCLUSIONS

To elucidate the possible mechanisms associated with the high abundance of *Pseudo-nitzschia* cells

and DA production in the natural environment, we must draw upon findings from both the laboratory and field settings. We suggest 3 conditions that, when met concurrently, could result in elevated DA levels in the Juan de Fuca eddy. First, the supply of nutrient concentrations with a low Si:N ratio may allow *Pseudo-nitzschia* spp. to out-compete other diatoms if Si is utilized to low or limiting concentrations. Waters with a low Si:N ratio are most commonly found in areas with high N loading, typical of estuarine waters and other coastal regions influenced by freshwater run-off. A low Si:N ratio in source waters may also be typical of natural episodic events such as El Niño, which reduces upwelling in this region. Second, the N supply, with respect to Si and P, must be high to ensure that toxigenic *Pseudo-nitzschia* cells, once abundant, do not experience N-limitation and thus, are able to produce DA. The third condition conducive to DA accumulation is a long residence time of toxin-producing *Pseudo-nitzschia* cells in well-lit surface waters, either in the eddy or during the horizontal transport to the coast.

The Juan de Fuca eddy provides a sharp spatial gradient in nutrient supply between this feature and the surrounding waters. Our findings suggest that sufficient NO_3^- assimilation rates, resulting in balanced DIC:NO_3^- uptake ratios in the phytoplankton community, coincide with DA production by *Pseudo-nitzschia* in the eddy. To verify this as an absolute requirement for toxin production, continued spatial and temporal sampling of this region is warranted, particularly at times when *Pseudo-nitzschia* is in high abundance and DA production is at a maximum. Nutritional status of *Pseudo-nitzschia* cells appears to be an important factor influencing DA production. In attempts to forecast harmful algal blooms in the future, we must go beyond traditional environmental parameters typically measured in monitoring programs and examine the physiology of the phytoplankton community. The determination of biological rate processes will assist in our understanding of the cellular status leading to DA production. Such measurements may better indicate the potential for toxic events.

Acknowledgments. The authors would like to thank R. Thomson and F. Whitney of the Institute of Ocean Sciences (IOS), Sidney, British Columbia for allowing us to participate on their research cruises. We thank N. Adams, K. Baugh and B. Bill of Northwest Fisheries Science Center, Seattle, WA as well as J. Barwell-Clarke and W. Richardson of IOS for their help with sample collection and analysis. We are grateful to D. Harris of the University of California, Davis for N isotope analysis and M. Maldonado for insights and comments during manuscript revision. A special thanks to the officers and crew of the CCGS J.P. Tully for their efforts at sea. This research was partially funded by NSERC through a grant to P.J.H.

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Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: October 31, 2003; Accepted: July 13, 2004
Proofs received from author(s): October 18, 2004