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Blooms of *Pseudo-nitzschia* and domoic acid in the San Pedro Channel and Los Angeles harbor areas of the Southern California Bight, 2003–2004

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Abstract

Abundances of *Pseudo-nitzschia* spp. and concentrations of particulate domoic acid (DA) were determined in the Southern California Bight (SCB) along the coasts of Los Angeles and Orange Counties during spring and summer of 2003 and 2004. At least 1500 km^2 were affected by a toxic event in May/June of 2003 when some of the highest particulate DA concentrations reported for US coastal waters were measured inside the Los Angeles harbor (12.7 μg DA L^{-1}). Particulate DA levels were an order of magnitude lower in spring of 2004 (February and March), but DA concentrations per cell at several sampling stations during 2004 exceeded previously reported maxima for natural populations of *Pseudo-nitzschia* (mean = 24 pg DA $cell^{-1}$, range = 0–117 pg DA $cell^{-1}$). *Pseudo-nitzschia australis* dominated the *Pseudo-nitzschia* assemblage in spring 2004. Overall, DA-poisoning was implicated in >1400 mammal stranding incidents within the SCB during 2003 and 2004. Ancillary physical and chemical data obtained during our regional surveys in 2004 revealed that *Pseudo-nitzschia* abundances, particulate DA and cellular DA concentrations were inversely correlated with concentrations of silicic acid, nitrogen and phosphate, and to specific nutrient ratios. Particulate DA was detected in sediment traps deployed at 550 and 800 m depth during spring of 2004 (0.29–7.6 μg DA (g sediment dry weight)$^{-1}$). The highest DA concentration in the traps was measured within 1 week of dramatic decreases in the abundances of *Pseudo-nitzschia* in surface waters. To our knowledge these are the deepest sediment trap collections from which DA has been detected. Sinking of the spring *Pseudo-nitzschia* bloom may constitute a potentially important link between DA production in surface waters and benthic communities in the coastal ocean near Los Angeles. Our study indicates that toxic blooms of *Pseudo-nitzschia* are a recurring phenomenon along one of the most densely populated coastal stretches of the SCB and that the severity and magnitude of these events can be comparable to or greater than these events in other geographical regions affected by domoic acid.

Keywords: Domoic acid; Harmful algal blooms; Los Angeles; *Pseudo-nitzschia*; Southern California

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1. Introduction

Phytoplankton blooms consisting of toxic species of the diatom genus *Pseudo-nitzschia* are a common occurrence along the western US coast (Buck et al., 1992; Villac et al., 1993; Walz et al., 1994; Fryxell et al., 1997; Horner et al., 1997; Trainer et al., 2000; Kudela et al., 2004). Members of this genus are known producers of the neurological toxin domoic acid (DA) which, when accumulated through trophic activities, has lead to sickness or mortality in sea mammals, seabirds and humans (Amnesic Shellfish Poisoning, ASP) (Bates et al., 1989; Scholin et al., 2000; Gulland et al., 2002). These harmful algae have become a focal point of numerous ecological studies and monitoring efforts in recent years. This work has provided information on the spatial and temporal dynamics of blooms, concentrations of DA in plankton and higher organisms, and the identity of *Pseudo-nitzschia* species that are the likely producers of toxin within specific geographical regions (Hasle, 2002; Trainer et al., 2002; Hickey and Trainer, 2003; Costa and Garrido, 2004).

Considerable research has been conducted in an effort to understand the environmental factors that promote toxic blooms of *Pseudo-nitzschia*. Through these studies, coastal upwelling and river runoff have been implicated as factors that may create physical and chemical conditions (e.g., high nutrient concentrations) that are conducive to promoting phytoplankton blooms (Bates et al., 1999; Trainer et al., 2000, 2002; Kudela et al., 2005). However, linking these processes to blooms of *Pseudo-nitzschia* species and to toxin production has been problematic. Not all *Pseudo-nitzschia* species are capable of producing DA, and toxic species do not produce DA constitutively. Laboratory studies have demonstrated that toxin production in some species of *Pseudo-nitzschia* may increase under silicate or phosphate limitation (Bates et al., 1991; Pan et al., 1996a, 1996b; Fehling et al., 2004). In addition, DA can chelate iron and copper, and thus the molecule may affect trace metal acquisition or metal detoxification by phytoplankton (Rue and Bruland, 2001; Maldonado et al., 2002; Wells et al., 2005). Thus, the scenario(s) under which *Pseudo-nitzschia* blooms and DA is produced in nature may be varied and complicated, thwarting the development of a broadly applicable theory explaining these toxic events.

Most studies of *Pseudo-nitzschia* spp. along the west coast of the US have been conducted in the region from Washington state through central California (Buck et al., 1992; Villac et al., 1993; Walz et al., 1994; Scholin et al., 2000; Horner, 2003; Trainer and Suddleson, 2005). Limited information is available on blooms of *Pseudo-nitzschia* along one of the most populated coastal stretches of the Southern California Bight (SCB), the greater Los Angeles area (California Department of Health Services: http://www.dhs.ca.gov/ps/ddwem/environmental/Shellfish). The goal of this study was to establish the extent to which this latter geographical region is impacted by blooms of *Pseudo-nitzschia* and to characterize the timing and magnitude of these events. The data presented here document that a large area of the coastal waters within and around the Los Angeles harbor and adjacent San Pedro Channel experience toxic blooms. The concentrations of particulate and cellular DA observed within this region during 2003–2004 were among the highest ever recorded for Californian coastal waters. These events resulted in significant impacts on marine mammal populations and may have important, albeit presently uncharacterized, effects on benthic communities in the area.

2. Materials and methods

2.1. Study area and sample collection

This study combined measurements of the spatial extent (regional surveys) and seasonality of *Pseudo-nitzschia* and toxin occurrence, as well as sediment trap analyses to examine the fate of toxic blooms and potential linkages to benthic food webs. Samples were collected during four regional surveys in early 2003 and 2004 to determine the spatial distribution of *Pseudo-nitzschia* and DA in coastal waters near Los Angeles (Fig. 1). Samples were also obtained throughout 2004 in close proximity to the Los Angeles harbor to monitor seasonal changes in the abundances of *Pseudo-nitzschia* (time-series location shown in Fig. 1). Finally, sediment trap material collected during the spring of 2004 was tested for DA to investigate the potential export of *Pseudo-nitzschia* cells and toxin from surface waters (sediment trap location starred in Fig. 1).

A regional survey consisting of 17 stations south of Palos Verdes Peninsula and the Los Angeles harbor was conducted on 8 May 2003 (Fig. 1). Locations inside the breakwater of the harbor were occupied as well as stations out to ~15 km offshore. The sampling grid was expanded for a subsequent survey (2–4 June) to include Santa Monica Bay northeast of Palos Verdes and Newport Beach to the southeast (53 stations; Fig. 1). Surveys were also conducted on 27/28 February 2004 (completion of the sampling grid required 2 days due to weather) and 1 March 2004, and consisted of 27 stations

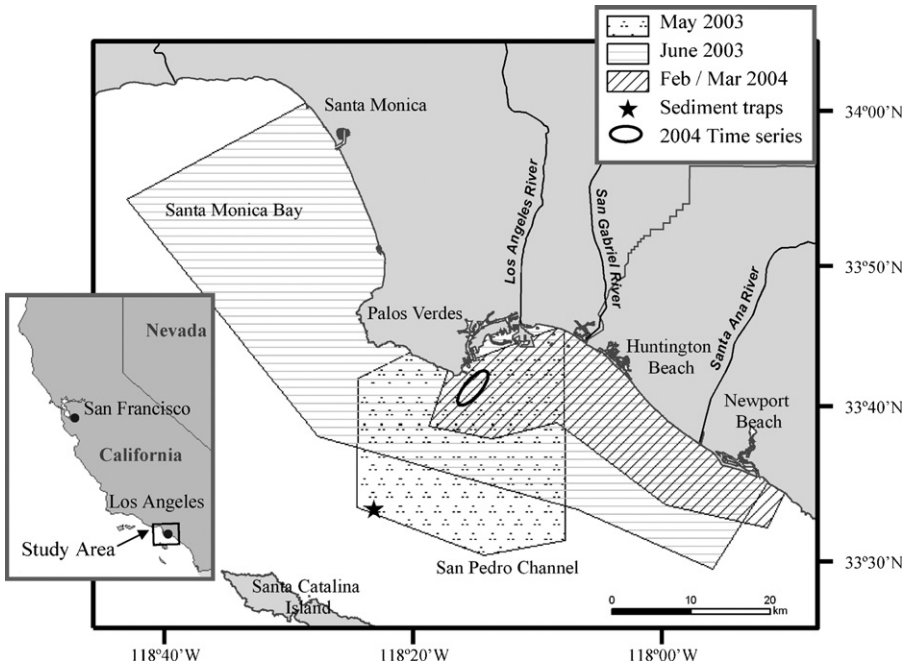

Fig. 1. Map of the study area showing the Los Angeles harbor and adjacent coastal waters of the San Pedro Channel and Santa Monica Bay. Surveys were conducted on 8 May and 2–4 June of 2003 and 27/28 February and 1 March of 2004. Surface waters were sampled approximately weekly during 2004 to determine *Pseudo-nitzschia* abundances south of the LA harbor (2004 time-series indicated by ellipse). Three sediment trap samples (6-day deployments) were collected in the San Pedro Channel during spring 2004.

from the southern tip of Palos Verdes to Newport Beach (Fig. 1). Seasonal changes in the abundance of *Pseudo-nitzschia* were monitored (weekly-to-monthly) at a site approximately 3 km south of the LA harbor throughout 2004 (Fig. 1). All seawater samples analyzed were collected from the surface (0–1 m). Samples were returned to the laboratory in 1 L polycarbonate bottles, chilled on ice and protected from sunlight during transport.

Sediment trap material was collected in spring of 2004 during deployments of a single moored sediment trap array in the San Pedro Channel (∼15 km offshore; 33°33′N and 118°24′W, Fig. 1). The deployment period encompassed a significant bloom of *Pseudo-nitzschia* in the Channel from the end of February until mid-April. DA analyses were performed on a total of three samples; one sample from a trap located at 800 m and two samples from a trap located at 550 m depth. Each sediment trap (McLane, Mark 78-H21 Parflux) was equipped with multiple collecting cups that were rotated into position to collect sinking particles for a period of 6 days and then rotated into a closed position. Sedimenting particles were preserved in formaldehyde solution (2% final concentration) in the sample cups. The trap arrays remained at depth for 4–11 weeks before they were recovered and samples processed. Material from each sediment trap cup was sieved through a 1 mm screen prior to DA analyses.

2.2. Particulate domoic acid concentrations and cell counts of Pseudo-nitzschia

Concentrations of particulate DA (μg DA L^{-1}) were measured by filtering 220–1000 mL of seawater onto GF/F Whatman filters. Filtration took place onboard (2003) or as soon as the bottles were returned to the laboratory (2004). The filters were stored frozen at −20 °C until analyzed using an Enzyme Linked ImmunoSorbent Assay (ELISA kits; Biosense™ Laboratories, Bergen, Norway). The limit of detection for the ELISA assay was either 0.001 μg DA L^{-1} (May 2003) or 0.01 μg DA L^{-1} (all other samples) and depended on the volume of sample filtered. Approximately 2 mL of sediment trap material were analyzed to determine particulate DA using the ELISA assay. Values were converted to μg DA per g dry weight of material collected in each cup (μg DA (g sed dry wt)$^{-1}$). The dry weight of sediment material was determined after rinsing, drying and weighing aliquots of sediment trap contents ($n = 4$).

Total abundances of *Pseudo-nitzschia* spp. in seawater samples were determined using inverted light

microscopy and standard settling techniques for samples (25–50 mL) preserved with acid Lugol's solution (10% final concentration) (Utermöhl, 1958). Individual *Pseudo-nitzschia* species were identified using scanning and transmission electron microscopy (SEM/TEM; see below).

2.3. Environmental parameters

Physical (salinity and temperature), chemical (PO_4^{3-}, NO_3^-, NO_2^{2-}, $Si(OH)_4$) and biological (chlorophyll *a*) factors were determined during the spring surveys in 2004. A total of 61 stations were sampled including the locations for which abundances of *Pseudo-nizschia* and measurements of DA were conducted. The datasets also provided some detailed information on environmental conditions near the river mouths of the Los Angeles, San Gabriel and Santa Ana rivers.

Temperature and salinity data were obtained from vertical profiles using a CTD sensor package. Seawater samples for nutrient analyses (PO_4^{3-}, $NO_3^- + NO_2^{2-}$, $Si(OH)_4$) were prefiltered through GF/F syringe filters onboard and the filtrates frozen at −20 °C. Nutrient analyses were conducted on thawed samples using an Alpkem RFA AutoAnalyzer (Gordon et al., 1993). Seawater samples for the determination of chlorophyll *a* (100 mL) were collected onto GF/F filters onboard. The filters were stored frozen at −20 °C until measured fluorometrically (Turner Design 10-AU Fluorometer) following standard protocols (Parsons et al., 1984).

2.4. Culture establishment and Pseudo-nitzschia species identification

Individual cells of *Pseudo-nitzschia* or single chains were micropipetted from natural samples using a dissecting microscope. Each cell or chain was transferred through 2–3 washes of f/2 medium (made from 0.2 μm filtered seawater) (Guillard, 1975). New micropipettes were used after every transfer to avoid carry over of more than one specimen or other phytoplankton. Successfully established cultures were grown in f/2 medium at 15 °C and approximately 165 μEinstein m^{-2} s^{-1} (10:14 h *L:D*).

Natural samples and aliquots of cultures of *Pseudo-nitzschia* spp. established by micropipetting were preserved with acid Lugol's solution (10% final concentration) and prepared for SEM (model ISI WB-6) and TEM (model JEOL JEM1200EX) (Miller and Scholin, 1998).

2.5. Statistical analyses

Pseudo-nitzschia abundances, particulate DA and cellular DA concentrations were examined for their relationships with physical parameters (salinity and temperature), biological (chlorophyll *a*) or chemical (PO_4^{3-}, $NO_3^- + NO_2^{2-}$, $Si(OH)_4$) constituents and nutrient ratios (Pearson product–moment correlations, Table 1). All data analyses were conducted using the Software package Statistica (StatSoft, 2002).

Table 1
Results from linear regression analyses

	Particulate DA	Salinity	Temperature	Chl	PO_4^{3-}	*N*	$Si(OH)_4$	$Si(OH)_4$:PO_4^{3-}	N:PO_4^{3-}	N:$Si(OH)_4$	*n*
27/28 February 2004											
P. abundances (cells L^{-1})	**0.61**	**0.83**	−0.07	−0.31	−0.10	**−0.53**	**−0.55**	**−0.47**	**−0.50**	−0.23	27
Particulate DA (μg L^{-1})		**0.68**	−0.21	0.14	0.10	**−0.51**	**−0.59**	**−0.71**	**−0.66**	−0.10	27
Cellular DA (pg $cell^{-1}$)		0.37	−0.21	0.35	0.18	−0.32	**−0.41**	**−0.61**	**−0.52**	0.01	27
1 March 2004											
P. abundances (cells L^{-1})	**0.59**	**0.46**	0.37	0.03	**−0.58**	**−0.50**	**−0.52**	**−0.46**	**−0.43**	0.20	22
Particulate DA (μg L^{-1})		**0.63**	**0.68**	**−0.42**	**−0.68**	**−0.63**	**−0.70**	**−0.63**	**−0.58**	0.20	24
Cellular DA (pg $cell^{-1}$)		**0.55**	**0.69**	**−0.53**	**−0.67**	**−0.64**	**−0.62**	**−0.55**	**−0.58**	0.12	22

Individual correlations between *Pseudo-nitzschia* abundances, particulate DA concentrations and cellular DA levels with physical, biological and chemical parameters were examined for surveys conducted on 27/28 February and 1 March 2004. Values shown in bold are significant at $p = 0.05$. *n* = number of observations; $N = NO_3^- + NO_2^{2-}$.

3. Results

3.1. Regional surveys during 2003

The highest particulate DA concentrations in this study were observed in the LA harbor region during the survey in May 2003 (Fig. 2). A maximum of 12.7 μg DA L^{-1} was observed in the northeast region of the harbor approximately 1 km from the Los Angeles River mouth and within the outer breakwater of the harbor. Concentrations of particulate DA were high throughout the harbor at that time ranging from 5.6 to 6.6 μg DA L^{-1}, but decreased offshore by 2–3 orders of magnitude.

Concentrations of particulate DA were substantially lower (nearly 50-fold) within the survey area less than 1 month later, with a maximum of 0.29 μg DA L^{-1} measured near the Palos Verdes Peninsula (Fig. 3; note different scale from Fig. 2). Similar to distributions observed in the previous month, however, concentrations within LA harbor during June 2003 were high relative to values outside the harbor (mean inside the harbor = 0.18 μg DA L^{-1} ($n = 7$) compared to mean = 0.06 μg DA L^{-1} ($n = 46$) outside the harbor). Overall, particulate DA was detected throughout the sampling area on both sampling dates in 2003 with only a few exceptions. DA concentrations in the Santa Monica area were below detection at nearly half the sampling sites (lower limit of detection for the ELISA assay was <0.01 μg DA L^{-1}; Fig. 3 open circles).

Scanning and transmission electron microscopy (SEM and TEM) performed on cultures confirmed the presence of *P.* cf. *cuspidata* (Fig. 4a and b) in the San Pedro Channel during 2003.

3.2. Regional surveys during 2004

The survey on 27/28 February of 2004 revealed a maximal concentration of 1.94 μg DA L^{-1} off the southern tip of Palos Verdes (mean = 0.34 μg DA L^{-1}, Fig. 5a). A similar range of DA concentrations was observed on 1 March with a maximum of 1.04 μg DA L^{-1} south of Newport Beach (mean = 0.22 μg DA L^{-1}, Fig. 6a). In contrast to the distributions observed in 2003, concentrations of particulate DA within Los Angeles harbor during 2004 were generally low in comparison to stations outside the breakwater. Some stations within the harbor on 1 March had DA concentrations below the limit of detection (<0.01 μg DA L^{-1}; Fig. 6a, open circles). *Pseudo-nitzschia* spp. abundances averaged 20 × 10^3 cells L^{-1} (range = 2–53 × 10^3 cells L^{-1}) on

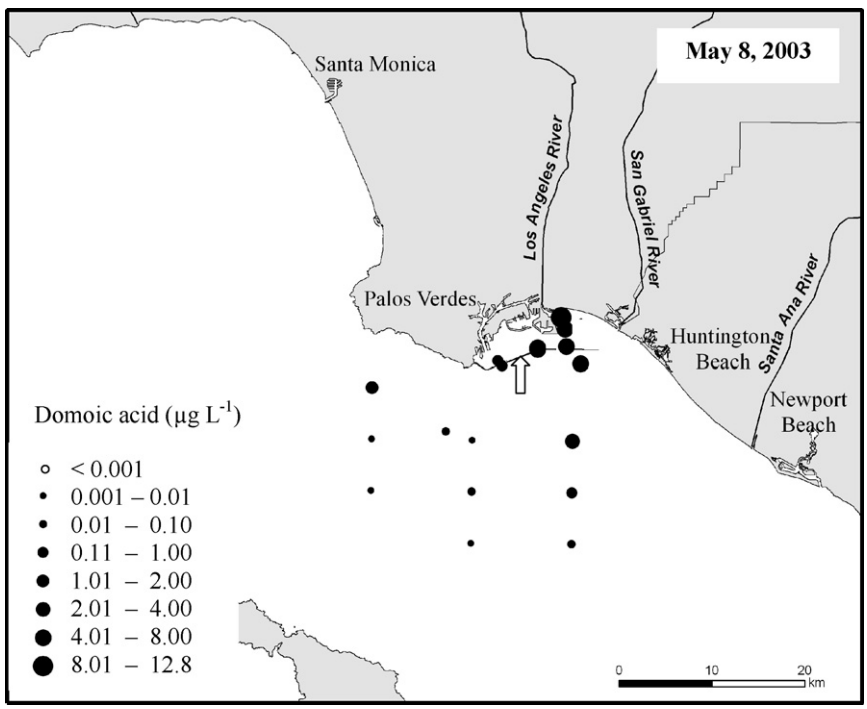

Fig. 2. Particulate domoic acid concentrations (μg DA L^{-1}) in surface waters of the San Pedro Channel and LA harbor during May 2003. The breakwater of the LA harbor is visible as the black lines near the top of the study area (illustrated by the arrow).

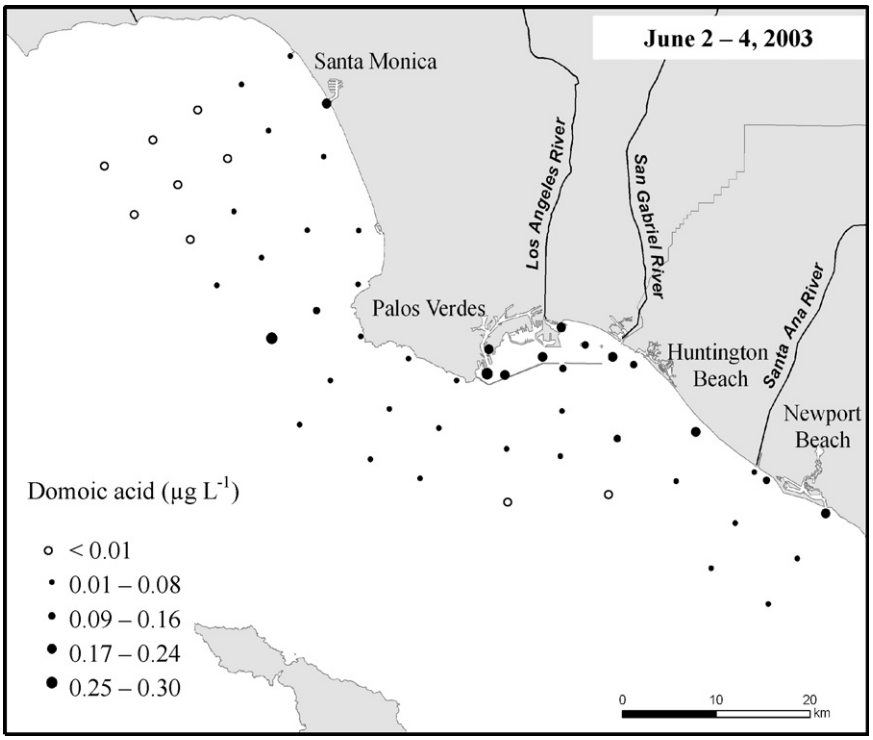

Fig. 3. Particulate domoic acid concentrations (μg DA L^{-1}) in surface waters of the San Pedro Channel and LA harbor during June 2003.

27/28 February and 6×10^3 cells L^{-1} (range = <1–34×10^3 cells L^{-1}) on 1 March (Figs. 5b and 6b). Particulate DA concentrations during 2004 were positively correlated with cell abundances of *Pseudo-nitzschia* (correlation coefficients (r) = 0.61 and 0.59 for 27/28 February and 1 March, respectively; $p < 0.01$; Table 1; Fig. 7). *Pseudo-nitzschia australis* (Fig. 4e and f) and *P.* cf. *cuspidata* were identified in isolates and natural samples from February/March of 2004, with *P. australis* dominating the assemblage. Also present were *P. delicatissima* (Fig. 4c and d) and *P. multiseries* (not shown).

Cellular DA concentrations (pg DA $cell^{-1}$) for the 2004 regional surveys were calculated from total abundances of *Pseudo-nitzschia* and particulate DA concentrations. These values averaged 15.6 pg DA $cell^{-1}$ on 27/28 February and 32.5 pg DA $cell^{-1}$ on 1 March, but a wide range of values was obtained (range = 0–88 pg DA $cell^{-1}$ and 0–117 pg DA $cell^{-1}$, for February and March, respectively).

Heavy rainfall prior to our sampling surveys in 2004 resulted in substantial river discharge from the Los Angeles, San Gabriel and Santa Ana rivers into the San Pedro Shelf region (USGS database at http://waterdata.usgs.gov/ca/nwis). Salinity levels <32.9 ppt were measured in surface waters on 27/28 February and 1 March in and around Los Angeles harbor and Newport Beach. These values are characteristic of river plume water in the Southern California Bight (Washburn et al., 2003). *Pseudo-nitzschia* abundances and particulate DA concentrations were positively correlated with salinity during both surveys in 2004 (r = 0.46–0.83; $p < 0.05$; Table 1). A significant positive relationship was also observed between cellular DA and salinity on 1 March (r = 0.55, $p < 0.01$). Surface temperatures ranged from 13.3 to 14.6 °C on 27/28 February and from 13.6 to 15.6 °C on 1 March. High particulate and cellular DA concentrations co-occurred with higher temperatures on 1 March (r = 0.68 and 0.69, respectively, $p < 0.01$; Table 1).

Concentrations of chlorophyll *a* in surface waters across the survey area ranged from 0.06 to 5.58 μg L^{-1} (mean = 1.91 μg L^{-1}) on 27/28 February and from 0.23 to 10.89 μg L^{-1} (mean = 1.67 μg L^{-1}) on 1 March 2004. Highest concentrations on both sampling dates were observed adjacent to or inside the Los Angeles harbor breakwater. Abundances of *Pseudo-nitzschia* spp., however, did not correlate with chlorophyll *a* concentrations (Table 1). Microscopy confirmed that other phytoplankton taxa (mainly *Prorocentrum* spp.) contributed significantly to phytoplankton biomass in the harbor during the study period. Further, particulate

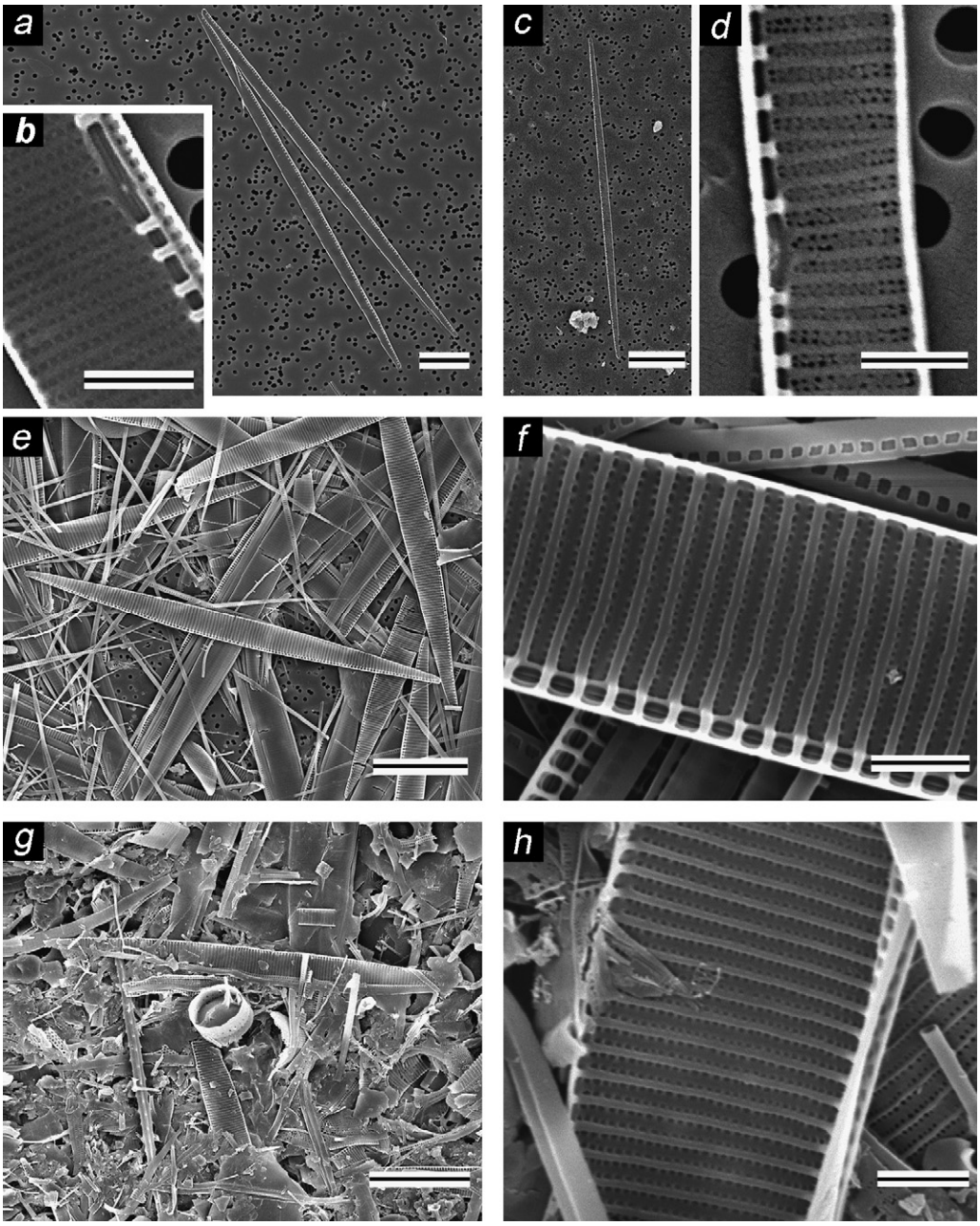

Fig. 4. Scanning electron micrographs of *Pseudo-nitzschia* spp. from the San Pedro Shelf area. (a) *P.* cf. *cuspidata* cultured during spring 2003. Scale bar = 10 μm. (b) Detail of *P.* cf. *cuspidata* showing central area of the valve. Scale bar = 2 μm. (c) *P. delicatissima* from a natural sample collected during March 2004. Scale bar = 10 μm. (d) Detail of central area of *P. delicatissima* valve in (c). Scale bar = 1 μm. (e) *P. australis* from a natural sample collected February 2004. Scale bar = 20 μm. (f) Detail of *P. australis* valve in (e). Scale bar = 1 μm. (g) *P. australis* from a sediment trap sample collected 16–22 April from 500 m depth in the San Pedro Channel. Scale bar = 20 μm. (h) Detail of *P. australis* valve in (g). Scale bar = 2 μm.

and cellular DA levels were inversely correlated with chlorophyll *a* levels on 1 March ($r = -0.42$ and -0.53, $p < 0.05$; respectively; Table 1).

High nutrient concentrations were detected near the river mouths and associated with areas of lower salinity. Samples collected on 27/28 February revealed that the Los Angeles River had the highest nutrient concentrations followed by the San Gabriel River, the Newport Bay region and the Santa Ana River. Ranges of nutrient concentrations at these locations were 1.1–5.7 μM

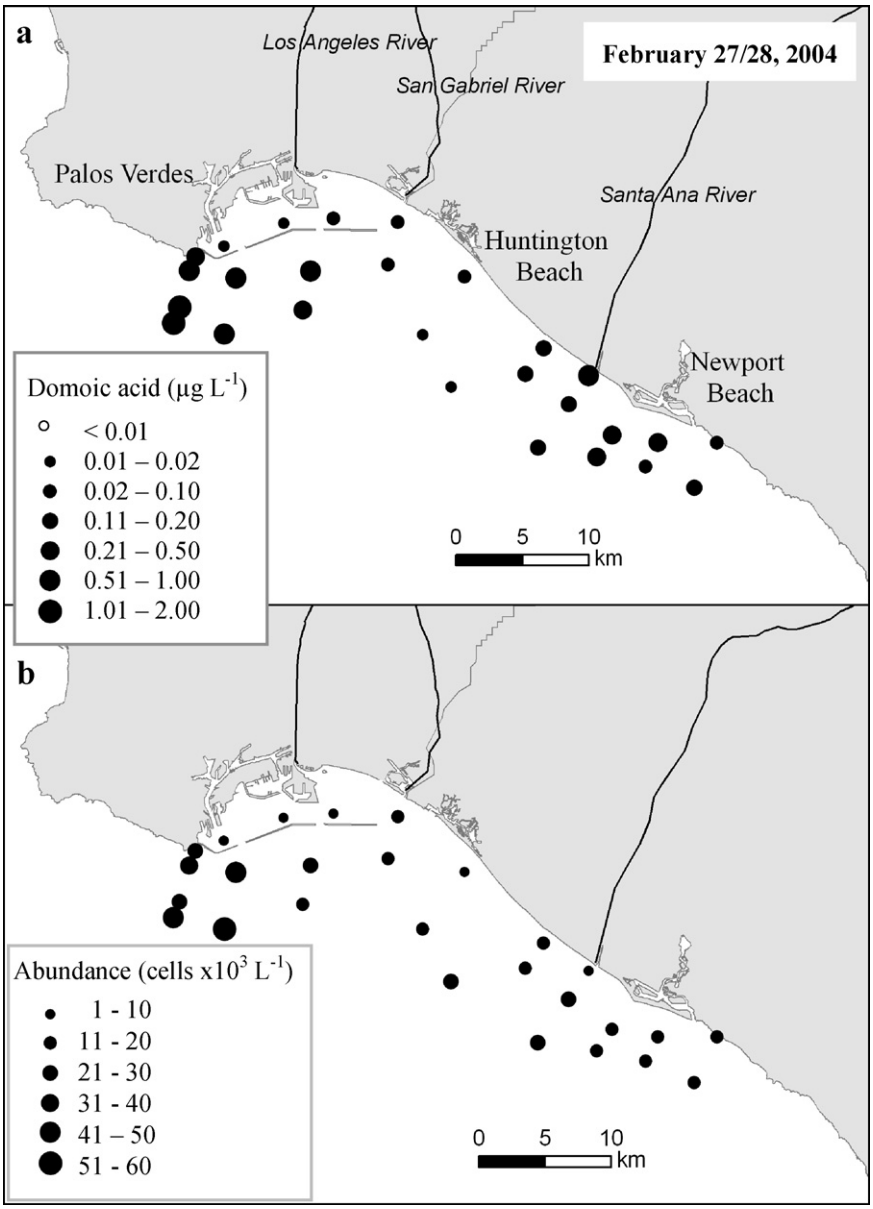

Fig. 5. Particulate domoic acid concentrations (a) and abundances of *Pseudo-nitzschia* spp. (b) in surface waters of the San Pedro Channel and Los Angeles harbor during February 2004.

PO_4^{3-}, 7.7–39.8 µM $NO_3^- + NO_2^{2-}$ and 10.8–52.0 µM $Si(OH)_4$ and decreased by 1–2 orders of magnitude from the river mouths to the offshore stations.

Abundances of *Pseudo-nitzschia* and DA concentrations (particulate and cellular) generally were inversely correlated with concentrations of phosphate, nitrogen ($NO_3^- + NO_2^{2-}$) and silicic acid in the water (Table 1). Exceptions to this generality were phosphate on 27/28 February ($p < 0.05$, Table 1) and between cellular DA and nitrogen during February ($p < 0.05$, Table 1). *Pseudo-nitzschia* abundances, particulate DA concentrations and per cell toxin levels were also inversely correlated with $Si(OH)_4$:PO_4^{3-} ratios and N:PO_4^{3-} ratios ($p < 0.05$, Table 1; shown for cellular DA in Fig. 8a–d). Overall, $Si(OH)_4$:PO_4^{3-} and N:PO_4^{3-} ratios in the water were well below values typical of nutrient replete phytoplankton biomass with averages of 5.4 (range = 0.5–16.6) and 5.0 (range = 0.2–30.1), respectively (February and March combined). No significant relationships were noted between *Pseudo-nitzschia* abundances or DA concentrations and N:$Si(OH)_4$ ratios (mean = 1.0, range = 0.1–4.4; Fig. 8e and f; Table 1).

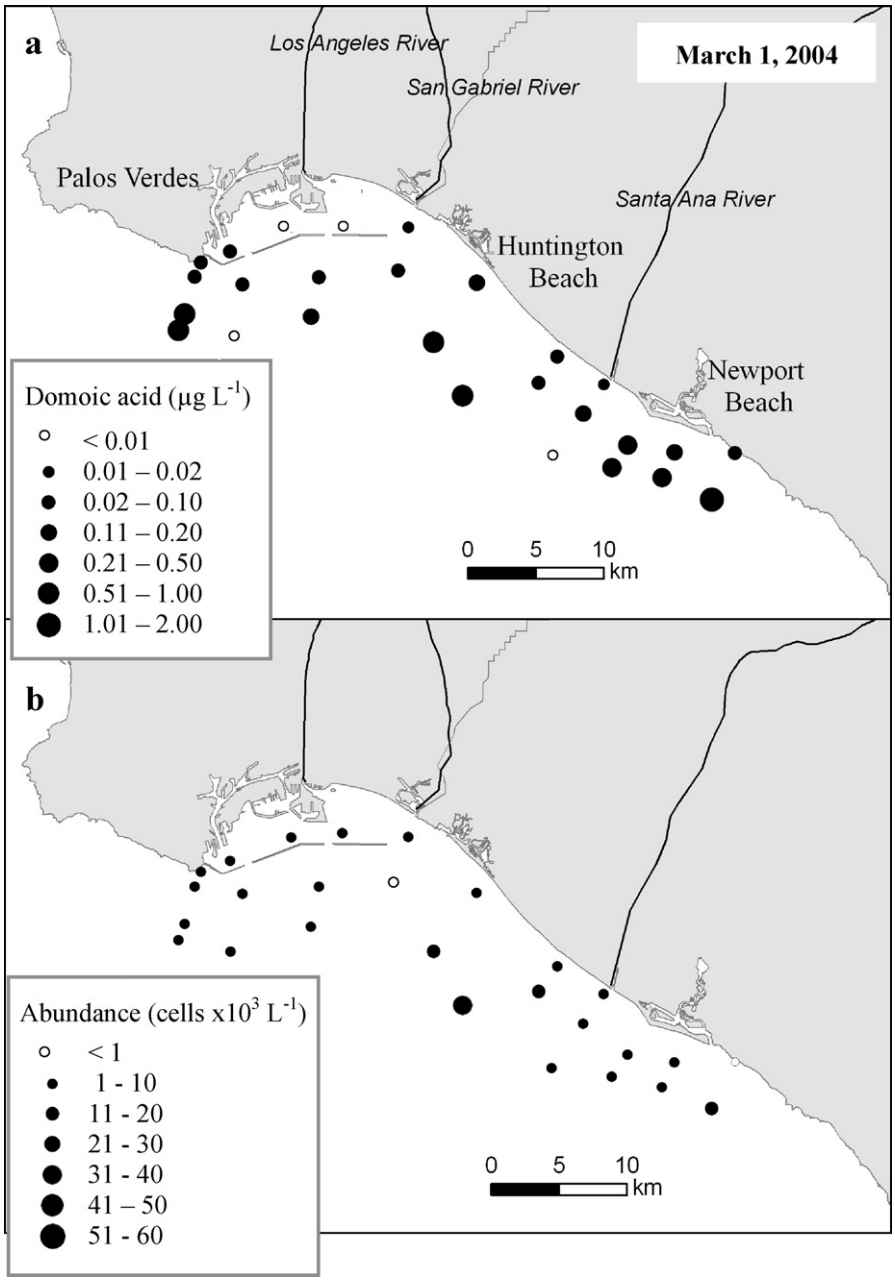

Fig. 6. Particulate domoic acid concentrations (a) and abundances of *Pseudo-nitzschia* spp. (b) in surface waters of the San Pedro Channel and Los Angeles harbor during March 2004.

3.3. Time-series and sediment trap samples in 2004

A time-series of surface seawater samples was collected at weekly-to-monthly intervals south of the LA harbor to establish the temporal pattern of *Pseudo-nitzschia* spp. throughout 2004 (Fig. 9; location indicated by ellipse in Fig. 1). A peak in the abundance of total *Pseudo-nitzschia* cells occurred during late March/early April at this study site (567×10^3 cells L^{-1}) and abundances remained high for approximately 10 days (Fig. 9). Concentrations of particulate DA during this time period were 1.73 µg DA L^{-1} on 31 March (490×10^3 cells L^{-1}) and 0.03 µg DA L^{-1} on 12 April (30×10^3 cells L^{-1}). These values corresponded to 3.5 and 1.1 pg DA $cell^{-1}$, respectively.

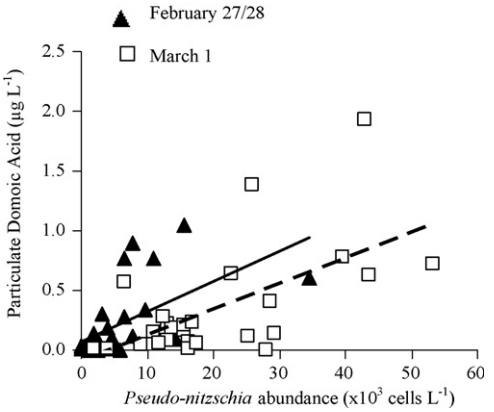

Fig. 7. Scatter plot of domoic acid concentrations during 2004 and corresponding abundances of *Pseudo-nitzschia* spp. in the samples. Data for the February survey are shown as triangles (solid line, $y = 0.0254x + 0.0708$, $R^2 = 0.331$), data for the March survey are illustrated as squares (broken line, $y = 0.0215x - 0.0904$, $R^2 = 0.388$).

Three sediment trap samples were collected in the San Pedro Channel during spring 2004 encompassing the seasonal peak in *Pseudo-nitzschia* spp. abundance (location of mooring is starred in Fig. 1). The highest particulate DA concentration observed for these three samples was 7.6 μg (g sed dry wt)$^{-1}$ for material collected from 16 to 22 April at 550 m, immediately following the highest abundances of *Pseudo-nitzschia* in the time-series data set (Fig. 9). DA concentrations of 1.5 and 0.29 μg (g sed dry wt)$^{-1}$ were detected for material collected in traps prior to and following the maximal value (corresponding to collection periods of 28 February to 5 March (800 m) and 23–29 March (550 m), respectively). Frustules of *P. australis* were observed in the sediment trap material using SEM (Fig. 4g and h).

4. Discussion

4.1. *Pseudo-nitzschia spp. and domoic acid in the Los Angeles harbor and adjacent coastal ocean*

Plankton assemblages in the Southern California Bight (SCB) commonly include *Pseudo-nitzschia* species. *P. australis*, *P. delicatissima*, *P. cuspidata*, *P.* cf. *cuspidata*, *P. multiseries*, *P. pseudodelicatissima*, *P. pungens*, *P. fraudulenta*, *P. heimii*, and *P. subpacifica* have been identified in the area (Villac et al., 1993; Lange et al., 1994; Fryxell et al., 1997; Trainer et al., 2000; this study). Concomitantly, the presence of domoic acid (DA) in coastal SCB waters has been documented (Trainer et al., 2000; Busse et al., 2005; California Department of Health Services). Nevertheless, *Pseudo-nitzschia* blooms in the SCB near Los Angeles have not gained the public and scientific attention garnered by blooms along the US west coast north of Santa Barbara (Table 2) where DA contamination of shellfish has been recognized as a major concern for human health (Horner and Postel, 1993; Horner et al., 1996; Trainer et al., 1998; Trainer and Suddleson, 2005).

The results of the present study corroborate and expand previous observations on *Pseudo-nitzschia* and DA in the SCB, and indicate that toxic blooms of *Pseudo-nitzschia* are a major concern and recurring phenomenon in coastal waters near Los Angeles. Field data from 2003 and 2004 revealed that these events can be distributed over a large area of the coastal ocean in this region (at least ∼1500 km^2). Moreover, concentrations of particulate DA and cellular DA attained values as high as have been observed in other geographical regions experiencing DA toxicity events (Table 2).

Los Angeles harbor constituted a 'hot spot' for particulate DA during May 2003 with concentrations in surface waters ranging from 5.6 to 12.7 μg L^{-1}. Previous studies have reported maximal values of particulate DA typically below 8 μg L^{-1} whereas toxin concentrations greater than 12 μg DA L^{-1} have been documented rarely (Table 2). DA in the present study was also detected approximately 1 month later in June of 2003 throughout most of the ∼1500 km^2 survey area between Santa Monica and Newport Beach. Abundances of *Pseudo-nitzschia* were not determined during 2003, and therefore DA cellular concentrations were not calculated. *Pseudo-nitzschia* cf. *cuspidata* was identified from the *Pseudo-nitzschia* isolates cultured from the San Pedro Channel in 2003, but it remains unclear if *P.* cf. *cuspidata* was the main source of DA in those surveys.

Particulate DA was detected again during February and March 2004 along the San Pedro Shelf from Palos Verdes to Newport Beach where it attained maximal levels of ∼2 μg L^{-1} (total study area ∼500 km^2). Cellular concentrations of DA ranged from 0 to 117 pg DA cell^{-1}. These values generally fell within the range documented for natural assemblages of *Pseudo-nitzschia* in other studies (0–78 pg DA cell^{-1}; see Table 2), but a few values did exceed maximal estimates previously reported (Scholin et al., 2000; Trainer et al., 2000). *P. australis* was identified as the primary source of DA in these latter studies, and *P. australis* also dominated the *Pseudo-nitzschia* assemblage during our study although *P.* cf. *cuspidata* was also common in spring 2004.

Our time-series of samples off the Palos Verdes Peninsula showed that our surveys may have missed the

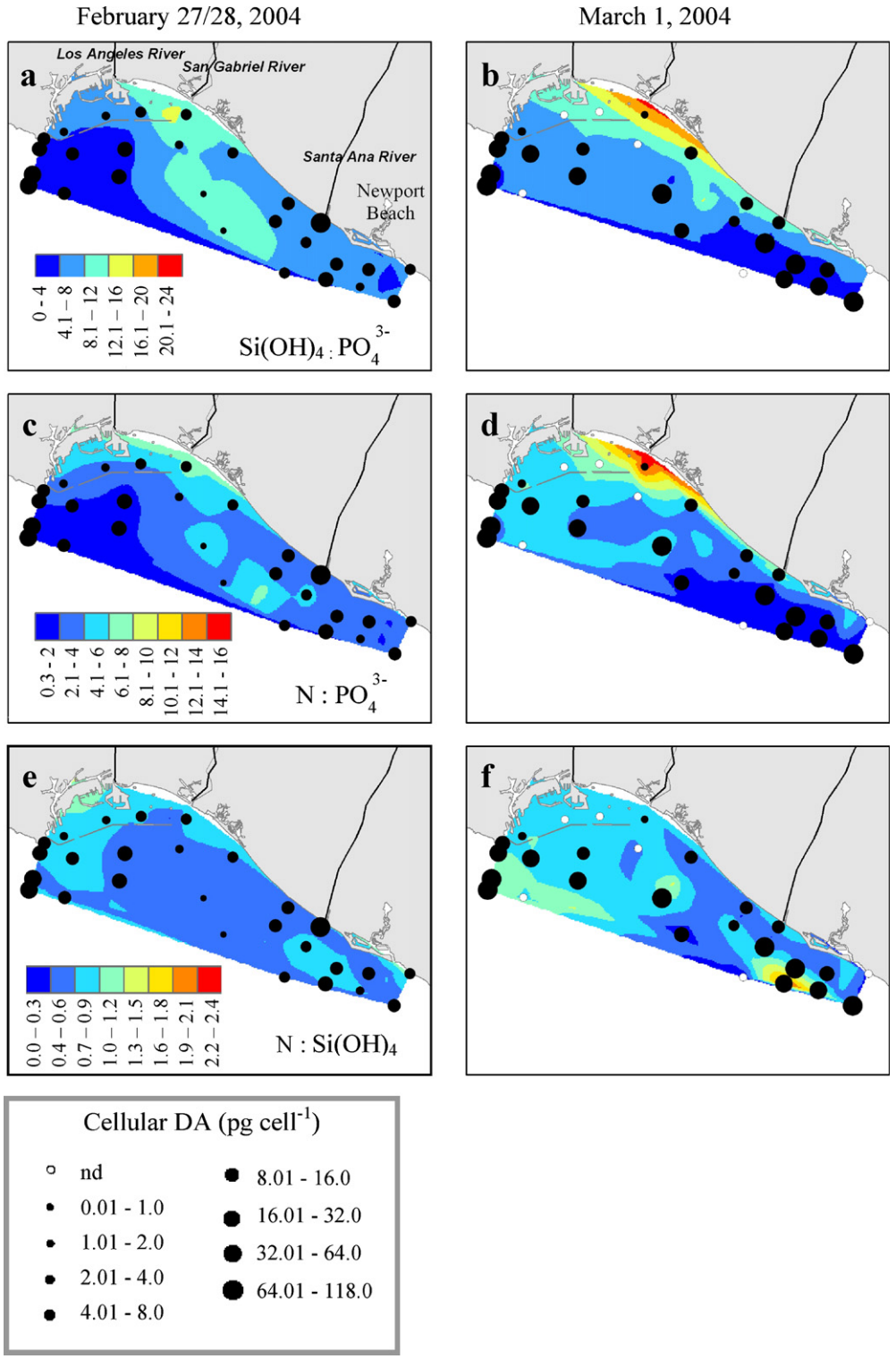

Fig. 8. Nutrient ratios ($Si(OH)_4$:PO_4^{3-}, N:PO_4^{3-} and N:$Si(OH)_4$) in surface waters on 27/28 February (a, c, and e) and 1 March (b, d, and f), 2004. Cellular DA concentrations for each of the sampling stations are indicated by filled circles. Cellular DA could not be calculated for sampling stations where *Pseudo-nitzschia* abundances or particulate DA concentrations were below the limit of detection (empty circles).

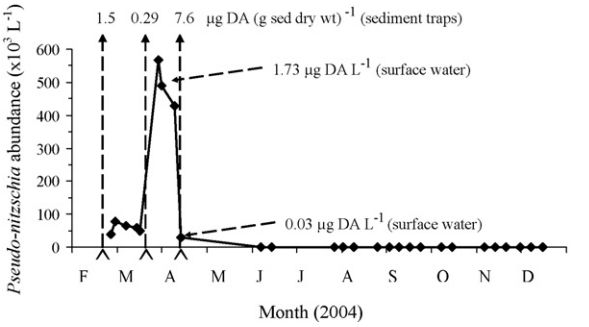

Fig. 9. *Pseudo-nitzschia* spp. abundances in surface waters monitored throughout 2004 ~3 km south of the LA harbor. Concentrations of particulate DA in surface waters was measured on 31 March and 12 April. Sediment trap material analyzed for particulate DA was collected in traps from 28 February to 5 March (800 m), 23–29 March (550 m) and 16–22 April (550 m). Arrows along the *x*-axes indicate dates when sediment trap collection ended for each of the three sediment trap cups.

peak of the bloom in 2004 since abundances of *Pseudo-nitzschia* increased in that area towards the end of March. Toxin analyses were not routinely possible from the time-series samples but analysis of the sample collected on 31 March confirmed that particulate DA concentrations at that time were still substantial (1.73 µg DA L^{-1}, Fig. 9). Taken together, the results of our study indicated that toxic blooms in coastal waters near Los Angeles are of a severity and magnitude that warrants further investigation.

4.2. Relationships between environmental variables, abundances of Pseudo-nitzschia and domoic acid

Changes in environmental conditions due to river discharge and coastal upwelling have been implicated as possible causes for the development of *Pseudo-nitzschia* blooms (Horner and Postel, 1993; Dortch et al., 1997; Trainer et al., 1998, 2002; Pan et al., 2001; Parsons and Dortch, 2002; Kudela et al., 2005). Although these coastal processes may play a role in the development of toxic blooms, the exact relationships between the growth of *Pseudo-nitzschia* species, the production of DA, and specific environmental forcing factors have been difficult to establish.

Laboratory experimentation has improved our general understanding of how the availability of macro- or micronutrients can affect toxin production in cultures of *Pseudo-nitzschia*. High toxin content in cells of *P. seriata* and *P. multiseries* has been demonstrated under silica and/or phosphate stress (Bates et al., 1991; Pan et al., 1996a, 1996b; Fehling et al., 2004). Domoic acid production also has been stimulated experimentally in *P. australis*, *P. multiseries* and *P. fraudulenta* by iron and/or copper limitation (Rue and Bruland, 2001; Maldonado et al., 2002; Wells et al., 2005). The multitude of cause–effect relationships between nutrient availability (macronutrients and trace metals) and DA production reported from laboratory studies imply that there may be multiple scenarios in nature that could stimulate toxin production by *Pseudo-nitzschia*.

Observational studies of natural ecosystems that obtain measurements of chemical and physical properties concurrently with bloom dynamics play a fundamental role in attempting to link cause and effect of toxic blooms. For this reason, nutrient concentrations, temperature, salinity and chlorophyll concentrations were determined during our regional surveys on 27/28 February and 1 March of 2004. Our analysis of these data indicated that chlorophyll concentrations were higher in regions with high nutrient loading (PO_4^{3-}, $NO_3^- + NO_2^{2-}$, $Si(OH)_4$) from river discharge inside and adjacent to the Los Angeles harbor. The taxonomic composition of the phytoplankton assemblage, however, was mainly algae other than *Pseudo-nitzschia*. Abundances of *Pseudo-nitzschia*, concentrations of particulate DA and cellular DA were higher at offshore stations and not immediately associated with the highly elevated nutrient concentrations and chlorophyll peaks characteristic of the coastal waters immediately affected by the river plume. In contrast, toxin levels were inversely correlated with concentrations of silicic acid, nitrogen and phosphate in the study area, possibly implicating the drawdown of some of these nutrients due to the development of populations of *Pseudo-nitzschia* and the production of DA. In a general sense this speculation agrees with the results of laboratory studies demonstrating that silica and phosphate stress increased toxin production in *Pseudo-nitzschia* (Bates et al., 1991; Pan et al., 1996b; Fehling et al., 2004). Low $Si(OH)_4$:PO_4^{3-} and N:PO_4^{3-} ratios (but not N:$Si(OH)_4$ ratios) also correlated with high cellular DA concentrations in the present study. The exact relationship between these ratios, *Pseudo-nitzschia* growth and the production of DA will require further investigation, but clearly elemental ratios as well as the absolute concentrations of specific elements play a role in the success of these diatoms and DA production.

The SCB region is typically influenced by river discharge as well as coastal upwelling during the winter and spring (Horner et al., 1997; Schiff et al., 2000; Kudela et al., 2005). Both processes tend to be highly episodic, and both affected the study area during January and February 2004 (USGS database at

Table 2
Summary of reported particulate DA concentrations and *Pseudo-nitzschia* spp. abundances along the West coast of the US

Location	Month, year	Depth (m)	Particulate DA ($\mu g\ L^{-1}$)	Cellular DA ($pg\ cell^{-1}$)	*P.* abundances (10^3 cells L^{-1})	*P.* species present	Source
Monterey Bay, CA	October–November, 1991	Surface	0.29–12.3	0.4–33	100–1000	*P. australis* dominant, *P.* f. *pungens multiseries* and *P. pseudodelicatissima* present	Walz et al. (1994)
Penn Cove, WA	July–August, 1997	Surface[a] - 27	bd - 0.8	nd	700–13,000	*P. pungens*, *P. multiseries*, *P. australis, P. pseudo-delicatissima*	Trainer et al. (1998)
Monterey Bay, CA	May, 1998	Surface	nd	7.2–75	0–130	*P. australis* dominant	Scholin et al. (2000)
San Francisco to Santa Barbara, CA	June, 1998	Surface[a] - 40	0.1–7.3	0.1–78	49–11,000	*P. australis*[b] and *P. multiseries* dominant	Trainer et al. (2000)
Washington coast, CA	July, 1997	Surface	1.0–2.7	1.0–4.6	600–900	*P. pseudodelicatissima* and/or *P. delicatissima* dominant, *P.* cf. *heimii* present	Trainer et al. (2002)
	June–October, 1998	Surface	bd - 4.7	0–0.7	100–17,100	*P. pseudodelicatissima* and/or *P. delicatissima* dominant, *P.* cf. *heimii* present	Trainer et al. (2002)
Juan de Fuca Eddy, WA	September, 2001	Surface[a] - 11	0.01–0.03	0.5–3.4	9–19	*P.* cf. *pseudodelicatissima* dominant *P.* cf. *australis* present	Marchetti et al. (2004)
San Diego, CA	February, 2004	Surface	bd - 2.3	5–43	0–77	*P. australis* dominant, *P. multiseries* common	Busse et al. (2005)
Los Angeles area, CA	May–June, 2003	Surface	bd - 12.7	nd	nd	*P.* cf. *cuspidata* present	This study
	February–March, 2004	Surface	bd - 1.9	0–117	<1–53	*P. australis* dominant, *P.* cf. *cuspidata* common	This study

Note: Different methods were used to determine toxin concentrations in these cited studies. bd: below detection limit; nd: no data available.

[a] Indicates depth at which maximum particulate DA concentration was detected if vertical sampling occurred.

[b] Species associated with maximum levels for cellular DA.

http://waterdata.usgs.gov/ca/nwis and Pacific Fisheries Environmental Laboratory, http://las.pfeg.noaa.gov). In addition, the Los Angeles metropolitan area is one of the most densely populated coastal regions in the United States. Thus, this region constitutes a unique and complex experimental site for examining the interplay between natural and anthropogenic factors influencing the development of harmful algal blooms in coastal waters.

4.3. Implications of toxic blooms for coastal waters in the Los Angeles area

Toxic blooms of *Pseudo-nitzschia* in Los Angeles coastal waters pose a significant threat to the health of this coastal ecosystem. Higher trophic levels within marine communities that are commonly affected through food web transfer of DA include sea mammals and sea birds (Fritz et al., 1992; Work et al., 1993; Gulland, 1999; Scholin et al., 2000; Gulland et al., 2002; Silvagni et al., 2005). During 2003 and 2004 DA-poisoning was implicated in >1400 mammal stranding incidents within the SCB (San Luis Obispo area through Orange County; California Marine Mammal Stranding Network, J. Cordaro, pers. commun.). This estimate included 1115 California sea lions and 122 common dolphins in 2003, and 231 sea lions in 2004. DA-contaminated planktivorous fish have been identified as vectors for toxin transfer to marine mammals and sea birds (Lefebvre et al., 2001, 2002; Costa and Garrido, 2004) but key details are lacking regarding how DA concentrations in plankton relate to toxin body burden in fish and their consumers (marine mammals, seabirds and humans).

Pseudo-nitzschia cells consumed in surface waters by herbivorous zooplankton and planktivorous fish constitute a risk to higher trophic levels feeding in the water column. However, the aggregation of toxic diatoms into rapidly sinking macro-particles (sinking rates > 100 m d^{-1}) (Smetacek, 1985; Alldredge and Gotschalk, 1989) may also constitute an ecological threat to benthic communities. Intact *Pseudo-nitzschia* cells and/or frustules have been observed in sediment traps and in samples of sediments (Dickman and Glenwright, 1997; Dortch et al., 1997; Trainer et al., 2000; Parsons and Dortch, 2002). At present it is poorly understood how important the sinking of cells to depth might be for the delivery of toxin to benthic environments.

In this study, sediment trap material collected from 550 and 800 m depth in the San Pedro Channel during spring of 2004 tested positive for particulate DA. To our knowledge these are the deepest sediment trap collections in which DA has been demonstrated. The highest DA concentration (7.6 μg (g sed dry wt)$^{-1}$) was measured in a trap from 550 m that collected material 1 week after abundances of *Pseudo-nitzschia* decreased dramatically in surface waters. Electron microscopy confirmed the presence of *P. australis* in the trap sample; the same species that dominated surface assemblages during March and April. This record of DA in deep sediment traps indicates a potentially important mechanism linking toxic algal blooms in the euphotic zone to DA contamination of benthic organisms (Bates et al., 1989; Langlois et al., 1993; Goldberg, 2003).

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