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# Coastal upwelling linked to toxic *Pseudo-nitzschia australis* blooms in Los Angeles coastal waters, 2005–2007

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Harmful algal blooms dominated by the diatom *Pseudo-nitzschia* spp. have become a perennial but variable event within surface waters near the greater Los Angeles area. Toxic blooms during spring seasons from 2005 to 2007 varied strongly in their overall toxicity and duration. Differences in bloom dynamics were linked to differences in storm-induced river discharge following episodic rain events and coastal upwelling, both major coastal processes that led to the injection of nutrients into coastal surface waters. Heavy river runoff during early 2005, a record-rainfall year, favored a phytoplankton community mainly comprised of algal taxa other than *Pseudo-nitzschia*. The spring bloom during 2005 was associated with low domoic acid surface concentrations and minor contributions of (mainly) *P. delicatissima* to the diatom assemblage. In contrast, highly toxic *P. australis*-dominated blooms during spring seasons of 2006 and 2007 were linked to strong upwelling events. River discharge quotas in 2006 and 2007, in contrast to 2005, fell well below annual averages for the region. Surface toxin levels were linked to colder, more saline (i.e. upwelled) water over the 3-year study, but no such consistent relationship between domoic acid levels and other physicochemical parameters, such as macronutrient concentrations or nutrient ratios, was observed.

**KEYWORDS:** domoic acid; *Pseudo-nitzschia*; southern California; coastal upwelling; river discharge

## INTRODUCTION

Southern Californian coastal waters near Los Angeles have witnessed the appearance of toxic blooms of *Pseudo-nitzschia* spp. during the last decade, and the San Pedro/Long Beach harbor region has been identified as a “hot spot” for the neurotoxin, domoic acid (DA) (Schnetzer *et al.*, 2007; Caron and Schnetzer, unpublished data). The autecology of *Pseudo-nitzschia* species and DA poisoning events have become a research focal point not only along the US west coast (Kudela *et al.*, 2004; Cochlan *et al.*, 2008; Anderson *et al.*, 2009) but worldwide, as geographical locales and maximal toxin levels associated with *Pseudo-nitzschia* blooms appear to be on the rise (Hasle, 2002; Casteleyn *et al.*, 2008; Trainer *et al.*, 2012). DA is a water-soluble neurotoxin that accumulates in filter-feeding organisms, most notably shellfish and planktivorous fish such as anchovy and sardine (Lefebvre *et al.*, 2002; Costa and Garrido, 2004; Krogstad *et al.*, 2009). Humans consuming DA-contaminated seafood experience Amnesic Shellfish Poisoning (ASP) whose symptoms may include vomiting, confusion, memory loss, coma and even death (Bates *et al.*, 1989; Wright *et al.*, 1989). Although fatal ASP cases in humans have been rare, DA poisoning has resulted in a large-scale mortality in marine animal populations including sea lions and seabirds (Work *et al.*, 1993; Gulland *et al.*, 2002; Bejarano *et al.*, 2008), and shellfish closures along US coasts have caused significant economic losses as a consequence of DA contamination (HARRNESS, 2005; Bauer, 2006).

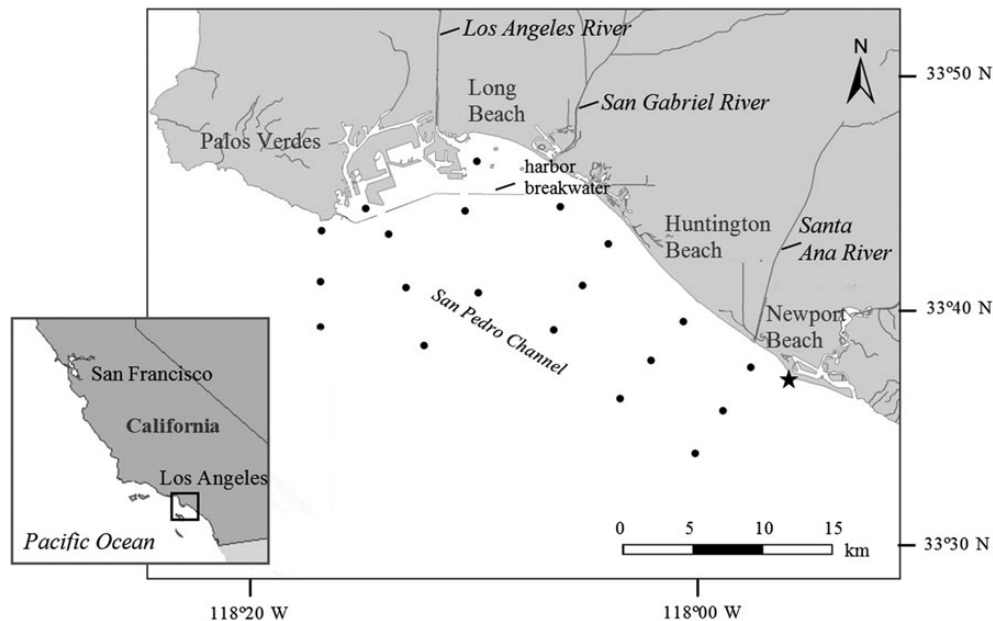
Coastal upwelling events, mesoscale eddies and anthropogenic inputs of nutrients from river discharge and land runoff have all been implicated as possible causes for *Pseudo-nitzschia* blooms (Trainer *et al.*, 2000; Parsons *et al.*, 2002; Kudela *et al.*, 2005; Anderson *et al.*, 2008). Determining the specific factors that regulate toxin production during bloom events, however, has been challenging because a variety of environmental triggers appear to initiate or stimulate DA production (Lelong *et al.*, 2012). Laboratory and field studies have demonstrated that macronutrient levels (i.e. silicate and/or phosphate limitation; Pan *et al.*, 1996a; Pan *et al.*, 1996b; Fehling *et al.*, 2004), nutrient ratios (Schnetzer *et al.*, 2007; Heisler *et al.*, 2008), the form of available nitrogen (Howard *et al.*, 2007; Cochlan *et al.*, 2008; Thessen *et al.*, 2009), trace metal concentrations (iron deficiency and copper surplus; Rue and Bruland, 2001; Wells *et al.*, 2005; Silver *et al.*, 2010), bacterial associations (Bates *et al.*, 1995; Kaczmarek *et al.*, 2005) and partial CO<sub>2</sub> concentration (Tatters *et al.*, 2012) may affect toxin production.

*Pseudo-nitzschia* bloom dynamics and DA concentrations within the San Pedro and Long Beach harbors (“Los Angeles Harbor”) and in the adjacent San Pedro Channel were characterized during a 3-year period to examine the temporal relationship between these events, coastal upwelling and river discharge from the highly urbanized greater Los Angeles metropolitan area. We conducted a total of 39 surveys between March 2005 and December 2007 and collected surface samples from 20 locations within a ~400-km<sup>2</sup> study region. Concentrations of particulate DA (pDA), cellular DA (cDA) and *Pseudo-nitzschia* abundances in surface waters were measured in relation to physical (temperature and salinity), biological (chlorophyll *a*) and chemical factors (inorganic macronutrients) to investigate environmental controls that may play a role in bloom development. Overall, we observed a strong seasonal pattern of *Pseudo-nitzschia* abundance for all the three years, but each year’s event varied in the degree of toxicity and duration. Significant levels of toxin in surface waters during spring were preceded by strong upwelling events in 2006 and 2007, years that were otherwise characterized by low river runoff load into the San Pedro Basin. In contrast, toxicity in surface waters during spring was low in the aftermath of heavy river discharge during winter–spring of 2005 although a dramatic increase in algal biomass (chlorophyll *a*) during this time was associated with phytoplankton taxa other than *Pseudo-nitzschia*. Maximal toxin concentrations were observed inside the Los Angeles Harbor and immediately outside the breakwater in 2006 and 2007 and were among the highest reported for any geographical region to date.

## METHOD

### Study site and sampling

Weekly-to-monthly surveys were conducted between March 2005 and December 2007 (total of 39) to investigate the spatiotemporal relationship between *Pseudo-nitzschia* bloom dynamics (toxin concentrations and *Pseudo-nitzschia* abundances in surface waters) with pertinent environmental parameters (temperature, salinity, inorganic nutrients and chlorophyll *a*), river discharge and coastal upwelling. The study area encompassed 20 sampling stations that spanned over ~400 km<sup>2</sup> of the coastal Southern California Bight region between the Palos Verdes peninsula and Newport Beach and included sampling sites inside the San Pedro and Long Beach harbors (Fig. 1). Sampling frequency increased from monthly to weekly during months when phytoplankton biomass was high and



**Fig. 1.** Map of study region ( $\sim 400 \text{ km}^2$ ) encompassing twenty surface sampling sites (black dots) along the coast of southern California between the Palos Verdes peninsula and Newport Beach. The location of the three major rivers that discharge into the San Pedro basin are shown (Los Angeles, San Gabriel and Santa Ana River). Newport Pier sampling site shown as star.

when *Pseudo-nitzschia* cells were detected. Seawater samples were collected from the surface (0–1 m) using a bucket and brought to the laboratory in 1 L polycarbonate bottles, which were chilled on ice and protected from sunlight during transport.

### Physiochemical measurements, river discharge and coastal upwelling data

Surface temperature (digital Fisherbrand thermometer) and inorganic nutrient concentrations were measured for all surveys during the 3-year study period. Seawater samples to determine nutrient concentrations ( $\text{PO}_4^{3-}$ ,  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{Si}(\text{OH})_4$ ) were stored at  $-20^\circ\text{C}$  until analyses using an Alpkem RFA segmented flow analyzer (Gordon *et al.*, 1993). Samples for the determination of surface salinity were obtained for 2006 and 2007 and analyzed on a Guildline AutoSal laboratory salinometer following the manufacturer's recommended technique (Technical Manual). Salinity was not measured during surveys in 2005, but salinity information at a sampling location near Newport Pier (Fig. 1) was obtained from the Southern California Coastal Ocean Observing System website (<http://www.sccoos.org>). Chlorophyll *a* (chl *a*) was measured fluorometrically (Turner Design 10-AU Fluorometer) following standard protocols on seawater samples (50–100 mL) filtered onto GF/F filters (Parsons *et al.*, 1984). Average rates of river discharge into the San Pedro basin were obtained from the USGS database

(<http://waterdata.usgs.gov/ca/nwis>) and a time series of temperature adjacent to the Newport Beach Pier was obtained from the Southern California Coastal Ocean Observing System website (<http://www.sccoos.org>). Discharge data for the Los Angeles, San Gabriel and Santa Ana River were analyzed as the main contributors of river discharge into the Los Angeles harbor and adjacent San Pedro Channel (Fig. 1). Upwelling indices for the central Southern California Bight ( $33^\circ\text{N}$   $119^\circ\text{W}$ ), indicative of periods when upwelling was more likely to occur in response to overall wind patterns, were obtained for 2005 to 2007 from the National Oceanic and Atmospheric Administration (NOAA) Pacific Fisheries Environmental Laboratory (PFEL; <http://las.pfel.noaa.gov>).

### Particulate DA concentrations and *Pseudo-nitzschia* abundances

Concentrations of pDA ( $\mu\text{g pDA L}^{-1}$ ) were measured from 200 mL seawater samples filtered onto Whatman GF/F filters immediately after the samples were returned to the laboratory (within  $\sim 2 \text{ h}$ ) to minimize DA degradation. The filters were stored frozen at  $-20^\circ\text{C}$  until analyzed using an Enzyme Linked Immunosorbent Assay (ELISA kits; Biosense<sup>TM</sup> Laboratories, Bergen, Norway). Optimization and ground-truthing of the method to determine DA in particulate phytoplankton had been achieved during an earlier study (Schnetzler *et al.*, 2007). Filtration of 200 mL of seawater yielded a detection limit

of  $0.01 \mu\text{g pDA L}^{-1}$  for the ELISA assay. Each sample was run at several dilutions and in duplicate. Plankton tows in surface waters (0–1 m) were conducted at 10 of the 20 sampling stations during each survey using a net with 20- $\mu\text{m}$  mesh size (every other station was sampled throughout the grid). The relative contribution of *Pseudo-nitzschia* to the overall phytoplankton community was determined using aliquots ( $\sim 0.1 \text{ mL}$ ) from each tow sample via light microscopy. Samples in which *Pseudo-nitzschia* cells were detected were further examined using scanning and transmission electron microscopy (SEM/TEM; typically 5–10 stations per sampling event) to facilitate the identification of *Pseudo-nitzschia* species (Miller and Scholin, 1998; Lundholm *et al.*, 2003). Absolute *Pseudo-nitzschia* abundances were determined for all surveys in 2006 and during the peak of the bloom in 2007 (survey during which maximal surface toxin levels were detected during that particular year) using inverted light microscopy and standard settling techniques after sample preservation (25–50 mL) with acid Lugol's solution (10% final concentration; Utermöhl, 1958). An approximate lower limit of detection of 5 cells  $\text{mL}^{-1}$  for the microscopic counts resulted from a total settled volume of 25 mL seawater and counting at least 150 cells per sample or 20 fields of view at  $40\times$  magnification. Concentrations of cDA (pg cDA  $\text{cell}^{-1}$ ) were derived from pDA concentrations in a sample and abundances of *Pseudo-nitzschia* whenever measurable quantities of both parameters were obtained.

#### Statistical analyses

Abundances of *Pseudo-nitzschia*, pDA and cDA concentrations were examined for their relationships with physical parameters (salinity and temperature), biological (chlorophyll *a*) and chemical ( $\text{PO}_4^{3-}$ ,  $\text{NO}_3^- + \text{NO}_2^{2-}$ ,  $\text{Si(OH)}_4$ ) constituents and nutrient ratios. The data were log-transformed prior to correlation analyses using the Software package Statistica (StatSoft, 2002). Missing data points were disregarded in pair-wise comparisons and values below detection (ELISA results for DA concentrations) were assigned a zero value.

## RESULTS

### River discharge and coastal upwelling

Rainfall, and consequent river discharge, is highly seasonal in southern California but also exhibited strong interannual variability during this three-year study (2005–2007). Heavy rainfall during the winter and spring of 2005 resulted in substantial river discharge from the Los Angeles, San Gabriel and Santa Ana rivers into the San Pedro Shelf region prior to our sampling surveys (gray shaded area in Fig. 2A). In contrast, precipitation during

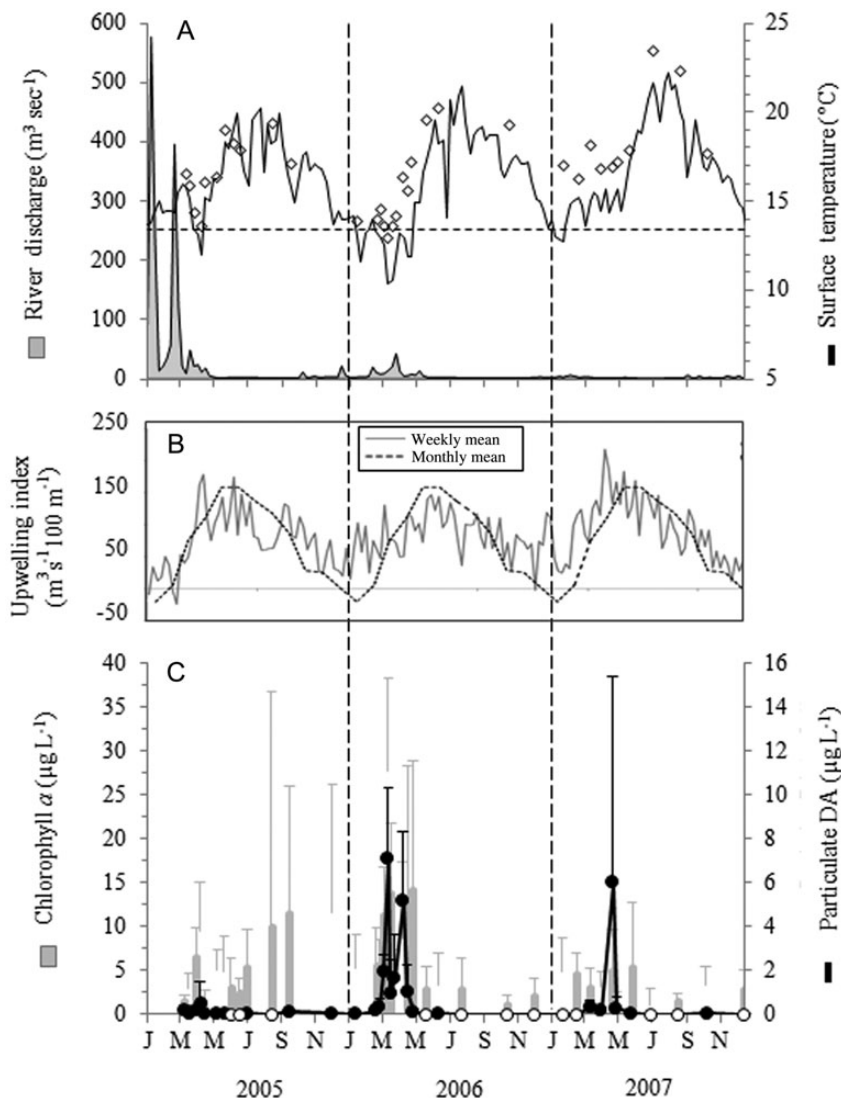
2006 and 2007 was well below previously recorded annual averages (USGS database at <http://waterdata.usgs.gov/ca/nwis>; Fig. 2A). Accordingly, only 18 of 424 surface salinity values obtained during 2006 and 2007 were  $< 32.9$ . These low values are characteristic of discharged river water in the Southern California Bight (Washburn *et al.*, 2003). More than half of these lower salinity measurements during 2006 and 2007 were obtained at the two stations closest to the Los Angeles River outlet (Fig. 1). Overall, surface salinities ranged from 22.8 to 33.8 during spring seasons in these two years (average = 33.2;  $n = 215$ ), from 30.8 to 33.9 during summers (average = 33.5;  $n = 94$ ) and from 21.5 to 35.0 during autumn and winter months (average = 33.3;  $n = 128$ ).

Annual changes in temperature (survey averages shown as diamonds in Fig. 2A) as well as continuous temperature records from a monitoring site at the Newport Pier (at the southeastern end of our sampling grid; black line in Fig. 2A) indicated the appearance of cooler water ( $\leq 13.5^\circ\text{C}$ ) along the coastline during winter–spring periods ( $13.5^\circ\text{C}$  being the nominal temperature of the  $25 \text{ kg/m}^3$  isopycnal). Minimum temperatures in surface waters tended to occur in late winter through spring when the basin-wide upwelling index increased (NOAA PFEL) (Fig. 2B). The upwelling index was lower during January–March 2005 than in the following 2 years, but intensified in early April. The index during the spring season of 2005 and 2006 appeared to be somewhat below the 45-year average (1967–2012) but was above the average during winter–spring 2007. Overall, surface temperatures ranged from  $11.7$  to  $19.2^\circ\text{C}$  during spring seasons (average for all 3 years =  $15.4^\circ\text{C}$ ;  $n = 311$ ), from  $15.0$  to  $24.6^\circ\text{C}$  during summers (average =  $19.0^\circ\text{C}$ ;  $n = 179$ ) and from  $12.5$  to  $19.7^\circ\text{C}$  during autumn and winter months (average =  $16.1^\circ\text{C}$ ;  $n = 179$ ).

### Phytoplankton dynamics

*Pseudo-nitzschia* was a member of the spring diatom assemblage in each of the years studied and typically co-occurred with other chain-forming genera such as *Chaetoceros*, *Asterionellopsis* and *Leptocylindrus*. Electron microscopy revealed the presence of *P. delicatissima*, *P. fraudulenta* and *P. pungens* in 2005 with *P. delicatissima* being most common. *P. australis* dominated the *Pseudo-nitzschia* assemblage during 2006 and 2007, and other *Pseudo-nitzschia* species were only rarely detected ( $< 1\%$  of all examined *Pseudo-nitzschia* cells using SEM/TEM).

A succession in the composition of the phytoplankton assemblage was observed each year from dominance by diatoms during winter and early spring to dominance by a mixed dinoflagellate community by May/June. The latter assemblage had strong contributions from the



**Fig. 2.** Three-year record of (A) weekly averages of river discharge rates ( $\text{m}^3 \text{s}^{-1}$ ; gray area, primary y-axis) and surface temperature ( $^{\circ}\text{C}$ ) at Newport Beach city pier (black line, secondary y-axis) located at the southeastern corner of the survey grid. Average temperatures recorded during the sampling surveys (diamonds, secondary y-axis) mirrored temperature that was continuously recorded at the Newport Beach city pier; Surface temperatures below  $13.5^{\circ}\text{C}$  indicate periods of coastal upwelling (dotted line). (B) Weekly means for the upwelling indices [ $\text{m}^3 \text{s}^{-1}$  ( $100 \text{ m coastline}^{-1}$ )] for the San Pedro coastal region (solid line) and monthly means computed over the past 45 years (broken line). (C) Averages for chlorophyll *a* concentrations ( $\mu\text{g L}^{-1}$ ; gray bars, primary y-axis) and for particulate toxin levels ( $\mu\text{g pDA L}^{-1}$ ; black line, secondary axis) are shown with standard deviations in relation to each survey average. Filled circles indicate detectable concentrations of pDA at one or more sampling stations. Open circles indicate that all pDA concentrations were below the ELISA limit of detection for all the sampling stations of that survey. Vertical dashed lines delineate each of the sampling years.

genera *Prorocentrum*, *Ceratium* and *Cochlodinium*. Surveys between May and December of 2005 were unique from surveys during the same period in 2006 and 2007 in that 2005 was characterized by blooms of the red tide-dinoflagellate *Lingulodinium polyedrum*. Abundances of this species in excess of  $3 \times 10^6 \text{ cells L}^{-1}$  were observed, and in some regions, the bloom was nearly monospecific.

Annual maxima of chl *a* were associated with samples dominated by *L. polyedrum* in August/September 2005, by

*Pseudo-nitzschia* spp. during March/April 2006 and by a mixed dinoflagellate community in May of 2007 (survey averages of chl *a* levels concentrations are gray bars in Fig. 2C). High spatial variability in phytoplankton biomass throughout the sampling area (indicated by high standard deviations for chl *a* concentrations in Fig. 2C) was observed on several survey dates. This high variability during the summer/autumn of 2005 was mainly due to exceptionally high cell densities of *L. polyedrum* at stations closest to shore,

and during spring of 2006 and 2007 when *Pseudo-nitzschia* bloomed. High spatial variability of chl *a* for the latter years was a consequence of the diatom occurring at high abundance at stations inside and immediately adjacent to the harbor breakwater (Fig. 1). Individual surface concentrations of chl *a* ranged from 0.1 to 72.2  $\mu\text{g L}^{-1}$  during spring periods (mean for all 3 years = 8.0  $\mu\text{g L}^{-1}$ ;  $n = 328$ ), from 0.1 to 120.2  $\mu\text{g chl } a \text{ L}^{-1}$  during summers (mean = 4.3  $\mu\text{g chl } a \text{ L}^{-1}$ ;  $n = 230$ ) and from 0.4 to 65.2  $\mu\text{g L}^{-1}$  in autumn and winter months (mean = 5.1  $\mu\text{g L}^{-1}$ ;  $n = 177$ ).

### Particulate DA

Particulate DA (pDA) in surface water samples was detected at one or more stations during 27 of the 39 surveys conducted between March 2005 and December 2007 (Fig. 2C indicates average concentrations of pDA for each survey, together with the standard deviation across all samples within a survey). Some of the surveys (3–5 each year) yielded samples that exhibited no detectable pDA throughout the sampling area (open circles in Fig. 2C).

High concentrations of pDA in surface waters during spring 2006 and 2007 were preceded or co-occurred with decreases in temperature to  $<13.5^{\circ}\text{C}$ , indicative of upwelling of water from beneath the nutricline (Fig. 2). Detectable but low concentrations of pDA in surface waters occurred on the leading edge of the single cooling event during spring 2005, and following high river discharge in the preceding weeks. Concentrations of pDA were highest during spring, with low or undetectable levels observed during summer and variable but generally low concentrations in autumn and winter (Fig. 2C).

Variability in the maximal concentrations of pDA attained in the samples, and the duration of toxic events, was substantial among the three years (Fig. 2C). The highest concentration of pDA among all samples during 2005 was observed during mid-April (2.91  $\mu\text{g L}^{-1}$ ) and coincided with the highest survey average for pDA during this year (0.38  $\mu\text{g L}^{-1}$ ;  $n = 20$ ). Toxin was detected during 10 consecutive surveys between January and early May 2006 (Fig. 2C), with maximal concentrations of 14.4  $\mu\text{g L}^{-1}$  observed during a bloom in mid-March, and 11.9  $\mu\text{g L}^{-1}$  during a second bloom in mid-April. Averages of pDA for all samples within a survey for these dates were 7.1 and 5.3  $\mu\text{g L}^{-1}$ , respectively ( $n = 20$  each). Similarly, toxin was detected during 5 consecutive surveys during 2007 between mid-March and May (Fig. 2C). The overall duration of the 2007 bloom was shorter than in the previous year, but it was characterized by maximal pDA concentrations that were nearly twice the maximal concentrations of all samples in previous years (up to

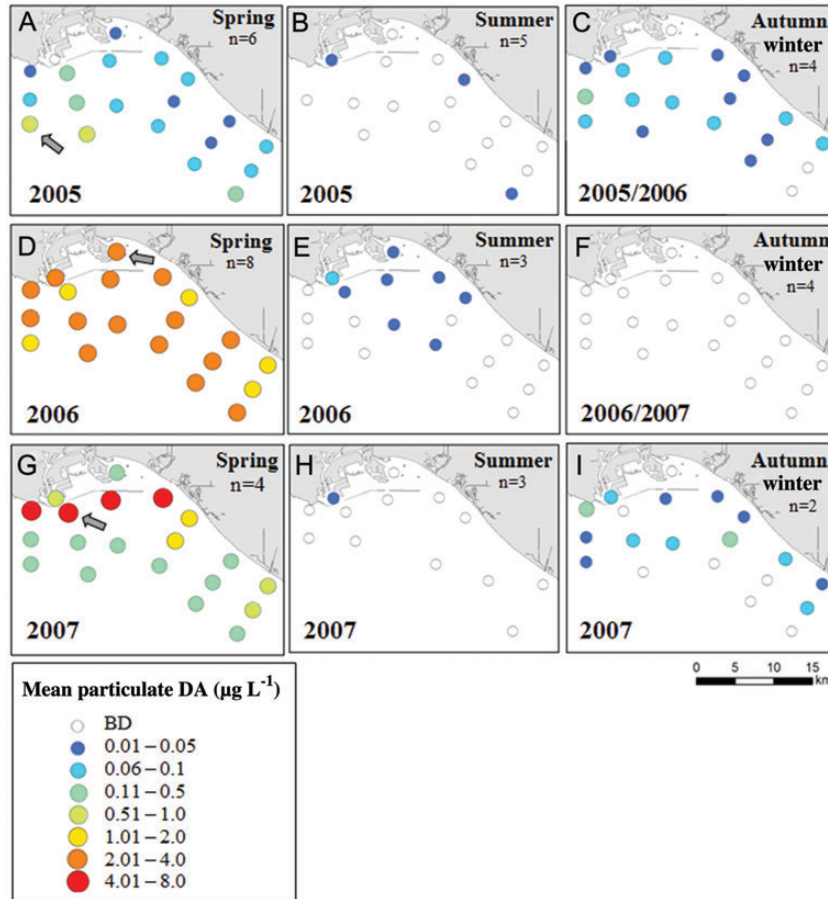
27.0  $\mu\text{g L}^{-1}$  in April; the overall average among all samples in that survey was 5.7  $\mu\text{g L}^{-1}$ ;  $n = 20$ ; Fig. 2C).

### Seasonal patterns of toxin distribution

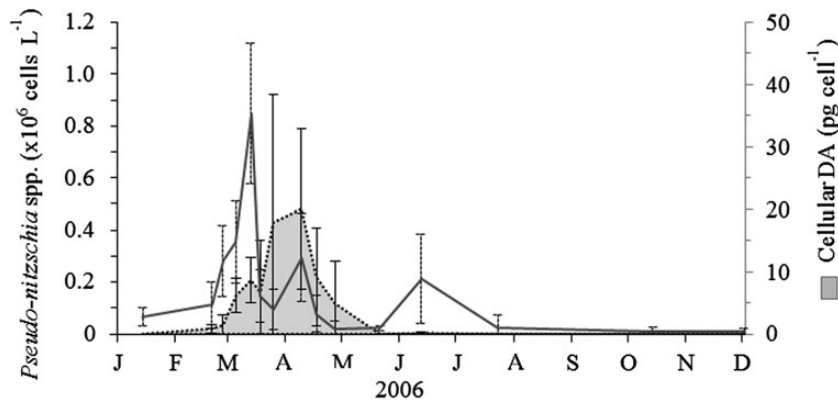
Seasonal patterns of pDA concentrations averaged for spring, summer and autumn/winter for each of the three years illustrated strong seasonality of DA events in surface waters of the study area (Fig. 3). These plots also indicate the generally lower toxin levels observed during the spring bloom in 2005 compared with the following two years (Fig. 3A, D and G). The highest toxin concentrations observed during spring were observed southwest of the San Pedro harbor during 2005 (arrow in Fig. 3A), inside the LA harbor in 2006 (Fig. 3D) and immediately outside the harbor breakwater during 2007 (Fig. 3G). High concentrations of pDA were more uniformly distributed throughout the sampling grid during spring of 2006 (Fig. 3D) and were typically in excess of 2.0  $\mu\text{g L}^{-1}$ . The majority of the remaining sampling sites during all three years averaged  $\leq 1.0 \mu\text{g L}^{-1}$  during the spring sampling campaigns. Low but measureable concentrations of pDA during the summer were detected in surface waters within or just outside the harbor in all three years (Fig. 3B, E and H) while relatively low levels of pDA in the autumn/winter were detected throughout most of the sampling area during 2005 and 2007 but not 2006 (Fig. 3C, F and I).

### *Pseudo-nitzschia* abundances

*Pseudo-nitzschia* cell abundances were obtained for 2006, a major bloom year in the region during which these diatoms always constituted a significant portion of the total diatom community. Absolute abundances of *Pseudo-nitzschia* cells were not determined during 2005, but abundances of *Pseudo-nitzschia* relative to the overall diatom assemblage were estimated at  $<10\%$  for all sampling dates. Abundances of *P. australis* during 2006 ranged from  $<5$  to  $1.44 \times 10^6$  cells  $\text{L}^{-1}$  ( $n = 166$ ; Fig. 4). These abundances of *P. australis* represented 17 to 77% of all diatoms present in the samples. Cell abundances indicated a major bloom of *P. australis* during mid-March (average abundances across the study site of  $847 \times 10^3$  cells  $\text{L}^{-1}$ ;  $n = 20$ ), and a minor bloom again during mid-April (survey average =  $294 \times 10^3$  cells  $\text{L}^{-1}$ ;  $n = 20$ ). Another minor bloom occurred during June (survey average =  $212 \times 10^3$  cells  $\text{L}^{-1}$ ) following a period in which the phytoplankton community was dominated by dinoflagellates. CDA for the *Pseudo-nitzschia* cells in the 2006 surveys was calculated based on *Pseudo-nitzschia* abundances and pDA concentrations (Fig. 4). Average values of cDA for each survey ranged from undetectable to 66.0 pg DA cell $^{-1}$  ( $n = 169$ ) and peaked during April



**Fig. 3.** Seasonal plots of particulate domoic acid (pDA) concentrations in surface waters averaged for each sampling station for spring (**A D** and **G**), summer (**B, H** and **E**) and autumn/winter (**C, F** and **I**) during 2005 (**A–C**), 2006 (**D–F**) and 2007 (**G–I**). The number of surveys that were conducted during each season and for which averages are plotted is indicated in top right corner of each panel. Arrows in panels **A, D** and **G** indicate the sampling stations at which maximal pDA levels were observed in each year. BD = below detection (limit of detection = 0.01  $\mu\text{g L}^{-1}$ ). Number of surveys averaged during each season is shown in the upper right corner of each panel.



**Fig. 4.** Abundances of *Pseudo-nitzschia* spp. (black line) and cellular domoic acid (cDA) concentrations (gray shaded area) in surface waters observed during surveys in 2006. Concentrations are survey averages of all 20 sampling stations. Error bars are standard deviation based on all 20 samples. The *Pseudo-nitzschia* assemblage during 2006 was almost entirely comprised of *P. australis*; other *Pseudo-nitzschia* species were rarely detected (<1% of all SEM/TEM examined *Pseudo-nitzschia* cells).

(survey on April 12) near the end of the major March bloom of *P. australis* and during the first minor bloom during April. The minor bloom of *P. australis* during June corresponded to very low cDA values for the diatom.

The relative contribution of *P. australis* to the diatom assemblages during 2007 was estimated at <5% during all surveys except the one on April 26 when *P. australis* made up ~60% of the diatom community (data not shown). Floating aggregations of *Pseudo-nitzschia* cells were visible at stations within the sampling grid during this survey (Fig. 5). Concentrations within the aggregates (up to ~20 cm in diameter) were not determined; however, overall averages of cell abundances in surface samples (with homogenized densities including previously aggregated cells) were documented at <5 to  $1.12 \times 10^6$  cells  $L^{-1}$  throughout the sampling grid (survey average =  $3.75 \times 10^5$  cells  $L^{-1}$ ;  $n = 15$ ). Concentrations of cDA for this survey were calculated at undetectable to 118 pg DA cell $^{-1}$  ( $n = 15$ ).

### Inorganic nutrients

Surface nutrient concentrations ( $PO_4^{3-}$ ,  $NO_3^- + NO_2^-$  and  $Si(OH)_4$ ) within the sampling region were generally highest during spring and autumn/winter periods (Fig. 6). Nutrient concentrations decreased 1 to 2 orders of magnitude from nearshore to offshore station locations; high values were observed sporadically in time and space during the study. Orthophosphate was highest during spring 2007 ( $9.6 \mu\text{mol L}^{-1} PO_4^{3-}$  during May) at stations off Huntington Beach at the southeastern edge of the sampling grid. Maximal concentrations of nitrogen ( $102 \mu\text{mol L}^{-1} NO_3^- + NO_2^-$  during April 2006) and silicate ( $137 \mu\text{mol L}^{-1} Si(OH)_4$  during March of 2005)

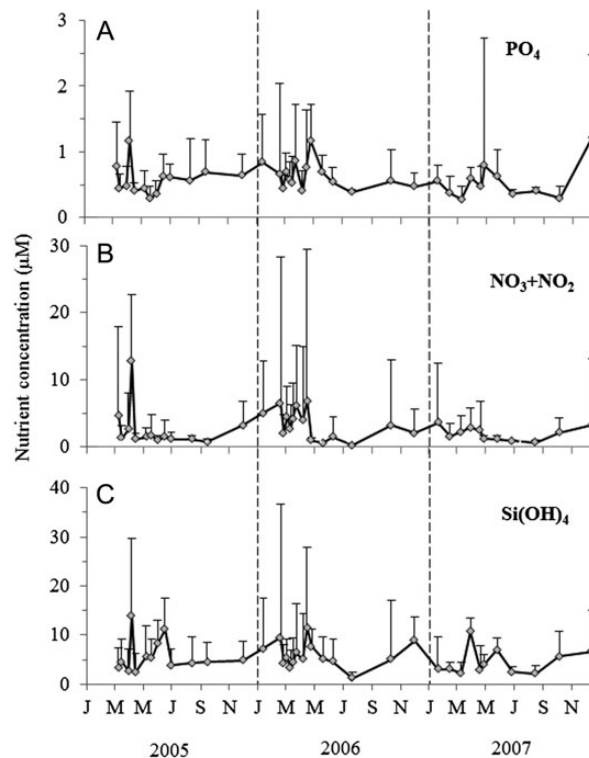


**Fig. 5.** Surface aggregations of *Pseudo-nitzschia* spp. during the April 2007 bloom. Aggregates measured up to ~20 cm in diameter and were observed at several survey locations.

were observed inside the harbor and immediately south of the Los Angeles and San Gabriel river outlets. Throughout the 3-year study period, Si:P ratios averaged 9.2 (range = 0.1–49.7;  $n = 753$ ), N:P ratios averaged 3.7 (range = 0.9–69.5;  $n = 753$ ) and N:Si ratios averaged 0.6 (range = <0.1–33.9;  $n = 746$ ; Fig. 6). Strongest deviations from mean Si:P and N:Si ratios were observed during river runoff periods in 2005, while the lowest N:P and N:Si ratios were observed during 2007.

### Correlation analysis

Statistical analyses were conducted to examine the relationships between pDA, cDA and *Pseudo-nitzschia* abundance and individual physiochemical parameters for the entire study period and for each individual year (data points for which pDA levels fell below the detection limit of the ELISA assay were not included). Cell abundance and pDA were negatively correlated with temperature, and together with cDA positively correlated with chl *a* (Table 1). In addition, increased cDA was associated with increased N:Si ratios and *Pseudo-nitzschia* abundance negatively correlated with  $PO_4^{3-}$  and  $Si(OH)_4$  (Table 1). Detailed analyses on a yearly basis showed that these overall trends were strongly driven by the 2006



**Fig. 6.** Nutrient concentrations ( $\mu\text{M}$ ) measured during in the study area 2005–2007. Values are averages of all 20 sampling stations for each survey and are shown with standard deviations across all stations.



Table 1. Results for Spearman rank correlations.

	<i>P.</i> abund.	Salinity	Temperature	Chl <i>a</i>	PO <sub>4</sub> <sup>3-</sup>	N	Si(OH) <sub>4</sub>	Si:P	N:P	N:Si	<i>n</i>
All years											
pDA	<b>0.57</b>	0.09	-0.29	<b>0.38</b>	-0.01	0.00	-0.1	<b>0.13</b>	0.00	0.11	175–184
cDA		0.07	-0.03	<b>0.18</b>	0.12	0.14	-0.04	0.16	0.12	<b>0.25</b>	135–138
<i>P.</i> abund.		0.09	-0.22	<b>0.48</b>	-0.26	-0.09	-0.18	0.05	0.02	0.06	139–284
2005											
pDA			-0.11	-0.07	-0.09	<b>-0.27</b>	-0.23	-0.23	<b>-0.32</b>	-0.01	65
2006											
pDA	<b>0.6</b>	0.15	-0.32	<b>0.3</b>	-0.09	0.05	-0.21	-0.05	0.12	0.03	124–164
cDA		0.03	-0.12	<b>0.2</b>	0.14	0.15	0	-0.12	0.13	<b>0.23</b>	120–124
<i>P.</i> abund.		0.09	-0.28	<b>0.5</b>	-0.26	-0.11	-0.18	-0.24	0.02	<b>0.28</b>	124–153
2007											
pDA	0.38	0.07	-0.23	<b>0.64</b>	0.16	0.09	-0.21	-0.42	-0.5	<b>0.35</b>	15–61
cDA		0.64	-0.13	<b>0.50</b>	0.16	0.06	0.07	0.00	-0.03	-0.04	15
<i>P.</i> abund.		0.34	-0.05	<b>0.46</b>	0.29	0.22	0.18	-0.07	0.13	0.07	15

Individual correlations between pDA, cDA and *Pseudo-nitzschia* cell abundances (*P.* abund.) and physical, biological and chemical parameters, examined for the entire study period and for each study year separately. Values in bold are significant at  $P=0.05$ .  $N = NO_3^- + NO_2^-$ ;  $n$  = number of pair-wise comparisons.

observations. Interestingly, however, no single parameter was consistently correlated with pDA for all three years nor were correlations for cDA or *Pseudo-nitzschia* abundances consistent (Table 1). Concentrations of pDA in surface waters were negatively correlated with N (=  $NO_3^- + NO_2^-$ ) and N:P ratios for 2005 ( $P < 0.05$ , Table 1), but these factors were not correlated for 2006 or 2007. Toxin concentrations were negatively correlated with temperature and Si(OH)<sub>4</sub> for 2006, negatively correlated with Si:P and N:Si ratios in 2007, and positively correlated with chl *a* for both 2006 and 2007 ( $P < 0.05$ , Table 1). *Pseudo-nitzschia* abundances were negatively correlated with temperature, PO<sub>4</sub><sup>3-</sup>, Si(OH)<sub>4</sub> and Si:P, and positively correlated with chl *a* and N:Si. For 2007, no linkage was observed between either cell number or cDA and any of the physicochemical factors (Table 1). In 2006, cDA in 2006 was positively correlated with chl *a* and N:Si (Table 1). Additional analyses using data summed by individual survey or pooled by season also indicated that correlations to chemical/physical parameters were highly variable (data not shown).

One trend was an association between pDA concentrations (and for *Pseudo-nitzschia* abundance; not shown) and colder, more saline water. This relationship is clearly illustrated when pDA was plotted against temperature and salinity (Fig. 7).

## DISCUSSION

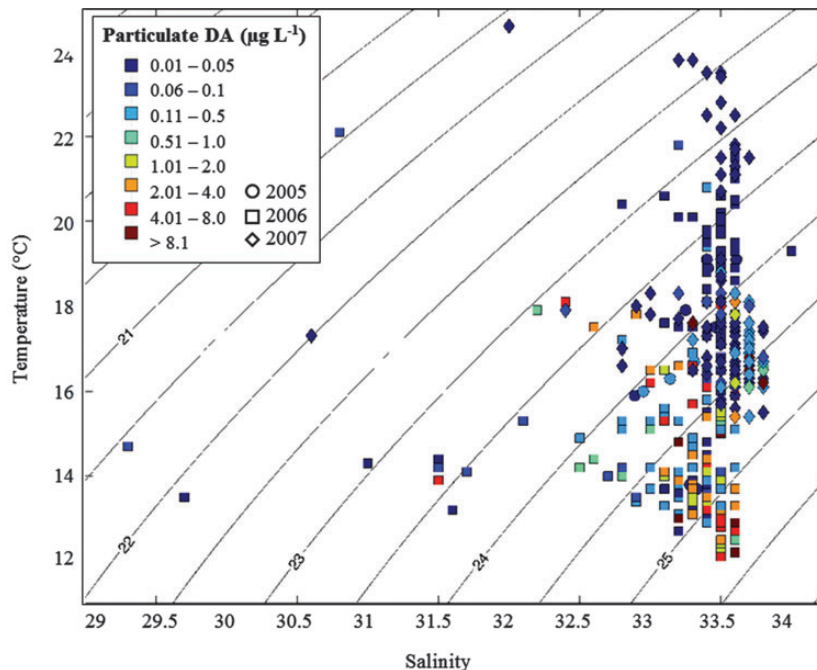
Coastal upwelling and rainfall-dependent river discharge are highly episodic events in southern California. They can provide substantial nutrient injections into coastal waters, although the quality and quantity of these injections varies with the particular type and magnitude of the event. The temporal variability and magnitude of

these events, and their qualitative differences, can affect the extent and type of phytoplankton bloom resulting from such nutrient injections. The goal of this study was to examine the relative contribution of these processes to *Pseudo-nitzschia* blooms and DA production in the region.

### *Pseudo-nitzschia* blooms and DA in the Los Angeles area

Concentrations of pDA observed in surface waters during this study were among the highest reported to date in this region and elsewhere where these events have been documented. Monitoring for abundances of *Pseudo-nitzschia*, bloom dynamics and DA has indicated an apparent increase in recent years of toxic *Pseudo-nitzschia* blooms along the coastline of the Southern California Bight (Schnetzer *et al.*, 2007). The region bordering the Los Angeles/San Pedro harbor region, in particular, has emerged as a perennial “hot spot” for *Pseudo-nitzschia* blooms and DA events. In the present study, the maximum observed pDA for a *Pseudo-nitzschia* assemblage dominated by *P. delicatissima* was 2.9  $\mu\text{g L}^{-1}$  during 2005, while for *P. australis*-dominated, blooms during spring of 2006 and 2007 maxima observed were 14.4 and 27.0  $\mu\text{g L}^{-1}$ , respectively (while *P. delicatissima* outnumbered all other *Pseudo-nitzschia* species in 2005, DA production was not confirmed for this species). Visible accumulations of *Pseudo-nitzschia* were noted at the sea surface at several sampling stations during the 2007 spring bloom, which to our knowledge is the first report of *Pseudo-nitzschia* surface aggregates (up to ~20 cm in diameter).

The blooms documented in our study area extend our knowledge of these events within the same study region reported for 2003 and 2004 when *P. cf. cuspidata* and *P.*



**Fig. 7.** Concentrations of particulate domoic acid (pDA) in surface water samples collected in 2005, 2006 and 2007 plotted on a temperature-salinity plot. Note the relationship between pDA concentrations ( $\mu\text{g L}^{-1}$ ) in surface waters with relatively low temperature ( $^{\circ}\text{C}$ ) and high salinity. Isolines show densities ( $\text{kg m}^{-3}$ ).

*australis* were the primary DA producers. Maximal concentrations of pDA observed during those years were  $12.7$  and  $1.9 \mu\text{g L}^{-1}$ , respectively (Schnetzer *et al.*, 2007). Extremely high values (up to  $52.3 \mu\text{g L}^{-1}$ ) were recently documented from samples collected during March 2011 from surface waters of the San Pedro Channel 5–10 km south of our study grid (Stauffer *et al.*, 2012). Maximal concentrations of pDA for northern and central California have been reported at  $\sim 27 \mu\text{g L}^{-1}$  for the Juan de Fuca region (Trainer *et al.*, 2009) and up to  $49 \mu\text{g L}^{-1}$  for the Santa Barbara Channel in 2005 (Anderson *et al.*, 2009).

The maximal concentration of cDA calculated for *Pseudo-nitzschia* cells collected from coastal waters in our study area fell within the range of the highest values published to date;  $\sim 120 \text{ pg cell}^{-1}$  for both 2004 and 2007 (Schnetzer *et al.*, 2007). Cellular DA values of  $75$  and  $78 \text{ pg cell}^{-1}$  have been reported for Monterey and Morro Bay, respectively (Scholin *et al.*, 2000; Trainer *et al.*, 2000), and  $216 \text{ pg cell}^{-1}$  for natural assemblages from the Santa Barbara Channel (Anderson *et al.*, 2009). The absolute values of cDA must be considered crude estimates of maximal cellular toxin concentrations because these calculations attribute all pDA in a sample to intracellular content of the *Pseudo-nitzschia* cells in the sample. DA may also be contained in grazing zooplankton or microzooplankton, or in detrital material within the sample. Nevertheless, the relative magnitude of these

values indicates that the blooms in our study region represented highly toxic bloom events.

The concentrations of DA we observed off the coast of southern California constituted a significant health risk for marine animals in the region (Caron *et al.*, unpublished data; California Marine Mammal Stranding Network). Moreover, the rapid transport of toxic *Pseudo-nitzschia* cells to depths of  $> 800 \text{ m}$  within the Los Angeles coastal region has raised questions regarding the potential impact of these annual toxic events on toxin accumulation in benthic food webs in the area (Schnetzer *et al.*, 2007; Sekula-Wood *et al.*, 2009).

### Coastal upwelling, river discharge and *Pseudo-nitzschia* spp

Storm events during winter and early spring in the Southern California Bight can lead to significant rainfall followed by episodic river discharge into the coastal ecosystem, particularly within our study area. The San Pedro Bay receives output from three of the largest rivers in Southern California, whose watershed includes highly urbanized terrestrial environments that have a high percentage of impervious terrain (Fig. 2). Wind events in the region that result in upwelling, however, are usually not accompanied by significant rainfall. Differences in the magnitude and timing of these two processes (wind-driven upwelling and river discharge) among the three

years of this study allowed us to examine their relative importance in stimulating spring blooms of *Pseudo-nitzschia* and DA production within the Southern California Bight region (Horner *et al.*, 1997; Schiff *et al.*, 2000). Inputs of nutrient-rich water from river discharge or upwelling generally favor bloom formation by diatoms during the spring in this region (Venrick, 1998; Schnetzer *et al.*, 2007), but their specific contributions to blooms of toxic *Pseudo-nitzschia* species have not been clearly established.

Record rainfall during early 2005 contributed massive amounts of freshwater discharge into the San Pedro basin (Fig. 2; <http://waterdata.usgs.gov/ca/nwis>). Substantial upwelling or vertical mixing did not occur until mid-spring (Fig. 2A and B) following the earlier rain and runoff events. Chlorophyll concentrations of that year were indicative of phytoplankton abundances similar in magnitude to subsequent years (chl *a*, Fig. 2B), but *Pseudo-nitzschia* spp. (mainly *P. delicatissima*) remained a minor component of the phytoplankton community during 2005, and pDA concentrations in the region were the lowest for the three years investigated (Fig. 3A).

In contrast, river discharges into the San Pedro basin during 2006 and 2007 were well below annual averages (Fig. 2A; <http://waterdata.usgs.gov/ca/nwis>), yet these two years were characterized by repeated cooling events (particularly 2006) when low-temperature water ( $\sim 13.5^{\circ}\text{C}$ ) was observed in surface waters during late winter and spring. Interestingly, high concentrations of pDA in surface waters throughout the study area were also observed in both years (Fig. 3D and G). *P. australis* dominated spring assemblages during the ensuing blooms that were observed in 2006 and 2007.

The different responses of the phytoplankton community within our study area during these three years raise the probability that nutrient input to this coastal ecosystem, per se, was not a complete explanation for blooms of *Pseudo-nitzschia* and outbreaks of DA. We observed correlations between nutrient concentrations (or nutrient ratios) and pDA, cDA and *Pseudo-nitzschia* abundances but these relationships were not consistently observed among the three years (Table 1). A clear link was apparent, however, between high toxin concentrations in water samples with both relatively low temperature and high salinity (Fig. 7). This relationship presumably indicates a strong relationship of toxic *Pseudo-nitzschia* cells with denser, upwelled water, but generally not with periods of warmer (summer) or less saline (river discharge) water. This linkage between upwelling to the surface of denser water and *Pseudo-nitzschia* bloom emergence within the Southern California Bight agrees with previous field studies along the Northern and central California coast that have associated pDA in surface waters with upwelling events (Trainer *et al.*, 2000; Kudela *et al.*, 2005;

Anderson *et al.*, 2006; Lane *et al.*, 2010). Studies have also shown the role that mesoscale eddies play in the transport and concentration of toxic *Pseudo-nitzschia* along the California shelf (Anderson *et al.*, 2006; Trainer *et al.*, 2009). How small-scale eddies that form within the San Pedro Channel (DiGiacomo and Holt, 2001) impact *Pseudo-nitzschia* dynamics in the Los Angeles area remains to be determined.

A general relationship between upwelling events along the coast in our study area and the presence of DA is indicated by our results (Fig. 7), but the exact nature of the relationship was not fully resolved by the present study. Several explanations are possible, one being that specific macro- or micro-nutrients are supplied from deep water during upwelling events that specifically and uniquely favor the growth of *Pseudo-nitzschia* spp. and within this group *P. australis* as one of the most potent species (Trainer *et al.*, 2009). Similarities in the absolute concentrations of macronutrients ( $\text{PO}_4^{3-}$ ,  $\text{NO}_3^- + \text{NO}_2^-$  and  $\text{Si}(\text{OH})_4$ ) during years with toxic and non-toxic blooms do not appear to support this contention (Fig. 6), although some organic nutrients (e.g. urea) and micronutrients (trace metals, vitamins) that might play a role in establishing community composition were not measured in this study. Moreover, significant correlations between nutrient ratios and pDA were observed for some subsets of the overall dataset (Table 1), suggesting that very subtle changes in environmental conditions may stimulate toxin production. Alternatively, there is growing evidence that *Pseudo-nitzschia* populations occur in subsurface phytoplankton maxima within our study region, and these populations rise into the surface waters during upwelling events providing “seed populations” to initiate toxic blooms (Noble *et al.*, 2009; Seegers, Los Angeles, pers. comm.). Given this notion, strong river discharge in early 2005 could have delayed such seeding(s) resulting in a phytoplankton (and *Pseudo-nitzschia*) assemblage that differed strongly from both subsequent years. Gaining a better understanding of these cause-effect relationships will be essential as field programs for HABs are typically restricted to monitoring algal abundance changes and commensurate physiochemical conditions in surface waters. The continued and increased deployment of remote sensing technology (i.e. Autonomous Underwater Vehicles) has begun to provide high spatial and temporal scale data to close this knowledge gap (Smith *et al.*, 2010). Most significantly, perhaps, our study does not indicate a direct link between river discharge during episodic winter rain events and the stimulation of toxic blooms of *Pseudo-nitzschia* spp. following the runoff events. This finding is somewhat surprising given that these buoyant discharges can contribute significantly to nutrient enrichment of surface waters and stratification of the water

column in coastal waters, and given the growing recognition of a general relationship between coastal eutrophication and harmful algal blooms globally (Glibert *et al.*, 2005). We speculate that ratios of macronutrients (or perhaps micronutrient availability) within the plume of river water acted to stimulate species of phytoplankton other than *Pseudo-nitzschia* spp. during our study. Whether this relationship is unique to this study region, or to the period over which our surveys were conducted, is difficult to conclude until a longer time series of measurements can be examined.

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