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# *Pseudo-nitzschia* species composition varies concurrently with domoic acid concentrations during two different bloom events in the Southern California Bight

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The San Pedro Shelf (SPS) region of the Southern California Bight has witnessed an increase of *Pseudo-nitzschia* spp. blooms during the past decade, although the domoic acid (DA) concentrations observed during these events have varied considerably. This study compared the extent, timing and environmental controls of *Pseudo-nitzschia* blooms that were observed in two consecutive years on the SPS. Environmental conditions were characterized during ship-board surveys during spring 2013 and 2014 along an onshore–offshore transect at surface and subsurface depths. A *Pseudo-nitzschia* bloom of similar cell abundances was observed during each year, yet maximal DA concentrations differed by nearly two orders of magnitude. Environmental parameters were favorable for *Pseudo-nitzschia* spp. growth in both years, but few factors could be identified that specifically pertained to DA, with the exception that toxicity correlated negatively with dissolved silicic acid concentrations. Automated ribosomal intergenic spacer analysis for *Pseudo-nitzschia* species indicated that the relative abundance of toxin-producing species had a strong influence on DA concentrations between years, with high-DA concentrations corresponding to *Pseudo-nitzschia* communities dominated by *P. australis*/*P. seriata*. Factors explaining the preferential growth of particular *Pseudo-nitzschia* species remain enigmatic but are important for predicting toxic events attributable to these taxa.

**KEYWORDS:** domoic acid; *Pseudo-nitzschia*; southern California; harmful algal bloom; coastal upwelling

## INTRODUCTION

*Pseudo-nitzschia* is a cosmopolitan genus of pennate diatom that is known to produce the water-soluble neurotoxin domoic acid (DA) (Bates *et al.*, 1989). The genus is commonly observed in upwelling systems, where it can form toxic blooms (Trainer *et al.*, 2012). DA accumulates in the food web via trophic transfer, contaminating fisheries and presenting human health hazards. Consumption of contaminated seafood can cause Amnesic Shellfish Poisoning (ASP) in humans, which manifests clinically with symptoms of diarrhea, gastrointestinal pain, disorientation and memory loss, and in extreme cases, can cause death. Closures of DA contaminated fisheries can result in significant economic impacts (Wessells *et al.*, 1995). Toxic *Pseudo-nitzschia* blooms can also have a negative impact on animal populations in marine ecosystems, causing sickness and mass mortality events in marine animals, including sea lions, sea otters, whales and seabirds (Fritz *et al.*, 1992; Scholin *et al.*, 2000; Lefebvre *et al.*, 2002; Kvitik *et al.*, 2008).

There are more than 40 described species of *Pseudo-nitzschia*, although not all species are toxigenic. At least 14 species have exhibited the ability to produce DA under a variety of conditions (Lelong *et al.*, 2012; Trainer *et al.*, 2012), but DA production is not constitutive. Multiple environmental stressors appear to stimulate toxin production and DA production among and within toxigenic species can range several orders of magnitude (Thessen *et al.*, 2009; Lelong *et al.*, 2012; Trainer *et al.*, 2012). Therefore, it has been difficult to establish a simple relationship between *Pseudo-nitzschia* cell abundance and bloom toxicity in nature (Seubert *et al.*, 2013).

The availability and the form of macro- and micronutrients influence DA production in many toxigenic *Pseudo-nitzschia* species. Culture and field studies have indicated that macronutrient limitation (specifically phosphorus and silicate limitation) can trigger or enhance DA production (Pan *et al.*, 1996a, 1996b; Fehling *et al.*, 2004). The form of nitrogen can also modulate both toxin production and cell growth and this effect can vary by species and strain (Thessen *et al.*, 2005, 2009; Howard *et al.*, 2007; Cochlan *et al.*, 2008; Kudela *et al.*, 2008). Iron limitation, copper toxicity (Rue and Bruland, 2001; Maldonado *et al.*, 2002; Wells *et al.*, 2005) and increased partial CO<sub>2</sub> concentrations (Tatters *et al.*, 2012) have also been reported to increase DA production. Additionally, studies have shown that combined environmental stressors such as silicate limitation and increased partial CO<sub>2</sub> concentrations can synergistically enhance cell toxicity (Tatters *et al.*, 2012). Physical factors, such as upwelling (Kudela *et al.*, 2005; Schnetzer *et al.*, 2013), mesoscale eddies (Anderson *et al.*, 2006) and water column structure (Ryan *et al.*, 2014), have also been

correlated with toxic bloom formation. Overall, the environmental cues controlling bloom formation and toxin production appear to be quite complex, involving a suite of chemical, physical and biological factors.

The presence of *Pseudo-nitzschia* species has been documented in the Southern California Bight since the 1930s (Lange *et al.*, 1994); however, only recently has there been documentation of DA and other HAB events in the region (Schnetzer *et al.*, 2007, 2013; Caron *et al.*, 2010). The San Pedro Shelf (SPS) is an emerging DA “hot spot” based on the increasing frequency and concentrations of observed toxic events (Schnetzer *et al.*, 2013). Some of the highest recorded particulate DA (pDA) concentrations (52.3 µg L<sup>-1</sup>) were measured in the San Pedro Channel in 2011 (Stauffer *et al.*, 2012). The coastal areas surrounding the SPS are densely populated and are impacted by urban discharge. Anthropogenic nitrogen inputs in the SPS region, in particular, ammonium from wastewater discharge, can rival the input of new nitrogen from upwelling (Howard *et al.*, 2014). This may play a role in regional bloom formation (Nezlin *et al.*, 2012) and perhaps the upsurge in DA events due to the influence of nitrogen form on both toxin production and cell growth (Howard *et al.*, 2007). The region is also characterized by seasonal upwelling-favorable wind patterns (Nezlin and Li, 2003) and highly episodic rain and river discharge events (Schnetzer *et al.*, 2013; Seubert *et al.*, 2013). Upwelling has been linked to the formation of blooms in the region, resulting in toxic *Pseudo-nitzschia* blooms that show strong seasonality, typically occurring during spring (Schnetzer *et al.*, 2013; Seubert *et al.*, 2013). Nevertheless, while these general triggers for bloom initiation are known, the specific factors leading to toxic events are not well constrained.

The goal of this study was to examine the extent, duration and toxicity of *Pseudo-nitzschia* blooms during spring on the SPS in the waters proximal to Newport Beach, CA, USA. The physiochemical and biological variables associated with the *Pseudo-nitzschia* blooms were measured at surface and subsurface chlorophyll maximum (SCM) depths on a near-weekly basis during the springtime period of March–April in 2013 and April–May in 2014. The relationships between *Pseudo-nitzschia* composition, abundance and toxicity were examined in relation to environmental factors.

## METHOD

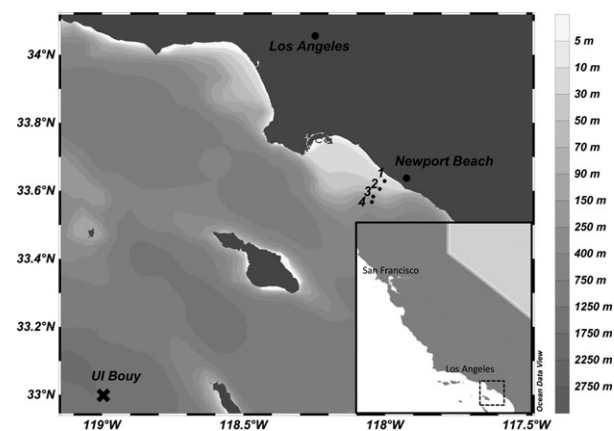
### Study site description

Eleven shipboard surveys were conducted during springtime in 2013 and 2014 on the SPS near Newport Beach,

CA to compare the biological and physiochemical parameters that influence the extent, duration and DA concentrations of *Pseudo-nitzschia* blooms. The study consisted of four sampling stations along an onshore–offshore transect (Fig. 1). Shipboard surveys were conducted in 2013 on 8 March, 15 March, 17 March and 5 April on the *R/V Yellowfin* and on 22 March on the *R/V Rachel Carson*. Surveys in 2014 were conducted on 2 April, 7 April, 25 April and 5 May on the *R/V Yellowfin* and 14 April and 18 April on the *R/V Rachel Carson*.

### Discrete sample collection

Discrete water samples were collected from the surface (~1–2 m depth) and the SCM (depths of the latter varied, Table 1) using a SBE32 water-sampling carousel. A total of 80 discrete samples, 36 in 2013 and 44 in 2014, were collected and processed for a variety of analyses during the study. Vertical profiles of environmental parameters were conducted at each station to locate the SCM, which was defined as the highest chlorophyll fluorescence within the water column. Profiles of *in situ* parameters were obtained using a Sea-Bird SBE911plus CTD equipped with a Wet Labs ECO-FLNTU(RT)D fluorometer, SBE3plus temperature sensor and SBE4C conductivity sensor (Sea-Bird Electronics, Bellevue, WA, USA) (Supplementary Fig. 1). Salinity data were not available for 7 April, 25 April and 5 May from the 2014 surveys due to instrument failure.



**Fig. 1.** Map of shipboard survey stations in the waters of the San Pedro Shelf near Newport Beach, CA. The inset map depicts the California coastline with a dashed black box outlining the study site within the Southern California Bight. Depth contours are shown on the legend. Water depths at Stations 1–4 were 15, 30, 57 and 190 m, respectively. Wirewalker profiling instruments were moored at Stations 2 and 3. The location of the NOAA/NMFS/PFEG buoy used to calculate the regional Bakun UI data is indicated with a black x.

### Chlorophyll *a* and microalgal community composition

Total chlorophyll *a* (chl *a*) was analyzed fluorometrically via the non-acidification method using a Trilogy Turner Designs fluorometer (Turner Designs, Sunnyvale, CA, USA). Samples for chl *a* analysis were collected by filtration onto glass fiber filters (Sterlitech, grade F, Kent, WA, USA). Sample volumes varied depending on the *in situ* chlorophyll fluorescence and ranged from 50 mL to 100 mL. Filters were extracted in 100% acetone at  $-20^{\circ}\text{C}$  in the dark for 24 h.

Seawater samples were collected and preserved with 1% formalin (final concentration) for the determination of phytoplankton community composition (cells  $> 10\ \mu\text{m}$  in size). Preserved samples were stored at  $4^{\circ}\text{C}$  until analysis. Phytoplankton were enumerated using a Leica DM IRBE inverted light microscope (Leica Microsystems, Buffalo Grove, IL, USA) at  $400\times$  after settling 25 mL of the sample in Utermöhl chambers for approximately 24 h (Utermöhl, 1958). The counting method as applied yielded a limit of detection of  $\sim 3.0 \times 10^3\ \text{cells L}^{-1}$ . Cells were categorized according to four groupings: diatoms, dinoflagellates, grazers and other. Organisms within each group were identified to genus whenever possible.

### Measurements of particulate and cellular DA

Subsamples were collected in duplicate from all discrete water samples for pDA analysis via gentle vacuum filtration of 200 mL of sample water onto glass fiber filters. Filters were stored at  $-20^{\circ}\text{C}$  in the dark until analyzed. Filters were extracted in 3 mL of 10% methanol, sonicated for 15 s and centrifuged for 15 min at 4 000 rpm. The supernatant was analyzed via Mercury Science, Inc., DA Enzyme-Linked ImmunoSorbant Assay (ELISA: Mercury Science, Durham, NC, USA) according to the methods described in Litaker *et al.* (2008). The method had a detection limit of  $2.0 \times 10^{-2}\ \mu\text{g L}^{-1}$  as applied in our samples. Samples below detection were assumed to be zero for all calculations and statistical analyses. Cellular DA concentrations (cDA) were calculated from pDA concentrations and abundances of *Pseudo-nitzschia* cells whenever there were detectable quantities of each parameter.

### Analysis of *Pseudo-nitzschia* species composition using automated ribosomal intergenic spacer analysis

Six filters archived for pDA were opportunistically analyzed for *Pseudo-nitzschia* species community composition via automated ribosomal intergenic spacer analysis

*Table I: Dissolved nutrient concentrations and ratios for all stations at the surface and the SCM during spring 2013 and 2014 on the San Pedro Shelf. Dissolved nutrient concentrations:  $\text{NO}_x^-$  (nitrate+nitrite), ammonium, urea, phosphate and silicic acid are reported in  $\mu\text{M}$ . Ratios of  $\text{NO}_x^-$ :phosphate (N:P),  $\text{NO}_x^-$ :silicic acid (N:Si) and silicic acid:phosphate (Si:P) are reported as atom:atom. The mean, standard deviation (SD), range, number of samples (n), number of samples below the detection limit (BD) and average depths with standard deviation are shown*

	Mean	SD	Range	n	BD	Mean	SD	Range	n	BD
	Surface 2013 (1.8 ± 0.4 m)					Surface 2014 (1.8 ± 0.4 m)				
$\text{NO}_x^-$	0.97	1.60	0–6.09	18	4	1.18	1.46	0.05–4.96	21	0
$\text{NH}_4^+$	0.18	0.33	0–1.36	18	5	0.18	0.15	0.03–0.68	22	0
Urea	0.09	0.03	0.04–0.16	18	0	0.11	0.07	0.05–0.35	22	0
$\text{PO}_4^{3-}$	0.27	0.16	0–0.67	18	2	0.23	0.11	0.09–0.41	21	0
$\text{SiO}_4^{2-}$	1.65	2.08	0–7.62	18	3	3.12	2.47	0.75–7.08	21	0
N:P	4.68	10.7	0–36.3	18	6	3.81	3.71	0.32–13.5	21	0
N:Si	0.63	0.77	0–2.60	18	6	0.29	0.25	0.02–0.89	21	0
Si:P	4.84	5.81	0–17.0	18	5	12.1	5.98	4.65–23.5	21	0
	SCM 2013 (12.9 ± 3.6 m)					SCM 2014 (15.0 ± 1.2 m)				
$\text{NO}_x^-$	2.55	2.50	0–8.11	18	1	4.28	4.65	0.21–14.6	21	0
$\text{NH}_4^+$	0.44	0.62	0–2.51	18	4	0.37	0.56	0.02–2.39	22	0
Urea	0.09	0.09	0–0.44	18	1	0.10	0.04	0.04–0.18	22	0
$\text{PO}_4^{3-}$	0.37	0.13	0.13–0.64	18	0	0.49	0.29	0.18–1.17	21	0
$\text{SiO}_4^{2-}$	2.93	1.61	0–5.78	18	1	5.81	4.14	1.18–14.3	21	0
N:P	8.30	9.14	0–26.5	18	1	6.86	4.82	0.76–15.3	21	0
N:Si	0.77	0.63	0–2.09	18	1	0.58	0.34	0.08–1.02	21	0
Si:P	8.92	6.09	0–25.2	18	1	11.2	3.53	4.82–16.6	21	0

(ARISA). ARISA is a community analysis technique that utilizes the ITS1 region as a molecular marker to resolve *Pseudo-nitzschia* taxonomy in natural samples (Hubbard *et al.*, 2008). Samples were collected at Stations 2 and 4 on 15 March 2013, Stations 1 and 4 on 5 April 2013, Station 3 on 7 April 2014 and Station 3 on 5 May 2014. All samples were collected at the surface. Samples were collected via gentle vacuum filtration of 200 mL of sample water onto glass fiber filters and archived at  $-20^\circ\text{C}$  in the dark until extraction. Environmental DNA was extracted using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) and amplified in preparation for ARISA using the *Pseudo-nitzschia*-specific ITS1 primer set PnAll F/R according to the methods outlined in Hubbard *et al.* (2014). Purification of PCR products for ARISA was conducted using MultiScreen PCR $\mu$ 96 filter plates (EDM Millipore, Darmstadt, Germany) and 1 ng of product was analyzed on an ABI 3730 XL using a LIZ600 size standard. Electropherogram analysis with DAX software (Van Mierlo Software Consultancy, Eindhoven, Netherlands) used the same peak calling criteria outlined in Hubbard *et al.* (2014). To identify the novel 140 bp ARISA peak, direct sequencing was conducted on PCR product resulting from successive PCRs with the PnAll primer pair. Products were visualized on a 3% agarose gel following electrophoresis. The target band was excised and purified using the QIAgen Gel Purification Kit (Qiagen Inc., Valencia, CA, USA), and sequenced bidirectionally with the PnAll primers by

Eurofins Genomics. Complementary sequences were aligned and edited using Sequencher<sup>®</sup> version 5.1 DNA sequence analysis software (Gene Codes Corporation, Ann Arbor, MI, USA) and identified by querying the Genbank nucleotide (nr/nt) database with the BLAST function (Altschul *et al.*, 1990); a consensus sequence was submitted to GenBank (accession # MG195950). Other sequence data from U.S. west coast *Pseudo-nitzschia* species (Hubbard *et al.*, 2008, 2014; Marchetti *et al.*, 2008; Smith *et al.*, 2012; Carlson *et al.*, 2016) were utilized for ARISA peak assignments (Supplementary Table I).

### Dissolved nutrient measurements

Nitrate + nitrite (hereafter referred to as  $\text{NO}_x^-$ ), phosphate, silicic acid, ammonium and urea samples were collected via filtration through 0.22  $\mu\text{m}$  acetate luer-lock syringe filters from all discrete samples. Samples collected for analysis of  $\text{NO}_x^-$ , phosphate and silicic acid were collected in acid-rinsed plastic scintillation vials. Samples for urea and ammonium analysis were collected in separate containers following the same procedure. Samples were frozen at  $-20^\circ\text{C}$  until analyzed.  $\text{NO}_x^-$ , phosphate and silicic acid were measured on a QuickChem 8500 Flow Injection Analysis system (Lachat Instruments; Hatch Company, Loveland, CO, USA). Urea was measured spectrophotometrically according to the method described in Mulvenna and Savidge (1992) on a Varian Cary 50 Bio UV/Visible Spectrophotometer (Varian Medical Systems,

Palo Alto, CA, USA). Ammonium was measured fluorometrically on a TD-700 fluorometer (Turner Designs, Sunnyvale, CA, USA) according to the methods described in Holmes *et al.* (1999). Atom:atom nutrient ratios were calculated for  $\text{NO}_x^-$ :phosphate (N:P),  $\text{NO}_x^-$ :silicic acid (N:Si), silicic acid: $\text{NO}_x^-$  (Si:N) and silicic acid:phosphate (Si:P).

### Regional upwelling estimates

Regional upwelling was determined using Bakun Upwelling Index (UI) values from a NOAA/National Marine Fisheries Service (NMFS)/Pacific Fisheries Environmental Group (PFEG) buoy located at 33°N 119°W (Fig. 1), which is ~100 km offshore to the southwest of the study region (the station closest to the study area). The UI provides an estimate of upwelling strength based on the calculated upwelling-favorable wind forcing on the ocean surface. Indices are expressed in units of cubic meters per second along each 100 meters of coastline. Data were obtained from the PFEG online database (<http://www.pfeg.noaa.gov/>).

### Instrumented measurements using wirewalker vertical profilers

Wirewalker vertical profilers (Rainville and Pinkel, 2001; Pinkel *et al.*, 2011) were moored near Station 2 (33.604°N, -118.023°W) and Station 3 (33.582°N, -118.038°W) during the study (Fig. 1). A wirewalker is a profiling platform that uses wave energy to gain downward motion of a positively buoyant instrument array along a moored wire. The data provided continuous vertical profiles of pertinent environmental variables as defined by attached sensor packages. Wirewalkers were deployed during both years of this study but data are only presented from the 2014 deployment. The 2013 deployment period ended prior to the bloom event in April (data not presented here). Each wirewalker was outfitted with a WETLabs water quality monitor (WQM) that was equipped with a Sea-Bird CTD (SBE-37) that yielded depth, temperature and conductivity, a SBE-43 dissolved oxygen sensor and a WETLabs optical chlorophyll *a* and turbidity sensor. The WQM sampled at a rate of 1 Hz, yielding a vertical resolution of  $\approx 1$  m following data analysis. The WQM had a 40-min sampling regime during each 1-h period. The sensor package at Station 2 collected profiles from 2 to 25 m. Data were collected from 31 March until 5 April (2014), after which time the instrument package malfunctioned. Data collection resumed on 16 April after replacement of the instrument package, and data collection continued until 5 May. A total of 871 profiles were

collected prior to sensor malfunction and 3965 profiles were collected after the sensor package malfunction was corrected. The sensor package at Station 3 collected profiles from 2 to 47 m. A total of 2545 profiles were collected at Station 3 from 31 March until 16 April, after which time the data collection failed.

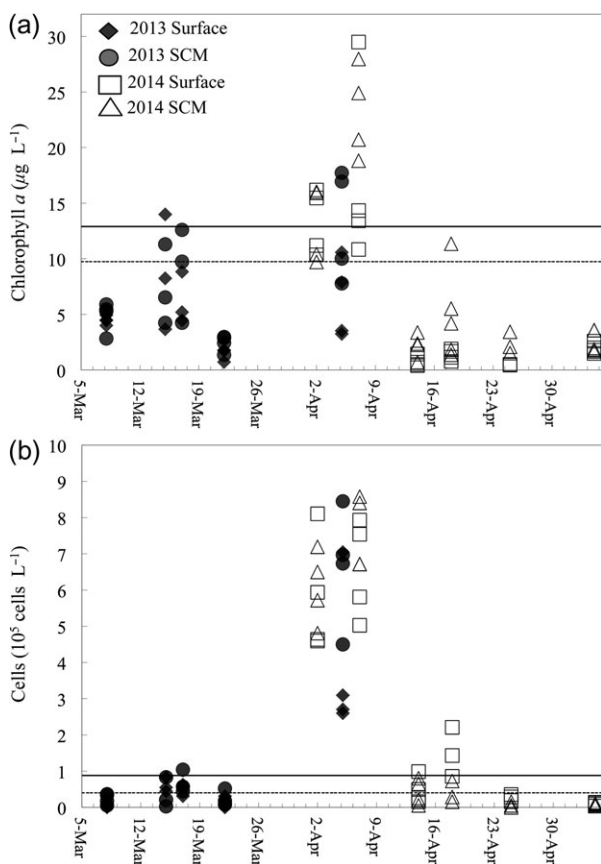
### Statistical analysis

Temperature, salinity, dissolved nutrients, nutrient rations, chl *a*, *Pseudo-nitzschia* cell abundances, pDA and cDA concentrations were organized into data sets that were categorized by all data, data by year and data by year and depth. Pair-wise comparisons were made between parameters in these data sets to test for bulk differences between years (e.g. 2013 vs. 2014) and year by depth (e.g. 2013 surface vs. 2014 surface). The Mann–Whitney rank sum test was used to compare parameters and statistically significant differences were determined at  $P < 0.05$  (Mann & Whitney, 1947). Spearman rank order correlation analysis was used to determine the strength of association between pDA, cDA and *Pseudo-nitzschia* abundances and environmental variables. The analysis was performed on the entire data set (2013 + 2014) and by each individual year. A significant correlation was defined as variables with a positive or negative Spearman's correlation coefficient ( $\rho$ ) with a  $P < 0.05$ . Statistical analyses were performed in SigmaPlot (v.11.0.0, Systat Software, Inc.).

## RESULTS

### Chlorophyll *a* concentrations and phytoplankton community composition

Chlorophyll *a* was measured in a total of 80 discrete samples during the study. In 2013, a total of seven samples exceeded chl *a* concentrations of a minor bloom ( $\geq 10 \mu\text{g L}^{-1}$ ; as defined by Seubert *et al.* (2013) for the region) and three additional samples exceeded major bloom values ( $\geq 13 \mu\text{g L}^{-1}$ ). In 2014, five samples exceeded minor bloom concentrations and additional eleven samples exceeded concentrations of a major bloom (Fig. 2a). Overall, chl *a* concentrations between the 2 years overlapped, and there were no significant differences in these values when averaged for each of the 2 years for samples collected at the surface or SCM ( $P > 0.05$ ). The average chl *a* concentration in surface samples during 2013 was  $5.19 \pm 3.46 \mu\text{g L}^{-1}$ , while the average during 2014 was  $6.04 \pm 7.67 \mu\text{g L}^{-1}$ . Chl *a* in the SCM averaged  $7.23 \pm 4.87 \mu\text{g L}^{-1}$  and  $8.30 \pm 8.40 \mu\text{g L}^{-1}$  during 2013 and 2014, respectively.



**Fig. 2.** (a) Chlorophyll *a* concentrations ( $\mu\text{g L}^{-1}$ ) versus sampling date for 2013 and 2014. Solid line marks the criteria for a major phytoplankton bloom ( $\geq 13 \mu\text{g chl } a \text{ L}^{-1}$ ) and the dotted line shows the criteria for a minor bloom ( $\geq 10 \mu\text{g L}^{-1}$ ) according to Seubert *et al.* (2013). Closed diamonds represent 2013 surface chl *a* concentrations, closed circles represent 2013 SCM chl *a* concentrations, open squares represent surface 2014 chl *a* concentrations and open triangles represent SCM 2014 chl *a* concentrations. (b) *Pseudo-nitzschia* cell abundances ( $10^5 \text{ cells L}^{-1}$ ) from each sampling date in 2013 and 2014. Solid line marks the criteria for a major *Pseudo-nitzschia* bloom ( $\geq 8.80 \times 10^4 \text{ cells L}^{-1}$ ) and the dotted line shows the criteria for a minor bloom ( $\geq 4.00 \times 10^4 \text{ cells L}^{-1}$ ) according to Seubert *et al.* (2013). Closed diamonds represent 2013 surface *Pseudo-nitzschia* abundances, closed circles represent 2013 SCM *Pseudo-nitzschia* abundances, open squares represent 2014 *Pseudo-nitzschia* abundances at the surface and open triangles represent SCM *Pseudo-nitzschia* abundances.

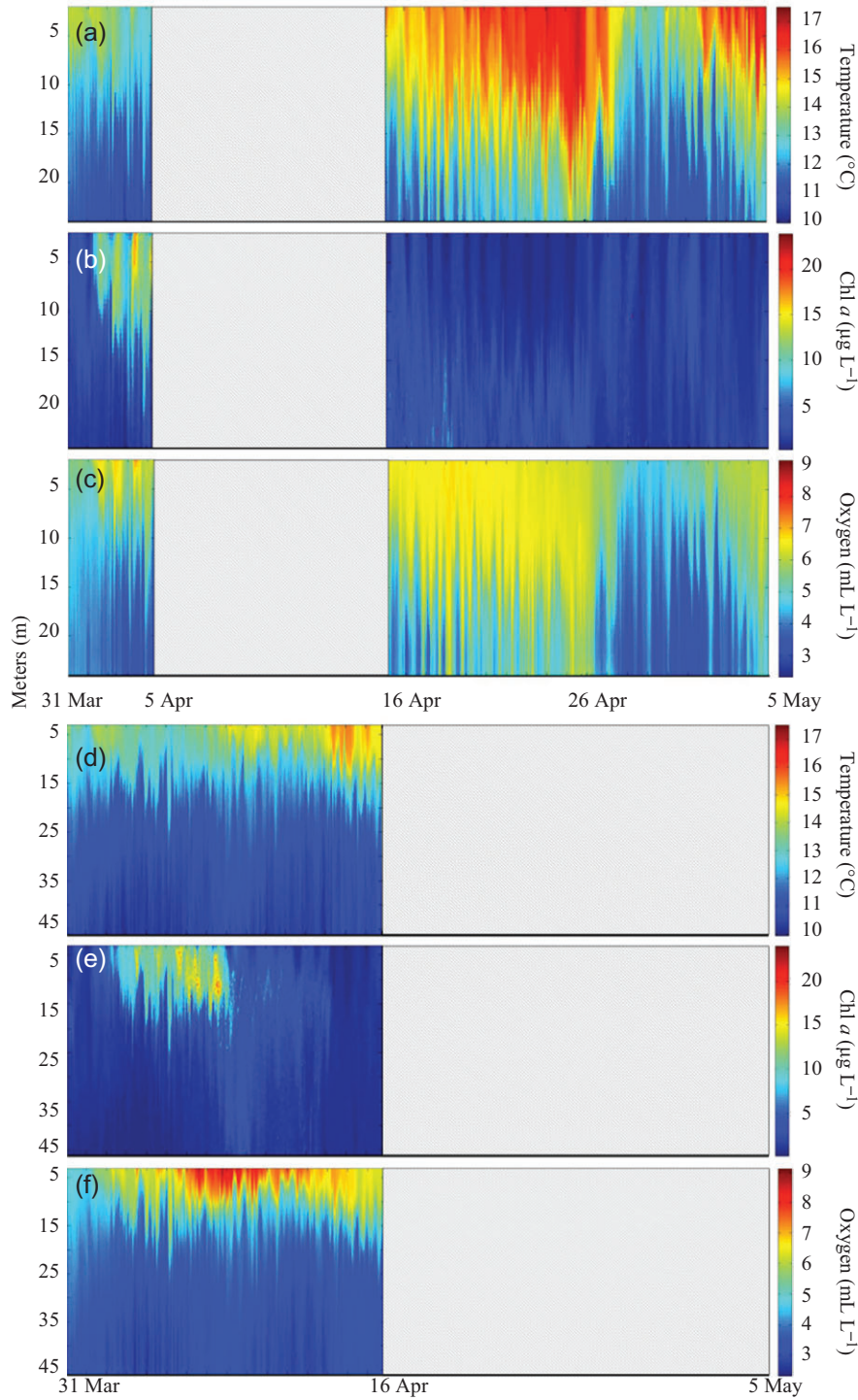
Phytoplankton biomass was maximal each year during early April (Fig. 2a). The highest chl *a* concentrations in 2013 were observed on 5 April when several surface and SCM samples exceeded major bloom concentrations (Fig. 2a). Chl *a* in March 2013, prior to the 2013 bloom, was generally  $< 5 \mu\text{g L}^{-1}$  during each survey cruise, with a few peaks of  $> 5 \mu\text{g L}^{-1}$  at offshore stations on 15 and 17 March. During the 2014 major bloom, concentrations of chl *a* on 2 April and 7 April, generally exceeded the maximal concentration measured in 2013, except for a few stations (Fig. 2a). Chl *a* in 2014 was generally low following the 7 April survey;

chl *a* was  $\leq 2.5 \mu\text{g L}^{-1}$  at the surface and  $\leq 5.5 \mu\text{g L}^{-1}$  at the SCM for the remaining survey cruises, with the exception of one high value at the SCM on 18 April.

The vertically profiling instruments deployed at Stations 2 and 3 (Fig. 1) in 2014 documented the development of a phytoplankton bloom that co-occurred with near-surface temperatures of  $13\text{--}14^\circ\text{C}$  at both stations (Fig. 3a,b,d and e). Water temperature in the upper 10 m of the water column at Station 2 was  $13\text{--}14^\circ\text{C}$  at the time of deployment on 31 March (Fig. 3a). An increase in chl *a* concentrations from  $\leq 2$  to  $\geq 10 \mu\text{g L}^{-1}$  occurred between 1 and 5 April in the upper 10 m of the water column (Fig. 3b). By 16 April, surface waters were relatively warm ( $\sim 15\text{--}16^\circ\text{C}$ ) until 27 April–2 May (end of deployment), when temperatures were  $13\text{--}14^\circ\text{C}$ . Chl *a* was consistently  $\leq 5 \mu\text{g L}^{-1}$  after 16 April at Station 2. Water temperatures of  $13\text{--}14^\circ\text{C}$  were observed at Station 3 at a depth of 2–15 m at the time of deployment on 31 March (Fig. 3d). Chl *a* concentrations in that depth range increased from  $\leq 2$  to  $\geq 6 \mu\text{g L}^{-1}$  between 1 April and 9 April (Fig. 3e) and attained a maximum of  $\geq 10 \mu\text{g L}^{-1}$  within the depth range 5–15 m. Concurrently with the presence of elevated phytoplankton biomass, dissolved oxygen ranged from 10 to  $15 \text{ mL L}^{-1}$  within the chl *a* feature, compared to a background concentration of  $\leq 5 \text{ mL L}^{-1}$  (Fig. 3f). Chl *a* profiles after 7 April indicated pre-bloom concentrations of  $\leq 2 \mu\text{g L}^{-1}$ . The decrease in chl *a* biomass occurred at the same time that surface waters were  $15\text{--}16^\circ\text{C}$ .

Absolute abundances of *Pseudo-nitzschia* cells overlapped between years, with similar maximal abundances observed each year (Fig. 2b). A major *Pseudo-nitzschia* bloom was observed each year based on the definition of Seubert *et al.* (2013) of cell abundances  $\geq 8.80 \times 10^4 \text{ cells L}^{-1}$ . Bloom abundances of *Pseudo-nitzschia* were observed on the 5 April survey at all stations in 2013, and at all stations during the surveys conducted on 2 April and 7 April in 2014 (Fig. 2b). Maximal cell abundances of  $8.45 \times 10^5 \text{ cells L}^{-1}$  were observed in 2013 and  $8.58 \times 10^5 \text{ cells L}^{-1}$  in 2014, on 5 April 2013 and 7 April 2014, respectively. Surveys conducted prior to 5 April 2013 and following 7 April 2014 showed *Pseudo-nitzschia* abundances that were generally much lower than the abundances observed during the bloom events. Overall, abundances of *Pseudo-nitzschia* ranged from BD (below detection) to  $7.05 \times 10^5 \text{ cells L}^{-1}$  at the surface and from BD to  $8.45 \times 10^5 \text{ cells L}^{-1}$  at the SCM in 2013. Cell abundances ranged from 0.03 to  $8.11 \times 10^5 \text{ cells L}^{-1}$  in surface waters, and from BD to  $8.58 \times 10^5 \text{ cells L}^{-1}$  at the SCM during 2014.

Diatoms were the dominant group within the microplankton community during the study periods in both years. However, the relative contribution of *Pseudo-nitzschia* cells to the total microplankton community varied between



**Fig. 3.** *In situ* data from the wirewalkers moored at Station 2 (nearshore) and Station 3 (offshore) in 2014. **(a)** Temperature (°C), **(b)** Chlorophyll *a* (µg L<sup>-1</sup>) and **(c)** Dissolved oxygen (mL L<sup>-1</sup>) at Station 2. **(d)** Temperature (°C), **(e)** Chlorophyll *a* (µg L<sup>-1</sup>) and **(f)** dissolved oxygen (mL L<sup>-1</sup>) at Station 3. Data gaps are shown as gray areas.

years. Common members of the phytoplankton community during the 2013 study included *Chaetoceros*, *Skeletonema*, *Thalassiosira*, *Thalassionema*, *Navicula* and *Cylindrotheca*,

and those genera typically outnumbered *Pseudo-nitzschia* cells during the surveys conducted in March. However, *Pseudo-nitzschia* was the numerically dominant member

of the phytoplankton community ( $\geq 50\%$  of microplankton) during the 5 April survey at both depths (data not shown). In 2014, *Pseudo-nitzschia* was never the dominant species in the microplankton community ( $\sim 14\text{--}36\%$  of the community). Other chain-forming diatoms including *Chaetoceros*, *Thalassiosira* and *Leptocylindrus* generally outnumbered *Pseudo-nitzschia* (data not shown). Given the small cell volume of *Pseudo-nitzschia* cells ( $\sim 1000 \mu\text{m}^3$ ), they never dominated the biovolume of the phytoplankton community.

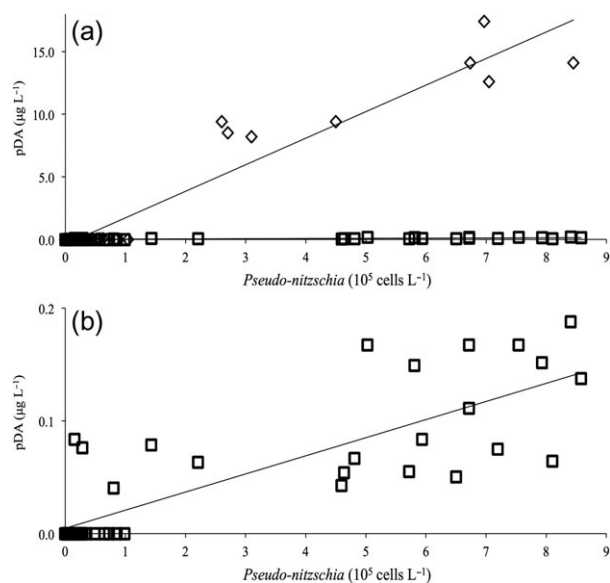
### DA in the Plankton during 2013 and 2014

Particulate DA (pDA) concentrations showed considerable differences in maximal values and ranges between years. The maximal pDA concentration detected during the 5 April 2013 bloom was two orders of magnitude higher than the pDA concentration detected during the bloom in 2014 (Fig. 4), despite similar maximal *Pseudo-nitzschia* cell abundances during both blooms. The maximal pDA concentration observed in 2013 was  $17.4 \mu\text{g L}^{-1}$  (5 April survey, SCM), while the maximal value in 2014 was only  $0.19 \mu\text{g L}^{-1}$  (7 April survey, SCM). The average pDA concentration of all samples collected in 2013 was  $2.61 \pm 5.21 \mu\text{g L}^{-1}$ , while the average value in 2014 was  $0.05 \pm 0.06 \mu\text{g L}^{-1}$ . A  $>50$ -fold difference between average pDA concentrations was observed between years,

however, the difference in these averages was heavily influenced by maximal pDA concentrations observed during bloom periods. Toxin was not detected in every sample collected (samples below the detection limit were entered as 0). pDA showed considerable temporal variability throughout the study periods in both years, with 44% of the 36 samples from 2013 with detectable concentrations (4 out of 5 surveys) and 46% of the 44 samples (4 of the 6 surveys) in 2014. pDA concentrations during 2013, excluding non-detects, ranged nearly three orders of magnitude with concentrations ranging  $0.02\text{--}12.6 \mu\text{g L}^{-1}$  in the surface and  $0.03\text{--}17.4 \mu\text{g L}^{-1}$  in the SCM (Fig. 5a). Detectable concentrations of pDA in 2014 ranged  $0.06\text{--}0.17 \mu\text{g L}^{-1}$  in the surface and  $0.05\text{--}0.19 \mu\text{g L}^{-1}$  in the SCM (Fig. 5a).

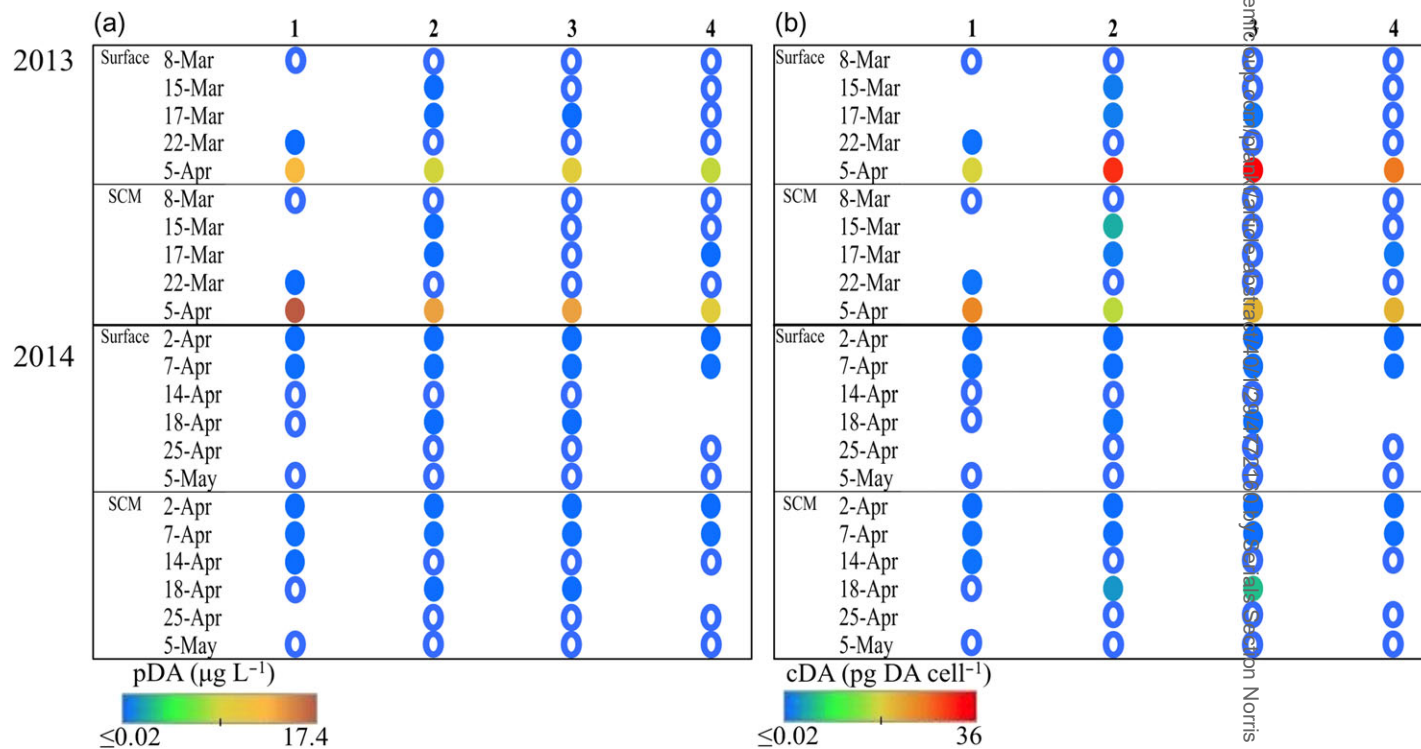
*Pseudo-nitzschia* cells had higher DA quotas, on average, in 2013 than in 2014 (Fig. 5b). The average cDA in all samples collected in 2013 was  $5.71 \pm 10.6 \text{ pg DA cell}^{-1}$ , while the average cDA of all samples collected in 2014 was  $0.26 \pm 0.87 \text{ pg DA cell}^{-1}$ . The difference in yearly cDA averages was skewed by the large difference in toxin quotas calculated for the 2013 bloom compared to the 2014 bloom (Fig. 5b). Cellular toxin concentrations in samples with detectable cDA spanned two orders of magnitude in 2013, similar to the trend observed in pDA. Cellular toxin concentrations in samples collected near the surface ranged from  $0.36$  to  $36 \text{ pg DA cell}^{-1}$  and from  $0.62$  to  $21 \text{ pg DA cell}^{-1}$  in the SCM. cDA during 2014 were  $0.08\text{--}0.55 \text{ pg DA cell}^{-1}$  at the surface, while cDA concentrations in the SCM ranged from  $0.08$  to  $5.7 \text{ pg DA cell}^{-1}$  (Fig. 5b).

Particulate and cDA showed similar temporal patterns between years with maximal toxin concentrations during each year occurring in early April (Fig. 5), despite large differences in absolute values between years (Fig. 4). No consistent lateral or vertical spatial patterns in toxin were detected between years along the transect or between depths (Fig. 5). Samples from the SCM generally had higher concentrations of toxin than those at the surface during 2013, but not in 2014 (Fig. 5a). The average pDA concentration was 2–3 orders of magnitude higher between the surveys conducted in March (pDA  $\leq 0.1 \mu\text{g L}^{-1}$ ) and the 5 April 2013 survey when pDA concentrations ranged from  $8.20$  to  $17.4 \mu\text{g L}^{-1}$  (Fig. 5). Toxin concentrations were similar (and low) between depths in 2014. Low concentrations ( $0.06 \pm 0.01 \mu\text{g L}^{-1}$ ) of pDA were detected on 2 April and showed little difference among stations or depths (Fig. 5). pDA concentrations were higher, by comparison to 2 April, on 7 April and particulate and cellular toxin concentrations did not vary much by stations or depth (pDA:  $0.15 \pm 0.02 \mu\text{g L}^{-1}$ ). Toxin was not detected in any of the samples from the remaining surveys after 7 April with



**Fig. 4.** (a) Particulate DA concentrations versus corresponding *Pseudo-nitzschia* cell abundances for 2013 (data shown as open black diamonds) and for 2014 (data shown as open black squares). The equation of the 2013 regression line is  $y = 2.1201 \times 10^{-5} x - 0.3938$ ,  $R^2 = 0.9236$ ,  $P < 0.0001$ . (b) Plot of 2014 pDA versus *Pseudo-nitzschia* cell abundances on an expanded y-axis (data shown as open black squares). The equation of the 2014 regression line is  $y = 1.6283 \times 10^{-7} x + 0.0047$ ,  $R^2 = 0.6984$ ,  $P < 0.0001$ .



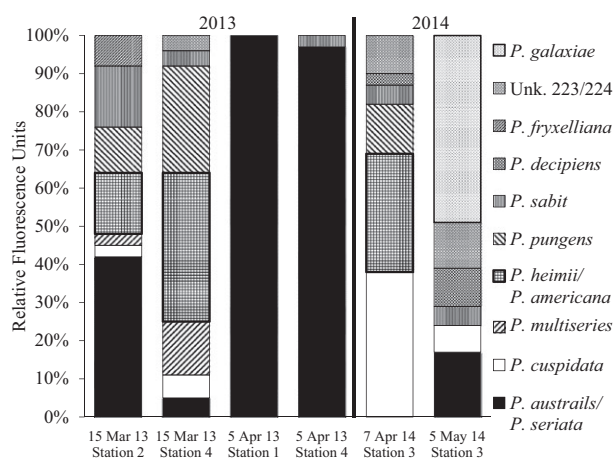


**Fig. 5.** (a) Heat map of particulate DA concentrations ordered by station number (columns) and date (rows) for samples collected at the surface and at the depth of the SCM, showing the spatial and temporal trends in pDA concentrations. The color key (lower left) indicates pDA concentrations in  $\mu\text{g DA L}^{-1}$  ranging from  $\leq 0.02$  to 17.4. An open circle indicates that the sample was below detection (BD) and no circle indicates the absence of a measurement. (b) Heat map of cDA ordered by station (columns) and date (rows) for samples collected at the surface and at the depth of the SCM. The color key (lower left) indicates cDA concentrations in  $\text{pg DA cell}^{-1}$  ranging from 0 to 36.2  $\text{pg DA cell}^{-1}$ . An open circle indicates that the sample was below detection and no circle indicates the absence of a measurement.

the exception of low concentrations ( $<0.1 \mu\text{g L}^{-1}$ ) measured at two stations on 18 April (Fig. 5a and b).

### *Pseudo-nitzschia* species composition changes and toxin concentrations

*Pseudo-nitzschia* species composition was assessed using ARISA, a genus-specific, PCR-based method that identifies taxa based on amplicon sizes and estimates their relative abundance based on the proportion of ITS1 copies from different species in the starting template (Hubbard *et al.*, 2014). This approach demonstrated shifts in community composition during both years and between years (Fig. 6). Ten fragment sizes were observed (Supplementary Table I), representing seven species (*P. sabit*, *P. galaxiae*, *P. pungens*, *P. multiseriis*, *P. fryxelliana*, *P. decipiens* and *P. cuspidata*), two potential species complexes that cannot be discriminated based on fragment size (*P. australis/P. seriata* and *P. heimii/P. americana*), and one fragment that could not be attributed to *Pseudo-nitzschia* species found along the US west coast (unknown amplicon of 223/224 base pairs). The 140 base pair sequence (MG195950) was 98% similar to *P. galaxiae*, the closest species match in GenBank (*e.g.* DQ336158), and was differentiated only by two short deletions. Several taxa observed with ARISA have never before been reported in the coastal waters of the SCB, including *P. sabit*, *P. decipiens* and *P. fryxelliana*, indicating the importance of further taxonomic study in this area. Most species detected were potential toxin-producers except *P. heimii/P. americana*, *P. sabit*, *P. fryxelliana* and *P. decipiens* (Supplementary Table I).



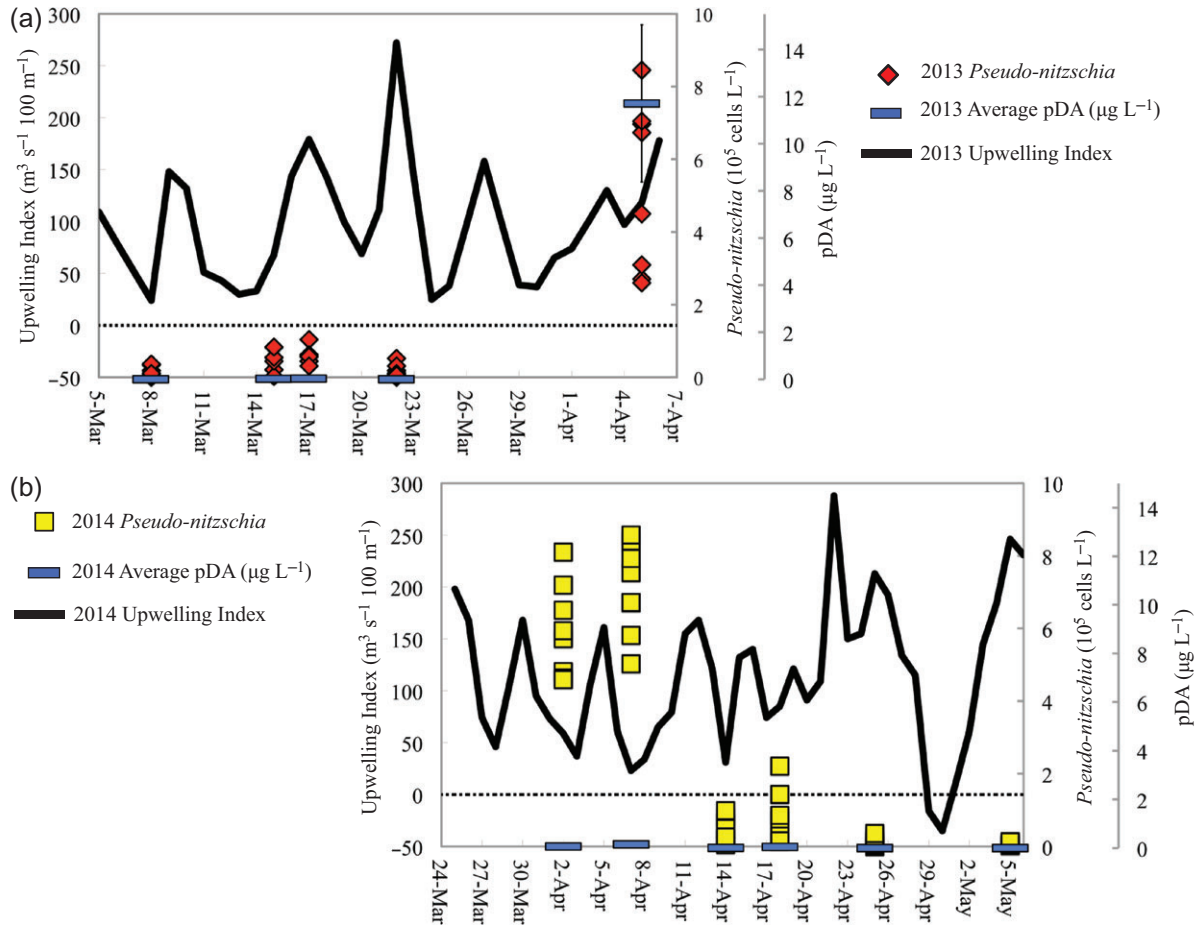
**Fig. 6.** Relative proportions of *Pseudo-nitzschia* species from ARISA (in relative fluorescence units) for each of the six samples analyzed from 2013 and 2014. Each bar is labeled with the date and station where the sample was collected. Unk. 223/224 refers to an ARISA fragment of 223/224 base pairs that does not correspond to any known *Pseudo-nitzschia* species.

ARISA was performed on three bloom samples collected during this study, when cell abundances exceeded  $1.0 \times 10^5$  cells  $\text{L}^{-1}$ . pDA concentrations differed considerably between these samples along with marked differences in *Pseudo-nitzschia* species composition. Samples collected from Stations 1 and 4 during the bloom on 5 April 2013 showed dominance of *P. australis/P. seriata* (100% and 96% of the ARISA signal, respectively) (Fig. 6). Concurrently, high abundances ( $7.0 \times 10^5$  cells  $\text{L}^{-1}$  and  $3.1 \times 10^5$  cells  $\text{L}^{-1}$ ) and elevated pDA concentrations ( $12.6 \mu\text{g L}^{-1}$  and  $8.20 \mu\text{g L}^{-1}$ ) were observed at Stations 1 and 4, respectively. The 7 April 2014 sample had comparable cell abundances relative to samples collected on 5 April 2013; however *P. australis/P. seriata* was not detected. The ARISA profile collected at Station 3 in 2014 indicated a mixed assemblage comprised mainly of *P. cuspidata* (38%) and *P. heimii/P. americana* (31%) with smaller proportions ( $< 15\%$ ) of *P. pungens*, *P. sabit*, *P. decipiens* and the 223/224 base pair amplicon (Fig. 6). The pDA concentration corresponding to this sample,  $0.17 \mu\text{g L}^{-1}$ , was the highest concentration observed in surface samples collected in 2014.

Community composition was also assessed for three samples collected when cell abundances were below the major bloom criterion, and pDA concentrations were either low or below detection. ARISA performed on samples collected on 15 March 2013 showed an assemblage comprised of *P. fryxelliana*, *P. cuspidata*, *P. pungens*, *P. multiseriis*, *P. sabit*, *P. heimii /P. americana* and *P. australis/P. seriata*, but the proportions of these species detected in the offshore and nearshore samples differed. The offshore ARISA profile (Station 4) showed high proportions of *P. heimii /P. americana* (39%) and *P. pungens* (28%) while the more nearshore Station (2) showed a high proportion of *P. australis/P. seriata* (42%). Concurrently, low concentrations of pDA ( $0.05 \mu\text{g L}^{-1}$ ) were detected at Station 2, while pDA was not detected at Station 4. The ARISA profile collected on 5 May 2014 indicated higher proportions of *P. galaxiae* (49%) relative to *P. australis/P. seriata* (15%). No toxin was detected during the 5 May survey and cell concentrations were  $<1.4 \times 10^4$  cells  $\text{L}^{-1}$ .

### *Pseudo-nitzschia* cell abundances, DA and upwelling events

The UI was used as a metric to compare wind-driven upwelling between years, as a possible driver of *Pseudo-nitzschia* blooms and/or DA events. The overall magnitude of regional upwelling was similar between years despite large differences in maximal pDA concentrations (Fig. 7a and b). The UI averaged  $102 \pm 61 \text{ m}^3 \text{ s}^{-1}$   $100 \text{ m}^{-1}$  over the period of 7 March–6 April in 2013,



**Fig. 7.** *Pseudo-nitzschia* cell abundance, average pDA concentration and the calculated UI versus date for the study periods in (a) 2013 and (b) 2014. Panels (a) and (b) are offset to allow for the overlap of dates that occur in both years. (a) The UI (Bakun Index Values from NOAA/NMFS/PFEG for: 33°N 119°W) for 2013 is shown with a black line, *Pseudo-nitzschia* cell abundances are shown by diamonds and average pDA concentrations for each survey date are shown by horizontal bars. (b) UI for 2014 is shown with a black line, *Pseudo-nitzschia* cell abundances are shown with squares, and average pDA concentrations for each survey date are shown by horizontal lines. The black horizontal dashed lines in **a** and **b** indicate delineation between upwelling and downwelling meteorological events in both panels.

*Table II: Spearman rank order correlation results between Pseudo-nitzschia abundance, pDA or cDA and measured physiochemical parameters. These analyses were conducted using data sets of all data combined, and by individual year*

	PN	Chl <i>a</i>	Temp	Sal	NO <sub>x</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>	Urea	PO <sub>4</sub> <sup>3-</sup>	SiO <sub>4</sub> <sup>2-</sup>	N:P	N:Si	Si:P	<i>n</i>
2013 + 2014													
PN		<b>0.654</b>	<b>-0.243</b>	<b>-0.227</b>	0.094	-0.198	<b>-0.311</b>	-0.210	<b>-0.225</b>	-0.071	-0.022	-0.218	56-80
pDA	<b>0.775</b>	<b>0.663</b>	<b>-0.304</b>	-0.150	-0.086	<b>-0.314</b>	<b>-0.224</b>	-0.179	<b>-0.248</b>	-0.063	0.043	-0.195	56-80
cDA		<b>0.529</b>	<b>-0.287</b>	-0.009	-0.109	<b>-0.279</b>	-0.130	-0.120	<b>-0.285</b>	-0.101	0.044	<b>-0.255</b>	56-80
2013													
PN		<b>0.653</b>	-0.187	0.117	0.022	-0.235	-0.066	0.205	-0.145	-0.110	-0.041	<b>-0.434</b>	36
pDA	<b>0.761</b>	<b>0.393</b>	-0.023	0.017	-0.144	<b>-0.485</b>	-0.022	-0.073	<b>-0.401</b>	-0.172	-0.054	<b>-0.474</b>	36
cDA		0.319	0.056	0.002	-0.190	<b>-0.456</b>	0.045	-0.118	<b>-0.472</b>	-0.199	-0.037	<b>-0.529</b>	36
2014													
PN		<b>0.664</b>	<b>-0.368</b>	<b>-0.766</b>	-0.226	-0.168	<b>-0.536</b>	<b>-0.507</b>	<b>-0.396</b>	-0.050	0.004	-0.130	20-44
pDA	<b>0.803</b>	<b>0.818</b>	<b>-0.559</b>	<b>-0.444</b>	-0.016	-0.010	<b>-0.416</b>	<b>-0.321</b>	-0.150	0.126	0.153	0.102	20-44
cDA		<b>0.684</b>	<b>-0.541</b>	-0.293	0.001	0.008	-0.260	-0.194	-0.068	0.087	0.107	0.095	20-44

*Pseudo-nitzschia* abundance; NO<sub>x</sub><sup>-</sup> = NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>; Temp, temperature; Sal, salinity. Bolded values are significant at *P* ≤ 0.05; *n* is the number of pairwise comparisons.

while the UI averaged  $111 \pm 73 \text{ m}^3 \text{ s}^{-1} 100 \text{ m}^{-1}$  during the 2014 period of 1 April–6 May.

Each year's maximal *Pseudo-nitzschia* abundances occurred after a series of moderate UI peaks (Fig. 7a and b). The highest *Pseudo-nitzschia* abundances, DA concentrations and chl *a* concentrations observed in 2013 were on 5 April. Multiple UI peaks occurred over the period preceding that date. Similarly, a succession of UI pulses occurred during 2014 prior to the 7 April 2014 survey, when the yearly maximal *Pseudo-nitzschia* abundances and chl *a* concentrations were observed (Fig. 7b).

### Relationships between environmental conditions, *Pseudo-nitzschia* abundance and DA

Spearman rank correlation was used to examine the associations between *Pseudo-nitzschia* cell abundance, pDA, cDA and measured biological and physiochemical parameters for the entire data set (2013 + 2014), and for each individual year (2013 and 2014). The strongest correlation ( $P \leq 0.05$ ) was the positive correlation between *Pseudo-nitzschia* cell abundance and pDA concentration (Table II). Positive correlations were also observed between *Pseudo-nitzschia* abundance and chl *a* across the study (Table II). Silicic acid negatively correlated with *Pseudo-nitzschia* abundance, pDA and cDA in the entire data set. When examined by individual year, silicic acid negatively correlated with pDA and cDA in 2013, and with *Pseudo-nitzschia* abundance in 2014. cDA negatively correlated with the Si:P ratio across the entire data set, while *Pseudo-nitzschia* abundance, pDA and cDA negatively correlated with the Si:P ratio in 2013, but not in 2014. *Pseudo-nitzschia* abundances and pDA negatively correlated with  $\text{PO}_4^{3-}$  concentrations in 2014, but not in 2013 or the combined years data set. *Pseudo-nitzschia* abundances, pDA and cDA correlated negatively with temperature in the entire data set and in 2014, but not in 2013. No correlations were determined between *Pseudo-nitzschia* cell abundance, pDA or cDA and N:P ratios, N:Si ratios or  $\text{NO}_x^-$  concentrations.

Environmental conditions were compared between survey periods in 2013 and 2014 and revealed differences in silicic acid concentrations between high- and low-toxin years. Surface silicic acid concentrations were significantly higher in 2014 than in 2013 ( $P \leq 0.05$ ; Table I). Specifically, average silicic acid concentrations were particularly low on 17 March and again on 5 April compared to other cruises in 2013 (Supplementary Fig 2). The Si:P ratio was also significantly lower in 2013 than in 2014 at the surface ( $P \leq 0.05$ ), indicating that silicic acid limitation was more prevalent in surface

waters in 2013 when maximal pDA concentrations were concurrently higher. No significant differences were detected between years at either depth for concentrations of any of the measured nitrogen species,  $\text{PO}_4^{3-}$ , or N:P and N:Si ratios, however, variations in these parameters were observed within each year, particularly during bloom and non-bloom periods (Table I and Supplementary Fig 2).

## DISCUSSION

The present study investigated the extent, duration and DA concentrations of two *Pseudo-nitzschia* blooms during spring 2013 and 2014 on the San Pedro Shelf (SPS) along the coast of the central Southern California Bight. *Pseudo-nitzschia* blooms have become an annual occurrence on the SPS during the last decade (Lewitus *et al.*, 2012), yet the magnitude of these events has varied greatly and the environmental drivers leading to toxic blooms in the region are still not well resolved. A major bloom was observed in both years of this study with maximal abundances each year of up to  $\sim 8 \times 10^5 \text{ cells L}^{-1}$  (Fig. 2b). Prior studies on the SPS reported bloom abundances ranging from  $10^4$  to  $10^6 \text{ cells L}^{-1}$  (Schnitzer *et al.*, 2007, 2013; Stauffer *et al.*, 2012; Seubert *et al.*, 2013; Seegers *et al.*, 2015). Particulate toxin concentrations in the plankton during the present study were within the range of those previous findings. Nonetheless, maximal pDA concentrations during 2013 and 2014 differed by approximately two orders of magnitude ( $17.4$  and  $0.19 \mu\text{g L}^{-1}$ , respectively). Particulate DA concentrations as high as  $52.3 \mu\text{g L}^{-1}$  have been observed in the Southern California Bight (Stauffer *et al.*, 2012), although detectable pDA concentrations have more commonly ranged between  $\sim 0.05$  and  $\sim 27 \mu\text{g L}^{-1}$  (Schnitzer *et al.*, 2007, 2013; Seubert *et al.*, 2013).

Overall, our results indicate a strong correlation between *Pseudo-nitzschia* abundances and pDA during each year, yet the absolute values of the relationship between *Pseudo-nitzschia* abundances and maximal pDA concentrations varied greatly between years (Fig. 4). Moreover, the differences in maximal pDA concentrations between years appeared to be a consequence of differences in *Pseudo-nitzschia* species assemblages, which were quite different during the 2013 and 2014 blooms (Fig. 6). Maximal pDA concentrations during the study were observed during a bloom dominated by *P. australis*/*P. seriata* on 5 April 2013. The bloom during 2014 was comprised of a mixed assemblage of multiple species with *P. cuspidata* and *P. heimii*/*P. americana* detected in the highest proportions. Concurrently, pDA concentrations in 2014 were two orders of magnitude lower than values observed in 2013 (Fig. 6).

Table III: Summary of literature reports of *Pseudo-nitzschia* species, maximal DA concentrations and maximal cell abundances associated with blooms occurring in the San Pedro Shelf region.

Year	Dominant <i>Pseudo-nitzschia</i> species	Maximal pDA concentration ( $\mu\text{g L}^{-1}$ )	Maximal cell abundances (cells $\text{L}^{-1}$ )	Reference
2003	<i>P. cf. cuspidata</i>	12.7	Not reported	Schnetzer <i>et al.</i> (2007)
2004	<i>P. australis</i>	1.94	$5.3 \times 10^4$	Schnetzer <i>et al.</i> (2007)
2005	<i>P. delicatissima</i>	2.91	Not reported	Schnetzer <i>et al.</i> (2013)
2006	<i>P. australis</i>	14.4	$1.44 \times 10^6$	Schnetzer <i>et al.</i> (2013)
2007	<i>P. australis</i>	27.0	$1.12 \times 10^6$	Schnetzer <i>et al.</i> (2013)
2013	<i>P. australis/P. seriata</i>	17.4	$8.45 \times 10^5$	This study
2014	<i>P. cuspidata/P. heimii/P. americana</i>	0.19	$8.58 \times 10^5$	This study

Published reports on toxigenic *Pseudo-nitzschia* cells have indicated that DA production can vary several orders of magnitude between different species, as well as among different strains of the same species (Bates *et al.*, 1998). Studies conducted under laboratory conditions have reported DA quotas from *P. australis* and *P. seriata* ranging from 0.0027 to 37 pg DA cell<sup>-1</sup>. In contrast, *P. cuspidata* has a relatively low-cellular DA quota ranging from 0.019 to 0.031 pg DA cell<sup>-1</sup> and *P. heimii* and *P. americana* are considered to be non-toxigenic species (reviewed in Trainer *et al.*, 2012). Based on this information, the *P. australis/P. seriata* bloom during 2013 presumably possessed a greater potential for toxin production than a bloom of a mixed assemblage of low-DA and non-DA producing species (Supplementary Table I) of similar magnitude, partially explaining the observations of the present study. Cellular DA quotas during the 5 April 2013 bloom ranged from 14 to 36 pg DA cell<sup>-1</sup>, which are in the upper range of quotas observed in laboratory studies of *P. australis* and *P. seriata* (reviewed by Trainer *et al.*, 2012).

High-DA quotas in the *P. australis/P. seriata* population in the present study may also indicate that the cells during the 2013 bloom event were nutrient limited. DA production by toxigenic species is not constitutive and published studies have demonstrated that a range of environmental conditions can regulate the DA quotas of toxigenic *Pseudo-nitzschia* cells (reviewed in Lelong *et al.*, 2012). Silicic acid drawdown in surface waters that occurred during the 5 April 2013 bloom likely led to silicic acid limitation of *P. australis/P. seriata* cells that dominated the assemblage (Table II, Supplementary Fig. 2), implicating this factor in high-DA production. Maximal pDA concentrations on 5 April 2013 were observed in surface water samples when silicic acid concentrations were below detection (Supplementary Fig. 2). Previous work has shown that silicic acid limitation is strongly linked to DA production, both in the field and in culture (Pan *et al.*, 1996a; Fehling *et al.*, 2004; Anderson *et al.*, 2006; García-Mendoza *et al.*, 2009).

Year-to-year variability in maximal pDA concentrations observed on the SPS, therefore, appears to indicate

a combined influence of species composition of the *Pseudo-nitzschia* assemblage and the effect of physiochemical conditions on toxin production. Natural communities are typically populated by a mix of different *Pseudo-nitzschia* species (Bates *et al.*, 1998), or a mix of different strains of the same species (Parsons *et al.*, 1999), which may have varying rates of DA production in response to physiochemical conditions. Studies conducted in the SPS have attributed blooms with elevated pDA concentrations to several toxin-producing species (Table III). The majority of bloom events in the region with high-pDA concentrations ( $>10 \mu\text{g L}^{-1}$ ) have coincided with high abundances ( $>1.0 \times 10^5$  cells  $\text{L}^{-1}$ ) of *P. australis*, with the exception of a bloom of *P. cf. cuspidata* in 2003 (Table III; Schnetzer *et al.*, 2007, 2013). Blooms with lower maximal pDA concentrations ( $<3 \mu\text{g L}^{-1}$ ) during 2004 and 2005 were dominated by *P. australis* and *P. delicatissima*, respectively (Schnetzer *et al.*, 2007, 2013).

Year-to-year variability in maximal pDA concentrations has also been observed in other bloom hot spots along the US west coast, including the Juan de Fuca eddy region of the Washington coast, Monterey Bay, CA, USA and the Santa Barbara Channel, CA, USA (Walz *et al.*, 1994; Trainer *et al.*, 2000, 2009a, 2009b; Anderson *et al.*, 2006, 2009; Bargu *et al.*, 2012; Lewitus *et al.*, 2012). Blooms in those regions have shown wide variations in particulate DA concentrations, similar to the SPS, ranging from below detection to  $\sim 25 \mu\text{g L}^{-1}$ . Blooms in those regions have also been populated by a variety of toxigenic species including *P. australis*, *P. cf. pseudodelicatissima*, *P. cuspidata*, *P. multiseriata*, *P. fraudulenta* and *P. pungens*. Particulate DA concentrations observed in those studies have been linked to a range of different environmental conditions, including low-dissolved nutrient concentrations, imbalances in nutrient ratios, temperature anomalies and circulation patterns. As observed in the present study, high variances in pDA concentrations do indeed appear to be partly a consequence of a “generalized” *Pseudo-nitzschia* response to physiochemical parameters, but we speculate that pDA concentration is also a consequence of how physiochemical conditions select for particular species or strains within

the overall *Pseudo-nitzschia* assemblage. Therefore, understanding the interplay between environmental factors and their effects on *Pseudo-nitzschia* strain/species composition (and attendant physiological capabilities) should substantively improve our ability to predict future DA events.

The development of *Pseudo-nitzschia* blooms along the US west coast has historically been linked to upwelling (Trainer *et al.*, 2000; Lewitus *et al.*, 2012). Schnetzer *et al.* (2013) correlated upwelled water to toxigenic *P. australis* blooms on the SPS (see their Fig. 7), and elevated *Pseudo-nitzschia* abundances have been shown to co-occur with upwelling conditions in many bloom “hot spots” along the west coast of the USA (Lange *et al.*, 1994; Bates *et al.*, 1998; Trainer *et al.*, 2000; Kudela *et al.*, 2005; Schnetzer *et al.*, 2013). Wilkerson *et al.* (2006) found that short periods of upwelling-favorable wind conditions followed by periods of relaxation appear to specifically favor diatom growth. This is likely due to the time that cells need to respond physiologically to upwelled nutrients (Kudela *et al.*, 1997), and manifests itself as time-lagged growth response to upwelling events. Our study supports those observations showing that both the timing and magnitude of upwelling events is an important factor in the accumulation *Pseudo-nitzschia* cells. Specifically, in both years, a series of upwelling and relaxation events favored *Pseudo-nitzschia* bloom development, as revealed by the regional UI (Fig. 7). Our results do not indicate, however, that upwelling controlled differences in toxin concentrations observed between years.

Our work indicates that specific, though perhaps subtly distinct, physiochemical parameters resulted in the differences in *Pseudo-nitzschia* species dynamics between years. Previous studies examining *Pseudo-nitzschia* species dynamics have concluded that different *Pseudo-nitzschia* species appear to be associated with specific environmental factors such as temperature, nutrient concentrations and nutrient ratios (Bates *et al.*, 1998; Fernandes *et al.*, 2014; Hubbard *et al.*, 2014; Guannel *et al.*, 2015; Ruggiero *et al.*, 2015). Upwelling clearly stimulated *Pseudo-nitzschia* growth in the SPS region during our study, but the specific conditions that promoted the dominance of *P. australis*/*P. seriata* in 2013 and a mixed *Pseudo-nitzschia* assemblage in 2014, and, therefore, the overall toxin levels appearing during these blooms remain unclear. nMDS analysis was unable to resolve clear differences in physiochemical conditions between the 5 April 2013 and 7 April 2014 cruises when the species compositions were very different (Supplementary Fig. 3). This result may indicate that the physiochemical conditions leading to differences in *Pseudo-nitzschia* assemblages may have been extremely subtle or potentially related to an unmeasured parameter

such as iron or vitamins. It also may indicate that the conditions measured at the time of peak toxin concentrations of the blooms were not representative of the conditions that favored the development of the toxigenic or non-toxigenic populations, as has previously been suggested by Schnetzer *et al.* (2007). Alternatively, or additionally, upwelling could have advected an outside or deep water mass containing a toxin-producing seed population into the local environment, which then developed into a bloom in surface waters where conditions were more favorable for growth (Trainer *et al.*, 2000; Anderson *et al.*, 2006; Seegers *et al.*, 2015). Such time-lagged relationships or advective influences are difficult to resolve from ship-based sampling programs.

## CONCLUSIONS

Our study demonstrates that the species composition of the *Pseudo-nitzschia* assemblage strongly affected the concentrations of DA that occurred during blooms in successive years in the same location. Similar abundances of *Pseudo-nitzschia* were observed during both blooms, yet maximal DA concentrations differed by almost two orders of magnitude between 2013 and 2014. High-DA concentrations corresponded with a bloom overwhelmingly dominated by *P. australis*/*P. seriata*, while a bloom with a mixed *Pseudo-nitzschia* assemblage had very low-DA concentrations. In both years, the growth of *Pseudo-nitzschia* cells seemed to be driven by periods of upwelling followed by relaxation. Low-dissolved silicic acid concentrations, which have been previously linked to enhanced DA concentrations, were correlated with high-pDA concentrations and cellular DA quotas in 2013. Our study points to the importance of understanding not only the factors that lead to *Pseudo-nitzschia* bloom development but also the factors that control species dynamics within the *Pseudo-nitzschia* assemblage. Given the importance of species composition on potential DA concentrations, it is fundamentally important to understand the subtle chemical, physical and biological factors that determine *Pseudo-nitzschia* composition. Without a better understanding of the factors driving blooms of toxigenic *Pseudo-nitzschia* species, prediction of the timing and magnitude of toxigenic blooms in the region will remain elusive.

## SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Plankton Research* online.

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