Pseudo-nitzschia and domoic acid fluxes in Santa Barbara Basin (CA) from 1993 to 2008

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A R T I C L E   I N F O

Article history:
Received 11 September 2010
Received in revised form 11 April 2011
Accepted 13 April 2011
Available online 21 April 2011

Keywords:
Pseudo-nitzschia
Domoic acid
Sediment traps
Santa Barbara Basin
Climate change
NPGO

A B S T R A C T

Blooms of domoic acid (DA) producing Pseudo-nitzschia, regularly occur off the coast of California. Although it has been hypothesized that these blooms are increasing in frequency, the lack of historical records limits our understanding of potential causal mechanisms. In this study, an 15-year time-series (1993–2008) of sediment trap samples collected from the Santa Barbara Basin (SBB) at 540 m were analyzed for Pseudo-nitzschia (n = 196, microscopy and SEM) and DA (n = 206, LC–MS/MS) concentrations and fluxes. Results suggest that there was an abrupt shift towards greater frequency and higher magnitude Pseudo-nitzschia blooms and toxic DA flux events in the SBB after the year 2000. SEM analysis of sediment trap material indicates that these events were mainly blooms of P. australis, with cell fluxes increasing by an order of magnitude from a maximum of 4.5 × 106 cells m–2 d–1 pre–2000, to as high as 3.2 × 108 cells m–2 d–1 thereafter. Similarly, sediment trap DA fluxes increased by an average of 13.4 μg m–2 d–1, with only one large event (≥5 μg m–2 d–1) from 1993 to 1999 versus 16 large DA events from 2000 to 2008. While the causes of this abrupt shift remain ambiguous, we suggest that this shift may be related to natural climate variability associated with a change in phase of the North Pacific Gyre Oscillation (NPGO) and its potential influence on the composition and magnitude of waters that are upwelled into the SBB.

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1. Introduction

Toxic Pseudo-nitzschia were first recorded in the Santa Barbara Basin (SBB) in 1998 during a wide-spread bloom that affected the central California coastline (Trainer et al., 2000). The species responsible was identified as Pseudo-nitzschia australis and its production of the neurotoxin domoic acid (DA) resulted in the mass mortality of over 400 California sea lions (Scholin et al., 2000). Since then, toxic Pseudo-nitzschia blooms have occurred regularly in SBB, prompting regulatory monitoring of shellfish DA concentrations in SBB shellfish by the California Department of Public Health beginning in 2002 (Langlois, Biotxin Monthly Reports).

The environmental drivers of toxic Pseudo-nitzschia blooms remain enigmatic. Several studies off central and southern California suggest that toxic Pseudo-nitzschia blooms are associated with the upwelling of colder, high-nutrient waters (Anderson et al., 2006; Kudela et al., 2004), whereas others report blooms after periods of high-nutrient river runoff (Bates et al., 1998; Dortch et al., 1997; Fisher et al., 2003; Lane et al., 2009; Pan et al., 2001; Trainer et al., 2000; Van Dolah et al., 2003; Wells et al., 2005), or due to resuspension of seeding populations into the euphotic zone during upwelling and storms (Garrison, 1981; Trainer et al., 2000). These studies are further complicated by the fact that peaks in Pseudo-nitzschia cell abundance and DA concentrations may be loosely coupled in time. For example, increases in toxin production may be a function of silicic acid or phosphate stress (Bates et al., 1991; Fehling et al., 2004; Pan et al., 1996a,b), as well as iron and copper limitation that may occur prior to and after Pseudo-nitzschia abundance maxima (Maldonado et al., 2002; Rue and Bruland, 2001; Wells et al., 2005).

Coastal eutrophication is often cited as driving the observed increase in HAB frequency worldwide (see reviews by Anderson et al., 2002; Gilbert et al., 2005; Hallegreaff, 1993), but this may also be due to enhanced awareness and monitoring. In the case of Pseudo-nitzschia, for example, a number of the species now...
recognized to be toxic were previously identified as “Nitzschia seriata (Cleve) Peragallo”, a composite taxon that may contain both toxic and non-toxic species (e.g. Bates, 2000). Off the coast of California, a recent examination of SBB sediment cores suggests that a rise in the relative abundance of Pseudo-nitzschia in SBB is a modern phenomenon with significant increases beginning in 2000–2001 (Barron et al., 2010).

Sediment trap studies in SBB and San Pedro Basin (SPB), also located off the coast of southern California, have demonstrated that sediment traps are effective tools for documenting both Pseudo-nitzschia and DA fluxes to deep waters in coastal regions (Schnetzer et al., 2007; Sekula-Wood et al., 2009). These studies indicate that high concentrations of surface water Pseudo-nitzschia and DA are temporally coupled to those measured in sinking particles collected in sediment traps located as deep as 800 m, providing a pathway for toxin exposure in deep water and benthic ecosystems. As such, the transport of DA to depth is theorized to be particle-mediated: (i) retained intracellularly within Pseudo-nitzschia frustules, (ii) incorporated into the biomass and fecal pellets of biota (e.g. zooplankton and planktivorous fish) which have fed on toxic Pseudo-nitzschia blooms, and/or (iii) adsorbed onto particles (Burns and Ferry, 2007; Lail et al., 2007). Aggregation of particles and fast sinking rates may decrease exposure of DA to photodegradation in surface waters and potentially increase the retention of this toxin for transport to deep water ecosystems.

Building upon previous sediment trap work in SBB, a major goal of this study was to investigate long-term changes in Pseudo-nitzschia and DA fluxes at ~550 m from 1993 to 2008. Bottom sediments were also obtained to contrast Pseudo-nitzschia and DA water column fluxes, as well as to investigate variability of DA retention in surface sediments at varying depths within the basin. Furthermore, as the advent of using sediment trap samples for DA detection is recent, we more closely describe one method for DA determination in sediment trap samples which focuses on quantifying DA in both the particulate and dissolved phases of the collected trap material.

2. Materials and methods

2.1. Sample collection

The SBB sediment trap mooring has been stationed near the center of the basin (34°14′N, 120°2′W) since August 1993 (Thunell, 1998) (Fig. 1). Over the course of the trapping program, the deepmoored Mark VI sediment trap has been positioned between 490 and 540 m water depth (total water depth ≈590 m). Deployments last ~6 months, such that each of the 13 trap cups continuously collect material for ~2 weeks. Cups are prepared with filtered seawater amended with preservatives and buffers to a final concentration of 10% sodium azide and 1% sodium borate to retard grazers during collection and aid in sample preservation. The seawater used in the initial trap solutions was collected and filtered from surface waters (0–5 m) in the San Pedro Harbor at the Southern California Marine Institute. When available, trap solution blanks were obtained to examine possible DA contributions to trap cups. Post-collection processing of sediment trap samples and geochemical analyses is detailed in Thunell (1998) and Thunell et al. (2007). Briefly, immediately after collection, samples are transported to the University of South Carolina for processing. Supernatants are decanted (with a portion saved and frozen) and the remaining trap material split with a buffered sodium borate solution using a precision rotary splitter. A “wet” split of the buffered sediment trap sample is then stored. A second sample split is immediately freeze-dried and ground for geochemical analyses. All sample splits are stored refrigerated and in the dark before and after analyses. SBB sediment trap materials collected from August 1993 to January 2008 were analyzed for Pseudo-nitzschia and DA fluxes. Within this time range, periods of missing data are primarily attributed to trap failure due to clogging associated with intervals of high mass flux. To a much lesser extent, data are also absent when total mass fluxes were very low and there was insufficient material for analysis.

Archived wet splits of sediment trap cups (n = 196) were freeze-dried, and the ungrounded material used for Pseudo-nitzschia cell counts (cells g-1 dry mass) and species identification. The SBB sediment trap samples (n = 206) and trap cup supernatants (n = 206) were used to measure particulate (pDA) and dissolved DA (dDA) concentrations, respectively. Using the supernatants, the potential loss of DA to the dissolved phase (i.e. solubilization) associated with sediment trap collection methods was also investigated. Please note that given the length of the time-series program, pDA (freeze dried and ground) and dDA (supernatants) have been stored frozen in the dark for variable periods of time that range from several weeks to more than a decade.

Bottom sediment samples were obtained from throughout the SBB to examine Pseudo-nitzschia cell and DA concentrations (Fig. 1). In April 2005, a box core was obtained at the location of the sediment trap mooring. Sediments were laminated, suggesting minimal bioturbation, and the top 5 cm (0.25 cm resolution) were analyzed for Pseudo-nitzschia cell and DA concentrations. Additional surficial sediment samples (0–2 cm, n = 28) were collected using a sediment grab during the California Bight 2008 campaign (CA-B08) of the Southern California Coastal Water Research Project (SCCWRP). These bulk samples were analyzed for DA concentration only.

2.2. Pseudo-nitzschia cell counts and species identification

Pseudo-nitzschia frustules within sediment trap and bottom sediment samples were enumerated using settling chambers and light microscopy. For sample preparation, wet splits of sediment trap and bottom sediment samples were freeze-dried, and the unground material (6–25 mg for sediment trap samples and 40–80 mg for bottom sediments) was gently resuspended in 5 mL deionized water with subsequent dilutions for counts performed as needed. Aliquots of the resuspensions were pipetted into settling chambers (Utermöhl or Sedgwick-Rafter) and allowed to settle for a minimum of 1 h. Samples were counted using an Olympus BX51 (Tokyo, Japan) inverted light microscope (200×) or an Olympus CX31 compound microscope (100×) using standard techniques (Andersen and Throndsen, 2003; Hasle, 1978).
For selected samples, *Pseudo-nitzschia* species identification was determined by SEM (JEOL 5600LV) performed at the NOAA Center for Coastal Environmental Health and Biomolecular Research (Charleston, SC). Freeze-dried unground samples were placed on an aluminum stub using double stick tape and sputter-coated with gold-platinum using a Denton Vacuum Desk II Sputter Unit (Moorestown, New Jersey, USA).

### 2.3. Domoic acid extraction and analysis

DA was extracted from freeze-dried and ground (<250 μm) sediment trap (~100 mg) and bottom sediment (~200 mg) samples by adding 1.3 mL of 50% methanol, followed by 12 h on a wrist action shaker (Burrell wrist action shaker, Model 78). Samples were then centrifuged (15 min at 2700 rpm) and decanted. The liquid phase was centrifuged a second time to separate out any remaining particles. No extraction step was necessary for sediment trap cup supernatants. Frozen archived trap supernatants were thawed at room temperature, shaken to homogenize, and then filtered through 0.7 μM Whatman glass fiber filters (GF/Fs) prior to analysis.

DA concentrations were determined by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) using the modified methods described in Sekula-Wood et al. (2009). Briefly, samples were analyzed in multiple reaction mode using an Agilent 1100 HPLC with autosampler coupled to a Micromass–Quattro mass spectrometer equipped with an electrospray ion-spray. Chromatographic separation was achieved using an Aqua Sep 5 μm particle size, 10 cm × 2.1 mm (i.d.) column (ES Industries, West Berlin, NJ) in conjunction with a corresponding Aqua Sep 5 μm particle size, 1 cm × 3.2 mm (i.d.) guard column. The mobile phase was a mixture of 0.1% aqueous formic acid in deionized water (A) and 0.1% aqueous formic acid in acetonitrile (B). The initial condition was 95:5 A:B for 4 min, followed by a linear gradient over 11 min ending at 5:95 A:B. The ratio of A and B was reset to the initial condition over the following 7 min to reestablish initial conditions. A 4-min solvent diversion was used to avoid salt contamination of the ion source. Additional modifications included a sample injection volume of 20 μL and the use of caffeine as an internal standard. Retention time of DA in samples was determined based on the retention time of a certified DA reference standard from the Institute for Marine Biosciences, NRC Canada (Halifax, NS, Canada). DA was identified and quantified from the signal of the parent mass (m/z 312) and two daughter fragments (m/z 326 and m/z 161). Caffeine was used as an internal standard and monitored from the parent mass (m/z 195) and two daughter fragments (m/z 138 and m/z 110).

### 2.4. Physical and climatic forcing

To examine potential drivers of variability in the *Pseudo-nitzschia* and DA flux patterns, we examined several climate indices and used standard correlation methods to compare them with the sediment trap time series data. These are the upwelling index at 15 N, 119 W from Pacific Fisheries Environmental Laboratory (PFEI, [http://www.pfeg.noaa.gov/products/PFEL modeled/indices/upwelling/upwelling.html](http://www.pfeg.noaa.gov/products/PFEL modeled/indices/upwelling/upwelling.html)), the Multivariate El Niño Southern Oscillation (ENSO) Index (MEI), the Pacific Decadal Oscillation (PDO), the North Pacific Gyre Oscillation (NPGO), and United States Geological Survey (USGS) daily stream discharge data for the Ventura River, a main source of allochthonous input to the Santa Barbara Channel. The monthly upwelling index from PFEI is an estimate of wind-driven coastal upwelling and offshore Ekman transport from which we calculated monthly anomalies using the global climatology from 1993 to 2008 (our trap time series record). For interannual climate variability, we used the MEI, a sliding bi-monthly index of ENSO activity produced by the NOAA Physical Sciences Division. For a given month (n) in the sediment trap record, we matched the corresponding MEI month (n – 1)/month (n) according to [http://www.esrl.noaa.gov/psd/people/klaus.wolter/MEI/table.html](http://www.esrl.noaa.gov/psd/people/klaus.wolter/MEI/table.html). The PDO and NPGO were retrieved from The Joint Institute of the Atmosphere and Oceans ([http://www.jisao.-](http://www.jisao.-)

![Fig. 2](http://www.jisao.-)

**Fig. 2.** SEM images of SBB sediment trap material. Images are from samples collected in 1 May 2002 (a), 15 May 2002 (b), 2 April 2004 (c), and 10 March 2006 (d). In all cases, the *Pseudo-nitzschia* species identified was *P. australis.*
3. Results

3.1. Pseudo-nitzschia in sinking particles and bottom sediments

Light microscopy of sediment trap material revealed that *Pseudo-nitzschia* was present as broken fragments and whole frustules, and in some cases as intact chains of two to four frustules in length. Other diatom genera commonly present included Skeletonema, Ditylum, Coscinodiscus, Chaetoceros, Thalassionema, Navicula. Other plankton frequently observed were foraminifera and coccolithophores (intact or as individual coccoliths). Closer inspection of *Pseudo-nitzschia* within the trap cups using both light microscopy and SEM showed excellent frustules preservation with no evidence of silica dissolution throughout the time-series (Fig. 2). Post-2000, the *Pseudo-nitzschia* species identified using SEM was *P. australis*, which is considered to be one of the most toxic *Pseudo-nitzschia* spp. commonly found in California waters (Trainer et al., 2000). Images in Fig. 2 correspond to samples with high *Pseudo-nitzschia* cell abundances in May–August 2002 (Fig. 2a and b) and June 2004 (Fig. 2c) collected at 500 m depth. Fig. 2d corresponds to a sediment trap sample collected in January 2006 at the initial stages of a *Pseudo-nitzschia* bloom and is a good representation for the varied composition of material observed in most trap samples. In rare instances, some frustules (never dominant) may have been of a different *Pseudo-nitzschia* species. However, positive identification could not be confirmed due to limited exposure (covered by other trap debris or present in only small fragments) or poor orientation of the frustule on the stub. Prior to 2000, the largest *Pseudo-nitzschia* flux event, which occurred in May 1995, was comprised of both *P. australis* and the second most toxigenic species, *P. multiseries*. Large blooms in 1994 appear to be monospecific *P. australis* blooms. Thus it is not clear that the shift towards larger, more frequent and more toxic blooms in 2000 represents a significant shift in *Pseudo-nitzschia* species composition. It is important to note here that analysis of silica within the sediment trap samples suggests that less than 2% of the total biogenic silica measured within the trap cup was lost to the supernatant phase. Combined, these lines of evidence strongly suggest that minimal dissolution of *Pseudo-nitzschia* frustules occurred during sediment trap sample collection and storage.

Cell counts resulted in *Pseudo-nitzschia* concentrations ranging from $3.1 \times 10^3$ to $1.6 \times 10^8$ cells g$^{-1}$ sed. There is a distinct increase in *Pseudo-nitzschia* concentrations beginning in late 2000. Prior to 2000, peaks in *Pseudo-nitzschia* concentrations were an order of magnitude lower and did not exceed $3.5 \times 10^6$ cells g$^{-1}$ sed. Since 2000, there have been six instances where *Pseudo-nitzschia* fluxes in the trap were greater than 2.0 $\times 10^7$ cells m$^{-2}$ d$^{-1}$: December 2000; May 2002; October 2002; April 2004; September–October 2005; and April 2007.

*Pseudo-nitzschia* cell fluxes (Fig. 3) were calculated as the product of cell concentration and total mass flux (ranging from $0.1$ to $9.1$ g m$^{-2}$ d$^{-1}$). Trends in flux were similar to those observed with concentrations, such that *Pseudo-nitzschia* cell fluxes are consistently larger beginning in 2000, reaching as high as $3.2 \times 10^8$ cells m$^{-2}$ d$^{-1}$ in December 2000. Prior to 2000, the largest fluxes ($\sim 4.5 \times 10^7$ cells m$^{-2}$ d$^{-1}$) occurred in early 1994, April 1995, and as a sharp peak in late 1997.

![Fig. 3. Climate, riverine, and upwelling indices relative to Pseudo-nitzschia fluxes from 1993 to 2008. Panel (a) MEI $\times 100$ index, (b) Ventura River Discharge ($\times 100$ ft$^3$ s$^{-1}$), (c) PMEL UI Anomaly, (d) PDO, and (e) Pseudo-nitzschia flux ($\times 10^6$ cells m$^{-2}$ d$^{-1}$). See text for details. Also plotted on panel (e) is the NPGO index (grey dashed line) and an inset of Pseudo-nitzschia fluxes from 1993 to 2000 with a higher resolution scale.](image-url)
Blooms of *Pseudo-nitzschia* were present during both upwelling and non-upwelling periods, but were more frequent when upwelling anomalies were positive (PFEL UI Anomaly) (e.g. 2002, 2004, 2005, 2006, and 2007). The increase in *Pseudo-nitzschia* in September–October 2005 did not occur during a local upwelling event, but coincided with a small cooling event during these months associated with upwelled waters near Point Conception advected into the western channel during episodes of high summer wind stress curl (Anderson et al., 2009). Notably, the largest cell flux (\(\sim 3 \times 10^6\) cells m\(^{-2}\) d\(^{-1}\) in December 2000) occurred at the end of a particularly long La Niña event lasting from 1998 through early 2001 (Fig. 3, MEI index). While the 1995 bloom occurred immediately following an upwelling event (consistent with more recent trends), the 1994 increase occurred immediately prior to upwelling at the end of a prolonged downwelling period. There is no correlation between *Pseudo-nitzschia* fluxes and riverine discharge. An abrupt increase in *Pseudo-nitzschia* fluxes after 2000 may be related to a basin wide shift in climate as reflected in the NPGO index (see Section 4).  

Compared to the sediment traps, core samples had significantly less biogenic material. Plankton material was less abundant and mostly composed of centric diatoms (either as whole frustules or girdles) and foraminifera. When present, *Pseudo-nitzschia* concentrations in SBB-MC1 samples ranged from 300 to 2000 frustules g\(^{-1}\) sed, orders of magnitude lower than those observed in the sediment traps and similar to previous work in SBB (Barron et al., 2010). *Pseudo-nitzschia* frustules preserved in the core were typically whole, suggesting that fragments either did not preserve well or were too small to be identified as *Pseudo-nitzschia* using light microscopy.

### 3.2. Dissolved versus particulate DA in sediment trap samples

DA was detected in both trap cup supernatants (dDA) and particulates (pDA) beginning in 1994 and continuing throughout the time series with peaks in DA concentration occurring in 1997, 2000–2001, 2002, 2004, 2007, and 2008. Concentrations of pDA ranged from <0.1 to 35.6 \(\mu\)g DA g\(^{-1}\) sed, with the maximum concentration occurring in May 2002. Concentrations of dDA commonly exceeded 600 \(\mu\)g DAL\(^{-1}\) with a maximum concentration of 4190 \(\mu\)g DAL\(^{-1}\) occurring in February 2004. Increases in pDA were often synchronous with increases in dDA concentrations. While possible that dDA could have been present in the seawater used to fill the sample cups, dDA was not detected in any archived trap solution blanks. For cruises where blanks were unavailable, a baseline dDA concentration would have been expected in all 13 trap cups from that deployment had dDA been initially present in the collected seawater. However, this was never observed and suggests dDA in supernatants was sourced solely by materials entering trap cups during trap deployment.

Comparison of the contribution of dDA versus the total amount of DA measured in trap cups (sum of dDA and pDA, volume and mass corrected) shows that in nearly all cases, the majority of DA present in the trap cups was detected in the dissolved phase (88 ± 21%), and the proportion of dDA increased with increasing amounts of total DA. At low amounts of total DA (<20 \(\mu\)g DA), solubilization generally ranged from 40 to 100% (minimum ∼10%). This proportion increased to 80–100% when the total amount of DA in trap cups exceeded 20 \(\mu\)g DA. Based on the assumption that the source of both dDA and pDA is associated with sinking particles entering the trap, the sum of dDA and pDA (volume and mass corrected) was used to calculate total particulate DA, denoted as pDA+.

A number of studies have shown rapid DA degradation with sample storage (Smith et al., 2006; Wright and Quilliam, 1995). In order to examine possible diagenetic effects associated with DA preservation within supernatants over time, a known concentration of certified DA reference standard was added to the supernatant solution used during the SBB trap deployments; filtered seawater amended with preservatives and buffers to a final concentration of 10% sodium azide and 1% sodium borate. The spiked supernatant was then stored refrigerated and in the dark in the same manner as the supernatant samples and DA concentrations measured at various time intervals throughout the course of almost a year.

Results from this experiment demonstrate that DA is rapidly degraded (<51% of initial concentration) within the sediment trap solution over the first 180 days, the average length of sediment trap deployment in the SBB (Fig. 4). This degradation rapidly slows thereafter, such that when using a modeled regression to the data, DA concentrations decrease to ∼25% of initial concentrations at 6 years and to 15% by year 16. Please note that these are for samples stored refrigerated and our samples, once recovered from the sediment trap, were stored frozen. Therefore, they are likely to have undergone less degradation. These results suggest that the DA concentrations measured within the SBB sediment traps are minimum estimates, and while gradual changes in DA fluxes may have occurred throughout the entire time-series, abrupt changes due to degradation are highly unlikely. This is particularly true of this study, as all supernatant samples (which contain 88 ± 21% of the DA within the sediment trap) collected from 1993 to 2005 were measured at last two years after sample collection and over a period of several months. It is interesting to note here that there is no evidence of DA isomerization in the degraded DA trap samples or in the spiked DA degradation experiment.

### 3.3. DA in sinking particles and bottom sediments

Concentrations of pDA+ in sinking particles, uncorrected for possible degradation, ranged from below detection to ∼320 \(\mu\)g g\(^{-1}\) sed, and in 6 instances after 2000, exceeded the U.S. Federal shellfish limit of 20 ppm, by ranging from 31 to 313 ppm. Prior to 2000, only one event in February 1997 (185 ppm) had a DA concentration that exceeded 10 ppm Large pDA+ fluxes (product of total mass flux and pDA+ concentrations) typically ranged from 50 to 200 \(\mu\)g DA m\(^{-2}\) d\(^{-1}\), with a maximum flux of 520 \(\mu\)g DA m\(^{-2}\) d\(^{-1}\) occurring in late January 2004 (Fig. 5). The first large pDA+ flux event (∼180 \(\mu\)g DA m\(^{-2}\) d\(^{-1}\)) occurred in February 1997, during upwelling that preceded the 1997–1998 El Niño. Smaller fluxes (<15 \(\mu\)g DA m\(^{-2}\) d\(^{-1}\)) are detectable prior to 1997, with the earliest occurrence of DA (7 \(\mu\)g DA m\(^{-2}\) d\(^{-1}\) in

![Fig. 4. Simulated DA trap supernatant degradation experiment. SBB sediment trap supernatant amended with a known DA concentration and measured repeatedly over time. The dashed and dotted line represents the initial concentration (339.3 \(\mu\)g L\(^{-1}\)) and the black line depicts the best fit to the data. Error bars are the standard deviation of replicate measurements.](image-url)
January 1994. Analysis of all trap data suggests that there was an abrupt shift in the frequency and magnitude of DA flux events, with one large event (>5 µg m⁻² d⁻¹) from 1993 to 1999 versus 16 large DA events from 2000 to 2007. On average, DA fluxes to sediment traps have increased by 13.4 µg m⁻² d⁻¹ since 2000.

DA concentrations in shellfish have been monitored since 2002 in SBB by the California Department of Public Health (http://www.cdph.ca.gov). Most shellfish samples are obtained from intertidal areas and a few from sentinel bags that hang off piers (ranging from 0 to 10 feet water depth depending on tides). In SBB, samples have also been collected from shellfish grown just offshore at ~30 ft water depth (Gregg Langlois, written comm., 2009, Bernard Friedman, Santa Barbara Mariculture Company). Comparison of shellfish DA concentrations in SBB with DA detected in the sediment trap reveal coincident events in April–May 2002, April 2004, and April 2007 (Fig. 5).

To examine the retention of DA in bottom sediments, DA was analyzed in SBB-MC1 and CA-B08 samples. Analysis of the top 5 cm of SBB-MC1 showed no evidence of DA except in the top interval (0.0–0.25 cm) with a concentration of 19 ng g⁻¹ sed, previously reported in Sekula-Wood et al. (2009). Of the CA-B08 samples from SBB analyzed, DA was detected in 8 samples with concentrations ranging from 1.2 to 8.0 ng g⁻¹ sed (Fig. 6). The majority of these samples were collected near shore in waters < 100 m deep. Notably, concentrations in bottom sediments were several orders of magnitude less than those detected in sinking particles.

4. Discussion

4.1. Quantifying DA in sediment trap samples

In previous studies measuring DA in sediment trap samples, DA was determined by either: (1) methanol extraction of a wet split of the sediment trap sample which collectively accounts for toxin in the particulate and dissolved phases (Schmetzer et al., 2007; Sekula-Wood et al., 2009) or (2) separate analyses of trap particulates and aliquots of trap cup solution (Sekula-Wood et al., 2009) which were then summed (given mass and volume corrections). Both approaches are reasonable for determining the total amount of DA in the trap cup. However, only in the second method can solubilization of DA be resolved since the dissolved and particulate pools of DA are determined independently.

In this study, measurement of DA in sediment trap samples by the latter method revealed that the vast majority of DA detected was present in the dissolved phase (88 ± 21%). Of the DA entering the trap entrained within particles, it is likely that intracellular DA is released to the surrounding supernatant by the increased permeabil-
ity ("leakiness") of Pseudo-nitzschia cells with age (i.e. senescence and death). In addition, given that DA is highly water soluble, agitation (during trap recovery or shipment of samples) may promote this exchange and desorption of DA from particulates, namely organic matter. Thus, DA fluxes in traps will likely be substantially underestimated if DA in supernatants is not measured.

A number of studies have found that DA degrades in methanol extracts from shellfish or acidified samples over time (Smith et al., 2006; Wright and Quilliam, 1995). Our experiment with known DA concentrations added to a mixture of sodium azide and sodium borate buffered seawater solution confirms that DA degradation has also likely occurred within the SBB sediment trap supernatants. Most of this degradation (51%) occurred within the first 6 months of collection. In contrast, degradation rates slowed to less than 4% per year of initial DA concentrations, two years after collection. As such, while all DA measurements presented in this study should be viewed as minimum estimates, we argue that trends in DA concentrations measured more than two years after collection are reflective of overlying water column processes and are not an artifact of measurement. For example, the first time SBB sediment trap concentrations exceeded 20 ppm DA (U.S. Federal shellfish limit) is in December 2000, with a pDA of 35 ppm. This sample was already more than 6 years old by the time it was measured for DA. If this sample was allowed to stand for an additional 6 years (1994), DA concentrations would still be 28 ppm according to the supernatant degradation model (Fig. 4). Please note that all DA concentrations presented herein remain uncorrected for degradation.

4.2. Pseudo-nitzschia and DA flux time series in SBB

Analysis of sediment trap materials in this study show that toxic Pseudo-nitzschia blooms in SBB have occurred as early as January 1994, four years prior to the 1998 mass mortality of California sea lions along the central coastline that was attributed to P. australis (Scholin et al., 2000; Trainer et al., 2000). Throughout the sediment trap time-series, P. australis blooms and DA events were more frequent during upwelling, but also occurred during the relaxation phase as well (Figs. 3 and 5). This is consistent with previous studies that suggest increased nutrient availability, as well as silica stress (low silica/nitrogen and silica/phosphorus ratios) favor blooms of lightly silified Pseudo-nitzschia (Anderson et al., 2006, 2009, 2011; Bates et al., 1998; Marchetti et al., 2004; Trainer et al., 2002). With respect to ENSO events in SBB, the only relationship observed was a large increase in cell abundance occurring at the end of a long La Niña (1998–2001). Although rivers are thought to be an important source of nutrients in SBB (McPhee-Shaw et al., 2007), it was difficult to ascertain any relationship with river discharge since large discharge events (>100 ft³ s⁻¹) generally overlapped upwelling periods. In April 2007, shellfish DA concentrations in SBB reached ~600 ppm and were the highest recorded since the start of the monitoring program. This high concentration event coincided with the death of a minke whale attributed to DA-poisoning in SBB (Fire et al., 2010). Sediment trap collections began to detect particulate DA during the March 13–31, 2007 time period, with fluxes increasing from 3.9 to 177 µg m⁻² d⁻¹ by May 4, 2007 (Fig. 5). It is important to note here that while DA events were often detected in both sediment trap samples and shellfish, there were numerous occurrences of DA in shellfish that did not occur in the sediment trap record. This may in part be the result of the high particle filtration rates of shellfish relative to the concentrations of DA and particles offshore. In contrast, higher fluxes of DA within the sediment traps, such as in early 2004 (Fig. 5), are likely due to a geographic disconnect between the nearshore environment and the center of the basin, which can be attributed to the large spatial variability of blooms structured by nutrient availability, winds, and mesoscale eddies in the channel (Anderson et al., 2006).

Most notable in the sediment trap records of both Pseudo-nitzschia and DA is the pronounced increase in frequency and magnitude of events beginning in 2000–2001 and perhaps as early as 1998. Some of this temporal difference may be related to the preservation of DA in sediment trap samples stored in excess of five years. However the presence of significant sediment trap DA concentrations as early as 1994, and the abrupt rather than gradual increase in DA concentrations over the past 15 years, suggests that this change is not an artifact of sample collection and measurement.

The increase in DA and Pseudo-nitzschia cell abundance after 2000–2001 coincides with sediment core observations by Barron et al. (2010). Their analysis of a box core collected near the center of SBB showed that prior to 2000, P. australis typically constituted <1% of the diatom assemblage with smaller peaks of 3% (1986–1987) and 5% (1990–1991). Beginning in 2000–2001, P. australis increased to 13% suggesting that there was a significant shift towards conditions favoring proliferation of toxic Pseudo-nitzschia species, which Barron et al. (2010) suggest is a result of changes in the intensity of the California Current potentially associated with the Pacific Decadal Oscillation (PDO).

The PDO describes ocean-atmosphere climate variability in the Pacific basin as identified by changes in sea surface temperature (SST) and has been linked to biological regime shifts in the North Pacific (Peterson and Schwinger, 2003). An abrupt shift towards a negative PDO (lower SST) occurred in 1998. While this is earlier than that observed changes in DA and Pseudo-nitzschia in the sediment trap records, it is important to note that sediment trap clogging precluded us from making measurements during that time period. Another abrupt shift with positive PDO (warmer SST) from 2003 to 2006 also occurred. Direct correlations between PDO and Pseudo-nitzschia and DA fluxes are poor, although a significant relationship with Pseudo-nitzschia flux exists when PDO leads by 18 months (r = −0.34 at the 5% significance level). Alternately, the North Pacific Gyre Oscillation (NPGO), an independent mode of sea surface salinity and sea surface temperature variability, may have a more direct impact on conditions in SBB. When positive, the NPGO represents an intensification of the geostrophic circulation and an increase in the southward transport of both the Alaskan Coastal Current and California Current systems. This in turn results in increased wind-forcing that favors upwelling conditions in the SBB (Di Lorenzo et al., 2008). When lagged by two months, the NPGO significantly correlates with the sediment trap times series of Pseudo-nitzschia (r = 0.28 at the 5% significance level), such that blooms peak when the rate of change of the NPGO index also peaks (Fig. 3). In contrast, DA fluxes peak 12 months after the NPGO peak (r = 0.23 at the 5% significance level), suggesting a possible delayed response in toxin production (Fig. 5). Combined, these results suggest that seasonal changes in Pseudo-nitzschia and DA abundances in the SBB may be influenced by the NPGO, which modulates basin scale upwelling strength and nutrient availability, particularly when the PDO shifts in unison with the NPGO as occurred in 1998 (Di Lorenzo et al., 2008).

In order to further investigate the temporal history of Pseudo-nitzschia and DA presence in SBB, we examined a number of surface sediment samples. Comparison of Pseudo-nitzschia cell concentrations in sediment trap and core samples (SBB-MC1) indicated poor preservation of Pseudo-nitzschia frustules in bottom sediments. Unlike the sediment trap samples that were preserved in buffered solutions immediately after sediment trap recovery, SBB surficial sediments have likely been exposed to overlying waters for periods of more than year. As a lightly silicified genus, Pseudo-nitzschia is likely more susceptible than other diatom species to dissolution processes at the sediment-water interface and upper portion of
bottom sediments, particularly those exposed to saline and slightly alkaline anaerobic sediments as found in SBB (Dickman and Glenwright, 1997). In SBB-MC1 core samples, light microscopy revealed substantially different planktonic assemblages dominated by foraminifera and centric diatoms. The dominating presence of these structurally robust forms suggests that more lightly silicified and easily fragmented diatoms had been effectively removed by dissolution or fragmented to sizes too small for positive identification.

Concentrations of pDA in SBB bottom sediments were approximately three orders of magnitude lower than concentrations observed in the overlying sediment trap samples (<100 m depth difference). The loss of DA supports the degradation of frustules that would result in the release of intracellular DA and diffusion to surrounding porewaters. This suggests that the potential exposure of pelagic and benthic feeders to the toxin is highly dependent on how tightly coupled the foodweb is to surface production. The distribution of DA-positive CA-B08 samples implies that there is greater DA retention in bottom sediments at shallower depths (Fig. 6), and suggests that oxygen content of sediments does not increase DA retention within cells.

Although historical sediment records of *Pseudo-nitzschia* abundance were unsuccessful in this study, several other authors have demonstrated that *Pseudo-nitzschia* spp. may be well-preserved in other settings and have used cell abundance to understand temporal variability of *Pseudo-nitzschia* blooms in coastal systems (Lundholm et al., 2010; Parsons et al., 2002). For example, Parsons et al. (2002) used 210Pb-dated sediment cores from the northern Gulf of Mexico to demonstrate that several spp. of *Pseudo-nitzschia* had increased in abundance since the 1950s, coincident with anthropogenic increases in riverine nitrogen fluxes and decreases in the dissolved Si:N ratio. More recently, Lundholm et al. (2010) used 210Pb-dated sediment cores from the Mariager Fjord, Denmark to identify a shift in *Pseudo-nitzschia* species from *Pseudo-nitzschia multiseriis* to *Pseudo-nitzschia pungens* around 1950. This shift was also coincident with an increasing relative abundance of *Pseudo-nitzschia seriata* and *Pseudonitzschia americana* and positively correlated to nitrogen loading and perhaps increasing water temperatures. Therefore, the shift in increasing *Pseudo-nitzschia* abundance and DA toxicity in the SBB is not unique, and demonstrates how both anthropogenic and natural climate oscillations may promote *Pseudo-nitzschia* blooms, and hence DA production, in coastal settings.

5. Conclusions

Long time-series measurements of sediment trap material collected at ~500 m water depth suggests that toxic blooms of *Pseudo-nitzschia* have occurred annually in SBB since at least 1994 and have increased in magnitude beginning in 2000. These *Pseudo-nitzschia* blooms are dominated by *P. australis*, one of the most toxic *Pseudo-nitzschia* spp. in the California region. The vertical transport of DA-laden particles represents a significant sink of the toxin to deeper waters and a source of toxin for potential bioaccumulation in pelagic and benthic ecosystems. Significant decreases in cell and DA concentrations in bottom sediments suggests that *Pseudo-nitzschia* frustules are quickly degraded at the sediment–water interface, resulting in loss of intracellular toxin. Thus, the greatest threat of toxin below surface waters is likely to affect biota feeding in mid- and deep-water environments as opposed to benthic communities. *Pseudo-nitzschia* and DA events have increased in both frequency and magnitude over the past decade coincident with an ecosystem shift in chlorophyll and nutrients associated with the California Current system and the NPGO. These conclusions, however, remain speculative given the lack of strong correlational relationships.

Surface water sampling and shellfish monitoring are invaluable tools for the early detection of DA events and are thus essential for minimizing human exposure to the toxin. However, sediment traps can provide insight into the fate of DA produced in surface waters and help assess the threat of toxin accumulation to deeper water ecosystems. Continuous collections at fine-scale resolution from sediment traps can also provide long-term records of *Pseudo-nitzschia* and toxin events. Results from this study illustrate that such datasets can be used to examine changes in frequency and magnitude of such events and help in assessing historical field conditions favorable for blooms and toxin production.

Acknowledgements

We thank Eric Tappa for his outstanding contributions to the success of the SBB sediment trapping program and the crew of the R/V Yellowfin for their efforts in both sediment trap deployments and recovery. We also wish to thank Steven Bograd for his advice on the NPGO. Two anonymous reviewers helped to significantly improve the manuscript. This work was supported in part by the National Science Foundation Chemical Oceanography Program, Grant #OCE0854025.

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