Title

Delivery to and presence of domoic acid in the surface sediments of the Santa Cruz Municipal Wharf, Santa Cruz, California, USA

Author

Ziccarelli, Lisa Marie

Publication Date

2014

UNIVERSITY OF CALIFORNIA

SANTA CRUZ

Delivery to and presence of domoic acid in the surface sediments of the Santa Cruz Municipal Wharf, Santa Cruz, California, USA

A thesis submitted in partial satisfaction
of the requirements for the degree of

MASTER OF SCIENCE

in

OCEAN SCIENCES

by

Lisa Marie Ziccarelli

June 2014

The Thesis of Lisa Marie Ziccarelli
is approved:

Professor Raphael Kudela

Professor Emeritus Mary Silver

Professor Alan Shanks

Tyrus Miller
Vice Provost and Dean of Graduate Studies

Table of Contents

List of Figures and Tables

ABSTRACT

Lisa Marie Ziccarelli

Retention of domoic acid in the surface sediments of the Santa Cruz Municipal Wharf, Santa Cruz, California, USA

Seasonal blooms of *Pseudo-nitzschia* spp., along with corresponding seasonal increase/decrease in domoic acid concentrations, consistently occur in the waters surrounding the Santa Cruz Municipal Wharf. Domoic acid has been intermittently observed in the water column when *Pseudo-nitzschia* spp. are not present, and presence or absence of cells is generally a poor indicator of toxin concentration in the water and sentinel mussel samples. Chlorophyll *a* and domoic acid values from seawater, sediment and Solid Phase Adsorption Toxin Tracking (SPATT) samples from February through December 2013 were analyzed and compared to a long-running weekly time series at the Santa Cruz Municipal Wharf, part of the California Department of Public Health monitoring program, to investigate retention of domoic acid in the sediments. We hypothesized that domoic acid concentrations in the sediment would increase immediately following a *Pseudo-nitzschia* bloom and increased domoic acid concentrations in the water column would follow a mixing event due to sediment and bottom water resuspension. Despite the lack of a significant toxic algal bloom during the study period, domoic acid was consistently observed at the sediment-water interface. Peaks of domoic acid concentrations in SPATT samples preceded peaks of particulate domoic acid from seawater samples, suggesting that the domoic acid source was more closely associated with the sediment-water interface. Sediment resuspension is a likely origin, suggesting that the sediment is potentially acting as a reservoir for domoic acid. It may be important for public health monitoring programs to include a sediment toxin analysis, particularly given the known presence of domoic acid in commercially harvested benthic organisms, such as crabs and flatfish.

To

Ma and Dodie

For *always* being there for me

ACKNOWLEDGEMENTS

First and foremost, I want to thank my advisor, Raphe Kudela. Whenever I required advice or needed to be pointed in the right direction, he was ready and willing. He not only conducted the HPLC analysis of all my samples, but also addressed my many questions with patience and brilliance. I want to thank my committee members, Alan Shanks and Mary Silver. Alan took a chance on me and I am forever grateful. Mary provided unlimited inspiration, as she allowed me to explore all my ideas. Their support and guidance was essential to my success.

I want to thank my lab manager, Kendra Negrey. Her tenacity and patience to teach me proper lab techniques has made me a better scientist. In the Kuldela lab, the support and friendship offered by Jennifer Broughton was instrumental. She was always willing to offer advice and lend a helping hand. I also want to thank Laura Peteiro, Cori Gibble and Regina Radan for their experience and generosity.

To those in my cohort, Claire Parker, Taylor Brooke, Kane Cunningham, James Shope (honorary), Ralph Till and Natasha Vokhshoori, I owe a great deal. During my time at UCSC, they provided friendship and fond memories. To my SCUBA instructors, Cecil Shin and Steve Clabeusch, thank you for showing me a new world and teaching me how to sample it safely. To my department manager and graduate advisor, Lezlie Ward, you do the work of many and I am forever in your debt. I must thank the Friends of Long Marine Lab for their monetary support of my project through their Student Research and Education Award program. Finally, I need to

thank my family. Their support and encouragement to pursue my passions, despite many challenges, fuels my determination to solve the puzzle.

Chapter 1: Background & Introduction

The phytoplankton is essential to nearly all life on this planet. As the base of the marine food web, the phytoplankton supports marine life and, as prolific photosynthesizers, these single-celled protists provide the oxygen necessary for terrestrial life. A stark increase in phytoplankton abundance, known as a bloom, occurs in the absence of limiting factors such as grazing zooplankton and fungal infections and the abundance of light and nutrients. Harmful algal blooms (HABs) occur when substantially greater concentrations of toxic phytoplankton cells are centered in one location. HABs occur in both saltwater and freshwater environments and cause harm through two primary mechanisms: 1) production of toxins that may kill animals directly or may cause illnesses following ingestion of intoxicated prey, and 2) alteration of food webs due to toxin accumulation. HAB events can lead to illness and death in humans, fish, seabirds, marine mammals and other marine life. They can also cause damage to ecosystems, fisheries resources and recreational facilities.

HABs occur on nearly every coastline and reports of HAB events have drastically increased in the past few decades (Trainer et al. 2012). This strong increase is attributed to excessive nutrient pollution of the water, as well as enhanced detection of HABs by coastal monitoring programs (Van Dolah 2000). The impacts of these naturally occurring phenomena are extensive and vary depending on the species involved. Twelve species of *Pseudo-nitzschia*, a cosmopolitan genus of pinnate diatoms, can produce a neurotoxin known as domoic acid (DA) (Lundholm

2011). DA causes the neurons of the brain to fire chaotically and over stimulate the receptors, causing the brain to essentially loose use of those inflicted neurons (Mos 2001). Particularly vulnerable are neurons are found in the amygdala and the hippocampus, which are critical for memory and navigation (Todd 1993). Symptoms of DA poisoning in mammals can include disorientation, seizures, short-term memory loss, permanent brain damage and coma (Grant et al. 2010).

On the Pacific coast of the United States, the first documented toxic *Pseudo-nitzschia* bloom occurred in 1991 (Work et al. 1993). More than 100 brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) died in Monterey Bay, California, after eating anchovies contaminated by *Pseudo-nitzschia australis*, one of the most problematic species in this area (Trainer et al. 2012). Monterey Bay is situated along the central California coast in a region dominated by coastal upwelling and subject to recurring blooms of toxic *Pseudo-nitzschia* (Lane et al. 2009).

Previous work on *Pseudo-nitzschia* has been generated primarily during relatively short, episodic events within Monterey Bay, CA (Buck et al. 1992, Fritz et al. 1992, Work et al. 1993). More recent work has employed long-term monitoring approaches (Jester et al. 2009, Lane et al. 2009, Langlois 2012). While the majority of these studies have focused on cell and toxin concentrations in surface waters, interest in downward transport and impacts on benthic food webs has yielded alluring results.

In shallow coastal environments, toxic blooms of *Pseudo-nitzschia* can encompass the entire water column (Kvitek et al. 2008). Evidence from sediment traps suggests rapid downward export of individual cells and chains, as well as DA, following a bloom event (Alldredge and Gottschalk 1989, Buck et al. 1992, Kvitek et al. 2008, Sekula-Wood et al. 2009). Retention of significant DA concentrations in *Pseudo-nitzschia* cells at depth (Trainer et al. 2008), along with adsorption of DA to sediments (Burns and Ferry 2007), poses potentially long-lasting impacts to the benthic food web. The benthic environment can thus serve as a source of DA contamination even after a surface bloom has subsided.

Research and monitoring programs at the University of California, Santa Cruz, currently collect water samples from the Santa Cruz Wharf (SCW) to provide information about bloom activity, toxin levels and changing ocean conditions, but do not analyze the sediments for DA (Lane et al. 2009). This study explores the potential for the sediments at SCW to act as a DA reservoir.

References

Alldredge, AL and CC Gotschalk. (1989) Direct observation of the mass flocculation of diatom blooms: Characteristics, settling velocities and formation of diatom aggregates. Deep Sea-Research I 36:159–171

Buck, KR, L Uttalcooke, CH Pilskaln, DL Roelke, MC Villac, GA Fryxell, L Cifuentes and FP Chavez. (1992) Autecology of the diatom *Pseudo-nitzschia australis*, a domoic acid producer, from Monterey Bay, California. Marine Ecology Progress Series 84:293-302

Burns, JM and JL Ferry. (2007) Adsorption of domoic acid to marine sediments and clays. Journal of Environmental Monitoring 9:1373-1377

Fritz, L, MA Quilliam, JLC Wright, AM Beale and TM Work. (1992) An outbreak of domoic acid poisoning attributed to the pinnate diatom *Pseudo-nitzschia australis*. Journal of Phycology 28:439-442

Grant, KS, TM Burbacher, EM Faustman and L Gratttan. (2010) Domoic acid: Neurobehavioral consequences of exposure to a prevalent marine biotoxin. Neurotoxicology and Teratology 32(2):132-141

Jester, R, K Lefebvre, G Langlois, V Vigilant, K Baugh and MW Silver. (2009) A shift in the dominant toxin-producing algal species in central California alters phycotoxins in food webs. Harmful algae 8:291-298

Kvitek, RG, JD Goldberg, GJ Smith, GJ Doucette and MW Silver. (2008) Domoic acid contamination within eight representative species from the benthic food web of Monterey Bay, California, USA. Marine Ecology Progress Series 367:35-47

Lane, JQ, PT Raimondi and RM Kudela. (2009) Development of a logistic regression model for the prediction of toxigenic Pseudo-nitzschia blooms in Monterey Bay, California. Marine Ecology Progress Series 383:37-51

Langlois, GW. (2012) Marine Biotoxin Monitoring Program Annual Report. Retrieved from California Department of Public Health http://www.cdph.ca.gov/HealthInfo/environhealth/water/Pages/Shellfishreports.aspx

Lundholm, N. (2011) Bacillariophyta. IOC-UNESCO Taxonomic reference list of harmful micro algae. Retrieved from http://www.marinespecies.org/HAB

Mos, L. (2001) Domoic acid: A fascinating marine toxin. Environmental Toxicology and Pharmacology 9:79-85

Sekula-Wood, E, A Schnetzer, CR Benitez-Nelson, C Anderson, WM Berelson, MA Brzezinski, JM Burns, DA Caron, I Cetinic, JL Ferry, E Fitzpatrick, BH Jones, PE Miller, SL Morton, RA Schaffner, DA Siegel and R Thunell. (2009) Rapid downward transport of the neurotoxin domoic acid in coastal waters. Nature Geoscience 2(4): 272-275

Todd, ECD. (1993) Domoic acid and amnesic shellfish poisoning – A review. Journal of Food Protection 56(1):69-83

Trainer, VL, BM Hickey and SS Bates. (2008) Toxic diatoms. In: PJ Walsch, SL Smith, LE Fleming, H Solo-Gabriele, WH Gerwick (Eds.), Oceans and human health: Risks and remedies from the seas. Elsevier Science Publishers, New York 1:219-237

Trainer, VL, SS Bates, N Lundholm, AE Thessen, WP Cochlan, NG Adams and CG Trick. (2012) Pseudo-nitzschia physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14:271-300

Van Dolah, FM. (2000) Marine algal toxins: Origins, health effects, and their increased occurrence. Environmental Health Perspectives 108(suppl 1):133-141

Work, TM, AM Beale, L Fritz, MA Quilliam, M Silver, K Buck and JLC Wright. (1993) Domoic acid intoxication of brown pelicans and cormorants in Santa Cruz, California. In: TJ Smayda, Y Shimizu (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier, Amsterdam 3:643–649

Chapter 2: Delivery to and presence of domoic acid in the surface sediments of the Santa Cruz Municipal Wharf, Santa Cruz, California, USA

Introduction

Monterey Bay is situated along the central California coast in a region dominated by coastal upwelling and subject to recurring blooms of toxic *Pseudo-nitzschia* (Lane et al. 2009). While Monterey Bay is historically described as an open embayment, upwelling dynamics are closely correlated with local wind patterns (Graham and Largier 1997). Persistent northwest winds, along with Ekman transport, force deep nutrient-rich waters up to the surface. This upwelling regime drives the high biological productivity that characterizes the region (Graham and Largier 1997). Monterey Bay is thus an ideal site to study phytoplankton bloom dynamics, as it provides a representative area of the nearshore California Current system, with an extended upwelling period.

The major patterns of phytoplankton growth represent a biological response to water-column stratification, nutrient availability, the intensity and persistence of upwelling conditions and the initial phytoplankton stock size (Reynolds 2006). Within the California Current upwelling system, phytoplankton growth fluctuates seasonally, as maximum chlorophyll *a* (the photosynthetic pigment contained in all phytoplankton cells) concentrations are routinely observed during summer upwelling, compared to relatively low concentrations during winter upwelling relaxation and storms (Kudela et al. 2005). The phenology of coastal upwelling also influences phytoplankton community structure (Jester et al. 2009).

In the spring, more sunlight is readily available and nutrients have mixed into the surface layer of the ocean. The water column stratifies as rising temperatures warm the surface waters, inhibiting vertical mixing of phytoplankton and nutrients. This combination of environmental conditions promotes rapid production and accumulation of phytoplankton, known as blooms. Exceptional blooms, dominated by one or several species, can cause major ecological disturbance, sometimes leading to large economic losses and illness (Trainer et al. 2012). As is typical of the California Current system, phytoplankton growth within Monterey Bay fluctuates seasonally from high biomass during spring and summer to relatively low levels during winter (Pilskaln et al. 1996).

Marine diatoms of genus *Pseudo-nitzschia* produce domoic acid (DA), a neurotoxin responsible for amnesic shellfish poisoning, symptoms of which include gastroenteritis, dizziness, headache, seizures, disorientation, short-term memory loss and coma in humans (Grant et al. 2010). DA is therefore important for both public and ecosystem health, with documented severe impacts to marine mammals and birds (Trainer et al. 2012). As toxic species of *Pseudo-nitzschia* senesce or die, cells sink to the benthos where the toxin may accumulate in surface sediments and pore waters. During mixing events, the accumulated toxin may be resuspended into the water column and could account for high levels of toxin in the absence of *Pseudo-nitzschia*. Retention of DA in sediment and pore water may alter public health monitoring programs, which currently do not sample the benthos for toxins.

Research and monitoring programs at the University of California, Santa Cruz, currently collect water samples from the Santa Cruz Wharf (SCW) to provide information about changing ocean conditions, such as water quality and bloom activity (Lane et al. 2009). Previous studies have documented both export of intact cells to great depths (Sekula-Wood et al. 2009; Kiørboe et al. 1996) and accumulation of DA in benthic invertebrates and flatfish (Vigilant et al. 2007). As part of the time series at SCW, Solid Phase Adsorption Toxin Tracking (SPATT) samplers have been deployed approximately weekly since July 2008 (Lane et al. 2010). SPATT frequently record DA when there are few or no *Pseudo-nitzschia* cells in the water column, suggesting either the presence of dissolved DA, an inherent bias in the phytoplankton observations or the presence of DA in the sediments (Lane et al. 2010).

Although the high solubility of DA in seawater (Maldonado et al. 2002) would seemingly reduce the amount of toxin reaching the benthos, there is evidence to suggest that DA not only reaches the benthos, but may also be preserved after a *Pseudo-nitzschia* bloom occurs (Sekula-Wood et al. 2009). Transfer of DA to the benthos may also be enhanced by the formation of cell aggregates, which have higher sinking rates than individual cells (Shanks and Trent 1980; Alldredge and Gotschalk 1989; Thunell et al. 2007). Sinking, therefore, provides one possible mechanism for transfer of DA to the benthos.

If *Pseudo-nitzschia* cells do accumulate in the sediment, then sample analysis should reveal evidence of DA retention in the sediments and pore waters. To investigate DA retention in sediment, this study reports on whole water, pore water and sediment samples collected throughout spring and summer 2013, when seasonal blooms of *Pseudo-nitzschia* are most common. Sample data are compared to the SCW chlorophyll, particulate DA, dissolved DA and Solid Phase Adsorption Toxin Tracking (SPATT) measurements collected as part of the California Harmful Algal Bloom Monitoring and Alert Program (HABMAP) time series conducted by UCSC from the same location. Two hypotheses are posed: first, DA concentrations in the sediment are expected to increase immediately following a *Pseudo-nitzschia* bloom, and second, following a bloom when Pseudo-nitzschia abundance is too low, DA concentrations will increase in the water column during a mixing events. If these hypotheses are borne out, the DA sequestered in the sediments would suggest the need for inclusion of sediment toxin analysis in public health monitoring programs and predictive models for impacts from harmful algal blooms.

Methods

1. Field Sampling

1.1 Study area and water sample collection

Samples for this study were collected in Monterey Bay from the Santa Cruz Municipal Wharf (36° 57.48'N, 122° 1.02'W), approximately daily between February 17 and September 2, 2013, and then every approximately 3 days from September to December 3, 2013, providing a total of 195, 203 and 215 samples for water, sediment and SPATT (out of 289 days total). Surface water samples were collected using a rinsed bucket. Bottom water samples were collected using a weighted 1.5 L Niskin bottle. The bottle was lowered until it touched the sediment and the line went slack. The bottle was then triggered and returned to the surface. Both surface and bottom water samples were transferred into separate 1 L Nalgene bottles. Temperature was recorded using a YSI Model 30 (YSI Inc., OH, USA). Water sampling was not aligned with tidal cycles; as a result, the time-series includes sampling at multiple tidal elevations and flow rates. Santa Cruz Wharf sampling occurs approximately weekly, with data from SPATT maintained at approximately 3 meters above the sediment and whole water, available from March 2010 through December 2013. SCW sampling uses slightly different methods than used for the intensive daily sampling. Specifically, that water samples for particulate DA and chlorophyll are collected from an integrated whole water sample from approximately 0, 1.5 and 3 meters, with 250 mL of water collected for pDA. For historical and logistical reasons, sampling locations were also different for the SCW whole-water sampling, SPATT deployments and the benthic sediment sampling conducted as part of this study. The SCW samples are collected on the north (water) and south (SPATT) sides of the wharf, while the daily water and sediment samples were initially collected near the SCW SPATT, but the location was subsequently moved closer to the location of the SCW water samples (9 March 2014). All of the sites are within approximately 100 m of each other, but were presumably subject to variability in local conditions.

1.2 Sediment and SPATT collection

Sediment was collected using a sediment trap, consisting of a 1 L Niskin bottle in a weighted milk crate, on an approximately daily interval until September and an approximately 3 day interval from September to December, but there was also a 7 day interval (7-14 March) where no sediment was collected. The bottle was supported in the center of the crate vertically by 4 PVC pieces 2.54 cm x 45.72 cm placed through the holes of the crate. Four “feet” were constructed by filling plastic containers with quick-dry cement. Another 45.72 cm piece of 2.54 cm PVC was pushed into the cement before drying. U-bolts attached these PVC pieces to the crate. Leaving the bottom opening of the bottle closed and the top open, the unit was lowered from the platform down to the benthos. For collection, a drop messenger triggered the bottle. The contents of the bottle were transferred to a glass jar. The bottle was then re-set and the sediment trap lowered to the benthos for the next collection day. Between 0.01 and 14 grams (mean=2.423 g; Figure 1) SPATT rings were constructed following standard procedure (Lane et al. 2010). Resins used include DIAION® HP20 and SEPABEADS® SP207. SPATT rings were attached to the bottom of the outside of the milk crate with reusable zip ties, such that they hung about 15 cm above the sediment. SPATT were not deployed or were lost for 83 days during the entire study period (24-28 Feb, 6-13 Mar, 7-9 Apr, 12 May, 21-22 May, 1 Jun, 19-23 Jun, 25 Jun, 27 Jun, 29-30 Jun, 6 Jul, 13 Jul, 2 Sep, 5 Sep, 7 Sep, 10 Sep, 12 Sep, 14-15 Sep, 17 Sep, 19 Sep, 21 Sep, 24 Sep, 26 Sep, 28 Sep-1 Oct, 3-6 Oct, 8 Oct, 10 Oct, 12-15 Oct, 17 Oct, 19-20 Oct, 22 Oct, 24-25 Oct, 27-28 Oct, 30-31 Oct, 2-3 Nov, 5 Nov, 7-10 Nov, 12 Nov, 14 Nov, 16-17 Nov, 19-21 Nov, 23 Nov, 25 Nov).

1.3 Diver surveys

If DA was observed in all sediment samples, then DA should also have been observed in sediment samples collected in diver surveys. To test whether the sediment trap was missing some huge reservoir in the sediments, a team of divers also collected sediment samples weekly from 17 July to 28 August 2013. Divers followed a transect line along the wharf between 300º and 120º, with the sediment collection unit as the center of the transect line. Divers used 50 ml Corning centrifuge tubes to scrape surface sediment at 7 locations 5 m apart. Divers also collected samples on the other (North) side of the wharf. All samples were immediately transported to the lab for processing following the methods described for sediment samples.

2. Chlorophyll *a* analysis

Aliquots (25 ml) of seawater were filtered through GF/F filters (nominal pore size 0.7µm) and polycarbonate, 10 µm filters for chlorophyll *a* (Chl *a*) analysis. Filters were placed in glass extraction tubes with 7 ml 90% acetone, covered with foil and stored in a -20ºC freezer. Samples were analyzed after 24 h using the standard fluorometric method (Welschmeyer 1994) with a Turner Designs 10-AU fluorometer. Chl *a* concentrations were determined by application of a standard calibration factor determined using pure Chl *a* (Sigma-Aldrich).

3. DA extraction and analysis

Domoic acid concentrations were determined for three sample types: SPATT, particulate DA from seawater and sediment pore water from the sediment trap, as well as diver surveys. SPATT samples were processed using standard methods (Lane et al. 2010). All DA concentrations from SPATT are reported as micrograms of DA per gram of resin. 250 ml of both surface and bottom water was filtered through GF/F filters. Filters were placed in a plastic tube with 3 ml of 10% MeOH before sonication using a probe sonicator for 30 sec at approximately 6 W power. The extracts were then filtered using a Durapore membrane (0.22 µm pore size; Millipore, Billerica, MA, USA). Aliquots (1.5 ml) of the filtered extract were subsequently extracted following the method of Wang et al. (2007). DA from seawater is reported as ng/L particulate domoic acid (pDA). Sediment samples underwent sonication for 2 min. Sediment pore water was then decanted into a glass test tube. Processing of sediment pore water samples and dissolved DA from filtered seawater also followed the extraction methods described by Wang et al. (2007). Following extraction, sediment samples were dried at 50°C for >24 h, and weighed to determine dry weight. DA from sediment pore water is reported as ng/g dry sediment.

Domoic acid was analyzed by liquid chromatography/mass spectrometry (LC/MS) using an Agilent 6130 system in SIM mode. A standard curve and blank samples were prepared and run with each batch of samples, using CRM DA-f (National Research Council Canada). The MDL depends on the sample volume, equating to 1.0 µg/L for dissolved DA (seawater and sediment pore water), 20 ng/L for pDA, and 4 ng/g for SPATT.

4. Statistical Methods

Statistical analysis of data used the MySTAT statistical package and the routines included in Microsoft Excel. Significance values were set at 0.05.

Results

1. *Chlorophyll a*

From the chlorophyll *a* data, one can see three pulses in phytoplankton biomass during 2013 when concentrations exceeded 1 standard deviation from the mean: 17 Feb - 13 Mar, 10 Apr - 8 May and 22 May - 12 Jun (Figures 2 and 3). Overall, concentrations range from 49.35 to 0 μg/L. On a daily basis, concentrations processed from a GFF were generally greater than those from a 10 μm filter. For both surface and bottom, GFF samples have a moderate positive correlation, but are statistically different (R^2=0.532; p=$3.124*10^{-7}$) (Table 1). Similarly, for both surface and bottom, 10 μm samples have a moderate positive correlation and are statistically different (R^2=0.495; p=$3.667*10^{-9}$) (Table 1). Approximately 60% of the surface phytoplankton community structure is >10 μm, while 70% of the bottom community is >10 μm. Surface measurements obtained using GFF are very strongly correlated with, and statistically different from, surface measurements using 10 μm filters (R^2=0.939; p=$1.405*10^{-42}$) (Table 1). Similarly, bottom water measurements made using GFF are very strongly correlated with, and statistically different from, measurements using 10 μm filters (R^2=0.940; p=$3.328*10^{-32}$) (Table 1).

Daily surface (p=0.133) and bottom (p=0.304) chlorophyll concentrations measurements obtained using GFF filters are not statistically different from the corresponding SCW concentrations (Figure 4). Similarly, daily surface (p=0.137) and bottom (p=0.194) water measurements made using 10 μm filters are not statistically different from the corresponding SCW concentrations (Figure 4). Interestingly, daily surface water concentrations exhibited strong positive correlations with SCW concentrations for GFF (R^2=0.793) and 10 µm (R^2=0.793), while daily bottom water concentrations exhibited only weak positive correlations with SCW concentrations for GFF (R^2=0.377) and 10 µm (R^2=0.344) (Table 3).

2. *Surface and bottom water pDA*

Surface and bottom water pDA concentrations reveal three pulses during the study period with values at least one standard deviation above the mean: 17 February - 16 March, 28 April - 6 May and 31 May - 6 July (Figure 6). The first pulse reached 32.097 ng/L, while the second reached 65.715 ng/L. The third pulse was smaller than the first two, as concentrations did not exceed 13.925 ng/L. Surface pDA concentrations exhibited a moderate positive correlation with bottom pDA concentrations (R^2=0.524) (Table 1). These pulses were not observed in the pDA concentrations of the weekly dataset (Figure 7). As a result, daily surface and bottom water concentrations were generally greater than weekly wharf concentrations. Additionally, daily surface pDA concentrations exhibited no correlation with SCW concentrations (R^2= -0.070), while daily bottom pDA concentrations had a moderate positive correlation with SCW concentrations (R^2=0.628) (Table 3).

3. Sediment

DA in the sediment reached a maximum on 24 February 2013, with a concentration of 40.73 ng per g sediment, and with corresponding lower non-zero values on 23 and 25 February 2013 (Figure 8). Subsequent DA concentrations remained relatively low or undetectable during the remainder of the study, with ~9% of samples positive for DA (19 of 201 samples). Sediment DA concentrations are significantly different from surface pDA concentrations ($p=0.005$) or bottom pDA concentrations ($p=7.611*10^{-5}$). Sediment DA concentrations had negligible correlations with all other measurements, except for bottom water pDA ($R^2=0.332$) (Table 1). Diver surveys were conducted during a period when samples collected from the sediment trap were less than the limit of detection. The diver survey samples were also non-detect for all but one sample (0.105 ng/g), suggesting that DA was not present in the immediate vicinity of the sampling location.

4. SPATT

DA concentrations from SPATT samples exceeded the alert level (20 ppm ≈ 50-70 ng/g DA from SPATT) at least once each month (Figure 9). Measurable concentrations of DA were found in 196 HP20 and SP207 samples during 2013. Concentrations of DA above the regulatory limit were detected in 44% of HP20 samples and in 14% of SP207 samples. DA concentrations in HP20 and SP207 SPATT samples were not correlated ($R^2=0.059$) (Table 1). In fact, daily SPATT DA concentrations had no significant correlation with any other daily variable. Averaged daily and SCW DA concentrations from SPATT samples are not statistically different for SP207 ($p=0.301$), while the HP20 sample concentrations are significantly different ($p=0.018$). Daily concentrations of both HP20 and SP207 tend to be higher than the SCW concentrations (Figures 10 and 11), but, when daily SPATT samples are averaged to match the duration of the SCW SPATT, moderate to strong correlations were evident for both HP20 ($R^2=0.575$) and SP207 ($R^2=0.941$) (Table 2).

Additionally, HP20 concentrations ($p=7.532*10^{-20}$) and SP207 concentrations ($p=2.614*10^{-5}$) were statistically different from sediment DA concentrations.

Discussion

Although there was a persistent *Pseudo-nitzschia* bloom inside Monterey Bay during 2013, DA concentrations above 0.3 ppm were not detected in shellfish samples. California Department of Public Health (CDPH) annual reports show decreasing means of DA concentrations since 2010 (Langlois 2010-2012). Relatively low DA concentrations were observed during this study. Indeed, 2013 exhibited the lowest DA concentrations in both mussels and water samples at the Santa Cruz Wharf since 2002, despite the high relative abundance of *Pseudo-nitzschia* cells throughout the year. Whole-cell probing (Lane et al. 2009; Miller and Scholin 2002) showed that the *Pseudo-nitzschia* were not the typical toxigenic species seen in Monterey Bay (*P. australis* and *P. multiseries*), and were most likely low-toxicity or non-toxic strains, possibly *P. pungens* and *P. hasleana* (H. Bowers, pers. comm.).

Three periods of increased pDA concentrations were observed in the surface and bottom water samples. Of these pulses, the first and third pulses lasted four and five weeks, respectively. The second pulse lasted only one week and exhibited the highest pDA concentrations during the study. The SCW dataset did not capture these pulses, suggesting that sampling one day per week may not provide sufficient resolution of DA pulses at this site.

Daily surface chlorophyll concentrations exhibited very strong positive correlations with SCW concentrations, while daily bottom chlorophyll concentrations exhibited only weak positive correlations with SCW concentrations. These results are not unexpected, considering the water collected for SCW samples is integrated over the upper 3 meters of the water column. Since chlorophyll concentrations for surface and bottom water were not strongly correlated water at the study site is presumably not consistently well mixed. These results may be due to a resuspension mechanism, such as bioturbation, bottom flow and long period waves.

Following a maximum (27.156 ng/g) on 24 February, sediment DA concentrations remained low (less than 10 ng/g). These low values may indicate that DA has a short residence time of hours to days in the sediment at the Santa Cruz Wharf. Sediment concentrations, however, are conservative estimates. DA concentrations were obtained using a 1.5 mL aliquot of a variable larger volume. Approximately 10 to 30 mL of pore water was collected for each sample, providing a minimum range of sediment DA concentrations from 3.318 to 271.565 ng/g.

Since sediment DA concentrations were generally greater than total (particulate and dissolved) SCW DA concentrations, DA may have accumulated in the sediments over a short period of time. Wind stress and current intensity likely have a strong influence on the residence time of DA in the sediments. Winds can stimulate *Pseudo-nitzschia* blooms of substantially greater concentrations of cells centered in one location and can be especially important for transporting toxic cells (Trainer et al 2000, 2002) or providing mixing necessary to bring nutrients into the photic zone (Lund-Hansen and Vang 2004). Wind-driven mixing may also cause settled, intact cells to mix into the water column. Depending on cellular integrity, wind intensity and current strength, intact cells may break and release DA.

Despite the lack of a toxic *Pseudo-nitzschia* event at the study site, several interesting trends were observed. HP20 DA concentrations suggest that DA concentrations are chronically above potentially dangerous levels for human exposure at the sediment-water interface. Sediment resuspension by bioturbation, moving lagan, bottom flow or waves could provide a continuous source of DA. SP207 samples provide similar results, but to a lesser extent when compared to HP20. This disparity is likely due to the stronger adsorptive character of SP207, which brings the resin to equilibrium with the water column more quickly than that of HP20 (Lane et al 2010).

Daily and SCW SPATT datasets are not statistically different for SP207 ($p=0.168$), while the HP20 datasets are significantly different ($p=0.008$). As previously mentioned, this disparity is likely due to the stronger adsorptive character of SP207. Therefore, HP20 may be the better choice for SPATT rings that are deployed for longer periods of time. Daily HP20 DA concentrations tend to be higher than the SCW concentrations, suggesting that there is either more toxin at the benthos compared to the surface or that the DA in the SCW SPATT degraded during long storage periods. SCW concentrations may be underestimating the concentration of DA in the water column.

Similar trends were evident for the weekly chlorophyll, SPATT and pDA when compared to daily data of the same time intervals. The daily data provide a higher temporal resolution of both the *Pseudo-nitzschia* blooms and the pulses of DA. While SCW sampling is sufficient to capture seasonal and interannual patterns (Lane et al. 2010), it clearly introduced bias during the relatively low toxicity period encountered during this study. In particular, the three pDA events were poorly resolved from SCW sampling, and the ephemeral nature of the DA in sediment samples suggests that it would be easy to miss the potential importance of DA accumulation in the sediment. SPATT deployed near the sediment-water interface demonstrate that there is a persistent source, either from senescent cells in bottom waters or from resuspended cells in a sediment reservoir of DA. This suggests that benthic organisms may be consistently exposed to DA, as well as occasional intense pulses (e.g. mid-February) of the toxin.

Sedimentation of *Pseudo-nitzschia* cells may occur by several mechanisms: 1) cells or chains my settle individually, 2) cells may coagulate to form sinking aggregates, and 3) cells may be transported by grazing (Kiørboe et al. 1996). The *Pseudo-nitzschia* valve has a vermiform shape that lends itself more easily to fragmentation than the centric diatoms (Turner 2002). Valve shape, along with the high solubility of DA in seawater (Maldonado et al. 2002), should reduce the amount of DA reaching the benthos, as well as the impact on benthic ecosystems. Nonetheless, sinking rates of *Pseudo-nitzschia,* if flocculated into marine snow can exceed 50 meters per day (Shanks 2002; Alldredge and Gotschalk 1989). These flocculates may be ingested, resuspended by bioturbation, bottom flow and waves, or preserved in the sediment.

Domoic acid can accumulate in benthic organisms, which serve as crucial vectors of toxin transfer in the marine food web (Kvitek et al. 2008). Consistent exposure of benthic organisms to DA due to *Pseudo-nitzschia* sedimentation, suggests that DA concentrations at the top of the water column may not serve as a reliable proxy for DA concentrations to which benthic organisms are exposed.

Conclusions

Daily SPATT sampling provides the ability to construct high temporal resolution time-series of changing toxin levels at the sediment-water interface. The generally good correlations between daily and SCW samples, suggest that longer deployments of approximately 3 days would provide similarly useful time-averaged results. The difference in SPATT and daily versus SCW testing suggests ways in which public health programs can improve DA testing efforts. However, the most significant observation is that DA was frequently seen in the benthos and at the sediment-water interface, despite the lack of a classic toxic event at the surface. Peaks of DA in both SPATT resins preceded peaks of pDA from water samples, suggesting that the DA source came from the bottom during this study, or that SPATT is more sensitive to onset of a DA event compared to traditional sampling of particulate material (c.f. Lane et al. 2010). Persistence of DA at the sediment-water interface and presence of DA in the sediment itself suggests that the source of this DA may be resuspension due to bioturbation, bottom flow or wave action, and that the sediment is potentially acting as a reservoir for DA. We conclude that near-surface blooms are not the only source of DA that leads to trophic transfer, and that monitoring programs focused on human or wildlife health should consider the potential for the sediments to act as a reservoir and potential concentrating mechanism leading to DA accumulation in benthic organisms and subsequent intoxication of predators, such as otters, sea lions and flatfish.

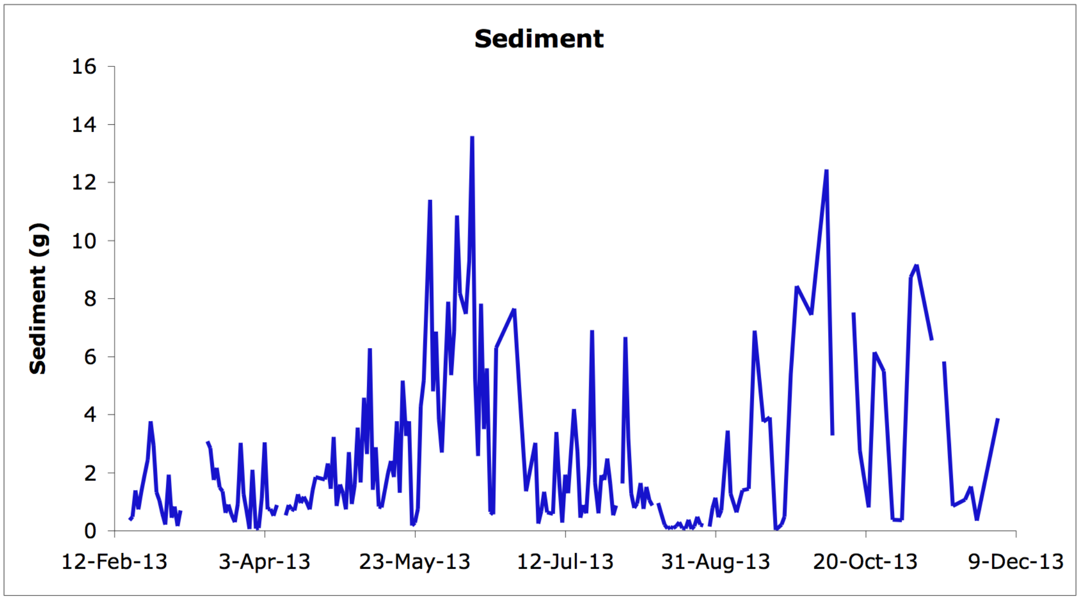

Figure 1. Dry weight of sediment collected from sediment trap (mean=2.423 g).

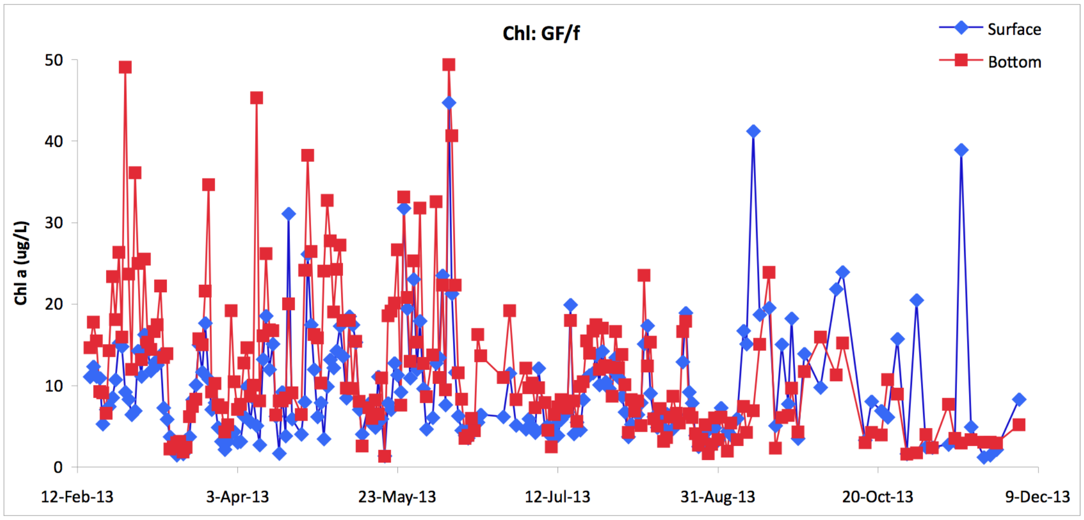

Figure 2. Chlorophyll *a* concentrations (µg/L) for surface and bottom water samples processed with GFF filters.

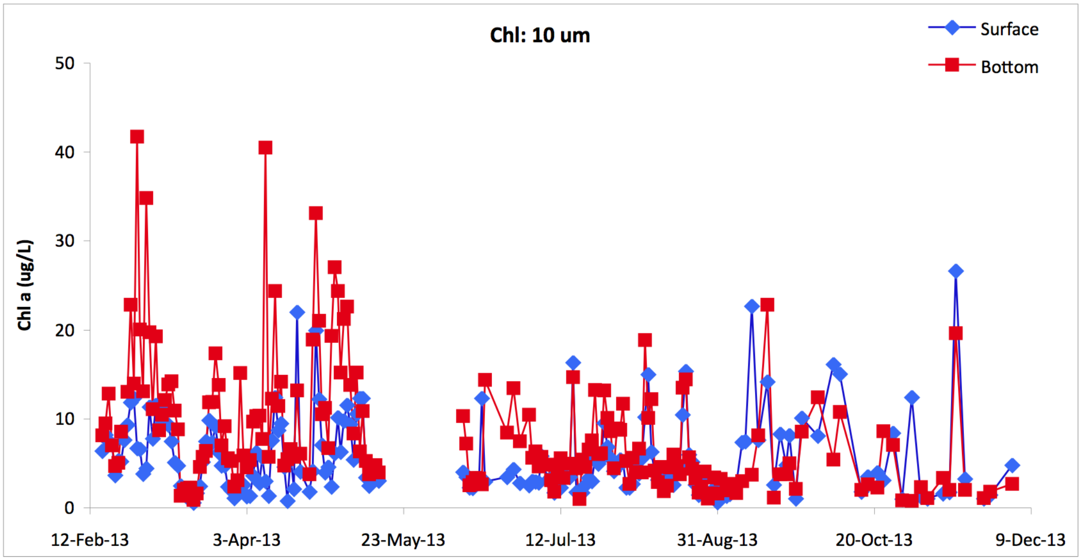

Figure 3. Chlorophyll *a* concentrations (µg/L) for surface and bottom water samples processed with 10 µm filters.

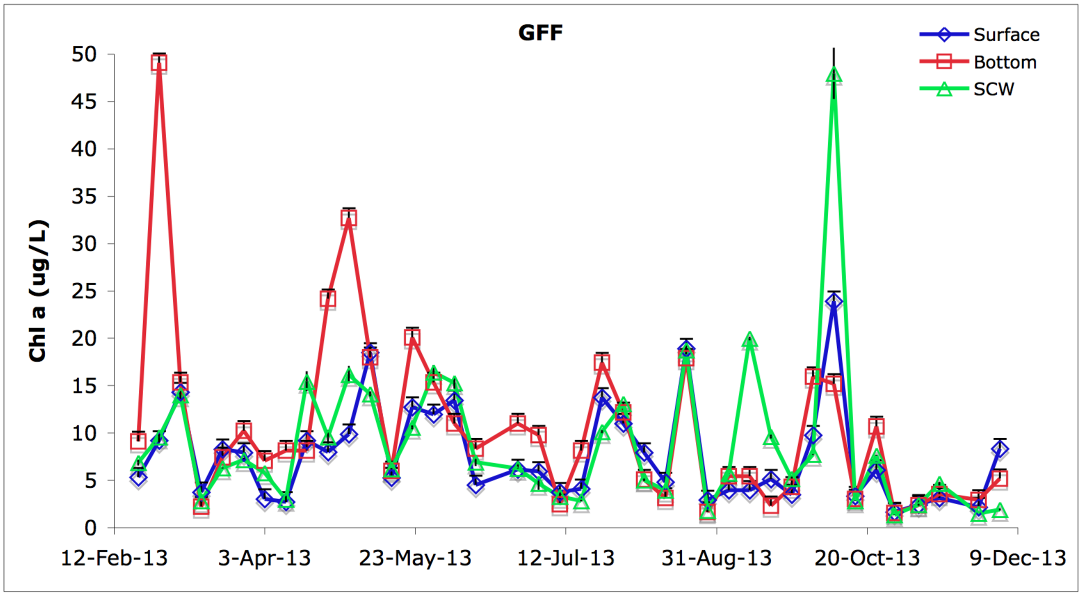

Figure 4. Daily surface and bottom chlorophyll *a* measurements obtained using GFF filters are not statistically different from the analog weekly values (p=0.133; 0.304).

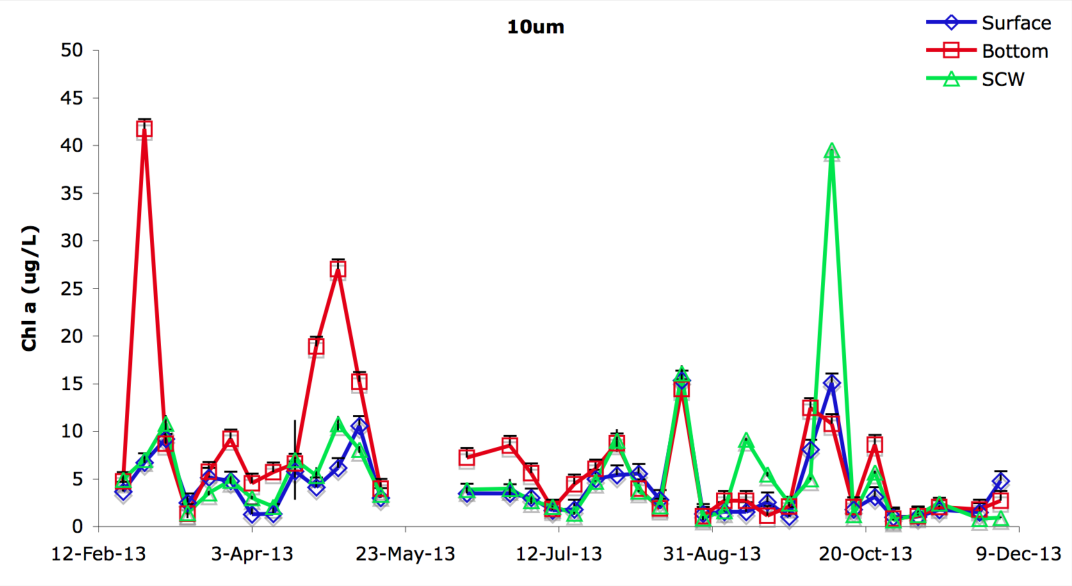

Figure 5. Averaged daily surface and bottom water measurements obtained using 10 µm filters are not statistically different from the analog weekly wharf values (p=0.137; 0.194).

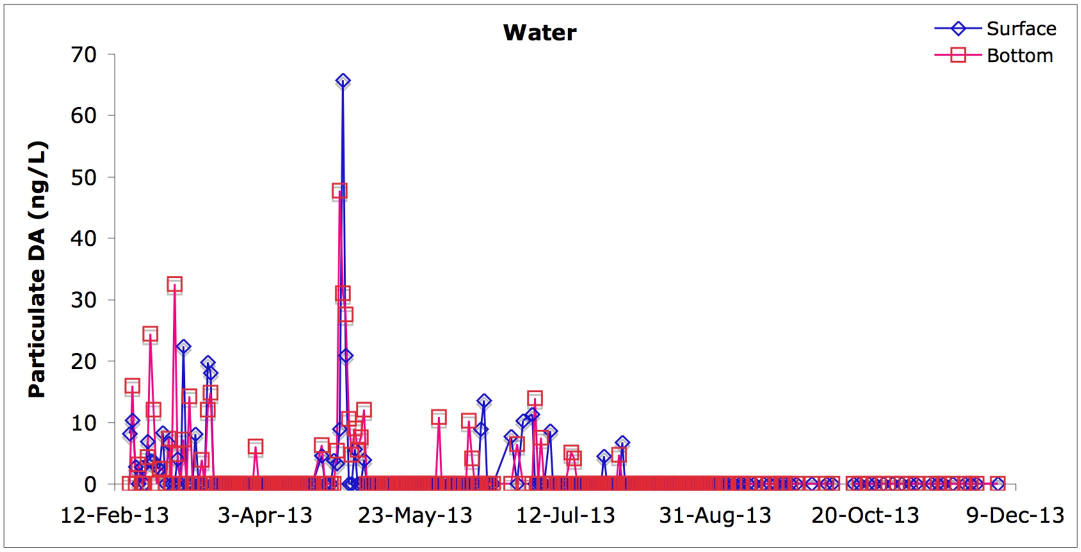

Figure 6. Three pulses of pDA concentrations in surface and bottom water samples occurred in 2013 between 17 February - 16 March, 28 April - 6 May and 31 May - 6 July.

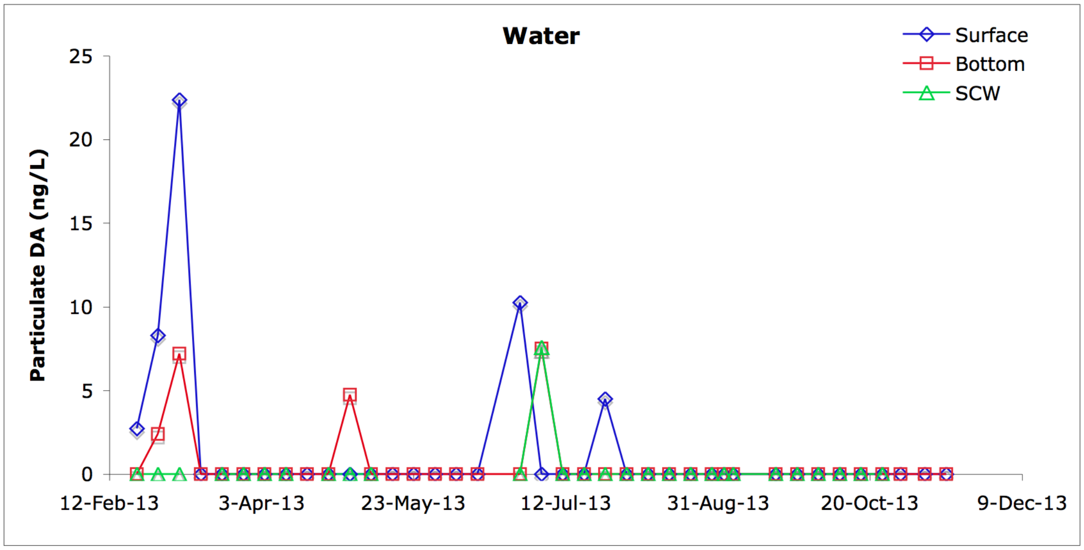

Figure 7. Daily surface and bottom water pDA concentrations matched with respective weekly pDA concentrations. Weekly pDA concentrations do not exhibit the three pDA pulses in the surface and bottom water. Weekly sampling does not capture daily fluctuations in DA.

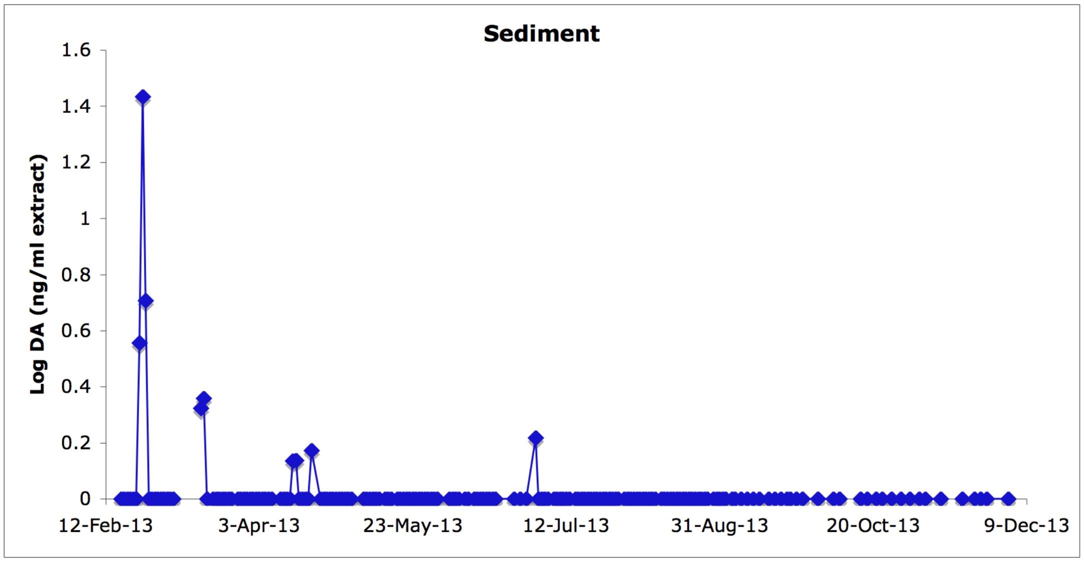

Figure 8. Log-transformed dry sediment pore water DA values (ng/g) collected from sediment sampler.

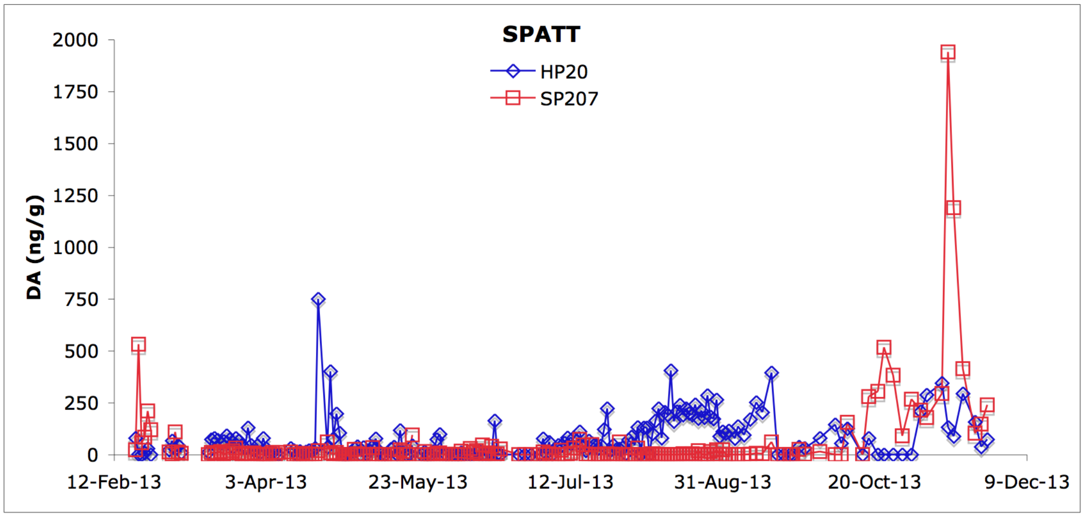

Figure 9. DA concentrations (ng/g) for SPATT resins HP20 and SP207. Values indicate chronic DA exposure at the sediment-water interface.

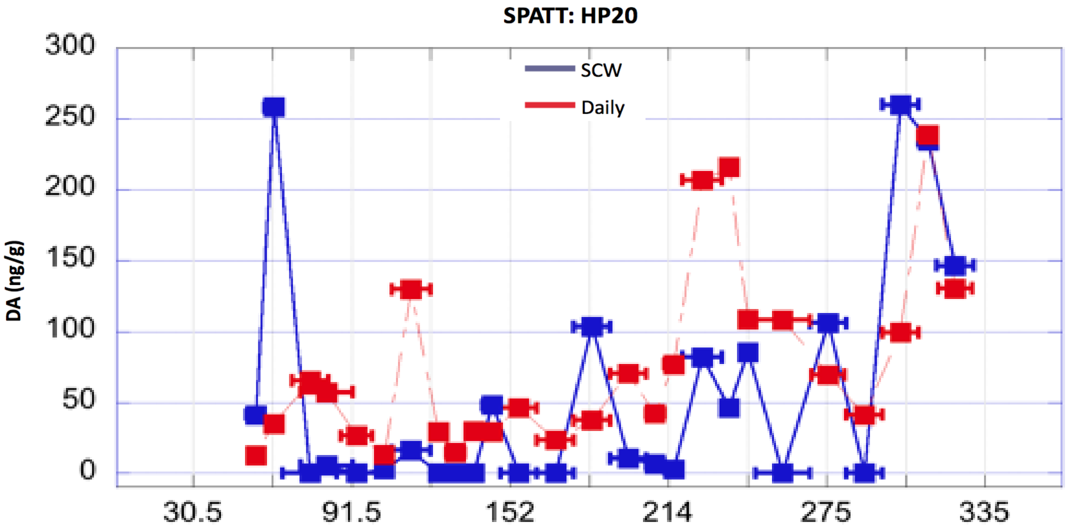

Figure 10. Averaged daily DA concentrations from HP20 and SCW DA concentrations from HP20 are statistically different (p=0.018).

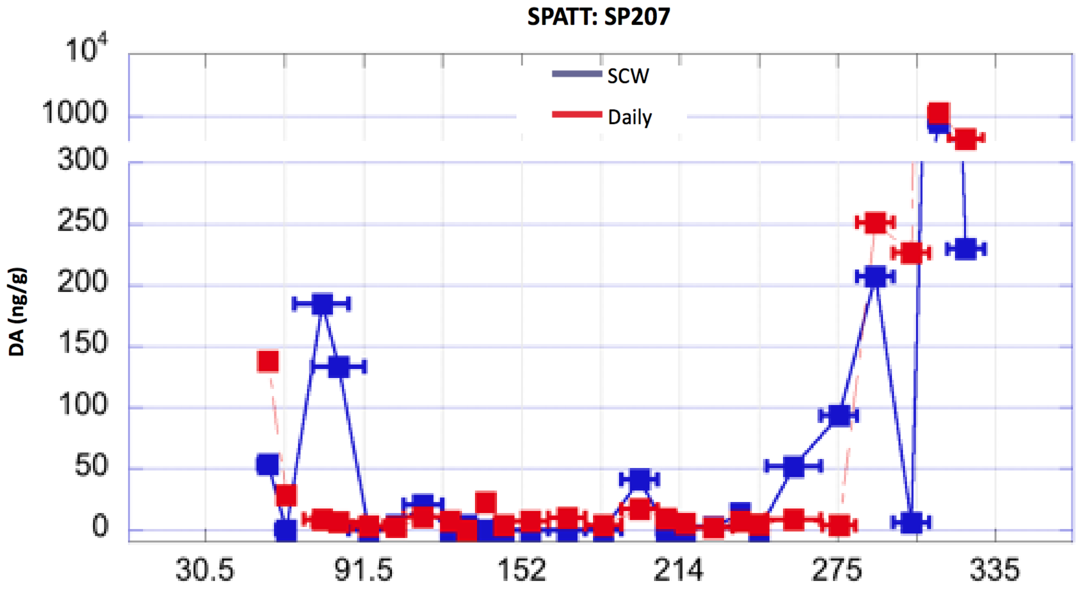

Figure 11. Averaged daily DA concentrations from SP207 and SCW DA concentrations from SP207 are not statistically different (p=0.301).

	HP20	SP207	pDAsur	pDAbot	ChlGFFsur	ChlGFFbot	Chl10µmsur	Chl10µmbot	DAsed
HP20		0.059	-0.102	-0.139	0.065	-0.155	0.145	-0.089	-0.048
SP207			-0.044	-0.026	0.068	-0.150	0.115	-0.045	0.070
pDAsur				0.524	-0.017	0.044	0.002	0.048	0.072
pDAbot					0.093	0.207	0.079	0.190	0.322
ChlGFFsur						0.532	0.939	0.442	0.006
ChlGFFbot							0.458	0.940	0.095
Chl10µmsur								0.495	0.064
Chl10µmbot									0.052
DAsed									

Table 1. R^2 values for correlations between all daily measurements.

	SCW HP20	SCW SP207
Daily HP20	0.419	0.551
Daily SP207	0.527	0.951

Table 2. R^2 values for correlations between averaged daily and weekly SPATT measurements.

	SCW Chl GFF	SCW Chl 10µm	SCW pDA
Daily Chl GFF sur	0.793	0.807	-
Daily Chl GFF bot	0.377	0.368	-
Daily Chl 10µm sur	0.749	0.793	-
Daily Chl 10µm bot	0.339	0.344	-
Daily pDA sur	-	-	-0.070
Daily pDA bot	-	-	0.628

Table 3. R^2 values for correlations between daily and SCW chlorophyll and pDA measurements.

References

Alldredge, AL and CC Gotschalk. (1989) Direct observation of the mass flocculation of diatom blooms: Characteristics, settling velocities and formation of diatom aggregates. Deep Sea-Research I 36:159–171

Graham, WM and JL Largier. (1997) Upwelling shadows as nearshore retention sites: the example of northern Monterey Bay. Continental Shelf Research 17(5):509-532

Grant, KS, TM Burbacher, EM Faustman and L Gratttan. (2010) Domoic acid: Neurobehavioral consequences of exposure to a prevalent marine biotoxin. Neurotoxicology and Teratology 32(2):132-141

Jester, R, K Lefebvre, G Langlois, V Vigilant, K Baugh and MW Silver. (2009) A shift in the dominant toxin-producing algal species in central California alters phycotoxins in food webs. Harmful algae 8:291-298

Kiørboe, T, JLS Hansen, AL Alldredge, GA Jackson, U Passow, HG Dam, DT Drapeau, A Waite and CM Garcia. (1996) Sedimentation of phytoplankton during a diatom bloom: Rates and mechanisms. Journal of Marine Research 54(6):1123-1148

Kudela RM, G Pitcher, R Probyn, F figueiras, T Moita and V Trainer. (2005, June) Harmful Algal Blooms in Coastal Upwelling Systems. Oceanography 18(2):184-197

Kvitek, RG, JD Goldberg, GJ Smith, GJ Doucette and MW Silver. (2008) Domoic acid contamination within eight representative species from the benthic food web of Monterey Bay, California, USA. Marine Ecology Progress Series 367:35-47

Langlois, GW. (2010-2012) Marine Biotoxin Monitoring Program Annual Report. Retrieved from California Department of Public Health http://www.cdph.ca.gov/HealthInfo/environhealth/water/Pages/Shellfishreports.aspx

Lane, JQ, PT Raimondi and RM Kudela. (2009) Development of a logistic regression model for the prediction of toxigenic Pseudo-nitzschia blooms in Monterey Bay, California. Marine Ecology Progress Series 383:37-51

Lane, JQ, CM Roddam, GW Langlois and RM Kudela. (2010) Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. Limnology and Oceanography: Methods 8: 645-660.

Maldonado, MT, MP Hughes, EL Rue and ML Wells. (2002) The effect of Fe and Cu on growth and domoic acid production by *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis*. Limnology and Oceanography 47:515–526

Miller, PE and CA Scholin. (2002) Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filter-based whole cell hybridization. Journal of Phycology 32(2):371-382

Pilskaln, CH, JB Paduan, FP Chavez, RY Anderson and WM Berelson. (1996) Carbon export and regeneration in the coastal upwelling system of Monterey Bay, central California. Journal of Marine Research 54(6):1149-1178

Reynolds, C. (2006) Ecology of phytoplankton. New York, NY: Cambridge University Press.

Sekula-Wood, E, A Schnetzer, CR Benitez-Nelson, C Anderson, WM Berelson, MA Brzezinski, JM Burns, DA Caron, I Cetinic, JL Ferry, E Fitzpatrick, BH Jones, PE Miller, SL Morton, RA Schaffner, DA Siegel and R Thunell. (2009) Rapid downward transport of the neurotoxin domoic acid in coastal waters. Nature Geoscience 2(4): 272-275

Shanks, AL. (2002) The abundance, vertical flux, and still-water and apparent sinking rates of marine snow in a shallow coastal water column. Continental Shelf Research 22(14):2045-2064

Shanks, AL and JD Trent. (1980) Marine snow – Sinking rates and potential role in vertical flux. Deep-sea research Part A. Oceanographic Research Papers 27(2):137-143

Thunell, R, C Benitez-Nelson, R Varela, Y Astor and F Muller-Karger. (2007) Particulate organic carbon fluxes along upwelling-dominated continental margins: Rates and mechanisms. Global Biogeochemical Cycles 21:GB1002

Trainer, VL, NG Adams, BD Bill, CM Stehr, JC Wekell, P Moeller, M Busman and D Woodruff. (2000) Domoic acid production near California coastal upwelling zones, June 1998. Limnology and Oceanography 45(8):1818-1833

Trainer, VL, SS Bates, N Lundholm, AE Thessen, WP Cochlan, NG Adams and CG Trick. (2012) Pseudo-nitzschia physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14:271-300

Turner, RE. (2002) Sedimentological evidence of an increase in Pseudo-nitzschia (Bacillariophyceae) abundance in response to coastal eutrophication. Limnology and Oceanography 47(2):551-558

Vigilant VL and MW Silver (2007) Domoic acid in benthic flatfish on the continental shelf of Monterey Bay, California, USA. Marine Biology 151(6): 2053-2062

Wang, Z, KL King, JS Ramsdell and GJ Doucette. (2007) Determination of domoic acid in seawater and phytoplankton by liquid chromatography–tandem mass spectrometry. Journal of Chromatography 1163: 169-176.

Welschmeyer, NA. (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnology and Oceanography 39: 1985-1992.

Chapter 3: Summary & Conclusions

During the study period, chlorophyll concentrations were approximately 50 micrograms per liter, which is relatively high for this location (Lane et al. 2010). I also observed a persistent non-toxic *Pseudo-nitzschia* bloom with small pulses of toxic species. These results are not unexpected, as CDPH annual reports indicate decreasing means of DA concentrations since 2010 (Langlois 2010-2012). DA concentrations from mussel tissue never exceeded the regulatory limit during this study. Indeed, 2013 exhibited the lowest DA concentrations in both mussels and water samples at the Santa Cruz Wharf since 2002 (Langlois 2010-2012). Additionally, whole-cell probing (Lane et al. 2009; Miller and Scholin 2002) showed that the *Pseudo-nitzschia* observed were not the typical toxigenic species seen in Monterey Bay (*P. australis* and *P. multiseries*).

Despite the lack of a classic toxic *Pseudo-nitzschia* event, DA was observed in water, sediment and SPATT samples. Three pulses of pDA were observed in both the surface and bottom water samples. Surface pDA concentrations were strongly correlated to SCW pDA concentrations, while bottom pDA concentrations were not. Surface chlorophyll concentrations were also strongly correlated to SCW chlorophyll concentrations, while bottom concentrations were not. These results suggest that the water column was not well mixed during the study period.

Since sediment DA concentrations were generally greater than total (particulate and dissolved) SCW DA concentrations, DA may have accumulated in the sediments over a short period of time. Furthermore, HP20 DA concentrations suggest chronic DA exposure at the sediment-water interface. Persistence of DA at the sediment-water interface, along with presence of DA in the material collected in the sediment trap, suggests that the source of this DA may be resuspension due to bioturbation, bottom flow or wave action, and that the sediment is potentially acting as a reservoir for DA.

Current monitoring programs, including the SCW sampling program do not analyze the sediments for DA. Sampling time intervals may also be important for monitoring programs. When compared to daily sampling, pulses in pDA were poorly resolved in the approximately weekly SCW dataset, suggesting that sampling intervals of less than a week are more effective at capturing changes in DA concentrations. Daily sampling provides greater resolution of changing DA concentrations in SPATT samples. HP20 DA concentrations suggest a chronic presence of DA at the sediment-water interface. Daily concentrations of HP20 DA were generally greater than the approximately weekly SCW values, suggesting that toxin concentrations are greater near benthos compared to surface, or that the near-surface SPATT samplers are diluted by exposure to waters of low DA concentrations during the approximately weekly deployment. Therefore, weekly values may be underestimating the concentrations of DA in the water column at the Santa Cruz Wharf. Since the local hydrodynamics of the study site may influence these differences, monitoring programs should also consider the appropriate sampling interval. It is important to note that organisms will be exposed to DA on a daily (or shorter) time scale, suggesting that the weekly integrated DA values from the near-surface SPATT may be underestimating exposure.

The most significant observation from this study is that DA was frequently seen in the benthos and at the sediment-water interface. This was observed despite the potential limitations of this study, such as the lack of a classic toxic event at the surface. To track DA from the surface to the sediment, this study would ideally be continued so as to capture a high-DA event in the water column. Differences in the depths of daily and SCW SPATT deployments limited direct comparisons. Due to variability in the SCW sampling intervals, along with gaps in the SCW dataset, SCW SPATT DA concentrations could be reliably compared to daily variables other than daily SPATT DA concentrations. Differences in sampling locations may have also influenced results.

To enhance this work, I would recommend comparing body burdens of DA in benthic organisms before, during and after a toxic event. I would also investigate possible wharf effects. Since bottom flow and grain size vary between the North and South sides of the Santa Cruz Wharf, DA concentrations in the water column and in the sediments may also vary. Measuring the total sediment pore water would provide more accurate estimates of sediment DA concentrations, since concentrations obtained in this study are conservative.

Measuring the total amount of sediment collected by the sediment trap would provide essential information about the downward flux of both sedimenting material and DA at this site. Sediment cores would provide an insight into how well toxic cells are preserved and would provide information about the residence time of DA in the sediments. It is likely that preservation is dependent on chemical factors, such as oxygen concentration and pH; these relationships would be worth pursuing in future studies. Persistence of DA at the sediment-water interface and presence of DA in the sediment itself suggests that the source of this DA may be resuspension due to bioturbation, bottom flow or wave action, and that DA may accumulate in sediments over short periods of time. To explore these mechanisms of resuspension, I suggest comparing these data with wind and upwelling indices.

Since SP207 samples did not capture the greater fluctuations of DA when compared to HP20 samples, I suggest deployments of only HP20. Alternatively, both SPATT resins could be compared at longer time intervals. Since the variability in SPATT DA concentrations were poorly resolved by the approximately weekly SCW dataset, sampling on a twice-weekly basis might help determine appropriate sampling intervals for each resin; alternatively, the daily time series could be examined to identify decorrelation scales and underlying temporal patterns, which would help to inform optimal sampling intervals. Finally, I would include additional sampling locations. Comparing these data amongst difference sites would provide a broader understanding of the transport of DA to depth and the possible retention properties of different sandy coastlines.

Despite the potential limitations of this study, several significant conclusions can be reached. I consistently observed more DA at the sediment-water interface than at the surface, most likely due to resuspension by long period waves. The sediment is potentially acting as a reservoir for domoic acid. I conclude that near surface blooms of toxic *Pseudo-nitzschia* are not the only source of DA that could lead to trophic transfer.

References

Langlois, GW. (2010-2012) Marine Biotoxin Monitoring Program Annual Report. Retrieved from California Department of Public Health http://www.cdph.ca.gov/HealthInfo/environhealth/water/Pages/Shellfishreports.aspx

Lane, JQ, CM Roddam, GW Langlois and RM Kudela. (2010) Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. Limnology and Oceanography: Methods 8: 645-660.

Miller, PE and CA Scholin. (2002) Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filter-based whole cell hybridization. Journal of Phycology 32(2):371-382