

Capstone Project Presented to the Faculty of Earth Systems Science & Policy  
In Partial Fulfillment of the Requirements for the Degree of Bachelor of Science

California State University, Monterey Bay  
Center for Science, Technology, and Information Resources

***Emerita analoga* (Stimpson)- Possible New Indicator Species for  
the Phycotoxin Domoic Acid in California Coastal Waters**

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## **Table of Contents**

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Cover Letter	3
Capstone Paper	6
Abstract	7
Introduction	8
Materials and Methods	10
Results	13
Discussion	14
Conclusion	15
Acknowledgements	16
Literature Cited	17
Caption for Figures	20
Figure 1-4	21
Appendix A: Extraction Protocol	25

**To the Faculty of Earth Systems Science and Policy:**

This work began with a short conversation I had with my advisor, Dr. Rikk Kvitek, late in the fall of 1998. At the time we were monitoring changes in PSP levels for two intertidal invertebrates, sand crabs (*Emerita analoga*) and sea mussels species (*Mytilus* spp.), and wondered if the foraging patterns of shorebirds changed with seasonal toxicity of these prey items. Concurrent to our field research another group at UCSC, collaborating with NOAA researchers in Charleston, South Carolina, was investigating the cause of sea lions deaths along the central coast. The "red-tide" toxin domoic acid (DA), a potent neuro-agent, was strongly suspected in these deaths. The NOAA group became interested in our *Emerita* and wanted to test them for the toxin. When Rikk asked if I wanted to follow up on the DA question I said, "sure" and the beginnings of a Capstone began to emerge.

The Charleston group subsequently detected DA from *Emerita* specimens that were originally sent for the development of an extraction procedure. We later learned that a localized bloom of DA producing diatoms had occurred in Monterey Bay adjacent to where *Emerita* had been collected. While the crabs were hot with toxin, mussel samples collected at the same time were not. This information, plus the analysis of additional samples, combined to support what we had suspected: *Emerita* retain marine toxins.

The implication that a more successful indicator species for DA had been identified was exciting, however, we didn't know how long *Emerita* retained the toxin or even if it did so in association with DA producing diatoms. Our detection of DA in *Emerita*, while significant compared to mussels- the standard bivalve indicator for a suite of natural and anthropogenic toxins- was, however, discrete. The co-occurrence of DA producing diatoms nearshore may have been a coincidence. So, to test our initial findings, and possibly support our *Emerita*-DA-Diatom suspicions, I designed my research to incorporated field and laboratory components to test two hypotheses: 1) *Emerita* retain detectable levels of DA in association with *Pseudo-nitzschia* sp. (diatom group which produce DA) and 2) DA in *Emerita* is comparable to mussel uptake of the same toxin. My basic approach was two-fold: field collection of targeted species and laboratory analysis for the DA toxin using HPLC-UV detection. DA loads were then compared with the occurrence of *Pseudo-nitzschia* spp. in Monterey Bay.

### ***Capstone Format***

This Capstone document is presented in three basic parts: Cover Letter, Capstone Paper, and Appendix. The paper component is a formal presentation of methodologies, results and discussion of the project described in this letter and is scientifically formatted for journal submission. Due to the collaborative nature of the project seven authors are listed as contributors to the work along with me, the primary author. With the exception of the Abstract, which was prepared with co-authors in advance for conference publication, the paper presented here is original work written in consultation with my advisor, Dr. Rikk Kvitek, with additional content contributions by Carrie Bretz, and represents the final stages toward journal submission pending co-author approvals.

### ***Practical Application for New DA Indicator Species***

As a harmful algal bloom toxin (HABT's), DA is relatively new. It was first identified as a potent neuro-agent after 107 people experienced gastrointestinal and neurological dysfunction 48 hours after consuming cultured mussels (*Mytilus edulis*) harvested from Prince Edward Island, Canada in 1987. While most patients recovered (some experienced significant memory loss 5 years after the event) the consumption of contaminated shellfish resulted in three mortalities and the first clinical description of amnesic shellfish poisoning (ASP). The DA toxin was subsequently attributed to a species of diatoms (*Pseudonitzschia* sp.) previously unknown to produce HABT's.

The Canadian example is a textbook illustration of ecological food web transfer. Mussels cultured in an estuary were exposed to and consumed toxic diatoms, which bloomed naturally over a period of weeks, and then transferred the toxin (sequestered in mussel tissue) to human consumers. The transferability of DA to higher trophic tiers has also been documented in other mortality events affecting marine mammals, fish, and shore birds along the Pacific coast.

The regulatory limit for DA in seafood was established (20 ppm) after the Canadian case with no additional reports of human poisoning documented. However, the potential impact to human health, fisheries, and marine life posed by DA in the environment is mediated by our ability to successfully detect the toxin. Despite the use of sophisticated detection techniques to identify HAB species and monitor the toxins they produce, indicator species such as mussels and clams are still the fundamental tools for many State agencies monitoring along our coast. While such application has been instrumental in forewarning against consuming PSP contaminated shellfish, the use of bivalve indicators to detect DA

has met with varying degrees of success (see Capstone Introduction). For State agencies like the California Department of Health Services (CDHS) the challenge of inadequate indicators is compounded by the unpredictability of toxic DA bloom events. Along densely populated coastal areas like Monterey Bay, where DA related mortalities of marine life have occurred, access to reliable indicators can reduce the likelihood of humans being affected. With this research we propose the utility of *Emerita* as an indicator for the DA toxin. The synchrony in occurrence of DA producing diatoms nearshore and DA measured in *Emerita* suggests accumulation sensitivity not presently observed in bivalve indicators.

The need to compliment current monitoring practices with a more successful indicator is clear. While this work is by no means exhaustive (accurate depuration rates, precise localization of DA source and storage in tissue are just a few logical "next steps" which others are pursuing), the research presented here strongly supports *Emerita* as a viable, cost effective tool for HAB monitoring. For this study the average cost to prepare one sample for analysis, calculating only the cost for chemicals, filters, and SAX columns, was \$5.00 per sample. I'm assuming, of course, that the costs we incurred purchasing additional supplies and equipment necessary for the process are not issues with bio-toxin labs equipped for the procedure. *Emerita* tissue is easily amenable to HPLC-UV analysis. Since this analytical approach is already incorporated into current detection practices, *Emerita* can easily fit into any existing monitoring program utilizing available supplies and equipment alongside mussel analysis with little additional cost.

***Emerita analoga* (Stimpson)- Possible New Indicator Species for the  
Phycotoxin Domoic Acid in California Coastal Waters**

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### Abstract

Blooms of domoic acid (DA) synthesizing diatoms (*Pseudo-nitzschia* spp.) have been associated with the death and injury of hundreds of marine shorebirds and mammals, exposed humans to potentially serious health risks, and threatened to significantly impact coastal fisheries and economies dependent on marine resources. While indicator organisms are widely utilized to monitor for marine biotoxins like paralytic shellfish poisoning (PSP) toxins, a reliable intertidal indicator species to monitor DA remains to be identified. Here we evaluate and confirm the utility of the common sand crab (*Emerita analoga*) as an indicator for DA in comparison with mussels (*Mytilus californianus*), a general sentinel indicator. Mussels and sand crabs, collected from natural populations in Santa Cruz, California (Apr. 1999 - Feb. 2000), were tested for DA using the HPLC-UV method. Toxin loads in sand crabs ranged from 0.07 to 10.4  $\mu\text{g DA g}^{-1}$  and coincided with abundance of DA producing *Pseudo-nitzschia* species nearshore. The toxin was not detected in any of the mussel samples collected during the study period. The rise and fall of DA in sand crabs in synchrony with *Pseudo-nitzschia* abundance, combined with this common intertidal species' accessibility and ease of DA extraction, recommend sand crabs as a reliable, cost-effective monitoring tool for DA in the coastal environment.

**Keywords:** Domoic acid, Harmful algal blooms, HPLC-UV, Indicator species, Mussel toxicity, *Pseudo-nitzschia*, Sand crabs

## Introduction

The occurrence of harmful algal blooms (HAB's) in coastal waters world-wide can adversely impact the health of human and marine communities, the viability of local fisheries, as well as the safety of commercial and recreational seafood products (Shumway 1990; Bates 1997). While improved field and laboratory analytical tools have substantially contributed to rapid detection and identification of HAB species and the toxins they produce, the use of bivalves by agencies monitoring marine toxins is still the fundamental approach in safeguarding public health in North America (Altwein *et al.* 1995). However, the sensitivity of sentinel indicators, although sufficient for tracking paralytic shellfish poisoning (PSP) toxins (Price *et al.* 1991), has been less than reliable for the more recently identified biotoxin, domoic acid (DA) (Langlois *et al.* 1993; Altwein *et al.* 1995).

The health threat posed by DA was first documented in 1987 when three people died and over 100 became sick after eating cultured blue mussels (*Mytilus edulis*) harvested from Prince Edward Island, Canada (Perl *et al.* 1990). The source of the potent neurotoxin was subsequently localized to a species of diatom (*Pseudo-nitzschia* spp.) not previously known to produce marine toxins (Bates *et al.* 1989). As a result of this event, regulatory limits for DA (20ppm) in seafood were established after which no additional cases of DA intoxication in humans, clinically recognized as amnesic shellfish poisoning (ASP), have been documented (Todd 1990; Altwein *et al.* 1995). However, reoccurring mortality events in Monterey Bay, California, involving shorebirds in 1991 and marine mammals in 1998 and 2000, have raised general concerns regarding the apparent ease with which DA is naturally transferred across trophic tiers (Work *et al.* 1993; Lefebvre *et al.* 1999; Scholin *et al.* 2000).

DA has been detected in numerous, commercially valuable, marine species including razor clams (*Siliqua patula*), Dungeness crabs (*Cancer magister*), anchovies (*Engraulis* spp.) and mussels (*Mytilus* spp.) (Altwein *et al.* 1995). Although HPLC-UV analysis of DA is well established, monitoring agencies using mussels and razor clams as general sentinel organisms for DA along North America's Pacific coast have met with varying degrees of success (Langlois *et al.* 1993; Altwein *et al.* 1995; Horner *et al.* 1997). Razor clams, for example, have been useful in warning the public against collecting and consuming clams in Washington State; however, razor clams can retain DA six months after initial contamination independent of bloom conditions nearshore (Horner *et al.*

1993; Wekell *et al.* 1994). Thus, as an indicator, razor clams remain fishery-specific, and since the species is marginally present along the California coast, their utility is geographically restricted (Ricketts *et al.* 1985). Mussels, by contrast, can depurate DA quickly (Novaczek *et al.* 1992) and, when collected from natural populations, may not harbor toxins during periods of toxic *Pseudo-nitzschia* blooms (Walz *et al.* 1994; Scholin *et al.* 2000). Furthermore, since mussels occur naturally on rocky outcrops or other hard substrata like wharf pilings, their utility for DA monitoring is limited to discrete geographic locations. In California, where long sandy beaches dominate 60% of the state's 1900 km coastline (Habel and Armstrong, 1977), this means many coastal areas are unmonitored due to the absence of an appropriate and reliable indicator organism.

In an effort to bridge the monitoring gap observed along California's coast, Bretz *et al.* (unpublished data) detected seasonal variations of PSP toxins in the common sand crab, *Emerita analoga* -Stimpson. Originally suggested by Sommer and Meyer (1937) as a suitable indicator for PSP, sand crabs are widely distributed on the west coasts of North and South America with a genus distribution worldwide (Efford 1976; Tam *et al.* 1996). In California, sand crabs are the most common intertidal macro invertebrate on exposed sandy beaches and are easily accessible within the swash zone throughout the year (Efford 1965; Dugan *et al.* 1994).

In October of 1998, sand crabs were collected from Natural Bridges State Park in Santa Cruz, California, to test their suitability as an indicator for DA and were subsequently confirmed to contain the toxin by LC-MS/MS. However, DA in mussels collected at the same time, from an adjacent site, were below detectable limits. A localized bloom of *Pseudo-nitzschia australis* ( $\leq 1.7 \times 10^4$  cells L<sup>-1</sup>) coincided with these collections.

The purpose of this research was to test the general hypothesis that sand crabs naturally incur DA in association with toxic *Pseudo-nitzschia*. Paired samples of sand crabs and mussels were collected from natural populations over an eleven-month period in Santa Cruz, CA, and analyzed for DA using the HPLC-UV method. Toxin loads were then compared to nearshore *Pseudo-nitzschia* abundance. Here we demonstrate the utility of the common sand crab to indicate the presence of DA in comparison with mussels. We describe the methods for sample collection, laboratory preparation of tissue samples for toxin extraction, and analysis of DA by HPLC-UV.

## Materials and Methods

### Field Samples and Collection Sites

Sand crabs and mussels were collected once every two weeks at Natural Bridges State Park and Lighthouse State Beach in Santa Cruz, California, between April 1999 through February 2000 (Figure 1.). Sites were chosen based on the availability and accessibility of both species from rocky and adjacent sandy beach locations. Natural Bridges is also a site where mussels are routinely collected by the California Department of Health Services (CDHS) for monitoring marine toxins (Price *et al.* 1991). The Lighthouse site was chosen due to its proximity to Santa Cruz pier where seawater samples are routinely collected and monitored for plankton abundance.

Twenty-five to 30 individual mussels up to approximately 5 cm in length were selected from natural populations at each of the two intertidal sites. Sand crabs were sampled randomly from aggregated populations within the sandy swash zone adjacent to mussel bed locations. Thirty to 50 individual sand crabs (of varying size classes) were collected by perturbing the surface sand with a shovel or bare feet until liquefaction occurred. Sand crabs were then captured with a hand held rectangular fish net ( $0.1\text{m}^2$ , mesh size 4mm) as they emerged and flowed with a receding wave.

Sand crabs and mussels were transported in an iced cooler to California State University, Monterey Bay (CSUMB) where they were stored at  $-70\text{C}$  for later DA extraction and HPLC-UV analysis. Control samples for spike and extraction efficiency experiments were collected from natural populations at Natural Bridges on September 17, 1999 (mussels) and Pfeiffer State Beach on May 20, 1999 (sand crabs).

Abundance of *Pseudo-nitzschia australis* and *Pseudo-nitzschia multiseries* were determined using species-specific LSU rRNA-targeted fluorescent probes described by Miller and Scholin (1998). Between 10 and 30 ml of sample were prepared on 1.2 mm Isopore polycarbonate filters (Millipore Corporation, Bedford, MA01730) and cell counts were then made on a Zeiss Standard 18 compound microscope, equipped with epifluorescence. Water samples were collected each week from the Santa Cruz Municipal Wharf and biweekly from Lighthouse State Beach beginning October 1999 through February 2000. The Monterey Bay Aquarium Research Institute (MBARI) provided additional *Pseudo-nitzschia* abundance data for the months of April 1999 through September 1999.

### Sample Extraction

Samples were prepared for DA analysis using the extraction procedure described by Quilliam *et al.* (1995). Briefly, 100g of shucked mussel tissue were pooled and homogenized for 1 to 2 min. in a blender (Oster model 6634, Sunbeam Ltd., Brampton, Ontario, Canada). A 4g sub-sample was weighed from the homogenate and combined with 50% aqueous methanol (MeOH) at a 1:4 ratio and homogenized for 2 min. using a sonicating probe (Fisher Sonic Dismembrator F60, Fisher Scientific, Pittsburgh, PA, USA). The resultant slurry was centrifuged at 3,000-x g for 10 min. then filtered through a 0.45 $\mu$ m Whatman GD/X nylon filter (Fisher Scientific) attached to a 10 ml glass syringe. The clarified filtrate was collected in an amber vial for solid phase extraction clean-up.

Sand crabs were prepared for DA extraction by squeezing whole animals ( $n = 5$ ), selected randomly from each collection, through a garlic press for tissue separation. Each sample was homogenized for 1 min. with a sonicating probe and then an aliquot of 4g was weighed for aqueous methanol addition.

Extraction efficiency experiments were conducted using control samples of each tissue type. Mussels and sand crabs were homogenized, as described above, and 4g sub-samples were spiked with varying concentrations of DA standard (Sigma, St. Louis, USA). Standards were made using DA obtained as crystalline powder (1mg, 90% pure) and dissolved in 1 ml of Milli-Q water. Standards were refrigerated and kept in the dark when not in use.

### Solid Phase Extraction (SPE)

Extracts were further treated to strong anion exchange (SAX), solid phase extraction (SPE) clean-up using the method described by Hatfield *et al.* (1994). SAX cartridges from J. T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA; lot no. N40550) were mounted to single sample processors (Supelco) and preconditioned with 6 ml of Milli-Q water, 3 ml MeOH, and 3 ml 50% aqueous MeOH. Each solution was added when the meniscus of the previous solvent touched the top of the column packing, thus never allowing the column to go dry. Filtered samples were then vortexed and 2 ml from the sample were passed through the column, approximately one drop per second, followed by 5 ml of 0.1M sodium chloride (NaCl; analytical grade) wash in 10% acetonitrile

(MeCN). Lastly, 5 ml of 0.5M NaCl in 10% MeCN was applied to elute DA from the column. The eluate was collected in an amber vial for DA analysis by HPLC-UV. Aqueous solutions of methanol and acetonitrile (HPLC grade; Fisher Scientific) were prepared using Milli-Q water. SAX columns were tested for DA recoveries prior to sample application and resulted in  $97 \pm 2\%$  ( $n = 3$ ) recoveries.

#### High Performance Liquid Chromatography (HPLC-UV)

Sand crab and mussel extracts were analyzed for the presence of DA using the method described by Lefebvre *et al.* (1999). Briefly, samples were separated isocratically using a Hewlett-Packard 1090 auto injection HPLC equipped with a diode array detector (DAD) set at 242 nm and 280 nm with a bandwidth of 10 nm (reference signal set at 450 nm with a bandwidth of 10 nm). A reverse phase Vydac C<sub>18</sub> column (2.1 mm x 25 mm, # 201TP52, Separations Group, Hesperia, CA, USA) with a Vydac guard column (5  $\mu\text{m}$ ) was used. The mobile phase consisted of water/MeCN/TFA (90/10/0.1, v/v/v) and was degassed with helium for 10 min. prior to analysis. Sample injections were 20  $\mu\text{l}$  with a flow rate of 0.3  $\text{ml min}^{-1}$  at 40°C. A DA calibration curve using the DACS-1C certified reference standard (Canadian National Research Council, Institute for Marine Biosciences, Halifax, NS, Canada) at concentrations of 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, and 16  $\mu\text{g ml}^{-1}$  ( $r = 0.99$ ) resulted in a retention time (RT) of 6.8 min. with the limit of detection (LOD) at 0.1  $\mu\text{g DA ml}^{-1}$  (or 1.25  $\mu\text{g DA g}^{-1}$ ).

DA concentration per gram of mussel or sand crab tissue was calculated using the following equations:

1.  $X \mu\text{g DA ml}^{-1} = (\text{area} - 6.18)/88.35$
2.  $(X \mu\text{g DA ml}^{-1}) (5 \text{ ml}/2 \text{ ml}) (20 \text{ ml}) = \text{total } \mu\text{g DA}$
3.  $\text{Total } \mu\text{g DA} / 4 \text{ g mussel or crab tissue} = \mu\text{g DA g}^{-1}$

Equation (1) was generated from the calibration curve calculating DA concentration X in a 20  $\mu\text{l}$  injection and then (2) multiplied by the dilution factor of extracted samples for total DA. Equation (3) calculated DA concentration per gram of mussel or sand crab extract.

## Results

### Sand Crab and Mussel Samples

Extraction efficiency experiments for mussel and sand crab controls spiked with 50 and 25 µg DA g<sup>-1</sup> resulted in 97 ± 1.9% (n = 3) and 99 ± 2.2% (n = 3) DA recovery for sand crabs and 84 ± 0.1% (n = 3) and 93 ± 0.6% (n = 3) for mussels. HPLC chromatograms showed no interference with DA detection in either tissue type with retention times of ~ 6 to 6.3 min. (Figure 2A, B & Figure 3A, B).

Sand crabs from Lighthouse State Beach in Santa Cruz, California, collected on December 24<sup>th</sup>, 1999 contained 10.4 ± 3.0 µg DA g<sup>-1</sup> (Figure 3C). Mussels collected from the same site on the same day contained no detectable DA as determined by HPLC-UV (Figure 2C). DA concentration decreased 5.6 folds for sand crabs collected two weeks later on January 7<sup>th</sup>, 2000 to 1.8 µg DA g<sup>-1</sup>. Measurable amounts of the toxin were also found in samples from Natural Bridges on April 4<sup>th</sup>, 1999 and January 7<sup>th</sup>, 2000 with concentrations of 0.4 ± 0.6 µg DA g<sup>-1</sup> and 0.07 µg DA g<sup>-1</sup> respectively. DA was not detected in any of the mussel samples collected (Figure 4A, B).

### *Pseudo-nitzschia*

During the study period *Pseudo-nitzschia* (P-n), reported here as *P. australis* and *P. multiseries*, were present in nearshore waters beginning in mid March to early April (1999) and again in December (1999) until the first week of January (2000) (Figure 4C). P-n density ranged from 4.0 × 10<sup>2</sup> to 9.8 × 10<sup>3</sup> cells L<sup>-1</sup> during March through the first week of April with peaks on April 2<sup>nd</sup> at 9.8 × 10<sup>3</sup> and falling to 8.3 × 10<sup>3</sup> and 4.0 × 10<sup>2</sup> on April 5<sup>th</sup> and 8<sup>th</sup>, respectively. After this time, cells were either low, not exceeding densities greater than 1.4 × 10<sup>3</sup> cells L<sup>-1</sup>, or absent until activity rose dramatically from 3.6 × 10<sup>3</sup> on the December 10<sup>th</sup> to 2.0 × 10<sup>4</sup> and 3.4 × 10<sup>4</sup> cells L<sup>-1</sup> on December 16<sup>th</sup> and 23<sup>rd</sup>. *P. australis* comprised ~87% of the total P-n cells observed for the December bloom period. P-n activity declined rapidly after December 23<sup>rd</sup> with a relatively short-lived peak (≤ 1.0 × 10<sup>4</sup> cells L<sup>-1</sup>) on January 7<sup>th</sup> of the following year.

## Discussion

DA concentrations in sand crabs varied with local abundance of DA producing P-n diatoms (Figures 4A, B, C). From February 1999 to March 2000, 70% of the total P-n cells observed were contributed by *P. australis*. During the month of December, when diatoms dramatically peaked and sand crabs from Lighthouse contained relatively high DA levels, *P. australis* was the most abundant P-n species observed. *P. australis* is the primary producer of DA occurring along central California's nearshore marine environment (Buck *et al.* 1992; Fritz *et al.* 1992; Villac 1993; Horner *et al.* 1997; Lefebvre *et al.* 1999; Scholin *et al.* 2000). During a two-year survey in which DA producing P-n were monitored in Monterey Bay, California, Waltz *et al.* (1994) identified *P. australis* as the main source of DA in nearshore plankton samples, even though other species capable of producing the toxin were also observed. Their data further suggest that *P. australis* may produce DA independent of cell density, this would imply toxin presence whenever that species is found in the coastal environment. The sudden uptake and release of DA in sand crabs, linked with the occurrence and disappearance of *P. australis*, strongly suggests that *P. australis* was the source of the toxin in our study.

The presence of DA in sand crabs associated with relatively low *P. australis* ( $\leq 10^4$  cells L $^{-1}$ ) suggest DA accumulation capabilities not characteristic in the standard bivalve indicator. The failure of mussels as a general sentinel organism for DA along the west coast of North America may be attributed to a variety of factors including high water solubility of the toxin, mussels' ability to depurate the toxin quickly, and/ or the intensity and duration of exposure to toxic blooms (Novaczek *et al.* 1992; Altwein *et al.* 1995). How these factors (or others) contribute to DA retention and depuration in mussels is presently unclear. It is possible that duration of DA exposure may partially explain uptake differences observed between sand crabs and mussels. Both sand crabs and mussels filter nearshore water for food; however, unlike mussels, sand crabs can migrate with tidal cycles and this suggests multiple if not continuous exposure to ocean water either interstitially or when swimming. This enhanced frequency of submergence may permit sand crabs to ingest more dissolved or particulate DA (i.e. from *P. australis* cells) than mussels, which can become separated from their food source due to tidal variations.

Although DA was not detected for mussels collected during this study, which was not unexpected, it is worth noting that mussels have shown low to moderate levels of the toxin during other periods (Langlois *et al.* 1993). Therefore, their value as a fisheries specific indicator is warranted only if they adequately guard against public consumption of contaminated shellfish.

The ease with which DA was extracted from and detected in sand crabs confirm the amenability of this new tissue matrix to DA analysis using the HPLC-UV method. Although the recovery yields from the extraction efficiency experiments were high (> 95%), the total extractable DA from samples using the garlic press method maybe as low as 50% (Powell *et al.*, submitted). Toxin concentrations reported here are, therefore, likely to be conservative estimates of total DA loads for sand crabs.

### **Conclusions**

The potential impact to human health, fisheries, and marine life posed by DA in the coastal environment is dependent upon our ability to successfully detect the presence of this toxin. The complications resulting from inadequate indicators, exemplified by bivalve sentinel species, are further compounded by the unpredictability of toxic DA bloom events. HPLC-UV detection of DA in the common sand crab (*E. analoga*), in synchrony with the rise and fall of toxic *Pseudo-nitzschia* abundance nearshore, provides compelling evidence that this species represents a more reliable, cost-effective alternative to mussels as the indicator of choice for DA along the California coast.

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### Captions for Figures

Figure 1. Map of Monterey Bay with intertidal collection sites located at (A) Natural Bridges State Park and (B) Lighthouse State beach in Santa Cruz, California. Water samples collected at pier.

Figure 2. HPLC chromatograms of mussel tissue: (A) Control sample collected from Natural Bridges on September 17, 1999; (B) Control sample spiked with 25  $\mu\text{g DA g}^{-1}$ . Retention time (RT) = 6.359 min.; (C) Sample collected December 24, 1999 from Lighthouse State beach during a *Pseudo-nitzschia* bloom nearshore to Santa Cruz pier.

Figure 3. HPLC chromatograms of sand crab tissue: (A) Control sample collected from Pfeiffer State beach on May 20, 1999; (B) Control sample spiked with 50  $\mu\text{g DA g}^{-1}$ . Retention time (RT) = 6.008 min.; (C) Sample collected December 24, 1999 from Lighthouse State beach. RT = 5.705 min. RT differences is due to guard column change.

Figure 4. Comparison of domoic acid concentration ( $\mu\text{g DA g}^{-1}$ ) in sand crab (*Emerita analoga*) and mussel (*Mytilus californianus*) tissue collected April 1999 through February 2000 from two intertidal locations in Santa Cruz, California: (A) Natural Bridges State Park and (B) Lighthouse State beach. HPLC-UV limit of detection (LOD) of 1.25  $\mu\text{g DA g}^{-1}$  is indicated by dashed horizontal line. Note scale differences. (C) Time series for *Pseudo-nitzschia* spp. (*P. australis* and *P. multiseries*) monitored during February 1999 through March 2000 from Santa Cruz pier. Break in lines indicate no data available. *Pseudo-nitzschia* is reported here as cells  $\text{L}^{-1}$ .

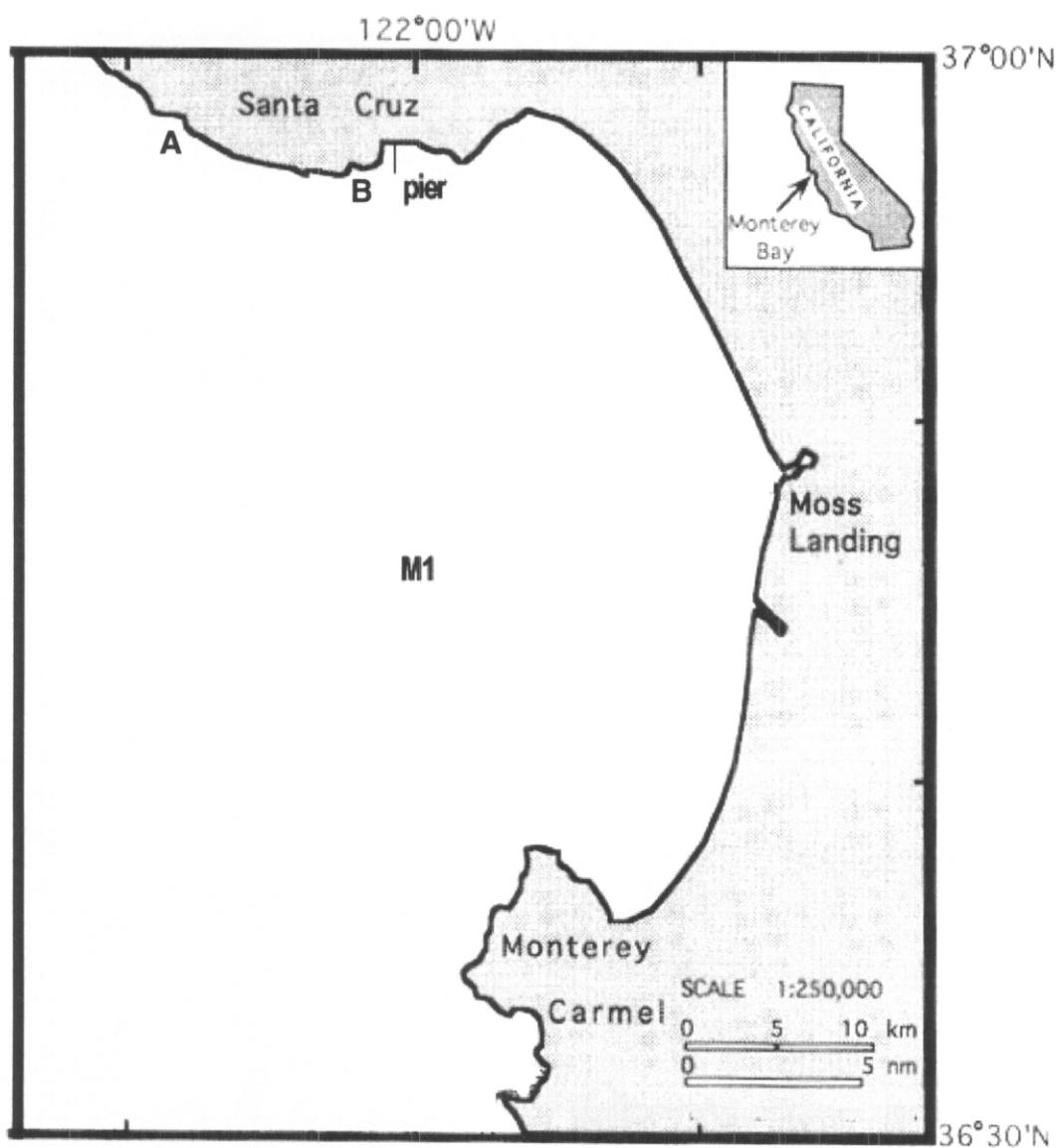
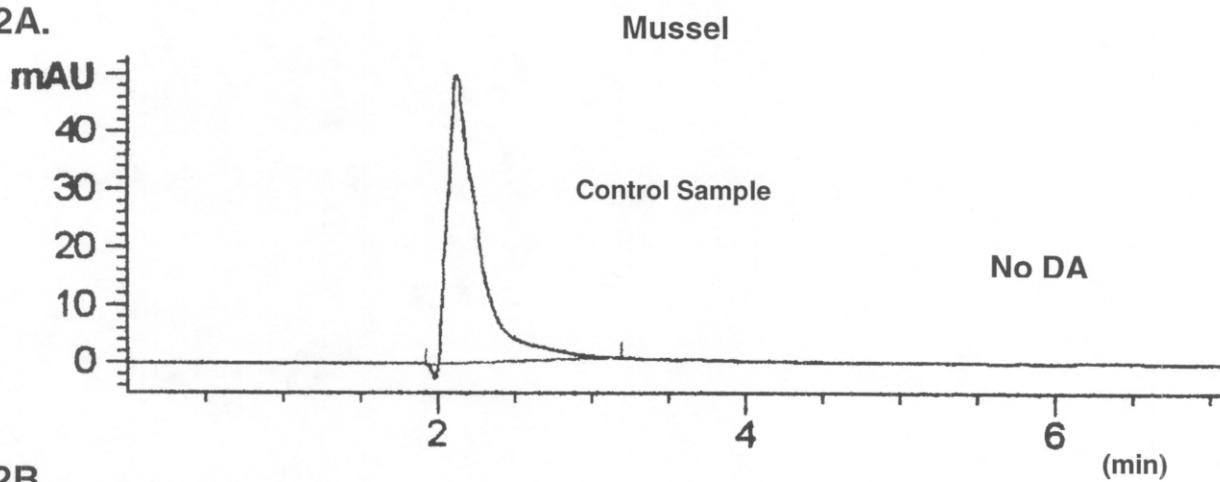
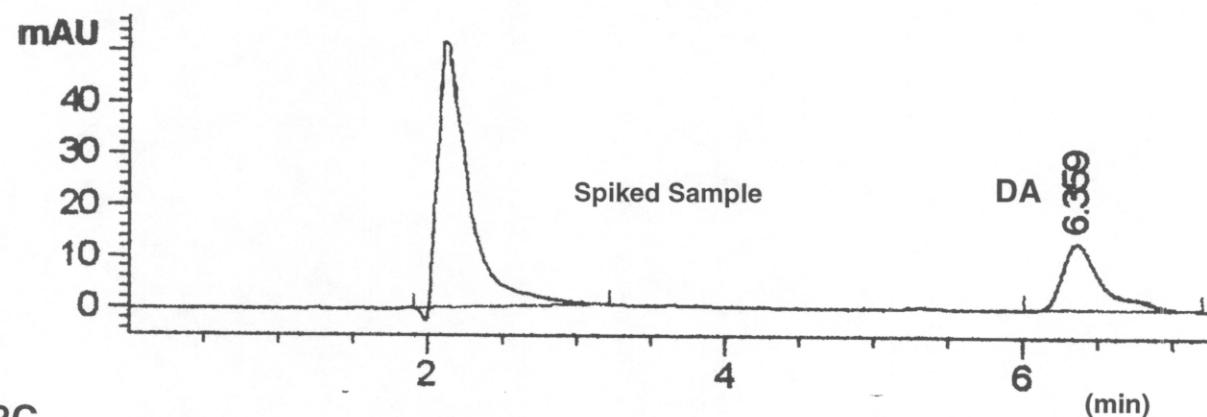


Figure 1.

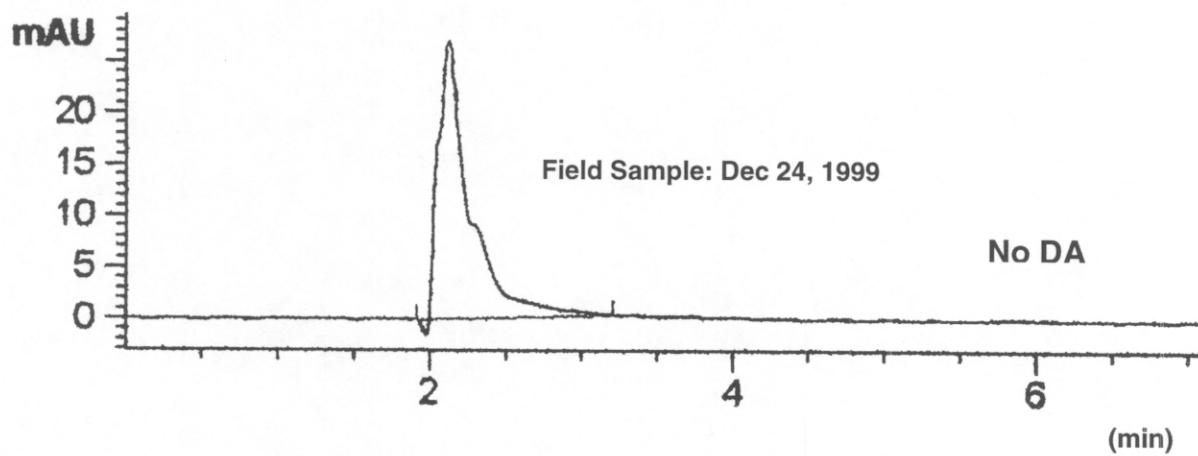
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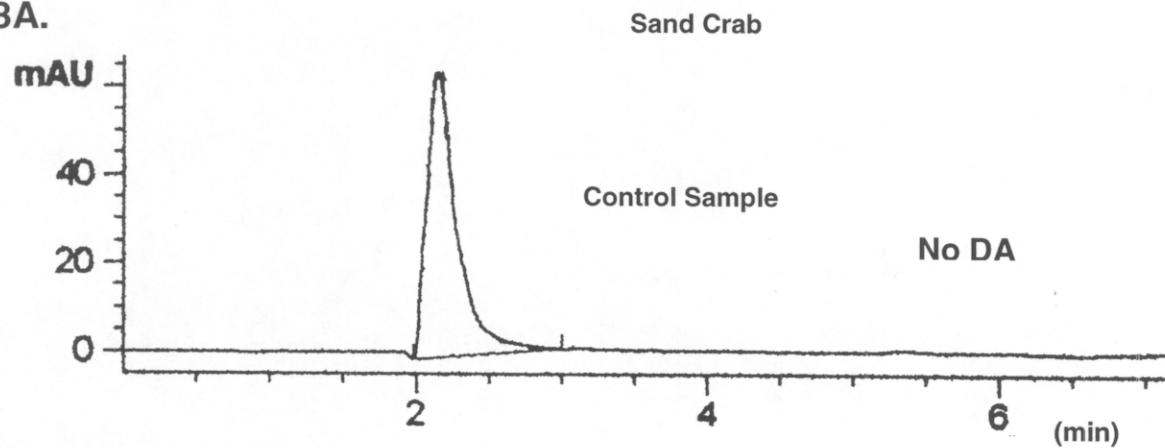
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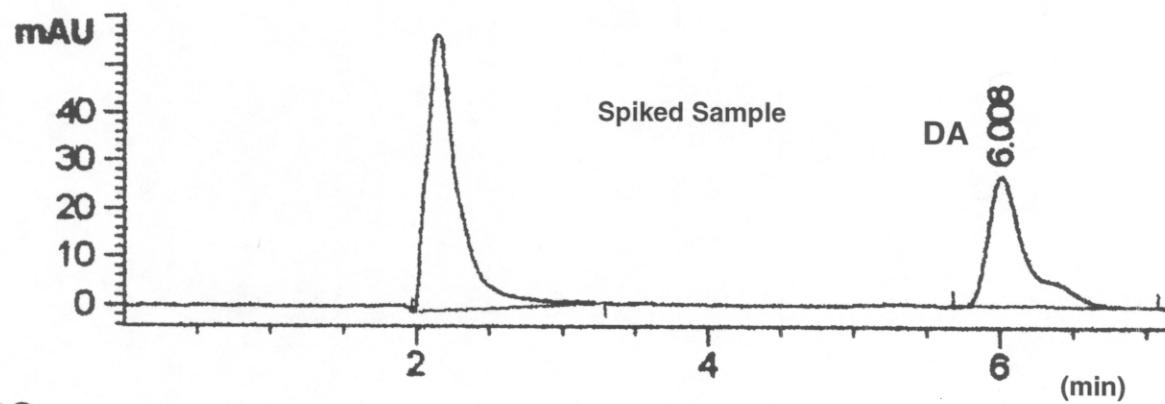
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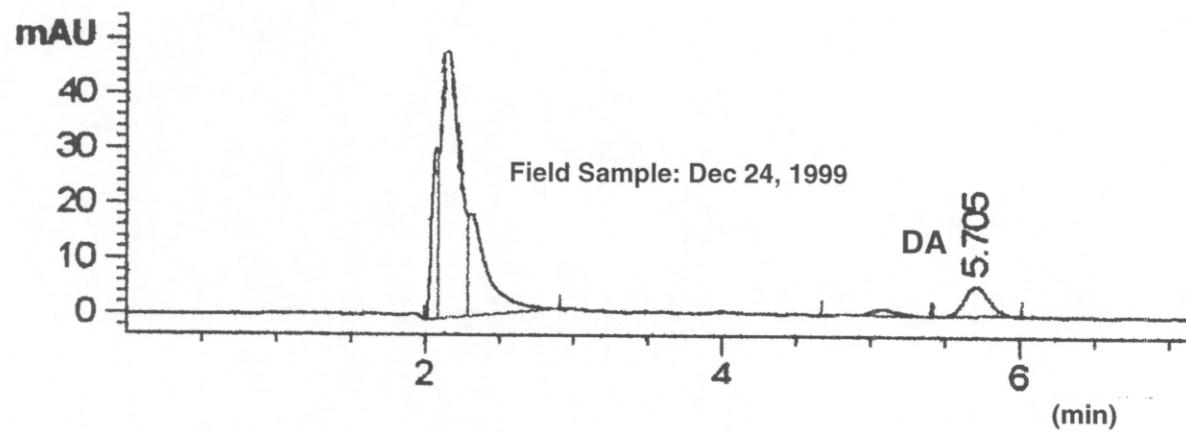
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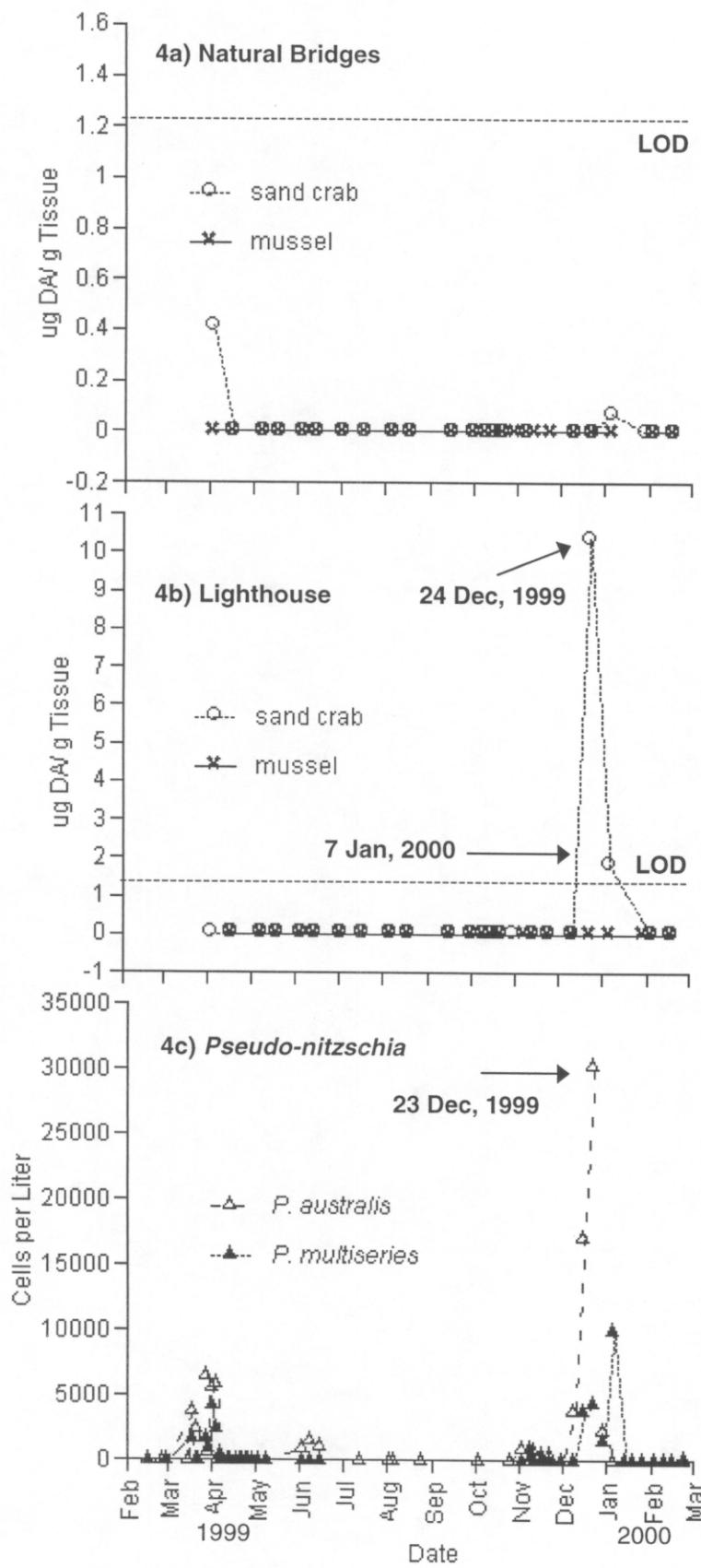


3B.



3C.





## Appendix A: Sample Extraction

### I. Homogenization

1. Pool 100g of mussel tissue in blender and homogenize
  - A. Pool 5 individual sand crabs into garlic press and squeeze tissue into 50 ml centrifuge tube
  - B. Record weight
  - C. Blend sample with sonicating probe for two minutes
2. Accurately weigh 4g from each homogenized tissue types into separate 50 ml centrifuge tubes
3. Record weight
4. Dilute 4g samples with methanol : water solution (1:1) up to 20 ml
5. Blend sample with sonicating probe for 1 to 2 minutes or until fully mixed
6. Cap centrifuge tube and centrifuge at 3,000g (or higher) for 10 minutes and let stand for an additional 10 minutes
7. Tare weight of 16 ml amber glass vial
8. Filter **all** clarified supernatant through a 0.45-micron disposable filter using a glass syringe
9. Collect filtrate in a clean 16 ml amber glass vial
10. Record weight of filtrate
11. Dispose excess mussel and sand crab slurry

### II. Sample Clean-up: Strong Anion Exchange (SAX), Solid Phase Extraction (SPE)

Filtered samples will undergo a final clean-up step to reduce matrix interference. A single use strong anion exchange (SAX), solid phase exchange (SPE) cartridge (J.T. Baker) will be used for this purpose.

The cartridge is not to go dry during this process. When meniscus of one solvent reaches the top of the column packing add the next solvent. **All solvents should be eluted at a rate of 1 drop/sec.**

1. Precondition strong anion exchange cartridge:
  - a. pass 6 ml nano pure water through column
  - b. pass 3 ml methanol through column
  - c. pass 3ml 50% methanol (aqueous)
2. Elute 2.0 ml of filtered tissue sample through cartridge (1 drop per second). When sample meniscus touches top of column elute 5.0 ml of wash solution through column. Discard effluent. **Wash is 0.1 M NaCl in 10% Acetonitrile (MeCN) in nano water.**
3. Elute DA with 5.0 ml of 2<sup>nd</sup> eluting solution 1 drop per second. Collect ALL 5ml into a tared vial. Record weight. Mix solution with vortex and pipette 1ml into 1.5ml autosample vial. Transport to UCSC for HPLC. **2<sup>nd</sup> eluting solution is 0.5M NaCl in 10% Acetonitrile (MeCN) in nano water.**