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Toxicon 40 (2002) 1259–1265

TOXICON

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Emerita analoga (Stimpson)—possible new indicator species for the phycotoxin domoic acid in California coastal waters

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Received 21 December 2001; accepted 12 April 2002

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 commerce dependent on marine resources. While indicator organisms are widely utilized to monitor for marine biotoxins like paralytic shellfish poisoning 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 sea mussels (*Mytilus californianus*). Mussels and sand crabs, collected from natural populations in Santa Cruz, California (April 1999–February 2000), were tested for DA using the HPLC–UV method. Toxin loads in sand crabs ranged from below detectable limits to 13.4 μg DA g^{-1} and coincided with the abundance of DA producing *Pseudo-nitzschia* species nearshore. Toxin levels in mussels collected during the study period were below HPLC–UV detectable limits. 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, clearly indicate the utility of sand crabs as a reliable, cost-effective monitoring tool for DA in the nearshore coastal environment.

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

1. Introduction

The occurrence of harmful algal blooms (HABs) 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 most common approach to 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 reliable for the 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 traced 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 (20 ppm) in seafood were established after which no additional cases of DA intoxication in humans, clinically recognized as amnesic shellfish poisoning, have been documented

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0041-0101/02/$ - see front matter
PII: S0041-0101(02)00129-0

(Todd, 1993; Altwein et al., 1995). However, recurrent mortality events in Monterey Bay, California, involving shorebirds in 1991 and marine mammals in 1998 and 2000, have raised 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 sea mussels (*Mytilus* spp.) (Altwein et al., 1995). Although HPLC–UV analysis of DA for the above mentioned species is well established, the use of mussels and razor clams by monitoring agencies as general sentinel organisms for DA along the Pacific coastal environment 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 6 months after initial contamination independent of nearshore bloom conditions (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* (P-n) 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 (California Department of Navigation and Ocean Development, 1977), this means many coastal areas are left unmonitored due to the absence of an appropriate and reliable indicator organism.

In an effort to bridge this monitoring gap along California's coast, Bretz et al. (2002) 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, during a localized bloom of *Pseudo-nitzschia australis* ($\leq 1.7 \times 10^4$ cells l^{-1}), 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 (Powell et al., 2002). However, DA in mussels collected at the same time, from an adjacent site, was below detectable limits.

The objective of the study presented here was to test the hypothesis that sand crabs accumulate and depurate DA in synchrony with the abundance of toxic *Pseudo-nitzschia* and that their reliability as an indicator species is superior to *Mytilus californianus* (mussels). Paired samples of sand crabs and mussels were collected from natural populations over an 11 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 receptor binding assay (RBA) and HPLC–UV.

2. Materials and methods

2.1. Field samples and collection sites

Sand crabs and mussels were collected once every 2 weeks at Natural Bridges State Park (36°57′, 122°03′) and Lighthouse State Beach (36°57′, 122°01.5′) in Santa Cruz, California, from April 1999 to February 2000 (Fig. 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 PSP toxins (Price et al., 1991). The Lighthouse site, approximately 2.4 km south of Natural Bridges, was chosen due to its proximity to Santa Cruz pier where seawater samples are routinely collected and monitored for plankton composition and abundance (Silver, pers. com.).

Twenty-five to 30 individual mussels up to approximately 5 cm in length were selected from natural

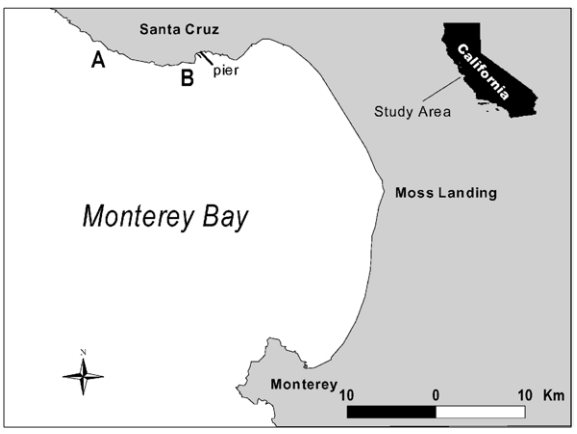

Fig. 1. Map of Monterey Bay coastline with intertidal collection sites located at (A) Natural Bridges State Park and (B) Lighthouse State beach in Santa Cruz, California.

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. Sand crabs (30–50 individuals 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 m^2, mesh size 4 mm) 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 °C for later DA extraction and HPLC–UV analysis. Control samples for extraction efficiency experiments, collected from natural populations at Natural Bridges on 17 September 1999 (mussels) and Pfeiffer State Beach on 20 May 1999 (sand crabs), were tested by HPLC–UV prior to sample analysis (Section 3).

Abundances of the DA producing *P. australis* and *Pseudo-nitzschia multiseries* in surface waters were determined using species-specific LSU rRNA-targeted fluorescent probes described by Miller and Scholin (1998). Sample of 10–30 ml were passed through 1.2 μm pore diameter. Isopore polycarbonate filters (Millipore Corporation, Bedford, MA01730) and cell counts were then made on a Zeiss Standard 18 compound microscope, equipped with epifluorescence. Whole water samples (1–2 l) were collected each week from October 1999 to February 2000 from the Santa Cruz Municipal Wharf (SCMW). Additional *Pseudo-nitzschia* abundance data for the time period between April 1999 and September 1999, also collected at SCMW, were provided by the Monterey Bay Aquarium Research Institute (MBARI).

2.2. Sample extraction

Mussels were prepared for DA analysis using the extraction procedure described by Quilliam et al. (1995). Briefly, 100 g of shucked mussel tissue were pooled and homogenized for 1–2 min in a blender (Oster model 6634, Sunbeam Ltd, Brampton, Ontario, Canada). A 4 g subsample 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 3000 × *g* for 10 min then filtered through a 0.45 μm 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 (SPE) clean-up.

Sand crabs were prepared for DA extraction by squeezing whole animals, 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 4 g was weighed for 50% aqueous MeOH addition and processed as described earlier. Three replicates consisting of five homogenized sand crabs were processed from each collection if available.

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

2.3. Solid phase extraction

Extracts were further treated by strong anion exchange (SAX), SPE clean-up using the method described by Hatfield et al. (1994). SAX cartridges from Supelco (Supelco Park, Bellefonte, PA 1823, USA; lot number SP2028B) were mounted on 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 0.1 M sodium chloride (NaCl; analytical grade) wash in 10% aqueous acetonitrile (MeCN). Lastly, 5 ml of 0.5 M NaCl in 10% aqueous 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 MeOH and MeCN (HPLC grade; Fisher Scientific) were prepared using Milli-Q water. SAX columns were tested prior to sample application and resulted in a 94 ± 2% SD ($n = 3$) DA recovery in spiked 50% MeOH.

2.4. 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 set at 242 nm with a bandwidth of 10 nm (reference signal set at 450 nm with a bandwidth of 10 nm). A reverse phase Vydac C18 column (2.1 mm × 25 mm, #201TP52, Separations Group, Hesperia, CA, USA) with a Vydac guard column (5 μm) was used. The mobile phase consisted of water/MeCN/trifluoroacetic acid (TFA) (90/10/0.1, v/v/v) and was degassed with helium for 10 min prior to analyses. Sample injections were 20 μl with a flow rate of 0.3 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 μg ml^{-1} ($r = 0.99$) resulted in a retention time (RT) of 6.8 min. RT for samples in this study ranged between 6.3 and 6.8 min

and changed from 5.7 to 6.0 min after the guard column was changed. The LOD for samples, based on three times the standard deviation of the lowest detectable standard, was 0.2 μg DA ml^{-1} or 2.5 μg DA g^{-1}. DA concentration per gram of mussel or sand crab tissue was calculated using the following equations:

$$X\ \mu g\ DA\ ml^{-1} = (area - 6.18)/88.35 \quad (1)$$

$$(X\ \mu g\ DA\ ml^{-1})(5\ ml/2\ ml)(20\ ml) = total\ \mu g\ DA \quad (2)$$

$$Total\ \mu g\ DA/4\ g\ mussel\ or\ crab\ tissue = \mu g\ DA\ g^{-1} \quad (3)$$

Eq. (1) was generated from the calibration curve calculating DA concentration X in a 20 μl injection and then Eq. (2) multiplied by the dilution factor of extracted and SAX cleaned samples for total DA. Eq. (3) was used to calculate DA concentration per gram of mussel or sand crab extract.

2.5. Receptor binding assay

Selected SAX cleaned extracts prepared as described in this paper were sent to the NOAA/NOS Marine Biotoxins Program (Charleston, SC, USA) to be tested for DA-like activity using a RBA described by Van Dolah et al. (1997) with modifications described by Lefebvre et al. (1999). Briefly, this assay is performed in a 96 well micro-titer plate format, and is based on competing a known amount of [^{3}H] kainic acid (a DA analog) against unlabelled toxin contained in a sample or standard for a given number of receptor sites in a preparation of cloned glutamate receptors. The percent of labeled toxin bound to the receptors is inversely and quantitatively related to the amount of unlabelled toxin in a sample. For each sample extract μg DA equiv. g^{-1} was determined with an LOD of 10 ng equiv. ml^{-1} or 50 ng DA equiv. g^{-1}.

3. Results

3.1. Sand crab and mussel samples

DA in mussel and sand crab controls was undetectable as determined by HPLC–UV (Figs. 2(A) and 3(A)). Control homogenates spiked with 25 and 50 μg DA g^{-1} resulted in 99 ± 2.2% SD ($n = 3$) and 97 ± 1.9% SD ($n = 3$) DA recovery for sand crabs and 93 ± 0.6% SD ($n = 3$) and 84 ± 0.1% SD ($n = 3$) for mussels. HPLC chromatograms showed no interference with DA detection in either tissue type with RTs of 6–6.3 min (Figs. 2(B) and 3(B)).

A total of 86 mussel and sand crab samples were collected at Natural Bridges State Park and Lighthouse State Beach from April 1999 to February 2000 and analyzed for DA by HPLC–UV. The strongest response for DA was detected from sand crabs collected from the Lighthouse site on 24 December, 1999 which contained 13.4 ± 3.11 μg DA g^{-1}, ($n = 3$). DA for the paired mussel sample was undetectable. Analysis of these samples by RBA yielded

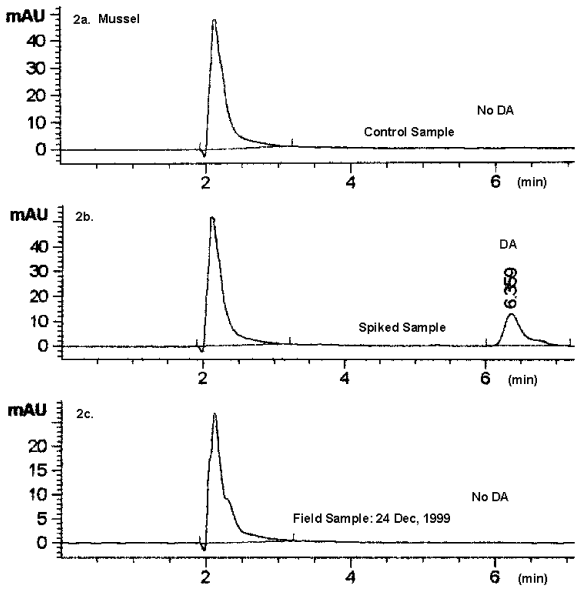

Fig. 2. Representative HPLC chromatograms of mussel tissue: (A) Control sample collected from Natural Bridges on 17 September 1999; (B) Control sample spiked with 25 μg DA g^{-1}. RT = 6.359 min; (C) Sample collected 24 December, 1999 from Lighthouse State beach during a *P. australis* bloom nearshore to Santa Cruz pier.

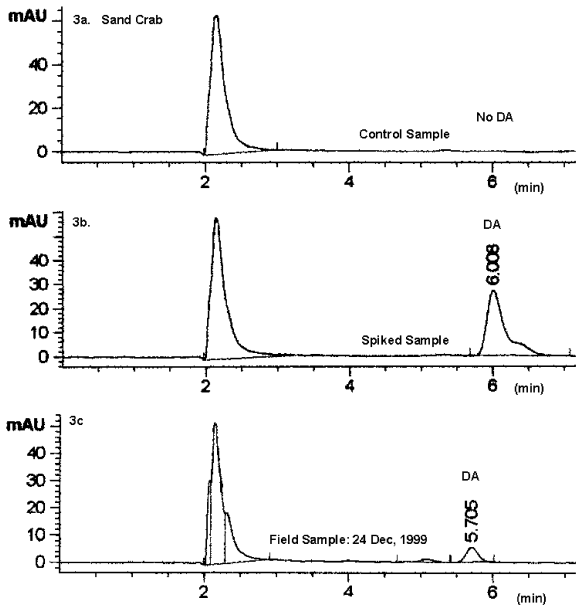

Fig. 3. Representative HPLC chromatograms of sand crab tissue: (A) control sample collected from Pfeiffer State beach on 20 May, 1999; (B) control sample spiked with 50 μg DA g^{-1}. RT = 6.008 min; (C) sample collected 24 December, 1999 from Lighthouse State beach. RT = 5.705 min. RT differences are due to guard column change.

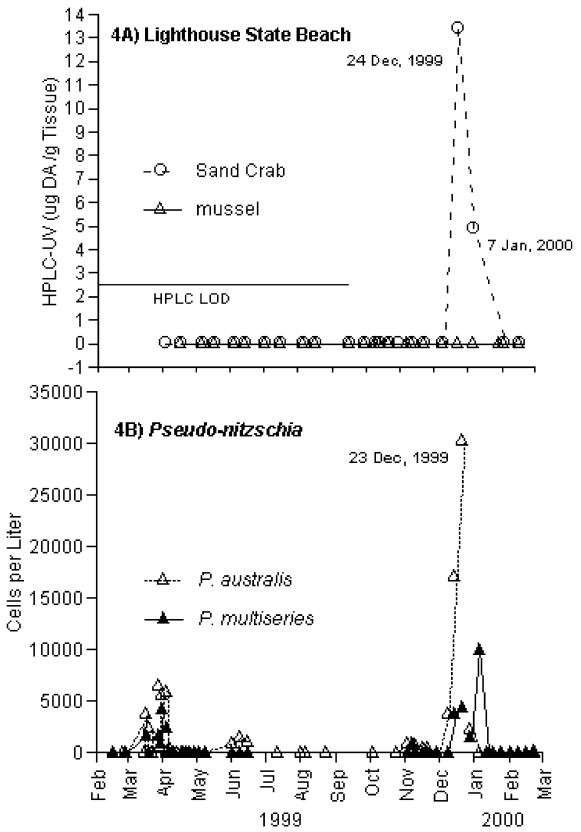

Fig. 4. Comparison of DA in sand crab (*Emerita analoga*) and mussel (*M. californianus*) tissue collected from April 1999 to February 2000 in Santa Cruz, California as determined by HPLC–UV: (A) lighthouse State beach. HPLC–UV limit of detection (LOD) is indicated by solid horizontal line. Values given are the means of three replicates of five individuals. (B) Time series for *Pseudo-nitzschia* spp. (*P. australis* and *P. multiseries*) monitored in surface waters during February 1999 through March 2000 from Santa Cruz pier. Break in lines indicate no data available. *Pseudo-nitzschia* is reported here as cells l^{-1}.Values given are the means of three replicate samples.

comparable results for sand crabs at 11.40 ± 4.53 μg DA equiv. g^{-1}, ($n = 3$), while the mussel sample contained 0.31 μg DA equiv. g^{-1}. Sand crabs collected at the same site 2 weeks later, on 7 January, yielded DA concentration corresponding to 4.9 μg DA g^{-1} (no replicate available) (Fig. 4(A)). DA in sand crabs and mussels collected from Natural Bridges was undetectable by HPLC–UV. However, analysis of the 4 April sand crab sample by RBA yielded 1.46 ± 0.74 ($n = 3$) μg DA equiv. g^{-1}.

3.2. *Pseudo-nitzschia abundance*

During the study period *Pseudo-nitzschia* (P-n), reported here as *P. australis* and *P. multiseries*, were present in waters near the SCMW beginning in mid March to early April (1999) and again in December (1999) until the first week of January (2000) (Fig. 4(B)). P-n density ranged from 4.0×10^2 to 9.8×10^3 cells l^{-1} from March to the first week of April with peaks on 2 April at 9.8×10^3 and falling to 8.3×10^3 and 4.0×10^2 on 5 and 8 April, respectively. After this time, cell densities were either low, not exceeding 1.4×10^3 cells l^{-1}, or not detected until December. After that time, P-n abundance rose dramatically from 3.6×10^3 on 10 December to 2.0×10^4 and 3.4×10^4 cells l^{-1} on 16 and 23 December. *P. australis* comprised 87% of the total P-n cells observed for the December bloom period. P-n abundance declined rapidly after 23 December with a relatively short-lived peak ($\leq 1.0 \times 10^4$ cells l^{-1}) on 7 January of the following year.

4. Discussion

DA concentrations in sand crabs varied with local abundance of DA producing P-n diatoms (Fig. 4(A) and (B)). From February 1999 to March 2000, *P. australis* contributed over 70% of the total P-n cells. Cell density was most concentrated during the December period when sand crabs from Lighthouse contained relatively high DA levels. Independent analysis of DA produced by P-n cells confirmed toxin presence in nearshore waters for this time period (Silver et al., unpublished data).

Pseudo-nitzschia spp. have been identified as DA sources affecting coastal regions along Washington, Oregon, California and Mexico (Horner et al., 1997; Hernandez-Becerril, 1998; Trainer et al., 2000). The observance of *P. australis* as a recurrent component in Monterey Bay's plankton assemblage has been confirmed by numerous sources (Buck et al., 1992; Fryxell et al., 1997). DA poisoning events affecting regional nearshore trophic chains, in association with *P. australis* blooms, are also well documented (Fritz et al., 1992; Work et al., 1993; Lefebvre et al., 1999; Scholin et al., 2000). During a 2 year survey in which DA producing P-n were monitored in Monterey Bay, Waltz et al. (1994) identified *P. australis* as the main source of DA in nearshore plankton samples, even though other potentially toxic species were observed. The sudden accumulation and disappearance of DA in sand crabs, linked with the abundance of *P. australis*, is consistent with *P. australis* as the source of the DA toxin.

The presence of DA in sand crabs associated with relatively low *P. australis* densities ($\leq 10^4$ cells l^{-1}) reveals DA accumulation capabilities not characteristic of the standard bivalve indicator. The failure of mussels as a sentinel organism for DA along the west coast of North America may be attributed to a variety of factors including lower ingestion rates or feeding inhibition in the presence of toxic *Pseudo-nitzschia* blooms 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. With the PSP toxins, many bivalve species have shown remarkable differences in their accumulation and depuration depending on their inherent sensitivity to the toxins. For example, the mussel, *M. edulis*, which appears to be insensitive to the PSP toxins readily feeds on toxic cells and accumulates high toxin loads; in contrast, the oyster, *Crassostrea virginica*, which is highly sensitive to the toxins has shown low toxin accumulation by altering feeding rates or complete shell closure (Bricelj and Shumway, 1998). In addition, 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 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) compared to mussels, which can become separated from their food source due to tidal variations. However, this interspecific difference was not seen with respect to PSP toxins, suggesting that tidal exposure may not be a factor. 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 confirms the amenability of this species to DA analysis using the HPLC–UV method. However, while recovery yields from extraction efficiency experiments were high (>95%), preparation of tissue for DA extraction using the press method may not be as efficient as anticipated. The press method requires semi-soft tissue samples that are either thawed from archival material or processed quickly after collection. The release of fluids during this process, considering the hydrophilic nature of DA, may account for the high variability observed among sand crab replicate samples. Powell et al. (2002) report separation of gut tissue from the carapace body may reduce total extractable DA by as much as 50%. Toxin concentrations reported here are, therefore, likely to be conservative estimates of total DA loads for sand crabs.

5. Conclusions

The potential impact of DA to human health, fisheries, and marine life in the coastal environment may best be managed by 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 reliable, cost-effective alternative to mussels as an indicator of choice for DA along the California coast.

Acknowledgments

We would like to thank Cheri Everlove, Lonnie Lundsten, Talitha Manouki, and Margaret Geissler (CSUMB) for laboratory support and assistance; Shonna Dovel (UCSC) for running samples on the HPLC–UV; William Head, Chris Hasagawa, and Sharon Anderson (CSUMB) for administrative support; Ron Rinehart at Monterey Peninsula College for additional laboratory space; Peter Miller (MBARI) for *Pseudo-nitzschia* data and technical assistance; Dan Mills and Gregg Langlois (CDHS) for technical assistance on shellfish testing protocol; the crew at the Sea Floor Mapping Lab (CSUMB) for field assistance; Chris Scholin (MBARI) and Mary Patyten (CSUMB) for reviewing this manuscript. This research was supported with funds from the National Science Foundation's interagency ECOHAB Program, Grant #OCE-9726263, and NOAA Grant #NA96OP0475.

References

Altwein, D.M., Foster, K., Doose, G., Newton, R.T., 1993. The detection and distribution of the marine neurotoxin domoic acid on the Pacific coast of the United States 1991–1993. J. Shell. Res. 14, 217–222.

Bates, S.S., 1997. Toxic phytoplankton on the Canadian east coast: implications for aquaculture. Bull. Aquac. Assoc. Can. 97, 9–18.

Bates, S.S., Bird, C.J., de Freitas, A.S.W., Foxall, R., Gilgan, M., Hanic, L.A., Johnson, G.R., McCulloch, A.W., Odense, P., Pocklington, R., Quilliam, M.A., Sim, P.G., Smith, J.C., Subba Rao, D.V., Todd, E.C.D., Walter, J.A., Wright, J.L.C., 1989. Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can. J. Fish. Aquat. Sci. 46, 1203–1215.

Bretz, C.B., Manouki, T.J., Kvitek, R.G., 2002. *Emerita analoga* (Stimpson) as an indicator species for paralytic shellfish poisoning toxicity along the California coast. Toxicon in press.

Bricelj, V.M., Shumway, S.E., 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. Rev. Fish. Sci. 6 (4), 315–383.

Buck, K.R., Uttal-Cooke, L., Pilskaln, C.H., Roelke, D.L., Villac, M.C., Fryxell, G.A., Cifuentes, L., Chavez, F.P., 1992. Autecology of the diatom *Pseudonitzschia australis*, a domoic acid producer, from Monterey Bay, California. Mar. Ecol. Prog. Ser. 84, 293–302.

California Department of Navigation and Ocean Development, 1977. Assessment and Atlas of Shoreline Erosion along the California Coast.

Dugan, J.E., Hubbard, D.M., Wenner, A.M., 1994. Geographic variation in life history of the sand crab *Emerita analoga* (Stimpson) on the California coast: relationships to environmental variables. J. Exp. Mar. Biol. Ecol. 181, 255–278.

Efford, I.E., 1965. Aggregation in the sand crab *Emerita analoga* (Stimpson). J. Anim. Ecol. 34, 63–75.

Efford, I.E., 1976. Distribution of the sand crabs in the genus *Emerita* (Decapoda, Hippidae). Crustaceana 30, 169–183.

Fritz, L., Quilliam, M.A., Wright, J.L.C., Beal, A.M., Work, T.M., 1992. An outbreak of domoic acid poisoning attributed to the pennate diatom *Pseudonitzschia australis*. J. Phycol. 28, 439–442.

Fryxell, G.A., Villac, M.C., Shapiro, L.P., 1997. The occurrence of the toxic diatom genus *Pseudo-nitzschia* (Bacillariophyceae) on the West Coast of the USA, 1920–1990: a review. Phycologia 36, 419–437.

Hatfield, C.L., Wekell, J.C., Gauglitz, E.J., Barnett, H.J., 1994. Salt clean-up procedure for the determination of domoic acid by HPLC. Nat. Toxins 4, 206–211.

Hernandez-Becerril, D.C., 1998. Species of the planktonic diatoms genus *Pseudo-nitzschia* of the Pacific coasts of Mexico. Hydrobiologia 379, 77–84.

Horner, R.A., Kusske, M.B., Moynihan, B.P., Skinner, R.N., Wekell, J.C., 1993. Retention of domoic acid by Pacific razor clams, *Siliqua patula* (Dixon, 1789): preliminary study. J. Shell. Res. 12, 451–456.

Horner, R.A., Garrison, D.L., Plumley, G.F., 1997. Harmful algal blooms and red tide problems on the US west coast. Limnol. Oceanogr. 42, 1076–1088.

Langlois, G.L., Kizer, K.W., Hangsen, K.H., Howell, R., Loscutoff, S.M., 1993. A note on domoic acid in California coastal molluscs and crabs. J. Shell. Res. 12, 467–468.

Lefebvre, K.A., Powell, C.L., Busman, M., Doucette, G.J., Moeller, P.D., Silver, J.B., Miller, P.E., Hughes, M.P., Singaram, S., Silver, M.W., Tjeerdema, R.S., 1999. Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. Nat. Toxins 7, 85–92.

Miller, P.E., Scholin, C.A., 1998. Identification and enumeration of cultured and wild *Pseudo-nitzschia* (Bacillariophyceae) using species-specific LSU rRNA-targeted fluorescent probes and filtered whole cell hybridization. J. Phycol. 34, 371–382.

Novaczek, I., Madhyastha, M.S., Ablett, R.F., Donald, A., Johnson, G., Nijjar, M.S., Sims, D.E., 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Can. J. Fish. Aquat. Sci. 49, 312–318.

Perl, T.M., Bedard, L., Kosatsky, T., Hockin, J.C., Todd, E.C.D., Remis, R.S., 1990. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. New Engl. J. Med. 322, 1775–1780.

Powell, C.L., Ferdin, M.E., Busman, M., Kvitek, R.G., Doucette, G.J., 2002. Development of a protocol for determination of domoic acid in the sand crab (*Emerita analoga*): a possible new indicator species. Toxicon 40, 481–488.

Price, D.W., Kizer, K.W., Hansgen, K.H., 1989. California's paralytic shellfish prevention program, 1927–1989. J. Shell. Res. 10, 119–145.

Quilliam, M.A., Xie, M., Hardstaff, W.R., 1995. A rapid extraction and clean-up procedure for the liquid chromatographic determination of domoic acid in unsalted seafood. J. AOAC Int. 78, 543–554.

Ricketts, E.F., Calvin, J., Hedgpeth, J.W., 1985. Between Pacific Tides, Stanford University Press, Stanford, p. 257.

Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, S., Chavez, F.P., Cordaro, J., DeLong, R., DeVogelaere, A., Harvey, J., Haulena, M., Lefebvre, K., Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin, R., Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowles, T., Silvagni, P., Silver, M., Spraker, T., Trainer, V., Van Dolah, F.M., 2000. Mortality of sea lions along the central California coast linked to toxic diatom bloom. Nature 403, 80–84.

Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and aquaculture. J. W. Aquac. Soc. 21, 65–104.

Sommer, H., Meyer, K.F., 1937. Paralytic shellfish poisoning. Arch. Pathol. 24, 560–598.

Tam, Y.K., Kornfeld, I., Ojeda, F.P., 1996. Divergence and zoogeography of mole crabs, *Emerita* spp. (Decapoda: Hippidae), in the Americas. Mar. Biol. 125, 489–497.

Todd, E.C.D., 1993. Domoic acid and amnesic shellfish poisoning—a review. J. Food Prot. 50, 69–83.

Trainer, V.L., Adams, N.G., Wekell, J.C., Lesoing, M., 2000. Domoic acid production by *Pseudo-nitzschia pseudodelicatissima* off the central Washington coast is linked to record levels of toxin in razor clams. J. Shell. Res. 19, 638.

Van Dolah, F.M., Leighfield, T.A., Haynes, B.L., Hampson, D.R., Ramsdell, J.S., 1997. A microplate receptor assay for the amnesic shellfish poisoning toxin, domoic acid, utilizing a cloned glutamate receptor. Anal. Biochem. 245, 102–105.

Walz, P.M., Garrison, D.L., Graham, W.M., Cattey, M.A., Tjeerdema, R.S., Silver, M.W., 1994. Domoic acid-producing diatom blooms in Monterey Bay, California: 1991–1993. Nat. Toxins 2, 271–279.

Wekell, J.C., Gauglitz, E.J., Barnett, H.J., Hatfield, C.L., Simons, D., Ayres, D., 1993. Occurrence of domoic acid in Washington State razor clams (*Siliqua patula*) during 1991–1993. Nat. Toxins 2, 197–205.

Work, T.M., Beal, A.M., Fritz, L., Quilliam, M.A., Silver, M., Buck, K., Wright, J.L.C., 1993. Domoic acid intoxication of brown pelicans and cormorants in Santa Cruz, California. In: Smayda, T.J., (Ed.), Toxic Phytoplankton Blooms in the Sea, Elsevier, Amsterdam, pp. 643–649.