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

M. E. Ferdin[1*], Rikk G. Kvitek[1], Carolyn Bretz[1], Christine L. Powell[2], Gregory J. Doucette[2], Mary W. Silver[3], Christopher A. Scholin[4]


Common Sand Crab (*E. analoga*)

Abstract

We evaluate and confirm the utility of the common sand crab (*Emerita analoga*) to monitor the algal toxin domoic acid (DA) in the coastal environment. *Emerita* and sea mussels (*Mytilus* sp.), a general sentinel indicator for DA, were collected from natural populations over an 11-month period in Monterey Bay, California, and tested for DA using the HPLC-UV method. DA levels in Emerita ranged from 0.07 to 10.4 ug DA g^{-1} and coincided with observed density trends in *Pseudo-nitzschia* sp nearshore. The toxin was not detected for any of the mussels collected for this study.

Table 1. Extraction Efficiency Experiments

Tissue Type (N=3)	Concentration	% Recovery
Sand Crabs (±2.19)	25 ug DA/g	99%
(±1.91)	50 ug DA/ g	97%
Mussels (±0.63)	25 ug DA/ g	93%
(±0.83)	50 ug DA/ g	94%

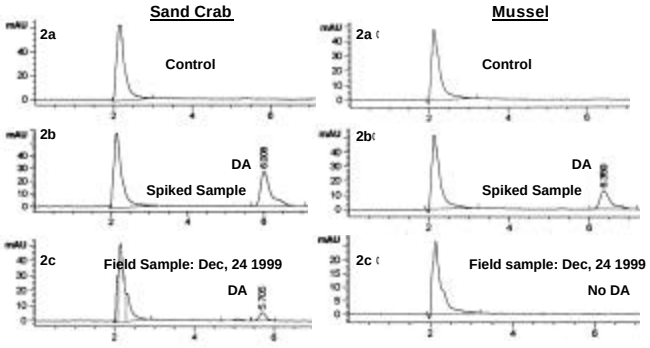


Figure 2. HPLC-UV Chromatographs of Sand Crab and Mussel

Control tissue collected from natural populations **(2a,a)** and spiked with Sigma DA showed no interference for toxin detection in either tissue types with retention times of 6.0-6.3 minutes **(2b, b)**. A strong elution peak for DA was observed for sand crabs collected Dec, 24 1999 **(2c)** while the peak was absent for mussels collected from the same sites, at the same time **(2c)**.

Introduction

The potential impact to human health, fisheries, and marine life posed by harmful algal blooms (HAB's) is mediated by our ability to successfully detect HAB species and the toxins they produce. While sophisticated analytical tools have greatly aided our efforts in the field and in the laboratory, monitoring for the presence of natural marine toxins with general sentinel indicators is still the fundamental approach in safeguarding public health for government agencies tracking marine toxins in North America (Altwein *et al*, 1995). Bivalve indicators like sea mussels (*Mytilus* sp.) and clams (*Siliqua* sp.), although effective for monitoring paralytic shellfish poisoning (PSP) toxins along the Pacific coast (Price *et al*. 1991), have been less than reliable for the more recently discovered HAB toxin **domoic acid (DA)** (Langlois *et al.* 1993). For State agencies like the California Department of Health Services (CDHS) the challenge of inadequate indicators for DA, a diatom synthesized neuro-toxin, has been compounded by the motility of DA to cross trophic tiers (Work *et al.* 1993; Lefebvre *et al* 1999; Scholin *et al*. 2000).

In 1998 the were found positive for domoic acid, which was incidentally associated with a bloom of DA producing *Pseudo-nitzschia* diatoms nearshore. As a result, field trial comparisons of mussels and sand crabs were conducted to test the hypotheses that 1) sand crabs retain DA in synchrony with toxin source and 2) that DA loads in sand crabs are comparable to sea mussels (*Mytilus* sp.), the standard bivalve indicator. DA loads in both species were then compared to P-N activity nearshore.

RESULTS

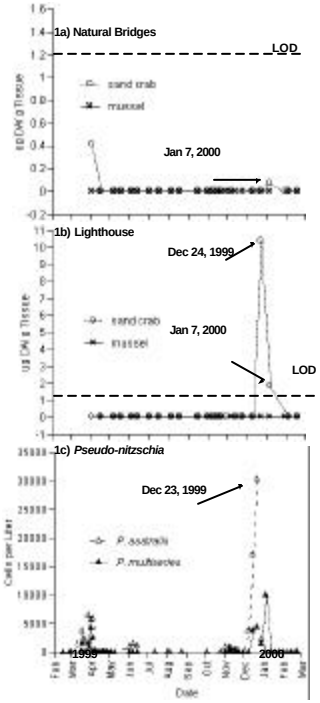


Figure 1. Field Trial Comparison of DA Loads and Toxin Source for Sea Mussels and Sand Crabs.

HPLC-UV analysis of DA showed differential concentrations of the toxin in sand crabs compared to mussels collected April 1999 through February 2000 from Natural Bridges State Park (**1a**) and Lighthouse State beach (**1b**) in Santa Cruz, California. Instrument limit of detection (LOD; 1.25ug DA/g) is denoted by dashed line. Note scale differences for each site.

The appearance of DA in sand crabs co-occur with the abundance of DA producing *Pseudo-nitzschia* sp.(P-N). During the study period P-N (*P. australis and P. multiseries*) was present in nearshore waters from mid march to early April 1999 and again in mid December to early January 2000 **(1c)**. P-N activity was most pronounced between December 10th through the 23rd when observed cells increased from 3.6×10^3 to 3.4×10^4 cells L^{-1}. *P. australis* contributed over 80% of the total cells observed for the December "bloom" period and ~70% over the P-N monitored period.

Figure 3. Sample Location and Schedule

Sand crabs and mussels were collected as pairs once every two weeks beginning early April 1999 through late February 2000 from two intertidal locations in Santa Cruz, California:

- Natural Bridges State Park **(A)**
- Lighthouse State Beach **(B)**

Targeted species were sampled from natural

Sand Crabs

- up 50 Individuals (varying size classes)
- Haphazard Collection From Swash Zone

Mussels

- Twenty-five to 30 Individuals (~7 cm)
- Adjacent Rocky Location

Samples were transported in iced coolers to CSU Monterey Bay where they were stored at -70C for later toxin extraction and HPLC-UV analysis.

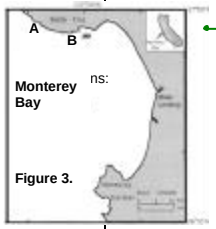
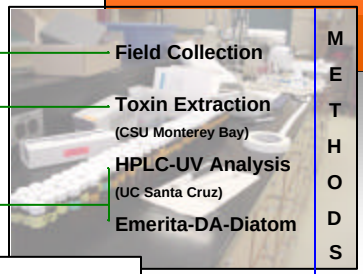


Figure 3.

METHODS



Plankton Sampling

DA loads in targeted species were compared to the presence of DA producing Pseudo-nitzschia (P-N) diatoms nearshore. Sea water samples from Santa Cruz pier were monitored for P-N abundance and species (P. australis and P. multiseries) using genetic probes described by Scholin *et al* (1995?).

HPLC-UV Analysis

SAX Extracts were analyzed for DA isocratically using the method described by Lefebvre *et al* (1999).

Hewlett-Packard 1090 auto Injection Specifications:

Detector: DAD set at 242nm and 280nm

Column: Vydac C_{18} with Vydac guard column (5um)

Mobile phase: Isocratic , water/MeCN/TFA (90/10/0.1, V/V/V)

Flow rate: 0.3 ml^{-1} at 40°C

Injection: 20 ul

Limit of Detection: 0.1 ug DA ml^{-1} (1.25 ug DA g^{-1})

Retention Time: DA eluted 6-7 mins and 5-6 mins after guard column was changed.

Figure 4. Toxin Extraction for Sand Crabs and Mussels

Tissue samples were extracted for the DA toxin using the methanol procedure described by Quilliam *et al.* (1995) with clean-up modifications by Hatfield *et al.* (1994).

Further modification:

Sand Crab

- Tissue separated from carapace (garlic press used)
- Sufficient Weight for analysis (5 individuals pooled)

Extraction Efficiency Experiments

Controls of each tissue type were treated to two series of spike and recovery experiments (50 and 25 µg DA g^{-1}) to evaluate:

- Method of DA Recovery From Sample Matrix
- Detection efficiency of DA From Sand Crab Tissue
- Efficiency of SAX, SPE columns

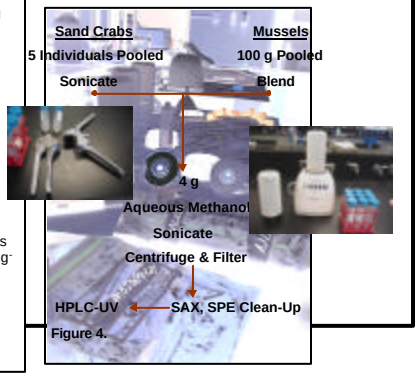


Figure 4.

Conclusion

The change in DA loads detected for the common sand crab (*E. analoga*) in synchrony with the rise and fall of *Pseudo-nitzschia* sp. strongly suggests real-time compatibility to nearshore diatom activity. This coupled with the ease with which DA was extracted from sand crab tissue, recommends this intertidal invertebrate as a more successful, cost effective alternative to the widely utilized bivalve indicator.

Acknowledgements

We would like to thank Shonna Dovel and Kathi Lefebvre at UC Santa Cruz for technical expertise in HPLC-UV; Cheri Everlove and the crew of the Sea Floor Mapping Lab at CSU Monterey Bay for field and laboratory support. This research was funded by the National Science Foundation's interagency ECOHAB Program.

[1]California State University, Monterey Bay, 100 Campus Center, Seaside, California 93955, U.S.A.; [2]Marine Biotoxin Program, NOAA/National Ocean Service, 219 Fort Johnson Rd., Charleston, South Carolina 29412, USA; [3]University of California, Santa Cruz, California 95064, USA; [4]Monterey Bay Aquarium Research Institute, 770 Sandholdt Rd., Moss Landing, California 95039, USA; * Corresponding Author.