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Emerita analoga (Stimpson) as an indicator species for paralytic shellfish poisoning toxicity along the California coast

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Abstract

Paralytic shellfish poisoning toxins (PSPT) produced by certain harmful algal blooms (HABs) pose a serious threat to public health worldwide. Along the West coast of North America, *Mytilus californianus* (California sea mussel) has long been used as the primary indicator species for monitoring levels of PSPT in the environment. However, because the natural distribution of this species is limited to rocky shores, vast stretches of coastline bordered by sandy beach habitat are not regularly monitored for PSPT presence. This insufficient spatial coverage greatly reduces the ability to track and predict HAB development and movement along the open coast. Earlier studies on *Emerita analoga* (common mole or sand crab) demonstrated this species' potential to sequester the neurotoxin saxitoxin (STX) and its derivatives. The goals of this study were to (1) develop a practical and efficient PSPT extraction protocol for sand crabs, and (2) compare the utility and reliability of this species as an indicator of PSPT with that of mussels. Laboratory spike and recovery results showed that STX extraction efficiencies were very similar for both species. Field comparisons of natural accumulation and depuration rates of PSPT for the two species revealed that PSPT events detected using mussels were also identified with sand crabs.

Keywords: Extraction protocol; Harmful algal blooms; Indicator species; Sand crab; Sea mussel; Toxin accumulation

1. Introduction

The incidence of harmful algal blooms (HABs) in California has increased over the past two decades. HABs, although somewhat seasonally predictable, often vary in toxicity from year to year. The most significant HAB toxins in terms of public health are commonly known as the paralytic shellfish poisoning toxins (PSPTs), a group of saxitoxin (STX) derivatives produced by dinoflagellates belonging primarily to the genus *Alexandrium* (Schantz, 1986; Van Egmond et al., 1994). Once ingested, the PSP toxins act to block voltage-gated sodium channels in nerve tissues, which can result in respiratory paralysis and ultimately death given a sufficient dose (Narahashi, 1988). Because PSP toxins have been shown to enter the marine food web via filter-feeding invertebrates, particularly molluscs and crustaceans, these compounds can present a serious health hazard to higher predators such as fish, birds, marine mammals, and humans (Quayle, 1969; White, 1981; Jonas-Davies and Liston, 1985; Potts and Edwards, 1987; Anderson and White, 1992; Smayda, 1992; Cembella et al., 1994).

In California, the Department of Health Services (CDHS) Marine Biotoxin Monitoring and Control Program is responsible for monitoring and notifying the public about the local occurrence of harmful algal blooms and their associated toxins. The CDHS relies on the collection and testing of sea mussels (*Mytilus californianus*, hereafter mussels) from natural populations, and outplants (Price et al., 1991), as well as, observations generated by phytoplankton monitoring programs which fill temporal and spatial gaps in shellfish toxicity data (Conrad et al., 1996). Each county along the coast is requested to submit samples to the CDHS at bi-weekly intervals during the mussel quarantine season (normally May 1 through October 31; CDPH, 1942), and monthly, during the off season. Sample analysis is based on the standard mouse bioassay, where shellfish PSPT concentration is correlated with the time elapsed between an intraperitoneal injection of a

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homogenized mussel extract and resulting mouse death (Laycock et al., 1994). The mouse bioassay, adopted as an official AOAC method (#959.08) in 1965, still remains the accepted procedure for detecting and measuring PSP toxicity in commercial and recreational shellfish (Bricelj and Shumway, 1998). The Food and Drug Administration (FDA) quarantine limit for PSPT in shellfish is set at 80 μg STX equivalents/100 g tissue, and values between 80 and 160 μg, typically signal the onset of a PSPT producing bloom. The CDHS shellfish monitoring program is part of a larger nation-wide effort to prevent the human consumption of contaminated marine organisms, track blooms as they migrate along the coast, as well as increase our understanding of the sources and ecology of harmful algal blooms.

Sea mussels are an ideal monitoring species for PSPT producing HABs along the west coast of North America because they are abundant, widely distributed, easily obtainable from rocky intertidal habitats, and are known to rapidly concentrate and release PSPT (Price et al., 1991). However, in California, sandy beaches constitute 64% of the coastline (Office of Emergency Services (OES), 1994) and are unsuitable for mussel recruitment and colonization. Consequently, much of California's shoreline is left unmonitored making it difficult or impossible to track or predict HABs as they spread along the coast. Without advanced warning of elevated toxicity, there is an increased potential for serious economic damage to marine fisheries and bivalve industries, and a greater risk to public health (Ahmed, 1991; Burgess, 1997; Van Dolah, 2000). In addition, both temporary and prolonged closure of local beaches and harvesting operations can have far-reaching economic consequences (Shumway, 1990; Anderson et al., 2000).

As a result, there is a pressing need for an indicator species suitable for monitoring PSP toxins along sandy shores. The variety of organisms commonly found on California's beaches offer a diverse range of candidates for use in toxicity testing. The goals of this study included the identification of candidates for use in toxicity analyses, and the development and testing of one of these species as an appropriate and reliable bioindicator for PSPT monitoring. The common mole or sand crab, *Emerita analoga* best fulfilled the criteria, based on Wenner's (1988) guidelines for selecting bioindicators for sandy beach environments.

E. analoga (hereafter sand crab) occurs intertidally on sandy beaches along the western coast of North America from Kodiak Island, Alaska to Bahia de San Francisquito, Baja California, and along the west coast of South America from San Lorenzo, Equador to False Bay, Argentina (Efford, 1976). Sand crabs tend to aggregate in dense patches, visibly filter feeding with their long secondary antennae (Fusaro, 1980; Ricketts et al., 1985). Specimens are easily collected within the swash zone throughout the year using simple nets (Efford, 1965; Ricketts et al., 1985; Dugan et al., 1994).

Sand crabs have previously been used for testing environmental pollutants such as DDT and heavy metals (Wenner, 1988), and the HAB toxin domoic acid (Ferdin et al., 2002; Powell et al., 2002). Sommer (1932) was the first to conduct a study on the occurrence of PSP toxins in sand crabs. Through a series of experiments he was able to document the concurrent rise and fall of PSPT concentrations within sand crabs and sea mussels. Sommer's extraction method, however, involved the extremely tedious dissection and removal of the crab's liver, a technique not amenable to the development of a routine protocol for monitoring laboratories responsible for processing large numbers of weekly samples. Sommer and Meyer (1937) published an additional study comparing the toxicity levels of a variety of marine organisms at several sites over a 3-year period. Results revealed PSPT retention and release rates in sand crabs and mussels (*M. californianus*) to be comparable. Curiously, despite Sommer's provocative findings, there has apparently been no further research published on the use of sand crabs as indicators for PSP toxicity.

The evaluation of sand crabs as a monitoring tool for PSPT involved determining the patterns and concentrations of PSPT accumulation as well as the rate of toxin uptake, retention, and depuration as compared to mussels; and the development of a practical and efficient procedure for routine PSPT extraction for use with the standard mouse bioassay.

The general approach taken was two-fold. In the laboratory, the FDA-accepted mussel extraction protocol was used as a baseline procedure, and modified for sand crabs using spike and recovery verification methods. Simultaneous field sampling of natural populations of mussels and sand crabs at five pairs of rocky and sandy shore sites exposed to the same HAB conditions were used to compare PSPT concentrations in a time series for both species. The results from these paired samples were then used to evaluate the effectiveness of sand crabs as an indicator of PSPT presence in relation to mussels, the currently employed sentinel species, by testing the following hypotheses:

H_0: Sand crabs do not retain PSPT in body tissues when exposed to a PSPT-producing HAB
H_1: Sand crabs do retain PSPT
H_2: Sand crabs do retain PSPT at concentrations similar to sea mussels
H_3: Sand crabs accumulate PSPT at the same rate as sea mussels
H_4: Sand crabs depurate PSPT at the same rate as sea mussels

2. Methods

2.1. Modification of standard PSPT extraction procedure for use with E. analoga

2.1.1. Sample collection and preparation

Sand crab and mussel samples were collected from a paired rocky and sandy beach site at Pebble Beach, CA in

early October of 1998. Sand crab specimens of varying size classes were randomly collected in 100 g quantities (approx. 30–40 medium-sized individuals), within the beach swash zone, by perturbing the sand with a small shovel and capturing emerging animals in a 1/4 in. mesh net. Whole animals were rinsed of noticeable particulates (such as sand or algae) using ambient seawater and stored in pre-labeled plastic bags, transported to California State University Monterey Bay (CSUMB), and frozen at −70 °C. Mussel samples ($n = 25–30$, 6–9 cm length) were collected and prepared according to California Department of Health Services Marine Biotoxin Monitoring and Control Program protocols (Price et al., 1991). Intact specimens were rinsed before shucking to remove any foreign material (e.g. limpets or encrusting bryozoans). The soft tissue was separated from the shell by severing the adductor muscle from the inside of the shell on either side, and pooled with other individuals collected at the same site and date. Tissue samples were frozen at −70 °C.

2.1.2. Extraction trials

Several revisions to the original extraction procedure used by the CDHS (AOAC, 1995; SOP#WSL-510-TM, 1997) were applied to sand crabs. Initially we included both the exoskeleton and soft tissues for homogenization, an approach also prescribed by Powell et al. (2002) for the extraction of domoic acid from sand crabs. However, because the PSPT extraction protocol uses hydrochloric acid to release the STX, there was difficulty controlling the pH of the homogenate due to the reaction between calcium carbonate in the exoskeleton and the acid. Removal of the exoskeleton from the soft tissue during the preparation of crab tissue greatly reduced the pH variability. A potato ricer was employed to compress whole crab bodies and extrude the viscera and ova. The tissue was then homogenized, and a subsample of 50 g was transferred to a glass beaker for acidification with an equal mass of 0.18 M hydrochloric acid (HCl). The pH of the acidified homogenate mixture was determined, and if necessary, adjusted with 5 M HCl to a pH between 2 and 4. If the sample reached a pH of less than 2 or more than 4, any toxins present could either become enhanced, or be deactivated, respectively (Park et al., 1986). The acidified tissue was boiled for 5 min to ensure the proper breakdown of cells in order to release the STX. Antifoaming agents were not used.

Another difficulty encountered was that during the boiling of the acid/tissue mixture, the sand crab homogenate often reached boiling point very quickly, and at lower temperatures than the mussel homogenate. Samples had to be diligently monitored in order to prevent the mixture from erupting out of the beaker, and constant stirring by hand was often required until the 5 min boiling time had elapsed. After cooling, the sample was again adjusted for pH changes and brought back to its' original weight with 3 mM HCl. Sand crab samples tended to have a higher lipid content than mussels, which made separating the solids from the supernatant challenging. As a result, centrifuge times (6 min at maximum speed) were doubled for the sand crab protocol. The resulting supernatant was isolated and stored at −70 °C until shipped to the California Department of Health Services Laboratory for replicate testing with a mouse bioassay. Extraction efficiency results were reported in μg STX equiv./100 g of tissue.

2.2. Spike and recovery experiments

The goal of the spike and recovery experiments was to determine what, if any, matrix effects sand crab tissue would have on STX extraction and analysis. Mussel and sand crab specimens were collected and prepared as previously described, on June 21, 1999 from Natural Bridges State Park, CA. Triplicate 50 g samples each of sand crab, mussels, and deionized (DI) water were spiked at five different toxicity levels with 50, 80, 200, 500, and 1000 μg of FDA-approved purified (>95%) STX stock generously provided by Dr Sherwood Hall (FDA-DHHS). DI water samples were added to act as controls to determine if the procedure produced any by-products that might interfere with mouse bioassay interpretation. Samples were spiked by pipette into samples prior to homogenization and then immediately processed following the conventional (mussel) and modified (sand crab) extraction protocols. Negative controls ($n = 3$ replicates each) for sand crab and mussel samples were also included to test for STX integrity by verifying the absence of native STX in the tissue matrix. Resulting extracts were kept at −70 °C. Correlation curves from mouse bioassay analyses were adjusted for reduced sample volume. Comparison of mean recovery for each of the three extraction types were tested using ANOVA.

2.3. Field samples for interspecific comparison

Between the months of April and September in 1998, and April and November in 1999, samples of sand crabs and mussels were collected from five pairs of adjacent rocky and sandy beaches along the California coast (Fig. 1). The study sites from north to south: Limantour Beach, Muir Beach, Pescadero Beach, Pebble Beach, Pfeiffer State Beach, have historically exhibited a latitudinal gradation in toxicity from the north to the south (Price et al., 1991). During the 1998 and 1999 sampling periods, both species were collected, as paired samples, bi-weekly, and within 24 h of each other. When possible, collections were made on days with particularly low tides. CDHS (G. Langlois) provided information on developing or existing algal blooms within the range of our study sites. Sand crab and mussel tissues were handled, extracted, and analyzed via the protocols and methods described earlier.

Fig. 1. Map of California showing locations where sand crab (*E. analoga*) and mussel (*M. californianus*) samples were collected from five pairs of adjacent rocky and sandy beaches during the study. Arrow indicates PSP toxicity gradient based on historical accounts and monitoring data.

3. Results

3.1. Spike and recovery

Results from the spike and recovery experiment (Fig. 2) with mussel, sand crab, and DI water samples inoculated at five different toxicity levels (50, 80, 200, 500, and 1000 μg) demonstrated that saxitoxin recovery for sand crabs closely parallels that of sea mussels. There was no statistically significant difference (ANOVA, $p = 0.27$) between the recovery levels for the three sample types. Sand crabs yielded a slightly higher level of saxitoxin than mussels at the lower toxicity levels (50, 80, 200, and 500 μg), while at the 1000 μg level, mussels had a higher recovery. Overall percent recovery rates for mussel and sand crabs were 69.9 and 72.3, respectively. As expected, DI control samples had the highest percent recovery rate of the three sample types at 84.1. Results for all negative controls (unspiked tissue) were below the detection limit of the mouse bioassay ($\sim < 38$ μg STX equiv./100 g) (Price and Kizer, 1990).

3.2. Two-year field comparison

Throughout the study, PSPT levels for both species rose above the 80 μg STX/100 g state quarantine level only at Muir and Pebble Beach in 1998, and Limantour, Muir and Pescadero in 1999. Toxicity for the other sites in 1998 and 1999 remained well below 80 μg/100 g, rarely exceeding the minimum detection limit ($\sim$38 μg STX/100 g) of the mouse bioassay test. Data clearly show that our study commenced (April 1998) during a degrading toxic bloom. Limantour Beach samples, a historically toxic region (Price and Kizer, 1990; Price et al., 1991), showed evidence of low toxicity during this early sampling period, suggesting that bloom maturation may have followed the north to south pattern typical for this region.

At Limantour Beach (Fig. 3(A)), sand crab and mussel toxicity rose and fell in tight synchrony demonstrating comparable toxicity signals at low toxin levels. Samples in 1999 show a distinct spike in toxicity starting in early September and ending in late October. Sand crabs were the first to detect the initial onset of PSPT activity (83 μg) in late August. Two weeks later mussel toxicity was still below the state quarantine limit at 79 μg, while sand crab levels had reached 293 μg. PSPT levels in mussels peaked 2 weeks later in late September at 810 μg, the highest toxicity recorded during the study. Sand crab toxicity also peaked in late September, at 419 μg. PSPT in both species dropped below 80 μg by mid to late October.

Muir Beach mussel and sand crab toxicity values also became elevated during the same period, but with sand crabs being much higher than mussels during the spring of 1998 (Fig. 3(B)). This difference may have been due to mussel

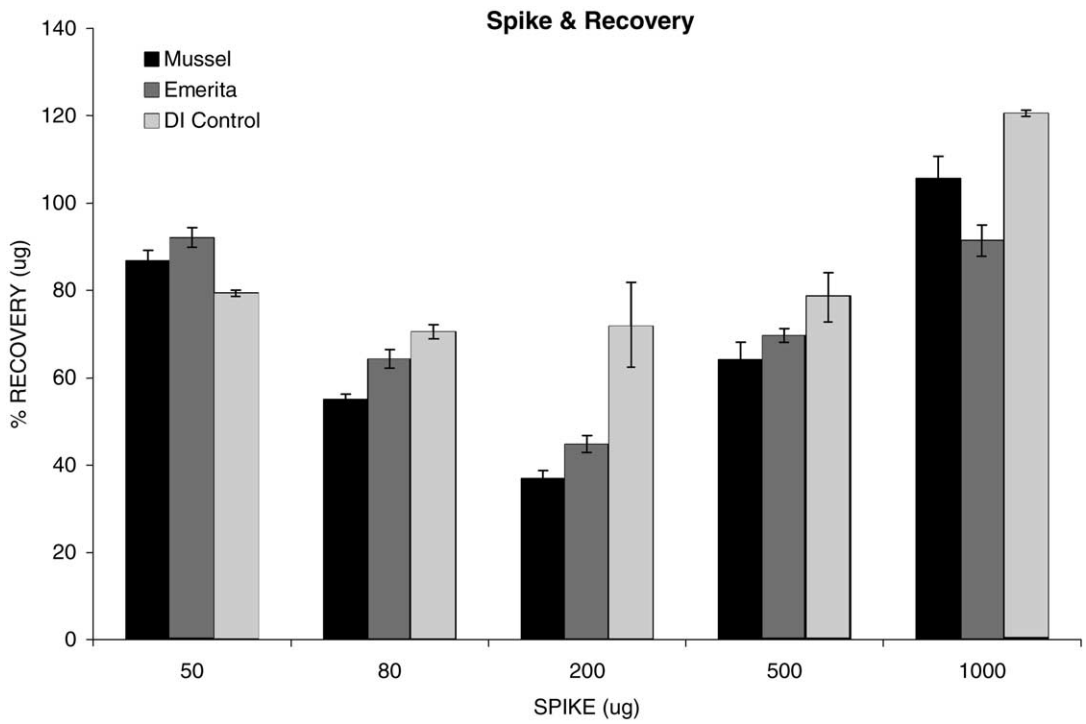

Fig. 2. Spike and recovery experiment with mussel, sand crab, and deionized water (control) samples inoculated at five different toxicity levels (50, 80, 200, 500, and 1000 μg STX). Bar graph represents percent STX recovery (± standard error) for each spike level.

toxicity already having peaked prior to the start of sampling for this project.

Peak PSPT activity for Pescadero Beach in 1998 was 49 μg in sand crab tissues, slightly higher than that detected during the same time interval in mussels (Fig. 3(C)). In 1999, toxicity levels remained near the minimum detection limit until late August, when they began to rise rapidly. The absence of sand crab data in late September was due to the collection of a very small size class (which yielded inadequate tissue volumes). As a result, the toxicity peak for sand crabs during that period may have exceed 468 μg measured the following week. Both mussel and sand crab toxicities declined rapidly after the measured peak falling below 80 μg the following week.

Pebble Beach (Fig. 3(D)) and Pfeiffer State Beach (Fig. 3(E)) exhibited similar levels of non-detectable to low toxicity throughout the study, with the exception of the first sampling date in early 1998. At Pebble Beach, mussel toxicity (83 μg STX equiv./100 g, respectively) exceeded that of sand crabs (55 μg STX equiv./100 g). It is impossible to ascertain whether this discrepancy was due to a time lag in mussel depuration, or reduced uptake on the part of sand crabs.

4. Discussion

Evaluating the role of sand crabs as a bioindicator for PSP toxicity stems from an acute need for a more comprehensive monitoring program for harmful algal blooms and their toxins. HAB distributions are patchy due to environmental conditions such as winds, tides, and currents that can either concentrate, or disperse toxic cells of an algal bloom along coastal habitats. The variable toxicity that results from the influences of oceanographic conditions on HABs makes monitoring for these biological variances challenging. Price and Kizer (1990) stated that "(California) data are insufficient (in determining if) major blooms grow from single locations, develop through coalescence of smaller discrete blooms, (or develop) offshore and move toward land in an already advanced stage, thereby causing rapid increases in shellfish toxicity". Unfortunately, even recent, focused research has failed to definitively answer these basic questions regarding bloom development (Anderson, 1995). Without the ability to forecast HAB induction and maturation, standard monitoring programs remain the best option for protecting the public from toxin exposures due to consumption of contaminated seafood. At present, however, >60% of the California coastline is not being monitored for PSPT due to the restricted distribution of the California sea mussel to rocky shores. Without expanding our existing monitoring programs to include reliable indicator species for sandy beaches, our ability to track the growth and movement of PSPT producing HABs will remain temporally and spatially limited. The addition

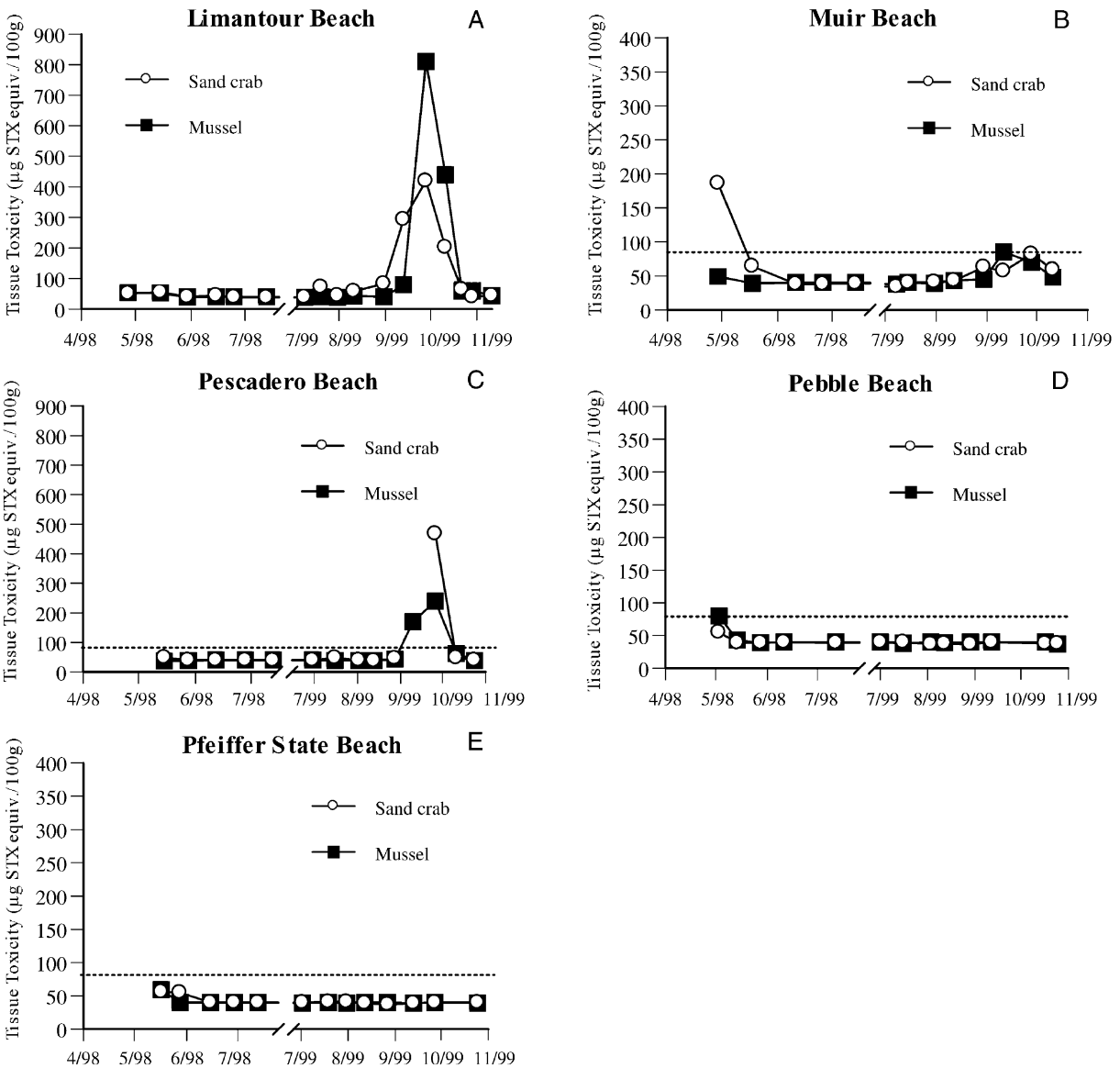

Fig. 3. Time series comparison of PSP toxicity in sand crab (*E. analoga*) and mussel (*M. californianus*) tissue collected at (A) Limantour Beach (Marin County, CA), (B) Muir Beach (Marin County), (C) Pescadero Beach (San Mateo County), (D) Pebble Beach (Monterey County), and (E) Pfeiffer State Beach (Monterey County). The horizontal line at 80 µg denotes the FDA imposed quarantine limit for PSP toxicity in commercially and recreationally harvested shellfish. The limit of sensitivity at $\sim < 38$ µg is basically nondetect. Note difference in scale of *y*-axis. Time series not intended for intersite comparisons.

of sand crabs as bioindicators in coastal monitoring regimes can bridge this critical gap.

Comparisons of natural field populations from our two-year study indicate that sand crabs do indeed uptake and retain PSPT, within their tissues (H_1: sand crabs do retain PSPT), at levels comparable with those found in adjacent mussel beds (H_2: sand crabs retain PSPT at concentrations similar to mussels). These results are consistent with those of Sommer and Meyer (1937) comparing PSPT found in mussels, sand crabs, and Washington clams (*Saxidomus nutallii*) at six different sites along the central coast of California. Their data indicated that PSPT levels of sand crabs generally paralleled those of mussels.

Though difficult to confirm with data near the mouse bioassay limit of detection, increases in PSPT levels for both mussels and sand crabs appeared very similar, and occurred during the same sampling interval (H_3: sand crabs accumulate PSPT at the same rate as mussels). Overall,

there were few examples in this study where the sand crab rate of uptake did not correspond to the rate of uptake in mussels. At Limantour Beach in 1999 (Fig. 3(A)), the overall pattern of sand crab and mussel PSPT accumulation and depuration paralleled one another. And though increasing levels of PSPT were detected in sand crabs several weeks before mussels, both species retained high toxicity for the same length of time. Toxicity levels for both species dropped to below the quarantine limit over the same period (mid-October). Sommer and Meyer (1937) also documented PSPT levels declining at approximately the same rate, but noted that higher levels of PSPT in sand crabs were often still detectable even when mussel samples no longer had an apparent toxin signal.

These field studies, strongly support the general hypothesis that mussels and sand crabs are functionally equivalent in their uptake, retention and loss of saxitoxins when exposed to PSPT producing HABs. Furthermore, these results support the assertion that sand crabs are a highly suitable PSPT monitoring species for use along the Eastern Pacific where rocky headlands are frequently separated by many miles of sandy beaches. However, because field studies aimed at testing the comparability of HAB bioindicator species with different habitat preferences are necessarily confounded to some degree by site differences, unequivocal quantification and testing of these hypotheses will require controlled, laboratory feeding experiments. In the meantime, the total lack of PSPT monitoring along California's sandy shores, and the mutually consistent results presented by Sommer and his colleagues in the 1930s and these authors here, combined with the development of a reliable, easy and cost effective STX extraction protocol for sand crabs, makes a strong argument for the immediate addition of *E. analoga* to California's highly respected PSPT monitoring program.

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