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An evaluation of the health status of the Lavaca Bay, Texas ecosystem using *Crassostrea virginica* as the sentinel species

Wesley Bissett Jr. [a,*], Roger Smith [b], L. Garry Adams [b], Robert Field [a], William Moyer [a], Tim Phillips [c], H. Morgan Scott [c], Terry Wade [d], Stephen Sweet [d], James A. Thompson [a]

[a] Texas A&M University, College of Veterinary Medicine, Department of Large Animal Clinical Sciences, 4475 TAMU, College Station, TX 77843-4475, USA
[b] Texas A&M University, College of Veterinary Medicine, Department of Veterinary Pathobiology, 4467 TAMU, College Station, TX 77843-4467, USA
[c] Texas A&M University, College of Veterinary Medicine, Department of Veterinary Integrative Biosciences, 4458 TAMU, College Station, TX 77843-4458, USA
[d] Texas A&M University, College of Geosciences, Geochemical and Environmental Research Group, 3149 TAMU, College Station, TX 77843-3149, USA

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ABSTRACT

Locational risks for compromised ecosystem health for the eastern oysters (*Crassostrea virginica)* harvested from Lavaca Bay, Texas were estimated. Flow cytometric evaluation of variations in DNA content and the lysosomal destabilization assay were used for evaluation of genotoxicity and stress, respectively. Bayesian geo-statistical methods were utilized to estimate and evaluate spatial effects. For models with spatial risks, continuous surface maps of predicted parameter values were created to evaluate risk location. Lysosomal destabilization assay results were spatially oriented whereas flow cytometry results were fit best with the random effects model. While not spatially oriented, the highest levels of variations in DNA content were also present near industrial facilities. Locational risks of increased biomarkers of genotoxicity and stress in the eastern oyster (*Crassostrea virginica)* were increased with proximity to industrial facilities

1. Introduction

The health of marine organisms is highly dependent on the health of the ecosystem in which they live. The quality of the marine environment has become a concern in the United States with significant contamination resulting from industrial, agricultural, and urban development. The diversity and persistence of pollutants has resulted in contamination of the marine environment with complex mixtures of chemicals (Smolders et al., 2003; Fent, 2003; Brooks et al., 1992; Crocker and Young, 1990). Evaluation of adverse responses associated with exposure to these complex mixtures using traditional dose-response analyses has been problematic because of the complexity of the interactions that occur when biological systems experience complicated exposures (Long et al., 2006; Donnelly et al., 2004).

Lavaca Bay, a secondary bay located along the mid-Texas Gulf Coast, is an example of a marine ecosystem affected by industrial, urban, and agricultural activities. Numerous anthropogenic chemicals have been found in the bay including heavy metals, polycyclic aromatic hydrocarbons (PAHs), and persistent organo-chlorine pollutants (Gill, 2004; O'Connor, 2002; Sager, 2002; TDH, 2000; Hutchinson et al., 1996). Part of the bay has been classified as a Superfund site due to past activity at the Aluminum Company of America's (ALCOA) aluminum smelting facility located along the bay's eastern shoreline (USEPA, 2006). The Superfund program was established in 1980 and allows the United States Environmental Protection Agency (USEPA) to address hazardous waste sites through either government or corporate remediation efforts (USEPA, 2008). The USEPA classified this area as a Superfund site due to elevated levels of mercury as well as persistent elevations in PAH. Elevated mercury levels were the result of the release of mercury-contaminated wastewater originating from a chlor-akali unit formerly in operation at the ALCOA facility (USEPA, 2006). The PAHs were thought to originate from past activity at a Witco Corporation facility formerly located at the ALCOA site. Activities by these two companies resulted in pollution of groundwater zones with mercury and PAHs. One of these polluted zones communicates directly with Lavaca Bay and has resulted in a continuing source of pollutants long after industrial activities have ceased and corrective measures initiated (USEPA, 2006). The bay also receives effluent from an urban wastewater treatment plant and wastewater discharges from a plastics production facility (USEPA, 2004a,b).

Public health authorities, recreational fishermen, and commercial fishermen have expressed concern over the possibility of adverse effects in Lavaca Bay resulting from the discharge and drainage of polluted waters into the bay (Lewis, 2007; Wilson,

* Corresponding author. Tel.: +1 979 845 3541; fax: +1 979 847 8863.
E-mail address: wbissett@cvm.tamu.edu (W. Bissett).

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2006). The Texas Department of State Health Services (TDSHS, 2000) closed a portion of the bay located adjacent to ALCOA for the harvest of seafood for human consumption. This closure was the result of elevated levels of mercury found in various marine species typically harvested for human consumption. The closure currently applies to crab and finfish but does not include oysters and shrimp (Prosperie et al., 1999).

Addressing the concerns about the health of Lavaca Bay has been difficult. Numerous studies have estimated chemical concentrations in Lavaca Bay waters, sediments, and marine species with most of the work focusing on food safety issues arising from elevated mercury and PAH levels in seafood (O'Connor, 2002; Sager, 2002; Evans et al., 2000; Palmer-Locarnini and Presley, 1996; Palmer and Presley, 1993). Evaluation of adverse response in marine species occurring as a result of these chemical exposures has received less attention. The complex mixture of chemicals present in Lavaca Bay has complicated the evaluation of adverse response occurring as a result of proximity to industrial activities (Donnelly et al., 2004; Carr et al., 2001).

2. Objectives

The objectives of this study were two-fold. The first objective was to evaluate the health of Lavaca Bay marine organisms as measured by biomarkers associated with the general health status and genotoxicity using the eastern oyster (*Crassostrea virginica*) as the sentinel organism. The second was to determine the spatial distribution of these biomarkers.

3. Materials and methods

3.1. Sample collection

Oysters were harvested either by a standard oyster dredge or by hand from all identified oyster reefs in Lavaca Bay. Viable oyster reefs were located in the vicinity of the Texas State Highway 35 causeway which separates Lavaca Bay roughly in half and in the southern portions of the bay. Oyster collection locations are provided in Fig. 1.

Viable Reefs were identified as Matagorda Bay – Lavaca Bay (MBLB), Matagorda Bay – Lavaca River (MBLR), Matagorda Bay – South of Causeway (MBSC), Matagorda Bay – Witco (MBWC), Matagorda Bay – Turning Basin (MBTB), Matagorda Bay – Gallinipper Reef (MBGR), Matagorda Bay – Gallinipper Point (MBGP), Matagorda Bay – Harbor Refuge (MBHR), and Lavaca Bay – Port Lavaca (LBPL). MBSB, MBWC, and MBTB were located within the USEPA Superfund site and the Texas Department of State Health Services closure area. MBLB was sampled for the lysosomal destabilization assay however; it was not sampled for genotoxicity analysis. There was a significant amount of time between collection for lysosomal destabilization assay analysis and collection for genotoxicity evaluations due to complications associated with establishment of an effective protocol for maintaining hemocytes for flow cytometric evaluation. The reason for this omission was that multiple attempts to harvest oysters for genotoxicity analysis from this location were unsuccessful. Harvested oysters were placed in watertight plastic bags and kept on ice prior to delivery to the laboratory.

3.2. Lysosomal destabilization assay

After delivery to the laboratory, the lysosomal destabilization assay was performed as per the procedure described by Hwang et al. (2002). A 1 ml syringe containing physiological saline solution with a 25 gauge needle was used to aspirate hemolymph from the oyster's pericardium. Fifty microliters of the saline and hemolymph solution was placed on a microscope slide and incubated for 30 min in a light-proof humidity chamber to allow adhesion of cells to the slide. Excess solution was removed and slides were incubated in neutral red solution for 1 h at room temperature then slides were evaluated using light microscopy. A minimum of 100 cells per sample were counted and the percentage of destabilized cells determined. Destabilization was indicated by a color change of the cytosol resulting from the movement of the neutral red solution from the lysosomes to the cytosol in damaged cells. Destabilization values of 50% or less is considered to be indicative of a healthy status. Values exceeding 50% destabilization are indicative of increasing levels of damage. Two different collections were performed 42 days apart with a minimum of six oysters per location evaluated. All lysosomal destabilization assays were performed within 48 h of collection.

3.3. Genotoxicity

After collection, the oysters were opened and 200–500 μls of hemolymph was obtained via pericardial aspiration with a 25 gauge × 0.3 cm needle on a 1 ml syringe. Sample processing was completed within 48 h of collection of oysters. Fixation and storage of the hemolymph was performed as per the protocol established by Darzynkiewicz and Juan (1997) and the ethanol/hemolymph mixture stored at temperatures between 0° and −40 °C prior to analysis. The ethanol suspended cells were then centrifuged at 200*g* and the ethanol decanted. The cells were rinsed in Physiological Buffered Saline (PBS), centrifuged at 200*g* and the PBS removed. The cells were then re-suspended in 1 ml of Propidium Iodide and Triton X-100 staining solution with RNase. The mixture was placed on ice and protected from light prior to flow cytometric analysis.

A Becton-Dickson FACSCalibur was then set for excitation with blue light and detection of propidium iodide at red wavelengths. Cells were gated on side scatter, forward scatter, and the ratio of peak to integrated fluorescence. Ten-thousand cells meeting all gating parameters were measured per sample and the variation in DNA content reported as the half-peak coefficient of variation.

3.4. Statistical analysis

Oyster reefs were identified by latitude and longitude. These coordinates were used to plot the location using a commercial GIS software program.[1]

The map was then projected into Universal Transverse Mercator 1983 (UTM83), Zone 14 units. The UTM83 coordinates were exported and used for all statistical analyses. The spatial distribution of the oyster DNA full peak-half max coefficients of variation and the percentage of lysosomal destabilization were each modeled using generalized linear kriging expanded to include a nugget, or "random" effect at each location (Diggle and Ribeiro, 2007). The model used a Bayesian method of inference, with vague prior beliefs and Markov Chain Monte Carlo (MCMC) implementation. The MCMC implementation was performed by use of a readily available software package (Spiegelhalter et al., 2003). The prior beliefs included a non-informative normal distribution for the intercept with mean = 0 and precision = 0.0001, and vague gamma priors (Gamma[0.01, 0.01]) for variance components, including the range and nugget (spatially random location effect) and spatial effects (spatially dependent location effect). For all models, the distance-based variance function was exponential with the covariance between location_i and location_j modeled as a function of the

[1] ArcGIS, Version 9.1, Environmental Research Systems research Institute, Redlands, Ca.