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Short communication

Integrating contaminant responses in indicator saltmarsh species

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Abstract

A challenge in environmental management is to provide both methodology and a framework for assessing effects of pollutants in resident species and then applying the findings to management. The Pacific Estuarine Ecosystem Indicator Research (PEEIR) consortium advocates the development of an integrated portfolio of techniques using indicator species selected for various habitat types. We developed such a portfolio for California salt marsh ecosystems and evaluated the feasibility of our approach in management applications. PEEIR is employing a suite of biomarker responses in two indigenous species, the lined shore crab (*Pachygrapsus crassipes*) and the longjaw mudsucker (*Gillichthys mirabilis*). Detrimental effects such as apoptosis, endocrine disruption, and ovarian tumors have been observed in *G. mirabilis* at a site where toxicity test responses were relatively low. With *P. crassipes*, developmental abnormalities and several markers of decreased reproductive performance were quantified at the same site. Multivariate statistical techniques are used to examine the relationships between the responses and multiple contaminant and natural stressors. For the fish, findings are related to population-level parameters using dynamic energy budget (DEB) models.

Keywords: Saltmarsh; Indicators; Biomarkers; Ecological risk assessment; Wetlands; Endocrine disruption

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1. Introduction and approach

Assessment of ecological condition, *diagnosis* of specific stressors, and *forecasting* of potential changes in populations are the most significant applications of ecosystem indicators (Pretti and Cognetti-Varriale, 2001). Integrating responses of exposure to contaminants across different levels of biological organization is key to understanding mechanistic linkages and indicator utility (Clements, 2000). These applications require measurement of both stressor and ecosystem responses; yet, options for integrating such responses remain limited (Strobel et al., 2000; Chevre et al., 2003; Galloway et al., 2004). A key goal of the PEEIR consortium is to develop a suite of indicators of saltmarsh condition using indicator organisms widely distributed in saltmarsh ecosystems of the Pacific coast. We selected the mudsucker, *Gillichthys mirabilis*, as an indicator fish species because it inhabits mud burrows and remains in the same sites (~30 m range) for its juvenile and adult phases. The shore crab, *Pachygrapsus crassipes*, was chosen as the representative invertebrate as it is abundant along the entire West coast, inhabits mud burrows, and can be found in a reproductive state during spring and summer. Additional investigations with plants and microbes that are integrated in the conceptual design of the program are beyond the scope of this discussion.

Our approach involves quantifying molecular, biochemical, and cellular responses in individual organisms collected from stressed and less-stressed wetlands, and along gradients within wetlands, in conjunction with chemical, organismal, and population measures. To accomplish this goal, we have implemented an integrated program of field sampling as well as field and laboratory experiments. The program facilitates synoptic sampling by all investigators at several sites in Northern and Southern California. Field experiments involve outplanting of fish and crabs at specific stations for up to 3 months, followed by analysis of parameters that are directly linked to growth/condition indices at the level of individual organisms. We are also measuring contaminant levels both at the sites and in tissues of organisms. Biomarkers investigated in one or both species include: (1) DNA strand breaks in blood cells, (2) acetylcholinesterase activity, (3) apoptosis in liver and ovarian tissues, (4) cytochrome p450 enzymes, (5) choriogenin proteins in male/non-reproductive fish, and (6) histopathology. In addition, extracts of sediment are shared among analytical chemists and a molecular biology laboratory where screening for endocrine disrupting chemicals is on-going. Multivariate analyses relating biomarker responses to growth and condition impairment are a key part of the integration effort.

For the fish, growth rates and body condition are being compared at contaminated and reference sites (as well as among fish with varying biomarker responses) using body morphometrics and daily growth increments measured in otoliths. Mark and recapture studies are used to verify growth increments. In addition, laboratory validation studies have been completed to pinpoint selected mechanistic relationships between biomarkers and growth (Rose, 2005). We are developing new approaches based on dynamic energy budget models (Fujiwara et al., 2005) to analyze variability in growth and condition among individuals and assess implications for individual survival and population size structure and dynamics. For crab, extensive field surveys have been conducted to characterize relationships between reproductive impairment and demographic parameters such as crab size and population size.

2. Results

The spatial distribution of biomarker responses in *G. mirabilis* at multiple sites have been analyzed individually and in combination. For example, the frequency of apoptosis in liver of fish collected in 2003 was significantly different among sites (Fig. 1), with the most contaminated site (Stege Marsh) exhibiting the highest incidence. Hence, this technique may be a useful "early warning" indicator of contaminant stress. However, greater scope of inference can be attained when multiple biomarkers are considered and related to fish condition. When principal components analysis (PCA) is used to evaluate interrelationships among multiple datasets, the first three components explain 41%, 15%, and 10% of the variation among morphometric and biomarker measurements on the fish. Examination of the component loadings indicates that principal component 1 accounts primarily for size variation among fish, whereas components 2 and 3 reflect variation in biomarker responses (Fig. 2). Thus, apoptosis can be related to other biomarker responses and measurements linked to fish growth and condition. Ultimately, proportions of fish in different health categories can be discerned and associated with multiple contaminant stressors. These findings and additional data are now being used to parameterize population models.

For *P. crassipes*, several measures of reproductive performance were related to contaminant exposure at the sites, but biomarker responses were less informative. Specifically,

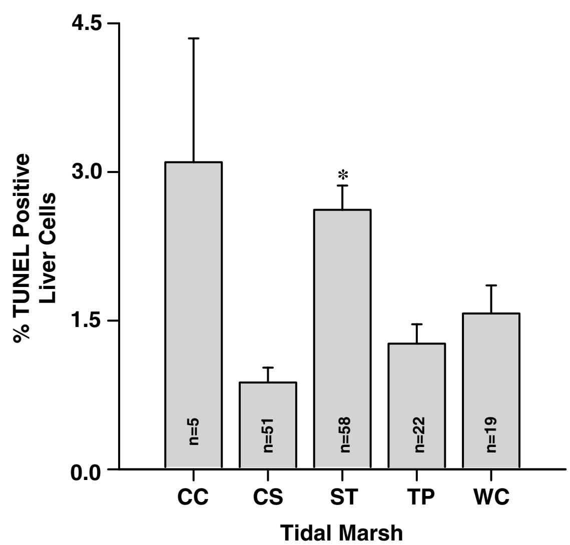

Fig. 1. Mean percent of TUNEL positive cells (apoptosis) ±1 SE in liver cells of *Gillichthys mirabilis* collected from California tidal marshes including China Camp (CC), Carpinteria Salt Marsh (CS), Stege Marsh (ST), Tom's Point (TP), and Walker Creek (WC) in August of 2003. Liver sections were examined for TUNEL positive cells using modifications of Gavrieli et al. (1992) and a commercial kit, the Dead-End Fluorometric TUNEL System (Promega). At least 300 liver cells, excluding red blood cells, were analyzed using at least five images of each fish liver to determine the percent of TUNEL positive cells. One-way ANOVA and SNK's multiple comparisons were used to examine differences in TUNEL positive cells among fish from different marshes. Sample size is shown within each bar, and the asterisk indicates significant differences relative to fish from the reference site, Tom's Point (TP) at $P < 0.05$.

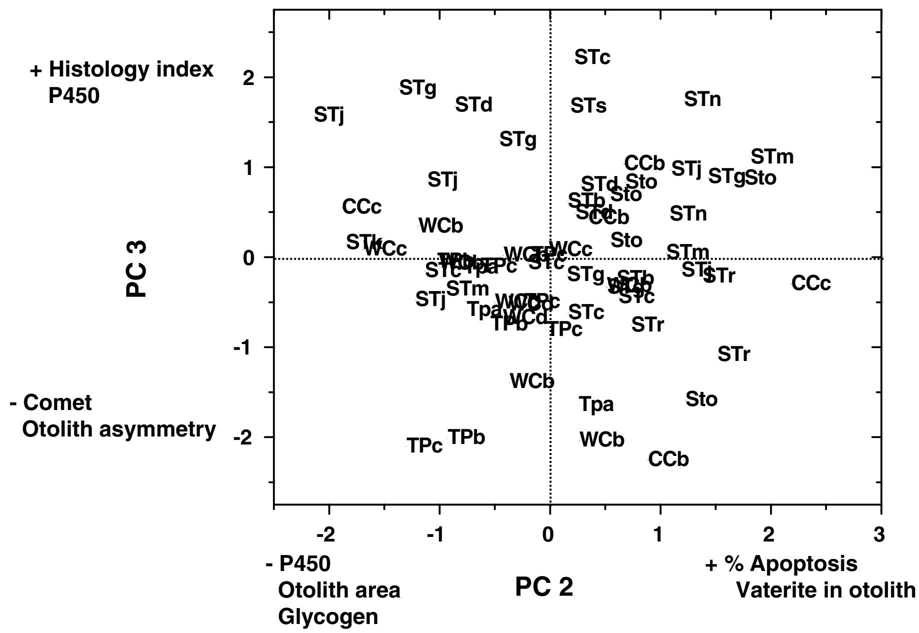

Fig. 2. Principle components analysis (PCA) of fish morphometrics and biomarkers. Factor scores from components 2 and 3 reflect relative condition of individual fish with upper case labels representing collection sites and lower case the sampling stations. Overall, fish condition is relatively distinct between sites in San Francisco Bay and Tomales Bay in Northern California. Component 2 shows a trend from low (negative scores) to high (positive) % cells apoptosis, and abnormal calcium development of otoliths (vaterite score), and high to low P450, otolith area, and glycogen score. Thus, fish from San Francisco Bay sites, Stege Marsh (ST) and China Camp (CC), tend to be smaller, have adequate glycogen, low P450, but show high apoptosis, and vaterite in comparison to fish from Tom's Point (TP) and Walker Creek (WC) in Tomales Bay. Component 3 also shows that fish from Stege Marsh have relatively poor liver condition, high P450, Stege fish also show a trend from the upper left to lower right in how they score for P450 (high to low) suggesting that fish from various locations may have been affected by different types of contamination.

fecundity, clutch size, and embryo abnormalities all were associated with contaminant exposure; yet, initial analyses of DNA strand breaks, metallothioneins and P450 enzyme levels revealed no significant variation among sites or in association with specific contaminants (submitted manuscript, data not shown). Further analyses are underway.

3. Conclusions

Organism-level indicators are the fundamental metrics needed to alert us to potential detrimental effects in a subpopulation at any given site. Yet, without supplemental information they cannot inform about the potential causes of stress (Carignan and Villard, 2002) or population-level consequences. For example, if an alteration in growth or reproduction is observed, managers have little information to determine why that alteration may have occurred or what its significance might be. We believe that if early warning, mechanistically-based indicators can be used to detect the stressors that managers are most concerned about, then improvements in the protection of aquatic life within a given wetland can be achieved (Cherr, 2002; Van Dam et al., 1998; Bennett et al., 1995). The indicators described here are part of an extensive set of integrated mechanistic indicators in marsh plants and animals at multiple spatial scales and levels of biological organization. These indicators are intended for various types of applications in marsh restoration, sediment quality protection, and management of specific contaminant inputs and threatened populations.

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