

ANNUAL REPORT

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Title: Pacific Estuarine Ecosystem Indicator Research (PEEIR) Consortium

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Research Category: EaGLE Program

Project Period: March 1, 2001 to February 28, 2005

Objective of the Research Project: The objective of our project is to develop indicators of wetland ecosystem health through evaluations of stressor-response patterns in wetland biota. We are creating an integrated suite of indicators to evaluate impacts of four types of stressors that are known to impair California wetlands. These include: toxic contaminants, nutrient enrichment, microbial contamination, and exotic species invasions. The PEEIR program is highly integrated with over 30 investigators working across levels of biological organization, trophic structure, life stage, time, biogeographic and disciplinary boundaries. Indicators will be expressed as simple data aggregations, as well as either more complex models or statistical expressions of overall ecosystem health.

Accomplishments:

Preliminary Sampling and Sampling Design

A major accomplishment of the first year is successful completion of preliminary sampling at all Northern and Southern California sites, as well as the refinement of our sampling design. **This year, study sites were located at seven sites in northern and southern California, including Walker Creek and Toms Point in Tomales Bay, Stege Marsh and China Camp in San Francisco Bay, Morro Bay, Carpinteria Marsh, and Mugu Lagoon. The sites span biogeographic boundaries, and the estuaries vary morphologically, providing a good test of the reliability of the indicators to assess wetland integrity across diverse environments. We began: 1) censusing the full spectrum of wetland communities, including microbes, plants, invertebrates, fishes, birds and parasites, and 2) characterizing sites for nutrient and toxic contaminants.**

The development of indicators critically depends on: 1) the initial establishment of an overarching sampling design that fully integrates the research of each of the five components of the project, 2) the vertical integration of investigations into the effects of contaminants on the wetland ecosystems, beginning with their bioavailability and working up the levels of biological organization from the subcellular to the landscape level, and 3) the development of sophisticated statistical approaches and new models that integrate the

diverse array of information that will be obtained during this multifaceted, four-year project. Because ecosystems subsume lower levels of biological organization, the Ecosystem Indicator Component (Morgan, Brooks, Bennett and Grosholz) has taken the lead, together with the Integration Component (Anderson, Nisbet, Bennett, Stewart-Oaten), to ensure that these three essential criteria are met. We made sure that representatives from all of the research groups participated in field sampling, and we subsequently spent a great deal of time discussing the best way to fully integrate our project based on our initial results and experience. This effort has led to the recommendation of a gradient-type design for the first two years of the PEEIR investigations. This approach will permit the further development and validation of more experimental methodologies.

During the second two years of the PEEIR investigations, we will expand our studies to examine more numerous sites in California, as well as a greater number of stations within existing sites. This approach will allow us to develop indicators using descriptive statistics and to elucidate patterns on a larger geographic scale. Probabilistic sampling approaches can be incorporated in this phase, helping to relate our techniques to EMAP-style monitoring. Various options for scaling at this level are being considered and will be debated in working groups with other EaGLE centers and with Dr. Tony Olsen of USEPA Corvallis.

Gradients or “near gradients” have been validated at three sites, and other sites are being characterized during the spring and summer. Dr. Page has documented varying levels of dissolved inorganic nitrogen (DIN) in a gradient-type pattern at Morro Bay and in Carpinteria Salt Marsh. Varying levels of heavy metals contamination have been documented using ICP-MS on sediment and plants at Stege Marsh (Green, Higashi, Fan, Hollibaugh), and pore water toxicity tests are now being conducted at this site (Cherr, Vines, and Anderson) (see below).

Numerous mechanisms exist to enhance integration among groups, and selected examples of our most highly integrated achievements are described below. A summary of responses being assessed in the PEEIR center is presented in Figure 1.

Linking Toxicant Response to Reproduction and Population Viability

Development of biomarkers to reveal biological responses to contaminant exposure is a major focus of the PEEIR program. A goal is to develop a suite of cellular and molecular responses that can be related to reproductive performance and population viability in fish and invertebrates using highly integrated field sampling, laboratory exposures, and modeling activities (Figure 2). Responses are studied in serum and liver as well as in sectioned material. *Although the techniques are complicated, we believe some can be automated in the future for high-output monitoring and that such new immunological and biochemical screening tools are the “wave of the future” in pollution monitoring.*

For example, the incidence of apoptosis, or programmed cell death, in fish cells may have utility as a biomarker, and we propose that effects may be related to reproduction of fishes. Cell death in liver and gonad can directly impair reproductive output. Initially, we will utilize a well developed technique that measures DNA cleavage (the TUNEL assay) on field and laboratory samples. However, graduate student Wendy Rose is working with Drs. Cherr and Anderson to examine a second technique that screens for caspase activity in primary cultures of topsmelt liver cells. Caspase (cysteine aspartate protease) activity is an early sign of apoptosis and is specific to the apoptotic cellular pathway. This particular pathway has broader potential as an early-warning technique, because caspase 3 activity is a crucial bottleneck in the apoptotic cascade. Using a fluorogenic peptide substrate (Asp-Glu-Val-Asp-AMC) specific for caspase 3 activity (Promega), we examined caspase activity in topsmelt hepatocytes exposed to cadmium (0, 10 and 100 ppb concentrations) for 12 hours. We detected significantly higher caspase 3 activity levels with increasing cadmium dose in topsmelt liver cell extracts. In addition to the TUNEL assay, we will use the caspase technique in laboratory studies and on selected field samples of mudsucker and topsmelt. The caspase technique is amenable to automation, once basic kinetics of response are better described.

As Figure 2 indicates, other immunologic assays of interest will be applied to the field collected samples. For example, postdoctoral researcher Dr. Carol Vines will employ choriogenin (egg shell protein precursors) antibodies as a routine tool to detect endocrine disruption in male fish. This approach is more broadly applicable than the more commonly used vitellogenin assay in fish, because the choriogenins are more highly conserved, and the peptide antibody can be used on a very broad range of fish species. All biomarkers will be related to development, growth, and fertility of aquatic species through synoptic field sampling and laboratory validation studies. For example, growth in model fish species (topsmelt, *Atherinops affinis*, and mudsucker, *Gillichthys mirabilis*) will be assessed using otolith analyses (Bennett and Brooks).

Biomarker responses such as apoptosis will be directly linked to reproductive outcome and population viability. Laboratory studies have been initiated (Anderson, Cherr, Nisbet, Muller, and graduate student Wendy Rose) to relate biomarker responses and growth alterations to reproductive outcome and population viability in a variety of models. For example, Dynamic Energy Budget Models (DEB) describe growth, development, and reproduction in single organisms in response to a specified food environment. Toxicant effects are represented through impacts on assimilation rates and efficiencies, and on respiration rates. Much of the first year effort has focused on testing the models against existing literature data. Following laboratory validation, field data can be entered into the model and population-level impacts projected.

An additional rapid screening tool is the CALUX bioassay for endocrine disruption, which has been developed in Dr. Denison's laboratory. This assay uses cells transfected with

constructs of dioxin-response element and fluorescent reporter genes. Studies this year have demonstrated an excellent relationship between TEQs of dioxin-like compounds and CALUX bioassay values. These and other data demonstrate the utility of this bioassay system for the detection and relative quantitation of dioxin-like compounds and related toxic/bioactive halogenated aromatic hydrocarbons (HAHs) in multiple media and tissue samples. The CALUX bioassay system provides a relatively rapid and more cost-effective approach for the detection and relative quantitation of TCDD-like HAHs in sample extracts; this bioassay can also be used to detect the presence of toxicants that elicit effects via the aryl hydrocarbon receptor pathway. Site sediments and pore waters are now being prepared for the CALUX system, and the final development of this assay for estuarine systems will be an important contribution to the PEEIR program. We envision that this assay could be used by other EaGLE centers that wish to screen for endocrine disrupting compounds at selected sites, and we have initiated discussions with the Great Lakes Ecosystem Indicator (GLEI) project on the use of this method, as well as the choriogenin antibody approach.

Other specific developments in the area of biomarker research include: 1) development of antibodies to specific peptide sequences within the cytochrome P450 enzymes that cross-react with fish and crustaceans-- these are potentially more powerful than currently available antibodies against purified protein due to their broad reactivity across phyla, as the domain used as the specific target is highly conserved (Snyder), 2) refinement of genotoxicity (COMET assay) techniques for topsmelt blood and liver (Anderson), and 3) initiation of studies to elucidate acetylcholinesterase enzyme inhibition in developing topsmelt embryos as an indicator of agricultural chemical effects in early lifestages (Wilson, graduate student Will Fry).

Relating Wetland Plant Health to Bioavailability of Toxicants at Multiple Scales

An exciting aspect of the PEEIR project is the effort to integrate measurements of toxicant bioavailability in plants with changes in wetland plant health using assessments from the molecular to the landscape scales. Investigators (Green, Fan, and Higashi) have determined that leaf salt exudates of native cordgrass and saltgrass harbor significantly elevated metals, including Cd and Pb, that are not normally translocated into shoots. This finding has triggered two plant collection campaigns at Stege Marsh and China Camp with the first samples undergoing broad metals scans by ICP-MS and phytochelator analysis.

Methods to evaluate health of the wetland plants are being developed and validated (Williams). For example, pulse amplified modified (PAM) fluorescence is being used to quantify stress in cordgrass (*Spartina foliosa*) and pickleweed (*Salicornia virginica*). PAM fluorimetry measurements are calibrated in the laboratory against measures of CO₂ uptake in these plants using toxicant spiking experiments. These studies are also designed to evaluate phytochelatin status (Fan, Higashi) and spectroradiometry (Ustin) as tools to further assess plant stress. Potential changes elucidated using spectroradiometry can later be discerned at the landscape scale using remote sensing.

The remote sensing component (Ustin) will also provide maps of the spatial extent and fragmentation of each marsh and will determine spatial patterns in the distribution and abundance of dominant species. These data will be related to field measurements of species composition, biomass, and leaf area index, extrapolating their spatial context and providing a basis for correlating the spatial distribution of bioindicators. The acquisition of high spatial resolution airborne hyperspectral imagery (AVIRIS) over northern and southern project sites in late summer 2002, will permit quantitative mapping of canopy pigments, water content, and dry standing litter. These measurements are expected to vary with site condition.

Products of this integrated effort could be useful in many applications in environmental monitoring and management. For example, plant exudates can be used to discern bioavailable contaminants in wetland plants, rather than simply revealing the amount present in sediments. Immediate linkage of these data to the health status of plants would be valuable in managing site cleanup, restoration activities, and assessing habitat quality. Tiered “aquatic life uses” of specific habitats can result from integrated measurement of habitat quality and the health of resident biota. The addition of the remote sensing techniques provides opportunity to cover much broader spatial scales and to relate findings to other forms of habitat destruction.

Other important advancements in the area of bioavailability include: 1) isolation and characterization of a class of bacteria that may contribute substantially to mercury methylation (Nelson)-- these are iron reducing bacteria, rather than sulfate reducers more commonly studied at mercury contaminated sites, and 2) initial development of a fluorine NMR technique to monitor for a class of pharmaceutical compounds, the fluoroquinolones (Scow and graduate student Ana Cordova).

Ecosystem Responses

Recent PEEIR data have explored the relationship between DIN (Page) and microbial community diversity (Holden, postdoctoral researcher Michael LaMontagne, and graduate student Yiping Cao) using a variety of techniques. Although variability within sites can be high, and is being cautiously characterized, we have obtained data indicating a negative relationship between microbial diversity and DIN at Mugu Lagoon using T-RFLP analyses which are based on analysis of PCR-amplified 16S rDNA using universal eubacterial primers. Using the same technique to assess microbial diversity, a negative relationship with DIN is not observed at either Morro Bay or Carpinteria Salt Marsh, suggesting the value of large scale field studies that can be conducted in the scope of the PEEIR Consortium. Measures of species richness from the T-RFLP method are highly transferable among sites and ecosystems, making them potentially valuable indicators; however, factors driving community composition need further study. Microbial community composition and diversity measurements (Holden) were expanded in the project recently to characterize these parameters along the contamination gradient at Stege Marsh in conjunction with plant exudate measurements and other biogeochemical indicators

(Higashi, Fan, Green).

Additional specific achievements in ecosystem responses include: 1) analysis of trematode parasites as an indicator of bird population diversity at numerous sites, with an important new NSF grant awarded to this group augmenting the geographic range of our investigations (Lafferty, Huspeni, and graduate student Ryan Hechinger), 2) assessment of nitrogen isotope ratios in selected biota at southern California sites to assess ecosystem responses to nutrient enrichment (Page), and 3) refinement of methods for trophic support and biogeochemical performance indicators such as ammonification, decomposition, and nitrification rates (Williams).

Data Integration and Management

It is increasingly recognized that single indices are not adequate measures of ecosystem health. Multimetric or integrative indices are composed of multiple key attributes and associated metrics that are shown empirically to change in value along a gradient of human disturbance. The choice of metrics can be strengthened by combining field research with the analysis of archival data sources. During the past year, through the The Bay Institute subcontract (Pawley), and with assistance from the San Francisco Estuary Institute and Ed Smith and Associates (Collins and Smith), we have developed a plan for the website and database system that will facilitate the development of integrative indicators of wetland ecosystem health. The database hub will be accessible via the website and house key PEEIR data and archival datasets that support the development of integrative indices -- indices (e.g. IBI and cross taxa indices) that require the association of data across disciplines and datatypes. This line of research has led to four main activities which are ongoing: 1) development of bibliographies of wetland indicator research applicable to the west coast, 2) documentation of archival data sets from our wetland sites, 3) design of a website which is both important as a conduit to the public and interested parties but also will serve as an important means for PEEIR scientists to communicate their work to their peers and 4) the development of a plan for a more detailed database design to house both metadata and key datasets from both PEEIR research activities and selected archival data.

Outreach, Agency Integration, and Pan-EaGLes Collaboration

Collaboration with EPA Region IX was initiated during the first year of this grant. EPA Region IX Science Coordinator, Dr. Bobbye Smith has worked closely with Dr. Anderson to define the most fruitful avenues for future interaction. Meetings and conference calls during this year have focused on developing partnerships and contacts that will help the PEEIR consortium accomplish

specific objectives more cost-effectively or with improved perspective on regulatory needs. In the coming year we look forward to more detailed technical discussions regarding the development and application of specific indicators and potential regulatory applications.

Discussions with EMAP investigators and their contractors (Drs. Steven Weisberg and Martha Sutula) involved in the Southern California Wetland Recovery Project (SCWRP) have resulted in a collaboration to study plant exudates. During the course of EMAP sampling along the west coast this summer, Dr. Peter Green of UC Davis will be funded by the PEEIR Consortium to sample plant exudates at a subset of wetland sites in California.

Dr. Josh Collins of SFEI has spearheaded efforts to integrate PEEIR consortium research with USEPA 104 grant research on wetlands in San Francisco Bay. He will work with Dr. Anderson to further these objectives in the coming year.

Collaboration among EaGLe centers is an important goal for the program overall. The Bodega Marine Laboratory sponsored visits by Dr. Hans Paerl (Atlantic Coast Ecosystem Indicator Consortium) and Drs. Gerry Niemi and Deb Swackhamer (Great Lakes Ecosystem Indicator Consortium) to discuss cross-center collaboration. Dr. Paerl and BML investigators outlined opportunities for graduate student exchanges in topics such as microbial ecology and remote sensing. Drs. Niemi and Swackhamer recognized an immediate benefit in applying the choriogenin antibody and CALUX assay techniques to some of their recently acquired samples. This will benefit the PEEIR Consortium because it will provide an opportunity to assess the applicability of the techniques on a broader geographic scale.

Publications

None expected in the first year.

Presentations

Anderson, "A Western Center for Estuarine Ecosystem Research", EMAP Partnerships in Coastal Monitoring, Pensacola Florida, April 2001.

Anderson and Page, presentation of PEEIR program at Southern California Wetland Recovery Project (SCWRP) Meeting attended by EMAP representative Walt Nelson, USEPA Corvallis, October 2001.

Anderson and Higashi, presentation of PEEIR program at EPA Region IX, November 2001.

Holden, Microbial Source Tracking, Bren School Water Quality Forum (UCSB), November, 2001.

Anderson, presentations of PEEIR program objectives to Gulf of the Farallones National Marine Sanctuary, Sea Grant Tomales Bay Watershed Coordinator, Point Reyes National Seashore, and San Francisco Bay Wetland Research and Monitoring Program (several dates in 2001/2002).

Holden. USEPA sponsored workshop in Microbial Source Tracking (Irvine, CA), February

2002.