

## YEAR 2 ANNUAL REPORT

**Period Covered by the Report:** March 1, 2002 to February 28, 2003

**Date of Report:** April 1, 2003

**EPA Agreement Number:** R-82867601

**Title:** Pacific Estuarine Ecosystem Indicator Research (PEEIR) Consortium:  
Administration & Integration Component

**Principal Investigator:** Susan Anderson<sup>1</sup>

**Co-Investigators:** Steven Morgan<sup>1</sup>, Gary Cherr<sup>1</sup>, Roger Nisbet<sup>3</sup>

**Institutions:** UC Davis Bodega Marine Laboratory<sup>1</sup>, UC Davis<sup>2</sup>, UC Santa Barbara<sup>3</sup>

**Collaborators:** Josh Collins, San Francisco Estuary Institute; Anitra Pawley, The Bay Institute;  
Ed Smith, Smith & Associates

**Research Category:** EaGLE Program

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

**Research Objectives:** The overarching goal of this project is to develop a suite of ecological indicators to rapidly assess the integrity and sustainability of wetlands in West Coast estuaries. We propose to develop an integrated suite of indicators to evaluate impacts of stressors across levels of biological organization, trophic structure, life stage, time and space.

### PEEIR OVERVIEW

The PEEIR Consortium is an integrated project to develop indicators of wetland integrity for the west coast. Our key goals are to develop synthetic indicators of contaminant and nutrient stress in salt marsh systems using: 1) fish and invertebrate model species at multiple levels of biological organization, and 2) wetland plants at multiple spatial scales. All PEEIR investigators participate in our common sampling design, which includes carefully selected stations in both northern and southern California. Furthermore, we conduct integrated field and laboratory experiments to characterize and validate indicators. The PEEIR integration team provides overall guidance and support. Our first year was devoted to method development and preliminary sampling. Accomplishments in our second year have included completion of the first full year of integrated sampling, organization of integrated experiments, analysis of key datasets, and further method development.

Progress in each of the project subcomponents is described in this compilation of four reports. The PEEIR integrated sampling effort is described in most detail in the Ecosystem Indicator Component (EIC) Report. Overall, initial data from our comprehensive sampling effort are encouraging. For example, a continuum of plant stress indicators that link biomass changes observed by remote sensing, ecological responses in wetland plants, and bioavailable contaminant fractions are being critically examined. In addition, markers of effect in fish and invertebrates have elicited statistically significant responses at the more contaminated sites, and the importance of these effects at the population level is being analyzed. The opportunity to work at multiple levels of biological organization and at multiple spatial scales across a broad geographic region is unique, and the PEEIR program fosters novel dialogue across disciplines.

### MODELLING AND INTEGRATION

An important aspect of PEEIR is the development of a theoretical component that may provide novel approaches to the integration of the numerous measurements we are undertaking. Investigators in various project components are involved in statistical integration activities. In particular, Dr. Stewart-Oaten and Dr. Bennett have explored a selection of multivariate procedures. However, integration efforts also include conceptual modeling and a critique of the use of indices of biotic integrity (IBI). The modeling research has focused on two themes: fish growth in variable environments, and the dynamics of stable isotopes of nitrogen in consumer species. This effort is being led by Dr. Roger Nisbet on the UCSB campus.

### **Conceptual Model**

As a part of the integration effort, Dr. Stewart-Oaten has been reviewing the contribution of individual investigations within PEEIR as potential components of an idealized wetland monitoring strategy that consists of three main steps: 1) initial survey, 2) confirmation of impairment, and 3) evaluation. Measures used in each stage differ in their specificity and costs. In the initial survey, measures should be very general over many potential problems and inexpensive, but they may produce false alarms. In the confirmation stage, measures should be more specific to types of problems but may be more expensive than in the initial surveys. Measures used in the final step can have different levels of specificity and cost depending on objectives of management actions. The objectives may be to meet the minimum conditions for some designated use of wetlands or to maintain hypothetical wetland “health” or “integrity”, if such condition can be defined.

This strategy identifies the chronological stages in the assessment of wetlands, and will help to translate our scientific efforts into actual management tools by placing each component of our project in the strategy. Furthermore, this strategy will also become a tool to integrate our work with efforts that exist outside of our project.

### **Evaluation of Index of Biotic Integrity (IBI)**

Multimetric indices such as IBI are becoming increasingly popular as a measure of indicator of ecosystem “integrity.” For example, the Ohio EPA uses the IBI as one of routine measurements for monitoring of rivers. During the past year we examined the feasibility of applying the IBI approach to PEEIR data on coastal wetlands.

In the IBI approach it is crucial that reference conditions of a system (i.e. conditions expected in a natural habitat without human disturbances) are identified in terms of abundance, species composition, and trophic structures of some macrofauna. However, all the coastal wetlands in California are disturbed to some degree by human activities. In addition, there is high natural temporal and spatial variability among and within the wetlands. These make identification of the reference conditions almost impossible. As a result, our current conclusion is that the IBI approach is not appropriate for our coastal wetland studies.

In addition to the specific difficulty applying the method to the wetlands, the IBI appears to have general problems. We are currently evaluating the literature that criticizes, as well as supports,

the IBI method so that these ideas are reflected in alternative approaches that we are currently developing.

### **Dynamic models of organismal growth in changing environments**

Field and laboratory studies within the EI and BRC components of PEEIR are providing data on the growth of fish and invertebrates in potentially stressed environments. Interpretation of the data and integration of findings from individual studies requires mechanistic models that relate variability in growth patterns to changes in the environment. For this purpose, we are studying the predictions of dynamic energy budget (DEB) models that describe growth, development and reproduction in arbitrary environments. Although there is a large body of theory for deterministic DEB models, our work requires stochastic models, which have been developed *ab initio* during Year 2. The models make predictions of changes in observed size distributions in a population, and will be used to estimate variability in observed growth rates from studies on individual organisms and from size distributions in a population.

### **Dynamics of stable isotope ratios**

Our decision to give priority to modeling the dynamics of nitrogen isotopes derived from initial findings of the EI component that show large within-year variation. We are using dynamic energy budget (DEB) theory to model the dynamics of the two nitrogen isotopes in individual organisms. Our initial aim is to construct models that reproduce the typical N-15 enrichment levels that are commonly observed across many taxa and ecosystems. Some short-term experiments on excretion rates are planned for summer or fall 2003 that will help with model parameterization. The models will predict the changes of the isotope ratio within organisms in response to changing food density and isotopic composition, and will then be used to interpret the large body of data that is being gathered by PEEIR and other investigators. Since nitrogen enrichment is such a ubiquitous issue, our models should be useful in many kinds of wetland systems both within and beyond California.

### **Future Activities**

Research on all the above themes will continue in Year 3. Late in that year, we anticipate construction of a model of nitrogen fluxes within a wetland. Models will be used to interpret results from field experiments planned for summer 2003. Plans for further statistical integration are evolving, and manuscripts for 2004 are under discussion.

## **ARCHIVAL DATA AND RELATED INTEGRATION**

To provide the most cost-effective development of wetland indicators, PEEIR is also investigating potential uses of archival data to characterize ecological condition in wetlands along the west coast. Dr. Anitra Pawley of The Bay Institute researched available resources to locate datasets associated with invertebrate density, community composition, and associated environmental contamination in and around PEEIR study sites. This involved queries of extant Federal and State databases, searching the primary and gray literature from the past two decades, and contacting researchers with specific connections to ongoing bio-monitoring programs (e.g., USGS, USFWS, CDFG, USEPA, local mosquito abatement districts, among others). While some data are available, in many cases they are not directly available for PEEIR study sites, are

of short duration, and/or measurements were not adequate for our specific needs. In some cases, limited data exist but are less readily available.

As a consequence, we are currently focusing on a more general investigation of stressors and effects upon the benthic invertebrate community of estuarine systems. In this way, we can look at more general responses to contaminant effects and provide a context for the west coast data that has been located. By compiling and documenting the available information using a wider set of data representing a larger area, the database is more robust and the analysis more powerful. Indeed, it is critical because of the data limitations for west coast sites.

The product generated from our current work plan will contain a qualitative component that incorporates a brief discussion of extant methodology for detecting stressor effects in the zoobenthic community, and also summarizes results from the literature to date. We envision that a quantitative meta-analysis will be completed to determine whether effects measured in the field are generally detectable, and the range of variables most sensitive to contaminant effects.

In addition to the effort described above, a summary of available datasets for San Francisco Bay has been completed by Dr. Pawley, and a similar report for Tomales Bay has been prepared by Dr. Ed Smith. In general, we have found the available data to be quite variable in nature and not adequate to provide any comprehensive or integrated view of salt marsh condition.

## **NASA-FUNDED REMOTE SENSING RESEARCH**

The NASA-funded remote sensing project (Dr. Susan Ustin, PI) is an integral component of the PEEIR project; and hence, we provide a brief update here in our report to EPA. Dr. Ustin and colleagues are active in our synthetic efforts to characterize stress responses in wetland plant communities. In addition, their work is vital to all site characterization and GIS activities. Key accomplishments for this year include:

- Acquired field spectra in all marsh sites except Mugu Marsh. Field data were acquired at transects where vegetation measurements were acquired but timed to occur at the time of the AVIRIS overflights.
- Requested and approved for AVIRIS overflights (Twin Otter) for all sites in September 2002. Coordinated selection of flightlines. Part of the Marin County flightlines were cloudy, making that data of lesser quality than at the other sites. Airspace permission over Mugu Marsh was not acquired by NASA during their flight schedule so it was not acquired. NASA approved additional funding to acquire data over these sites in May 2003.
- Completed a hydroponic greenhouse study of contaminant stress on the dominant salt marsh species (*Salicornia virginica*, *Spartina foliosa*, *Spartina alternifolia*, *Spartina* hybrid, *Scirpus robustus*, *Typha* ssp.). Plants were grown under cadmium, vanadium, heavy and light crude petroleum to investigate their response: leaf and canopy reflectance, photosynthesis, leaf fluorescence, chlorophyll, water and dry matter contents, and metal ion concentration.
- Calibrated the AVIRIS data to apparent surface reflectance. Ongoing GIS database

development to register the data to spatial coordinates and provide a basis for interpretation.

- Assumed responsibility for supporting the PEEIR GIS database. Provide training and space for PEEIR data manager, Webb Sprague.
- Presented a talk at the ASLO meetings describing the results of the cadmium study and preliminary results from the AVIRIS data over Stege Marsh (site of high soil cadmium concentrations) and China Camp. These results are consistent with lower live growth and accumulation of more dry plant litter at the Stege site.
- Coordinating LIDAR data acquisition and flightlines for the project with the National Park Service and the data provider.

Future activities include selection of new sampling locations for 2003 using AVIRIS data for two of the sampling sites. This will allow us to determine whether remote sensing can be used to provide cost-effective identification of sites with significant impairment of plant biomass. This will be an important step in our goal to develop indicators of stress in wetland plants at multiple spatial scales.

## **GEOMORPHOLOGY PROGRAM**

Each of the three PEEIR study sites consists of a number of biological and physio-chemical sampling stations arrayed within a single tidal drainage network. Some of the component channels of each network de-water at low tide, and others do not. Most of the sample stations are located within channels or on the adjacent marsh plain within 1-2 meters of channel banks. The hydro-geomorphic studies will empirically determine the high tide datums, and the frequency and duration of tidal inundation, for the chemical and biological sampling stations of the PEEIR. Equipment has been purchased and the installation sites have been selected. Data collection will begin in June 2003.

The hydro-geomorphic studies, in collaboration with the remote sensing group of the PEEIR, will also be used to ground-truth the LIDAR imaging, rectify the imaging to the local high tide datums, and develop predictive maps of the frequency and duration of tidal inundation and spatial patterns of sediment deposition for each site. The accuracy of the maps can be tested based on field observations of the distribution and residence time of surface water from over-bank tides. If the maps are accurate, then the distribution of conditions similar to the biological and phyio-chemical sample stations can be inferred across the extent of the imaging. If the imaging is vertically rectified to the local high tide datums, then the correlation between surface hydrology and spatial variations in plant community structure could be tested, and the vertically rectified LIDAR imaging might be used to test simple indices of channel density and topographic complexity that could be used in routine coastal wetland monitoring programs.

## **DATA MANAGEMENT ACTIVITIES**

The PEEIR data management component will serve all project investigators by providing centralized access of data for integrated statistical and modeling efforts needed to derive wetland indicators. Our plan for 2003 included the following: formulating a tested database, entering a

significant portion of 2002 data, providing working prototypes of data entry programs, and completing the specification of metadata formats.

All of the above projects are still in process. We are currently finalizing the conceptual design for the database based on samples of data submitted from the PIs. We will start entering 2002 data as soon as the database design is complete. Since we are such a heterogeneous group of researchers, with correspondingly heterogeneous data, we will use an informal, email and spreadsheet based data communication procedure, and not implement online data entry programs. We are still working on metadata specifications, pending directives from EPA. Perhaps most importantly, the data manager, Webb Sprague, is now familiar with much of the data and has developed working relationships with the researchers; this will be important as more data becomes available. The next step is to finish collecting data for 2002, to build a prototype application for viewing the data, and to build filters to convert the data into conformant data/metadata formats once we have decided on a metadata specification. We are also initiating a GIS integration effort under the direction of Dr. Ustin. Construction of a relational database will ensue later in the year.

## **ORGANIZATIONAL , PEER REVIEW, AND COLLABORATIVE ACTIVITY**

We have engaged in a number of activities that enhance the quality, scope and applicability of PEEIR findings. These include the following:

- We conducted our first Science Advisory Board (SAB) review at the November “All Hands” meeting. This review included the following members:
  - **Donald DeAngelis**, USGS/Biological Resources Division & Department of Biology, University of Miami, Coral Gables, FL
  - **John Knezovich** (Chair), Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA
  - **Richard Lee**, Professor of Oceanography, Skidaway Institute of Oceanography, Savannah, GA
  - **Charles Simenstad**, School of Fisheries & Aquatic Sciences, University of Washington, Seattle, WA
  - **Peter Thomas**, Marine Science Institute, The University of Texas at Austin, Port Aransas, TX
  - **Vern Vanderbilt**, Senior Research Scientist, NASA Amers Research Center, Moffett Field, CA

Responses to all SAB comments have been carefully scrutinized and will be fully implemented by Summer, 2003.

- We participated in numerous Cross-EaGLEs planning and assessment meetings, and Dr. Anderson has participated in the Science Advisory Board for the Gulf Coast EaGLE CEER-GOM.

- We have briefed Dr. Bobbye Smith, ORD liaison to EPA Region IX, on a regular basis regarding our activities and have developed various integration activities.
- We have developed collaborations with various agencies and institutions, including: the CALFED Bay Delta Program, which awarded PEEIR \$250,000 in matching funds, the San Francisco Bay Regional Water Quality Control Board for data to support regional decisions on marsh restorations, the UC Toxic Substances Research and Teaching Program which awarded a \$56,000 match for the restoration studies above, and the Southern California Coastal Water Research Project for investigations regarding microbial pathogens.
- We completed coordinated sampling with the west coast EMAP team and will provide them with data on endocrine disruptors and metals in marsh plants.

## **PUBLICATIONS & PRESENTATIONS**

### **Publications**

Pawley, A., S. Detwiler, C. Christian. 2002. A brief overview of historical data sources for the San Francisco Bay Estuary. The Bay Institute.

Smith, E. 2002. Tomales Bay water quality status and trends report. Smith & Associates.

Whitehead, A., K. Kuivila, J. Orland, B. Wilson, S. Anderson. 2003. Genotoxic effects of agricultural runoff in a California native fish. Submitted MS.

### **Presentations**

Anderson, S. Pacific Estuarine Ecosystem Indicator Research (PEEIR) Program. EPA Region IX briefing, San Francisco, CA. June, 2002.

Anderson, S. Pacific Estuarine Ecosystem Indicator Research (PEEIR) Program. CALFED briefing on PEEIR, Sacramento, CA. May, 2002.

Collins, Josh. Presentation at “Restore America’s Estuaries” Annual Meeting. Baltimore, MD. April, 2003.

Collins, Josh. Protecting coastal resources on the west coast: Annual meeting of West Coast National Estuaries Projects. Morro Bay, CA. May, 2003.

Ustin, S. Remote sensing of wetland conditions in west coast salt marshes. Talk presented at American Society of Limnology and Oceanography, Salt Lake City, UT. February, 2003.

Nisbet, R. M. Effects of food quality on growth and reproduction of individual organisms. Invited presentation at European Society for Mathematical and Theoretical Biology, Milan.

June, 2002.

Nisbet, R. M. The predictive power of simple population models. Plenary lecture at Society for Mathematical Biology, Knoxville, TN. July 2002.

Nisbet, R. M. From molecules to ecosystems with dynamic energy budget models. University of Chicago. March, 2003

**SUPPLEMENTAL KEYWORDS:** watersheds, estuary, ecological effects, bioavailability, ecosystem indicators, aquatic, integrated assessment, EPA Region IX

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**Principal Investigator:** Steven Morgan<sup>1</sup>

**Co-Investigators:** Roger Nisbet<sup>3</sup>, William Bennett<sup>1</sup>

**Institutions:** UC Davis Bodega Marine Laboratory<sup>1</sup>, UC Davis<sup>2</sup>, UC Santa Barbara<sup>3</sup>

**Collaborator:** Tim Hollibaugh, University of Georgia

**Research Category:** EaGLE Program

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

**Research Objectives:** The overarching goal of this proposal is to develop a suite of ecological indicators to rapidly assess the integrity and sustainability of wetlands in West Coast estuaries. We propose to develop an integrated suite of indicators to evaluate impacts of stressors across levels of biological organization, trophic structure, life stage, time, and space.

### PROGRESS SUMMARY

Four approaches were used by teams of investigators from UC Davis and UC Santa Barbara to determine the impacts of stress from nutrient loading, pollution, and exotic species on wetlands from northern and southern California: 1) physiochemical monitoring, 2) biological monitoring, 3) toxicity biomarkers and 4) statistical analysis and modeling. Research was conducted in concert with the Biochemistry & Bioavailability (BBC) team to characterize the physico-chemical environment, including temperature, salinity, oxygen, submergence times, sediment grain size, nutrient inputs and toxic contaminant loads; the Biochemical Response to Contaminants (BRC) team to conduct toxicity biomarker assays in the field; and the Remote Sensing Component (RSC) team to ground-truth measurements taken at the ecosystem level.

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. Sites span biogeographic boundaries and the estuaries vary morphologically, which provides a good test of the reliability of the indicators to assess wetland integrity across diverse environments. We 1) censused the full spectrum of wetland communities, including microbes, plants, invertebrates, fishes, birds and parasites, 2) characterized sites for nutrient and toxic contaminants in collaboration with the BRCC and BBC teams, and 3) developed modeling approaches that will enable us to determine whether our indicators responded significantly to measured stressors, the ability of indicators to distinguish between reference and impacted sites, and the effects of contaminants on individuals, populations and ecosystems across space and time. Specifically, indicators are being developed by contrasting conditions at previously characterized reference and impacted sites, following nutrient

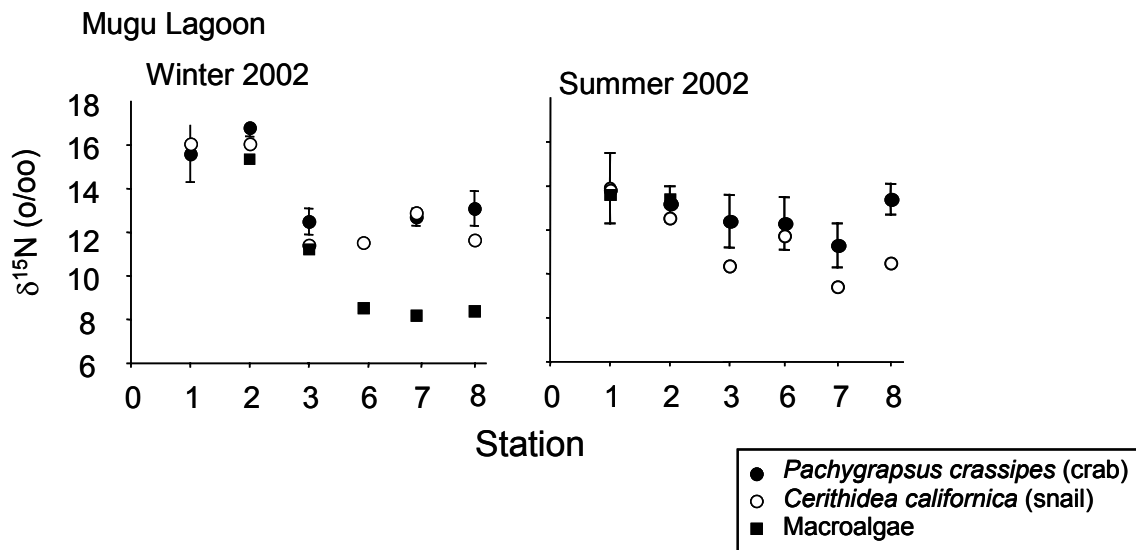
gradients at all seven sites and toxic contaminant gradients at three sites (Stege, Carpinteria, and Mugu).

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 and make sense of the enormous and diverse array of information that will be obtained during this multifaceted, four-year project. Because ecosystems subsume lower levels of biological organization, our component has taken the lead, together with the Integration component, to ensure that these three essential criteria are met. We made sure that representatives from all of the research components participated in field sampling, and then we spent a great deal of time discussing the best way to fully integrate our project based on our initial results and experience. This effort led to the incorporation of a gradient design of contamination at the study sites. The teams returned to field sites to characterize the bioavailability and toxicity of contaminants along the gradient. Invertebrate and fish communities were also characterized along the gradient. A fully integrated sampling scheme was developed and deployed at multiple stations within each of the seven sites.

Highlights of the preliminary analyses of data for the Ecosystems Indicator Component (EIC) are itemized below. However, it should be reiterated that all sampling is fully integrated with the other project components.

### Nutrient cycling

- Dissolved inorganic nitrogen concentration was orders of magnitude greater at Carpinteria than any other site, and it was twice as great at Stege than the rest of the sites.
- $\delta^{15}\text{N}$  values of macroalgae and selected consumers vary spatially and temporally within the study marshes.



- Crabs and snails may be useful “indicator” species since they are present year round at most stations
- \*<sup>15</sup>N values appear correlated with salinity suggesting incorporation of land derived N in marsh food webs.

**Primary productivity and trophic support**

- The dominant vegetation of marshes on the West Coast, *Salicornia*, is smaller, greener and denser where conditions are saltier and less toxic. In contrast, *Salicornia* has greater biomass, but more of it consists of brown stems, and plants are less dense where conditions are most toxic.
- *Spartina* was denser, taller, heavier and had greater percent cover where conditions were less toxic than at our most toxic site (Stege). Furthermore, the percentage of flowering shoots was low at the most toxic site.

<i>Spartina</i> Vegetation		
Mean +/- SD, n = 2 stations		
	<b>China Camp</b>	<b>Stege Marsh</b>
# Stems/m <sup>2</sup>	776 +/- 318	195 +/- 138
Canopy Height (cm)	870 +/- 310	460 +/- 140
% Cover	51 +/- 37	33 +/- 27
Biomass (kg/m <sup>2</sup> )	37.0 +/- 14.5	4.7 +/- 2.8
% Flowering Shoots	16 +/- 11	0.2 +/- 0.6

- Mats of cyanobacteria were prevalent at the most toxic site, unlike other sites.
- Ammonification rates, the first step in nutrient recycling, was highest at the most toxic site.
- Decomposition rates did not appear to be related to toxic exposure.

**Microbial communities**

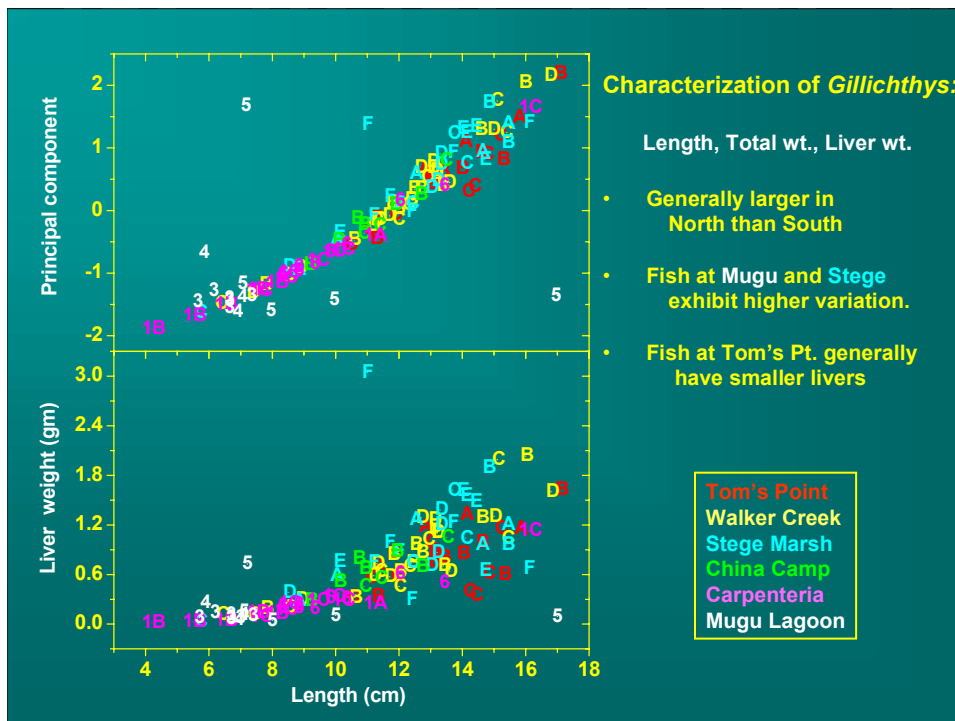
- Total coliform concentration was correlated with bacterial community composition and urbanization in the Santa Barbara area.
- Methods being developed appeared to accurately detect sources of contamination in laboratory-created “blind” samples. Dog, gull and human sources appear to contain different bacterial communities, and the bacterial community in blind trials appeared to be human in origin. This study was coordinated by the Southern California Coastal Water Research Project (SCCWRP), and forges an important collaboration between SCCWRP and PEEIR.

## Invertebrates

- Abundance and diversity of infauna appeared to be related to toxic exposure. Amphipods appeared to be particularly sensitive indicators of stress. Amphipods, especially *Corophium*, were much less abundant at our most toxic site and may vary with contaminant exposure within Stege and Walker. Interestingly however, one species of amphipod (*Lysanassidae*) was far more abundant at our most toxic site and it was found where contaminant exposure was greatest within the marsh. Contaminant exposure was greater in the marsh, where amphipods were most abundant, than in the channel, which may explain why amphipods were more susceptible to toxic exposure than other infauna.
- Several other potential indicators that were investigated appear to show less promise, including the prevalence of invasive species, fluctuating asymmetry in crabs, and imposex in snails. )
- Detritivore abundance holds considerable promise as an indicator.

## Fishes

- Fish size shows greater variation at our two most contaminated sites (Stege and Mugu) relative to other sites.
- Fish livers were larger at contaminated sites than at our reference site at Toms Point.
- Validation of growth rates from otoliths of our model fish species were accomplished and are being used to determine variation in growth rates with toxic exposure.



## **Parasites & birds**

- Trematode richness varies with bird richness and may be effective indicator of community diversity.
- Trematode frequency and richness are associated with general habitat quality when restoration sites were compared with natural marshes. It also appeared to be greatest at the most contaminated southern site (Mugu), intermediate at Carpinteria and least in Morro Bay.
- Fish ciliates have insufficient spatial variation to be used as indicators at this time and need to be compared with fish toxicology data.

We concluded that our field sites are appropriate, working at them is feasible, and our target species are sufficiently abundant. We detected significant differences in microbial populations between these sites. We have determined that a combination of a gradient design nested within reference and impacted sites are the most powerful design to detect the effects of contaminants on wetland ecosystems. We also concluded that contaminants are likely to be most concentrated in channels and along the margins of tidal creek, and we are targeting these areas. Additional stations within sites have been incorporated to put the gradient in context of the larger ecosystem. Further discussions of scaling up indicators of plant stress to the level of the landscape using remote sensing revealed that the approach still looks promising. We are developing and validating this indicator in collaboration with the RSC and BBC.

## **FUTURE ACTIVITIES**

Intensive discussions have been conducted to update our sampling plan for this year. Our plan consists of increasing the number of stations within two highly contaminated sites to increase our resolution of toxicant effects. Sampling will be continued at Stege Marsh, Carpinteria Marsh, and Walker Creek. Contaminant and toxicity exposure relative to population abundance and species richness will continue to be evaluated for multiple stations within each site in collaboration with the BRC and BBC. Outplant experiments will be conducted with crabs, fish and clams at selected stations with sites to measure reproductive and growth performance, biomarker responses and body burdens in collaboration with the BRC and BBC. Validation and initial field tests of plant stress relative to tissue burdens and bioavailability on the landscape level are being conducted in collaboration with the RSC and BBC. All other promising indicators described above will continue to be developed.

After intensive field work is completed this year, integration activities will include the synthesis of indicator data using both multivariate statistics and models. We will draft manuscripts reporting on the potential applicability of individual and aggregate indicators. Working teams will be initiated to formulate recommendations on 1) plant indicators at multiple spatial scales, 2) indicators for model animals that relate stressor measurements to changes in fitness, and 3) appropriate integrative indicators related to nutrient cycling and bird populations.

## **PUBLICATIONS & PRESENTATIONS**

### **Publications**

Hechinger, R. F. & K. D. Lafferty. 2002. Birds as determinants of parasite community structure in snails. Abstract and talk presented at the Tenth International Congress of Parasitology meeting, Vancouver, BC.

Huspeni, T. C. and K. D. Lafferty. 2002. Using larval trematodes that parasitize snails to evaluate a salt-marsh restoration project. Abstract and talk presented at the Tenth International Congress of Parasitology meeting, Vancouver, BC.

Lafferty, K. D. 2002. Host thresholds, extinction dynamics and trophic cascades. Symposium on the Changing Management of Wildlife Diseases. International Congress of Parasitology, Vancouver, BC.

Hollibaugh, J. T. and L. T. Vasquez. 2003. Composition of ammonia-oxidizing bacteria assemblages as indicators of estuarine processes. Abstract presented at American Society of Limnology and Oceanography, Salt Lake City, UT.

Huspeni, T. C. and K. D. Lafferty (in press). Using larval trematodes that parasitize snails to evaluate a salt-marsh restoration project. *Ecological Applications*.

Lafferty, K.D. (In press). Assessing estuarine biota in southern California. In Planning for Biodiversity Symposium Proceedings. US Forest Service Technical Report Series.

Lafferty, K.D., (In press). Is disease increasing or decreasing, and does it impact or maintain biodiversity? *Journal of Parasitology*.

Lafferty, K. D., and R. D. Holt. (In press). How should environmental stress affect the population dynamics of disease? *Ecology Letters*.

### **Presentations**

Hechinger, R., K. Lafferty, A. Brooks, & T. Huspeni. Abstract. Gill ciliate parasitism of wetland fish as an ecological indicator. UCTRS&TP 15th Annual Research Symposium. Long Beach, CA, April, 2002.

Hechinger, R. & K. Lafferty. 2002. Birds as determinants of parasite community structure in snails. Abstract and talk presented at 83<sup>rd</sup> annual meeting of the Western Society of Naturalists, Monterey, CA. November, 2002.

Brooks, A. The future of water quality assessment, the role of PEEIR. Carpinteria Valley Town Hall Meeting. January, 2003.

Hechinger, R. & K. Lafferty. What can snail parasites tell us about wetland biocomplexity? Poster presentation. UCTS&R Research Symposium, Oakland, CA. April, 2003.

**SUPPLEMENTAL KEYWORDS:** indicators, ecology, estuaries, wetlands, health, toxics, nutrients, exotic species.

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Biological Responses to Contaminants Component:  
Biomarkers of Exposure, Effect, and Reproductive Impairment

**Principal Investigators:** Gary N. Cherr<sup>1</sup>, Susan L. Anderson<sup>1</sup>

**Co-Investigators:** Michael S. Denison<sup>2</sup>, Frederick J. Griffin<sup>1</sup>, Roger Nisbet<sup>3</sup>, Mark J. Snyder<sup>1</sup>,  
Barry Wilson<sup>2</sup>

**Institutions:** UC Davis Bodega Marine Laboratory<sup>1</sup>, UC Davis<sup>2</sup>, UC Santa Barbara<sup>3</sup>

**Research Category:** EaGLE Program

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

**Research Objectives:** The objective in this proposal is to determine the efficacy of a suite of molecular, biochemical, cellular, and tissue level indicators to collectively predict ecosystem responses to contaminant stress. Biomarkers of reproductive impairment are important early warning indicators of ecosystem impacts, but they need complete characterization and validation in an ecosystem context as proposed in PEEIR. This section's particular emphasis is assessment of reproductive parameters. Because rapid and accurate techniques are not readily available, biomarkers associated with reproductive impairment can be early warning indicators of stress, and reproductive impairment can be directly linked to effects on populations through modeling efforts. The research proposed here is integral to the overall goals of PEEIR which are to establish indicators that environmental managers can use to: 1) develop an approach for synthesizing indicators into technically-defensible assessments of wetland health and integrity, 2) determine biotic integrity for fish and invertebrate populations within wetland communities, and 3) determine toxicant-induced stress and bioavailability for wetland biota.

### PROGRESS SUMMARY

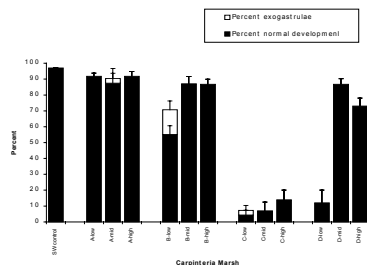
Research conducted in this component of the project is comprised of both laboratory experiments and field sampling. Each of these activities is coordinated with all components of the project. Integration is achieved through use of common sampling sites, model organisms, and modeling and statistical procedures common to the PEEIR Consortium. We completed our first intensive field sampling in the summer and fall of 2002. When analysis is completed, synthesis of multiple responses will ensue. Below are presented initial data for several project components.

#### Site Characterization and Developmental Toxicity

One of the first activities was to characterize wetland sites with respect to toxicity, since there was little or no background information available. Sea urchin embryo development, an accepted bioassay that is very sensitive, was used to evaluate sediment elutriates from stations at selected sites. Toxicity gradients were detected and were independent of sulfide and

ammonia levels (Figure 1). These data have been used in combination with chemistry values

and remote sensing outputs to inform site selection for the project.



**Fig. 1:** Example of toxicity of sediment elutriates from Carpinteria Salt Marsh. A decrease in normal development of sea urchin embryos indicates toxicity. Three samples at different elevations per station were assessed. Station C showed the most toxicity. Note the presence of abnormal development in the form of exogastrulae at “B-Low”.

In addition to providing basic toxicological data for subsequent PEEIR efforts, the data from these initial studies provided us clues as to what contaminants may be responsible for the observed toxicity. It was found that polycyclic aromatic hydrocarbons (PAHs) induce the specific developmental abnormality known as exogastrulation (Pillai et al., 2003). More recently, we found that phthalates also induce exogastrulation and they occur at high levels at Station B at the Carpinteria Salt Marsh. We plan to utilize the exogastrulation assay for *in situ* experiments and the molecular marker associated with this developmental abnormality,  $\beta$ -catenin, in other species since it is highly conserved.  $\beta$ -catenin may prove to be a universal marker applicable in many species.

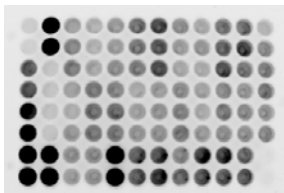
### **Biochemical and molecular responses**

An important area of research has been the development of the incidence of apoptosis, or programmed cell death, as an indicator of stress as well as decreased reproductive output in fish. Cell death in liver and gonad can directly impair reproductive output. We have utilized a well developed technique that measures DNA cleavage (the TUNEL assay) on field and laboratory samples, as well as caspase (cysteine aspartate protease) activity as indicators of apoptosis. This latter indicator 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, 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 indicating increased apoptosis in these cells. The caspase assay was also employed with livers of fish from several sites and it was found that sites with contamination showed increased caspase activity. While preliminary at present, further characterization of field samples is underway. In addition, the TUNEL assay is being used to corroborate the caspase data; for fish cells *in vitro*, the results are consistent with the caspase data.

For studies of endocrine disruption, we are applying immunologic assays to detect induction (estrogenic activity) of choriogenins (egg shell protein precursors) that are made by the liver in response to estrogenic compounds, including environmental estrogens and estrogen mimics. For this research, we are applying both commercial antibodies and antibodies we have developed as routine tools for detecting endocrine disruption in male and immature fish (Figure 2). This approach is more broadly applicable than the more commonly used vitellogenin assay in fish

because the choriogenins are more highly conserved, and the antibodies can be used on a very

broad range of fish species. By also utilizing data on the presence of estrogenic and/or



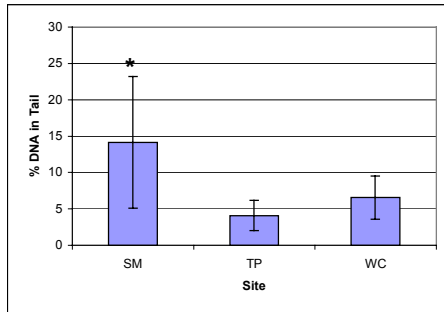
**Fig. 2:** Assessment of choriogenins in plasma from fish collected from several sites using an fluorescent ELISA microplate assay. The increased optical density indicates increased choriogenins. All samples were normalized to concentration of protein in the plasma. Initial studies suggest that fish from Stege Marsh, the most contaminated site we are studying, show increased circulating choriogenins. This suggests that estrogenic compounds may be present.

androgenic activities from sites determined by Dr. Denison's reporter bioassay (see below), subsequent chemical analyses, and demographic data collected by the EIC, we should be able to determine cause and effect relationships for reproductive impairment. These can be linked to populations in the modeling research underway in the Nisbet lab. We know of no other efforts that attempt to link endocrine disruptor screening tests to population-level effects in such a comprehensive manner.

A rapid screening tool for aryl hydrocarbon receptor (Ah receptor) activity (dioxin-like compounds) as well as for estrogenic and androgenic chemicals is the CALUX bioassay, which has been developed in Dr. Denison's laboratory. This assay uses cells transfected with constructs of the dioxin-response, estrogen-response, or androgen-response elements and fluorescent reporter genes. Studies have demonstrated an excellent relationship between TEQs of dioxin-like compounds and CALUX bioassay values (Fig. 6). 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 and endocrine disruptors in sample extracts; this bioassay can be related to or actually direct subsequent chemical analyses, as well as biomarker responses (P450s, choriogenins) and ultimately population level effects (reproductive impairment). 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. Discussion with the other EaGLE groups has taken place.

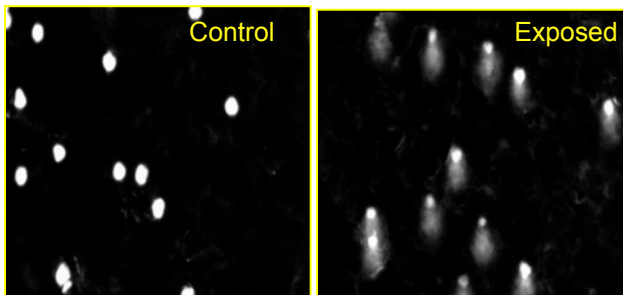
An indicator of contaminant stress can be DNA strand breaks. These strand breaks can be repaired but may lead to mutations or overall diminished energy budgets; unrepaired strand breaks will usually lead to cell death. We have assessed DNA strand breaks in blood cells from fish and crabs from the different marshes using the Comet assay that determines the percentage of DNA migrating from nuclei under electrophoretic conditions. This initial phase is for us to develop a baseline for DNA damage using an approach by which individuals may be repeatedly sampled. Figure 3 shows DNA damage in blood cells (hemocytes) from the shore crab (*Pachygrapsus*). Animals from Stege Marsh, the most contaminated site, show significantly elevated DNA stand breaks in their hemocytes. Similar results have been obtained in mudsucker

blood cells from the same marsh. We are now applying this assay to gametes and embryos from these species.

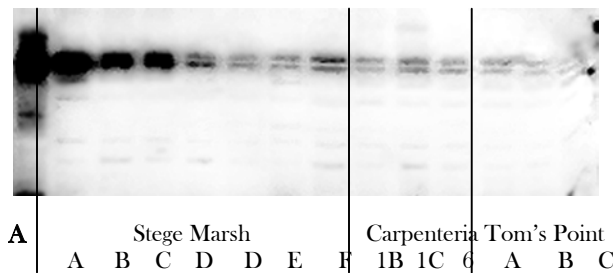


**Fig. 3:** DNA strand breaks in hemocytes from the shore crab. Increased DNA in the “tail” of the comet indicates increased DNA strand breaks. Stege Marsh (SM), the most contaminated of our sites, shows the greatest degree of DNA damage, as compared to Tom’s Point (TP) and Walker Creek (WC).

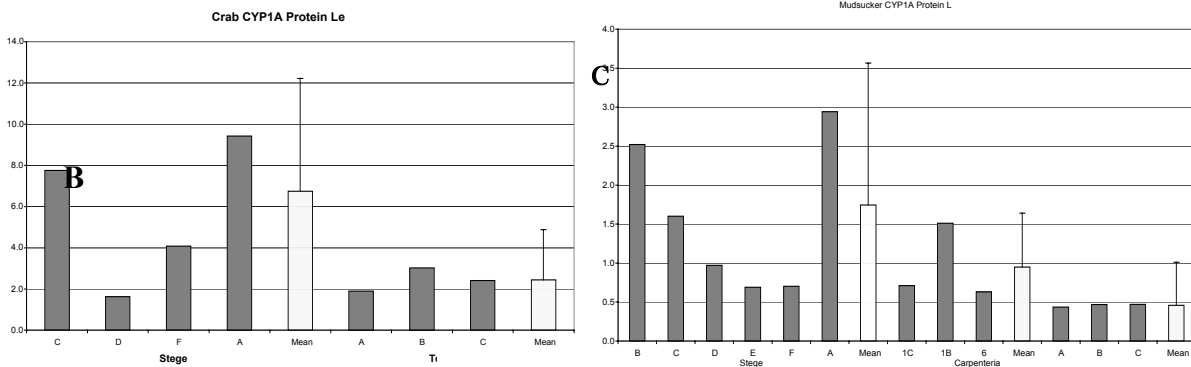
The fluorescence images below show control fish blood cells with little or no DNA strand breaks and cells from a fish exposed to metals showing the characteristic comet tail indicating high levels of strand breaks.



Biochemical biomarkers of exposure have been suggested as early warning indicators of marsh degradation once they are placed in the proper context, and other data sets are available regarding the overall condition of the organisms (Cherr, 2002). Our studies have included the analyses of P450 enzymes in tissues, as these tend to be responsive to exposure to many organic contaminants and have long been used as a biomarker in both laboratory and field studies. We have focused on CYP1A, which is involved in detoxication of many hydrocarbons and related chemicals. We have raised an antibody to a highly conserved peptide domain of CYP1A and have found that it cross-reacts with both vertebrate and invertebrate tissues. The PEEIR program provides a novel opportunity to place these data in a population context and further discern their utility as indicators.

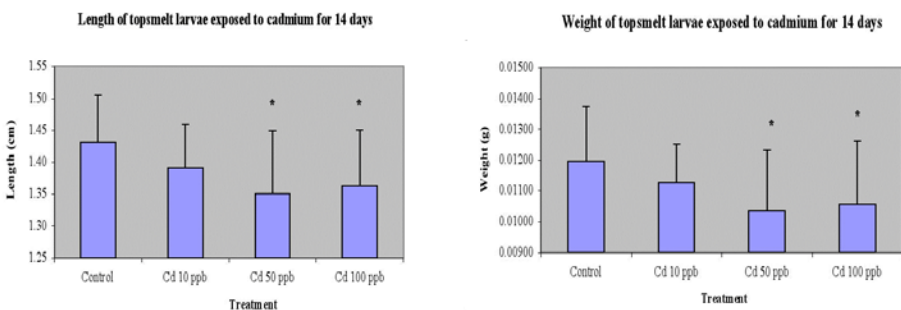


**Fig. 4A-C:** **A.** CYP1A levels in fish livers from Stege Marsh, Carpenteria Salt Marsh, and Tom’s Point. The stations at each marsh are designated by letter or number. Note the clear induction of CYP1A in fish from Stege Marsh, particularly at stations A, B, and C. Stations D and F also show elevated P450 as compared to Tom’s Point and Carpenteria Salt Marsh. **B.** Crab hepatopancrease CYP1A levels are higher at Stege Marsh than Tom’s Point. **C.** Similarly, mudducker liver CYP1A levels are higher at Stege as compared to Carpenteria or Tom’s Point, which were not different from each other.



### Relating biomarker responses to populations

Biomarker responses such as apoptosis and endocrine disruption can be directly linked to reproductive outcome and population viability. Laboratory studies are on-going (Anderson, Cherr, Nisbet, and graduate student Wendy Rose) to relate growth alterations to reproductive outcome and population viability in a variety of models. These will eventually include biomarker responses, as well. For example, Dynamic Energy Budget (DEB) models 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. We have been conducting sublethal exposures of young fish to cadmium, a contaminant present at several sites (Figure 5). By assessing growth, food intake, respiration, and metal loads in tissues, we will be able to develop a model that predicts individual and population effects of chronic exposure to contaminant stress.



**Fig. 5:** Effects of chronic Cd exposure (14 day) on topsmelt larval growth. 50 ppb and 100 ppb induce significant inhibition of growth based on length and weight.

### FUTURE ACTIVITIES

Analyses of samples will continue from both 2002 and 2003. We are initiating a large number of outplants of mudsuckers and crabs at four sites in order to directly assess biomarkers, growth, and tissue contaminants in a controlled experimental design. There are critical experiments for validation of annual sampling efforts. For reproductive studies, we will be assessing successful development in crabs as well as induction of spawning of sexually mature mudsuckers from different sites. This is the first time such approaches will have been attempted.

After results become available from the sampling and experiments conducted in 2003, synthesis efforts will include 1) integration of data with models; 2) statistical analysis relating biomarker responses to growth and reproduction of fish and invertebrates; and 3) the development of

methodology recommendations useful to EPA in development of sub-lethal indicators of contaminant stress in wetlands. Discussions are already underway with US EPA Region IX regarding potential technology transfer for our novel and widely applicable methods related to endocrine disruption. The Denison group has also accepted samples from West Coast EMAP to perform the CALUX bioassay on sediment samples. We will determine whether this rapid screening method is ready for broader application.

## **PUBLICATIONS & PRESENTATIONS:**

### **Publications**

Cherr, G.N. 2002. Can we develop and utilize indicators of ecological integrity to successfully manage ecosystems? In: *Managing for Ecosystem Health*, Third International Congress on Ecosystem Health, C.O. Qualset, D.J. Rapport, D. Ralston and B. Lasley, eds. CRC Press, in press. pp. 227-229.

Pillai, M.C., C.A. Vines, A.H. Wikramanayake and G.N. Cherr. 2002. Polycyclic aromatic hydrocarbons disrupt axial development in sea urchin embryos through a  $\beta$ -Catenin dependent pathway. *Molecular Biology of the Cell*, 13 (Suppl.):253A.

Pillai, M.C., C.A. Vines, A.H. Wikramanayake and G.N. Cherr. 2003. Polycyclic aromatic hydrocarbons disrupt axial development in sea urchin embryos through a  $\beta$ -Catenin dependent pathway. *Toxicology*, 186: 93-108.

### **Presentations**

Fry, W., Trainee, & B. W. Wilson, Abstract: Do organophosphate and pyrethroid insecticides affect the neuromuscular development of topmelt? UCTRS&TP 15<sup>th</sup> Annual Research Symposium, Long Beach CA. April, 2002.

Rose, W., Trainee, S. L. Anderson, & G. N. Cherr. Abstract: Cadmium induces apoptosis in fish hepatocytes and CHSE-214 cells. UCTRS&TP 15<sup>th</sup> Annual Research Symposium, Long Beach, CA. April, 2002.

Cherr, G.N. Biological responses to contaminants. CALFED briefing on PEEIR. Sacramento, CA. May, 2002.

Cherr, G.N. Indicators of environmental stress: natural and anthropogenic stressors. U.S. Geological Survey, Menlo Park, CA. September, 2002.

Cherr, G.N. Polycyclic aromatic hydrocarbons disrupt axial development in sea urchin embryos through a  $\beta$ -Catenin dependent pathway. Invited presentation at the 42<sup>nd</sup> Annual Meeting of the American Society for Cell Biology, San Francisco, CA. December, 2002.

Fairbairn, Elise, Trainee. Poster: "DNA Damage in Crab Hemocytes as an Indicator of Conaminant Exposure." UCTSR & TP Annual Research Symposium, Oakland, CA, April, 2003.

**SUPPLEMENTAL KEYWORDS:** aquatic, indicators, biomarkers, wetlands, reproduction, cellular, molecular, biochemical, bioavailability, ecosystem, ecological impacts, estuary

## YEAR 2 ANNUAL REPORT

**Period Covered by the Report:** March 1, 2002 to February 28, 2003

**Date of Report:** April 1, 2003

**EPA Agreement Number:** R-82867601

**Title:** Pacific Estuarine Ecosystem Indicator Research (PEEIR) Consortium: Biogeochemistry and Bioavailability Component

**Principal Investigator:** Richard M. Higashi<sup>1</sup>

**Co-Investigators:** Teresa Fan<sup>1</sup>, Patricia Holden<sup>2</sup>, Kathryn Kuivila<sup>3</sup>, Douglas Nelson<sup>1</sup>, Kate Scow<sup>1</sup>

**Institutions:** UC Davis<sup>1</sup>, UC Santa Barbara<sup>2</sup>, US Geological Survey, Sacramento, CA<sup>3</sup>

**Research Category:** EaGLE Program

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

### **Research Objectives:**

The overall aim of this section is to develop field indicators and the knowledge base to help assess the consequences of changes in chemical form of pollutants in tidal marshes. Most organic and inorganic pollutants relentlessly change chemical form due to biogeochemical processes. Different forms in turn define both toxicity and bioavailability. Our general objective is founded on two principles that involve biogeochemistry and bioavailability. Knowledge of factors involved in modifying chemical form and bioavailability is critical for accurately assessing and predicting organismal exposure and higher-order ecotoxicological effects.

This section's particular emphasis is assessment of metals and organic pollutant bioavailability in relation to sedimentary lower trophic level biomarkers. This emphasis on rooted plants and sediment microbes is because they are often the entry point of pollutants into the foodchain, and are major drivers of the biogeochemistry of the tidal marsh. The specific objectives of this section is to uncover the chemical, biochemical, and biotic markers that herald the bioavailability, transport, and/or bio-transformation of selected metal and organic pollutants. These markers, in appropriate concert with others generated by PEEIR, can comprise indicators of pollutant stress on marsh ecosystems.

## **PROGRESS SUMMARY**

### **Plant Indicators**

The development of plant stress and indicators at multiple spatial scales is an important goal of the PEEIR Consortium. In the PEEIR group sampling effort during summer 2002, *Spartina* and *Salicornia* shoots and roots were obtained from Stege, Carpinteria, and Walker marshes at stations established for all investigators within PEEIR. The tissues were cleaned, then preserved on-site by immersion in liquid nitrogen. Metabolite profiling by NMR and GCMS yielded patterns of over 40 metabolites that can be compared with other PEEIR data from these same stations. For example, relatively decreased sucrose stores were observed in plants at the same Carpinteria station (station "B") that exhibited considerable toxicity to sea urchin gametes, the unusual and strong response of exogastrulation (see BRC Report), and highest levels of phthalates. As stated below, these highest phthalate stations also correlated strongly with certain

peaks in the T-RFLP microbial community profiles (Holden project). In addition, suites of metals were analyzed in the plant samples, in collaboration with Peter Green. Interestingly, the tissue metals did not correspond to the leaf exudates metals, indicating that the transpiration stream for metal exudation interacted little with the plant cells. Manganese content of *Salicornia* woody and green stems related to microbial diversity (Holden project). Lastly, growth-chamber studies with *Spartina* and Cd exposure was initiated, and the first range-finding experiment was completed. The laboratory studies are an important aspect of our synthetic efforts to develop plant indicators at multiple scales.

Plants were grown and exposed to diuron in a growth-chamber study of the uptake and metabolism of diuron by wetland plants. Diuron is a widely-used herbicide which inhibits photosynthesis; thus, it impacts major functioning of plants that may be visible by remote-sensing. Although frequently detected in water, diuron also sorbs to sediments, and thus sediments may provide a continuous source of the pesticide to plants and aquatic organisms in wetlands. In conjunction with the Fan project, a series of laboratory studies were begun to measure the uptake and metabolism of diuron by native *Spartina* and *Salicornia*. The effects of diuron were estimated by measuring resulting changes in plant pigments and photosynthesis stress. The research with diuron will also help to validate metabolic indicators and their relationship to integrated responses of the plants.

### **Site characterization**

Analytical activity continued with the “Stressor Core” group, including Gary Cherr of the BRC and Peter Green. The principal aim is to characterize contaminant organics, metals, and embryonic toxicity along gradients at PEEIR-wide established marsh stations. Sediment elutriates were analyzed from Stege, Carpinteria, Walker, and Mugu marshes that correspond to samples being analyzed by Holden, Scow, and Cherr (BRC) groups, and were in close proximity to plants collected by the Fan project, shoot metal exudates by Green, and invertebrate survey cores (EIC projects of Grosholz, Morgan, and Lafferty). One example of a preliminary relationship was high elutriate Cu at Stege marsh stations that exhibited high gamete toxicity (BRC project of Cherr). Also at Stege marsh, the station (“C”) with the highest Cd leaf exudates also posted by far the lowest *Streblospio* and highest *Enchytraeidae* abundances (EIC projects of Grosholz and Morgan). Other examples of the Stressor Core findings and connections to other PEEIR research results are found in several descriptions above and below, as well as in the BRC section’s annual report.

### **Microbial responses**

Microbial community composition and diversity in sediments, as assessed by high-throughput T-RFLP analysis, was determined on sediment samples collected over two years of sampling. Interestingly, relationships with plant biochemical markers and contaminants were discerned; for example, certain T-RFLP peaks (identity currently unknown) associated strongly with dibutylphthalate in elutriates (Higashi project), which in turn were highest at Carpinteria Marsh stations that exhibited the highly unusual exogastrulation of embryos (BRC project of Cherr) and low plant sucrose stores (Fan project). As mentioned above, *Salicornia* woody/green

relationships of Mn (Fan/Williams project) also related to microbial diversity as estimated by T-RFLP. Relationships to PLFA data (Scow project) are being explored.

Focus continued on methods to assess inorganic mercury methylation in estuarine sediments, and to develop potential indicators of mercury exposure for the Walker Creek site. Pure cultures of another type of anaerobic bacteria (iron reducers) have been isolated, that are surprisingly active methylators, in addition to the sulfate reducers of current dogma. This gives a second target for assessment of the dominant methylators in the estuarine environments. Key analytical tools were added, including the ability to obtain very high quality DGGE gels from anoxic sediments, with sequencing of the resultant rRNA bands for phylogenetic and physiological perspectives on the dominant microbes. A major new analytical tool was obtained very recently using PEEIR funds: the Milestone DMA-80, which analyzes for total mercury via the EPA 7473 (“Thermal Decomposition Amalgamation with Atomic Absorption Spectrophotometry”). This is an automated instrument that requires little to no sample preparation for tissues and sediments, and is capable of single-digit ppb analyses of total mercury. Currently undergoing calibration and tests in the laboratory, this instrument is expected to support extensive surveys of marsh sediment and organism loads of mercury for PEEIR researchers, and together with DGGE, will enable a better understanding of estuarine mercury methylation by microbial communities.

Phospholipid Fatty Acid (PLFA) analysis was performed on sediment cores in order to examine the microbial communities. The fingerprinting was performed for sites at Stege, Carpinteria, Mugu and Walker marshes. Cores were taken at different sampling stations along the main channel, and at each station three elevation were sampled: Low (bottom of the channel), Med (slope) and High (vegetated area on top of channel). Sampling was performed by pooling 6 to 8 cores in a sterile glass jar. From this pooled sample, one core was retrieved for PLFA fingerprinting, and other cores were retrieved for T-RFLP and elutriate water analysis by other members of the BBC (Higashi project, BRC project of Cherr). The preliminary statistical analysis of the data was performed in CONOCO and Sigma Plot software.

A summary table of the PLFA information available to date is available upon request. Another way of looking at the PLFA data is to compare the mass of specific lipid biomarkers that are indicators of particular groups of microorganisms. The biomarkers obtained for some major groups of organisms in soil, as well as the average number of lipids and the total PLFA extracted, include the proportion of branched (Gram positive bacteria marker), cyclopropyl (aerobic bacteria markers), and linoleic acid (18:2 $\omega$ 6c: fungal biomarker) for subgroups of the samples. (Note: biomarker data need to be interpreted with caution; not all biomarkers are exclusive to a particular group, and it is possible that some members of a particular group may not have that biomarker). We are currently working with other PEEIR researchers to explore the data for correlations between pollutant gradients (Higashi project, BRC project of Cherr) and PLFA fingerprints. Further results of this study will be presented at the Estuarine Research Foundation Conference in Seattle (September 14-18, 2003).

## **FUTURE ACTIVITIES**

The BBC investigators have expended intense effort to provide analytical data and collaborations on chemistry research with the EIC and BRC components. Several examples of this are given

above, and many more instances are expected to emerge as the current data are cross-examined with that from other PEEIR sections. The cross-sectional Stressor Core activities will be brought to some sort of “closure” in 2003-04, although present plans call for a temporary increase in activity to support the animal transplant experiments planned for Summer 2003 by BRC and EIC.

A new organic chemistry component is being added to provide improved characterization of toxic compounds at our key sites. As field work is completed this year, further integration activities will include participation in working teams to forward recommendations on plant indicators at multiple spatial scales as well as to develop indicators for model animals that related chemistry to stressor measurements and ultimately changes in populations. Furthermore, methodologies useful to EPA will be detailed and technology transfer possibilities discussed. For example, Dr. Green has already participated in EMAP-West monitoring of wetland sites. His method of analysis of salt excretions in wetland plants as an indicator of toxic metal bioavailability may soon be ready for broader application.

## **PUBLICATIONS & PRESENTATIONS:**

### **Publications**

Steets, B. and P. A. Holden. 2003. A mechanistic model of runoff-associated fecal coliform fate and transport through a coastal lagoon. *Water Research* 37: 589-608.

LaMontagne, M. G. and P. A. Holden. 2002. Comparison of free-living and particle-associated bacterial communities in a coastal lagoon. *Microbial Ecology* (*in press*).

### **Presentations**

Cao, Y., LaMontagne, M. G. and P. A. Holden. Urbanization and coastal water quality: what can molecular fingerprinting tell us? Abstract presented at the UC TSR & TP 15<sup>th</sup> Annual Research Symposium, Long Beach, CA. April, 2002.

Curda, D., T. W. Fan, K. Kuivila (USGS). XAD efficiency. Abstract presented at the UCTRS&TP 15<sup>th</sup> Annual Research Symposium, Long Beach, CA. April, 2002.

Higashi, R. M., Briefing on PEEIR to EPA Region IX, San Francisco, CA. June, 2002.

Holden, P. A. and LaMontagne, M. G. Urbanization and coastal water quality: what can molecular fingerprinting tell us? American Society for Limnology & Oceanography, Victoria, BC. June, 2002.

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Green, P.G., Higashi, R.M., Fan, T.W. Mobilization of metals from salt marsh sediments by euryhaline plants. Presented at the Society for Environmental Toxicology and Chemistry Annual Meeting. November, 2002.

Green, P.G., Higashi, R.M., Fan, T.W. Mobilization of metals from salt marsh sediments by euryhaline plants. Presented at the American Society of Limnology and Oceanography Annual Meeting. February, 2003.

Holden, P. A. and LaMontagne, M. G. Microbial community composition and diversity as indicators of salt marsh ecosystem health. American Society for Limnology & Oceanography, Salt Lake City, UT. February, 2003.

Scow, K. and Cordova, A. L. PLFA results to be presented at Estuarine Research Foundation Conference in Seattle in September, 2003.

**SUPPLEMENTAL KEYWORDS:** bioavailability, transformations, biogeochemistry, selenium, mercury, pesticides