

## YEAR 3 ANNUAL REPORT

**Period Covered by the Report:** March 1, 2003 to February 29, 2004

**Date of Report:** April 1, 2004

**EPA Agreement Number:** R-82867601

**Title:** Pacific Estuarine Ecosystem Indicator Research (PEEIR) Consortium:  
Ecosystem Indicators Component

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**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 physicochemical 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 five sites in northern and southern California, including Walker Creek and Toms Point in Tomales Bay, Stege Marsh and China Camp in San Francisco Bay, and Carpinteria Marsh. 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 five sites, and toxic contaminant gradients at two sites (Stege and Carpinteria).

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 five 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**

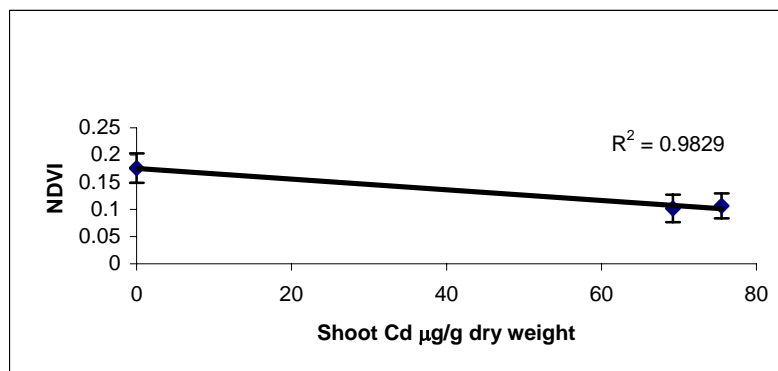
- Dissolved inorganic nitrogen concentration was orders of magnitude greater at Carpinteria than any other site, and it was twice as great at Stege than the rest of the sites.
- $\Delta^{15}\text{N}$  values of macroalgae and selected consumers vary spatially and temporally within the study marshes, and lower crab and snail  $\Delta^{15}\text{N}$  values at Carpinteria marsh may be related to nutrient inputs from intense agriculture.
- $\Delta^{15}\text{N}$  values appear correlated with salinity suggesting incorporation of land derived N in marsh food webs.
- Nitrogen isotopes may serve as a useful indicator of nutrient enrichment and as a diagnostic tool to assess land-derived sources.

### **Primary Productivity & Trophic Support**

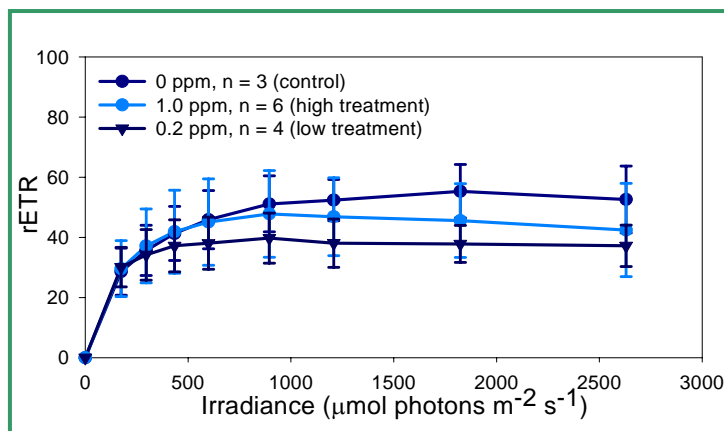
- The dominant vegetation of marshes on the West Coast, *Salicornia*, is smaller, greener and denser where conditions are saltier and less toxic. In contrast, *Salicornia* has greater biomass, but more of it consists of brown stems, and plants are less dense where conditions are most toxic.
- *Spartina* 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.
- Mats of cyanobacteria were prevalent at the most toxic sites, unlike at 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.

## Linking Plant Stress of Individuals to the Landscape Level

To determine whether physiological stress of plants can be detected at the landscape level using remote sensing, we calibrated plant physiological performance against known levels of contaminants as a first step to scaling up to the landscape level. Chlorophyll fluorescence and spectroradiometry provide measures of plant physiological performance and health, and can be applied to the landscape level as well as to individual plants. The Normalized Difference Vegetation Index (NDVI) and relative Electron Transport Rate (rETR) at the individual plant level were both sensitive indicators of plant response to Cadmium exposure in treatments. Cadmium effects seem to appear first as a decline in NDVI and rETR before a decrease in green biomass. Both parameters could be used as an indicator of plant stress prior to biomass decline, and further experiments are being conducted to confirm these preliminary results.



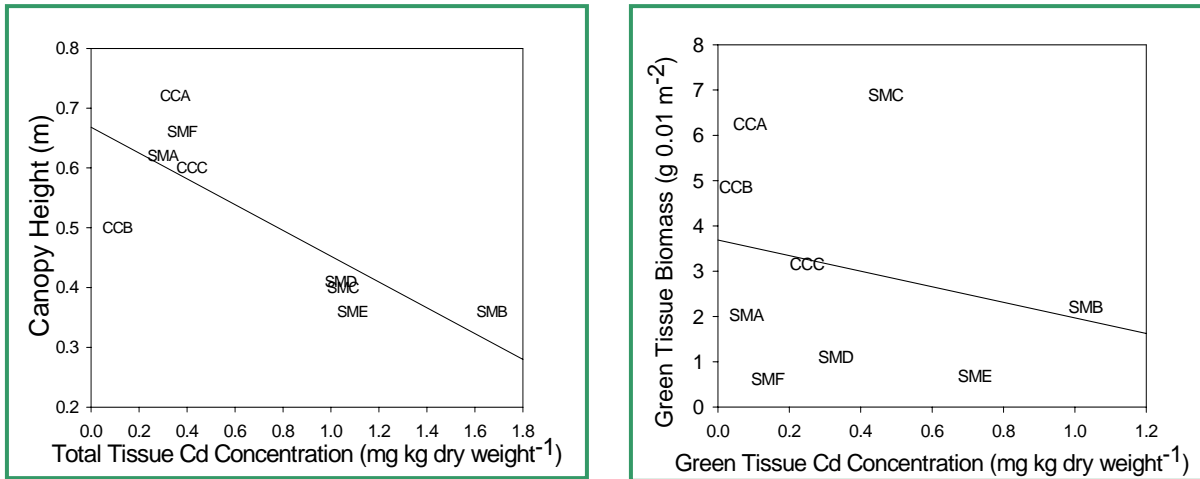
Correlation between week 5 NDVI and Cadmium concentration in shoot tissue



Photosynthetic performance was reduced in plants exposed to Cadmium. Comparison of relative Electron Transport Rate in young *Spartina foliosa* leaves responding to large, rapid changes in light intensity.

There were few relationships between vegetation structure and plant tissue cadmium concentrations, and it does not appear that vegetation structure is as sensitive an indicator as the physiological parameters investigated. However, there does appear to be a correlation between

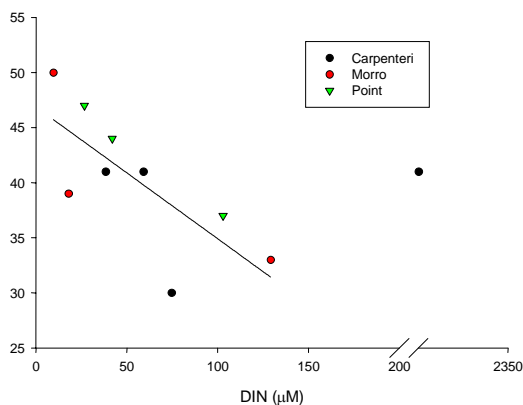
above ground biomass and Cd exposure in *Spartina foliosa* under controlled conditions, and a similar pattern was found in the field. Cd concentrations in *Salicornia* tissues are higher at a contaminated site (Stege), while biomass and canopy height are lower. Similarly, *Salicornia* tissue Cd concentrations were lower at a reference site (China Camp), while biomass and canopy height were higher.



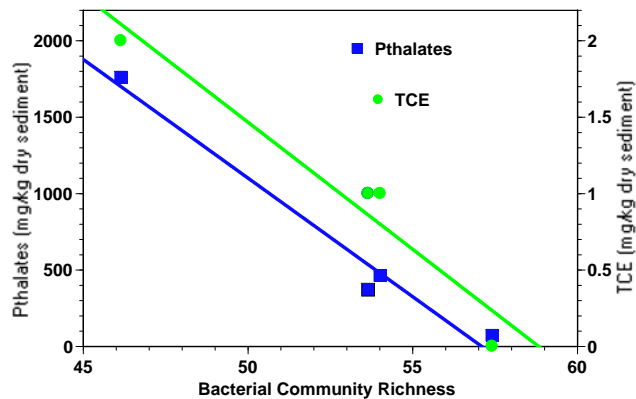
Vegetation Structure of *Salicornia virginica* across a Cadmium gradient at a reference site China Camp (CC) and a contaminated site Stege Marsh (SM); sampling stations are denoted by letter identification within sites

### Microbial Communities

- Bacterial diversity differed between polluted and reference sites and is a promising indicator of wetland health. The following results were found:
  - Nutrient (DIN) affected bacterial diversity
  - Elevation affected community composition
  - Metals affected bacterial diversity at Stege marsh
  - Organic pollutants affected bacterial diversity at Carpinteria marsh



Bacterial diversity versus overlying water nutrient concentration (DIN) at 3 sites.



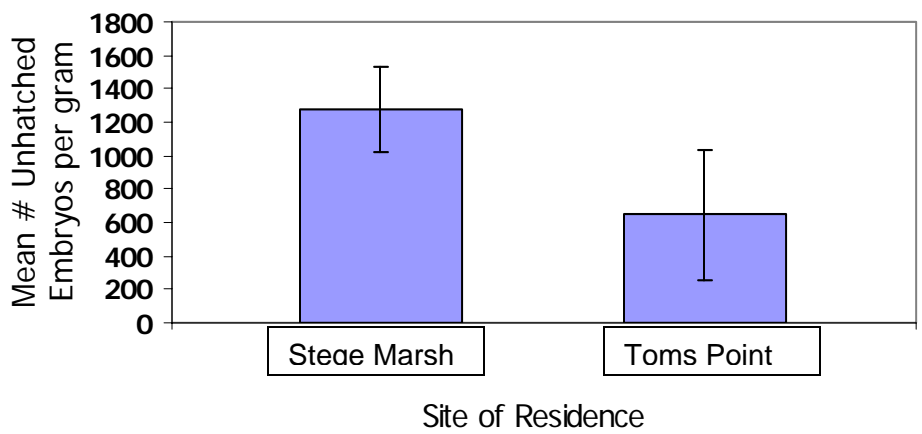
Affect of phthalates & TCE on bacterial diversity at Carpinteria

- Total coliform concentration was correlated with bacterial community composition and urbanization in the Santa Barbara area. Methods that are 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. Specifically, bacterial communities varied by host source, diversity increased with size of source, and there appeared to be source-specific peaks. These promising results indicated that *T-RFLP* could be a tool in microbial source tracking, but we still need to identify source-specific peaks. This study was coordinated by the Southern California Coastal Water Research Project (SCCWRP), and forges an important collaboration between SCCWRP and PEEIR.
- We are determining whether the microfauna in mudsucker digestive tracts collected from contaminant and reference sites differ. If we detect differences, we will determine whether the differences are related to diet (which might reflect a pollutant impact) or stress or other physiological disruption more directly related to the effects of pollutants.
- We are determining whether arsenate reduction could be used as possible indicators of impacted estuaries. Oxyanions of arsenic, selenium and other metalloids are actively cycled through microbial communities, where they serve as both electron acceptors and as electron donors for a select group of bacteria. We tested the hypothesis that microbial communities at contaminated and reference sites would in the rates at which these oxyanions were metabolized. We found that arsenate is reduced rapidly in all of the sediments we tested, and the reduction rate appeared slowest at a site from a contaminated marsh, Stege. There also were subtle differences in rates that might be detected by shorter incubations with more highly resolved sampling, and the protocol is being further refined.
- We are testing whether nitrification would be a potential indicator of impacted estuaries, by comparing ammonia oxidizer populations at various sites. 16S rRNA genes in DNA extracted from sediment samples was amplified with primers nit a and nit b gel. These amplicons were then used as templates for amplification with GC-clamped variable 3 region, universal primers AM1 and AM2. The amplicons were separated by denaturing gradient gel electrophoresis. Specific ribotypes were found to be shared among sites and overall patterns were quite variable. We were able to obtain ammonia oxidizer sequences from our samples, and the next step is to compare the results with contaminant load at the different sampling sites.

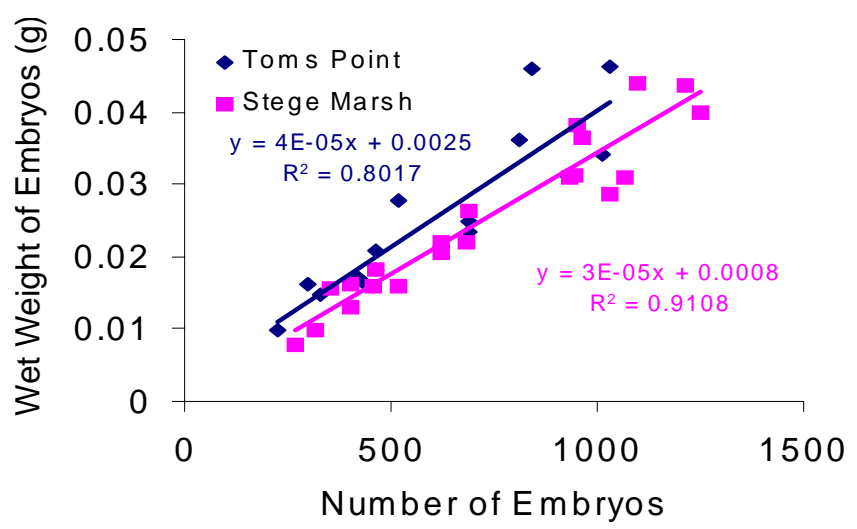
## **Invertebrates**

*Ecosystem condition.* Abundance and diversity of infauna appeared to be related to toxic exposure, and results were roughly comparable to those obtained the previous year. 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.

*Potential Indicator of Stress & Reproductive Impairment in Crabs.* Field outplants of crabs were conducted at sites along contamination gradients at impacted sites, as well as at reference sites in concert with the BRC team. Preliminary results indicate that crab reproduction may be impaired at impacted sites, and the project is being scaled up for the next field season. For example, females carried smaller broods at a contaminated site (Stege) than at a reference site (Toms Point), indicating that females produced fewer eggs or lost them. In another example, embryos weighed less at the contaminated site than at the reference site, indicating that females invested less energy per offspring, which in turn may affect hatching success and larval survival. Results for non-reproductive crabs, fish and clams also appear to be promising, and further studies will be conducted this summer.



Low clutch size at the contaminated site (Stege).



Low embryo weight at the contaminated site (Stege).

*Potential indicator of growth and reproductive impairment in clams.* Field experiments were conducted to determine whether growth and reproduction in the clam, *Macoma petalum*, were linked to biomarkers of stress (stress proteins, lysosome membrane damage, DNA damage) at different levels of exposure to contaminants. Preliminary data revealed that growth may not differ among the reference and contaminated sites, but it was markedly reduced at one of the stations at the contaminated site. Although the difference in growth was not related to temperature and salinity, it may be due to different levels of contamination, but this has yet to be examined.

*Potential indicator of ecosystem function.* We determined the link between detrital breakdown, an important ecosystem function, and levels of contaminants to test the hypothesis that the abundance of invertebrate decomposers would be reduced at contaminated sites, thereby slowing decomposition of plant litter. Our initial trial was conducted at a reference marsh and a contaminated marsh in San Francisco Bay. Station M at Stege Marsh, where high levels of soil contaminants occurred, had the slowest rates of decomposition, least richness and lowest abundance of invertebrate species.

## **Fishes**

*Censuses of individual health, population size & movement:* Censuses combined with mark-recapture studies provide valuable population size estimates, movement distances, and important population-wide trends in health (e.g. developmental abnormalities, sex ratios, disease). For example, fish size shows greater variation at our two most contaminated sites (Stege, Mugu) relative to other sites, and fish livers were larger at contaminated sites than at our reference site at Toms Point.

*Growth & Otolith Validation:* Efforts to accurately describe the development of *G. mirabilis* otoliths continue. Experiments are addressing the periodicity of microstructures and macrostructures, as well as how they relate to somatic growth. 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.

*Ecosystem Studies:* Combined with mark-recapture studies, whole ecosystem (community based) comparisons continue to be made among sites using a variety of taxa.

*Data Analysis and Integration:* Samples are continually being processed and analyzed. In the coming months these data will be integrated and used to develop population models that will help us evaluate the health of estuarine ecosystems.

## **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.

## **Conclusions**

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 creeks, and we are targeting these areas. Additional stations within sites have been incorporated to put the gradient in context to 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 decreasing intensive broad scale surveys to enable us to focus on two other priorities. First, we will continue to develop biomarkers during our field outplant experiments. 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 will continue to be conducted in collaboration with the RSC and BBC. All other promising indicators described above will continue to be developed. Second, we will concentrate on obtaining critical estimates of the population sizes, densities, reproductive seasons, and movements of our three model organisms. These data are needed to link individual measures of fitness to the population level and, ultimately, the ecosystem level. Sampling will be continued at the five sites sampled during this last field season.

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**

Ward, & KD Lafferty (2004), The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? *PLoS Biol* 2: 0542-0547.

### **Presentations**

Development of new estuarine ecosystem indicators at Carpinteria Salt Marsh, Sanibel-Captiva Conservation Foundation Estuarine Indicators Workshop, Sanibel, FL, A. Brooks.

Distinguishing human from natural influences on populations: dialectic diagnosis of environmental problems, California Estuarine Research Society, Bodega Bay, CA, S. Morgan, W. Bennett, L. Lewis, G. Cherr, R. Nisbet, S. Anderson.

Linking indicators of stress with reproductive impairment of crabs in West Coast wetlands, Gulf of Farallones National Marine Sanctuary Research Symposium, San Francisco, A. Briden, T. Visintainer, L. Judah, S. Spilseth, S. Morgan, G. Cherr.

Quantitative approach for assessing the ecological health of estuarine marshes: the Pacific Estuarine Ecosystem Indicator Research Consortium (PEEIR). Estuarine Research Federation, S. Morgan, B. Bennett, S. Williams, T. Grosholz, G. Cherr, R. Nisbet, S. Anderson.

### **SUPPLEMENTAL KEYWORDS:**

indicators, ecology, estuaries, wetlands, health, toxics, nutrients, exotic species.