

YEAR 3 ANNUAL REPORT

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

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Research Category: EaGLE Program

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

RESEARCH OBJECTIVES:

The overall aim of the proposed research 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. 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 food chain, and are major drivers of the biogeochemistry of the tidal marsh. The specific objective of this section is to uncover the chemical, biochemical, and biotic markers that herald the bioavailability, transport, and/or biotransformation 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 Biochemistry (Fan)

In our second full field campaign mounted in summer 2003, *Spartina* and *Salicornia* shoots and roots were again obtained from Stege, Carpinteria, and Walker marshes, including the expanded list of PEEIR-wide established stations. 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, and both the 2002 and 2003 data are being related now by several groups via the PEEIR data-sharing website. In general, the metabolites must be linked to biochemical pathway information, so we are primary end-users of this type of data, relating to other parameters observed by BBC, EIC, BRC, as well as the legacy contaminants data from the UC-Toxics grant to S.Anderson (analyses by the laboratories of T. Young and H. Hwang). In addition, suites of metals were again analyzed in the *Spartina* and *Salicornia* roots and shoots, in collaboration with Peter Green (Higashi project), and both the 2002 and 2003 data are uploaded to the data-sharing website. Analyses of the exposure marker, phytochelatin, is currently underway. Data analyses of all the above is also underway, in part focusing on the cues reported last year. For more definitive relationships of markers and indicators, growth-chamber studies with *Spartina* and Cd exposure in seawater-salinity sand culture was conducted, coordinating with the Williams group for IRGA and leaf reflectance spectra, Higashi and Green for shoot salt exudates collection and analysis, Cherr

group for toxicity testing of the exudates, and Scow group for microbial communities in the sand, essentially a type of “microcosm” experiment around the plant-soil system. Status and results from these collaborative analyses are reported by the appropriate research group. For our analyses, the phytochelatins show a clear induction by Cd treatment, which relates to the actual Cd accumulation as predicted, and the metabolites show some interesting trends, while chlorophyll content was not affected. Full pigment analyses to relate to reflectance spectra is underway. Also just started is an analogous experiment with *Salicornia*, while another set of *Spartina* is currently being raised at BML for a future Cd-nitrogen interaction experiment.

Organics & Metals Analyses & Role of DOM (Higashi and Green)

For the 2003 field sampling, sediment samples were collected from Stege, Carpinteria, China Camp, and Walker 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, Lafferty). Total metals analysis of these samples are currently underway, while corresponding *Spartina* leaf salt exudates (where appropriate) were sampled, analyzed, and uploaded to the PEEIR data-sharing website. Organic matter analyses revealed large variations in chemical substructure groupings, indicating vascular plant (lignic/cellulosic ratio), algal (cellulosic/peptidic ratio), and non-plant (peptidic/lignic+cellulosic ratio). Some of these relationships are easy to explain (e.g. vascular plant markers were much higher at vegetated stations), while many others must be further compared to the contaminants, plant, microbial, and invertebrate data that is emerging from the PEEIR 2003 field campaigns. In particular, comparison to PLFA data being generated by the Scow group would traditionally be considered complementary to organic matter structural analyses, and this is being pursued.

Bacterial Community Composition (Holden)

Terminal restriction fragment length polymorphisms (TRFLPs) were assessed to characterize bacterial community composition and diversity independent of culturing. Sediment cores were taken from low (within channel), medium (midpoint on bank) and high (on bank, in vegetated areas) locations at several stations within Stege, Walker, China Camp, Tom’s Point, and Carpinteria Marshes and were extracted for total DNA. Prior to extraction, cores were homogenized and split with other investigators. Extracted DNA was purified and phylogenetically conserved regions (encoding 16S rRNA) were amplified using fluorescently end-labeled universal primers. Amplicons were restricted using HhaI and sorted in an automatic sequencer. Restriction fragment lengths and peak heights comprise the raw data. All raw data from years 2001-2003 were normalized and aligned simultaneously so that community composition can be compared across sites and over time. Species richness and Shannon diversity were calculated. Generally, we observed that microbial communities differ with elevation in the marsh, where “high” communities are distinct from low and medium. Where chemical gradient data are available (mainly 2002), we see a decrease in microbial community species richness and diversity with increasing concentration in specific metals and organic pollutants; the patterns are not observed for all pollutants.

In Carpinteria Salt Marsh, we have also been studying denitrification, as was suggested by one of the original reviewers of the PEEIR proposal. Our work has specifically focused on

understanding the relative importance of particle-associated versus free-living bacteria in sedimentary denitrification. Our work shows that particle-associated denitrification populations are more abundant and possess higher denitrification capacity compared to their planktonic counterparts. Consistently with known controls in soil environments, total carbon and denitrifier population size are important determinants of denitrification potential in estuarine sediments. Furthermore, particle size appears to influence the population sizes of denitrifiers and denitrification potential. The graduate student conducting this work (Yiping Cao) is currently analyzing the phylogenetic and functional composition of denitrifier communities to discern genetic differences in space at the marsh that could control denitrification potential.

Occurrence, Bioavailability & Effects of Current-Use Pesticides (Kuivila)

The overall focus of the study is to examine the occurrence, bioavailability and toxic effects of current-use pesticides in wetlands. Both field sampling and laboratory studies are being used to determine the factors controlling pesticide occurrence and desorption from sediments and how these processes influence bioavailability and biological effects.

In terms of field sampling, sediment samples from all five marshes were analyzed for 34 current-use pesticides and DDT and its degradates to determine the role of toxic contaminants as stressors to wetland ecosystem health. Although current-use pesticides are typically more hydrophilic than historically-used organochlorine pesticides and would be expected to be primarily in the water, previous studies by the Kuivila laboratory have shown that sediment concentrations are often well-above equilibrium-predicted values. A variety of current-use pesticides were detected in sediments at both Carpinteria and Stege Marshes but the patterns were very different. Three insecticides were detected at Carpinteria Marsh: two pyrethroids (bifenthrin and permethrin) and one organophosphate (chlorpyrifos). A concentration gradient was observed with the highest concentrations at a site on the outer edge of the marsh and concentrations decreasing at downstream sites. The highest concentrations of bifenthrin, permethrin and chlorpyrifos were 24, 51 and 31 ng/g dry weight, respectively. The pyrethroid insecticides, bifenthrin and permethrin, are applied to lettuce, nurseries/greenhouses and strawberries in the watershed and are of concern because of their high toxicity to aquatic organisms, especially fish.

In contrast, a variety of herbicides were detected in the sediments of Stege Marsh. The two thiocarbamate herbicides found most frequently and at the highest concentrations were EPTC and molinate. The most inland site had the highest concentrations with EPTC at 205 ng/g dry weight and molinate at 61 ng/g dry weight. Other herbicides were detected include cycloate, napropamide, and pebulate. An analysis of potential sources of these herbicides using GIS and the California Department of Pesticide Use Database suggests that agricultural or urban use are not likely sources of these herbicides. Since registration information indicates that Stauffer or Zeneca is a registered producer of these herbicides, it is possible that these chemicals were manufactured at the plant located next to the marsh site and is the source of the sediment residues. These herbicides are relatively volatile and it is unusual to find elevated concentrations of any of these herbicides on sediments, let alone three or four of them. Very little information is available on the uptake and effects of these herbicides on plants.

No detectable levels of pesticides were found at China Camp, Tom's Point and Walker Creek. The lack of pesticides can be explained by the lack of agricultural or urban pesticide use in the watersheds for these marshes.

Further studies will examine the bioavailability and biological effects of the pyrethroid insecticides in collaboration with the BRC component (Cherr and Anderson). Pyrethroid insecticides are of concern because their use is increasing and they are toxic to aquatic organisms at much lower concentrations than the organophosphate and carbamate insecticides they are replacing. Bifenthrin and permethrin cause nervous system damage to invertebrates by blocking of sodium channels and inhibit ATPase enzyme production. The elevated concentrations of bifenthrin and permethrin at Carpinteria Marsh correspond with observed exogastrulation of embryos (BRC project of Cherr). Laboratory studies with pesticide spikes and elutriates will be used to determine if there is a cause and effect relationship.

At Stege Marsh, the two thiocarbamate herbicides detected, EPTC and molinate, could be adversely affecting *Spartina*. US EPA registration documents express concern that EPTC is toxic to both monocot and dicot plant species and that, although EPTC is a pre-emergent herbicide, it may cause some phytotoxic damage and growth effects on established plants. Further discussion is needed to determine what effects should be examined and how to appropriately design experiments to address this question.

Laboratory studies on wetland plants were focused on the effects of diuron, a urea herbicide. 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 this may provide a continuous source of the pesticide to plants and aquatic organisms in wetlands. In conjunction with the Williams (EIC) project, a series of laboratory studies are underway to measure the uptake and metabolism of diuron by native *Spartina* and *Salicornia*. Sand cultures are spiked with different concentrations of diuron and the effects estimated by measuring resulting changes in plant pigments and photosynthesis stress. Concentrations of diuron and its metabolites will be measured to estimate the uptake and metabolism of diuron by the plants. These results will be compared to the response of the plants in terms of phytochelatin (Fan), pigments of the violaxanthin cycle (Higashi), and Pulse-Amplitude Modulated (PAM) fluorescence (Williams, EIC).

The sediment samples previously analyzed for other current-use pesticides are being analyzed for diuron and its metabolites. Based on the sediment concentrations and the results of the laboratory studies, field samples of *Spartina* will be collected for further analysis of diuron and its metabolites.

Mercury Exposure & Bioaccumulation (Nelson)

Estuarine production of methyl mercury from inorganic mercury occurs mainly in anoxic sediments via the action of a spectrum of anaerobic bacteria. Methyl mercury may be "biomagnified" at higher trophic levels in food chains and can reach levels that trigger health warnings. The original goal of this study was to determine whether there existed differences in bioavailability of inorganic mercury or in Hg-methylation rates for our target estuaries, which included: Walker Creek, with known Hg-contamination from mining; Tom's Point, a putatively

non-impacted control site; Stege, impacted by the typical Hg-load from San Francisco Bay. An initial survey of sediment [Hg] at Walker Creek revealed unexpectedly high variability within and between stations. Therefore, a decision was made to redirect the study toward Hg concentrations in the shore crab, *Pachygrapsus crassipes*. Between various sites this indicator showed more than a 50-fold difference in concentrations, and there were very different tendencies for trophic magnification in larger crabs depending on the site. Stege crabs showed moderate Hg levels (200 ppb) and no tendency toward biomagnification in larger individuals. Similar concentrations occurred in small crabs from Walker Creek and Tom's Point, but larger individuals from these sites contained up to 1100 ppb and showed equivalent strong tendencies toward trophic magnification. These very different patterns of biomagnification may highlight food-chain differences that contribute to the relative impairment or health of a specific estuary. Additional *Pachygrapsus* surveys are being used to assess the impact of Walker Creek sediments on various estuarine and intertidal food chains throughout Tomales Bay.

Microbial Communities (Scow)

composition of sediment samples from the PEEIR estuaries. The method used was phospholipid fatty acid (PLFA) analysis, a fingerprinting method that analyzes methylated derivatives of primarily membrane-associated fatty acids. PLFA analysis provides an estimate of microbial biomass, biomarkers representing specific groups of microorganisms, as well as a fingerprint representing the composition of the microbial community. Advantages of this method are that the data are quantitative and thus lend themselves to multivariate statistical analysis, and the analytical techniques are relatively rapid and reproducible.

Estuaries sampled included Stege Marsh, China Camp, Walker Creek and Mugu Lagoon (2002 only). PLFA fingerprint patterns were statistically analyzed using multivariate statistical methods (correspondence analysis, constrained ordination analysis). Comparisons were made among samples collected in summer 2002 and 2003 at two spatial scales: (1) between estuaries, (2) within estuaries at different depths (e.g. within the channel ("low"), within the intertidal zone ("middle"), and the in unsaturated zone above the water table ("high")). Further comparisons were made between community fingerprints and environmental variables (pollutant concentrations, chemical and physical properties of the estuaries) within sites.

There were no strong differences in microbial community composition (by PLFA fingerprint) among estuaries, nor were there large differences within estuaries between the two years (Fig 1, 2). There were distinct differences, however, between the saturated channel and unsaturated zone samples in all estuaries. The PLFA fingerprints indicated that communities in the saturated channels across the sites were more similar to one another than were saturated and unsaturated samples within the same estuary. The intertidal zone samples, however, varied by site. In some cases they were identical to the channel samples and in other cases more similar to the unsaturated samples. At Stege, the intertidal samples were distinctly different from the two other depths as indicated by the clustering of these samples in the upper right hand quadrant of the Fig. 2A. These results suggest that environmental conditions associated with redox and relationship to the channel select for microbial communities that can be discriminated based on PLFA analysis. Thus unique biomarkers characteristic of each environment can be identified.

Within sites, pollutant concentrations correlated with the biomass of certain microbial groups (Fig. 3). In Stege and Carpinteria, Gram-positive biomarkers were negatively correlated to phthalate concentrations: in Carpinteria, sulfate reducer biomarkers positively correlated to TCE concentrations. Total PLFA (i.e. biomass) was negatively correlated to copper in the unsaturated samples and molybdenum at all locations in Stege Marsh. Also, at Carpinteria, fungal biomass increased with increasing concentrations of phthalate at high elevations; actinomycete biomass was negatively correlated to phthalate concentrations at low and medium elevations. Our results suggest small-scale variation in environmental factors (i.e. distribution in the channel profile) should be considered when interpreting potential effects of anthropogenic stressors.

The PLFA fingerprinting of microbial communities for both sampling years (2002 and 2003) has been completed and the data is available upon request. DNA fingerprinting data by T-RFLP have been generated for the same samples by Holden's group at UCSB and will be compared with the PLFA data to ascertain relative strengths of the two complementary approaches for analyzing microbial communities.

Fate and Effects of Ciprofloxacin in Estuarine Sediments

The bioavailability of contaminants in ecosystems is an important factor determining the impacts of these contaminants on biotic communities. The antibiotic ciprofloxacin is subject to adsorption to sediment particles via hydrophobic and ion exchange mechanisms. As part of our project on the fate of antibiotics in estuarine systems, we determined sorption coefficients for ciprofloxacin in Stege Marsh, China Camp, Mugu Lagoon and Walker Creek sediments. Sorption isotherm data were fit to Freundlich and Langmuir isotherm models. Ciprofloxacin sorbed strongly to all estuarine sediments (Fig. 4). Sorption was pH dependent because ciprofloxacin is a zwitterionic compound; thus pH must be considered when bioavailability is estimated. The National Reconnaissance of Pharmaceuticals, a recent survey of contaminants performed by the USGS in surface and groundwaters, failed to find significant concentrations of antibiotics in many places. The fact that ciprofloxacin (as well as other antibiotics) is strongly sorbed suggests that antibiotics may be present in the environment but associated with surfaces rather than in solution.

Additionally, microcosm experiments have been completed to measure the effects of a gradient of ciprofloxacin concentrations on the microbial communities of these same sediments. The PLFA fingerprints have been obtained and are currently being analyzed. These data, together with the results from the sorption experiments, will allow us to estimate potential effects of ciprofloxacin and on microbial communities in sediments. This project will be further extended to explore the distribution of antibiotic-resistant strains in the sampling sites. A manuscript describing the sorption data will be submitted shortly.

FUTURE ACTIVITIES:

The BBC group will spend much of the winter assisting with multivariate statistical analyses. Chemical and biochemical stressor data are a crucial underpinning of indicator assessment. *Fan*. We plan to conduct the above-mentioned *Salicornia* Cd experiment in sand culture, followed by the *Spartina* Cd/N experiment with the full complement of collaborators. The latter will test the effect of osmoregulation on Cd via the major osmolyte, glycinebetaine (a N-

containing compound) on Cd accumulation in plant tissues, also testing associated phytochelatin production, Cd-salt exudation, metabolite profiles, pigment composition and associated reflectance spectra, microbial communities, and other parameters as with the prior *Spartina* sand culture “microcosm”-type experiment. As these are intensive experiments and analyses, there are presently no plans for a third field sampling campaign for plants, and the focus will be on obtaining closure on indicators via the growth chamber experiments which are essential for interpretation of the field plant data, and completion of analyses and products (manuscripts), of which several are in the pipeline.

Higashi and Green. Plans for the coming year focus on completion of all scheduled metals analyses on currently archived samples, collaboration with the planned plant microcosm experiments, more detailed organic matter analyses from the archived sediments of 2002 and 2003, including analyses on the currently-being-installed GC-parallel analytical MS / isotope ratio MS (used to relate organic matter substructure to trophic level origins), and data mining/data relationships of the above with all appropriate PEEIR data. We are collaborating on several manuscripts, and these will be a key focus of the coming year.

Holden. We are currently in the process of co-evaluating TRFLP and other data (PLFA, toxicity, chemistry) to discern correlations along gradients in space at Stege and Carpinteria for a focused 2002 study. Our 3-year dataset will be used in a multi-year, multi-site comparison with other PEEIR data. When 2003 data on Carpinteria Salt Marsh chemistry are available, we will investigate possible relationships between denitrification and chemical gradients. One oral presentation has been given based on this work in the UC Natural Reserve System 2004 Mathias Symposium. Two manuscripts are under preparation, to be first-authored by Yiping Cao (doctoral candidate); tentative titles are “Significance of Particle-Associated Bacterial Communities in Denitrification in Carpinteria Salt Marsh” and “Phylogenetic and denitrification community analysis along chemical gradients in Carpinteria Salt Marsh”.

Kuivila. There will be a comparison of DDT and degradate concentrations with sediment analyses of legacy chemicals being conducted by Young and Hwang on the UC-Toxics funding. There are plans to collaborate with Carol Vines and Gary Cherr on laboratory studies to examine effects of bifenthrin and permethrin on sea urchin embryo development. We gave them standards for the two pyrethroids to use for initial testing. Depending on the results of laboratory tests, we would like to resample Carpinteria this summer and do elutriate studies on the sediments at sites A, B, C, and D.

With regard to diuron analyses, Seema Datta is currently developing and validating extraction and analysis methods for diuron and 3 metabolites in water, sediments/sand, and plant tissue. Testing of the current sediment method for applicability to diuron and its metabolites will occur in May. If it works, analysis of 2003 sediments will be completed by June. We are also finalizing details of the diuron exposure studies with Susan Williams and Jennifer Kunzelman. Results from the Fan growth chamber studies indicate that we need to use many more plants than originally planned to overcome the natural variability of the plants in culture. This requires the experiments to be conducted at BML whenever possible. In addition, the high mortality of *Spartina* collected in the fall means that the plant studies can only be conducted from spring to early fall. Planned for mid-May to mid-June are initial experiments with *Spartina* at 3 diuron concentrations and control conducted in growth chambers at UCD while the next batch of plants

acclimates at BML. Planned for mid-July to early-August is the next exposure study at BML so we can use more plants. Results from these studies will determine if we would repeat them a third time, switch over to *Salicornia*, or combine with other stressors.

Nelson. Because methyl mercury is the form that bio-accumulates in food chains and poses the greatest human health risk, it is logical for a researcher (or reviewer) to ask: “why are you measuring total mercury, rather than the methyl-form, in crab muscle?” One answer is that in muscle flesh of fish in excess of 90% of total mercury measured is methyl mercury. For our survey work we have assumed that this is probably true of crabs as well. Over this next year we test this assumption by making “splits” of a few of our crab muscle samples. One portion will be analyzed for methyl mercury by an outside lab (a time consuming and expensive process) and the other portion will be analyzed “in house” for total mercury using our DMA80 analyzer. Presumably mercury in crab leg muscle is in some sort of organo-mercury form. Regardless of whether this is as methyl mercury, mercury bound to –SH moieties of protein, etc. it is assumed that it will be available to bio-accumulate higher up in food chains, but it is important to gain more information on the exact form in the crab muscle.

To gain more insight on bioaccumulation of mercury in food chains in our estuaries of choice we propose the following plan for the coming year:

- In conjunction with Mark Page at UCSB we will have stored frozen crab legs that correspond to our Fall 2003 data for Tom’s Point and Stege split and analyzed in parallel for 15N and total mercury. This will be done over a full size spectrum of *Pachygrapsus* individuals from both sites and is focused on determining whether the very different [Hg] vs. size patterns at the two sites occur in spite of large crabs occupying the same trophic level at each site or whether food web differences contribute to the different patterns.
- We will finish analyzing roots and shoots of plants collected at different sites. We have strong preliminary data suggesting that roots of *Spartina* and *Salicornia* have much higher total mercury content than corresponding shoots. Whether there is significant accumulation difference between *Salicornia* vs. *Spartina* should be apparent by the conclusion of this analysis. This, in turn, may have implications for remediation strategies (high vs. low marsh) that would favor greater or lesser plant accumulation of mercury.
- We will analyze stored outplant clam samples for mercury in support of other PEEIR projects. Likewise, stored fish muscle samples (mud sucker) collected at our sample sites will be analyzed to determine whether bio-magnification differences detected in crabs are mirrored (or further exaggerated) at this trophic level.
- We plan to locate estuaries that are as comparable as possible to Tom’s Point and Walker Marsh in the upper reaches of Tomales Bay and on Point Reyes. These, plus our two normal study sites, will then be sampled over a relatively short time period (less than a week) for legs of *Pachygrapsus*, taking care to get -- in so far as possible -- a full size spectrum from each site. The goals here are: to continue to collect data to determine whether there is seasonal variation in biomagnification at our target sites and to determine the extent to which the presumed “point source” mercury contamination from Walker Creek has extended around Tomales Bay. This analysis will be done once in the spring and once in the fall of 2004.

Scow. This summer we expect to begin a study of the distribution of antibiotic resistant microbial communities in estuarine sediments. The project will involve sampling and performing antibiotic sensitivity testing. Sites contemplated for this effort are Stege Marsh, China Camp, Walker Creek and Tom's Point. The survey will involve both culture and non-culture based methods. We plan to target important antibiotics both in clinical and veterinary applications. The initial experiments will target antibiotics previously detected in streams and sediments, as reported in the USGS pharmaceutical survey.

Other activities for the coming year are:

- With Holden group (UCSB), we are making a comparison of estuarine sediment microbial communities across the PIEER sites using PLFA and T-RFLP data. This will also lead to an evaluation of biomarkers of microbial communities: what do different methods compare, and what are relative strengths and weaknesses of each.
- We have analyzed litterbag samples provided by Linda Judah. The raw data is now available and statistical analysis will be completed by the end of April. We expect to be able to correlate community composition to sites, rates of decomposition and litter quality.
- We have analyzed samples from the potted *Spartina* experiments (Sarah Norris and Teresa Fan). The raw data is available and statistical analysis will be completed by the end of April. We expect to correlate changes in PLFA fingerprints to a gradient of cadmium concentrations.

PUBLICATIONS & PRESENTATIONS

Publications

Fan, T.W-M., Lane, A.L., Chekmenev, E., Wittebort, R.J., Higashi, R.M. 2004. Synthesis and Physico-Chemical Properties of Peptides in Soil Humic Substances, *Journal of Peptide Research* 63: 1-12.

Fan, T.W-M., Lane, A.L., Higashi, R.M. 2004. An Electrophoretic Profiling Method for Thiol-Rich Phytochelatins and Metallothioneins. *Phytochemical Analysis*, in press.

Green, P.G., Fan, T.W-M., Higashi, R.M. Submitted to *Environmental Toxicology and Chemistry*. Salt Exudations from Coastal Wetland Plants as a Measure of Metal Mobilization from Sediment.

LaMontagne, M. G. and P. A. Holden. 2003. Comparison of free-living and particle-associated bacterial communities in a coastal lagoon. *Microbial Ecology* 46: 228-237.

LaMontagne, M.G., I. Leifer, S. Bergmann, L. Van De Werfhorst, P. A. Holden. 2004. Assessment of the effect of marine oil seeps on sediment bacterial diversity and community structure by PCR-TRFLP. *Environmental Microbiology*, in press.

Presentations

Fan, T.W-M. and Higashi, R.M. 2003. Metabolomic Applications of NMR and MS: Linkage to Proteomics and Functional Genomics. Invited talk at the Pittsburg Conference, Orlando, FL, March, 2003.

Fan, T.W-M., Higashi, R.M., Lane, A.L. 2003. Metabolomic Application of NMR and MS: Tools for Broad-Screening Metabolic Pathways. Platform presentation at the American Chemical Society National Meeting, New Orleans, April, 2003.

Fan, T.W-M. 2003. Approach to Regulatory Metabolomics. Invited talk to the National Institute of Environmental Health Sciences, Research Triangle Park, NC, November, 2003.

Fan, T.W-M., Higashi, R.M., Lane, A.L. 2003. In Vivo & In Vitro Application of NMR & Mass Spectrometry in Environmental Stress Metabolomics. Invited talk to the NIEHS-organized Metabolic Profiling: Application to Toxicology and Risk Reduction - An International Conference, Research Triangle Park, NC, May, 2003.

Higashi, R.M., Fan, T.W-M., Green, P.G. 2003. Vascular Plant - Pollutant Relationships in California Tidal Marshes: Implications for Ecosystem Indicators. Society for Environmental Toxicology and Chemistry, Austin, TX, November 2003.

Higashi, R.M. 2003. Prospects and Perspectives from Two Decades of Metabolite Profiling by Mass Spectrometry. Invited talk to the Univ. of Louisville Chemistry Dept. Seminar Series, February, 2003.

Holden, P.A. and M. G. LaMontagne. 2003. Bacterial communities as indicators of estuarine ecosystem health and links to watersheds. Estuarine Research Federation, Seattle WA. September 17, 2003.

Holden, P.A. Bacterial communities as indicators of estuarine ecosystem health and links to watersheds. Invited talk to the Coastal and Estuarine Processes seminar series, University of Maine, Orono, Soil Science Department. September 5, 2003.

Yiping Cao, Significance of Particle-Associated Bacterial Communities in Denitrification in Carpinteria Salt Marsh. Oral presentation and abstract. 2004 Mathias symposium, The University of California Natural Reserve System, February 27-29th, 2004. Bodega Marine Laboratory, California.

SUPPLEMENTAL KEYWORDS:

bioavailability, transformations, biogeochemistry, selenium, mercury, pesticides.