Interactive Effects of Metals and PAHs on Benthic Food Webs

Kevin R. Carman (PI)
Dept. of Biological Sciences
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-1761 fax: 225-578-2597 email: zocarm@lsu.edu

Co-PIs
John W. Fleeger
Dept. of Biological Sciences
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-1240 fax: 225-578-2597 email: zoflee@lsu.edu

Robert P. Gambrell
Dept. of Oceanography and Coastal Sciences
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-6426 fax: 225-578-6307 email: cowgam@unix1.sncc.lsu.edu

Ralph J. Portier
Institute for Environmental Studies
Louisiana State University
Baton Rouge, LA 70803
phone: 225-578-4287 fax: 225-578-4286 email: rportie@lsu.edu

Award Number: N00149910023
http://www.biology.lsu.edu/faculty_listings/fac_pages/kcarman.html

LONG-TERM GOALS

Our long-term goals are to understand how complex mixtures of contaminants influence benthic communities at the levels of microorganisms, microalgae, invertebrate grazers, and fish predators. In particular, we are interested in how contaminants influence foodweb interactions among these groups of organisms.

OBJECTIVES

Our research examines the interactive effects of metal (Cu, Cr, Cd, Hg, and Pb) and diesel-fuel contaminants on the benthic food web of a coastal salt marsh, the specific role that Cu plays in this suite of contaminants, and how hypoxia influences the manifestation of toxic effects. Specifically, we are examining how diesel and metal contaminants interact to influence the microbial (bacteria and microalgae), invertebrate, and juvenile fish components of the benthic community, and how their interactions influence trophic relationships among organisms. Previous studies have focused on either the ecotoxicological effects of metals *or* the effects of hydrocarbons, but essentially nothing is known about how these two classes of contaminants interact. The modes of toxicity of hydrocarbons and

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metals are quite different, and individually they may elicit different, sometimes opposite, ecological responses. Impacted field sediments, especially in harbors, are typically contaminated with both metals and hydrocarbons, and thus ecological impacts may be a consequence of their interactive effects. Our experimental approach to this problem will provide fundamental information on the ecological manifestations of metals-hydrocarbons interactions, and provide the basis for making ecologically sound decisions concerning appropriate bioremediation or mitigation strategies for contaminated field sites.

APPROACH

Experimental work is divided into two components: microcosm experiments to examine contaminant effects on benthic microbes and grazers, and laboratory experiments to examine contaminant effects on fish predation on benthic invertebrates. As proposed, two major microcosms experiments were performed in year 1 to examine responses of, and interactions between microbes and meiofauna when exposed to metals and diesel-metal combinations under normoxic conditions. In the past year (year 2), a microcosm experiment was performed to determine the specific role of Cu in mixtures of contaminants, and the influence of fish bioturbators (*Gobiosoma bosci*) on the influence of contaminants. Microcosms to which fish were added will also be used to examine the influence of diesel-metal mixtures on fish predation on meiofauna.

The microcosm approach used is one that we developed to study the influence of contaminants on interactions between microbes and meiofauna of sediment food webs (e.g., Carman et al. 1997). The microcosms (15.2 cm i.d.) represent minimally disturbed, natural assemblages of benthic organisms and the sediment in which they live. Experiments are well replicated (n = 4-5 per treatment) and include a complete set of uncontaminated controls, which allows rigorous hypothesis testing.

Microcosms of marsh sediment were collected by hand from mudflats associated with a *Spartina alterniflora* saltmarsh near the LUMCON facility in Cocodrie, LA and treated with diesel and metal contaminants as generally described by Carman et al. (1997). In the experiment conducted in May/June of 2000, experimental treatments were as follows. Sediments were contaminated with known concentrations of Navy-relevant metals - Cu, Cr, Cd, Pb, and Hg and or diesel-contaminated sediments. Metal concentrations were manipulated to simulate the relative abundances of metals in San Diego Harbor (SDH) (219 ppm Cu, 178 ppm Cr, 1 ppm Cd, 51 ppm Pb, 1 ppm Hg; Kennish 1997). Each treatment was represented by 4 replicate microcosms, and exposures were 30 days. The treatments were:

Treatment	Description
Controls	No contaminants
Diesel	Diesel only
High Metals	Cu, Cr, Cd, Hg, & Pb; 6x [SDH]
Low Metals	Cu, Cr, Cd, Hg, & Pb; 2x [SDH]
High Cu	Cu; 6x [SDH]
High Metals-Cu	Cr, Cd, Hg, & Pb; 6x [SDH]
High Metals + Diesel	Diesel and Cu, Cr, Cd, Hg, & Pb; 6x [SDH]
Low Metals + Diesel	Diesel and Cu, Cr, Cd, Hg, & Pb; 2x [SDH]
High Cu + Diesel	Diesel and Cu; 6x [SDH]
Low Cu + Diesel	Diesel and Cu; 2x [SDH]
High Metals-Cu + Diesel	Diesel and Cr, Cd, Hg, & Pb; 6x [SDH]
Low Metals-Cu + Diesel	Diesel and Cr, Cd, Hg, & Pb; 2x [SDH]

Natural photoperiods and temperature were maintained. Samples were collected to determine: (1) bacterial biomass and community composition (collaboration with D.C. White, Univ. of Tennessee), (2) microalgal abundance, community composition, and productivity, (3) meiofaunal abundance and community composition, (4) meiofaunal grazing on microalgae, (5) sediment hydrocarbon concentrations (6) bulk sediment and acetate-extractable concentrations, (7) pore-water Cu and Cu speciation, (8) sediment carbohydrate and EPS concentration. In addition, we monitored O_2, and NH_4^+ concentrations in water overlying microcosms, and determined vertical profiles of oxygen and RedOx in sediment.

WORK COMPLETED

We are in the latter stages of processing samples, and early stages of processing data from the 2000 experiment. More complete results will be presented at the ASLO and Benthic Ecology Meetings in spring 2001. Results presented below are a few highlights of what we have found thus far.

We also used recently available Cu-speciation data to re-examined results from the first experiment in this project (Nov. 1998) concerning the influence of metals alone on the benthic community (Rodney Powell, Louisiana Universities Marine Consortium, is examining Cu speciation in microcosm sediments). A manuscript on this experiment has been submitted for publication.

RESULTS

The influence of Cu on the toxicological effects of contaminant mixtures:

From an experiment conducted in 1999, we found clear evidence that combinations of metals (Cu, Cr, Cd, Hg, and Pb) and diesel yield ecotoxicological responses that are distinct from either diesel or metals alone. High metal-diesel combinations produced a synergistic effect in which algal biomass and productivity were significantly reduced relative to treatments contaminated only with diesel. Individually, both high-metals and high-diesel concentrations dramatically reduced copepod abundance. The effect was even more pronounced when metals and diesel fuel were present simultaneously. Neither low metals nor low diesel significantly reduced copepod abundance, but, in combination, copepod abundance was reduced approximately six-fold.

Results from our recent experiment provide compelling evidence that Cu plays a critical role in the ecotoxicological effects of metal, and metal-diesel mixtures. We examined the vertical zonation of benthic microalgae at 2-mm intervals down to 1 cm (Fig. 1 shows data for the top 6 mm) to determine if contaminant effects varied with depth. Diatoms are the dominant microalgal taxon in these sediments, and cyanobacterial biomass is a distant second. As observed in previously, metals alone, with or without Cu, did not significantly influence microalgal (diatom of cyanobacterial) biomass, nor did metals alone significantly alter the vertical distribution of microalgae. As also repeatedly observed in previous experiments, diesel contamination resulted in the formation of dense and visually apparent algal blooms, consisting primarily of diatoms. The enhancement of diatoms was most pronounced in the top 2 mm of sediment. When diesel was combined with metals, the stimulatory effect of diesel on diatoms was reduced. However, the reduction in diatom biomass was statistically significant ***only in treatments where Cu was present*** (i.e., the complete metal mixture (Cu, Cr, Cd, Hg, and Pb), or Cu alone). Metals alone did not significantly influence diatom or cyanobacterial biomass or vertical distribution. Diesel alone led to a surficial cyanobacterial bloom, and an inverted vertical distribution of cyanobacteria relative to controls. When present in combination with diesel, Cu, either alone or in combination with other metals (+Met), significantly reduced surficial cyanobacterial blooms, and qualitatively altered the vertical distribution of cyanobacteria.

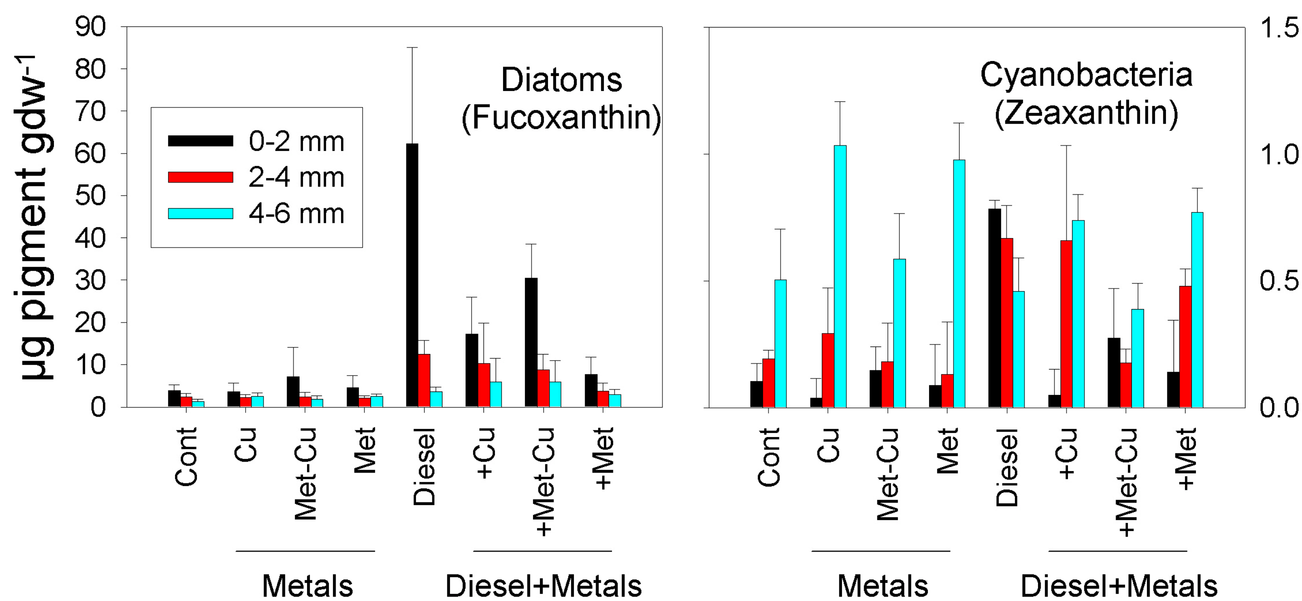

Figure 1: Vertical distribution of diatom and cyanobacterial biomass in microcosm sediments contaminated with various combinations of metals, diesel only, and diesel + metals.

Multivariate (Multi-Dimensional Scaling; MDS) analysis of benthic invertebrate major taxa indicated that Cu played an important role in the ecotoxicological effects of mixed metals, and metal-diesel combinations. MDS indicated that all invertebrate communities in treatments containing diesel (diesel alone, or diesel in any combination with metals) differed significantly from controls. Among metals-only treatments, High Cu and High Metals differed significantly from controls, but High Metals-Cu and Low Metals did not. In general, treatments contaminated with both diesel and Cu (High Cu + Diesel, High Metals + Diesel, and Low Metals + Diesel) differed significantly from the Diesel-only treatment; treatments lacking Cu (High Metals-Cu + Diesel and Low Metals-Cu + Diesel) and the Low Cu + Diesel treatment did not differ significantly from the Diesel-only treatment.

Metals Experiment:

Our initial experiment in this project was to examine the effects of metals *per se* on the benthic community. Four metal concentrations were considered: Control (no metals added), 1x [SDH], 3x [SDH], and 6x [SDH]. Metal concentrations of $\geq$ 3x [SDH] resulted in high mortality to deposit-feeding taxa (polychaetes in particular). Particle-feeding taxa (copepods and nematodes) were reduced in abundance only at 6x [SDH]. Essentially all dissolved Cu in sediment porewater was complexed with organic ligands in control and 1x [SDH] treatments, and thus very little Cu' (free inorganic Cu) was available (1.3-2.7 nM). In the 3x [SDH] treatment, organic ligands were saturated with Cu, and Cu' was higher in concentration (79.9 nM). In the 6x [SDH] treatment, [Cu'] was 199.5 nM, a direct proportional increase relative to total Cu added. A review of the literature revealed that [Cu'] in the 1x [SDH] treatment was below reported toxicity concentrations for copepods and nematodes, whereas [Cu'] in the 6x [SDH] treatment was within the range of reported toxic concentrations. These observations, in combination with our recent observations concerning the importance of Cu in toxicological effects (see above), support the hypothesis that Cu speciation in sediments is important in determining the toxicity of contaminant mixtures. The results also illustrate that particle-feeding taxa (e.g., copepods and nematodes) are strongly influence by exposure to Cu', whereas deposit-feeding taxa are more sensitive to metals associated with bulk sediments, or complexed with organic ligands.

IMPACT/APPLICATIONS

Our results indicate that metals and hydrocarbons interact to produce unique toxicological effects on benthic microalgae and invertebrates. Further, Cu is of particular importance to these metal-hydrocarbon interactions. It is also clear that Cu speciation in sediment porewaters strongly influences its toxicological effect. Preliminary data (not presented above) indicates that the presence of diesel in sediments enhances the concentration of Cu' in sediment porewaters, which may partially explain why metal-diesel mixtures are especially toxic.

TRANSITIONS

Roger Nisbet (UCSB) is heading a working group funded by the National Center for Ecological Analysis and Synthesis to develop models of contaminant effects on benthic food webs.
Results of our experimental work are being used in this on-going collaboration. We are also collaborating with D.C. White (Univ. Tenn.) to examine effects of contaminants on microbial community structure.

RELATED PROJECTS

1 - "How does produced water cause a reduction in the genetic diversity of harpacticoid copepods?" Minerals Management Service (J. Fleeger, PI and D. Foltz, Co-PI), $241,000, 1998-01. (An investigation into the influence of PAH's on genetic diversity of copepods.)

2 - "Relationships between benthic microalgae, grazers, and nutrients in a coastal salt marsh". NSF (K.R. Carman, PI.), $330,000. 1999-02. (Basic foodweb relationships in the system being studied in the ONR project).

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