

Ref. No.[UMCES]CBL 03-190

**Reporting Period:** 1/1/02-12/31/02 (year 3)**FUNDING:**

|                    |             |                |           |        |             |
|--------------------|-------------|----------------|-----------|--------|-------------|
| Current Sea Grant: | \$ 371,725  | Current match: | \$ 44,876 | TOTAL: | \$ 416,601  |
| Sea Grant to Date: | \$1,105,829 | Match to date: | \$126,681 | TOTAL: | \$1,232,510 |

**PROJECT TITLE:**

(CERP): Quantifying Ecological Risks of Contaminated Sediments on Living Resources in Supporting Decisions on Habitat Restoration Strategies in the Chesapeake Bay (Maryland Portion)

**PROJECT NUMBER:** R/CBT-38; NA96RG0501 SC7528007D

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**PROJECT OBJECTIVES:**

The CERP program seeks to develop capabilities to predict individual-level and population-level responses of estuarine animals to sub-lethal exposures of sediment-bound mixtures of contaminants. Specifically, we seek to relate individual biomarkers of exposure to population level consequences such as population growth rate or production. To achieve these goals we focus our work on the responses of two model species, the amphipod *Leptocheirus plumulosus* and the fish *Fundulus heteroclitus*, to sediments from two regions of concern within the Chesapeake Bay: Baltimore Harbor (MD) and the Elizabeth River (VA). The two species were chosen as model species to represent a suite of species that could be expected to respond similarly. For example, our research on *Leptocheirus* serves to document the cellular, organismal and population-level responses of a specific species to sediments containing complex mixtures of contaminants. Second the results for *Leptocheirus* are broadly significant as the life cycle of *Leptocheirus* is generally representative of a suite of benthic estuarine species that respond to the spring bloom by exhibiting a massive pulse of production that dominates trophic exchanges over the subsequent season. Similarly, *Fundulus* serves as a model fish species for many omnivorous species, and particularly for those that reproduce within the estuary. In all our work, we use an experimental approach involving exposure of populations of animals to sediments in large experimental mesocosms. The results

from these studies are analyzed and interpreted to yield predictive models of that integrate individual-level and population - level responses.

### **SUMMARY OF PROGRESS TOWARD PROJECT OBJECTIVES:**

In year three of the CERP project we initiated a series of experiments to test the effects of exposure to varying levels of sediment-bound contaminants on the individual-level responses and population dynamics of the estuarine fish *Fundulus heteroclitus*. These experiments mark the second phase of the work proposed for the CERP program. However, we note that analysis and interpretation of the results from the first phase, which focused on the amphipod, *Leptocheirus plumulosus* continue to provide insight.

Our work is a collaboration among scientists from three institutions from Maryland and Virginia; The University of Maryland Center for Environmental Science's Chesapeake Biological Lab, the Academy of Natural Sciences' Estuarine Research Center, and the Virginia Institute of Marine Sciences. The project is funded jointly by the Sea Grant Colleges in Maryland and Virginia. However, the individual research projects are fully integrated, and it is impossible to document our progress in reaching our research objectives without discussing all aspects of the research. Accordingly, in this annual report we summarize the progress toward all project objectives.

Research efforts in year three focused on a large experiment, F1, conducted from 6/17/02-10/15/02. The experiment sought to quantify the response of *Fundulus heteroclitus* cohorts to exposure to sublethal levels of contaminated sediments. In particular, we documented responses in the experiment with respect to (1) uptake of contaminants (2) behavior and bioenergetic partitioning, (3) production, (4) population dynamics, and (5) the expression of biomarkers. In related experiments we quantified the role of maternal transfer of contaminants in the early life history of *Fundulus*. The experiment involved exposing cohorts of *Fundulus* to a gradient (4 levels) of contaminated sediments at two different ration levels in 50 gal. flow through tanks. Each treatment combinations was replicated three times. The sediment treatment was created by mixing "control" sediments from Fishing Bay, MD with contaminated sediments from the Atlantic Woods site in the Elizabeth River, VA., to create a gradient of exposure. Food was provided at either 6% or 12% g.g.d<sup>-1</sup>. Cohorts of *Fundulus* were introduced to each tank by adding 28 'small' (< 56 mm) and 28 'medium' fish (56-70 mm). Sub samples of fish were removed on days 0, 30, 60, 91 and 120 to determine contaminant burden, biomarker expression, behavior and bioenergetic partitioning and production. In addition, eggs were collected daily from each tank to quantify reproduction during the experiment.

**We documented significant effects of the contaminant treatment on overall mortality and growth of fish in the experiment. There was a significant effect of sediment treatment on adult growth. Fish from contaminated treatments grew less than those from control treatments. Most egg production occurred in the first 28 d, and appeared to vary with sediment treatment. Sediment treatment did not affect hatching success. Larvae from contaminated sediments were larger at hatch than those from control sediments, and maintained this size advantage for the first 14 d. We conclude that sub-lethal population-level effects of contamination in our system were expressed most in somatic production rather than reproduction.**

## SUMMARY OF ACCOMPLISHMENTS:

### A. BIOACCUMULATION OF CONTAMINANTS

Our objectives in the *Fundulus* experiments were to: 1) verify and quantify accumulation of target analytes throughout the experiment time course, 2) determine relative magnitudes of exposure pathways, 3) compare exposures with field conditions, and 4) relate burdens to sublethal effects.

#### 1. Organics: (Dr. J. E. Baker, Eileen Beard and Amy Merten, CBL ).

Two classes of organic chemicals were measured during the *Fundulus* experiment. Polycyclic aromatic hydrocarbons (PAHs) are present in high concentrations in the Elizabeth River sediments and, therefore, are the likely dominant stressor in the experiment. PAHs are metabolized and parent compounds do not accumulate in fish tissue. Polychlorinated biphenyls are also present in the sediments, although at quite low concentrations, and are used as a tracer of bioaccumulation. Concentrations of 42 PAHs were measured in the water (dissolved and particulate separately) of the mesocosms on Day 0 and on Day 120 (Fig. 1). PAH levels were low and uniform in the tanks on Day 0, and increased dramatically (nearly 100-fold) in the tanks containing diluted Elizabeth River sediments on Day 120. A majority of *t*-PAH (sum of 42 compounds) was associated with suspended particles on Day 120, reflecting the relatively high levels of suspended matter in the tanks. In contrast, PCB levels in the mesocosm water remained relatively low (<4 ng/L) throughout the 120 day experiment (Fig. 2). This likely reflects low levels of PCB contamination in the Elizabeth River sediment. Concentrations of *t*-PCBs (sum of 85 congeners) on suspended particles on a mass/mass basis did not vary among the treatments. At the end of the 120 day F-1 experiment, fish from each treatment were analyzed for PCBs to assess the extent of bioaccumulation. In all cases, *t*-PCB levels in *Fundulus* after 120 days were two times greater than those initially (Fig. 3). However, PCB concentrations did not vary among the treatments, indicating that neither sediment contamination nor food ration significantly affect PCB accumulation dynamics. **We conclude that the PCB levels in the Elizabeth River were low and that the main route of PCB exposure in all treatments was the commercial food. Therefore, fish in all mesocosms received the same PCB exposure and accumulated similar amounts. Potential subtle**

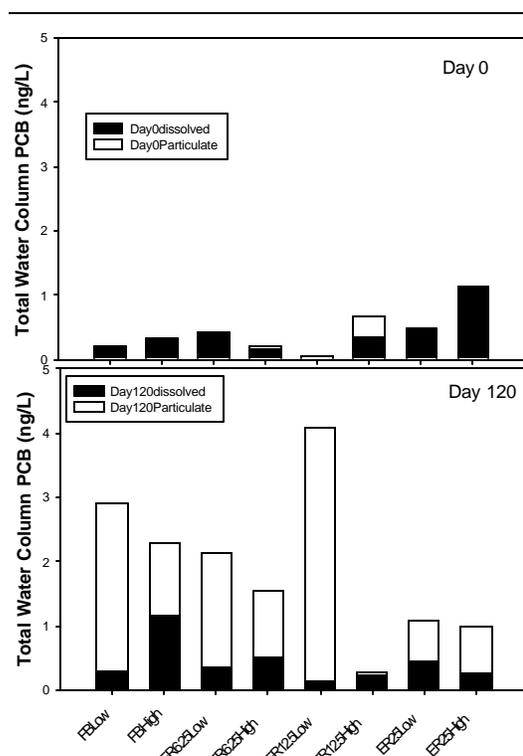


Figure 1 . PAH concentrations in CERP F1 mesocosm water

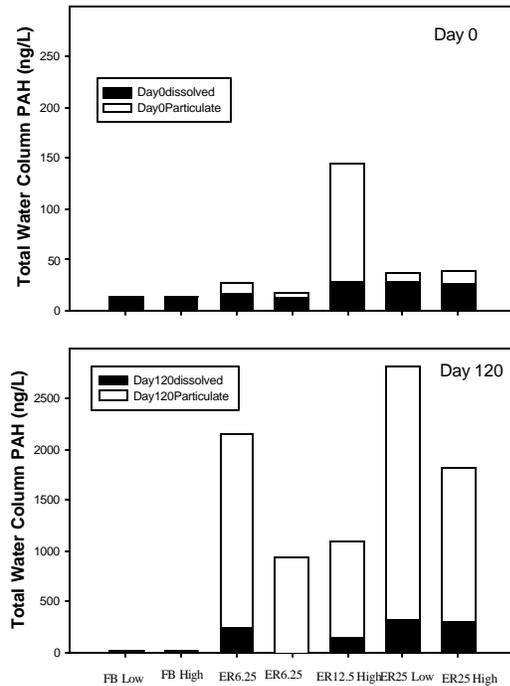


Figure 2. PCB concentrations in CERP F1 mesocosm water.

**effects on fish energetics caused by other stressors were apparently insufficient to alter bioaccumulation.**

In preparation for the second *Fundulus* experiment, clams were transplanted to the Elizabeth River to accumulate a realistic mixture of chemical contaminants. Replicate samples of clams deployed in the Elizabeth River are compared to control clams in Figure B4. PAH concentrations increased more than 100x, especially for the higher molecular weight PAH compounds (Fig. 4). **The resulting levels of PAHs in the clams should result in appreciable chemical challenge when the clams are fed to *Fundulus* in the CERP F2 experiment. Interestingly, the Elizabeth River clams accumulated modest levels of PCBs, only a three-fold enrichment over the controls. This is consistent with the PCB accumulation dynamics observed in the F-1 experiment, and supports the conclusion that PCB exposure from Elizabeth River sediments is small (analysis of sediments in underway).**

Day 120 F1-Fish  $\Sigma$ -PCB Accumulation

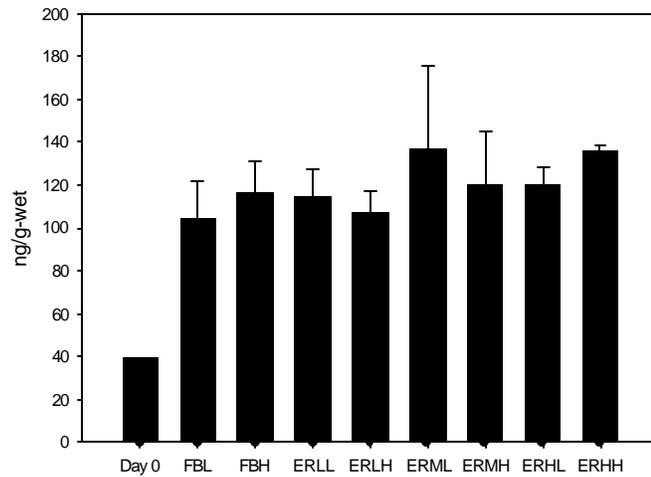


Figure 3. Concentrations of *t*-PCB in *Fundulus* after 120 days, CERP F1 experiment.

PCB Accumulation in Elizabeth River *Mercenaria*

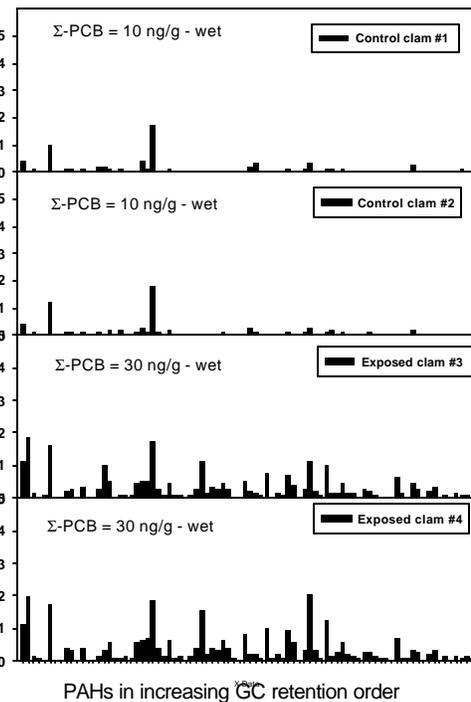
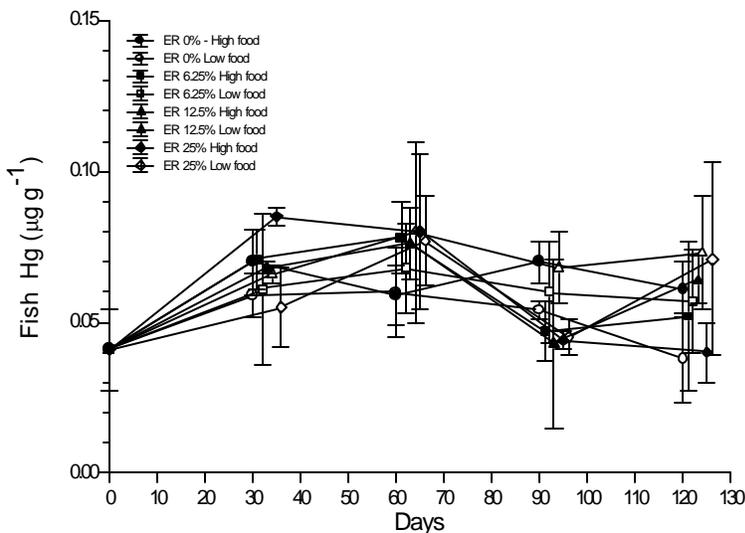


Figure 4. PAH concentrations in *Mercenaria* exposed in the Elizabeth River

## 2. Trace Metals: (Dr. G. Riedel, ANSERC).

Trace metal concentrations in the sediments were quantified. Arsenic in the control treatment (Fishing Bay sediment) averaged  $8.7 : \text{g g}^{-1}$ . Increasing the fraction of Elizabeth River sediment to the mixture to 25% raised the mean arsenic concentration to  $10.9 : \text{g g}^{-1}$ . There were no evidence of differences in the sediment between the two feeding levels. Mercury levels in the Fishing Bay sediment was ca  $16 \text{ ng g}^{-1}$ , and increased with increasing fractions of Elizabeth River sediment to ca  $90 \text{ ng g}^{-1}$  at 25% Elizabeth River sediment. Again there was no evidence for significant differences between the food levels.

We also quantified the concentration of trace metals in the overlying water. Arsenic(III) concentrations in the waters were generally minor, starting at ca  $0.05 : \text{g L}^{-1}$ , showing a maximum of ca  $0.1 - 0.2 : \text{g L}^{-1}$  near day 30, and declining later in the experiment, with no evidence for influences of the fraction of Elizabeth River in the sediment mix, or the food level. Arsenic (III+V) started in the range of  $0.5 : \text{g L}^{-1}$  and rose to  $0.8-1.1 : \text{g L}^{-1}$  by day 30, where it remained for the remainder of the experiment. Again, there was no evidence for influences of the fraction of Elizabeth River in the sediment mix, or the food level. Monomethyl arsenic (MMA) was a minor constituent of As, starting at ca  $0.04 : \text{g L}^{-1}$ , reaching a maximum of ca  $0.08$  from day 30 to 60, and declining back to ca  $0.03 : \text{g L}^{-1}$  by the end of the experiment. Again, there was no evidence for influences of the fraction of Elizabeth River in the sediment mix, or the food level. Dimethylarsenic was a slightly larger component, starting at ca  $0.1 : \text{g L}^{-1}$ , with some treatments reaching a peak of ca  $0.3 : \text{g L}^{-1}$  at day 30 and declining rapidly to ca  $0.05 : \text{g L}^{-1}$ . Again, there was no evidence for influences of the fraction of Elizabeth River in the sediment mix, or the food level.



**Figure 5.** Development of tissue mercury concentrations in *Fundulus* exposed to a gradient of contaminated sediments

Mercury in the water started at  $0.25 \text{ ng L}^{-1}$ , and showed high values of  $2-3 \text{ ng L}^{-1}$  in the 30-60 day period, and then declined to  $0.25 - 0.5 \text{ ng L}^{-1}$  at the end of the experiment. Curiously, there appeared to an effect of food level, with the high food treatments showing consistently higher Hg concentrations, particularly during the 30-60 day period.

**Over the course of the experiment, fish in the experiments showed increases in the trace metal concentration in their tissues. Initial samples of fish showed Hg concentrations of  $0.4 : \text{g g}^{-1}$**

(Figure 5). These rose to ca  $0.5 - 0.8 : \text{g g}^{-1}$  through the course of the experiment. Most of the change appears to have occurred within the first 30 d. of the experiment, suggesting that prolonged use of contaminated habitats is not required to uptake trace metals into fish tissues. There is some evidence that the higher food concentrations have higher Hg concentrations in the fish tissue, consistent with the observation that mercury in the water was higher at higher food concentrations.

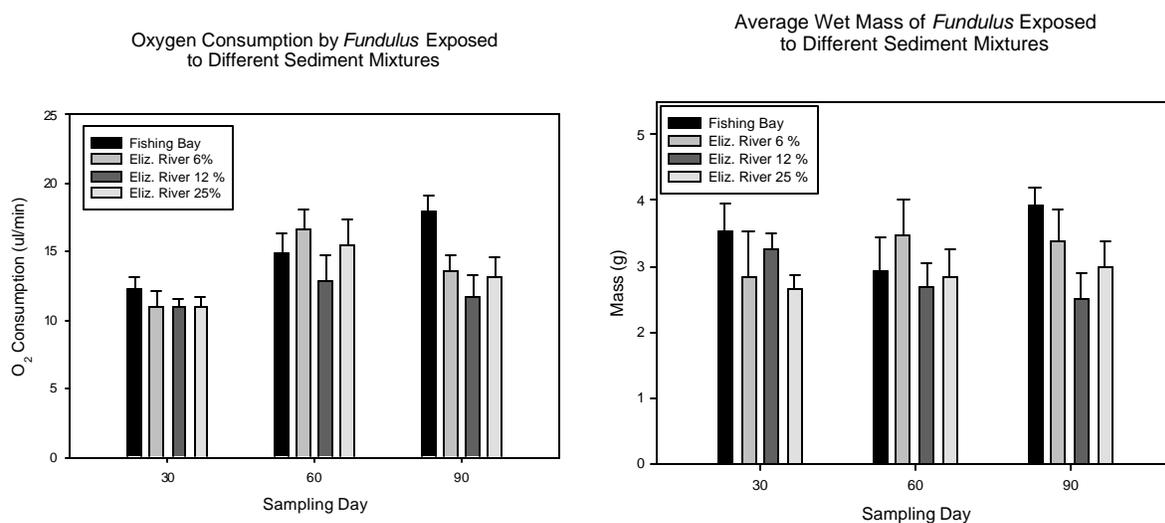
## B. BEHAVIORAL RESPONSES:

We sought to quantify energy allocation patterns in individual amphipods as a function of their exposure to contaminated sediments. We achieved this goal by conducting respirometry and behavioral observations.

### 1. Bioenergetic and Reproductive Responses: (Dr. C.L. Rowe, and T. Manyin, CBL).

On days 30, 60, and 90, oxygen consumption rates were individually measured for three individuals from each tank. Due to equipment failure, no measurements were obtained during the ensuing portion of the study. We hypothesized that sublethal effects of exposure to contaminated sediments would result in oxygen consumption rates differing from those for animals from the reference site (Fishing Bay). Despite slight differences in size by day 90, in which Fishing Bay

individuals were somewhat larger than contaminant-exposed animals, there was a reduction ( $P = 0.064$ ) in oxygen consumption by fish from the highest concentration of Elizabeth River sediments compared to Fishing Bay after 90 d of exposure (Figure 6). This result was verified statistically by an ANCOVA model



**Figure 6.** Average weight and oxygen consumption of fish exposed to a gradient of contaminated sediments on day 30, 60 and 90 of a 120 day experiment.

for repeated observations, in which wet mass was included as a covariate.

**These results, if substantiated, suggest that over a chronic period of exposure to contaminated Elizabeth River sediments, the test organisms experience a reduction in metabolism. Such a result would suggest that contaminants in the sediments (primarily PAHs), may operate narcotically, slowing metabolic processes and ultimately reducing energy assimilation and allocation to growth processes.**

## **2. Ecological and Behavioral Studies: (Dr. Linda C. Schaffner and Bruce Vogt, Department of Biological Sciences, VIMS).**

During 2002 we investigated the sublethal effects of contaminated sediments on fish (*Fundulus heteroclitus*) behavior. The basic protocol was to record the activity of fish in simple observation chambers soon after removing them from the mesocosms. An observation chamber consisted of a square glass dish with a grid placed in the bottom. Two fish were removed from each mesocosm and placed into holding containers. Fish were then randomly selected for video recording. An individual fish was placed into the monitoring chamber, allowed 1 min. to acclimate and then recorded for 1 min. This was repeated for each fish. Each video segment was then viewed and the number of lines crossed, the time spent in the center versus the edge of the chamber, and total time spent actively swimming was recorded. Analysis of these results indicated no significant effects of either food or sediment treatments on fish behavior or activity. High among-treatment variability and small sample size (N=6 for each treatment) made treatment effects difficult to discern. Additionally, stress resulting from the collection of fish from the mesocosms and the extra handling required to conduct the video recording may have masked behavioral effects. A new protocol to be used during F2 is being designed to increase sample size and decrease handling.

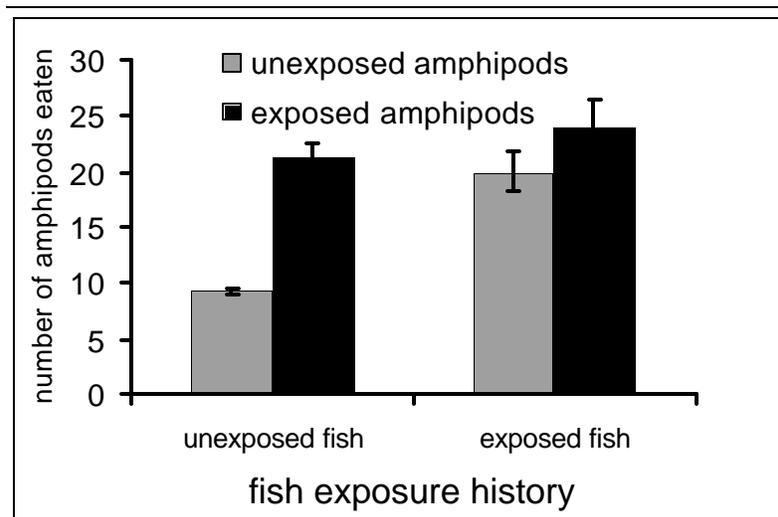


Figure 7. The total number of amphipods ingested in experimental systems containing amphipods, *Leptocheirus plumulosus*, and mummichogs, *Fundulus heteroclitus*, previously exposed to sediment from the Elizabeth River, Virginia (“previously exposed”) for 30 days, or maintained on sediment from Queen’s Creek, Virginia (“unexposed”), a relatively pristine site, for 30 days prior to the initiation of the experiment.

Further investigations of the influence of sublethal sediment contamination on amphipod and fish behavior have been conducted at VIMS. The objective of these

experiments was to assess the effect of sediment contamination on amphipod predator avoidance behaviors, as well as the ability of fish exposed to contaminated sediments to capture amphipods. The experimental design allowed both previously exposed and unexposed amphipods to interact with previously exposed and unexposed fish. Exposure systems were prepared for both fish and amphipods using 10 gal. aquaria and small 2 L plastic bins respectively. Exposure systems contained Elizabeth River, VA sediment (same collection site as used for the mesocosm studies) diluted to 7.5%, 15% and 30% or control sediment from Queens Creek, York River, VA. Amphipods and fish were maintained in these systems for a 30 day exposure period. At the end of the exposure amphipods and fish were collected and introduced into experimental chambers. An experimental chamber consisted of a 17 L plastic tub containing six condiment cups filled with sediment. Of the six condiment cups, three contained one of the Elizabeth River sediment dilutions and the remaining three contained Queens Creek sediment. Five amphipods were added to each cup and allowed 2 hrs to establish a burrow. At this point, the water level in the experimental chambers was raised to allow movement of amphipods between cups and fish were added.

Preliminary analysis of the experimental results indicates that previous exposure to contaminated sediment significantly affected both fish ( $F=10.52$ ,  $p<0.005$ ) and amphipod ( $F=14.47$ ,  $p<0.002$ ) behaviors and that there was no significant interaction that affected the total number of amphipods eaten in the experimental systems (Figure 7).

### **C. BIOMARKERS OF EXPOSURE:**

The second phase of experiments in CERP represent a period of increased opportunity for those PI's interested in the expression of biomarkers. In contrast to *Leptocheirus*, for which no biomarkers had been described previously, methods for analyzing expression of biomarkers in *Fundulus* are better developed and available for application or refinement. Accordingly, less effort has been needed for methods development, and CERP toxicologists have been capable of quantifying the expression of biomarkers in the experiments. Where validation of techniques was required, current progress has achieved the stage where samples archived from experiments on *Fundulus* can be analyzed.

#### **1. Metallothionein production: (G. Roesijadi, M. Hall, CBL).**

In the case of metallothionein in *Fundulus*, the approach taken was to analyze levels of MT mRNA as a biomarker of metal exposure. Because such analysis had not been conducted previously in this laboratory, there was a need for procedural development and validation of analytical techniques. This process was facilitated by the availability of the cDNA sequences of several teleost MTs and the high level of homology that existed in these sequences.

The initial work entailed primer design and verification of the amplification product as MT, thereby confirming the identify of the target sequence. Tissues examined were gill, liver, and intestine, which are considered of importance in uptake and accumulation pathways. Putative MT mRNAs were amplified by reverse transcriptase PCR using MT-specific consensus primers, yielding a 139 base pair fragment in all

cases. The amplification products were then provided to the University of Florida Genome Sequencing facility for analysis of the DNA sequences. The products derived from all three tissues had identical sequences showing close correspondence to those of other fish MTs. The deduced amino acid sequence for *Fundulus* MT is compared below with the rainbow trout MTA sequence:

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FUNDULUS      CECSKTGKSCGGSCSCTNC SCKSCKKS  CCPCCPSGCSKASGC
TROUT MTA     MDPCECSKTGSCNCGGSKCSNCACT SCKKASCCDCCPSGCSKASGCVCKGKTCDTSCCQ
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Having confirmed the identity of our amplification products efforts were then directed to development of a procedure for quantifying the level of expressed MT mRNA. The strategy was to develop a relative quantitative RT-PCR assay based on normalization of the MT mRNA to that of the 18S RNA, a housekeeping gene commonly used for this purpose. The Ambion QuantumRNA™ 18S Internal Standards kit was used for this purpose. This method entails constructing a first strand cDNA library from total RNA using random primers. Libraries were constructed for individual tissue samples and used as template for PCR-based amplification of the MT mRNA and 18S RNA using sequence specific primers. The signal strength of the abundant 18S RNA is attenuated during amplification with use of proprietary competitor technology (Ambion Inc.)

An experiment was then conducted in which individual fish were fed a commercial gelatinized fish food made into 10 mg pellets (wet weight) containing 0.04 mol added cadmium per pellet. Controls were fish fed pellets containing no added cadmium. Fish were individually fed to satiation once each day for six days and sampled on the seventh. Portions of gill, liver, and intestine were excised, weighed, and immediately frozen in liquid nitrogen. They were transferred to -70 C for storage. Samples of liver fish were used to develop and refine analytical procedures, prior to analysis of all tissues.

Unexpected interactions were found to occur in single tube PCR amplifications of MT mRNA and 18S RNA using a multiplexing protocol. Therefore, efforts were directed to a method in which amplifications were conducted in separate tubes containing replicate aliquots of the same *Fundulus* cDNA library as template for the PCR amplification. Amplified samples were

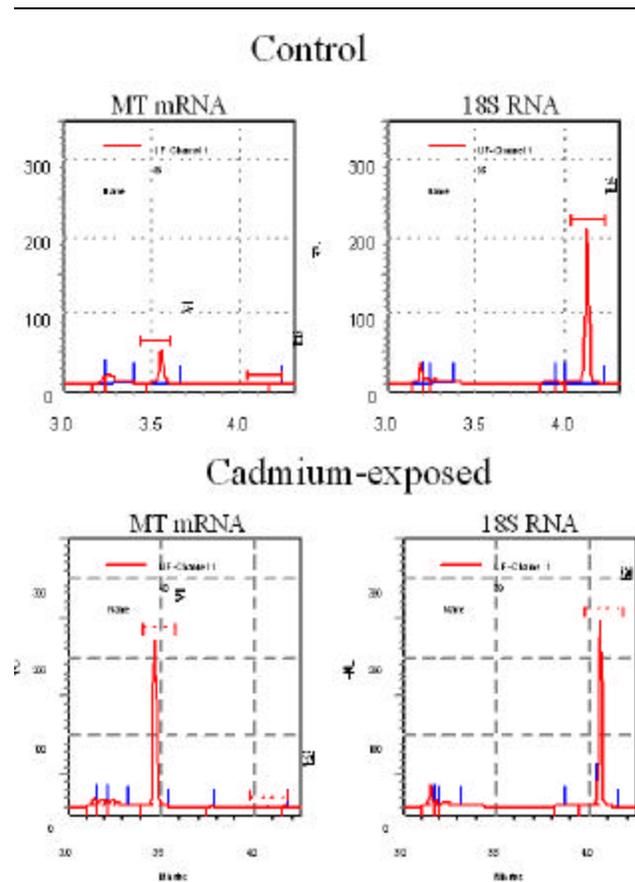


Figure 8. Representative capillary electropherograms of PCR products of separate amplifications for MT mRNA and 18S RNA off the same cDNA library of intestine from either a control or cadmium-exposed fish.

analyzed by capillary electrophoresis using laser-induced fluorescence of the intercalating dye, thiozol orange, for detection. Figure 8 shows a representative set of analyses of intestinal samples of control and exposed fish. The high MT peak of the cadmium-exposed tissues signifies induction. The 18S RNA peak is in a similar range as those for 18S RNA of the other samples. Following integration, the MT peak is corrected for the molecular weight differential when compared against the 18S peak, then normalized to that of the 18S. The MT mRNA is expressed as the MT mRNA:18S RNA ratio.

The results of analysis for MT mRNA levels in the different tissues of individual fish Table 1 indicated higher basal levels in liver and intestine in comparison with the gill, which was substantially lower. Exposure of fish to cadmium resulted in a five-fold induction of MT in the intestine, while little evidence of induction by cadmium was seen in either liver or gill. This pattern of MT induction is consistent with the dietary route of exposure used in this experiment. There was, apparently, insufficient time for translocation of cadmium from intestine to the other organs to cause induction of MT.

**These findings indicate that MT mRNA induction in the intestine is a good candidate as a biomarker for dietary exposure to cadmium and, likely, other divalent transition metals known to induce MT. Planned work to analyze MT mRNA of *Fundulus* in the experimental mesocosms of the CERP project will focus on the intestine as a target organ for exposure via contaminated sediments and food.**

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**Table 1.** MT mRNA levels in tissues of *Fundulus heteroclitus* (mean $\pm$ 1 S.D.) Levels are expressed as MT mRNA:18S RNA ratios.

| Treatment  | Liver           | Intestine       | Gill            |
|------------|-----------------|-----------------|-----------------|
| Control    | 0.15 $\pm$ 0.06 | 0.10 $\pm$ 0.06 | 0.04 $\pm$ 0.02 |
| Cd-exposed | 0.20 $\pm$ 0.08 | 0.50 $\pm$ 0.08 | 0.05 $\pm$ 0.04 |

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## **2. *Fundulus* cytochrome P450 1A (Cyp 1A) and vitellogenin (VTG): (Dr. P. Van Veld, VIMS).**

Hepatic cytochrome P4501A (CYP1A) was evaluated blot Western blot analysis using monoclonal antibody (MAb 1-12-3) a gift from Dr. John Stegeman (WHOI). CYP1A appeared to be mildly induced in mummichog exposed to 12.5 percent and 25 percent Atlantic Wood (Figure 9). Previous studies in our laboratory have yielded much more pronounced induction in fish exposed to Atlantic Wood sediment. Van Veld and Westbrook (1995) reported a 24-fold and 48-fold induction hepatic CYP1A in mummichog

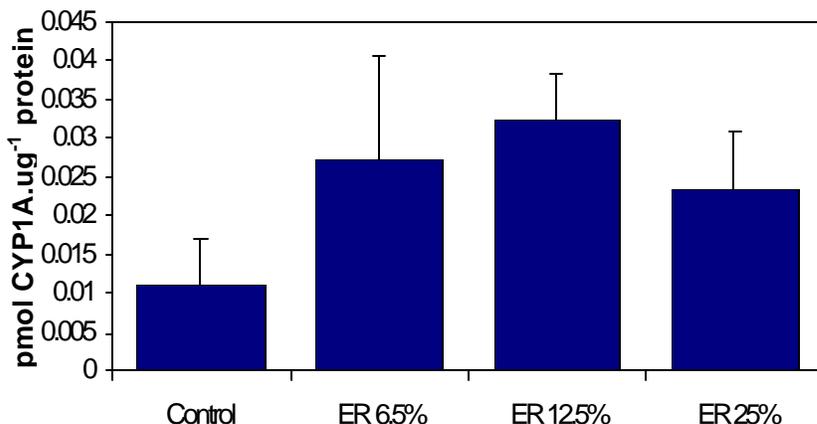


Figure 9. CYP1A expression in liver of fish exposed to Fishing Bay sediment and Elizabeth River (ER) sediment for 67 days.

in this study are unclear. Other investigators have reported down-regulation of CYP1A following

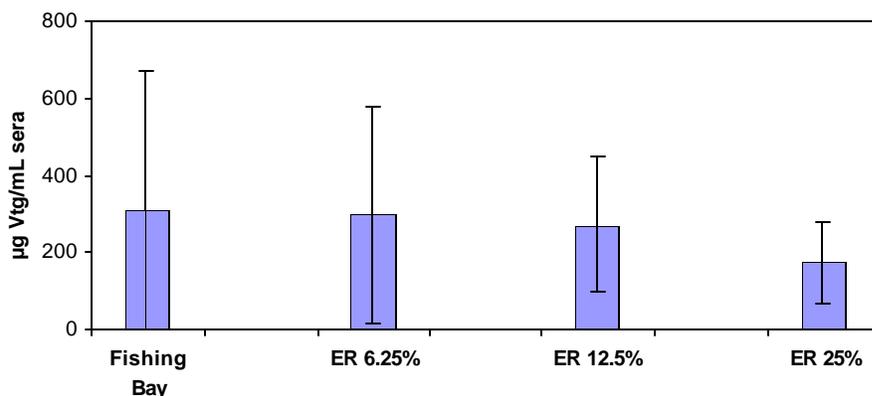


Figure 10. Induction of VTG in female *Fundulus* exposed to a gradient of contaminated sediments for 120 days.

of exposure during Experiment F2.

exposed for one week to 10 percent and 30 percent Atlantic Wood sediment respectively<sup>1</sup>. A more recent study in our laboratory resulted in 40-fold increase in CYP1A following one week exposure to Atlantic Wood sediment<sup>2</sup>. CYP1A induction is generally considered to be the most sensitive and reliable indicator of exposure to PAHs. The reasons for the modest induction observed prolonged exposure to high doses of organic contaminants such as PCBs. Our previous studies reported high level induction following one week exposures to Atlantic Wood sediment. It is possible that longer exposures such as that of the present study dampened the expected response. To address this possibility we plan to include samples at day 10

<sup>1</sup>Van Veld, P.A. and D.J.Westbrook (1995) Evidence for depression of cytochrome P4501A in a population of chemically resistant mummichog (*Fundulus heteroclitus*) Environ. Res. 3:221-234.

<sup>2</sup>Mirabilio, S.E (2001) Evaluation of vitellogenin as a biomarker of exposure to environmental estrogens in mummichog (*Fundulus heteroclitus*). Masters Thesis. The College of William and Mary. Williamsburg, VA. 66 pp.

Plasma vitellogenin (VTG) was measured by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibody against VTG of mummichog (a gift from Charles Rice, Clemson University). VTG is inducible by estradiol in male mummichog. However exposure of male mummichog to Atlantic Wood sediments during experiment F1 did not result in detectable VTG expression. Similar results were obtained with fathead minnow (*Promelas pimephales*) a species that responds to contaminants present in pulp mill effluent. We conclude from these results that there are no environmental estrogen-like compounds in creosote-contaminated sediments sufficient at concentrations sufficient to induce VTG. VTG production by male mummichog will not be useful as a biomarker of exposure or reproductive impairment in fish exposed to creosote.

VTG was detected in the plasma of all female mummichog that we collected from experiment F1 (Figure 10). There is a trend for lesser production of VTG in females exposed to Atlantic Wood sediment and studies are ongoing to determine if the difference is significant.

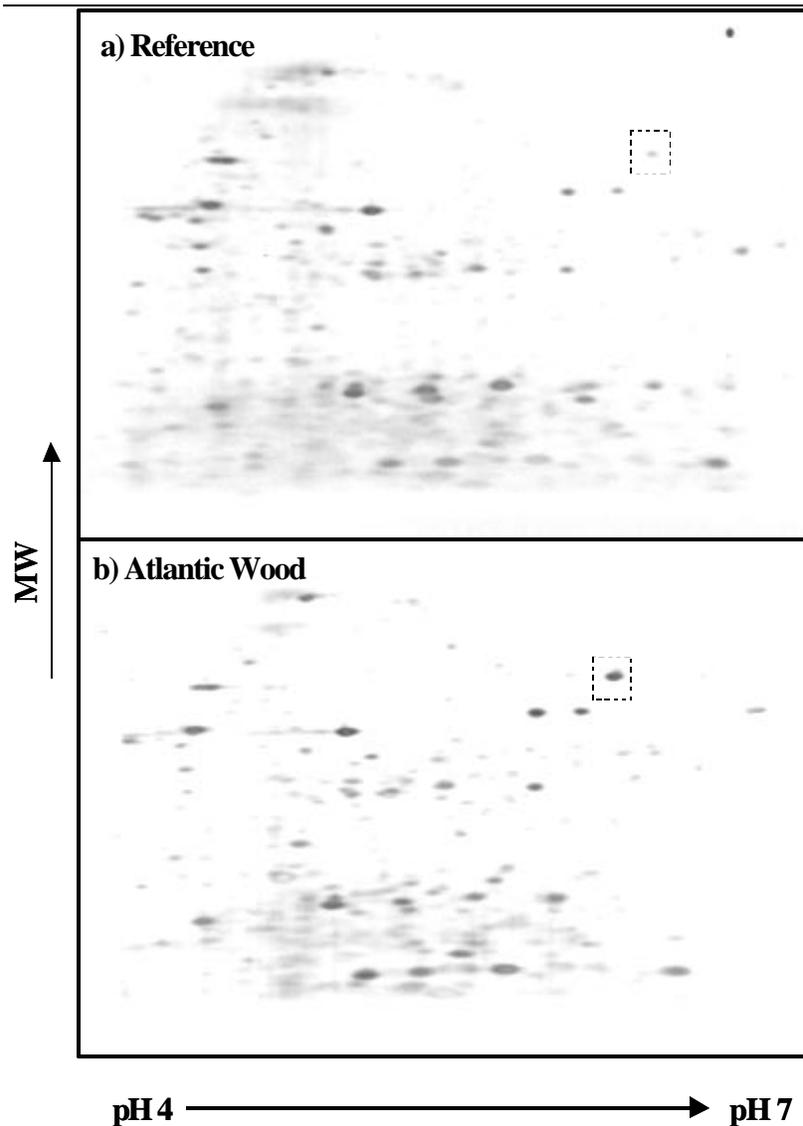


Figure 11. 2D-electrophoresis of reference site and Atlantic Wood mummichog cytosols indicating numerous differences in protein expression (e.g. see boxed area). These and other spots were excised for mass spec sequencing and identification.

Though not part of the original proposal, we are currently searching for new biomarkers of exposure and effects in Atlantic Wood mummichog using a two dimensional electrophoresis (2DE) and mass spectrometry proteomics. Samples of cytosolic proteins were obtained from reference site and Atlantic Wood mummichog and subjected to isoelectric focusing (1st dimension) followed by

polyacrylamide gel electrophoresis (2nd dimension). Silver staining of two dimensional gels revealed numerous distinct differences between reference site fish and Atlantic Wood fish in the expression of numerous proteins (e.g. see boxed area in Figure 11). Several of these spots were excised, trypsin-digested and taken to the proteomics facility at Eastern Virginia Medical School for sequencing and possible identification.

### **POPULATION LEVEL RESPONSES AND MODELING: (Dr. T. J. Miller, J. Nye and D. L. Davis, CBL)**

Research conducted under this theme had two principal foci. First we sought to explore the consequences of maternal exposure on the performance of offspring produced. Research in this area seeks to explore the effects of sub-lethal exposures in one generation on subsequent generations. The second goal is to develop population models of target species to explore the impact of sub-lethal exposures on potential population growth and population structure.

Many fish species can survive, grow, and reproduce in habitats of low water quality. Here, we examined the link between maternal exposure to contaminated sediment and the performance of their offspring. Mothers (M) were exposed to either contaminated (Elizabeth River, ER) or uncontaminated (Fishing Bay, FB) sediment. Larvae (L) from each of these groups were then transferred to either contaminated (ER) or uncontaminated (FB) sediment. The growth, survivorship, and nutritional condition in four treatments of larvae:  $M_{ER}L_{ER}$ ,  $M_{ER}L_{FB}$ ,  $M_{FB}L_{FB}$  and  $M_{FB}L_{ER}$  were compared. **There were significant maternal effects in the total length and yolk area at hatch. ER fish were larger at hatch, but had smaller yolk sacs. At the end of the 14-day experiment, larval growth was influenced by both maternal exposure and larval exposure to contaminated sediment. Larvae had slightly lower survivorship in the treatments where larvae from ER or FB mothers were switched to the opposite FB or ER treatments. Growth was significantly slower in larvae whose mothers were exposed to ER sediment, but that were then exposed to FB sediment after hatch. These results suggest that mothers provide materials other than genetic material that are important for larval growth and survival in the habitat in which they are spawned.**

We have continued to develop matrix projection models of both target species to explore the long term population-level consequences of sub-lethal exposures<sup>3</sup>. These models represent the population as being composed of a sequence of distinct stages. Transitions between the stages are governed by stage-specific rates of growth and survival, thereby permitting changes in growth rates brought about by sub-lethal levels of contamination to be explored. The models are used to integrate the results of the individual CERP experiments to quantify changes in population growth rates brought about by the exposure.

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<sup>3</sup>Salice, C. J. and T. J. Miller 2003. Population-level responses to long-term cadmium exposure in two strains of the freshwater gastropod *Biomphalaria glabrata*: Results from a life-table response experiment (LTRE). *Environmental Toxicology and Chemistry* 22:678-688.

**SUMMARY OF PROJECT USES:** Please list the companies, agencies, individuals, etc. who have requested or used the project's results and briefly describe the nature of the request/use.

The project has developed an extensive web site (<http://www.umsg.umd.edu/CERP>) that provides information to managers, stakeholders and the general public.

We are also finalizing arrangements for a workshop with stakeholders in the region to review "What are appropriate reference points for population-level ecotoxicology?". The field of toxicology is rapidly advancing from consideration solely of individual-level effects of contaminants on organisms to exploring effects at the population- and even the community-level, yet the reference points appropriate to these higher levels of biological organization remain uncertain. At the individual-level, researchers, managers and the general public have become accustomed to reference points, such as LD<sub>50</sub>'s, by which the level and seriousness of environmental contamination can be determined. A parallel development of reference points is occurring to deal with mixtures of contaminants. We will convene a structured working group in Fall 2003 in Solomons to produce a white paper that will review and evaluate potential population-level reference points. The objectives of the working group are to examine the range of possible reference points, assess their relevance to management, and to develop information to educate policy makers and the public regarding population-level reference points. The working group will comprise both scientists and managers from the Chesapeake region who have research expertise and management experience in the suite of environmental contaminants relevant to the region.

The resulting white paper will be written by working group participants and reviewed externally. The final document will be published by MD and VA Sea Grant through their joint funding of the Chesapeake Ecotoxicological Research Program (CERP).

**COOPERATING ORGANIZATIONS:** Please list the organizations that have provided financial, technical or other assistance to the project and describe the nature of the assistance.

The project is a cooperative project with colleagues at the Virginia Institute of Marine Sciences who are funded through a VA Sea Grant award.

**STUDENTS:** Please list the name(s) and department(s) of the student(s) who have worked on the project and describe the work done. If a thesis or dissertation is involved, list title. If a student has graduated, please indicate what he/she is doing.

Susan Klausterhaus (advisor J. Baker). Ph.D. Expected graduation 2005. Supported by CBL fellowship and CERP. Thesis title: To be determined.

Teresa Manyin (advisor C. Rowe): Ph. D. Expected Graduation 2003. Supported on CERP for 2 yrs, currently supported by EPA STAR Graduate Fellowship. Thesis title: A Multilevel Investigation of Contaminants of Concern on Chesapeake Bay Invertebrates.

Amy Merten (advisor J. Baker): Ph.D. Expected Graduation 2004. Supported by NOAA. Thesis title: Effects of contaminated prey exposures on bioenergetic costs and PCB accumulation in *Fundulus heteroclitus* "under the influence"

Janet Nye (advisor T. Miller). Ph.D. Expected graduation 2005. Supported by CERP. Thesis title: To be determined.

Timothy Tefteau (MD Sea Grant REU Student - Advisor. Dr. G. Riedel). The Use of the Stable Isotope  $^{111}\text{Cd}$  to Determine the uptake of Cadmium from three different uptake pathways on the estuarine fish *Fundulus heteroclitus*. Summer 2002.

**PUBLICATIONS:** Please give citations of published or submitted journal articles, technical reports, conference papers, etc. derived from the project.

Salice, C. J. and T. J. Miller 2003. Population-level responses to long-term cadmium exposure in two strains of the freshwater gastropod *Biomphalaria glabrata*: Results from a life-table response experiment (LTRE). *Environmental Toxicology and Chemistry* 22:678-688.

Manyin T, Rowe C. *In Prep*. Energetic and reproductive changes in burrowing amphipods (*Leptocheirus plumulosus*) resulting from chronic exposure to contaminated sediments from Baltimore Harbor, USA.

Schaffner, L., T. J. Miller, and K. R. Tenore *In prep*. Impacts of exposure to contaminated sediments on the production dynamics of *Leptocheirus plumulosus*.

Vogt, B. *In prep*. Effects of contaminated sediment exposure on predator-prey interactions using the fish (*Fundulus heteroclitus*) and the amphipod (*Leptocheirus plumulosus*).

Vogt, B. and L. Schaffner. *In prep*. Effects of contaminated sediment exposure burrowing behavior of the amphipod (*Leptocheirus plumulosus*).

**CONFERENCES AND SPECIAL PROGRAMS:** Please list all conferences, workshops, seminars, advisory activities, etc. attended or organized that were associated with the project.

Rowe C. Co-organizer and co-chair of session: Chesapeake Bay Research and Progress. 22<sup>nd</sup> Annual Meeting of the Society of Environmental Toxicology and Chemistry, Baltimore, MD.

Davis, D, D, and T. J. Miller. 2003 Population level effects of contaminated sediments on an estuarine fish, *Fundulus heteroclitus*. Annual Meeting of the American Fisheries Society. Quebec City, Quebec, Canada. August 2003.

Nye, J., and T. J. Miller. 2003. Maternal effects in *Fundulus heteroclitus* larvae exposed to contaminated sediment. Annual Larval Fish Conference. Santa Cruz, CA, August 2003

**COMMENTS OR ADDITIONAL INFORMATION:**

US EPA STAR Graduate Fellowship awarded to T. Manyin. Based upon research as part of the CERP project, Teresa Manyin developed a proposal to investigate sublethal, chronic effects of inorganic contaminants on benthic invertebrates in Chesapeake Bay.