Division of Science, Research and Technology

Research Project Summary

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Integrated Biomarkers for Assessing the Exposure and Effects of Endocrine Disruptors and Other Contaminants on Marine/Estuarine Fish

Authors

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Abstract

The development of fish biomarkers can be a powerful tool in assessing the status and trends of contaminants in estuaries, as well as the effectiveness of ongoing and future source control/cleanup. The goal of this research was to examine a suite of biomarkers and develop those that can provide an integrated measure of contaminant exposure from multiple contaminants including endocrine disruptors and provide a gauge of estuarine/marine water quality and ecosystem health. Mummichog or killifish (*Fundulus heteroclitus*), a common and abundant species, was collected from five sites including Newark Bay, Piles Creek, Sandy Hook, Union Beach and Tuckerton, New Jersey (NJ) for this study. These sites were selected to represent a contaminant gradient with elevated concentrations in Newark Bay to the reference location at Tuckerton. In addition, preliminary sampling was carried out examining biomarkers in White Perch (*Morone americana*) from the Delaware River, Hackensack River, Passaic River and near Tuckerton, NJ. Biomarkers included external examinations, blood parameters, organ to body weight ratios, histopathology, and biochemical endpoints. Vitellogenin concentrations were examined as a biomarker of endocrine disruption in both species.

The results support the need to conduct both classical toxicological evaluations (histopathology, organ to body weight ratios) along with biochemical endpoints (CYP1A1, metallothionein, vitellogenin) as biomarkers. The use of micronuclei was not found to be reliable as a biomarker, due in part to interference from cytosolic parasites and very low occurrence. Additional sampling and research is warranted due to the detection of neoplasms (i.e., tumors) in the livers of fish from several locations with nominally low levels of contamination. The biomarkers most useful in evaluating the health of the organisms include grossly visible lesions (external and internal), standard hematology (hematocrit, blood smears), standard body morphometrics, histopathology, biochemical endpoints (CYP1A1, vitellogenin) and bile fluorescence (specific polycyclic aromatic hydrocarbons [PAHs]). White perch is an appropriate biomarker species due to the number of biomarkers that can be successfully used on this species, as well as its widespread occurrence in estuarine areas of the state.

Introduction

Environmental measurements using fish (i.e., biomarkers) are an acceptable and recognizable indicator to the public and regulatory community. Use of biomarkers can provide an integrated measure of contaminant exposure from multiple contaminants, and provide an indicator of estuarine/marine water quality and ecosystem health. These relatively low-cost indicators are extremely sensitive measures of contaminant exposure and represent the cumulative impact of all inducing chemicals, whether or not they are detected analytically in the water column.

Measurement of accepted biomarkers in two fish species from NJ's waters were used to provide an integrated measure of exposure and bioavailability of toxic contaminants to the fish community. Both exposure/effect biomarkers (i.e., ethoxyresorufin-O-deethylase [EROD] activity) and reproductive biomarkers (i.e., vitellogenin) were used. The EROD (enzyme induction) biomarker assesses exposure and potentially the effects of PCBs, PAHs, and dioxins/furans. Measurement of reproductive biomarkers (i.e., vitellogenin) can assess the exposure and effects of endocrine disrupting chemicals. The feasibility of other biomarkers was also assessed, including metallothioniens (metal exposure).

The main objectives of this study were:

1. To develop a battery of biomarkers that can be used to evaluate fish health, which will correlate with levels and classes of toxic compounds in the estuary, and as an indicator of estuarine/marine water quality and ecosystem health.
2. To begin to establish a baseline data set concerning these biomarkers for comparison with

fish collected in latter years following remediation measures carried out in the NY-NJ Harbor and other state waters.

3. Make recommendations concerning an appropriate biomarker fish species, as well as the suitability of each specific biomarker.

Methods

In year 1 of the study, mummichogs (Fundulus heteroclitus) were collected in 2002 and 2003 from four locations including Newark Bay, Union Beach, Sandy Hook and Tuckerton, NJ. The mummichogs were collected using baited minnow traps and/or seine nets. Due to limited funding only a small number of biomarkers were measured to determine their effectiveness, as well as the usefulness of mummichogs as a biomarker species. In year 2 of the study additional biomarkers were evaluated, and fish were collected from 2004 through 2006. Mummichogs were collected from five locations including an additional station in Piles Creek (tributary to the Arthur Kill). White perch were collected from the Passaic River, Hackensack River, Delaware River, and Mullica River (reference location) near Tuckerton. The animals were transported back to the laboratory in aerated coolers, processed, and biomarker measurements performed. Table 1 lists the biomarker measurements collected on both species of fish.

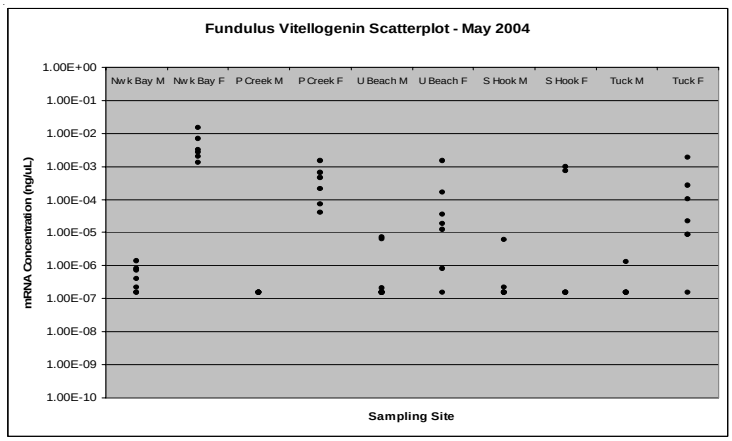

Figure 1. Vitellogenin mRNA scatterplot levels from *Fundulus heteroclitus* collected in May 2004.

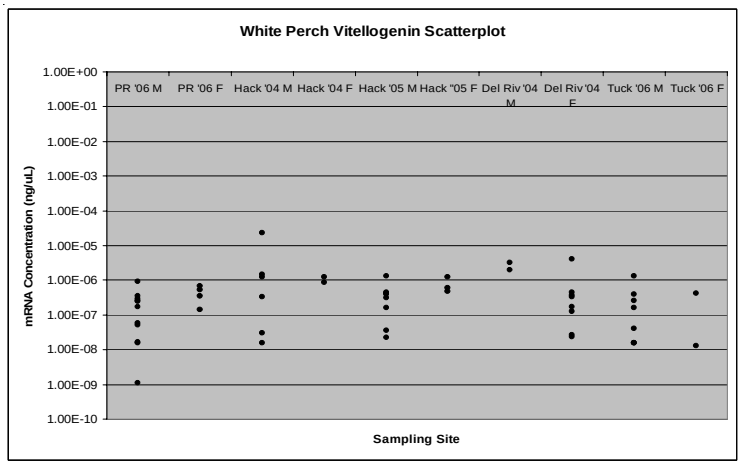

Figure 2. White Perch vitellogenin levels determined from hepatic tissue.

Table 1. Biomarker Endpoints Evaluated in These Studies.

Biomarker	Purpose	Endpoint
External Examination	▪Examine the fish for external lesions involving skin, fins, gills or eyes and internal color and shape of internal organs.	▪Incidence of external lesions and grossly visible lesions
Blood Smear	▪Morphological evaluation of red blood cells (RBCs) and white blood cells (WBCs) following staining with Wright/Giemsa stain and/or a DNA specific stain.	▪Micronuclei in RBC ▪WBC shift infection ▪Morphology of RBCs
Hematocrit	•Determine packed cell density	▪Anemia or altered RBC production
Total & Organ Weights	▪Size of organs is correlated with size of the organism (liver, spleen, gonads). •Gross morphological evaluation (color & shape) is an indicator of disease.	▪Stressors or diseased organs will have altered organ to body weight ratios.
Histopathology	▪Evaluate liver cellular structure at light microscopic level.	•Evaluation of normal vs. altered structures
Biochemical Endpoints including Endocrine Disruption	•Evaluate if populations inhabiting different locations have different levels of endogenous enzymes.	•Real time PCR for quantification of mRNA enzyme levels for P450 Cyp1A1, metallothionein (MT) and hepatic vitellogenin (VT) (Endocrine Disruption).
Fluorescent Activity	•Fluorescent activity in bile has been correlated with PAH activity.	•Increased basic fluorescence indicates increased aromatic contamination.

Table 2. Summary of Biomarker Endpoint Effectiveness and Other Recommended Biomarkers.			
Biomarker	**Purpose**	**Endpoint**	**Useful Biomarker**
External Examination	Examine the fish for external lesions involving skin, fins, gills or eyes and internal color and shape of internal organs.	•Incidence of external lesions and grossly visible lesions	**Yes**
Blood Smear	Morphological evaluation of RBC and WBCs following staining with Wright/Giemsa stain and/or a DNA specific stain.	•Micronuclei in RBC •WBC shift infection •Morphology of RBCs	**No:** Micronuclei **Yes:** RBC & WBC
Hematocrit	Determine packed cell volume	•Anemia or altered RBC production	**Yes**
Total & Organ Weights	Size of organs is correlated with size of the organism (liver, spleen, gonads). Gross morphological evaluation (color & shape) is an indicator of disease.	•Stressors or diseased organs will have altered organ to body weight ratios	**Yes:** Liver and spleen **Yes:** Gonad at specific spawning cycles
Histopathology	Evaluate liver cellular structure at light microscopic level.	•Evaluation of normal vs. altered structures	**Yes:** Expand to other organs: gill, kidney, etc.
Biochemical Endpoints	Evaluate if populations inhabiting different locations have different levels of endogenous enzymes.	•Real time PCR for quantification of mRNA enzyme levels for P450 1A1, metallothionein (MT) and hepatic vitellogenin (VT)	**Yes:** CYP1A1 & metabolism **No:** Hepatic MT **Yes:** Kidney MT **Yes:** Hepatic VT **Yes:** Circulating Hormones **Yes:** DNA adducts or protein adducts, or Comet assay
Fluorescent Activity	Fluorescent activity in bile has been correlated with PAH activity.	▪Increased basic fluorescence indicates increased aromatic contamination.	**Yes:** PAH fluorescence **Yes:** Metal & organic compounds

Results

The suite of biomarkers was tested in order to determine which ones would reflect exposure to and effects from contaminated conditions. In a similar manner, two common estuarine fish species were examined to determine their appropriateness as a biomarker species. The individual biomarker results varied by species, location and season. A few examples of biomarker results are provided in the following paragraphs. Full details can be found in the full report.

The vitellogenin levels for mummichogs and white perch are shown in Figures 1 and 2. The data set is more robust for the mummichogs, and only a relatively small number of white perch samples were collected. The mummichog data shows a clear difference between male and female levels of vitellogenin (i.e., lack of evidence of endocrine disruption) in most cases. In fact vitellogenin levels in female mummichogs from Newark Bay were significantly higher than all other sites. However, there was no statistical difference between male and female levels of vitellogenin in white perch. Some of this can be explained by the size differences and time of year, but the lack of difference indicates the need for additional samples and monitoring.

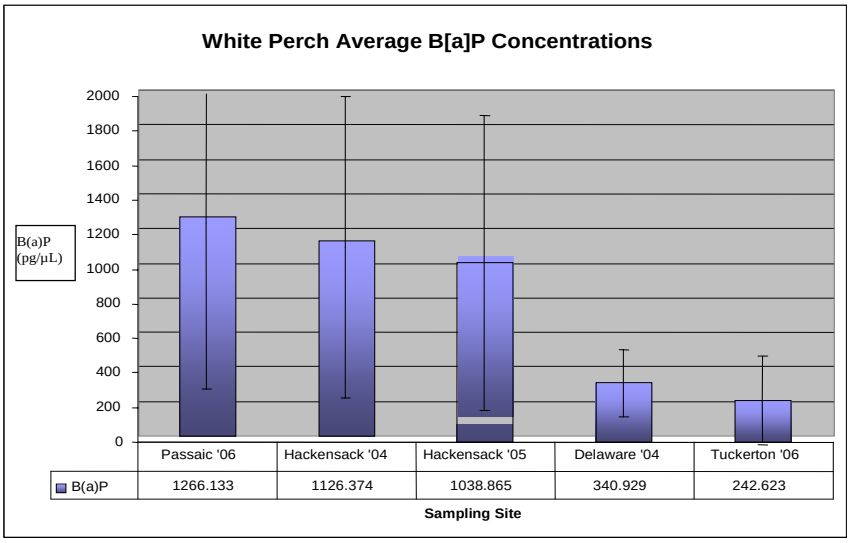

Figure 4. Comparison of White Perch bile fluorescence for B(a)P (mean ± SD).

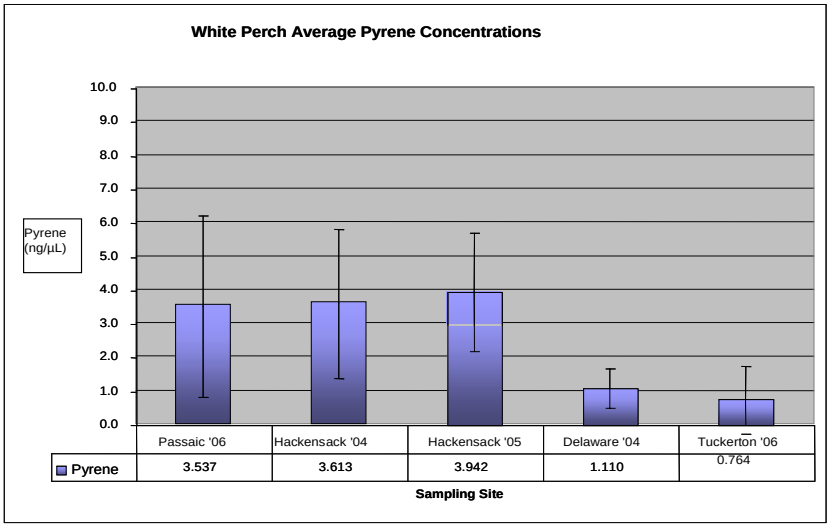

Figure 3. Comparison of White Perch bile fluorescence for pyrene (mean ± SD).

Another biomarker examined was fluorescent activity in bile as a measure of PAH exposure. Clear differences between sites were observed for pyrene and benzo(a)pyrene in white perch (Figures 3 and 4) with fish from the more urban areas exhibiting higher levels of PAH in the bile. Fluorescent activity in mummichog bile was more variable (Figure 5).

Table 2 lists a summary of the effectiveness for each of the biomarkers tested under this study.

Discussions and Conclusions

Fundulus heteroclitus is known for its ability to survive in heavily polluted environments. This ability to adapt may limit its usefulness as a sensitive species for biomarkers. There was substantial variability in several of the mummichog biomarkers on both a temporal and spatial basis, indicating that this fish may not be the ideal choice as a biomarker species. Although fewer fish were analyzed, the biomarker results indicate that the white perch appears to be the better biomarker candidate for estuarine waters.

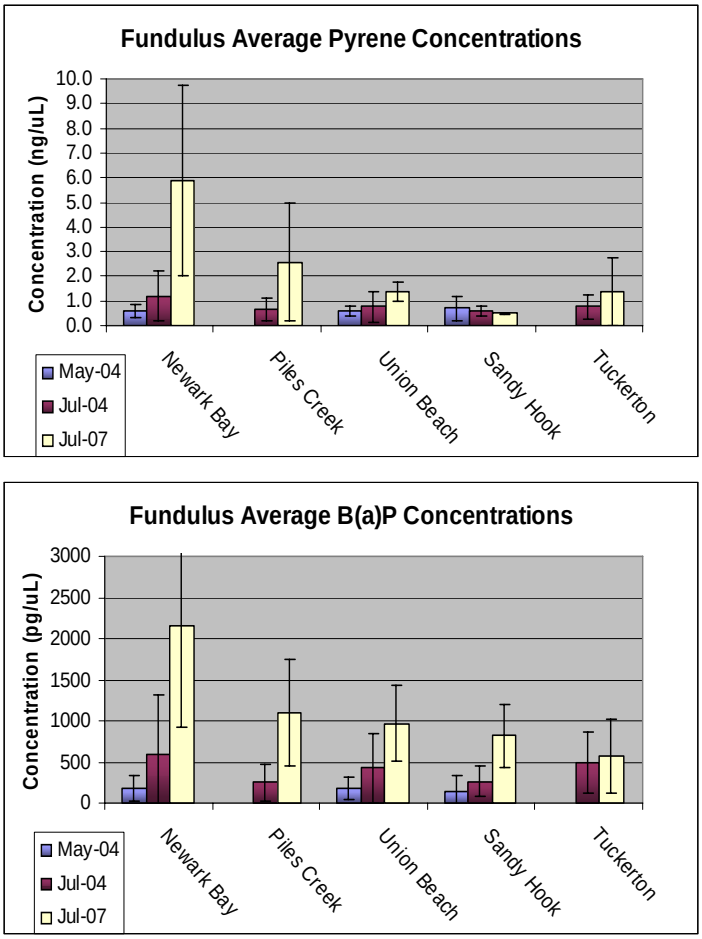

Figure 5. Comparison in *Fundulus heteroclitus* bile of pyrene and benzo(a)pyrene by site and collection date.

The following recommendations are based on the findings from these preliminary studies. Additional information and recommendations can be found in the full report.

1. The biomarkers that were useful in evaluating the health of the organisms included grossly visible lesions (external and internal), standard hematology (hematocrit, blood smears), standard body morphometric (length and weight), histopathology, biochemical endpoints (CYP1A1, vitellogenin) and bile fluorescence (specific PAHs).
2. Neither the micronuclei nor the hepatic metallothionein appeared to be useful biomarkers in differentiating among various populations.
3. Future studies could include, if available, species-specific gene activation based on gene chips, circulating hormones and proteins and alternative methods for evaluating DNA adducts.
4. White perch is an appropriate biomarker species due to the number of biomarkers that can be successfully used on this species, as well as its widespread occurrence in estuarine areas of the state.

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RESEARCH PROJECT SUMMARY

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