

2003 Progress Report: Development and Evaluation of Chemical Indicators for Monitoring Ecological Risk

EPA Grant Number: R828675- 05

Subproject: *This is subproject number 05, established and managed by the Center Director under grant R828675*

Center: [Great Lakes Environmental Indicators Project](#)

Center Director: [Gerald J. Niemi](#)

Title: Development and Evaluation of Chemical Indicators for Monitoring Ecological Risk

Investigators: Deborah Swackhamer¹, Matthew Simcik¹

Cooperators: David Mount², Gary Ankley², Philip Cook², Steven Diamond², Russell Erickson², Lawrence Burkhard²

Institutions: ¹University of Minnesota, Minneapolis; ² US EPA Mid-Continent Ecology Division, Duluth

EPA Project Officer: Barbara Levinson

Project Period: January 10, 2001 to January 9, 2005

Project Amount: \$6,000,000

RFA: [Environmental Indicators in the Estuarine Environment Research Program \(2002\)](#)

Research Category: [Ecological Indicators/Assessment/Restoration](#)

Description:

Objective: Our **overall goal** is to identify and validate effective contaminant indicators of adverse impacts on estuarine ecosystem health. Indicators will be developed in the Great Lakes, but will also be applicable to both marine and freshwater ecosystems. These contaminant indicators will be used to evaluate ecological condition. Specifically we will focus on the evaluation of two indicators: 1) indicator PAHs of photo-induced toxicity to fish and benthic organisms; and 2) organic chemical indicators of xenoestrogenic exposure to fishes.

The assessment of ecological condition in an effective manner is best accomplished using integrative indicators of condition. These indicators should be cost-effective, be applicable across multiple scales, and provide useful information for environmental managers. Within the omnibus project, this contaminants subproject focuses on contaminant indicators that will provide a measure of condition of the estuarine ecosystem. These indicators will also serve as diagnostic indicators that will identify the primary stressors affecting the specific ecological endpoint of concern. We have focused on PAH compounds and environmental estrogens since they are widespread in the environment and have existing sources, and thus are of current concern.

The **specific hypotheses** we are testing are: 1) Specific PAHs in combination with UV penetration are indicators of potential loss of vulnerable species within coastal fish and or benthic communities; and 2) specific chemicals are indicators of endocrine disruption in fish via the estrogen receptor. Data collected to test these hypotheses will be used to demonstrate the degree of usefulness of these two groups of indicator compounds as diagnostic indicators for estuarine ecosystems.

Our **overall approach** to this project is as follows. For both indicators, we will compare contaminant concentrations to a biological endpoint or condition across a gradient of non-degraded to highly degraded sites in approximately 20 locations being studied by the other indicator project groups in the program. For the PAH photo-induced toxicity indicator, we will collect the necessary field data to test the model developed in the lab by the collaborators at EPA-MED (Diamond, Mount, Erickson). These data include the concentrations of PAHs in sediment, larval fish, and oligochaetes (to determine the BAFs and to provide the doses for the model); sediment photo-induced toxicity potential (assayed in the lab using the aquatic annelid *Lumbriculus* [lab test organism] and field sediments); and UV dose (obtained from field measurements). The toxicity that is predicted from the model will be compared to that measured in the lab assay. Results will be used to calibrate the model, and independent field data will be used to validate the model. While photo-induced toxicity has been extensively studied and its acute toxicity demonstrated in the laboratory, this will be the first field test of such an indicator.

The xenoestrogen indicators will be identified in an analogous manner. A suite of potential xenoestrogens will be measured in fish tissue, sediment, and/or water and compared to vitellogenin induction in wild and caged male fish (a bioindicator of individual estrogen exposure) at the same gradient of sites. Using correlative statistical techniques, we will identify specific indicator xenoestrogens that are associated with vitellogenin induction. This would represent the first link of vitellogenin induction and chemical exposure in field sites other than near sewage treatment plants.

Progress Summary: First Year: Our goals for the first year of the project were largely focused on establishing and validating our field methods and assumptions.

Photo-induced PAH toxicity: Exposure of organisms to UV light and PAHs has the potential to excite certain PAHs that are internal to the organism (bioaccumulated) and cause acute cell damage and death. Larval fish are considered to be sensitive organisms because their transparency and behavior result in significant light exposure during this early life stage, and because they have the potential for high PAH exposure due to high lipid content and incomplete enzyme systems that are not yet capable of fully metabolizing and/or depurating PAHs.

Methods for sediment collection and UV dose measurements are established and were successfully tested during the 2001 pilot studies. The diatom/water quality and fish/macroinvertebrate subprojects are collecting these samples and data. Our efforts focused on methods for collecting larval fish in the field. In pilot studies in Green Bay and southern Lake Superior, light traps proved to be inefficient. Dip nets with mesh size of 500 um or less were the most efficient method tested, but require significant manual sorting of the sample. The methodology for indexing photo-induced toxicity potential using *Lumbriculus* exposed to field collected sediments has been well established at EPA MED. Summer research at MED included developing methods for exposing larval fish *in situ* at contaminated sites, which will be an integral piece establishing the connectivity of sediment chemistry, *Lumbriculus* response, and effects on larval fish.

Endocrine Disruption from Environmental Estrogen Exposure: The development of this indicator requires that we establish a link between the occurrence of specific chemicals in coastal

regions and environmental estrogen exposure to fish as indicated by a serum protein, vitellogenin (vtg). Vtg is a precursor to egg-yolk formation, and can be induced in both males and females in response to activation of the estrogen receptor. Vtg induction in males is an unambiguous indicator of exposure to exogenous and environmental estrogens. Our efforts focused on establishing methods to collect sufficient numbers of wild bullheads to demonstrate environmental estrogen exposure. Pilot studies revealed that Fyke nets were the most effective method for collections at sites where bullheads were plentiful. We also designed the necessary experiments needed to develop and validate the vtg assay for the coming field season.

Second Year: The second year has been devoted to site selection, and the collection of field samples. Some additional laboratory work at EPA MED was also accomplished.

Site Selection: The process of selecting sites using a random process that would test our indicators in a representative fashion proved to be challenging, time-consuming, and successful. The site selection for contaminants was slightly different from the other subprojects. We have the fewest number of sites (20), and we have specific gradients of condition that we wanted to capture in our sites that were not necessarily the dominant variables of interest to the other groups. We worked closely with Nick Danz and Ron Regal of NRRI/UMD, who developed a process that was a model for the others. More than 700 potential sites were identified (called segment sheds, which are defined as the watershed and shoreline length corresponding to the area draining second order tributaries and greater), and the associated stressor data for more than 200 variables were collected into a database. A principal components analysis of key variables relating to agricultural activity and industrial activity was completed, resulting in a 2-dimensional space accounting for about 70% of the variance. Sites were selected randomly from within given percentiles of the distributions, with a weighting of more contaminated sites relative to less contaminated sites. This resulted in sites containing high industry and high agricultural influence, those with high industry and low agricultural influence, those with low industry and high agricultural influence, and those with low industry and low agricultural influence. Sites were also stratified to be equally divided between the two eco-provinces, and to include all 5 Great Lakes. A few sites were handpicked to ensure the inclusion of a given type of pollution, or to overlap with other research in the area. A total of 22 sites were eventually compiled.

Sites were visited on two occasions – in the spring to collect larval fish, and again in mid-summer to collect wild fish and to deploy caged male fathead minnows.

PAH Photo-induced Toxicity Indicator: We successfully collected larval fish at 17 sites using dip nets from a small boat or by wading. The largest larval biomass in the nearshore region is thought to be yellow perch, so we concentrated our efforts during the post-spawning periods in each lake. Samples were protected from sunlight while processing and frozen for transport back to the UMN campus. Samples are currently being extracted and analyzed for a suite of 37 photoactive and non-photoactive PAHs. Some additional time has been spent on optimizing the PAH analytical methods.

Sediments were collected from approximately 50 sites (sample collections done by the fish/macroinvertebrate subproject) for the purpose of screening them for their PAH photo-

induced toxicity potency. This was to help bound the extent of this potential response, and to identify any “interesting” sites for more intensive investigation. These sediments are currently being assayed using the *Lumbriculus* assay at MED.

Water samples (a total of 28) were collected at all 22 contaminant sites, and from the absorbance readings of these samples the UV extinction coefficients were calculated. These data were then used to model the UV exposure at all sites for future use in the toxicity model. They were compared to the measured UV dose obtained from field measurements at 5 of the sites, and correlated well.

Additional experiments have been conducted at EPA MED to determine the kinetics and magnitude of PAH bioaccumulation in larval fish, and to evaluate responses of fish larva to ambient PAH and sunlight during in situ exposures at a PAH-contaminated site on the Great Lakes. Their preliminary findings indicate that accumulation of three waterborne PAHs by fathead minnows reached equilibrium within 24-48 hours regardless of lifestage (embryo v. larval). Benzo[a]pyrene appeared to be metabolized even in embryos. Based on these experiments, it appears that starting experiments with naive larva will not appreciably reduce PAH exposure relative to experiments with embryos. The in situ experiments showed reduced survival and growth in fish larva exposed to ambient conditions, with a dose-related reduction in these effects with decreasing sunlight exposure. These data and their interpretation are still being finalized.

Future Activities: The coming year will focus on three areas. The first is to determine the PAH concentrations in all fish larvae and sediment samples from the last field season, and to determine the toxicity potency of the sediments in the lab. Secondly, we will integrate and synthesize the PAH, UV, and sediment potency data into the toxicity model. Finally, additional field samples will be collected as needed for calibration and validation of the model.

Environmental Estrogen Indicator: Our pilot field activities indicated to us that we could not be certain of finding sufficient numbers of bullheads at all sites. Thus we added the exposure of caged male fathead minnows to our design. Our concerns were realized; fyke netting at 22 sites did not collect sufficient numbers of animals at any of the sites. This is partially due to the random site selection process (bullheads are not randomly distributed, and prefer certain habitats), the fact that populations move with changing water temperatures, and that we were limited in the amount of effort we could devote to net deployments (24-48 hr sets). The caged fish were a more controlled alternative to assay the potency of each site. Three cages per site, containing 10 fish each, were deployed for 8-day periods at 10 sites (there were not enough minnows commercially available to do all sites). Preliminary assessments of the minnows from 3 of the most contaminated sites indicated that there was no discernible vtg induction. Anticipating that exposures may be too short to detect vtg production, we also invested considerable resources in developing the methods to detect the mRNA for vtg production, which will be produced more quickly than the protein itself. This is a more complex technique, and we are working with Dr. Nancy Denslow at the University of Florida (of the Gulf EaGLE Center) to assess the applicability of this method to our study.

To this end, we are conducting a series of exposure experiments to determine the best endpoint for measuring estrogenic exposures in fish in the field, and these endpoints include vtg, mRNA for vtg, zona radiata protein (zrp, an eggshell protein), and the mRNA for zrp.

We have also devoted time to developing and validating the analytical methods needed to measure approximately 12 classes of estrogenic compounds.

Our future activities will be focused on both laboratory and fieldwork. First, we will be defining the best endpoint for determining estrogenicity in our field samples using laboratory and some controlled field experiments. Secondly, we will then deploy caged fish at all sites and determine their response. This will be followed up by intensive chemical characterization of the site for environmental estrogenic compounds.

Graduate Student Participation:

Randy Lehr's project title is: "Development and Validation of a Chemical Indicator for Endocrine Disruption in the Coastal Great Lakes" (University of Minnesota).

Andrew Adams' project title is: "Photo-Enhanced Toxicity of Polycyclic Aromatic Hydrocarbons to Larval Fish in the Coastal Great Lakes" (University of Minnesota).

Publications and Presentations: Total Count:

<u>Type:</u>	<u>Citation</u>
Publications	Danz, N.P., M.F. Simcik, D.L. Swackhamer, G.J. Niemi, R.R. Regal, A. Adams, R. Lehr, and T. Hollenhorst. Sampling design to capture contaminant gradients in the coastal Great Lakes. In preparation.
Presentation	Adams, A.D., R.A. Lehr, M.F. Simcik, D.L Swackhamer, S. Diamond, D.R. Mount. Photo-Induced Toxicity of Polycyclic Aromatic Hydrocarbons in Larval Fish of the Coastal Great Lakes. Is it Ecologically Relevant? SETAC meeting 11/9-11/13/03, Austin, TX. Scientific audience, 200 people
Presentation	Adams, A.D., R.A. Lehr, M.F. Simcik, D.L Swackhamer, S. Diamond, D.R. Mount. Photo-Induced Toxicity of Polycyclic Aromatic Hydrocarbons in the Coastal Great Lakes. IAGLR meeting 6/22-6/26/03, Chicago, IL. Scientific audience, 100 people
Presentation	Adams, A.D., R.A. Lehr, M.F. Simcik, D.L Swackhamer, S. Diamond, D.R. Mount. Photo-Induced Toxicity of Polycyclic Aromatic Hydrocarbons and Indicator Development Midwest SETAC 3/3-3/5/04, La Crosse, WI. Scientific audience, 100 people
Presentation	Erb, J.L., J.G Downward, E.M. Priuska, J.L. Wittliff, R.A. Lehr, D.L. Swackhamer, E. Garber, G. Larson, and J. Magner, Receptor-based Biosensor Responses Correlate with Fish Vitellogenin Production and Frog Developmental Abnormalities. Frontiers in Assessment Methods for the Environment (FAME), 8/10/03- 8/13/03, Minneapolis, MN Scientific Audience, approx. 100 people.

- Presentation Erb, J.L., R.A. Lehr, J.L. Wittliff, R. Allen, J.G Downward, R.H. Smith, D.L. Swackhamer and E.M. Priuska. Effects of Great Lakes water samples on estrogen receptor and aryl hydrocarbon receptor activity as measured by a biosensor. International Conference of Pharmaceuticals and Endocrine Disrupting Chemicals in the Water 3/19/03-3/21/03, Minneapolis, MN, Scientific Audience, approx. 120 people.
- Presentation Lehr, R.A., A.D. Adams, J.L. Erb, I. Knoebl, N.D. Denslow, G.T. Ankley, M.F. Simcik and D.L. Swackhamer, Monitoring Environmental Estrogens in the Great Lakes. Society for Environmental Toxicology and Chemistry (SETAC), 11/16/03-11/19/03, Austin, TX, Scientific Audience, approx 70 people.
- Presentation Lehr, R.A., J.L. Erb, A.D Adams, M.F. Simcik, and D.L. Swackhamer, Assessing the Potential for Endocrine Disruption in the Great Lakes. International Association for Great Lakes Research (IAGLR), 6/03, Chicago, IL, Scientific Audience, approx 20 people.
- Presentation Swackhamer, D.L. Developing Ecological Indicators of Chemical Stress to Nearshore Environments of the Great Lakes, Midwest Society of Environmental Toxicology and Chemistry, Stevens Point, WI, January 29, 2003 (Plenary Speaker).
- Presentation Swackhamer, D.L. Feminized Fish and Environmental Estrogens: Is There a Link?, Undergraduate Chemistry Lecture Series, University of Minnesota, Minneapolis, March 12, 2003.
- Presentation Swackhamer, D.L. Coffee Beans, Laundry Soap, and Fish Sexuality, Superior Science for You Speakers Series, Minnesota Sea Grant, U.S. EPA Laboratory, Duluth, MN, April 9, 2003.
- Presentation Swackhamer, D.L. Coffee Beans, Laundry Soap, and Fish Sexuality, Superior Science for You Speakers Series, Minnesota Sea Grant, North House Folk School, Grand Marais, MN, April 10, 2003.
- Presentation Swackhamer, D.L. Estrogen Mimics and Sex Education for Fishes SUNY-Environmental Sciences and Forestry, Syracuse, NY, January 2004, as part of their Women in Science Seminar Series.

A second manuscript based on the thesis listed below is completed and being readied for submission.

Graduate Students

Lehr, R. Use of estrogen equivalents to explain vitellogenin induction in fathead minnows (*Pimephales promelas*) exposed to a complex wastewater effluent, M.S. Thesis, Water Resources Science, University of Minnesota, February 2004.