NOVEL DIAGNOSTIC INDICATORS

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LTG 2 Poster 06

Science Questions

Long Term Goal 2, Multi-Year Plan Science Questions #2 - How can the sources and source strengths of stressors be inferred from in situ measurements? From stressor measurements? From biological indicators? From remotely-sensed observations and watershed properties?

Research Questions

– What diagnostic approaches are useful to identify the stressors affecting the nation's waters?

– What types and levels of biological indicators are useful for the identification of different classes and concentrations of stressors?

How Research Addresses the Water Quality MYP Goals

These research topics address the need for more sensitive indicators that can serve as diagnostic/predictive biomarkers for risk assessment. Furthermore, the combination of molecular and whole organism endpoints in these studies will allow us to identify and compare novel dose- and time-dependent indicators across multiple biological and taxonomic levels and enhance linkages from stressor sources to effects.

Water quality managers will have enhanced diagnostic capabilities for stressor identification. Additionally, the research results will be integrated into the Causal Analysis/Diagnosis Decision Information System (CADDIS) or similar tools to aid in stressor identification.

Research Objectives

The Nation's watersheds are constantly exposed to complex combinations of natural and anthropogenic stressors, and methods that identify the dominant stressors are required to guide management actions. While the ecological effects of many of these anthropogenic stressors are poorly understood, susceptibility due to species differences, biological activity at low concentrations, and bioaccumulation all increase the potential of many of the parent compounds and their metabolites for adverse effects on aquatic species. In addition to assessing the condition of the Nation's waterways and determining where impairments exist, efforts are also needed to determine what specific stressors are causing the impairments. Therefore, the objective of this program is to develop novel diagnostic indicators of waterway stressors across multiple biological levels.

The Exposure-Effect Hierarchy

Biological Level	Biological Effect
Community	Bio-assessment + Community Assessment
↑	↓
Population	Population Decline, Adaptation Population DNA Analyses
↑	↓
Organism	Toxicity Testing
↑	↓
Tissue	Histopathology
↑	↓
Subcellular/Cellular	Cell death/Mitosis and Molecular Changes

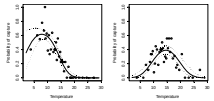
Research Methods & Results:
Individual - Population Biological Level

According to the Federal Insecticide, Fungicide, and Rodenticide Act, pesticides are defined as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest, and any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant. Pesticides in streams can have deleterious effects on aquatic life, as well as being a potential source of exposure for humans who may use the water.

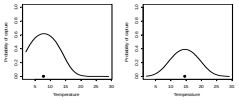
Water, macroinvertebrates, and sediments were sampled in Mid-Atlantic Coastal streams in 1999 and also in Midwest streams in 2003 with the addition of algae sampling. Water and stream sediment samples were analyzed for pesticides and metals, and macroinvertebrate samples were identified to species level. These data are being analyzed to determine aquatic macroinvertebrate indicator species for pesticides. Since all 50 states currently sample macroinvertebrates as part of their water quality assessment programs, pesticide indicator species would aid in stressor identification using their current sampling program.

Research Methods & Results:
Population - Community Biological Level

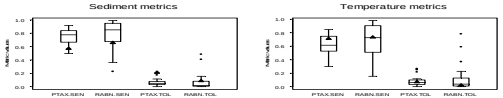
Invertebrates are frequently used in bioassessment and estimates of the tolerance or sensitivity of different species to different stressors provides a basis for determination of the causes of impairment in different streams. Using tolerance values, different species can be designated as sensitive or tolerant to a particular anthropogenic stressor. Then, to diagnose the causes of impairment, different tolerance metrics (e.g. the relative abundance of tolerant taxa) can be computed based on the observed assemblage of organisms. To determine the likely causes of impairment at a site, tolerance metrics can be compared to expectations.



Relationships between probability of capture and temperature for *Heterlimnius* (left plot) and *Malenka* (right plot). Solid lines: mean response determined by maximum likelihood estimation.



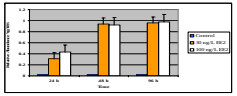
Tolerance values (shown as solid circles) for *Heterlimnius* (left plot) and *Malenka* (right plot). Here, the tolerance value is defined as the point along the environmental gradient where the probability of observing the species is maximized.



Comparisons of sediment (left) and temperature (right) tolerance metric values at a test site (solid triangle). Values are plotted for proportion of taxa that are sensitive (PTAX.SEN) or tolerant (PTAX.TOL), and relative abundance of sensitive taxa (RABN.SEN) and tolerant taxa (RABN.TOL).

Research Methods & Results:
Molecular – Individual Biological Level

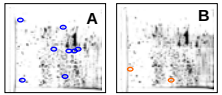
EDCs and pesticides affect fish species by disrupting numerous aspects of development, reproduction, and survival. Furthermore, new data have suggested that the reduced reproductive fitness resulting from these stressors may have an impact at biological levels beyond the individual, such as the population. Fish were exposed to various EDCs and pesticides individually or as mixtures for up to 96 hours and changes in the transcriptome and proteome were measured using microarrays followed by QPCR validation and 2-D gel electrophoresis followed by peptide analysis via mass spectrometry, respectively. These differential expression profiles will provide molecular diagnostic and predictive biomarkers for risk assessment and will enhance source-to-outcome linkages and support computational modeling.



Changes in Vg expression, relative to 18S, in male livers Following exposure to control water or EE_2 (30 or 100 ng/L) for up to 96 h as measured by QPCR.

Gene Expression	30 ng/L EE_2 Testis (48 h)	30 ng/L EE_2 Testis (96 h)	3.0 µg/L TRB Ovary (96 h)
Up-regulated	492 ± 339	875 ± 356	856 ± 955
Down-regulated	556 ± 380	822 ± 356	847 ± 759

Differentially-expressed genes in the testes or ovaries of zebrafish exposed to 30 ng/L EE_2 or 3.0 µg/L TRB, respectively, as determined by microarray analyses.

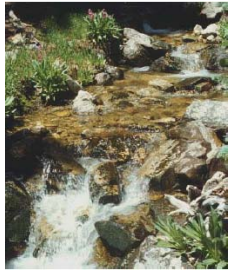


Representative protein expression profiles in 2-D gels from testes of zebrafish exposed to water (A) or 30 ng/L EE_2 (B) for 96 h.

Research Conclusions & Future Directions

The combination of techniques will allow us to identify and compare novel diagnostic indicators from a variety of waterways stressors, while simultaneously critically evaluating the relevance and linkage of biological data generated from multiple biological and taxonomic levels. Integration of these research capabilities has the potential to reduce the uncertainty in both ecological and human health risk assessments. This project has the additional potential of forming linkages across a considerable portion of the source-to-outcome continuum that is within the purview of EPA's risk assessment mission.

Conceptual Overview of Program



Interactions with Customers

The pesticide indicator taxa project received data from the long-term national research effort between the U.S. Environmental Protection Agency (USEPA) and the U.S. Geological Survey (USGS) termed Landscape Investigations for Pesticides Study (LIPS). The latest phase of LIPS was conducted in the Midwest Corn Belt (Corn Belt) and included roll-out meetings with EPA Region 5 personnel and State water quality officials in OH, IL, IN, and WI.

In February 2004, the US EPA and the Council of State Governments, with funding through the Region 9 RARE (Regional Applied Research Effort) Program and ORD NCEA (National Center for Environmental Assessment), convened a workshop of biologists from western state agencies, EPA and academia to review methods that are currently available for deriving and applying tolerance classifications.

EDC and pesticide research at the molecular level is being done in collaboration across ORD laboratories and in conjunction with partners at the USGS. Products from the pesticide research should directly and immediately benefit OPPTS during the re-/registration process.

How Research Contributes to Outcomes

- Water quality managers will have improved capabilities for diagnosing stressors.
- Research results can be integrated into the Causal Analysis/Diagnosis Decision Information System (CADDIS) or similar diagnostic tools to aid in stressor identification.
- The combination of molecular and whole organism endpoints in these studies will help illuminate linkages from stressor sources to effects.
- The research will help States use their existing macroinvertebrate and water quality monitoring databases to greatly enhance their stressor identification capabilities.

Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.