Compendium of the Results of the 1997-99 STAR Ecological Indicators Grants

Deliverable 2C: Compendium Report 1999 grants

U.S. Department of Commerce
COMMITS Contract No. 50CMAA900065

03 March 2005

Submitted to

Perot Systems Government Services
31 Schoosett Street
Pembroke, MA 02359

Submitted by

FTN Associates, Ltd.
3 Innwood Circle, Ste 220
Little Rock, AR 72211

1. INTRODUCTION

1.1 Background

The Environmental Protection Agency's (EPA) Office of Research and Development (ORD) provides leadership in science and conducts much of the EPA's research and development. To help EPA fulfill its mission of protecting human health and the environment, ORD conducts leading edge research and fosters the sound use of science and technology. Through its National Center for Environmental Research (NCER), ORD seeks to involve and support scientists in our nation's colleges and universities in research and educational efforts that will provide the sound science needed for environmental protection. As part of this effort, the EPA's external research program, Science to Achieve Results (the "STAR" program), funds research in a wide variety of environmental science disciplines. EPA's STAR program is unique among funding programs in that it advocates interdisciplinary research.

A significant part of the STAR program is Ecological Indicator research, which has the goal of developing the next generation of indicators for monitoring environmental condition. It is not practical to monitor all components of an ecosystem: the water, soil, air, plants, animals, and microorganisms, and their interactions. Ecological indicators are monitored as surrogates of overall ecosystem integrity and sustainability. The STAR program supports research to develop individual indicators, or "suites" of indicators, for a range of ecosystems. Research supported by the STAR program has resulted in development of a number of new indicators, as well as significant advancement of the science of ecological indicators.

In order for the information gathered from these research projects to be useful to decision makers, stakeholders, and the science community, it is beneficial to summarize the research results into comprehensive and easily accessible documents. In concordance with their commitment to communication, NCER is supporting a set of documents that highlight STAR program research results and successes related to ecological indicators. Because the product will be used directly by EPA, and to avoid any bias associated with the research projects, an extramural contract has been chosen as the appropriate vehicle to complete this task.

1.2 Objective

The objectives of this project are to produce three documents that outline the successes, results, and findings of each of the 1997-1999 Ecological Indicators grants: 1) a compendium of the 1997-1999 Ecological Indicators grants; 2) a synthesis of the 1997-1999 Ecological Indicators grants; and 3) a journal article based on the results of the 1997-1999 Ecological Indicators grants. The work presented here is a compendium for the 1999 Ecological Indicator grants.

1.3 Summary

Eight 1999 Ecosystem Indicator EPA STAR grants were reviewed in detail to determine how results of this research can or are being used by the larger community. The eight grants fell into one or more of the following themes and categories (Table 1, Appendix A):

Table 1: Themes and Categories for the 1999 EPA STAR Ecosystem Indicator Grants

Theme	Category	Number of Grants
Study System	Wetlands	2
	Freshwater	4
	Marine	3
Water Quality		6
Focus of Study	Forest	1
	Amphibians	2
	Fish	2
	Macroalgae	1
	Soil	1
	Algae	2
	Macroinvertebrates	4
	Bacteria	1
Stressor	Anthropogenic	3
	Nutrients	5
Genetics		4
GIS/Remote Sensing		3

Overall the eight grants produced two new genetic primers (the primers will allow future scientists to determine if fecal contamination can be traced to dogs or elk), three tools (including models and GIS coverages), 47 peer reviewed papers, and 135 oral presentations (Appendix B). The study areas for the eight grants were spread across the United States (U.S.) (Appendix C).

2. SUCCESSES, RESULTS, AND FINDINGS

2.1 STAR Grant R82-7637:

Developing an Indicator for Nutrient Supply in Tropical and Temperate Estuaries, Bays, and Coastal Waters Using the Tissue Nitrogen and Phosphorus Content of Macroalgae
Principal Investigator: Peggy Fong

Impacts:

Increases in the supply of nutrients are a critical worldwide issue and can lead to habitat degradation and harmful algae blooms. A bioindicator that can easily quantify nutrient supply to coastal ecosystems would be especially useful in systems that receive large amounts of nutrients in pulses or from non-point sources.

Successes and Lessons Learned:

- *Macroalgae can be valuable bioindicators of both quantity and source of excess nutrients, especially nitrogen.*
- *Factors that may confound this bioindicator, such as nutrient status of water or sediments, seasonal effects, and grazing pressure, can be controlled, further increasing the usefulness of this indicator.*
- *The long-standing hypothesis that algae can utilize nutrients stored in sediments was confirmed with experimental results.*

Indicators Developed/Tested:

- *Tropical Macroalgae: Five species of macroalgae were tested as potential indicators of the magnitude of nutrient supply.*
- *Temperate Macroalgae: Three species of macroalgae were tested as potential indicators of the magnitude of nutrient supply.*

Products:

- *Publications: 17 peer reviewed, 4 in review, 6 in preparation*
- *Presentations: 18 oral presentations*

User Groups/Community:

- *Environmental Protection Agency*
- *State water quality monitoring programs*

Themes:

marine, water quality, macroalgae, nutrients

Background

Eutrophication is caused by excess nutrients and can cascade into of a series of ecological problems such as shifts in marine populations and communities, degradation of habitat, and impacts on populations of economically important species such as fish and shellfish. Indicators quantifying nutrient supply and integrating impact over timescales of weeks to months are lacking and could be quite useful to systems that receive nutrients in pulses.

This study collected macroalgae species from a large geographic region covering the entire west coast of the continental U.S., Hawaii, and the Caribbean Sea. Nitrogen and phosphorus from the water column and sediment samples were compared to nutrient loadings found in macroalgae tissues. A series of elegant and complex experiments were set up to determine how a long list of environmental variables may affect this bioindicator: seasonal effects (dry versus wet), distance to shore (inshore versus offshore), initial nutrient status of the macroalgae (high versus low), macroalgae growth, water flow, salinity, light, nutrient origin (water versus sediment), grazing pressure by fish, and habitat (mangrove, seagrass, or coral).

Goals

- Identify and test several species for use as bioindicators of nutrient supply.
- Develop a relationship between nutrient supply and nitrogen/phosphorus in algal tissue.
- Develop quantitative relationships between ecological condition and nitrogen/phosphorus supply.

Findings

Tropical Macroalgae – Five species of macroalgae were collected from a wide variety of geographic areas: the Gulf of Panama and the Gulf of Chirqui (both in the Eastern Tropical Pacific), and Puerto Rico and Panama (both in the Caribbean); Hawaii and Honduras were used to test the successful bioindicator of excess nutrient supply. One particular species of macroalgae (*Acanthophora spicifera*) proved to be a valuable bioindicator of nitrogen and phosphorus, two elements strongly associated with eutrophication. Relationships between tissue nitrogen and water column nitrogen were on the whole stronger than phosphorus relationships. A series of experiments showed that tissue nitrogen is a function of nutrient availability (both in the field and in controlled laboratory settings); that the initial amounts of nutrients in macroalgae will affect this relationship (through different growth patterns); and that fish will preferentially graze on nutrient enhanced algae. Data from these experiments have quantified these relationships and suggested that the best bioindicator would be macroalgae grown in the laboratory under nutrient poor conditions and deployed in cages out of sediments to stop grazing and control nutrient uptake. The precision and accuracy of this bioindicator was tested in both Hawaii and Honduras to determine if the patterns found in the Caribbean and Eastern Tropical Pacific could be applicable to other regions; the results were favorable proving this bioindicator is transferable between regions.

Temperate Macroalgae – Three species of macroalgae were collected from nine California bays (stretching from Southern to Northern California) and ten Washington bays. All species were good indicators of tissue nitrogen and stable isotopes of nitrogen were used to determine the source of excess nutrients (either from the marine environment or the neighboring land). However, one species may not be usable throughout the region because each species density varies significantly along the coast. As in the tropical bioindicator, a series of experiments showed tissue nitrogen is a function of nutrient availability (both in the field and in controlled laboratory settings) and that the initial amounts of nutrients in macroalgae will affect this relationship (through different growth patterns). Similar to tropical macroalgae indicators, it was suggested that the best bioindicator would be macroalgae grown in the laboratory under nutrient poor conditions and deployed in cages to minimize grazing.

Implications

Simple bioindicators of excess nutrients in marine ecosystems are necessary given the quantity of nutrients supplied to these systems by human activities. This simple bioindicator based on macroalgae bridges the gap between immediate results from water quality sampling and long term results from monitoring programs.

Next Steps

This study developed a unique bioindicator that was tested in several areas where the nutrient regime was not well characterized. While this bioindicator is ready to be applied to most temperate and tropical ecosystems, local work may be necessary to successfully calibrate this indicator.

2.2 STAR Grant R82-7638:

Developing Effective Ecological Indicators for Watershed Analysis
Principal Investigators: Duncan Patten, W. Minshall, R. Lawrence, A. Marcus

Impacts:

Alteration of watersheds by grazing, fires, or logging may have important influences on the integrity of streams and associated riparian vegetation. Streams or riparian areas altered by watershed outputs may be useful indicators of watershed condition.

Successes and Lessons Learned:

- *High resolution hyperspectral imagery can be used to identify a number of stream features.*
- *Percentage of a watershed that burned was associated with stream power and bank failure rate.*

Indicators Developed/Tested:

- *River: River attributes can be expected to be related to watershed characteristics and may reflect a watershed's ecological integrity.*
- *Riparian: Riparian vegetation may integrate watershed level variables and may be indicators of watershed hydrologic processes and floodplain dynamics.*
- *Aquatic: Physical, chemical, or aquatic life data may be related to watershed condition.*
- *Scaling: Remote sensing technologies can be applied to quickly scale field based indicators "up" to the watershed level.*

Products:

- *Publications: 10 peer reviewed publications, 1 submitted, 2 Masters theses, 1 honors thesis*
- *Presentations: 20 oral presentations*

User Groups/Community:

- *National Park Service*
- *USDA Forest Service*
- *Natural resource managers*

Themes:

freshwater, water quality, forest, macroinvertebrates, anthropogenic disturbance, GIS/RS

Background

Human and natural alterations of watersheds are hypothesized to impact the rivers and riparian areas that drain them. Attributes of rivers and riparian areas in turn might be useful as indicators of overall watershed conditions. Remotely sensed data, especially hyperspectral imaging (sensors which measure up to 255 narrowly defined spectral channels, as opposed to multispectral remote sensing which have typically 5 to 7 broadly defined spectral regions) has the potential to rapidly and finely discriminate data pertaining to ecological indicators.

This study sampled four watersheds within the northern Greater Yellowstone Area of Montana and Wyoming. Forty-two watershed parameters were sampled and used to test associations with attributes of streams (e.g., depth, turbidity, stream power), riparian areas (e.g., vegetation structure, species), and aquatic life. Remote sensing technologies were used to evaluate three different sensors: Landsat (a multi-spectral satellite), AVRIS, and HYMAP (both of which are hyperspectral sensors mounted on aircraft).

Goals

- Determine how attributes of riverine systems are indicators of watershed condition.
- Determine if these indicators are spatially scaleable.
- Evaluate hyperspectral remote sensing technologies to measure and identify these indicators.

Findings

River Indicators – River attributes measured (e.g., stream power, channel shape, sedimentation, and bank stability) were linked to forty-two watershed variables (e.g., land cover and topography) with the goal of investigating how watershed scale disturbances of grazing, wildfire, and timber harvest affect streams. Results showed that grazing and timber harvesting cannot be linked to river attributes. However, stream responses such as changes in stream power or bank failure rates are related to general disturbance, not to the unique type of disturbance. Changes in stream power had the strongest response to fire-related disturbance but results showed that even measuring responses at greater than 10 sites per stream is inadequate to characterize watershed stream responses. It is, thus, impractical to use this indicator over large areas. Results indicate that stream bank failure is an important process and there is a strong relationship between the magnitude of failure and the frequency of failure–large failures are less frequent than small failures. This study also documented a relationship between stream power and the percentage of a watershed that was burned by the historic 1988 Yellowstone fires. These results implied that streams became more powerful in the aftermath of forest fire and that net incision has been the primary response in second- to fourth-order channels since these fires.

Riparian Indicators – This study documented a relationship between the spatial arrangement of processes developed by rivers (soil, floodplain development) and riparian vegetation. Watershed characteristics such as elevation, and proportion of barren and forestland cover types accurately classified coniferous herbaceous and deciduous riparian patches 70% of the time. Herbaceous vegetation seems to respond to differing stream processes and connectivity of riparian areas and may be a good indicator of watershed condition change. Riparian community factors appear to be related with watershed disturbance attributes; however, community structure of riparian areas does not appear to be related to watershed outputs.

Aquatic Indicators – Aquatic ecological variables were used to validate the relative pristine or altered nature of a watershed. Water quality data results indicated that all watersheds grouped independently, indicating that no one variable, or variable groupings, can determine watershed condition. However, chlorophyll *a* (a measure of algae activity) tends to be higher in streams that are designated “grazed.” Biological indicators showed that the presence or absence of macroinvertebrates was the best biological predictor of watershed condition. Results showed that every watershed had its own set of significant attributes that influence river and riparian processes. This uniqueness reduces the possibility of cross-regional indicators.

Scale Factors – Remote-sensing technologies can be applied to quickly scale field based indicators “up” to the watershed level. Analysis of hyperspectral imagery demonstrated that it could identify stream features such as depth, river habitats, and woody debris. However, analysis of all variables collected in this study showed that watersheds and rivers in the study area are relatively unique which reduced the cross-regional scalability of all indicators.

Implications

The use of high-resolution hyperspectral imagery has enormous potential in quickly and efficiently monitoring specific ecological condition variables or processes. As this discipline grows it may be possible to monitor large areas via aircraft and/or satellite systems at a faster and cheaper rate than field studies.

Next Steps

Further statistical analysis is necessary to integrate indicators for macroinvertebrates, river, and riparian zones. Additionally, with further development, specific river indicators may be identified and quantified using hyperspectral imagery.

2.3 STAR Grant R82-7639:

Molecular Detection of Anaerobic Bacteria as Indicator Species for Fecal Pollution in Water
Principal Investigator: Katherine Field

Impacts:

Contamination of water by fecal material is widespread and can cause beach closures, prohibit shellfish harvest, and destroy habitat. Current methods to detect bacterial contamination are slow (1-2 days), which reduces the effectiveness of measures to protect the public health.

Successes and Lessons Learned:

- *Two new genetic primers were discovered to distinguish dog and elk DNA from other fecal sources.*
- *A real-time quantitative DNA-based method of assessing fecal contamination of water was developed, shortening the time to obtain results from 1-2 days to 3-4 hours. In addition to being faster, this technique is much more sensitive than previous methods of detection.*

Indicators Developed/Tested:

- *Primers: Structure of the 16S ribosome DNA gene are similar in most life forms, making it easy to measure differences from distantly related organisms by precise aligning results.*
- *Subtractive Hybridization: Removal of bacteria related DNA and using hybridization techniques to isolate the leftover sequences for further analysis increases the power of detection of similar species.*
- *Real-time Q-PCR: Real-time DNA based measures of total fecal pollution and solely human fecal pollution were developed to decrease the time it takes to establish results.*

Products:

- *Methods: 2 new genetic primers were discovered to distinguish dog and elk fecal sources. A real-time quantitative DNA-based method of assessing fecal contamination of water was developed*
- *Publications: 7 peer reviewed publications, 6 published proceedings*
- *Presentations: 20 oral presentations*

User Groups/Community:

- *Southern California Coastal Water Research Project*
- *Environmental Protection Agency*
- *Health Canada*
- *Water Monitoring groups and agencies*

Themes:

freshwater, marine, water quality, bacteria, nutrients, genetics

Background

Bacteria contamination of water is the number one impairment of U.S. waters and currently affects 14% of waters reported by the EPA's 1998 303(d) list. Correcting this problem has been hampered by the difficulty in determining sources of contamination. Fecal contamination has long been suspected as a major source of impairment but quantification is difficult, as is distinguishing if the source is humans, livestock, or wild animals. Additionally, the standard indicator used to close beaches and shellfish harvesting (fecal coliforms) is slow (results take 1-2 days) and cannot distinguish between different mammalian species.

This study used two sophisticated molecular DNA techniques to attempt to determine sources of fecal contamination. In the first method, DNA libraries were developed for a specific portion of the ribosome DNA (the 16S rDNA gene), which is found in most life forms. DNA libraries are constructed by using primers—short, artificial DNA strands that exactly match the beginning and end of a DNA fragment to be

amplified. If DNA is present that matches a primer being used, DNA amplification results and a library can be constructed. Libraries were developed for elk, pigs, dogs, cats, gulls, and horses. In the second method, subtractive hybridization was used to determine new genetic markers of fecal source contamination. Subtractive hybridization is a technique that isolated genes without knowing their function. Water samples were collected in Tillamook Bay (Oregon) to test these newer DNA techniques alongside traditional measures of fecal contamination.

Goals

- Develop DNA markers to distinguish species that significantly contribute to pollution of Tillamook Bay.
- Identify strains of bacteria that are unique to different host species.
- Develop a quantitative indicator to estimate total pollution and proportion of different sources of fecal pollution.

Findings

Pwalrimers – Four elk primers were designed and tested; while one showed promise, the other three could not distinguish between ruminants (hoofed, even-toed mammals such as cattle, sheep, and goats). Five domestic pet (dog/cat) primers were tested; one showed promise while the others could not separate humans from pets. Three primers were tested for pigs, with one performing well. The results of this study showed that overall, it was not practical to use primers to construct DNA libraries to determine the source of fecal contamination.

Subtractive Hybridization – This study was the first to use subtractive hybridization to identify fecal contamination. DNA from the phylum Bacteroidetes (bacteria) was hybridized with dog fecal DNA (using cat and human subtractors) and with elk fecal DNA (using human and cattle subtractors) to determine unique primers. The new elk primer successfully distinguished elk from cattle or humans and the new dog primer successfully distinguished between dogs, humans, cats, pigs, and gulls. The use of this technique saved enormous amounts of time because large numbers of clones were not needed from each host to develop DNA libraries.

Real-time Q-PCR – Two new real time quantitative Polymerase Chain Reaction (Q-PCR) assays for the phylum Bacteroidetes were developed. The first was a general pollution index based on the entire phylum Bacteroidetes, the second targeted Bacteroidetes found only in humans. Bacteroidetes concentrations were highly correlated with the more traditional measures of fecal contamination—fecal coliform and *E. coli*. The test was sensitive, reproducible, and took 3-4 hours to complete. The quantitative range for which this test is applicable is four orders of magnitude greater than traditional measures, greatly increasing the sensitivity of fecal source tracking. Water samples from Tillamook Bay were used to test the field effectiveness of these new laboratory techniques. The new molecular approaches were less variable than traditional measures and sensitivity analysis concluded that the addition of one or two samples did not improve test results.

Implications

Bacterial contamination is a major water quality issue in the U.S. Rapid determination of levels of contamination will increase the effectiveness of public health departments closing beaches or fishing zones. Fine scale determination of the source of contamination could potentially help public officials improve water quality by targeting Best Management Practices at the offenders.

Next Steps

Epidemiological studies are currently being conducted to correlate human health outcomes with fecal contamination. The methods developed by this study will help identify sources of fecal contamination.

2.4 STAR Grant R82-7640:

Stream Plethodontid Assemblage Response (SPAR) Index Development, Application, and Verification in the MAHA
Principal Investigators: Robert P. Brooks, and G.L. Rocco

Impacts:

Small headwater streams are an important component of overall river water quality and account for up to 75% of the total stream length in the Mid-Atlantic Highland Area (MAHA). An ecological indicator that can distinguish headwater stream degradation based on amphibians could be useful to resource managers especially where fish assemblages are naturally poorly developed.

Successes and Lessons Learned:

- *Salamander assemblages within MAHA can be classified into 3 distinct groups; the stream types associated with these salamander classifications can be distinguished with environmental variables.*
- *Of 33 salamander metrics (biological measures) tested on all stream types, none performed extremely well.*
- *Of the 11 better-performing metrics that were assembled into 8 Indices of Biological Integrity (IBIs), none performed extremely well.*
- *Strong natural gradients within MAHA are affecting potentially useful metrics and IBIs.*
- *Training of volunteers can greatly improve their ability to identify salamanders and the use of volunteers can be a low cost method of data collection.*

Indicators Developed/Tested:

- *Index of Biological Integrity: A total of 8 IBIs were tested. Indices were constructed from 11 better-performing plethodontid (lungless) salamander metrics.*
- *Volunteer Training: Volunteers were trained and tested for effectiveness in identification of salamanders.*

Products:

- *Digital: A stream salamander sampling training manual was developed to train volunteers.*
- *Presentations: 3 presentations*

User Groups/Community:

- *Natural resource agencies*
- *Volunteer monitors*

Themes:

freshwater, water quality, amphibians, anthropogenic disturbance

Background

Headwater streams are an important component of overall stream health and have an important biological component. Amphibians are thought to be valuable indicators because they integrate stressors and pollutants throughout their complex life history, which encompasses terrestrial and aquatic habitats. Stream salamanders can provide valuable information in small headwater streams where traditional biological indicators, such as macroinvertebrates or fishes, may be absent.

This study sampled 138 streams from a large group of streams selected by the EPA Environmental Monitoring and Analysis Program (EMAP) to characterize the MAHA region. Five climate variables (ambient, surface, and soil temperature, and ambient and surface percent relative humidity), four field based water quality variables (stream water temperature, pH, conductivity, dissolved oxygen), and nine laboratory determined water quality variables (e.g., metals, sulfate) were measured at each stream along with physical variables pertaining to the stream (e.g., bank habitat, stream habitat). Salamanders of all

life-stages were captured and species identification occurred in the field; vouchers were collected and sent to the lab for verification of identification.

Goals

- Describe the range and variability of stream plethodontid assemblage responses (SPAR) throughout MAHA.
- Develop a SPAR based index to classify MAHA streams as reference, minimally degraded, and degraded.

Findings

Range and Variability of SPAR – Each of the 138 streams were classified into three MAHA disturbance categories (Degraded, Minimally-Degraded, or Reference) using nine variables relating to water chemistry, stream habitat and macroinvertebrates prior to data analysis of indicators. Results showed an incredible amount of variation in the 138 streams and several metrics tested were strongly tied to latitude, and to a lesser degree longitude. An attempt to normalize for latitude effects was employed by using a subset of 34 Reference and Minimally-Degraded streams and classify these streams based on salamander assemblage. Results of this normalization discovered three distinct geographic groups which were later used for indicator development. The predictive model was to classify the remaining 101 streams using latitude, boulder cover, water temperature, and stream gradient. Latitude accounted for 86% of the variability and the remaining 14% was related to stream environment. Cross validation seems to confirm this classification of three geographically distinct MAHA stream groups. The power of this classification is that variables are simple and easy to collect and are currently routinely recorded as part of stream assessments.

Index of Biological Integrity – Separate IBIs were developed and tested for each of the three geographic groups (outlined above) to determine if they could distinguish the three MAHA disturbance categories. Of an initial 33 metrics, preliminary analysis showed 22 with poor performance and 11 with potential usefulness. The 11 useful metrics were tested in combination with each other to determine if they could correctly classify streams into three MAHA disturbance groups. The best IBI correctly classified all streams 76% of the time. This IBI combined the number of salamander species found with the total number of individuals identified as headwater indicator species that remain larvae for more than one year. Another well performing IBI combined number of species, number of salamanders found, number of intolerant salamanders found, and number of terrestrial salamanders found. When separated into the three MAHA disturbance groups the performance of IBIs differed considerably. For instance, some IBIs distinguished Degraded streams, but not Reference or Minimally Degraded. It should be noted that no IBIs performed exceptionally well distinguishing Minimally Degraded streams (maximum accuracy was 68%). Generally, most IBIs could distinguish degraded sites better than minimally degraded sites, thus many streams of intermediate quality could be incorrectly identified as degraded.

Volunteer Training – Individuals from 41 volunteer groups were trained to identify salamanders. Most of the 65 individuals were biologists (70%) and had searched for amphibians in the past (84%) and cannot be considered representative of the general public. Training increased the average test scores by 32% and decreased the variability of scores significantly, indicating training greatly assisted volunteers. Volunteers were then sent to the field to implement the SPAR index. Volunteers correctly identified 88% of the vouchers submitted and confusion concentrated on larval stages of two similar species. This could be problematic given that the most successful IBIs are dependent on accurate species identification.

Implications

This study showed that stream salamanders might be a useful index to discriminate the health of headwater streams where other IBI species are not present (e.g., fish and/or macroinvertebrates). While there is considerable variability in MAHA with respect to degradation and stream salamanders, the

separation of streams into three geographic groups is not justified due to lower power of overall discrimination.

Next Steps

Future analysis of existing data and further data collection may provide stronger relationships between stream salamanders and response to stressors that would minimize geographic variation. Altering the training module to have greater emphasis on discriminating difficult species would be quite effective.

2.5 STAR Grant R82-7641:

Biogeochemical indicators of watershed integrity and wetland eutrophication
Principal Investigators: K.R. Reddy, J.P. Prenger, W.F. DeBusk, A. Ogram, W.D. Graham, E.F. Lowe, M.M. Fisher, and L.W. Keenan

Impacts:

Wetlands receive all the various inputs of adjacent uplands and thus should integrate stressors over a large area. Stresses to wetlands can affect nutrient cycling of carbon, nitrogen, and phosphorus which are basic processes and thus affect many species within an ecosystem.

Successes and Lessons Learned:

- *Total phosphorus is higher in impacted than unimpacted sites.*
- *Water level height and duration of flooding significantly influence nutrient dynamics.*
- *Phosphorus availability and vegetation type differentiate impacted from unimpacted sites.*

Indicators Developed/Tested:

- *Soil and Detritus: 12 unique parameters were tested in soil and detritus to discriminate between impacted and unimpacted sites.*
- *Microbial Communities: The composition of microbial communities in impacted and unimpacted sites could have an effect on a wetland's ability to cycle nutrients.*
- *Spatial and Temporal Dynamics: Time (flooded or not flooded) or space (where the sites were located) could possibly influence the explanatory power of indexes developed.*

Products:

- *Publications: 7 submitted, 6 in preparation, 4 theses and dissertations*
- *Presentations: 13 oral presentations*

User Groups/Community:

- *U.S. Army Corps of Engineers*
- *Environmental Protection Agency*
- *St. Johns River Water Management District*

Themes:

wetlands, soil, nutrients, genetics, GIS/RS

Background

An excess of nutrients (especially nitrogen and phosphorus), results in excessive plant growth and ultimately depletion of dissolved oxygen in the water. This can be a critical problem for wetlands due to their very nature (e.g., low elevation, placement within a riverine system). Ecological integrity of wetlands can be measured by their ability to sequester or convert these excess nutrients into forms easily uptakeable by plants or other life forms. Changes in the nutrient cycle are fundamental to the ecosystem and will affect all species which depend on them, unlike changes at higher levels which only affect a small portion of biota.

This study was conducted in east-central Florida on the Blue Cypress Marsh Conservation Area. Eighty sampling stations were distributed between three study sites (two impacted and one unimpacted) and 239 samples of soil and detritus (loose organic matter) were collected. Sixteen basic biogeochemical variables (e.g., pH, carbon, nitrogen, calcium) were tested in both soil and detritus to determine how effective they were in discriminating the difference between impacted and unimpacted sites. Microbial indicators (e.g., soil enzyme activity, sulfate reduction, denitrification) were measured at a sub-sample of sites to understand how they relate to basic biogeochemical parameters. Vegetation of these sites was classified into six groups to understand if vegetation influenced biogeochemical processes. Two sites were sampled, twice a month for 12 months to examine temporal variation.

Goals

- Develop sensitive, reliable indicators of ecological integrity for large-scale ecosystem management and restoration.

Findings

Soil and Detritus - Generally, variables tested in soil were better than detritus variables at discriminating between unimpaced and impacted sites. Total phosphorus in soil was one of the best explanatory variables. Soils at impacted areas were more basic than unimpacted sites, but the range and effect was small. Other variables which discriminated impacted from unimpacted sites included: Microbial Biomass Carbon, nitrogen and phosphorus (all in soil); ammonia concentrations in soil; and methane production of detritus. Generally, microbial activities varied with both nutrient levels and vegetation community. Phosphorus availability was the most important variable discriminating impacted from unimpacted; dominant vegetation was a secondary factor.

Microbial Communities - Microbial activities varied with both nutrient level and vegetation community. A high bacterial diversity was found and only a few similarities between impacted versus unimpacted sites were noted. Nutrient loading seems to enrich certain microbial communities. Genetic analysis of microbes found in impacted versus unimpacted sites showed clear differences.

Spatial and Temporal Dynamics - Temporal data showed many soil variables did not show differences throughout the year, but total phosphorus in soil did. Generally, detritus variables were poor discriminators. Several variables showed weak seasonality effects (that disappeared when time and sites were combined), but again, total phosphorus was clearly impacted by vegetation type, soil condition and hydrology. Spatial sampling across the study area showed relationships between soil properties and location. A spatial model was developed of total phosphorus and total carbon. Statistical analysis of the original 28 variables showed clear patterns of reducing variability (only 4 Principal Components were needed) and that new observations could be expected to be correctly classified into vegetation communities (75% of the time), impacted/unimpacted regions (85% of the time), or clusters (90% of the time). These models were tested independently on 12 wetland sites in Florida and Georgia and performed well.

Implications

The development of biogeochemical wetland indicators and their independent testing is an important undertaking. Soil biogeochemical measurements were indicative of vegetation types and could be used to determine the ecological integrity of the study area. Of most interest is the extrapolation of predictor equations to independent areas which showed that these indicators could be used in other wetlands throughout the region.

2.6 STAR Grant R82-7642:
Effects of Forest Fragmentation on Community Structure and Metapopulation Dynamics of Amphibians.
Principal Investigators: Lucinda Johnson, C. Johnson, R. Boone, J. Gross, J.H. Olker, and D. Breneman

Impacts:

Forested regions of the Great Lakes support a wide variety of woodland amphibians found in vernal (temporary) pools that may be impacted as fragmentation of forests surrounding these ponds increases due to timber harvesting.

Successes and Lessons Learned:

- *More amphibian species were found at pools in fragmented forests versus pools in unfragmented forests.*
- *Peak dispersal date for woodland frogs was 2-3 weeks later from pools in unfragmented forests.*
- *Canopy vegetation surrounding pools reduces UV-B radiation in the water column as much as particulate matter found in the water.*

Indicators Developed/Tested:

- *Amphibian Community Structure: The type of amphibians present at vernal pools should be related to the impact of forest fragmentation.*
- *Macroinvertebrate: Macroinvertebrate communities of vernal pools are poorly known but should be similar to macroinvertebrate communities found in streams.*
- *Vegetation: Vegetation patterns surrounding and in vernal pools may be related to the light regime and thus the amount of forest fragmentation.*
- *Amphibian Movements: Dispersing wood frogs may be influenced by the amount of forest fragmentation surrounding vernal pools.*
- *Vernal Pool Characteristics: Chemical and physical parameters related to vernal pools may be affected by the amount of forest fragmentation.*

Products:

- *Digital: A computer program, Individual-Based Movement, was created to model movement of vertebrates.*
- *Publications: 1 in review, 2 in preparation, 2 manuscripts*
- *Presentations: 8 presentations*

User Groups/Community:

- *Superior and Chippewa National Forest*
- *Boulder Lake Reserve, Duluth, MN*
- *USDA Forest Service*
- *Water resource managers*

Themes:

wetlands, amphibians, macroinvertebrates, anthropogenic disturbance, GIS/RS

Background

Amphibians are thought to be good ecosystem indicators of forest fragmentation because they are known to be susceptible to many stressors associated with fragmentation. Forest fragmentation reduces woodland habitat of amphibians, isolates habitat, changes water flows, and alters microclimates of vernal pools (e.g., light regime, nutrient dynamics). The affects of these stressors on amphibians breeding and living in these pools include increased mortality from habitat loss, road traffic, and predation.

This study sampled vernal pools in three ecoregions in Minnesota within fragmented and unfragmented forest patches. Thirty-seven pools were sampled over two years and amphibians and macroinvertebrates were recorded; physical habitat data pertaining to the pool (e.g., water level, UV radiation) were measured; quantitative vegetation sampling (e.g., percent cover, canopy cover) was conducted; and

landscape level data within two different buffers (1 and 3 miles) (e.g., land cover, road density) were determined.

Goals

- Determine the effect forest fragmentation has on amphibian communities.
- Assess how regionally and locally based indexes reflect amphibian habitats.
- Predict how changes in forest fragmentation may affect amphibian communities.

Findings

Amphibian Community Structure – Thirteen species were collected, ten of which were either very rare or occurred at all sites and thus were not useful for discriminating impacted from nonimpacted vernal pools. The presence or absences of only three species explained the majority of variation found. The presence of these species was negatively correlated with percent canopy cover and percent litter, while positively correlated with temperature. These three amphibian species were observed more at fragmented forest sites than at unfragmented forest sites.

Macroinvertebrates – Total number of species of macroinvertebrates was found to be negatively associated with percent forest. However, this relationship was not strong and explained a small amount of variation.

Vegetation – No significant effects of percent forest were found when compared to vegetation metrics. The lack of effect was attributed to the landscape effects of forest fragmentation.

Amphibian Movement – More than 14,700 frogs were captured, only 14% of which were adults. Most (80%) of the non-adult frogs were dispersing from their natal (original) ponds, while the remaining were transients from wetlands outside the study pools. Of the more than 6,000 dispersing frogs captured, peak dispersal date for pools in unfragmented forest sites was 2-3 weeks later than from pools in fragmented forest sites. Only 2.3% of the 6,000 dispersing frogs were captured again in a subsequent year and only 1.6% of the dispersing frogs were captured at a different site. Wood frogs originating from pools in unfragmented forests dispersed randomly while wood frogs originating from pools in fragmented forests dispersed away from open areas (fields). A landscape model was developed that described the movement patterns of wood frogs between vernal pools by combining slope with a satellite derived measure of spring wetness.

Vernal Pool Characteristics – The potential mutation effects of UV-B radiation in these vernal pools was determined to be in a narrow range of water depths (.2 to 1.5 inches). However, nearly all pools sampled had UV-B levels higher than a level expected to result in a population rate of 50% hind-limb malformations. Canopy vegetation reduced the risk of exposure almost as much as water column parameters (e.g., turbidity).

Implications

Forest fragmentation has certain effects on the characteristics of vernal pools and their amphibian communities. Of particular note is that vegetation canopies can protect vernal pools from UV-B radiation as much as particulates in the water column do. This finding is significant because UV-B radiation has been potentially linked to amphibian declines and mutation.

Next Steps

Indicator development could continue to better discriminate vernal pool condition. Results from this study show that potential indicators might include light regime, water temperature, and insect or frog community structure.

2.7 STAR Grant R82-7643:

Multi-level Indicators of Ecosystem Integrity in Alpine Lakes of the Sierra Nevada
Principal Investigators: James Oris, S.I. Guttman, A.J. Bailer, J.E. Reuter, and G.C. Miller

Impacts:

Alpine lakes are sensitive systems, but due to their picturesque setting have the potential to be highly impacted by humans. As these areas are settled, the likelihood of human caused stresses will increase over time.

Successes and Lessons Learned:

- *No strong relationships between zooplankton or benthic invertebrates and human impacts were seen.*
- *Some top predatory fish species of these lakes have the potential to accumulate mercury to levels above California state health guidelines.*
- *Molecular biomarkers of contaminant exposure in cells of fish gills and livers indicated biologically relevant chemical exposures in some lakes, despite extremely low levels of specific contaminants.*

Indicators Developed/Tested:

- *Benthic Invertebrates & Zooplankton: Human caused stresses could have measurable effects on populations of both benthic invertebrates and zooplankton.*
- *Fish Assemblage: Fish population variables such as number of individual and number of species have been shown by others to be altered by human impacts.*
- *Molecular Biomarkers: Changes in gene expression in response to contaminant exposures were measured and analyzed with five genes in the liver and three in the gills.*
- *Population Genetics: Genetic diversity of many species has been shown by others to decrease due to human impacts by differentially altering either survival or reproductive rates.*

Products:

- *Publications: 1 MS thesis, 1 PhD dissertation*
- *Presentations: 13 oral presentations*

User Groups/Community:

- *Tahoe Regional Planning Agency*
- *Donner Lake Property Owners Association*
- *California Fish and Game*
- *Nevada Department of Wildlife*
- *U.S. Fish and Wildlife Service*
- *U.S. Geological Service*
- *Community planning groups*
- *Watershed managers*

Themes:

freshwater, water quality, macroinvertebrates, algae, fish, nutrients, genetics

Background

Alpine lakes are typically characterized by nutrient poor waters and a resultant low assemblage of both plants and animals. These factors contribute to the sensitive nature of these systems and thus water quality of alpine lakes is of critical concern. The picturesque setting of alpine lakes has made them magnets for human settlement and human impacts can be considerable and varied (e.g., swimming, watercraft, septic tanks, water withdrawal).

This study conducted an environmental assessment on 16 Californian alpine lakes: five of which were pre-determined to be highly impacted by humans, seven moderately impacted, and four minimally

impacted. At each lake, physical habitat data (e.g., vegetation structure, human disturbance, water depth), physical water data (e.g., temperature, dissolved oxygen, pH), water quality (e.g., total phosphorous, mercury, ortho-phosphates), macroinvertebrates, sediment cores, zooplankton, and fish were collected. Over 1,100 metrics were collected and data were used to alter the pre-study assessment of human impact. This study also used sophisticated molecular techniques to determine biological repercussion of human impact. Gene expression is the process by which the information that lies in DNA is transcribed to messenger RNA and then to proteins. The incredible variation in populations that we see in nature is in part due to differential expression (creation of proteins) from the same DNA fragments. Gene expression often is altered as a result of toxicant exposure and thus is a sensitive, measurable endpoint for toxicity that can serve as an early warning of compromised health.

Goals

- Conduct environmental assessment on 16 alpine lakes with a defined range of human impacts.
- Conduct genetic tests on fish and invertebrates to determine genetic diversity.
- Conduct experiments to determine genetic change when fish are exposed to contaminants.
- Integrate these three goals with current monitoring protocols.

Findings

Benthic Invertebrates & Zooplankton – Analysis of the first two years of data from this study failed to produce enough benthic invertebrates to allow for statistical analysis, principally due to sampling technique. A new dipnet technique was used to collect over 8,000 invertebrates in one year from the 16 lakes. No traditional metrics of biological integrity based on benthic invertebrates used by other lake monitoring programs had any significant relationship with the pre- or post-assessment human impact categories. No strong relationships were found between zooplankton and assessment categories either.

Fish Assemblage – Fish sampling consisted of gill nets and fish traps and sampling of the 16 lakes produced 16 fish species, five of which were native species. Fish flesh was tested for accumulation of mercury and some large top predatory species were found to have mercury levels at or above California state health guidelines. Further analysis of data showed that fish diet is likely related to mercury levels, not just size.

Molecular Biomarkers –Fish were left in cages for 48 hours in each of the 16 study lakes. Cells from fish gills and livers were analyzed and three genes showed a strong relationship between their gene expression and the presence of recreational activity such as personal watercraft or boating.

Population Genetics – Genetic information at the population level for four species (two fish, one crayfish, one amphipod) was analyzed. Amphipods were unsuitable as bioindicators due to the presence of several cryptic species that confounded data analysis. There was no relationship between crayfish genetic structure and human impacts, hydrologic regime, nor geographic distance, indicating that the underlying genetic diversity of crayfish may be more related to the use of crayfish as bait (and thus human transport). The preliminary analysis of one fish species' genetic structure revealed that gene flow between lakes is very limited.

Implications

Based on the findings of this study in the 16 alpine lakes analyzed, nine of the pre-study assessments did not change, but seven of the pre-study assessments were incorrect (needing to be increased from Low to Moderate or from Moderate to High). This implies that previous methods of assessing alpine lakes were conservative. Unlike other lake monitoring programs, benthic invertebrates from alpine lakes do not seem to be impacted by humans, suggesting that new indicators may be necessary to track changes in alpine ecosystems.

Next Steps

Given the large amount of data collected, more analysis is necessary. In particular, sediment samples have yet to be analyzed and genetic analysis of both fish species is pending, as is their possible correlation with the large quantity of environmental variables collected.

2.8 STAR Grant R82-7644:

Integrative Indicators of Ecosystem Condition and Stress Across Multiple Trophic Levels in a San Francisco Estuary

Principal Investigators: Richard Dugdale, A. Arp, S. Bollens, D. Julian, W. Kimmer, J. Thompson, and F. Wilkerson

Impacts:

Shifts across multiple trophic levels due to environmental stress can have significant negative effects on ecosystem condition. Indicators that can detect environmental stress before ecosystems are affected are necessary to warn resource managers of impacts.

Successes and Lessons Learned:

- *A threshold value of 3 μM (microM is a measure of molarity, a measurement of gram-molecular weight per liter) of ammonium was discovered to be the upper limit of non-bloom growth conditions for phytoplankton.*
- *Condition of larval Pacific Herring is not related to food availability.*
- *Glycogen concentration in an exotic bivalve might be a potential indicator of freshwater stress.*

Indicators Developed/Tested:

- *Nutrient Status: The status of nutrients and productivity measurements of phytoplankton can assist in the determination of the relative contribution of diatoms to total biomass.*
- *Zooplankton: Reproductive rates of common species can give an indication of environmental stress.*
- *Benthic: Changes in the benthic community or individual parameters (such as condition) of key species can indicate environmental stress.*
- *Larval Pacific Herring: Physical characteristics of larval herring are sensitive to food availability.*
- *Stress Protein Analysis: Molecular tools to analyze stress proteins may give early indicators of environmental stress.*

Products:

- *Publications: 5 peer reviewed publications, 1 submitted, 1 in preparation, 1 MS thesis*
- *Presentations: 40 oral presentations*

User Groups/Community:

- *State water quality agencies*
- *Environmental Protection Agency*

Themes:

marine, water quality, macroinvertebrates, algae, fish, nutrients, genetics

Background

Indicators of stress to marine organisms at a variety of trophic levels are necessary to determine the extent of environmental stress throughout marine ecosystems. Phytoplankton, zooplankton, benthic species, and fish all respond differently to stress and useful indicators should take into account these differing responses.

This study was conducted within the open water of San Francisco Estuary and Bay where monthly measurements of temperature, salinity, nutrients, and chlorophyll were made for three years to characterize basic marine processes. A series of 36 experiments were made to determine how egg production varies according to food and/or salinity stress, larval Pacific Herring were collected monthly to determine how morphometric measurements vary with food availability, and the benthic community was sampled to determine how exotic invasive species can alter community patterns.

Goals

- Develop ecological indicators along a salinity gradient for nutrient status and benthic community, and use molecular tools to evaluate ecological condition.
- Investigate relationships between these ecological indicators and other physical and biological variables.
- Assess these ecological indicators in other locations.

Findings

Nutrient Status – Elevated biomass levels of the estuary were detected in all years and were dominated by larger species of phytoplankton. Nutrient status indicated that neither nitrate nor silicate is a limiting factor for phytoplankton growth. The variability of primary production is thought to be a result of high ammonium concentrations, which results in low nitrate availability. Ammonium concentration was discovered to be a primary indicator of phytoplankton productivity and a threshold of 3 μM was determined for healthy chlorophyll production.

Zooplankton – Egg production of four zooplankton species groups were measured as food and salinity were experimentally varied. Egg production of two species was unresponsive to food or salinity experiments but one species, *Acartia spp.*, exhibited a small response. Given this subtle response, it is unlikely this indicator will be useful.

Larval Pacific Herring – Six morphometric measurements were used to develop nine indices of larval Pacific Herring growth. Three indices showed promise as indicators of larval herring condition, but none had a strong correlation with either chlorophyll *a* biomass or zooplankton egg production rates.

Benthic – At least six exotic species have invaded the San Francisco Bay area with one, the bivalve *Potamocorbula amurensis*, classified as an aggressive invader with the potential for broad impacts. Carbohydrate storage in the form of glycogen was investigated as an indicator of freshwater stress. While general condition is not a useful indicator of estuarine stress, indications are that glycogen concentration might be. More research is necessary to test the indicator at several locations.

Stress Protein Analysis – Four stress proteins were analyzed from tissue samples of *P. amurensis*. Analysis of two of the four sample dates indicates that stress is spatially variable. Results need to be analyzed in conjunction with glycogen concentration and condition to determine if stress proteins might be potential indicators of organism health.

Implications

The determination of a threshold for ammonium concentrations is an important implication of this study. Recent changes to ammonium concentrations in the San Francisco Estuary and Bay have been attributed to the shift from primary to secondary treatment of sewer wastewater and agricultural runoff. The observed long-term decline of the fisheries of the San Francisco Estuary and Bay may be due, in part, to these shifts.

Next Steps

Data analysis of stress proteins needs to continue to determine if these variables might be useful indicators. New experiments that test glycogen storage across several locations and a larger gradient will assist in the evaluation of this potential indicator.

Grant	Study System	Water Quality	Focus of Study	Focus of Study	Focus of Study	Primary Stressor	Genetics	GIS/RS
R82-7637	Marine	Water Quality	Macroalgae			Nutrients		
R82-7638	Freshwater	Water Quality	Forest	Macro-invertebrates		Anthropogenic disturbance		GIS/RS
R82-7639	Freshwater Marine	Water Quality	Bacteria			Nutrients	Genetics	
R82-7640	Freshwater	Water Quality	Amphibians			Anthropogenic disturbance		
R82-7641	Wetlands		Soil			Nutrients	Genetics	GIS/RS
R82-7642	Wetlands		Amphibians	Macro-invertebrates		Anthropogenic disturbance		GIS/RS
R82-7643	Freshwater	Water Quality	Macro-invertebrates	Zooplankton	Fish	Nutrients	Genetics	
R82-7644	Marine	Water Quality	Macro-invertebrates	Zooplankton	Fish	Nutrients	Genetics	

APPENDIX B - PRODUCTS

Grant	New Method	Articles	Presentations	Tools/Models/GIS
R82-7637		17	18	
R82-7638		10	20	1
R82-7639	2	7	20	
R82-7640			3	1
R82-7641		7	13	1
R82-7642		1	8	1
R82-7643		0	13	
R82-7644		5	40	

APPENDIX C - GEOGRAPHIC DISTRIBUTION