

US EPA ARCHIVE DOCUMENT

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

**Deliverable 2B: Compendium Report 1998 grants**

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## **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 Agency'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. The EPA's external research program, Science to Achieve Results (the "STAR" program), funds research in a wide variety of environmental science disciplines and is unique in that it advocates interdisciplinary research.

A significant part of the STAR program are the Ecological Indicators grants, whose goal is to develop the next generation of indicators for monitoring the condition of the environment. It is not practical to monitor all components of an ecosystem, including the water, soil, air, plants, animals, microorganisms and their interactions. Consequently, it is important to learn which ecological indicators are best used as surrogates or markers of overall ecosystem integrity and sustainability. To meet this need the STAR program supports research to develop individual indicators, or "suites" of indicators, for a range of ecosystems. In some cases the science has greatly moved forward, and in other cases new indicators have been developed.

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, the NCER is supporting a set of documents that highlight research results and successes. 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 1998 Ecological Indicator grants.

### **1.3 Summary**

Twelve 1998 Ecosystem Indicator EPA STAR grants were awarded and eleven were reviewed in detail to determine how results of this body of research can or are being used by the larger community. The eleven grants fell into one or more of the following categories/themes (Table 1, Appendix A):

**Table 1: Themes/Categories for the 1998 EPA STAR Ecosystem Indicator Grants**

Theme	Category	Number of Grants
Study System	Forest	4
	Woodland	1
	Freshwater	4
	Marine	1
Water Quality		5
Focus of Study	Forest	2
	Individual Plants	3
	Soil	3
	Birds	2
	Fish	4
	Insects	1
	Macroinvertebrates	1
	Zooplankton	1
Stressor	Anthropogenic	4
	Pollutants	5
	Nutrients	1
Genetics		5
GIS/Remote Sensing		2

Overall the eleven grants were highly productive with a total of four new methods developed, five tools/models/GIS created, 67 peer reviewed papers, and 215 oral presentations (Appendix B). The eleven grants were spread across a wide geographical swath of the country (Appendix C).

Of special note are the four novel methods developed by this EPA sponsored research:

- A new method of counting pollen tubes to assess pollination success in a wildflower which is easier and non-destructive.
- A new technique to clone adjacent regions of the genome around a known piece of DNA.
- A new method for determining ammonium in soil extracts which is easier and more environmentally friendly than previous methods.
- A new method to determine the stomatal conductance (passage of water or carbon dioxide through stomata (small pores) of plants) was developed which could eliminate costly monitoring equipment.

## 2. SUCCESSES, RESULTS, AND FINDINGS

### 2.1 STAR Grant R82-6591

#### An Integrative Aquatic System Indicator

**Principal Investigators:** Richard S. Stemberger and E. K. Miller

**Impacts:**

While lake water quality monitoring has a long history, zooplankton have been largely ignored because of high cost and technical experience needs. Zooplankton graze on algae which can give water a characteristic green hue when present in large numbers. Alteration of these populations can result in dramatic decreases in lake water quality by decreasing grazing pressure.

**Successes and Lessons Learned:**

- A simple zooplankton body size index for predicting changes in lake transparency was developed which is more strongly related to water chemistry factors than other estimates.
- Zooplankton populations are primarily correlated to watershed factors while zooplankton size is primarily controlled by fish size structure which drives the intensity of predation on zooplankton by fish.

**Indicators Developed/Tested:**

- Body Length Indicator: The mean length of four common zooplankton were compared to water clarity. The indicator could in turn predict water quality changes.
- Watersheds & Tributaries: Key lake chemistry and zooplankton variables have a hierarchical relationship with land cover and stream tributary water chemistry.
- Nitrogen-Phosphorus Ratio: A low nitrogen to phosphorus ratio can increase cladocerans (zooplankton which feed on algae).

**Products:**

- Digital: Image processing software to calculate length and biomass.
- Publications: 2 peer reviewed publications, 3 undergraduate thesis, 3 manuscripts
- Presentations: 6 oral presentations

**User Community:**

- University of Maine at Orono
- University of New Hampshire
- Dartmouth College NIEHS Super Fund research group
- Maine Department of Environmental Protection
- Local Lake Associations which monitor water transparency

**Themes:**

freshwater, water quality, zooplankton, nutrients

#### Background

Many factors can cause degradation of lake water quality. While lake transparency is closely related to the amount and size of light scattering algae, no simple relationships exist to determine degradation cause. In fact competing hypotheses are equally plausible. For instance lake transparency reductions can be caused by an increase of nutrients (enabling light scattering algae populations to increase by providing them more nutrients) or by over harvesting large game fishes (eliminating control by predatory fish on small fish that eat large zooplankton grazers which ultimately allows more algae to survive).

Historically, the role of zooplankton in maintaining lake transparency has been largely ignored. This oversight is more related to time and expertise needed to measure zooplankton than the role they play in regulating lake water quality. One group of zooplankton (cladocerans) strongly prefer grazing on particles that have the greatest influence on backscattering, and cladoceran body size is determined by the

intensity of predation by small forage fish. This study sampled 60 lakes and watersheds in New Hampshire, New York, and Vermont in order to tease apart two competing explanations of degradation.

### Goals

- Develop an integrated indicator which can use zooplankton population parameters to determine lake water quality.

### Findings

*Body Length Indicator* – This study sampled zooplankton at a deep location of each lake by hauling a net from the bottom of a lake to the surface and found that water transparency (as measured by Secchi disks) can be predicted by a simple body size indicator which uses a mean of four common zooplankton groups. Calculating the index using the entire zooplankton assemblage did not perform any better than using these specific four groups. Body size is measured by connecting a dissecting microscope, video camera, digitizing board, and computer together. The authors of this study modified a public-domain image processing software package to enable an analyst to quickly measure up to 300 individuals an hour (10-20 times faster than manual methods). The digital image files are archived and quality control measures can quickly be used to check individual analysts' results. Body length indicator in conjunction with other water chemistry data strongly predict lake transparency.

*Watersheds & Tributaries* – Strong relationships between water chemistry variables (e.g., color, chlorophyll *a*, total phosphorus, total nitrogen) and watersheds and tributary water chemistry were found. Eight lake water chemistry models performed well (explaining 65-88 percent of the variance) using a wide variety of watershed and tributary water variables (e.g., proportion of watershed that the lake occupies, amount of total phosphorus tributaries have, land use/land cover percentages for watershed).

*Nitrogen-Phosphorus Ratio* – A low ratio of nitrogen to phosphorus should favor cladocerans while high ratios should favor calanoid copepods (a zooplankton which is a significant prey item for fish). Experiments in large tanks manipulated ratios of these elements and indeed showed the above relationship was true. However, field data collection in conjunction with laboratory experiments have not shown these relationships and thus fish predation on zooplankton must be overwhelming any potential effect that these elements may have on the zooplankton assemblage.

### Implications

A zooplankton body length indicator can be used with water chemistry data to predict transparency of lake water. This indicator incorporates the relationship between zooplankton (cladoceran) body size and intensity of fish predation which in turn allows for improved understanding of the health of fish populations, without costly direct sampling of fish.

### Next Steps

The integration of zooplankton, lake and tributary water chemistry, and watershed land cover is currently being analyzed and evaluated. A full analysis of these data will likely be hierarchical in nature. Early indications (see above) are that the relationships between lake water chemistry and tributary chemistry and watershed properties are stronger than originally thought. Quantifying the major sources of environmental variation as a percentage of each variable to the total explainable variance that affects zooplankton assemblage structure is necessary. This analysis will allow for a better understanding of the influence of spatial scale (i.e., watershed and tributary features) on water chemistry and zooplankton biomass and size structure. These analyses will identify the most effective indicators for predicting key features of the zooplankton assemblage that relate to water quality issues.

## 2.2 STAR Grant R82-6592

### Soil Enzyme Stability as an Ecosystem Indicator

Principal Investigators: Richard P. Dick

#### **Impacts:**

*The use of soil enzymes as indicators of past soil management practices (including tillage) has potential to promote sustainable use of forest and agricultural landscapes.*

#### **Successes and Lessons Learned:**

- *Confirmation that across soil types, air dried soil can be used to measure enzyme activity.*
- *Microwave Enzyme Stress (MWES) index was developed to standardize indicators across soil types and was a practical tool for isolating enzyme activity.*

#### **Indicators developed/tested:**

- *Enzyme Assay: Three enzymes were investigated for sensitivity to soil management: Arylsulfatase which converts organic sulfur and plant usable forms and also correlates with fungal biomass;  $\beta$ -glucosidase which is an important decomposer enzyme and releases sugars (glucose); and fluorescein diacetate (FDA) hydrolysis, a general indicator.*
- *Ergosterol: Fungal biomass is effective at decomposing highly carbon/nitrogen materials and ergosterol was tested as an indicator of fungal biomass.*
- *Relative Indexes: Soil indicators have a tendency to be strongly dependent on soil type thus a calibrated relative index is needed to standardize results across soil types. Two indexes were developed, enzyme activity-soil property ratios and microwave stress enzyme stress.*

#### **Products:**

- *Publications: 1 peer reviewed article, 1 Master of Science thesis, 2 senior theses.*
- *Presentations: 7 oral presentations.*

#### **User Community:**

- *Forest and agricultural land managers*

#### **Themes:**

*Forest, soil, anthropogenic disturbance*

### **Background**

Within human time scales, soil is not considered to be a renewable resource and thus development of an indicator which reflects ecosystem health and management actions would clearly have advantages. Soil chemical and physical properties (e.g., organic matter) have been shown to have relationships with soil management but change too slowly to be useful indicators of ecosystem health. Soil biota perform many functions such as decomposing plant and animal residue, fixing atmospheric nitrogen, transforming nitrogen and other nutrients among various organic and inorganic forms, and releasing plant available forms of nutrients. Soil characteristics related to biological activities such as microbial mass or enzyme activity play a role in nutrient cycling and have been shown to be potential sensitive indicators of ecosystem health.

Soil enzymes that are not associated with living cells are termed abiotic and are more sensitive to management actions than traditional measures such as total carbon analysis. This study collected soil samples from seven forested and ten agricultural sites across a wide range of soil types in Oregon. Forested sites were paired comparisons of old growth and Christmas tree farms while agricultural sites were paired undisturbed and cultivated sites (two sites were long term research areas).



### Goals

- Refine microwave enzyme stress test for maximum sensitivity in detecting soil management as a soil quality and ecosystem indicator.
- Determine temporal dynamics of selected soil enzyme activities across diverse agricultural systems.
- Develop an index that assesses soil quality independent of soil type considerations.

### Findings

*Enzyme Assay* –  $\beta$ -glucosidase was most effective as soil quality indicator of degraded agricultural soils. Arylsulfatase activity in forest soils was four times greater than agricultural soils and was effective at separating both agricultural and forest land management treatments. FDA hydrolysis was not an effective indicator of management activity.

*Ergosterol* – Old growth forest soils all had higher ergosterol levels than Christmas tree farms and ergosterol was determined to be a good indicator of fungal biomass. In addition, ergosterol was strongly correlated with arylsulfatase activity indicating this enzyme is a good indicator of fungal biomass.

*Relative Index* – The enzyme assays were generally good detectors of management techniques but were strongly dependent upon soil type. Two techniques were tested to standardize for soil type: enzyme activity per unit carbon and/or clay and the ratio of stressed enzyme activity (by microwaves) to total activity. Relative indexes had stronger relationships to agricultural soils and enzyme activity of  $\beta$ -glucosidase and arylsulfatase per unit clay were both effective in standardizing for soil types. FDA hydrolysis ratios, like their assay counterparts, were not relative indexes. A simple addition of the two  $\beta$ -glucosidase and arylsulfatase per unit silt and clay indexes was determined to be the most promising indicator of soil management independent of soil type.

*Quantifying the Stabilized Enzymes in Soils* – The microwave stress test was shown for 2 enzymes to be useful in quantifying the abiotic or stabilized form of enzymes in soils. This provides a low cost research tool that did not exist before.

### Implications

The confirmation of using air dried samples is critical as it will allow for easy storage of samples and allow for more time before an assay needs to be run, these factors will increase the usability of these suite of indicators. An indicator of soil health independent of soil type that is easy and cheap to measure was developed that seems to be relatively good discriminators of soil quality on agriculturally managed soils.

### Next Steps

Further research to develop indicator that can differentiate between forest soil management is still necessary. Further calibration of the Microwave Enzyme Stress index is necessary to tease out all the soil type effects in these ratios. Standardization for the power of a microwave is necessary, especially as the power of microwaves diminishes over the life of the device.

## 2.3 STAR Grant R82-6593

### Are Genetic Diversity and Genetic Differentiation Bioindicators of Contaminant Impact on Natural Populations? *Fundulus heteroclitus* as a Model Estuarine Species

**Principal Investigators:** Michael Newman, M. Mulvey, W. Vogelbein, M. Unger

#### **Impacts:**

*Animals exposed to toxic concentrations of pollutants can acclimate or experience genetic adaptation. Acclimation is not inherited by offspring, and may occur at the expense of other vital bodily functions. Genetic adaptation leaves more resistant genes in subsequent generations, but could come at a cost when a population is confronted with new stresses.*

#### **Successes and Lessons Learned:**

- *Genetic tests on a fish, (mummichog, *Fundulus heteroclitus*) are a robust indicator of population level effects of pollution.*
- *Tolerance to pollutants does not affect disease resistance nor genetic diversity.*

#### **Indicators Developed/Tested:**

- *Genetic Variation: Genetic markers and adaptation to pollutants vary with levels of contamination.*
- *Genetic Diversity: Pollution associated death could lead to loss of alleles.*
- *Population Parameters: Pollution caused adaptations could come at a cost of other biological parameters such as growth, longevity, fertility, and disease resistance.*

#### **Products:**

- *Publications: 3 peer reviewed articles*
- *Presentations: 8 oral presentations*

#### **User Community:**

- *State regulatory agencies,*
- *U.S. Environmental Protection Agency*

#### **Themes:**

*marine, water quality, fish, pollutants, genetics*

#### **Background**

The common coastal fish mummichog (*Fundulus heteroclitus*) is found in tidal creeks across eastern North America. Mummichogs are useful in examining pollutant toxicity because they complete their life cycle within a small area (approximately two acres). Genetic adaptation to pollution by this species has been demonstrated in both field and laboratory experiments, but relationships among exposure, tolerance, population parameters, and genetic markers are poorly understood. This study focused on pollution from the Atlantic Wood EPA Superfund site (Virginia) that is contaminated with several known toxic chemicals associated with processing wood (polycyclic aromatic hydrocarbons or PAHs were the focus). PAHs are a group of chemicals that are formed during incomplete burning of organic matter and are manufactured for industrial purposes but can also be found naturally. Many PAHs are carcinogens.

Genetic variation caused by environmental contamination can be measured by studying DNA inherited from the mother (mitochondrial DNA or mtDNA) and from proteins coded by DNA (allozymes) from many individuals across a pollution gradient. Pollution-associated genetic effects are indicated by correlations between level of contamination at a site and genetic qualities of associated populations.

### Goals

- Test whether genetic structure of mummichog reflect a mosaic of polluted and clean habitats.
- Determine if mummichog at polluted sites exhibit lower than expected genetic variability.
- Test whether mummichog have a contaminant tolerance that is lost if reared in clean habitats.
- Determine if mummichog at polluted sites are not replenished by migration from nearby clean sites.

### Findings

*Genetic Variation* – Both mtDNA and allozyme tests showed that mummichog genetic differences were determined more by differences in contamination than geographic distance. Additionally, genetic distance is not correlated with spatial distance at the scale studied.

*Genetic Diversity* – Both mtDNA and allozyme genetic analyses show that pollution does not have a significant effect on mummichog genetic diversity. This finding contradicts the general scientific opinion that contaminants often decrease genetic diversity through a variety of mechanisms including, low population numbers.

*Population Parameters* – Mummichog embryos were tested for tolerance to pollution and both field caught and laboratory raised embryos from contaminated sites were more tolerant (had less cardiac abnormalities) to contaminated sediments than mummichogs originating from clean sites. Embryos from sites with intermediate levels of PAH produced embryos of intermediate tolerance. Large groups of two generations of fish were raised in clean and polluted laboratory settings to test trade-offs between pollution tolerance and parameters such as growth, time-to-maturity, and fertility. A decrease in tolerance in fish reared in clean habitats for several generations was not found, indicating there was no cost associated with pollution tolerance. Genetic variation showed that population in polluted sites were locally adapted and did not depend on individuals migrating from clean sites to maintain the population.

### Implications

The general approach of using genetically based indicators to document effects of contamination in coastal fish has been shown. Additionally, these indicators are robust and have a potential further utility as a tool to monitor recovery associated with remediation activities.

### Next Steps

No differences in growth, time-to-maturity, fertility were found in fish exposed to clean and contaminated sediments and only higher sample sizes and more experimentation will determine if this relationship is accurate or a result of low statistical power.

## 2.4 STAR Grant R82-6596

### Demographic and genetic factors affecting population viability of *Lupinus perennis*, an indicator species of Oak Savanna.

**Principal Investigators:** Helen J. Michaels, R.J. Mitchell

#### **Impacts:**

*Perennial Lupine (*Lupinus perennis*) is a small wildflower that is thought to be an indicator of high quality midwestern oak savannas communities. This plant has suffered major population declines and is the only food source for three federally or state endangered butterfly species. Understanding the population dynamics and genetic factors that have contributed to this decline should aid in recovery of the vulnerable butterfly species.*

#### **Successes and Lessons Learned:**

- *A quick and inexpensive method to determine pollination success was developed.*
- *A new method was developed to determine genetic variation.*
- *A new perennial Lupine seed predator was discovered, perhaps explaining a mystery as to why many perennial Lupine seeds were germinating inappropriately during mid-summer.*

#### **Indicators Developed/Tested:**

- *Life History: A variety of life history traits were observed in the field and in the lab to test whether plants from small and large populations have differences.*
- *Pollination Success: A low impact indicator which tests whether pollination is successful based on counting pollen tubes in flowers was developed.*
- *Genetic Variation: Genetic variation as it relates to population size can contribute to reduced reproductive success.*

#### **Products:**

- *Methods: A novel application of chromosome walking (technique for cloning adjacent regions in the genome around a known piece of DNA) was developed for measuring genetic variation.  
A method of counting pollen tubes was also developed to assess pollination success.*
- *Publications: 5 manuscripts in progress or planned for submission, 2 thesis & dissertations.*
- *Presentations: 24 oral presentations.*

#### **User Community:**

- *U.S Fish & Wildlife Service*
- *Ohio Division of Natural Areas and Preserves*
- *Michigan Fish & Wildlife*
- *Toledo Metroparks*
- *Toledo Zoo*
- *The Nature Conservancy – Northwest Ohio*

#### **Themes:**

*woodland, plants, anthropogenic disturbance, genetics*

#### **Background**

The Perennial Lupine (*Lupinus perennis*) is a small wildflower found in dry openings within the savanna forests of the Midwest. It has suffered major population declines due to habitat destruction and is currently extinct or imperiled in nine states. It is the only food source for three federally and state endangered butterfly species and thus is included in federal recovery plans for those butterfly species. Understanding how this plant responds to their small and dispersed population will give ecologists increased insight into whether decreasing population size acts synergistically to further depress reproductive success.

This study monitored five small and five large populations of perennial Lupine in Northwestern Ohio and Southeastern Michigan.

### Goals

- Determine whether variation in population size and density explain reproduction patterns and whether pollinator visitation or foraging patterns have changed in response to population reductions.
- Determine whether reduced reproduction in small populations is associated with habitat degradation.
- Quantify whether differences in levels of genetic variation, inbreeding depression, and mating system occur as a function of population size and to examine their contributions to reduced reproduction in impacted populations.

### Findings

*Life History* – Neither the mean size of individual plants nor offspring vigor is related to whether plants were found within large or small populations, indicating that habitat quality does not vary with population size. Abortion rates were significantly higher for seeds from small versus large populations and cumulative fitness across four life stages (seed production, seed emergence, seedling survival, and seedling biomass) were significantly lower in small versus large populations. These results point to small populations suffering significant hardships and suggest that potential pollinators are changing behavior in response to small populations. A change in pollinator behavior has led to higher rates of self-pollinations and an attendant decrease in fitness.

*Pollination Success* – Observations of the number of visits by pollinators showed that visitation rates do not vary as a function of the plant population size. Direct observation of pollinators is time consuming, thus a new, quick, low impact method of assessing whether the perennial Lupine was pollinated was developed. This method counts the number of pollen tubes in a flower and performs exceedingly well as an indirect measure of successful pollination. Although population size and density did not affect the number of pollen tubes in a flower, the proportion of flowers with at least one tube increased with population size and density.

*Genetic Variation* -Genetic variation was tested at six different places on the genome and results showed that there are considerable genetic differences between plants originating from small populations versus large populations. Tests also showed that small populations had higher rates of self-pollination, indicating that the size of a plant population changes the behavior of pollinators. Pollination experiments showed that reproduction in both large and small populations suffers when flowers are self-pollinated, suggesting that inbreeding depression will continue to be a problem.

### Implications

The results of this study point to several factors consistently contributing detrimental effects to small populations of perennial Lupine. Additional conclusions include that these factors may be acting synergistically and thus may contribute to a rapid decline in population numbers. Research conducted by this study indicates that populations below a threshold between 200-700 perennial Lupines may not be sustaining.

### Next Steps

Data on soil characteristics (gravimetric water content, nutrients, pH, organic matter), light, and other habitat data are now being analyzed in order to directly test for environmental differences between large and small populations and evaluate their roles in determining reproduction of small populations. This question is also being tested experimentally through analyses of data from reciprocal transplants that compare the success of seedlings from large and small populations in each type of site. Analysis of three years of demographic monitoring data from ten populations to determine how survival, growth and

reproduction in the field contribute to differences in viability of plants in large and small populations is necessary.

## 2.5 STAR Grant R82-6597

### Land Use and Geomorphic Indicators of Biotic Integrity in Piedmont Streams

**Principal Investigators:** David Leigh, B. J. Freeman, M. C. Freeman, E. A. Kramer, C. M. Pringle, A. D. Rosemond

#### **Impacts:**

*The interplay between urbanization, land use and the landforms they occupy have direct impacts upon habitat quality and biological integrity of the nearby streams. These stressors interact at multiple time and spatial scales making the development of indicators a difficult task.*



#### **Successes and Lessons Learned:**

- *Models that predict stream quality which use more than two variables outperform models with one or two variables.*
- *Models that predict stream quality using a combination of field based and non-field based data outperform models with only non-field based data.*

#### **Indicators Developed/Tested:**

- *Landforms: This basin wide variable is static and can not be easily altered by humans, but exerts strong influences on the basin's aquatic systems.*
- *Stream Channel: While direct modifications of channels do occur, it is a rare event. Channel variables can act to limit habitat quality or biological integrity of streams.*
- *Sediment: Fine particles tend to be detrimental to organisms, and sediment transport (both magnitude and frequency) can significantly alter habitats.*
- *Land Cover: Most of the study area was at one time forested, extensive human modifications have decreased water and habitat quality of streams.*
- *Water Quality: Water quality can directly alter biological integrity.*

#### **Products:**

- *Publications: 4 peer reviewed articles, 8 papers in proceedings, 6 theses and dissertations.*
- *Presentations: 33 oral presentations.*

#### **User Community:**

- *Water quality managers*

#### **Themes:**

*freshwater, water quality, macroinvertebrates, fish, anthropogenic disturbance, GIS/RS*

### **Background**

Stream degradation has been linked to surrounding land cover and landforms, but two competing hypothesis explain this linkage. The Process Domain Concept (PDC) proposes that ecological conditions of streams respond to an underlying landform (geomorphic) template while the River Continuum Concept (RCC) proposes that streams are controlled by where they spatially lie within a gradient from headwater stream to larger rivers.

This study sampled 32 streams in the Etowah River basin (north of Atlanta, Georgia) and collected hundreds of variables in the field. Data collected included geomorphic (e.g., stream slope, channel), water quality (e.g., turbidity), habitat (e.g., NRCS Stream Visual Assessment Protocol, EPA Rapid Assessment Protocol), and biology (e.g., fish, macroinvertebrate) variables. Non-field based data such as land use (including change over time) and topography were used to assess relationships at the basin wide scale.

### **Goals**

- Determine if stream habitat and life (fish and macroinvertebrates) can be predicted from geomorphic and land cover measures.

- Determine the space and time scales at which these predictive measures operate.

### **Findings**

*Landforms* - In and of themselves landforms are minor indicators, however, landform indicators increase the predictive power of ecological condition models as secondary parameters. A key finding is that length of the trunk stream is an important factor, even with the small size range in the study area. While this gives credence to the RCC, other factors (see below) have higher predictive powers indicating the PDC is the overriding concept.

*Stream Channel* - Relationships between land cover and stream channels were varied, with few being strong. Stream slope was an important variable, and results showed local topography have overriding importance. Field measured slope was found to be far superior to non-field derived slope.

*Sediment* - Particle size is a powerful indicator of ecological condition. Additionally, a strong correlation between size of sediment particles and slope indicates landforms, not humans, are a direct influence. Current land cover has a correlation with particle size, indicating it exerts some degree of control but the dominant influence is stream slope.

*Land Cover* - Only current land cover (the study tested land cover since 1938) is a significant predictor of ecological condition. This relationship is stronger for habitat and macroinvertebrates than fishes. Only two variables (percent forest and percent water impounded) are needed to explain 80 percent of the variation in habitat scores. Land cover indicators were thought to be proxies for several stresses to aquatic ecosystems, not the stressors themselves.

*Water Quality* - Correlations between water quality and land cover were not strong, indicating that while land cover affects water quality (which in turn affect biota) other factors influenced by land cover (e.g., stream sedimentation) are probably more important. Dissolved oxygen, specific conductivity, and turbidity were all indicators of both habitat and ecological condition.

*Scale* - Four spatial scales of indicators were tested: basin wide, entire stream network within 330 ft of streams, only 0.6 miles of stream network within 330 ft of streams, extended reaches (only 100 times the width of a stream), and reaches (only 20 times the width of a stream). Results showed it is extremely important to have data at multiple scales and that the 1km scale is the weakest. Additional results showed in the absence of field data, it is important to include landform data. Finally, analysis showed that stream channel and sedimentation exert a fundamental importance.

### **Implications**

This study comprehensively tested hundreds of variables at a wide variety of spatial scales to develop a suite of indicators. Overall, the study supports the PDC as the driving force influencing these streams.

### **Next Steps**

This study was conducted within the Etowah River basin specifically because of the wide range of conditions found there. Regional applicability is possible because of the study design, however testing the model in other regions is the only way to validate this assumption. The models developed may not be appropriate if significant regional differences exist.



## 2.6 STAR Grant R82-6598:

### **Development and Testing Of A Multi-Resource Landscape-Scale Ecological Indicator: Forest Fragmentation, Structure, and Distribution Relative to Topography**

**Principal Investigators:** Steven W. Seagle and P.A. Townsend

**Impacts:** *The largest blocks of forests in the eastern United States are found in the Appalachian mountains. Ecological indicators which can track changes in the amount and quality of this forest are needed to determine the amount of stress this system is under.*

**Successes and Lessons Learned:**

- *A long term integrative measure of forest productivity (site index) was determined to be a combination of simple topographic variables.*
- *Development of lands by humans has preferentially selected the most productive breeding bird grounds.*
- *The use of RADAR in predicting vertical structure of forests was proven to be very useful.*

**Indicators developed/tested:**

- *Site index: Four indexes were tested as estimates of forest productivity and were based on age-height curves or height-diameter relationships of trees or topographic position.*
- *Bird Reproduction: Forest vertical structure and site index were evaluated as indicators of songbird density and breeding success.*
- *Remote Sensing: Synthetic aperture radar was evaluated as a method to predict forest vertical structure.*

**Products:**

- *Publications: 3 peer reviewed publications, 1 in review, 3 in preparation 2 dissertation*
- *Presentations: 23 oral presentations*

**User Groups/Community:**

- *US Fish & Wildlife Service*
- *Environmental Protection Agency*
- *State Agencies*

**Themes:**

*Forest, birds, GIS/RS*

### **Background**

The use of birds as indicators of ecological health have been long established but current techniques that measure reproductive success are time and money intensive. Breeding success of forest interior bird species is related to landscape condition and thus it may be possible to use easier to obtain forest landscape metrics as ecosystem health indicators. Topography in the Appalachian region is a strong influence on soil moisture and thus forest productivity and bird breeding success. Bird breeding success has long thought to be influenced by the vertical structure of forests, but this variable is difficult to measure at a landscape level.

This study measured tree height, age, and size to develop a site index for 22 sites within the Appalachian forests of western Maryland. Density and breeding success of forest songbirds was measured at these sites as was key habitat variables (e.g. percent canopy coverage) and food availability (litter invertebrates). Forest structure was measured in 180 field plots (e.g. canopy tree height, shrub height) and correlated to both active remotely sensed data (e.g. RADAR) and passive remote sensing imagery (e.g. Landsat, AVIRIS)

### Goals

- Evaluate topography as a factor influencing breeding productivity of forest interior songbirds.
- Use synthetic aperture radar to predict forest vertical structure.
- Develop a suite of metrics that can represent quality of habitat for birds and landscape integrity.

### Findings

*Site index* – Forest quality is thought to be controlled by moisture levels and available nitrogen. Four different site indexes which measure forest productivity were evaluated using statistics that estimated precision and accuracy of how each predicted the other. The site index based on age-height curves of oak trees provided reasonable estimates of site quality.

*Bird Reproduction* – Forest quality (site index) should also reflect the quality and availability of food for songbirds and thus be related to bird productivity (density and fledging success). Bird species were surveyed for two years on twenty sites. Seventeen species were found in the study area and songbird density was greater in wetter climate and in areas with high site indexes. Surprisingly, the site index was a stronger predictor of bird density than forest vertical structure. Site index was related to fledging success for one year (normal precipitation) but not during the dry year. Biomass of invertebrates found in the litter was related to both site index and fledging success, showing a strong bottom-up relationship from topography, site index, forest productivity, invertebrate biomass, and bird reproduction. Site index would thus be an important indicator of potential habitat quality but perhaps not the yearly variation that naturally occurs.

*Remote Sensing* – Forest vertical structure was measured in 180 field plots for statistical analysis in conjunction with satellite imagery. Both passive (Landsat, AVIRIS, Hyperion) and active (RADAR) sensors were evaluated. Significant relationships existed between RADAR imagery and several measures of forest structure (e.g. basal area, canopy height). Leaf-off passive imagery (Landsat) was extremely useful in determining understory layer communities, but the only hyperspectral passive imagery available was leaf-on and did not prove useful.

### Implications

This study clearly made great strides by linking topography, forest productivity, invertebrate biomass, and bird reproduction in a cohesive manner. Another key finding of this study is the ability to determine vertical structure of forest using RADAR satellite imagery, this discovery will facilitate mapping of this parameter across large regions.

### Next Steps

Further data analysis remains with respect to testing the regional validity of the forest site index and application of the forest site index and bird reproduction success models to other Mid-Atlantic sites.

## 2.7 Grant R82-6599

### Intraspecies genetic diversity measures of environmental impacts.

**Principal Investigators:** Daniel Krane, G.A. Burton Jr., and K.A. Grasman

#### **Impacts**

*The ability of a population to adapt to a changing environment is related to its genetic variation. Changes in this variability have been shown to be correlated to the extent these populations have been exposed to pollutants and human induced stresses.*

#### **Successes and Lessons Learned:**

- *Genetic variability in six test species are strongly correlated with traditional measures of assessing impacts to aquatic sites.*

#### **Indicators Developed/Tested:**

- *RAPD-PCR: Genetic variation within eight species from stressed and non-stressed sites were calculated and compared to the Index of Biological Integrity (IBI) and Invertebrate Community Index (ICI).*

#### **Products:**

- *Publications: 6 peer reviewed*
- *Presentations: 17 presentations*

#### **User Community:**

- *U.S. Environmental Protection Agency*
- *State agencies*

#### **Themes:**

*freshwater, water quality, fish, insects, pollutants, genetics*

### **Background**

Traditional measures of stress to the environment induced by humans include toxicity tests and biologically based indexes (such as counts of species or anatomical variation within contaminated sites). Toxicity tests offer results on immediate affects of pollutants and biological indexes are proxies measures of environmental stress. Stress and pollutants can cause significant decreases in populations which in turn often create genetic “bottlenecks” in which overall genetic diversity is lost. Genetic diversity loss can be measured even after a site’s species diversity or a populations density has reached pre-stress levels. Genetic tests thus have the potential of cheaply and easily measuring direct effects of anthropological stress. However, previous tests have focused on genetic markers which do not reflect the entire genetic variation of a species and thus have been less sensitive and poorly correlated with accepted indexes of water quality.

This study performed RAPD-PCR (Random Amplified Polymorphic DNA Polymerase Chain Reaction) tests on eight species (crayfish, snails, damsel fly, earth worms, pill bugs, pacific herring, *Hyallela azteca*, and garlic mustard). PCR is a technique used to amplify DNA samples to test for genetic variation and traditional PCR tests genetic variation on specific DNA segments. RAPD-PCR tests variation on unknown DNA segment which can provide results that can distinguish between even closely related individuals. Specimens were collected from streams in four Ohio sites which have been exposed to differing levels of stress. Ohio EPA has documented depressed aquatic systems in these streams which have been caused by polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs). One reference stream within each watershed was sampled as well.

### Goals

- Determine if RAPD-PCR can reliably estimate genetic diversity in test species.
- Evaluate if genetic diversity is correlated with acceptable ecological indicators.

### Findings

*RAPD-PCR* - Genetic similarities of crayfish were significantly lower at reference sites than impacted sites and genetic similarities of all sites were significantly correlated with calculated IBI and ICI scores for these sites. Previous genetic tests based on traditional PCR techniques have not shown strong correlations.

Of the eight species reported, four (crayfish, snail, damsel fly, and pill bug) showed lower genetic similarities for reference sites, three (earth worm, pacific herring, and *Hyallolela azteca*) showed higher genetic similarities for reference sites, and one (garlic mustard) showed no genetic variation at all.

This study showed that RAPD-PCR can be considered a sensitive measure of genetic diversity, and that it could be a viable alternative, or additional, measure of effects of environmental stress. However, genetic variation response to stress seems to be a species specific. Thus, the choice of a single indicator species should be made cautiously and several species, perhaps from different trophic levels, should be incorporated into this indicator.

### Implications

Measuring genetic diversity in populations is cheaper and less destructive than traditional ecological indicators and should be relatively insensitive to naturally occurring seasonal and habitat variability.

### Next Steps

Reductions in genetic diversity have many causes, and RAPD-PCR techniques can not experimentally distinguish between natural and human caused stresses, a rigorous sampling plan may be necessary. Additionally, reductions in genetic diversity are not the only response a population may have to environmental stress. These issues should be addressed before implementing this ecological indicator.

## 2.7 STAR Grant R82-6600

### Ecological Indicators for Large River-Floodplain Landscapes

Principal Investigators: Monica G. Turner and E. Stanley

#### **Impacts:**

*Most major river systems in the United States have been significantly altered by dams, reservoirs, and channelization. Development of ecological indicators for these systems has been hampered by their very definition; large rivers require landscape level indicators.*

#### **Successes and Lessons Learned:**

- *Physiography and flooding regimes predicted vegetation communities well.*
- *Land-use history was important in explaining current forest vegetation distribution.*
- *Bird communities were not related to habitat fragmentation but landscape variables have significant relationships with several individual species.*
- *Landscape metrics may compliment traditionally used riverine indicators.*
- *A new method for determining ammonium in soil which uses less toxic chemicals and less equipment than previous methods was developed.*

#### **Indicators Developed/Tested:**

- *Vegetation: Elevation relative to, and distance from, the river along with physiography was used to predict occurrence, community composition and abundance of trees.*
- *Birds: Habitat fragmentation and width of floodplain forest was used to predict avian communities and individual species.*
- *Soil: The amount and moisture of organic matter was used to predict the rate at which nitrogen is reduced and shifts in abundance of five tree species were used to predict soil microbe activity (both are measures of ecosystem health).*

#### **Products:**

- *Method: A new method for determining ammonium in soil extracts was developed.*
- *Publications: 10 peer reviewed, 4 in press, 2 articles in review, 2 dissertations, 3 theses.*
- *Presentations: 35 oral presentations.*

#### **User Community:**

- *State and regional resource managers*
- *River or riparian NGOs*
- *Ecological researchers.*

#### **Themes:**

*forest, birds, soil, anthropogenic disturbance*

#### **Background**

Due to their size, large rivers and the floodplains surrounding them have been historically neglected by science. Landscape level indicators of the health of these systems which provide many societal benefits (e.g., water source, navigation, ecological services) are lacking. There are serious scientific difficulties in extrapolating datasets collected in the field to broader scales.

This study evaluated landscape indicators for their utility in detecting changes in the structure and function of 250 miles of the Wisconsin River floodplain. Nine stream reaches were extensively sampled and field data concentrating on structure and composition of vegetation and birds communities was collected along with soil samples from over 600 sample locations. Microbial and nitrogen reduction soil processes were used as a link to ecosystem level dynamics. Current and historic land-use data was assembled from aerial photographs (1930s, 1960s and 1990s) to better understand how these factors may constrain the entire system.

### Goals

- Determine which landscape metrics are most useful for monitoring population, community, and ecosystem processes in large river-floodplain landscapes.
- Investigate extrapolating relationships between landscape metrics and ecological processes in large river-floodplain landscapes.

### Findings

*Vegetation* – Current landscape patterns explained little variation found for individual species presence or abundance. Physical geography and flooding regime (elevation with respect to the 100 year floodplain) were the only indicators which were important to predict the occurrence, composition or abundance of trees. Current land-use data did not help predict vegetation but as expected, historical land use was important to predict presence and abundance of tree species which inhabit mature and/or late-successional forests. Regional scale indicators of broad geographical province performed well indicating plot level data is not especially particularly necessary. The presence of levees did not affect tree growth rates but did affect the species of trees present.

*Birds* – Overall, most landscape level variables tested did not predict structure or composition of bird communities, only distance from the confluence of the Mississippi and Wisconsin rivers have an affect (negative) on species richness and abundance of some groups of birds. Landscape indicators did have some importance for individual species (e.g., some Woodpeckers, Ovenbird, Scarlet Tanager) with the strongest relationships being at regional scales.

*Soil* – Soil samples measured pH, concentrations of minerals, soil texture, percent organic matter, nitrogen, and nitrogen reduction. A new method for measuring the amount of ammonium found in soils was developed. This method uses less toxic chemicals and requires minimal equipment and has the potential to be valuable to other soil scientists. Nitrogen reduction was extremely variable in the study area, and no significant differences were found between the nine reaches. Significant effects of specific trees species (especially silver maples) were found when comparing microbial activity and organic matter to surrounding soils, indicating a change in the amount of maples over time could have important soil implications. The presence of levees significantly increased microbial activity but had not affect on the amount of organic matter or woody debris.

### Implications

The Wisconsin River is unusual in that it has a relatively high forest cover (40-60 %) which has been increasing since the 1930s. The landscape indicators tested in this study explained little of the variability of forest community structure, bird communities or soil properties. Broad (regional) scale indicators seemed to perform equally well and can be estimated using available digital sources, eliminating much field work if simple models are adequate. A basic framework for spatial extrapolation can be a powerful approach for testing hypotheses and evaluating the strength of relationships between indicators and responses.

### Next Steps

Analysis of extrapolating field based data to landscapes or regional levels is underway and should be extremely useful because many ecologists use similar techniques with varying levels of success. Analyses should also be extended to incorporate in-stream response variables and to evaluate the likelihood of non-linear responses to changes in indicators.

## 2.8 STAR Grant R82-6601

### Modeling Ozone Flux to Forests Across an Ozone Concentration Gradient in the Sierra Nevada Mountains, CA.

**Principal Investigators:** Allen H. Goldstein and J. A. Panek

#### **Impacts:**

*Ozone is a toxic air pollutant and pine species in California have the longest documented history of ozone stress of U.S. forested ecosystems. Ozone enters foliage through stomatal pores, thus understanding factors which influence stomatal conductance is key to understanding ozone stress.*

#### **Successes and Lessons Learned:**

- *As little as one third of ozone uptake occurs in summer, the time period over which current regulatory indices of vegetation ozone exposure are calculated, indicating the standard U.S. and European indices of ozone exposure are poor metrics of ozone uptake.*
- *When ozone concentrations were at their highest in summer, uptake by trees was lowest because of stomatal limitations imposed by drought stress.*

#### **Indicators Developed/Tested:**

- *Ozone Uptake: Direct and indirect measurements of ozone uptake were taken to determine correlation with physiological and environmental variables and with injury.*
- *Stable Carbon Isotope: The usage of a ratio of rare unstable (radioactive) carbon to its stable counterpart was tested as an indicator of stomatal conductance.*

#### **Products:**

- *Method: A stable carbon isotope method was developed to indicate stomatal conductance.*
- *Model: The model STOMATA was developed to predict leaf physiology of ponderosa pine.*
- *Publications: 13 peer reviewed publications, 1 submitted.*
- *Presentations: 13 oral presentations*

#### **User Community:**

- *National Park Service*
- *California Air Resources Board*
- *Forest managers*
- *UN/ECE Convention on Long-range Transboundary Air Pollution*

#### **Themes:**

*forest, plants, pollutants*

#### **Background**

Ozone is a strong oxidant toxic to plants. Current indices of ozone impacts on vegetation used in the U.S. and Europe (e.g. SUM0, SUM60, AOT40) are sums of concentrations above a threshold concentration. Because ozone must enter foliage through small pores (stomata) to cause damage, a standard created from atmospheric concentrations disregards the role of stomata in controlling ozone diffusion into the leaf. Stomata open and close in response to environmental variables, including drought stress.

This study measured ozone concentrations, uptake of ozone by ponderosa pines (*Pinus ponderosa*), various plant physiological parameters (e.g., stomatal conductance, net photosynthesis) and meteorology at four sites in the Sierra Nevada of California for three years. A model (STOMATA) was developed from this data which predicts leaf level physiological processes. An innovative approach using the ratio of stable to unstable forms of carbon (isotopes <sup>13</sup>Carbon to <sup>12</sup>Carbon) was investigated as an indicator of stomatal conductance.

### Goals

- Find an easily-measured indicator of stomatal conductance.
- Understand environmental controls on stomatal conductance in Californian Ponderosa Pine.
- Quantify ozone uptake in Californian Ponderosa Pine in California Sierra Nevada.

### Findings

*Ozone Uptake* – Atmospheric ozone concentration was poorly correlated with the amount of ozone entering (thus potentially injuring) trees. When ozone concentrations were at their maximum (late summer) ozone uptake was at a minimum because ponderosa pine stomata were closed to conserve water during the seasonal drought. Only 37 percent of annual ozone deposition occurred during summer, when ozone concentrations were highest, while 27 percent occurred in spring, indicating ozone deposition during the non-summer year is significant. The combination of mild California winters with trees remaining physiologically active and high winter ozone deposition (20 percent of annual deposition) indicate winter is a significant time period risk of ozone stress. The model STOMATA provides good estimates of leaf physiological variables and showed that water availability and climate had strong influences on the amount of ozone actually taken up by these pine trees.

*Stable Carbon Isotope* – The stable carbon isotope ratio was found to be a moderately good indicator of stomatal activity. When corrected for the effects of altitude, carbon isotope ratio explained 54 percent of the variance in mean daily stomatal conductance. Incorporating relative humidity, photosynthetically active radiation, and soil moisture significantly increased the utility of this indicator. Despite this strong relationship, the authors caution that there was still a large amount of within-site variation, and sample size may need to be increased for better results.

### Implications

Current vegetation exposure indices based on ozone concentrations are poor indicators of the amount of ozone that is damaging forests. A better index needs to be developed that incorporates plant physiological activity, and thus ozone uptake. The relationship between climate (water availability) and ozone uptake indicates that anticipated global climate changes could have significant effects on the amount of ozone injury in California forests.

### Next Steps

The use of stable carbon isotopes to indicate stomatal activity is promising. Increased sample size in future studies could improve the strength of this indicator. The model could be used to estimate ozone uptake historically at sites where ozone injury has been measured in the past and to predict ozone uptake under different climate change scenarios. Further research is necessary to enable scaling from the leaf level to the stand level and eventually to the landscape level, with the understanding that the heterogeneous distribution of water across the landscape could affect landscape-scale ozone uptake in forests.



## 2.9 STAR Grant R82-6602

### Ecosystem Monitoring via Genetic Diversity Surveys of Dandelions using VNTR Multilocus DNA Probes

**Principal Investigators:** Steven Rogstad and B. Keane

**Impacts:**

*The worldwide distribution of dandelions makes them a model organism to study the potential genetic effects of pollution or ecological stress. Differences in genetic population structure or mutation rates can be discriminated with new molecular genetic methodologies.*

**Successes and Lessons Learned:**

- *Particulate matter is a good indicator of soil contamination by metals.*
- *Genetic measures of dandelions appear to be good biomonitors of stress.*
- *Dandelions appear to be good biomonitors of several contaminant stresses, both through mutation rates that increase with increasing tissue concentrations of some pollutants, and through altered genetic variation of populations.*
- *Different dandelion clones occur on polluted versus non-polluted sites suggesting that different clones have different pollution tolerance responses and these have associated costs.*

**Indicators developed/tested:**

- *Particulate matter: Airborne particulate matter is known to contain trace amounts of metal elements, which could be related soil metal pollution at a site.*
- *Dandelion leaves: Metal contamination should be reflected in dandelion tissues.*
- *VNTR DNA: Several sites within the genetic code of organisms exhibit very high variation and these sequences potentially mutate easily in the presence of stressors.*

**Products:**

- *Publications: 21 peer reviewed, 6 submitted, 1 dissertation*
- *Presentations: 25 oral presentations*

**User Groups/Community:**

- *Environmental Protection Agency*

**Themes:**

*plants, soil, pollutants, genetics*

### Background

Dandelions (*Taraxacum officinale*) could potentially be powerful indicators of stressor caused genetic change. Factors that make dandelions good indicators include: worldwide distribution, ease of experimentation due to rapid growth, sequestration of pollutants in tissues, and U.S. dandelions reproduce asexually, thus offspring should be genetically identical unless mutation has occurred. Dandelions have certain portions of their genetic code at which very short genetic segments are tandemly repeated a random number of times: these sites are termed variable-number-tandem-repeats (VNTR). These repeated sequences exhibit high variation among individuals and it is possible to determine if the presence of trace metals changes repeat mutation rates or patterns of occurrence in populations.

This study used sampled dust, soil, and dandelions from 29 rural, industrial, and urban sites in five Midwestern states to determine if genetic changes due to trace metals can be detected. Airborne particle sample data were collected to determine if they can be used as an index of soil metal contamination. Soils were tested for the metals cadmium, chromium, copper, manganese, nickel, lead, and zinc. Dandelions were tested for presence of these metals in leaves, mutation rates at DNA repeat sequences, and genetic

diversity at polluted versus pristine sites. Clonal offspring from either pristine or contaminated soils were grown in pristine or contaminated soil to determine costs of pollution tolerance or intolerance.

### Goals

- Examine VNTR genetic markers in dandelions to determine whether effects of pollution on population genetics or mutation rates can be detected.
- Determine whether different dandelion clones respond differently to metal pollution.

### Findings

*Particulate Matter* – EPA monitors air pollution for the entire United States, including dust (particulate matter). Airborne particulate matter is known to contain trace metal elements and was investigated as an index of metal contamination. Analysis of 29 sites in five states (Colorado, Illinois, Indiana, Kentucky and Ohio) showed that the five year average of site's particulate matter is significantly related to the soil concentration of the eight metals tested. These results suggest that existing EPA data regarding particulate matter would be a good indicator of soil contamination.

*Dandelion Leave* – Analysis of 29 sites in five states showed that leaf concentration of four of the eight metals tested (chromium, manganese, lead, and zinc) was significantly related to the soil metal concentration. Additionally, fall versus spring sampling of leaves showed that leaf concentration of metals increases over the growing season. However, results indicate that the amount of contamination of a soil, does not predict the amount of other metals in dandelion tissue indicating that dandelions do not uptake these metals in direct proportion to air or soil presence.

*VNTR DNA* – Transmission of over 82,000 dandelion VNTR genetic markers from 120 parents (distributed across 16 of the 29 sites) to their offspring have been analyzed. The average site mutation rate varies 7.5 fold from the lowest to the highest, this being among the highest mutation rates for plants. Site airborne particulate matter and soil metal concentration was not related to average site mutation rate, but leaf concentration of four metals was significantly related to an increase in the mutation rate across sites. Additional results showed a genetic diversity decrease with increases in particulate matter as well as soil metal concentration. Reciprocal planting of dandelions from polluted and pristine sites in polluted or pristine soils showed that for five measures of productivity (such as shoot or root dry weight) that in every case, there is a cost associated with being either more pollution tolerant or being pollution intolerant. For instance, plants from pristine sites always did better than plants from polluted sites while grown in pristine soil, but did worse when grown in polluted soil. The converse was always true (plants from polluted sites did better in polluted soil than plants from pristine sites).

### Implications

Several human diseases can be traced to VNTR mutation, thus the demonstration that dandelion VNTR mutation rates have changed in the presence of trace metals has important ramifications to the potential causes of certain human genetic disorders. Mutation rates in dandelions have been increasing as tissue exposure to certain metals increases. Dandelions may be useful in examining the pollution mutation stress to which a site has been exposed.

### Next Steps

Data from all 29 sites will be analyzed. Additional activities will include testing genetic mutation rates under strict, laboratory controlled experiments and to try and determine if metals are the direct causal agents of mutation. Future work will include investigating the differing degrees of clonal variation of mutation rates in the presence of metals, and teasing out the separate relationships.

## 2.10 STAR Grant R82-6603

### Genetic Diversity in California Native Fish Exposed to Pesticides

**Principal Investigators:** Susan L. Anderson, D.E Hinton, B. May, K. Kuivila

#### **Impacts:**

*Organophosphate and carbamate pesticides were approved for use in the 1970s to decrease the likelihood of mortality to non-target species. However, the use of these pesticides remains controversial and documented affects have been shown to affect birds and fish.*

#### **Successes and Lessons Learned:**

- *Contaminated Central California water elicits a genetic change in native fish which cannot be attributed to organophosphates and carbamates.*
- *There is a need to characterize additional pesticides used in Central California.*
- *AFLP is superior to RAPD as a measure of genetic variation in fish.*
- *Sublethal effects in native fish improve the value of ecological risk estimates based on toxicity tests with non-native species.*

#### **Indicators Developed/Tested:**

- *Population Genetics: Long term pesticide exposure could induce measurable genetic change.*
- *Short-term Biomarkers: Two measures of short term response to pesticides were tested, DNA strand breaks and acetylcholinesterase enzyme activity (a pesticide target).*
- *Genetic Test Comparison: Two different genetic tests (AFLP and RAPD) were compared to determine their relative strengths and weaknesses in determining genetic differences.*

#### **Products:**

- *Publications: 4 peer review publications, 1 Masters Thesis, 1 PhD Dissertation*
- *Presentations: 24 oral presentations.*

#### **User Community:**

- *California Department of Fish & Game*
- *California State Water Quality Control Board*
- *California Bay Delta Authority*
- *U.S. Fish & Wildlife Service*

#### **Themes:**

*freshwater, water quality, fish, pollutants, genetics*

#### **Background**

Exposure to pesticides can have population level or individual level effects. Population level effects can be shown by alterations in genetic variation over time. Individual level effects can be shown by the number and type of DNA strand breaks – double strand breaks are harder for cells to repair and thus more likely to result in cell death than single strand breaks. Individual level exposure can also be shown by the level of enzyme activity in non-target organisms. A family of pesticides termed organophosphate/ carbamate were approved in the 1970s to stop the neurological enzyme acetylcholinesterase from working, thus causing death. This family of pesticides is thought to be short-lived and thus safer than previous pesticides, but mortality to birds has been documented and little work has been done to assess effects in native fish. Several methods are used to determine genetic variation, two are Amplified Fragment Length Polymorphism (AFLP) and Randomly Amplified Polymorphic DNA (RAPD). Both techniques rely on splitting DNA into smaller pieces, but RAPD is a more general method while AFLP methods are employed in strictly controlled laboratory settings to generate a large number of sites within the genetic code which exhibit high variation.

This study characterized long-term pesticide applications and collected over 1000 samples from Sacramento suckers (*Catostomus occidentalis*) in six California Central Valley watersheds to determine population level genetic information. Short-term pesticide concentrations and their effects on individuals were measured in the field and laboratory.

### Goals

- Determine population level genetic information for the native Sacramento sucker and if it has been altered by pesticide use.
- Determine strengths and weaknesses of two genetic tests.
- Determine the value of sublethal indicators of stress in native species in improving ecological risk assessment.

### Findings

*Population Genetics* – Two different genetic tests (AFLP and microsatellite markers) showed that exposure to pesticides was not a significant factor in increases in genetic mutation rate, decrease of genetic variations (bottlenecks), or any other induced selection in Sacramento suckers. Genetic diversity decreased as individuals were found upstream, regardless of contaminant exposure and genetic distance analysis clustered populations on the same river systems. Biogeography seems to play a larger role in determining genetic similarities than pesticide contamination.

*Short-term Biomarkers* – Sacramento suckers were enclosed in cages in the field as well as subjected to controlled laboratory exposures to field collected water samples to examine the sub-lethal responses to pesticide laden waters. Acetylcholinesterase enzyme activity and the number of DNA strand breaks were used to quantify response. Pesticide concentrations in 2000 and 2001 did not induce enzyme inhibition in exposed fish in the field or the laboratory but the number DNA strand breaks in Sacramento suckers were significantly higher in 2000 (field and laboratory) in exposed suckers than in reference populations. These contrasting results indicated that genetic changes are occurring at the individual level but that pesticides other than organophosphate/carbamates were involved. Additional testing of waters indicate that metals are not responsible for the effects observed.

*Genetic Test Comparison* – An assessment of the reliability of RAPD and AFLP genetic methods was performed using a well established genetic line of rainbow trout (*Oncorhynchus mykiss*). Results showed that while RAPD could discriminate a higher number of markers (bands) but that AFLP was superior in the reproducibility of experimental results, indicating that AFLP methods instill a greater confidence in findings.

### Implications

Population level genetic effects were not observed in Sacramento suckers but individual effects (DNA strand breaks) were. This study showed that simple chemistry analysis may not be sufficient to determine detrimental effects and that toxicological tests on native species should be used as well. Additionally, this study showed that pesticides of concern that are currently being controlled may not be responsible for these detrimental effects.

### Next Steps

Results showed that alterations to individual's genetic codes are occurring in the Central Valley of California but organophosphate and carbamate pesticides are likely not involved. Use of these pesticides is decreasing while pyrethroid insecticides (also a nervous system agent) is increasing and thus could be responsible. Future work is necessary to identify the toxic substance causing these measurable changes. In addition assessments of other types of sublethal responses such as nervous system damage are needed for native fish such as salmon and delta smelt.

**APPENDIX A – COMMON THEMES**

Grant	Study System	Water Quality	Focus of Study	Focus of Study	Focus of Study	Stressor	Genetics	GIS/RS
6591	Freshwater	Water Quality	zooplankton			nutrients		
6592	Forest		Soil			anthropogenic disturbance		
6593	Marine	Water Quality	Fish			pollutants	Genetics	
6595								
6596	Woodland		Plants			anthropogenic disturbance	Genetics	
6597	Freshwater	Water Quality	macro-invertebrates	fish		anthropogenic disturbance		GIS/RS
6598	Forest		Forest	Birds				GIS/RS
6599	Freshwater	Water Quality	Fish	Insects		pollutants	Genetics	
6600	Forest		Birds	Soil	Forest	anthropogenic disturbance		
6601	Forest		Plants			pollutants		
6602			Plants	Soil		pollutants	Genetics	
6603	Freshwater	Water Quality	Fish			pollutants	Genetics	

**APPENDIX B – PRODUCTS**

Grant	New Method	Articles	Presentations	Tools/Models/GIS
R82-6591		2	6	1
R82-6592		1	7	
R82-6593		3	8	
R82-6595				
R82-6596	2		24	
R82-6597		4	33	1
R82-6598		3	23	1
R82-6599		6	17	
R82-6600	1	10	35	1
R82-6601	1	13	13	1
R82-6602		21	25	
R82-6603		4	24	

**APPENDIX C – GEOGRAPHIC DISTRIBUTION**

