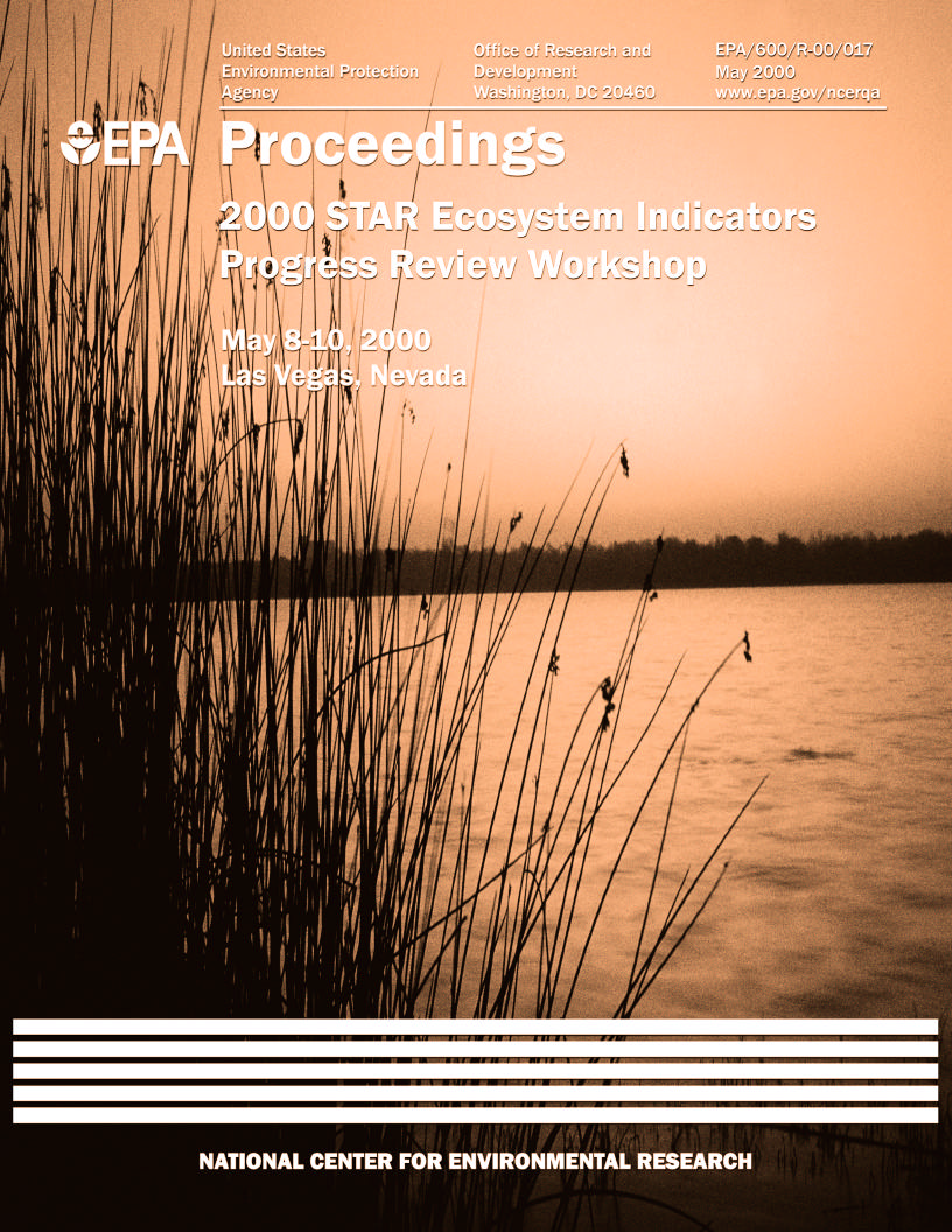

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NATIONAL CENTER FOR ENVIRONMENTAL RESEARCH

Introduction

The mission of the United States Environmental Protection Agency (EPA) is to protect public health and safeguard and improve the natural environmentCthe air, water, and land upon which life depends. Achievement of this mission requires the application of sound science to the assessment of environmental problems and to the evaluation of possible solutions. The National Center for Environmental Research (NCER) at EPA is committed to providing the best products in high-priority areas of scientific research through significant support for long-term research.

The Office of Research and Development's (ORD) Ecological Research Strategy identifies monitoring research focused on biological indicator development at the molecular, community, and landscape levels of biological organization as a high-priority research program. These indicators will be used for the monitoring of ecosystem condition as well as for exposure evaluation. The development of new characterization methods and the improvement of multiscale monitoring designs also are high-priority research components. This research represents the extramural component of ORD's Environmental Monitoring and Assessment Program (EMAP).

In 1997, NCERQA issued a Request for Applications (RFA) on Ecosystem Indicators. The purpose of this solicitation was to support research leading to the development of techniques and indicators that characterize and quantify the integrity and sustainability of ecosystems at local, regional, national, and/or global scales. In 1998 and 1999, the RFAs focused on molecular and landscape indicators, while maintaining their previous emphasis on integrity and sustainability. In 2000, the program's focus switched to estuarine indicators, with the expectation of funding groups of researchers on the East Coast, West Coast, Gulf of Mexico, and Great Lakes. Additionally, in FY 2000, new research on the development of ecosystem classification systems and associated reference conditions will be funded.

Annual progress reviews such as this allow investigators to interact with one another and discuss progress and findings with EPA and other interested parties. If you have any questions regarding the program, please contact the program manager, Barbara Levinson, by telephone at (202) 564-6911, or by e-mail at levinson.barbara@epa.gov.

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Section 1.

Molecular Genetics Research

Genetic Diversity in California Native Fish Exposed to Pesticides

Susan Anderson [1], Andrew Whitehead [1], Bernie May [2], Mark Bagley [2], Kathryn Kuivila [3], Barry Wilson [2], and David Hinton [2]

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The overall goal of this project is to determine the effect pesticide exposure may have on genetic variation in a California native fish. Objective 1 is to examine populations of Sacramento sucker (*Catostomus occidentalis*) exposed to landscape-scale pesticide releases to determine whether changes in genetic diversity are associated with indicators of pesticide exposure and are distinguishable from natural genetic variation. Molecular techniques to be utilized include Amplified Fragment Length Polymorphisms (AFLP) and microsatellites. Objective 2 is to compare the AFLP technique with the Randomly Amplified Polymorphic DNA (RAPD) technique to determine which produces the most informative and reproducible DNA fingerprints. Objective 3 is to evaluate potential linkages between any observed changes in genetic variation and fitness parameters in individuals and populations.

Pesticide exposure patterns are being characterized as one step in separating natural genetic variation from pesticide-related variation. An existing pesticide-use database is being used in combination with Geographic Information System (GIS) applications, as well as pesticide analysis in rivers and creeks, to quantify historical patterns of exposure, and thereby, to select sampling sites.

Fish are being sampled from sites inferred to be impacted by agriculture and from upstream clean reference populations (see Figure 1). Fin clips are archived from individual fish for genomic analysis. AFLP and microsatellite analyses will be performed on archived fin clips, and the resulting genomic patterns will be compared among exposed and reference populations.

Biomarkers indicating genetic damage (DNA strand breaks) and enzyme inhibition (acetylcholinesterase activity) in fish exposed to pesticides are being examined at selected reference and exposed sites as an additional method of discriminating pesticide-exposed populations from reference populations. An assessment of the infor- mativeness and reliability of RAPD and AFLP finger- printing methodologies has been completed for pop- ulation genetic analyses using fish with a well-established pedigree. Numbers of segregating bands were compared and tested for differences in reproducibility.

Whether exposed and reference populations differ in average sensitivity to pesticide exposure in laboratory experiments will be tested using fitness parameters and biomarker responses as endpoints. Correlations between genotype and tolerance also will be tested.

A definitive study design was devised using data on pesticide exposure and information regarding fish avail- ability. Seven hundred fin-clip samples of Sacramento sucker from multiple watersheds were archived. At this time, field sampling for AFLP and microsatellite anal- yses are approximately 75 percent complete. The DNA strand break assay has been further developed this year. The acetylcholinesterase enzyme inhibition assay also has been optimized with an examination of variation in response among tissues and characterization of different isoforms.

In comparing the relative merits of RAPD and AFLP techniques, it was found that the number of segre- gating bands is higher using RAPD than AFLP, but that the reproducibility of RAPD bands is far lower. Only 1 percent of the AFLP bands were judged to be irreproducible. In contrast, 16.1 percent of the RAPD bands were irreproducible. At present, there is no reason to believe that any criterion that can be developed will increase the reliability of RAPD methodology to a level that is comparable to AFLP analysis.

GIS analyses coupled with chemical analyses have resulted in the selection of sampling locations that together represent a strong field experimental design. This design allows for field replication, extensive characterization of background genetic variation, and examination of genetic differences over a very large geographic scale.

This study is unique because genetic variation associated with contaminant exposure has not been evaluated in fish populations on a large geographic scale. The RAPD technique is significant because it is used in ongoing U.S. EPA Environmental Monitoring and Assessment Program (EMAP) studies; however, the newly available AFLP technique permits examination of more of the genome per unit effort and has been demonstrat- ed to be more informative and reproducible.

Tasks for the year 2000 are directed entirely at progress on Objective 1. The first task is completion of approximately one-half of the AFLP and microsatellite analyses. The second task is evaluation of DNA strand breaks and acetylcholinesterase enzyme inhibition in lab- oratory exposures to diazinon as well as in field caging studies.

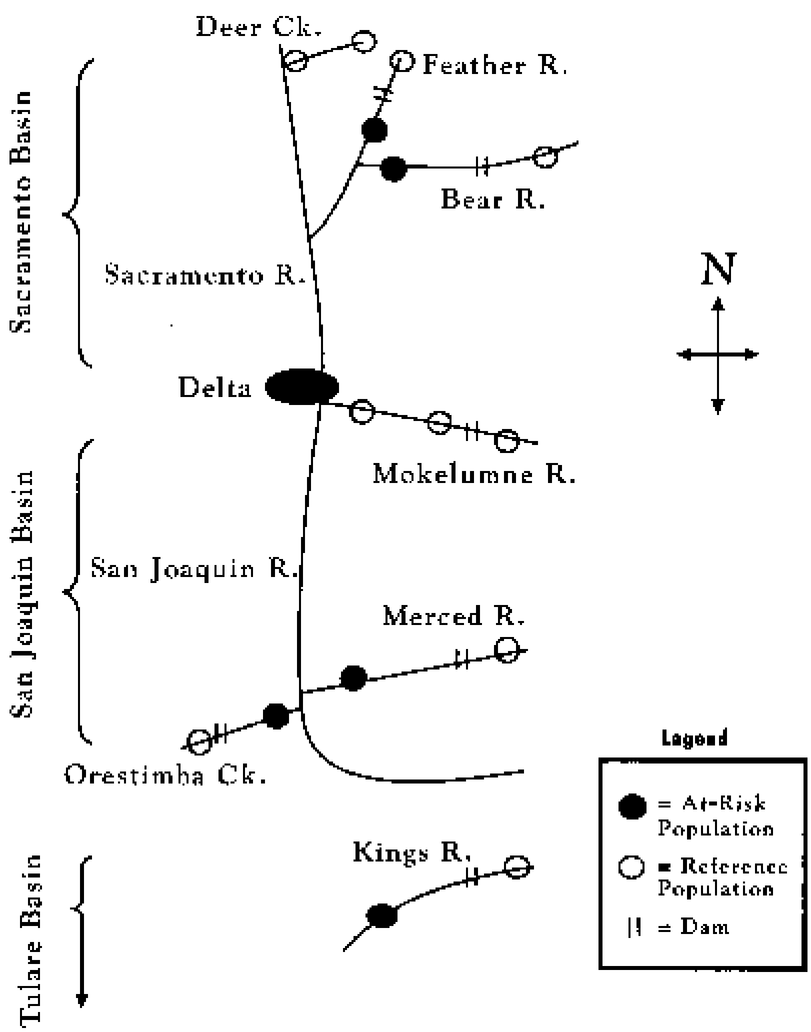

Figure 1. Locations of sampling sites in the Central Valley of California.

Molecular Detection of Anaerobic Bacteria as Indicator Species for Fecal Pollution in Water

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Fecal pollution in water is a threat to ecosystem integrity that also poses health risks to humans. Often the problem is not mitigated because the source of the pollution cannot be determined. For example, runoff from nonpoint sources such as farm manure and failing septic systems may be implicated. The standard indicators for fecal pollution, fecal coliforms, do not distinuish between human and animal sources.

An indicator system based on molecular markers from the anaerobic bacterial group *Bacteroides-Prevotella* was developed as part of this research project. The indicators are not grown, but instead, molecular markers amplified from filtrate from water samples are mea- sured. Using this method, human fecal pollution is distinguished from cow fecal pollution in estuarine and river waters (see Figure 1).

The objectives are to: (1) develop additional markers from other important polluting species; (2) identify the indicator strains or species; and (3) make the system quantitative, allowing for estimation of the proportions of different sources of fecal pollution.

Fecal genetic markers are amplified out of feces or water samples by polymerase chain reaction, using fluorescently tagged primers specific for 16S rDNA genes from *Bacteroides-Prevotella*. The amplification products are digested with restriction enzymes and rapidly screened on a DNA automated sequencer in GeneScan **J** mode, which estimates the proportions of each fragment based on relative fluorescence. Fluorescence data are converted into an electropherogram diagnostic for specific bacterial constituents. Work on this research project using this approach resulted in the identification of two *Bacteroides-Prevotella* diagnostic markers for human feces and three markers for cow feces.

Starting with fecal DNAs from other species, the same approach will be used to test for the cross-occurrence of markers among different species, and to develop markers from other species. 16S rDNA clone libraries will be prepared from *Bacteroides-Prevotella* amplification products and the marker strains in the clone libraries will be located and phylogenetically identified. The proportional contribution from cattle, human, and other sources will be measured by means of real-time quantitative PCR with a Perkin-Elmer TaqMan.

The project is in its first year and significant progress has been made towards the first two objectives. Fecal samples from 10 additional species have been tested for the presence of the human and cow markers. Of these, other ruminant species had some of the cow markers. None had human markers. Markers for additional species currently are being developed. 16S rDNA clone libraries were prepared from PCR products from *Bacteroides-Prevotella*-specific primers, and cow and human marker sequences were identified by row-and-column PCR followed by GeneScan **J** analysis. When the clones were sequenced, it was found that each marker represented a gene cluster rather than a single sequence. The human marker clones were related to *Bacteroides uniformis* and *B. vulgatus.* The cow markers comprised two novel gene clusters within *Bacteroides* but were unrelated to any previously characterized microorganisms.

Source identification of fecal pollution is a longstanding problem. Results from this research promise to provide a solution that will be rapid and economical. The next steps will be to continue developing *Bacteroides-Prevotella* markers from important polluting species, such as swine and waterfowl, and to survey the frequency of occurrence of the markers in individuals and populations, to make the assay quantitative. In addition, PCR primers specific to each individual marker are being developed to make the assay easier and more af- fordable for water-quality laboratories that do not have access to a DNA automated sequencer.

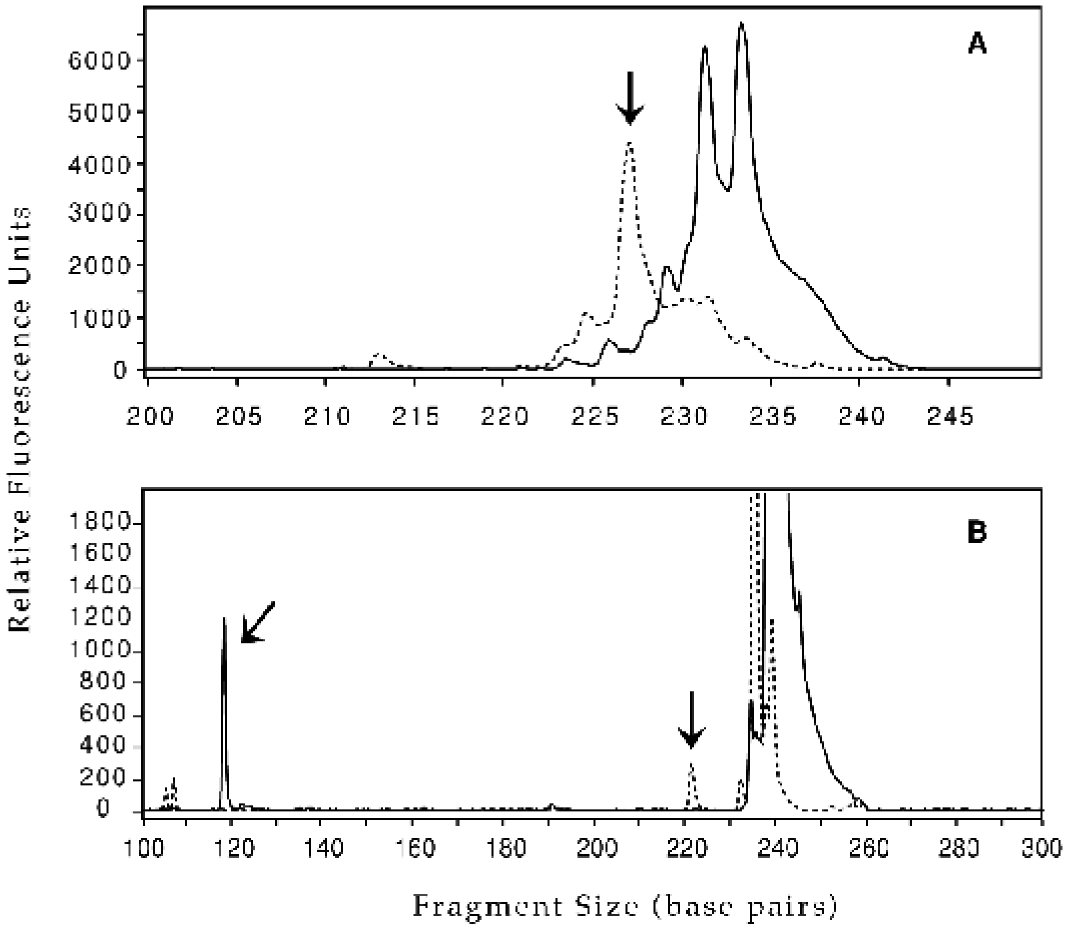

Figure 1. T-RFLP analysis of amplified *Bacteroides-Prevotella* 16S rRNA genes distinguishes human from cow fecal pollution. Solid lines represent combined human fecal DNAs; dotted lines represent combined cow fecal DNAs. The arrows indicate cow- or human-specific genetic markers.

Intraspecies Genetic Diversity Measures of Environmental Impacts

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This project's primary focus is an assessment of the suitability of RAPD-PCR (randomly amplified polymorphic DNA-polymerase chain reaction) genetic diversity measures as an alternative or adjunct for toxicity and bioaccumulation tests of ecological risk in a wide variety of resource types.

Initial efforts will assess the amenability of a set of organisms from a range of trophic levels to RAPD-PCR analysis and will identify organisms suitable for use at a variety of sites. Subsequent work will use these organisms to determine the impact of a broad range of environmental stressors upon overall genetic diversity levels within exposed populations.

The initial stages of this project have focused on refining the RAPD-PCR DNA profiling technique and determining which native organisms at a variety of local aquatic and terrestrial sites would be most suitable for analyses. Profiles from six different aquatic species (crayfish, snails, fathead minnows [*Pimephales promelas*], mosquitofish [*Gambusia affinis*], damsel flies, and *Hyalella azteca*) and five terrestrial species (pill bugs, earth worms, spiders, garlic mustard, and violets) have been generated and evaluated. All organisms studied to date have proved amenable to highly reproducible RAPD-PCR typing.

The important roles played by the species considered to date, in addition to their nonmigratory natures, normally high population densities, broad distribution, and sexual reproduction make them very well-suited for genetic diversity studies. The extremely high genetic similarity seen in garlic mustard (probably due to its recent introduction and invasive nature) both within and between collection sites makes it unsuitable for subsequent work. All other organisms display statistically significant differences in genetic diversity between populations collected at contaminated and reference sites (see Figure 1 for an example in crayfish). Populations collected at reference sites generally have had the highest levels of diversity, and these measures are correlated with the diversity measures of other species collected at the same sites as well as with other measures of ecosystem health, including IBI (the Index of Biotic Integrity) and ICI (the Invertebrate Community Index). Initial surveys of aquatic systems and their closely associated terrestrial sites have been made at Dick's Creek in Middletown, Ohio, and the Little Scioto River in Marion, Ohio. Analyses of the DNA profiles from the populations of organisms will be completed and used to determine which will be most suitable for use at other sites.

In Year 2 of the proposed study, genetic diversity levels of the most informative sentinel species will be considered in surveys of a third aquatic system that has been contaminated with a different set of pollutants. Specifically, organisms will be collected at one reference and three contaminated sites along the Clark Fork River in southwest Montana.

This river transects a variety of agricultural areas along its 120-mile reach. Mining, milling, and smelting activities have occurred extensively throughout its headwater and tributary streams, resulting in substantial heavy metal contamination of the watershed. The system is particularly interesting, due to its harboring of a pollution sensitive and high-quality benthic macroinvertebrate community despite extreme metal toxicity levels.

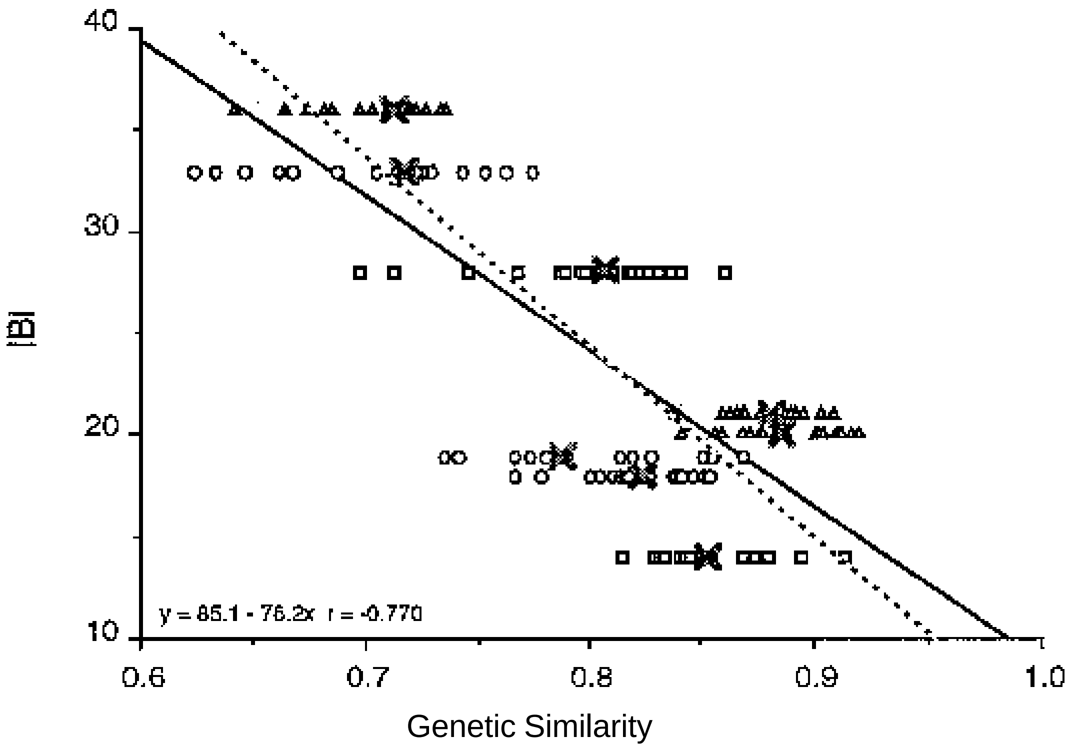

Figure 1. Index of Biotic Integrity (IBI) values are inversely correlated with RAPD-PCR based measures of genetic similarity. Average pairwise similarity of each organism relative to all others collected at its site are plotted against the independently obtained IBI value (Ohio EPA, personal communication) for that site (N=144, r=-0.770, p<<0.001) and a solid line shows the best fit linear regression. Crayfish collected from the impacted and reference Ottawa River sites are displayed as triangles (ª), those collected from sites along the Little Scioto River are displayed as circles (•) and those collected from Elk Creek and its reference stream, Dick's Creek, are shown as squares (~). Large Xs correspond to the mean pairwise genetic similarity of crayfish at each site (N=8, r=-0.804, p<0.01) and a dashed line corresponds to the best fit linear regression for those points.

Demographic and Genetic Factors Affecting Population Viability of *Lupinus perennis*, an Indicator Species of Oak Savanna

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Plants in small and sparse populations often have low reproductive success, indicating that fragmentation of populations by human activities may prevent populations from being self-sustaining. This research is intended to elucidate whether and how population decline develops for a model organism, Perennial Lupine *(Lupinus perennis: Fabaceae)*. This plant species, an important indicator species for the imperiled Oak Openings Savanna community of the Great Lakes ecosystem, not only suffers from habitat loss and fragmentation, but also is the only host plant for three endangered butterfly species. Thus, understanding how demographic and genetic factors contribute to decline of this species will improve understanding of this community and recovery efforts for imperiled butterfly species.

The causes of declines in plant reproductive success in small and sparse populations include decrease in pollinator services, loss of genetic diversity, inbreeding depression, and combinations of these factors. This research seeks to determine the importance of each factor, utilizing a blend of observational and experimental techniques and merging precise data from novel DNA-based indicators of genetic diversity with classical ecological data on reproductive ecology.

The observational studies focus on how existing variation in population size and density affect the factors above. Experimental studies involve a reciprocal transplant between large and small populations to confirm that population size is the cause of the observed patterns. These data will not only improve the understanding of fundamental biological principles in small populations, but also will be informative for designing Oak Savanna management strategies.

Pollinator visitation increased significantly in dense areas within populations, but did not differ significantly among populations varying in size. Significant inbreed- ing depression of seed set and offspring fitness was ex- hibited in a pilot experiment. A previously unnoticed seed predator (*Megalotomus quinquespinosus; Hemipt- era; Alydidae*) that mimics ant behavior and appearance was detected. Feeding damage from this bug disrupts Lupine seed dormancy, potentially affecting plant demography and population health. Several DNA microsatellite markers with which to assess inbreeding rates have been developed.

The pollinator visitation data indicates that ecological factors may influence population reproductive success on a fine scale, such that within-population density variation may be as or more important than population size itself (see Figure 1). Should these trends be substantiated in subsequent studies, management activities may need to consider plant density as well as population size to improve the status of *L. perennis*, the species that depend upon it, and the overall health of the Oak Savanna ecosystem.

The pilot study of inbreeding depression has helped refine the methods and experimental design to be em- ployed in the larger study during Year 2. The results also suggest that inbreeding depression will be detectable. The occurrence of an ant-mimicking seed predator appears to be unknown to most scientists and managers interested in Lupine and the Karner Blue. Work to disseminate this information is underway through conversations and a manuscript in preparation.

For Year 2 of the project (2000B2001), the tasks to be completed include: assessing genetic variation (isolating DNAs, running gels), analyzing the mating system (planting seeds, isolating DNAs, running gels), planting phytometers in the field, continuing pollinator observations, inbreeding depression pollinations, planting inbreeding depression seeds (fall), and analyzing and writing up research results. During Year 3 (2001B2002), it is planned to assess phytometer success, complete the mating system gels, assess inbreeding depression seedlings, and further analyze and write up the research results.

More information can be found at the following Web Sites: http://www.uakron.edu/biology/mitchell/lupine.html and at http://www.bgsu.edu/departments/biology/people/faculty/michaels/research.html.

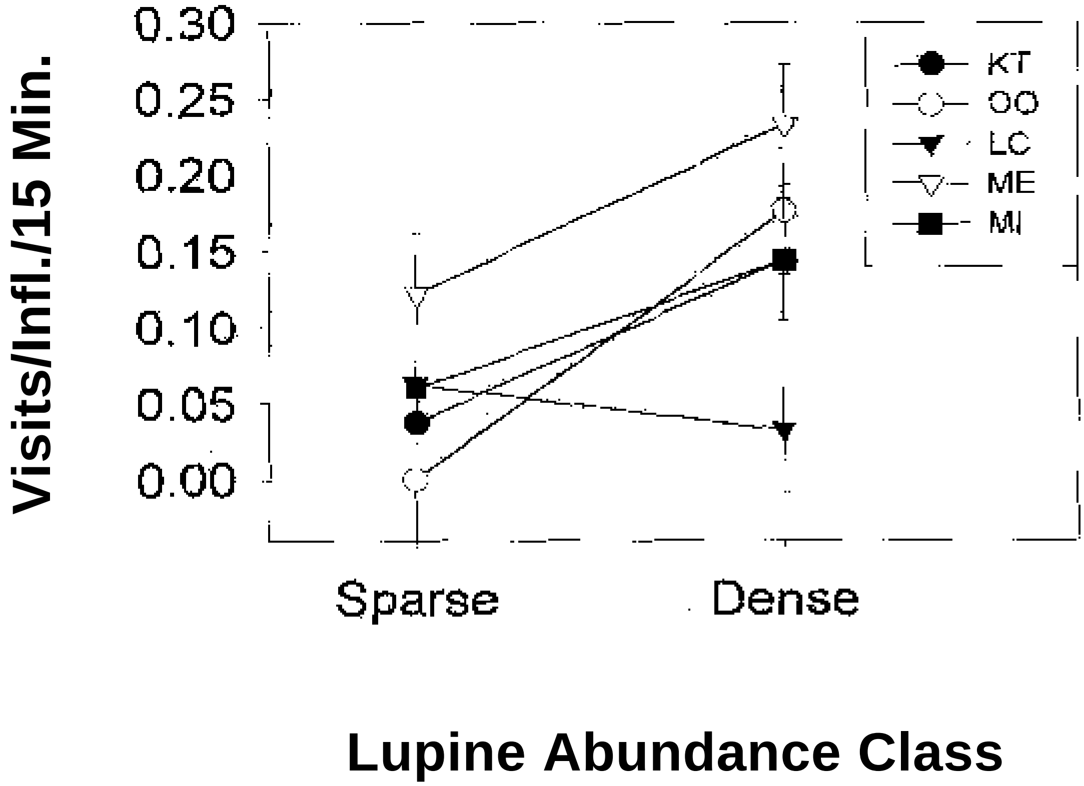

Figure 1. Inflorescence visitation rates by bumble bees to observation plots in 1999 (mean ± se). Lupine density significantly affected visitation rate ($F_{1, 80} = 9.6$, $P < 0.0027$).

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

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Molecular genetic traits of the mummichog are being evaluated as bioindicators of population-level effects of pollution. Mummichogs were sampled from nine localities along the Elizabeth River in Virginia. Sediment PAH concentrations span four orders of magnitude among these sites. Localities vary also in sediment concentrations of chlorinated hydrocarbons, tributyl tin, and metals. The prevalence of proliferative liver lesions was high in fish from one locality, the Atlantic Wood (AW) site, with a PAH concentration of 371,212 ng/g of sediment.

Molecular genetic data currently are being used to address several predictions regarding population response to contaminants: (1) populations residing in contaminated habitats are genetically distinct from populations in neighboring clean sites; (2) populations at polluted sites exhibit lower genetic diversity than populations taken from clean sites; and (3) the genetic structure of mummichog in the Elizabeth River reflects the mosaic of highly contaminated and clean habitat.

Genetic data consist of allozyme genotypes and DNA sequences for a 450 base pair segment of the d-loop of the mitochondrial genome. Preliminary analyses of allozyme data do not indicate correlation with level of contamination. Analyses suggest that overall genetic variability did not differ with PAH concentration. Highly polluted localities (AW, RS) were not genetically depauperate relative to neighboring relatively nonpolluted localities (SC, JC). Indeed, mummichog from the heavily PAH-contaminated AW site show high diversity in d-loop haplotypes.

Mummichogs from the AW site share the common haplotype found at other locations along the Elizabeth River as well as several highly divergent haplotypes (see Figure 1). Genetic analyses suggest that mummichog may move among localities more than previously reported.

Hypotheses regarding contaminant tolerance and individual and populations performance relative to genetic variability and contaminants will be examined in the next phase of the project. Additional survey work of mummichog inhabiting contaminated and clean habitats may be undertaken to evaluate the generality of these results.

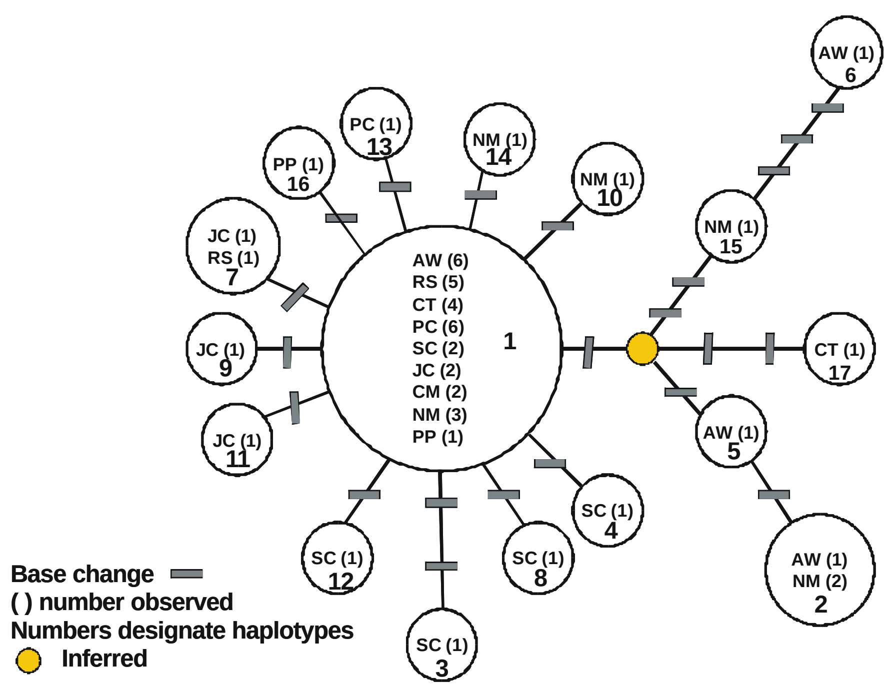

Figure 1. Relationships among d-loop haplotypes and distribution among Elizabeth River sites.

Multilevel Indicators of Ecosystem Integrity in Alpine Lakes of the Sierra Nevada

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The overall objective of the project is to develop protocols for environmental assessments of alpine lakes in the Sierra Nevada with a range of human impacts. These assessments will be conducted over the range of levels of biological organization (molecular to ecosystem), utilizing currently available assessment techniques and with the addition of two new ecological indicators.

The use of population genetics analysis as a response indicator and the use of molecular biomarkers of exposure to contaminants as a diagnostic indicator will be tested for incorporation into monitoring and assessment programs for surface waters. These indicators will provide information concerning the status of population diversity and stability as well as the exposure to nonpersistent, nonbioaccumulative contaminants. This information is missing from current monitoring and assessment protocols.

Over the 3-year project period, standard environmental assessments of a select group of alpine lakes with a defined range of human impacts will be conducted. There will be a total of 16 assessment sites (see Figure 1). Those selected for assessment include four minimal- ly impacted areas (Castle Lake, Eagle Lake, Marlette Lake, and Upper Angora Lake), eight areas with a range of moderate impacts (Fallen Leaf Lake, Gold Lake, Lake Tahoe at Sand Harbor, Prosser Reservoir, Spaulding Reservoir, Stampede Reservoir, Topaz Lake, and Twin Lakes), and four highly impacted areas (Boca Reservoir, Donner Lake, Lake Tahoe at Tahoe City, and Lake Tahoe at South Lake Tahoe).

In addition to the standard assessment, population genetic assessments will be conducted in fish and invertebrates at these same sites. Allozyme electrophoretic analysis and Randomly Amplified Polymorphic DNA (RAPD) analyses will be conducted on two organisms common to the lakes of the region (fish: Lahontan redside; invertebrate: Signal crayfish). Contaminant exposure assessments also will be conducted in fish using molecular biomarkers of exposure in the gills of fish. Five markers indicative of exposure to a wide variety of chemical contaminants (persistent and nonpersistent) and that can account for interactions among complex mix- tures of contaminants will be measured over time in the assessment areas using caged rainbow trout.

These additional techniques then will be applied to current assessment protocols. Because a group of lakes with defined levels of human impacts will be examined, the discriminatory ability of the assessment techniques may be analyzed using the current protocols compared to the protocols with the two new indicators added.

It is hypothesized that because current protocols do not account for genetic diversity or nonpersistent contaminants, the addition of these new indicators will greatly enhance the monitoring and assessment programs for surface waters.

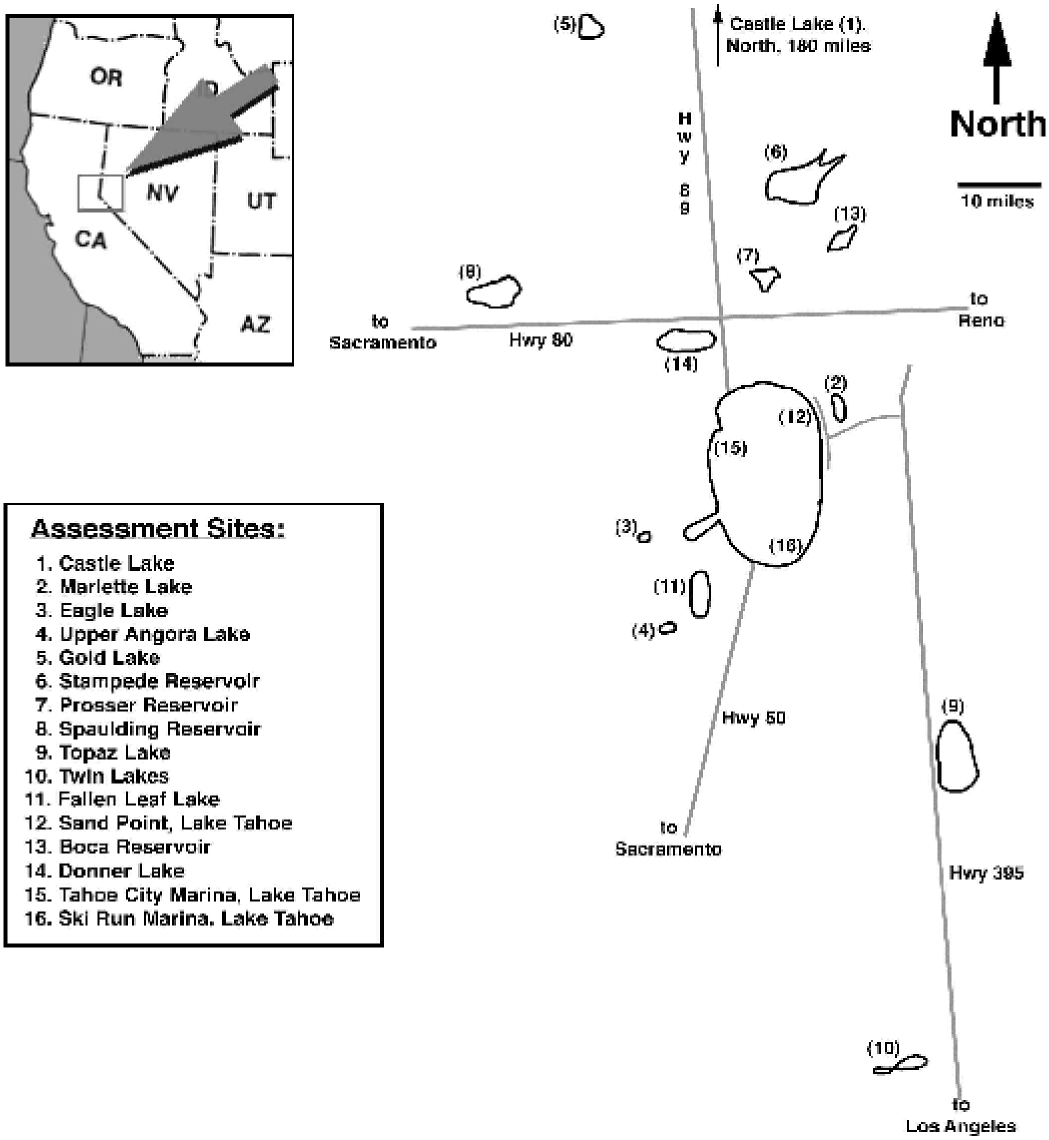

Figure 1. Map of assessment site locations within the Sierra Nevada.

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

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Department of Biological Sciences, University of Cincinnati, Cincinnati, OH

New methodologies in molecular genetics may provide novel types of ecological indicators for monitoring the integrity of natural ecosystems and the sustainability of ecosystems that are affected by anthropogenic influences. One type of ideal ecological indicator would be an organism that grows in a wide variety of habitats that could easily be used to monitor for the presence of mutagens or anthropogenic factors that alter population genetics.

Dandelions (*Taraxacum officinale:* Weber; *Asteraceae*) are a model ecological indicator organism because: (1) dandelions have an extremely wide ecological amplitude, growing almost worldwide from sea-level to alpine biomes, and from the tropics to north-temperate habitats; (2) dandelions grow rapidly, and populations can be easily manipulated and monitored; (3) it has been documented that a variety of pollutants can be sequestered in dandelion tissues; (4) dandelion seeds are produced asexually, rendering the detection of mutations easy; and (5) preliminary data have been generated using variable-number-tandem-repeat (VNTR) DNA probes that suggest that mutation rates and population genetic diversity parameters can be analyzed successfully with dandelions.

The specific hypothesis to be tested is that dandelions can be used as a sensitive ecological indicator species via comparative analyses of VNTR genetic markers used to examine anthropogenic changes in genetic diversity of both mutation rates and population genetics, at pollution-impacted versus nonimpacted sites.

Dandelions will be sampled from 12 sites: 6 relatively pristine sites versus 6 sites with chronic, high levels of contaminants. To determine whether rates of mutation differ between these two types of sites, leaf tissue and 8B10 seeds will be collected from each of 10 plants per site. Dandelion seeds are produced via agamospermy, a nonsexual process in which all seedlings from a maternal plant should be genetically identical to that maternal plant.

Preliminary results demonstrate that although the previous statement is true, a low level of mutation (about 0.005% of bands transmitted) is detectable. By surveying the parent-to-offspring transmission of tens of thousands of VNTR markers, whether mutation rates differ between pristine versus contaminated sites is being tested (see Figure 1).

These markers also will be used to examine whether populations differ in genetic diversity. Initial results demonstrate that very local populations harbor a large amount of genetic diversity, although certain clones may be widely distributed. Soil and dandelion tissue have been analyzed from more than 20 sites for metals, and 12 of these sites covering a range of contamination are being selected to compare dandelion mutation rates and population genetics.

It is expected that a determination will be made as to whether VNTR markers in dandelions can be used as sensitive indicators of anthropogenic changes in population genetic diversity due to either altered mutation rates or stressor-induced selection. If mutation rates or genetic diversity in dandelions are correlated with pollution history, dandelions provide an easily utilized biomonitor to survey the ecological integrity and sustain- ability of a wide range of habitats across multiple spatial scales throughout the world.

Besides completing the above projects, further experiments growing dandelions in controlled media spiked with mixtures of common pollutants will be conducted to investigate whether mutation rates increase with increasing pollutant concentrations. Further, studies have been initiated to determine whether dandelions from the most heavily polluted sites have higher fitness when competing with dandelions from more pristine sites when both are grown in polluted and nonpolluted soils.

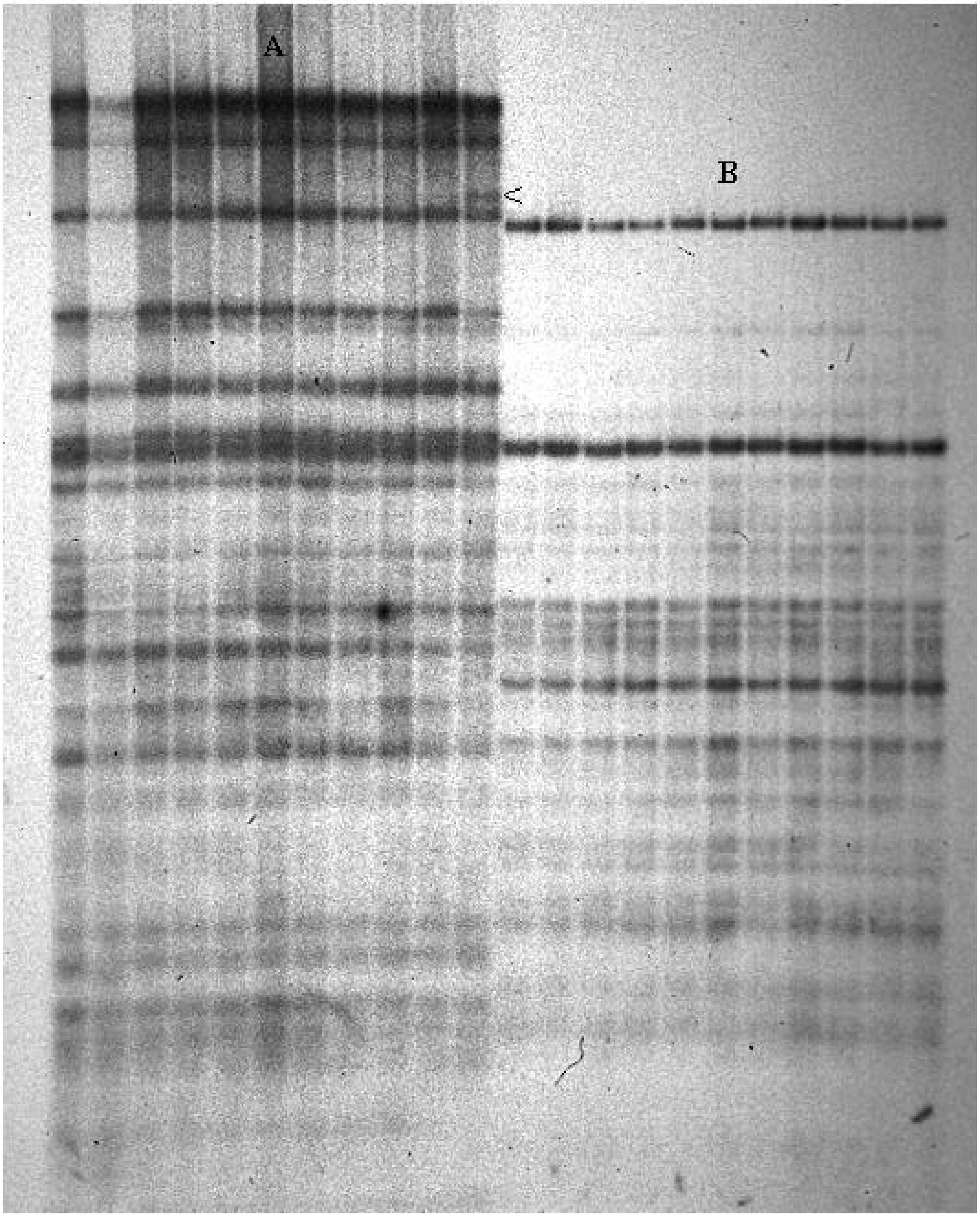

Figure 1. Maternal plant-to-offspring transmission of VNTR genetic markers in dandelion detected with the TTCCA (= core sequence) PCR-STR probe. Markers for two different maternal parents are shown (lanes A and B), with 10 clonal offspring of each maternal parent also shown (5 offspring on each side of each parent). More than 30 VNTR markers are shown across both parents, and parents clearly differ in the markers transmitted to their clonal offspring. One new mutant marker is detectable in the eleventh lane from the left (marked with a <). If an autoradiograph is interpreted to have a transmission of approximately 30 markers to 20 offspring (= 600 markers transmitted), with one novel marker, the detectable mutation rate is 1/601 = 0.00166 for that particular gel-probe autoradiograph. Three micrograms of genomic DNA per lane were digested with a five-fold excess of *Taq*I restriction endonuclease.

Section 2.

Multiscale and Landscape Indicators

Foliar Chemistry as an Indicator of Forest Ecosystem Status, Primary Production, and Stream Water Chemistry

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Monitoring the biogeochemical status of forest and stream ecosystems is a key component of assessing environmental quality in the Northeastern United States. Any monitoring system that requires spatially continuous capabilities will need to use some form of remote sens- ing. Because forest canopies are the only portion of the system accessible to optical reflectance remote sensing instruments, they offer the most likely target surface for monitoring forest health in this spatial mode.

It was hypothesized that forest productivity, soil mineralogy, and foliar chemistry at the whole-stand (not individual tree) level are all tightly linked to the biogeochemical status of a forest ecosystem. It was further hypothesized that the concentration of cations in forest canopies will be measurable by high spectral resolution remote sensing, as has been demonstrated for nitrogen and lignin, and that watershed-level stream chemistry, reflecting soil mineralogy, also will be predictable from watershed-level values of canopy chemistry derived by remote sensing.

The study area is the White Mountain National Forest (WMNF), a 300,000 ha area in northern New Hampshire. At the intensive plot scale, the long-term sampling program at the Bartlett Experimental Forest and the Hubbard Brook Experimental Forest, New Hampshire, will be used and augmented to examine and attempt to predict interannual variations in foliar chem- istry as well as woody and foliar production.

At the regional scale, canopy chemistry, soil mineralogy, and forest productivity will be measured at a series of existing experimental and monitoring research sites. Work on the spatially continuous monitoring scale across the White Mountain region will include the development of algorithms for predicting canopy cation concentrations using data from NASA's Airborne Visible-Infrared Imaging Spectrometer (AVIRIS). At the watershed scale, 50 streams will be sampled in the WMNF covering a range in estimated mineralogical richness. A nested approach will be used to determine the optimum scale at which stream water chemistry may be predicted. In addition, high spatial resolution (3**B**4 m) spectral data will be collected for sampled watersheds via low altitude AVIRIS data acquisition.

Preliminary findings include: (1) strong relationships between whole-stand level foliar canopy N, NPP, and forest floor C:N ratios exist; (2) foliar Ca can be mapped across the WMNF (300,000 ha) using remote sensing technology (see Figure 1); and (3) a GIS model has been developed for the WMNF that predicts element content of glacial till**C**the predictions for till Ca have been compared to plot level foliar Ca data, and a significant relationship exists.

Remote sensing technology can be used to estimate foliar chemistry (N, Ca) at a landscape scale. With this information, forest productivity estimates can be made. Soil mineralogical characteristics also can be modeled at the landscape scale. Relationships between stream water chemistry, mineralogy, foliar chemistry, and forest productivity can be determined with this information. This program then would establish the scientific basis for developing a satellite- or aircraft-based remote sensing program for monitoring forest health and stream water quality.

The next steps are to: (1) continue the collection and analysis of stream water from 50 streams across the WMNF; (2) process and analyze new AVIRIS imagery from a low altitude platform for selected watersheds**C**AVIRIS low altitude data (3**B**4 m resolution) for the summer of 2000 has been requested through NASA's Airborne Science Program; (3) collect data from new plots across the WMNF to validate foliar and glacial till element concentration maps; and (4) use soil and foliar element coverages to develop relationships with measured stream water chemistry and forest productivity.

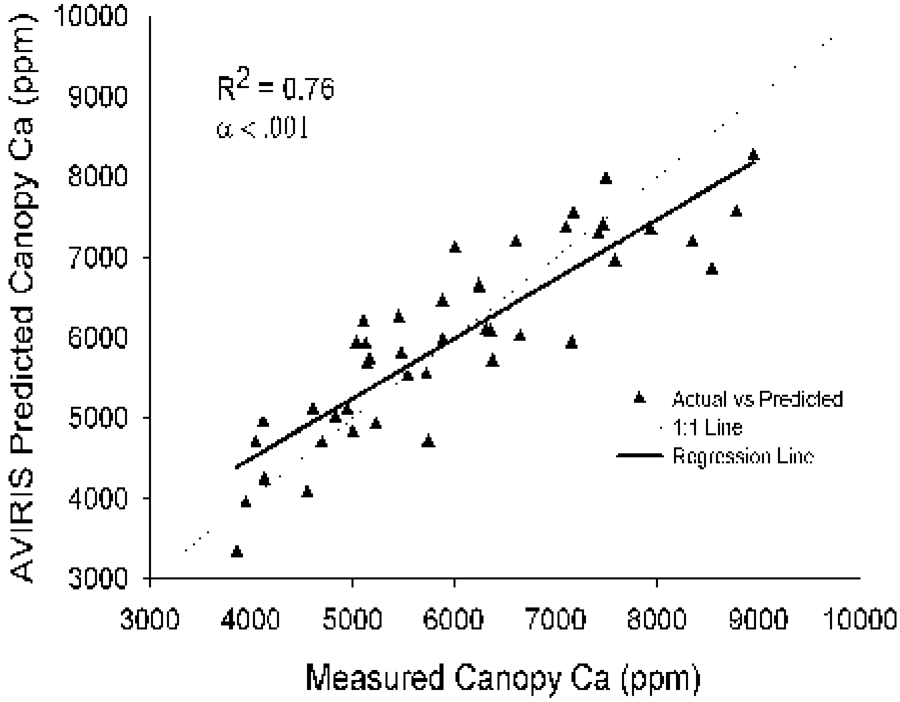

Figure 1. AVIRIS predicted plot level canopy Ca concentration plotted against measured canopy Ca concentrations.

Environmental Factors That Influence Amphibian Community Structure and Health as Indicators of Ecosystem Integrity

Val Beasley [1], Lucinda Johnson [2], Carl Richards [3], Patrick Schoff [2], Rebecca Cole [4], Camilla Lieske [1], Anna Schotthoefer [1], Cathy Johnson [2], Joseph Murphy [1], and Marvin Piwoni [5]

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The overall goal of this research is to assess the relative influence of landscape patterns, biotic interactions, water quality, and contaminants on amphibian health and community structure. The following will be evaluated: (1) the relative influence of wetland- and watershed-scale factors (i.e., landscape and ecological data) on amphibian community structure and health; and (2) whether amphibian community structure and health are indicative of ecological integrity.

During 1998, anuran community structure, wetland habitat, and landscape characteristics were quantified at 64 sites distributed throughout northern Illinois, southern-to-central Wisconsin, and southern-to-central Minnesota (see Figure 1). Site-specific surveys also were conducted to determine the incidence and types of anuran malformations and deformations.

Landscape data were summarized within a 2 km and 10 km radius/buffer surrounding each wetland. Macroinvertebrates were identified to the family level, and dominant vegetation was characterized for each wetland. Anuran data were summarized for 1998 and 1999, individual species-habitat relationships were examined, and site occupancy models were developed for several frog species based on local and regional characteristics. Logistic regression models were developed to predict the occurrence of the wood frog, spring peeper, and gray tree frog. Models incorporating data at several spatial scales were better predictors of frog presence/absence than those incorporating variables from only one spatial scale. During 1999, 36 wetlands in central Minnesota were analyzed for anuran and macroinvertebrate comm- unity structure, wetland vegetation, and physical/chem- ical attributes, including habitat structure, water, and sediment quality. Parasitological studies were conducted on tadpoles, metamorphs, adult frogs, and gastropods. In separate studies, full necropsies were performed on 485 frogs. A comprehensive set of tissues was collected, fixed in buffered formalin, and processed for histopathologic examination.

Malformations or deformations were identified in 13 of 559 frogs from 8 wetlands in 1998, and in 7 of 544 frogs from 6 sites in 1999. Among the malformations were webbed skin between the femur and tibio-fibula, skin lacking spots, and hemimely (partial absence) of the hind limb. Parasites identified included: *Echinostoma spp.*, *Rhabdias ranae*, *Haematolechus sp.*, *Megalodiscus sp.*, and *Ribeiroia sp.* *Echinostoma spp.* were found in the kidneys of frogs from all sites at prevalences of 40B100 percent (mean 82%) and intensities of 8B321 echinostomes/frog (mean 90).

In pathological studies on 485 frogs, intersex was identified in 6 percent of all frogs (from 11 sites). At least one lesion was identified in 55 percent of frogs. Condition scores from slightly poor to emaciated were noted in 8 percent of frogs from 12 sites. Parasitism was noted in the body cavity, GI tract, kidney, liver, lung, muscle, and skin. The most common abnormality for body cavity, kidney, liver, and lung was inflam- mation (52B84%); for GI tract it was no digesta (50%); for musculoskeletal system it was trauma (52%); and for spleen it was splenomegaly (73%). Diagnoses and sex determinations will be confirmed histologically.

Examinations of metamorphs, identification of parasites, histopathology studies, and chemical analyses from the 1999 field season will be completed in the coming months. Additional ecological, water quality, contaminant, and anuran community structure and health data will be collected in 2000 before the assessment of the potential use of amphibian community structure and health as indicators of wetland conditions.

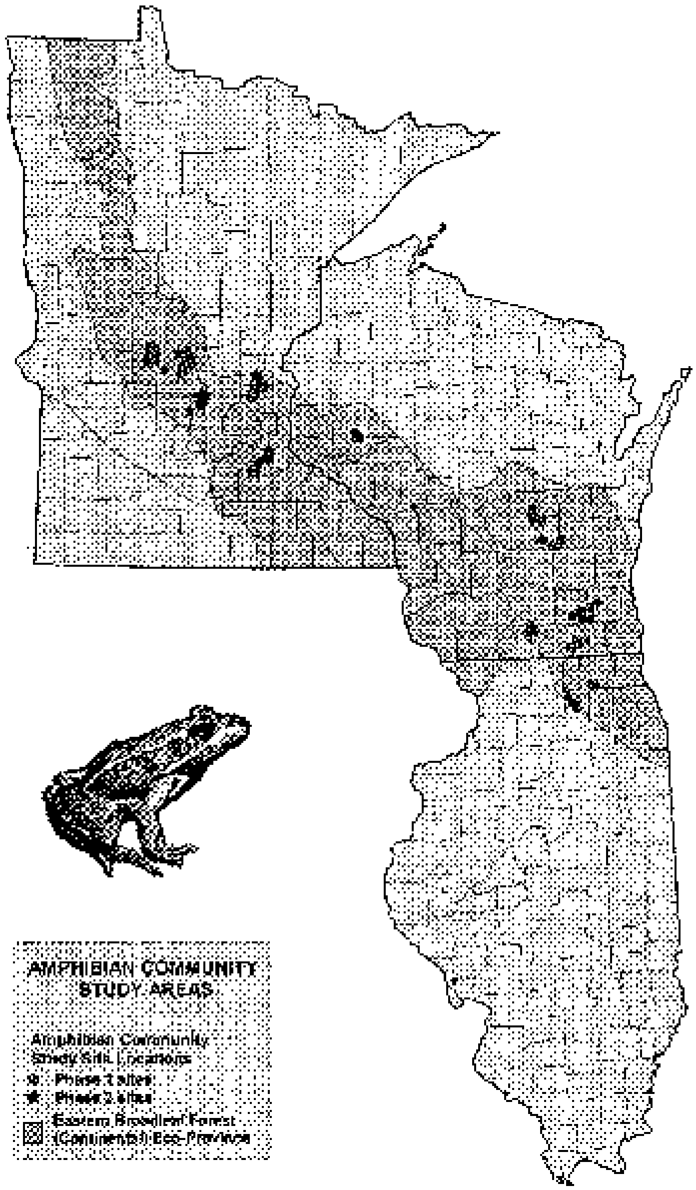

Figure 1. Amphibian community study areas.

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

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Tropospheric ozone is a pollutant that is responsible for forest damage worldwide. Extensive ozone monitoring networks currently measure ozone concentrations throughout the United States and Europe; however, the physiologically relevant measure of ozone for forest health is not concentration, but rather ozone *flux*, the amount of ozone that actually enters the foliage.

A model is being developed to estimate ozone flux from ozone concentration utilizing routinely measured ozone and meteorology. It is hoped that this model will be adapted for monitoring networks. The utility of *^{13}C as a proxy for stomatal conductance in the estimation of ozone deposition also is being explored.

In 1997 and 1998, an initial study was conducted at Blodgett Forest to investigate the influence of typical Mediterranean summer drought on the uptake of ozone and general physiological response of ponderosa pine. Fluxes of ozone, CO_2, water, and energy were measured by eddy covariance from May through October. Using this eddy flux data, typically reported ozone exposure metrics were compared against direct measurements of ozone deposition to document that ozone metrics are poor predictors of ozone uptake in California pine ecosystems.

In May 1999, the field campaign was expanded**C**sites were established at three additional locations along an ozone injury gradient in the Sierra Nevada Mountains, California. Site locations and characteristics are presented in Table 1. These sites take advantage of existing ozone monitoring (National Park Service and California Air Resources Board Environmental Protection Agency) and an ongoing forest damage assessment project. Year-round continuous measurements of ecosystem-scale ozone, carbon dioxide, water, and energy fluxes started in May at Blodgett Forest. Measurements of leaf-level physiology were made monthly at each site from May through September and included diurnal net photosynthesis, stomatal conductance and transpiration, predawn and afternoon water potential, photosynthesis response curves to light and carbon, and dark respiration. Foliage was collected for starch *^{13}C analysis every month. At the end of the season, foliage samples were collected for cellulose *^{13}C measurement

Another study at Blodgett Forest to gain further insights into the limitations imposed by typical summer drought on the uptake of carbon and ozone in the ponderosa pine ecosystem was completed. Two sites were set up**C**one control and one watered**C**in a ponderosa pine plantation. Carbon uptake in 1-year-old control foliage was reduced compared to the watered treatment during the 3 measurement days following treatment by 39 percent, 35 percent, and 30 percent, respectively, per unit leaf area. Stomatal conductance was lower at the control site, leading to a reduction in estimated ozone deposition (ozone concentration times stomatal conductance) of 36 percent, 46 percent, and 41 percent of the watered site, respectively. This experiment demonstrated that site moisture is one of the most important factors controlling ozone uptake in California forests. Expected changes in climate will profoundly affect the ozone uptake by California forest ecosystems.

The field campaign will continue during the growing seasons of 2000 and 2001. Models that estimate stomatal conductance and ozone flux into foliage will be evaluated and compared for their appropriateness in the Sierra Nevada ponderosa pine ecosystem. The selected model will be developed further to apply to this project's specific needs and compare modeled ozone deposition with direct measurements of canopy-scale ozone deposition at Blodgett Forest.

Table 1. Site locations and characteristics.

Site	Location	Slope	Elevation (m)	OII[1]	Ozone[2] (ppb)
Sequoia/King's Canyon National Park	N36E33'55" W118E46'36"	30%	1920	41.3	63[a]
Yosemite National Park	N37E42'43" W119E42'19"	10%	1220	14.7	41[a]
Blodgett Forest Research Station Control Site	N38E53'43" W120E37'58"	2%	1315	N/A	49[b], 57[c]
White Cloud	N39E19'00" W120E50'45"	10%	1326	27.3	62[a]

[1]Ozone Injury Index (OII) is derived from a combination of the primary effects of ozone on pine (Arbaugh et al.,1998).
[2]24 hour mean ozone concentration (in ppb) from June 1**B**October 31 were: (a) from Arbaugh et al.,1998, (b) from our measurements in 1998, and (c) from our measurements in 1999.

Effects of Forest Fragmentation on Community Structure and Metapopulation Dynamics of Amphibians

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The goals of this research project are to quantify the effects of forest fragmentation on amphibian community structure and metapopulation dynamics in vernal pools, and to develop appropriate ecosystem indicators for vernal pool ecosystems at multiple spatial scales.

The objectives of this study are to: (1) quantify the manner and extent to which forest fragmentation influences amphibian community structure of vernal pool ecosystems; (2) assess the extent to which landscape- and local-scale features reflect fundamental structural properties of vernal pool habitats and their biotic communities and, conversely, the extent to which indices of biotic integrity (e.g., amphibian community structure) reflect local and landscape properties; (3) develop predictive models that integrate landscape-scale factors with pond-scale attributes to quantify key compositional and structural attributes of the amphibian community, and derive ecosystem indicators at multiple spatial scales; and (4) develop predictive models to quantify the extent to which forest fragmentation influences the metapopulation dynamics of woodland amphibians and predict the consequences of landscape change on these metapopulations.

Recent evidence of declines and an increased rate of malformations in amphibian populations have prompted much interest and research into the potential anthropogenic stressors associated with these occurrences. Results of some of this research indicate that fragmentation may have a significant effect on the metapopulation dynamics of amphibian communities, which could result in declines and regional extinctions of populations. Forest fragmentation results in changes to forest landscapes and habitats that may have both direct and indirect effects on local and regional amphibian communities (e.g., direct habitat loss, disruption of dispersal corridors, altered habitat structure, and microclimate changes).

It was hypothesized that forest fragmentation can be directly related to changes in landscape structure and local habitats (e.g., vernal pools) that have a measureable effect on the integrity of amphibian communities. Landscape, local habitat, and biotic community variables will be quantified to examine the effects of forest fragmentation on pond-breeding woodland amphibians at three spatial scales: the landscape scale, the local or "pond-scape" scale (including ponds and surrounding terrestrial habitat), and the aquatic pond habitat. Relationships among these hierarchically nested scales will be quantified using an integrated series of empirical models. This approach of identifying relationships across scales and integrating them into a modeling framework will allow the development of multiscale ecological indicators of the effect of forest fragmentation on vernal pool eco- systems and on regional amphibian communities.

It also was hypothesized that indices of community diversity and structure and amphibian species traits can be used to assess effects of forest fragmentation on vernal pool systems and regional amphibian communities. Metapopulation models will be developed to assess the effects of fragmentation on local and regional populations, and to elucidate the mechanisms by which forest fragmentation may influence the composition and persistence of vernal pool communities. This is a new project, and to date there are no results to report.

Land Use and Geomorphic <u>Indicators of Biotic Integrity in Piedmont Streams</u>

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The overall goal and objective of this project is to define the predictive capabilities of scale-variable attributes of land cover (GIS-based) and geomorphology as risk assessment indicators of biotic integrity of stream ecosystems on the southern Piedmont.

The watershed under investigation is the upper Etowah River Basin north of Atlanta, GA. Given various aspects of historical landscape change, this research is investigating the following three ancillary questions: (1) Do physical stressors and the corresponding ecological response vary as a function of land cover in the watershed? (2) Is this relationship consistent within watersheds of vastly different sizes? (3) Do antecedent land cover conditions (>50 years ago) influence the physical stressor and ecological response relationship?

The approach to answer these questions involves two main projects over a 3-year period. The first pro- ject involves a comprehensive field survey of the geomorphic condition, habitat, water quality, and biological integrity in 30 streams draining watersheds of three distinct size classes of about 15, 50, and 100 km^2. These watersheds have variable land cover of 50B100 percent forest, based on the 1993 multiresolution land characteristic (MRLC) databases. The stream reaches are surveyed for a length of at least 15 stream widths. Regression analysis is used to develop predictive models of biotic conditions from geomorphic indicators, habitat assessment, water quality, and land cover characteristics. The second project involves a detailed analysis of geomorphic conditions, stream habitat, and biotic integrity of five streams (out of the original 30) that appear as unexplained residuals in the predictive models, which will be compared to five other streams that fit the models. This will involve an expansion of 10 surveyed reaches to a length of at least 1 km, and further assess- ment of the reach-scale versus watershed-scale controls on biotic integrity.

During January 2000, raw data from all aspects of the field survey were finalized and are being analyzed along with the land cover data. The preliminary findings suggest that reach-scale geomorphic and habitat assessments are somewhat better indicators of biotic integrity than the 1993 watershed-scale land cover characteristics. For example, the average particle size of the stream bed (in phi units) is highly correlated with the relative abundance of pool species of fish, exhibiting a correlation coefficient of 0.82 (see Figure 1). In contrast, the best correlation between pool species and land cover is found with percent forest, exhibiting a correlation coefficient of -0.48, and all other land cover classes are not significantly correlated.

The significance of the preliminary findings may be that objective reach-scale assessments of physical characteristics are better indicators of biotic conditions than watershed land cover. More analysis of linkages between the watershed-scale land cover and reach-scale physical conditions are needed to explore causal relation- ships and develop multiple regression models that may provide the best indicators of biotic conditions, however. The next steps involve continued analysis of the raw data from the 30 sites and identification of sites for the second phase of the analysis.

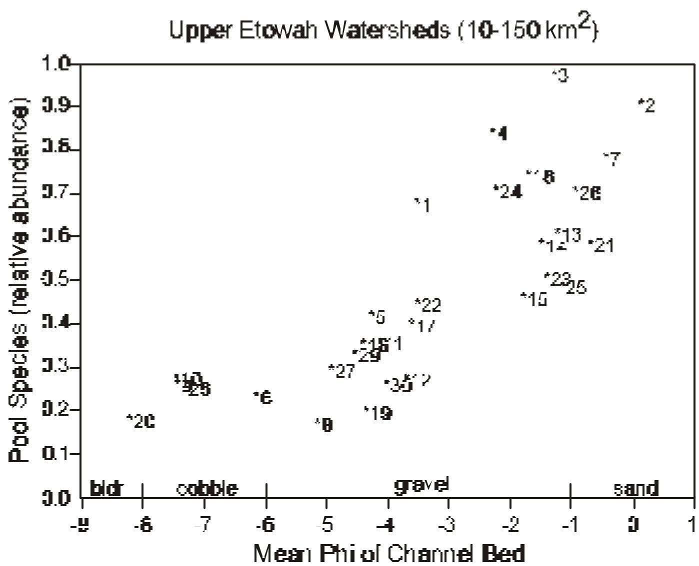

Figure 1. Relationship between mean Phi particle size and pool species (relative abundance) in streams of the upper Etowah River Watershed (10–150 km^2 basins).

Developing Effective Ecological Indicators for Watershed Analysis

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This project is designed to develop improved indicators and innovative techniques for assisting and monitoring ecological integrity at the watershed level in the Western United States. The specific objectives of this study are to develop practical, scientifically valid indicators that: (1) span multiple resource categories; (2) are relatively scale independent; (3) address different levels of biological organization; (4) can be rapidly and cost-effectively monitored by remote sensing; and (5) are sensitive to a broad range of anthropogenic and natural environmental stressors.

The study is based, in part, on the hypothesis that streams and riparian areas often reflect the ecological integrity of the associated watersheds. Due to a "funnel effect," these areas are the accumulation zones of environmental disturbances occurring in the watershed (see Figure 1). For example, logging, overgrazing, and forest fires influence sediment erosion rates that directly influence downstream stream and riparian conditions. Monitoring of key indicators in these accumulation zones may provide an efficient, cost-effective way to evaluate and monitor the ecological integrity and sustainability of the surrounding watershed.

The Upper Yellowstone River and its tributaries will be used because the watersheds of these streams incorporate a broad range of environmental conditions from relatively pristine in certain watersheds within Yellowstone National Park, to highly disturbed by forest fires and land uses such as mining, logging, and agriculture in other watersheds. Identification, assessment, and validation of effective indicators will involve the integration of results from research at various scales, including: (1) analysis of hyperspectral and traditional multispectral imagery from both aerial and satellite platforms; (2) field surveys of stream morphology and riparian habitat associated with remote sensing to assess indicators; and (3) intensive site-specific stream sampling of macroinvetebrate communities to validate the effectiveness of these indicators. Use and evaluation of remote sensing technologies is the primary research methodology. All indicators chosen must be able to be monitored by remote sensing.

Remote sensing techniques, which will enable selection of indicators to be used for rapid, cost-effective ecological monitoring on the regional and local scale, also will be used to help identify key ecological indicators in both streams and riparian areas. These also will be correlated with ecological indicators of disturbances in the surrounding watersheds, resulting in a set of effective ecological indicators and the development of innovative techniques for efficient watershed-level analysis at various scales in the Western United States.

As this project is in its initial phase, the sequential steps to be taken over the duration of this project include: (1) coarse watershed characterization for initial site selection; (2) stream and riparian site selection; (3) ground evaluation and selection of indicators at stream and riparian sites; (4) fine watershed characterization using remotely sensed data to determine stressors on study sites; (5) collection of remotely sensed data for study sites; (6) validation of watershed condition using aquatic biota; and (7) analysis of riparian and streams conditions as indicators of watershed condition, and remote sensing as measurement of indicators.

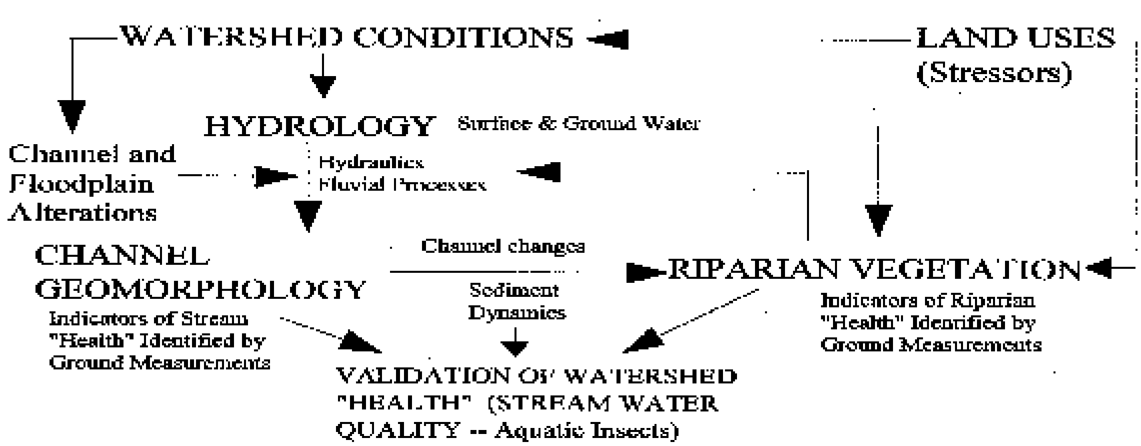

Figure 1. Conceptual model of interrelationships among watershed conditions, stream and riparian indicators, and watershed "health" indicators (aquatic insects), showing drivers and stressors. Remote sensing will be used for watershed characterization and evaluated as a technological tool for measuring ecosystem indicators in the upper Yellowstone River Watershed, the study area of this project which represents a western North American landscape.

Development and Evaluation of Multiscale Mechanistic Indicators of Regional Landscapes

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This project aims to develop suites of ecological indicators that cross spatial scales, mechanistically reflect ecosystem states and processes, are statistically robust, and are applicable across regional landscapes. These indicators also will be based on readily accessible information available in a real-time framework.

The specific objectives are to: (1) develop predictive models that integrate landscape-scale factors with reach-scale physical and chemical stream attributes to quantify key compositional and structural attributes of stream biota and derive ecosystem indicators at multiple spatial scales; (2) evaluate the appropriate scale of terrestrial and aquatic data necessary to resolve regional and local aquatic resource questions; (3) improve the ability to distinguish and quantify natural variation in indicators from that derived from anthropogenic stressors; (4) assess the extent to which regional and local-scale indices reflect fundamental ecosystem processes and structural properties of stream habitats and biota; and (5) quantify confidence limits and evaluate the geographic transferability of regional- and local-scale indicators.

A multitiered sampling and modeling strategy was used to integrate data collected at regional, local, and site scales. These data will be used to identify indicators at each scale that reflect critical ecosystem processes or state variables related to the integrity and sustainability of those ecosystems. These data also will be used to develop and test indicators representing fundamental driving variables and processes at multiple spatial scales, and integrate them into a system for identifying positive or negative trends in ecosystem health.

The basic configuration of the landscape varied substantially between the two primary study regions. The grain of the landscape was finer in Michigan with respect to both surficial geology and land cover characteristics. Watersheds in Minnesota were largely homogeneous with respect to these categories, while Michigan watersheds exhibit more variation.

Strong (albeit seasonal) relationships were found between landscape-scale features and dissolved nutrient concentrations; however, results of nutrient bioassays indicated that nutrients rarely limited primary production, and landscape-scale variables describe only a moderate amount of the variability in net primary production (less than 50%). This trend also was observed in macroinvertebrate communities that were weakly related to landscape-scale attributes but strongly related to local (reach-scale) attributes.

Different parameters of interest are driven by different scales and factors within the landscape. Landscape-scale information can be very useful in predicting differences in stream water nutrient concentrations. In contrast, it is difficult to predict major patterns of macroinvertebrate assemblages or primary production without understanding the reach-scale characteristics that have strongest influence on these variables; however, the reach-scale variables are themselves partially governed by landscape-scale variables. The project will continue by examining other stream assemblages, and employing correlative and mechanistic analyses to identify relationships among ecological indicators operating at local and regional scales. This approach will be directed by the conceptual model presented in Figure 1. A critical part of the next phase will be model evaluation, particularly with respect to placing confidence limits on predictive models. Following this will be an integration of individual models into a larger predictive framework.

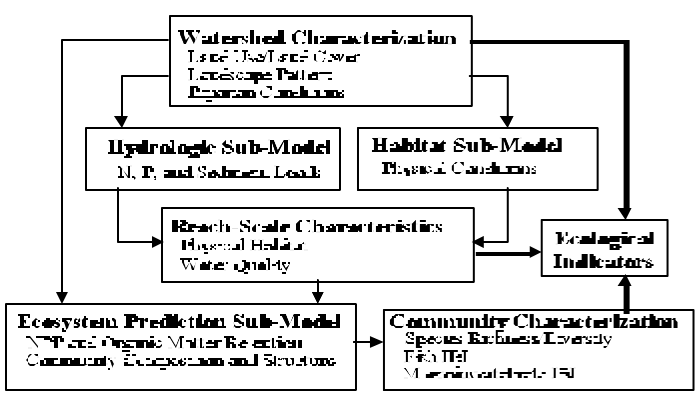

Figure 1. Conceptual model illustrating the organization of mechanistic factors operating at multiple spatial scales. Components summarize the development of predictive models, ecosystem attributes, and fundamental ecosystem processes. Indicators are derived at all spatial scales.

Development and Testing of a Multi-Resource Landscape-Scale Ecological Indicator: Forest Fragmentation, Structure, and Distribution Relative to Topography

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The goal of this project is to understand how water quality and avian habitat quality vary across landscapes as a function of forest distribution and topography (see Figure 1). More specifically, the project seeks to determine whether water and avian habitat quality covary so that they can be simultaneously extrapolated across the Mid-Atlantic Highlands using existing databases of topography, forest cover, and remotely sensed forest structure.

Efforts thus far have focused on: (1) taking an intensive spring/summer field season census of forest interior bird diversity and reproduction; (2) collecting field data on forest structure within intensive field sites; and (3) obtaining and analyzing radar and Landsat satellite imagery. Field sites were chosen to represent the climatic and topographic variation of the Mid-Atlantic Highlands. Twenty 10-ha study sites were est- ablished to equally represent dry and wet topographic positions within the Appalachian Plateau and Ridge-and-Valley provinces. Intensive studies on these sites will form the empirical basis to test the hypotheses con- cerning avian habitat quality, and then provide the stat- istical basis to extrapolate across the Mid-Atlantic High- lands.

During 1999, each study site was intensively surveyed for breeding bird reproductive success. An array of forest structural data also was collected for each site. Preliminary results from the avian survey indicate that forest interior bird reproductive success is higher on the Appalachian Plateau than in the Ridge-and-Valley, and within each province reproductive success is greater at lower slope positions. These results support the hypothesis that reproductive success, and thus habitat quality, is related to topography, with wetter provinces and topographic positions having higher success. Radar images of the intensive field sites are being analyzed to identify predictors of variation in forest structural characteristics. It is planned to collectively use degree of forest fragmentation, structural diversity, and topographic position to map the quality of avian habitat across the Mid-Atlantic Highlands.

Amount of forest cover within multiuse watersheds is negatively correlated with the export of nitrogen from the watershed. Watersheds within the Mid-Atlantic are being analyzed to test the hypothesis that topographic position of forest improves the correlation of forest cover with water quality. If this hypothesis is correct, and preliminary findings regarding the effect of topography on avian habitat quality withstand further statistical scrutiny, then forest cover and forest spatial distribution will to- gether form a powerful indicator of both water and avian habitat quality that can be mapped over large areas from existing databases.

Efforts currently are focused on three tasks. First, the relationship between forest structure and characteristics of remotely sensed radar images are being analyzed. This relationship will help refine predictions of avian habitat quality. Second, processing of Landsat land cover data and integration of forest cover with topographical data are aimed at developing simple measures that describe the relationship between forest spatial pattern and topography. These measures will be calculated at the watershed level and correlated with water quality parameters. Finally, a second field season to extend the understanding of spatial variation in avian reproduction and forest structure is being prepared.

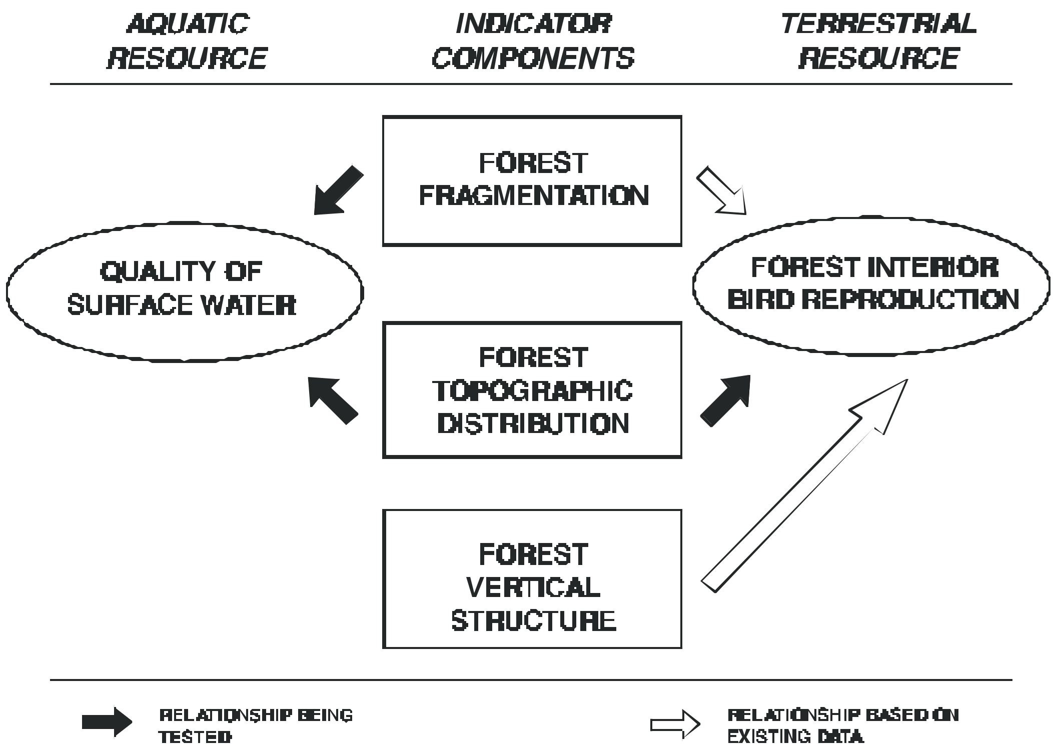

Figure 1. Multiple characteristics of forests in the Mid-Atlantic are being examined as indicators of both surface water quality and forest 0interior bird reproductive success. Hypothesized relationships (dark arrows) are being tested through either reanalysis of existing databases or field studies. In contrast, the effect of fragmentation on forest-interior bird reproduction is already quantified (white arrow). As indicator components, both forest fragmentation and forest topographic distribution are readily mapped from existing databases. The importance of forest vertical structure for forest bird diversity (white arrow) is well-known, but the relationship is difficult to extrapolate from existing data. Thus, remotely sensed radar imagery is being examined for extrapolating forest structure over large areas.

Ecological Indicators for Large River-Floodplain Landscapes

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Identifying landscape indicators that are well-correlated with specific ecological functions remains a crucial research need. Ecological indicators (population, community, ecosystem, and landscape) are being developed and tested along reaches of the Wisconsin River. A conceptual framework of this research is presented in Figure 1. Two questions are being addressed: (1) Which landscape metrics are most useful for monitoring population, community, and ecosystem processes in large river-floodplain landscapes? (2) What are the constraints on extrapolating relationships between landscape metrics and ecological processes in large river-floodplain landscapes?

Spatially extensive field sampling is being combined with landscape indicators to predict ecological variables over broad scales. Field sampling (n = 220 plots) was conducted during MayBJuly of 1999 within six 10B15 km study reaches. Vegetation (trees, shrubs, saplings, tree seedlings, and herbaceous cover), soils, and litter accumulation were sampled within each 10 x 20 m plot, and soil cores were obtained to estimate potential denitrification. A census of birds was taken twice at each plot using 8-minute point counts.

Preliminary results suggest that microbiological indices can be explained by ecological processes that operate at different spatial scales. At the landscape scale, microbial activity appears to be influenced by land cover, whereas the presence of levees may be a more important determinant at the scale of individual transects. At finer scales, different enzyme activities were found in soils under different types of trees. Microbial processes are important for the ecological integrity of floodplain ecosystems; these results suggest that changes in land use patterns, levee construction or removal, or tree species can affect these processes. Initial analyses of 4,870 bird observations (70 species) revealed similarity among the bird communities associated with each study reach, with an average of 70B80 percent of the species shared between reaches on a pairwise basis. However, numbers of individual birds were quite variable, with the three southernmost reaches containing approximately 30 percent more birds than the three northernmost reaches (e.g., the number of woodpecker species and individuals decreased from south to north). Some species generally associated with northern Wisconsin habitats were only detected at the southern-most reaches, suggesting a strong influence of land use or river flow modification. From a total of 39 tree species encountered, some floodplain species (e.g., *Acer-saccharinum*, *Fraxinus pennsylvanica*) were more abundant in the less human-modified southern reaches, whereas some upland species (*Populus tremuloides*, *Quercus velutina*) were more abundant in the more modified northern reaches.

During the coming year, the following activities are planned: (1) analyzing the field data obtained in six river reaches during the 1999 field season (bird censuses, vegetation sampling, denitrification, and microbial activity) in relation to landscape indicators; (2) interpreting historical and recent aerial photography for study reaches in the 1930s, 1960s, and 1990s; (3) conducting the second season of field sampling; and (4) beginning to address Question 2 by predicting and testing ecological indicators in new study landscapes, determining whether there are thresholds in landscape pattern beyond which ecological processes change qualitatively, and assessing the sensitivity of ecological indicators to landscape changes.

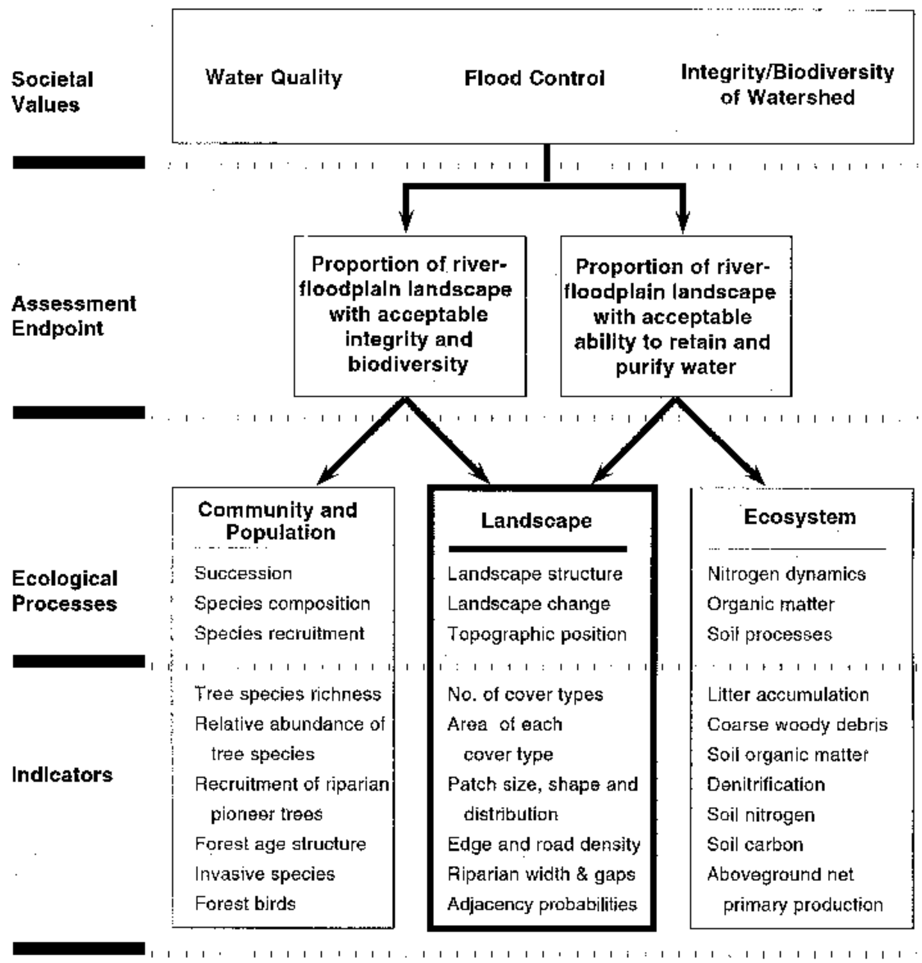

Figure 1. Conceptual framework for research in the Wisconsin River floodplain landscape showing relationships among societal values, assessment endpoints, ecological processes, and indicators.

Characterization of the Ecological Integrity of Commercially Grazed Rangelands Using Remote Sensing-Based Ecological Indicators

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The purpose of this retrospective study was to characterize the ecological integrity of a semi-arid landscape using satellite remote sensing and Geographic Information System (GIS) technologies.

The ecological basis of the research was the transition threshold hypothesis. A transition threshold is defined as the boundary in space and time between two seral states (e.g., the change of a grassland to woodland). A transition is the process (e.g., climate or grazing intensity) that brings about a change in state. An ecological risk assessment framework was used to determine the assessment endpoints of rangeland degradation as a change in plant growth form composition, a decrease in plant productivity, a reduction in soil quality, accelerated soil erosion, and a change in landscape composition and pattern.

Measurement endpoints, which relate to these five assessment endpoints, were derived from 27 years (1972B1998) of wet and dry season Landsat satellite imagery and 2 years of annual scenes (1985 and 1986). Measurement endpoints were the soil-adjusted vegetation index (SAVI), a surrogate for vegetation parameters and soil quality; the soil stability index (SSI), a surrogate for soil erosion; and thematic maps, where landscape met- rics were used to measure changes in landscape structure and configuration. The study site was 54,000 ha of the sagebrush steppe portion of the 108-year-old mixed grazing operation (wildlife and beef cattle) Deseret Land & Livestock Ranch (DLL) in northeastern Utah.

A GIS database of site biological, physical, and administrative characteristics, including historical and current ranch management records, was developed. The data were analyzed at the waterpoint, paddock, and landscape scales using graphical timeseries and multiple regression analyses. Sagebrush steppe is a two-phase mosaic of shifting dominance between shrubland and grassland due to herbivory, fire, and climate change. It was hypothesized that at the landscape-level, SAVI would respond to climate change (i.e., La Niña/El Niño). It also was hypothesized that at the community level, coincident periods of drought and intense grazing would lead to a landscape dominated by shrubland and increased erosion.

Two validation studies were conducted that confirmed that SAVI, an indicator of phenology, was consistent with field data and that interannual SAVI can discriminate between grazing effects. It was found that at the landscape-scale, the ranch had become more fragmented and was an unstable limit cycle responding to El Niño (wetting) and La Niña (drying), respectively.

At the community-level, shrubland dominated since 1974, grassland was declining, and there is no indication of increased erosion. Three-dimensional surface analysis suggests that in terms of SAVI response, increased grazing and climate are factors in shrub increase, but grazing appears to be the main factor in grass cover decline (see Figure 1).

Ecosystem measures such as SAVI can miss fundamental changes in composition that are important to ranch management. Limited field datasets from federal agencies had assessed the ranch in good condition. However, these datasets were temporally limited (6 years versus the 27 years of imagery), inconsistent, or measured inappropriate indicators. The following tasks remain to be completed: validation of the SSI, accuracy assessment of dry season thematic maps, comparison of metrics within 20 paddocks, and completion of the timeseries piosphere study.

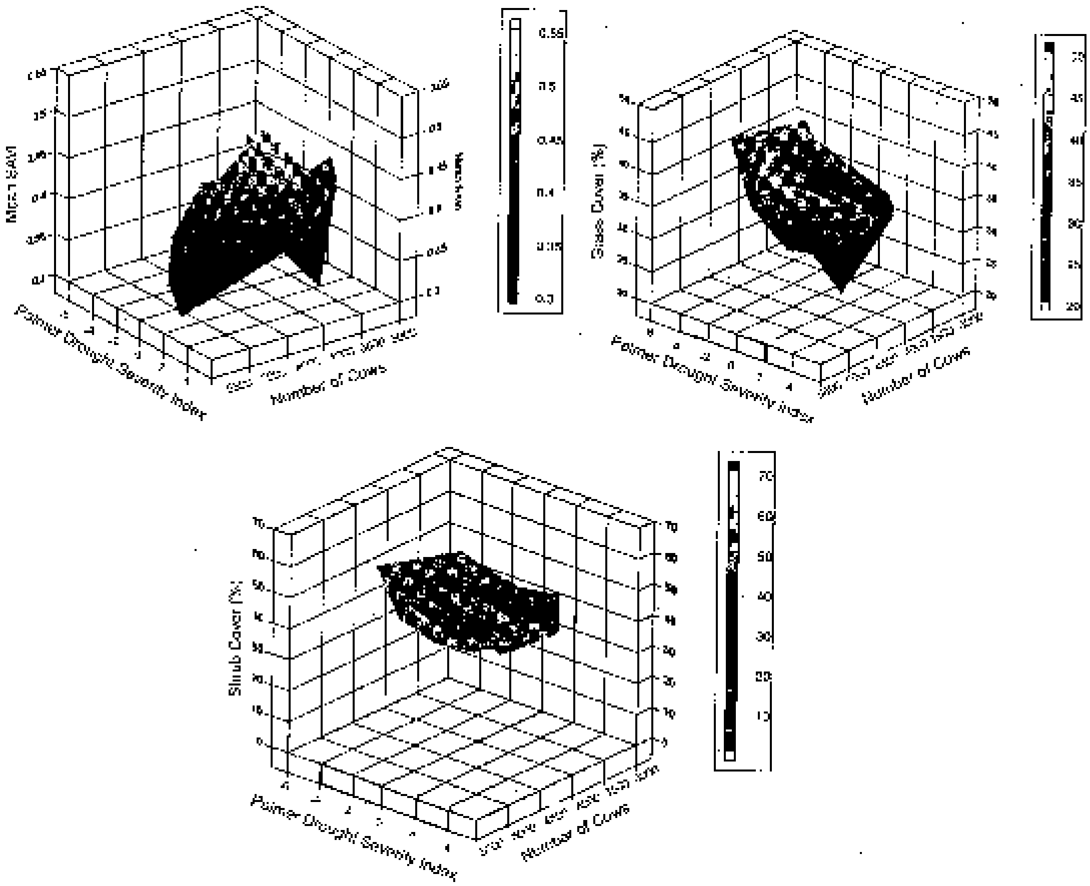

Figure 1. The top left figure suggests a fold catastrophe and is the relationship between the independent factors: number of cows and the Utah Region 5 Palmer Drought Severity Index (PDSI), and the dependant factor: landscape-level lagged wet season mean soil-adjusted vegetation index (SAVI). PDSI < 0 indicates drought conditions. The same independent factors are related to community measures of grass cover (top right) and shrub cover (bottom center).

Section 3.

Aquatic Indicators

Using Bioindicators To Develop a Calibrated Index of Regional Ecological Integrity for Forested Headwater Ecosystems

Robert P. Brooks [1], Robert S. Mulvihill [2], Terry Master [3], and Timothy J. O'Connell [1]

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The objective of this project is to develop a regional index of ecological integrity for forested headwaters in the Mid-Atlantic States, with particular emphasis on the Louisiana waterthrush (*Seiurus motacilla*), referred to as "LOWA." LOWA are forest-breeding warblers that also are riparian obligate. They are dependent both on large patches of mature forest and high-quality instream conditions. The index is intended to integrate and calibrate existing indicators of integrity that address stream health and upland forest condition individually, and at different scales.

Beginning in 1998, LOWA were monitored on 23 forested headwater streams in three physiographic provinces of Pennsylvania (see Figure 1). These streams represent either: (1) high water quality in large patches of interior forest; (2) high water quality in a relatively fragmented forest setting; or (3) low water quality in large patches of interior forest. Established indices of biotic integrity (IBIs) will be applied to the same streams where LOWA reproductive success already has been measured. The degree to which attributes of LOWA re- productive success can link small scale IBIs, such as macroinvertebrate IBIs, to landscape scale IBIs (i.e., the Bird Community Index) is being investigated.

Early analysis confirms that LOWA population variables correspond with large-scale condition as defined by the Bird Community Index. Correlates to instream condition are more complex to generate, and the results are pending. For example, the 1998 macroinvertebrate characterization for the Central study area included 9,862 individuals in 180 genera, 83 families, 18 orders, 9 classes, and 4 phyla.

In 1999, unusually high nest predation rates in all three study areas occurred. Nest predation rates were elevated on both reference and impacted streams. In the Central study area, the evidence suggests that mink (*Mustela vison*) are the culprit predators. It is speculated that mink have become locally (and perhaps regionally) abundant following a succession of mild winters.

Preliminary results of this research already have displayed the importance of "offstream" wetland seeps and springs as vital to the foraging efficiency of LOWA nesting on acidified streams. On acidified streams with abundant offstream foraging opportunities, LOWA are reproducing at a rate comparable to that observed in the most "pristine" streams.

This work also highlights the need to conduct research at large scales and across physiographic boundaries. For example, had all of the sites been located in one region in 1999, it would not be possible to demonstrate that the elevated nest predation observed was a widespread phenomenon. The 2000 field season is the third and final season on this project. In the upcoming year, the IBI will be developed, and its relationship to the land cover pattern and the habitat attributes that have been measured from the ground will be examined.

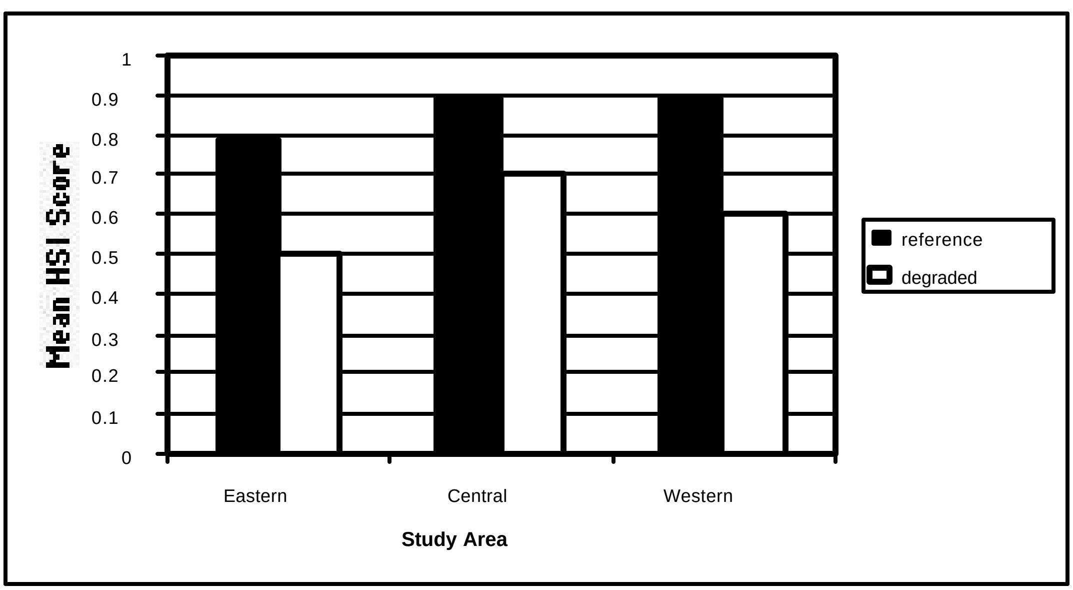

Figure 1. Mean habitat suitability index (HSI) scores for Louisiana Waterthrush on reference and degraded streams in three physiographic provinces of Pennsylvania.

Stream Plethodontid Assemblage Response (SPAR) Index: Development, Application, and Verification in the MAHA

Robert P. Brooks and Gian L. Rocco

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Amphibians generally are considered to be valuable response indicators (Vitt et al. 1991, Dunson et al. 1992). In small lotic systems that largely exclude predacious fish, small, lungless salamanders of the Family *Plethodontidae*, can exist in surprisingly high numbers, functioning as both invertebrate predator and vertebrate prey.

By virtue of their diverse and complex life histories and abundant and stable populations, stream salamanders offer the opportunity of serving as important ecological indicators for the assessment of headwaters, especially where traditional indicator species assemblages (fish, macroinvertebrates) may be poorly developed or absent.

In a study of 14 Central Appalachians headwaters in Pennsylvania, Rocco and Brooks (in preparation) document significant stream plethodontid responses to acidified and degraded stream conditions, attesting to their potential as bioindicators. Various useful metrics, based on species composition, abundance, and lifestage, also are proposed. In consideration of the effectiveness, relative ease, and low cost of the sampling technique, stream plethodontid assemblage responses (SPAR), used alone or in combination with other small stream assessment criteria, promises to be an effective assessment and monitoring tool for headwaters.

At the moment, however, the ability to interpret SPAR in similarly impaired streams across a larger geographical area is hampered by the relatively small area sampled. A reference base ideally would be geographically widespread, represent the entire range of conditions, and consist of a representative and unbiased sample of the population of headwaters in the region of interest.

To address this need, the primary objectives of this research are to: (1) describe the range and variability of SPAR across commonly encountered gradients of anthropogenic degradation (e.g., stream acidification, forest and riparian corridor fragmentation and degradation, pollution) in the Mid-Atlantic Highlands Area (MAHA); (2) develop and adjust SPAR for use in MAHA headwaters; and (3) evaluate the reliability and resolution of SPAR by application and verification.

The development, application, and verification of SPAR within MAHA will entail a two-phase process. Phase I aims to document and study plethodontid assemblage responses to as many different stream conditions as possible, across a large geographical area. This data- set will enable the development of the SPAR index for the MAHA. In Phase II, a random set of headwaters of unknown condition will be sampled and its impairment determined by the SPAR index. Once applied, an independent assessment of the stream by traditional methods (macroinvertebrates, water quality, surrounding landscape) will allow the verification of the reliability and consistency of the index, and if necessary, its calibration. The use of volunteers during the second phase will permit evaluation of the method for nonspecialists and long-term amphibian monitoring efforts.

The response composition of free-ranging, naturally occurring stream salamander populations in degraded and nondegraded watersheds will be studied by intensive sampling. Relevant abiotic and biotic variables at the plot, stream reach, and watershed will be measured and correlated to SPAR metrics.

The expected findings include: (1) description of stream salamander assemblage response along disturbance and pollution gradients commonly encountered in the MAHA Region; (2) identification of species most responsive to the environmental degradation investigated; (3) recommendations on the use of stream salamander assemblages in the assessment of headwaters; and (4) development and testing of stream salamander metrics that could be implemented separately or in con- junction with other criteria when performing small stream assessments.

Ultimately, it is hoped that this work leads to the improvement of existing headwater assessment protocols for the MAHA by the addition of a bioindicator that is abundant, widespread, ecologically important to several trophic levels, and interfaces between aquatic and terrestrial components of riparian areas.

Soil Enzyme Stability as an Ecosystem Indicator

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Sustainability assessment of ecosystems to assist land managers and policymakers in promoting long-term sustainability is a national priority, but quantifying environmental sustainability remains an elusive goal. One approach is to use the soil as an indicator of ecosystem "health."

Soil enzyme activity assays are advantageous as potential indicators because they are: (1) operationally practical; (2) sensitive integrative "biological fingerprints" of past soil management; and (3) apparently related to soil aggregation, linking enzymes with soil tilth.

In the first year of the project, the initial screening of three promising soil enzyme assays as indicators took place at three experimental sites in Oregon (located in divergent forest and agroecosystems) where detailed management history is known and soils vary widely in soil "health" (i.e., biological activity and organic matter content) because of past soil management. This included refinement of sample handling and laboratory protocols to increase sensitivity and reproducibility of the indicators. Samples were taken in early May, June, and September of 1999 to assess the seasonal temporal variability of these indicators. It was found that the forest soils generally have much higher activities than agricultural soils. This makes sense, because agricultural soils are regularly disturbed. Forest soils, even with recent logging, would only have had surface disturbance once, and even then, there would not be tillage. There is a very consistent pattern in the agricultural soils when within-site treatment effects are examined, in that those receiving greater C inputs (as cover cropping, manure, and green manure) have higher activities than the plots receiving lower C inputs.

Across agricultural soils, the biological measurements such as ergosterol, fungi biomass, and biomass C are very consistent with soil enzyme activities. Bacterial counts, however, show very few significant differences as a function of soil management in either forest or agricultural soils. This suggests that soil enzyme activities are good indicators of the soil biological component and are reflecting the differences due to fungal biomass.

The second year of the project will involve expanding to a wider range of soil types and soil management systems. A detailed data analysis will be performed and the potential for relative soil quality indicators that are independent of soil type will be investigated.

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

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The goal of this project is to evaluate a set of potential indicators of ecosystem condition for the San Francisco Estuary (SFE). The objectives of the research and field effort are to: (1) devise indicators of ecosystem condition; (2) investigate their relationships to stressors, including salinity, turbidity, nutrients, and introduced species; and (3) assess the utility of these indicators.

This research is being conducted on the open-water ecosystem of the SFE, including the portion of the landscape from freshwater to marine ecosystems. These potential indicators represent key population and individ- ual-level processes in a variety of trophic levels. The benthic community structure also is being examined for possible indicators of stress. All indicators are relatively simple to measure, significant to population ecology, expected to be sensitive to stress levels, and likely to be transportable to other aquatic habitats.

These indicators are: (1) nutrient status and productivity performance of phytoplankton; (2) relative con- tribution of diatoms to algal biomass and productivity; (3) reproductive rate of common copepod (zooplankton) species; (4) condition indices of larval herring; (5) con- dition indices of the dominant benthic bivalves; (6) sev- eral measures of benthic community structure; and
(7) expression of stress proteins in the indicator animal species.

This project is very new, funded in October 1999, so only preliminary findings are available. The field work has been initiated with monthly cruises to three sites in the SFE. Measurements of temperature, salinity, nutrients, and algal biomass as chlorophyll, particulate nitrogen, and particulate carbon were made. NO_3 and NH_4 uptake (fractionated by size class) with samples incubated under natural turbidity and clarified water conditions were obtained during each cruise.

Comparison nutrients and chlorophyll concentrations from December 1999 with previous observations in Central Bay suggest relatively low values, probably resulting from an exceptionally dry fall. Comparison of nutrient and chlorophyll concentrations from December 1999 in Central Bay with the two new up-estuary stations, San Pablo and Suisun Bays, shows nutrients, especially $Si(OH)_4$, increasing and chlorophyll decreasing in the freshwater direction. Copepod (zooplankton) reproductive rates (see Figure 1) were measured by collecting with gentle net tows, diluting the catch in surface bay water, and incubating individual females for 24 hours in 125 mL polycarbonate bottles filled with bay water with eggs strained out.

The first herring larval collections have just been made at the three sampling stations. The contents of each tow were split, with half preserved in formalin and half in ethanol for measurements of body characteristics including length, weight, head width, and total eye diameter.

The ethanol-preserved samples are stored for future studies outside the scope of this project. Sites have been selected for the benthos project at each of the three study areas and collections made for condition and glycogen analyses. Work on stress-induced proteins has been impacted by the departure of the post-doc involved, who has taken a faculty position at another institution. Planning for the prosecution of this aspect of the project is underway with the possibility of arrangements for collections to be made at our institution and the analyses at the individual's new post. Other options are being explored as well.

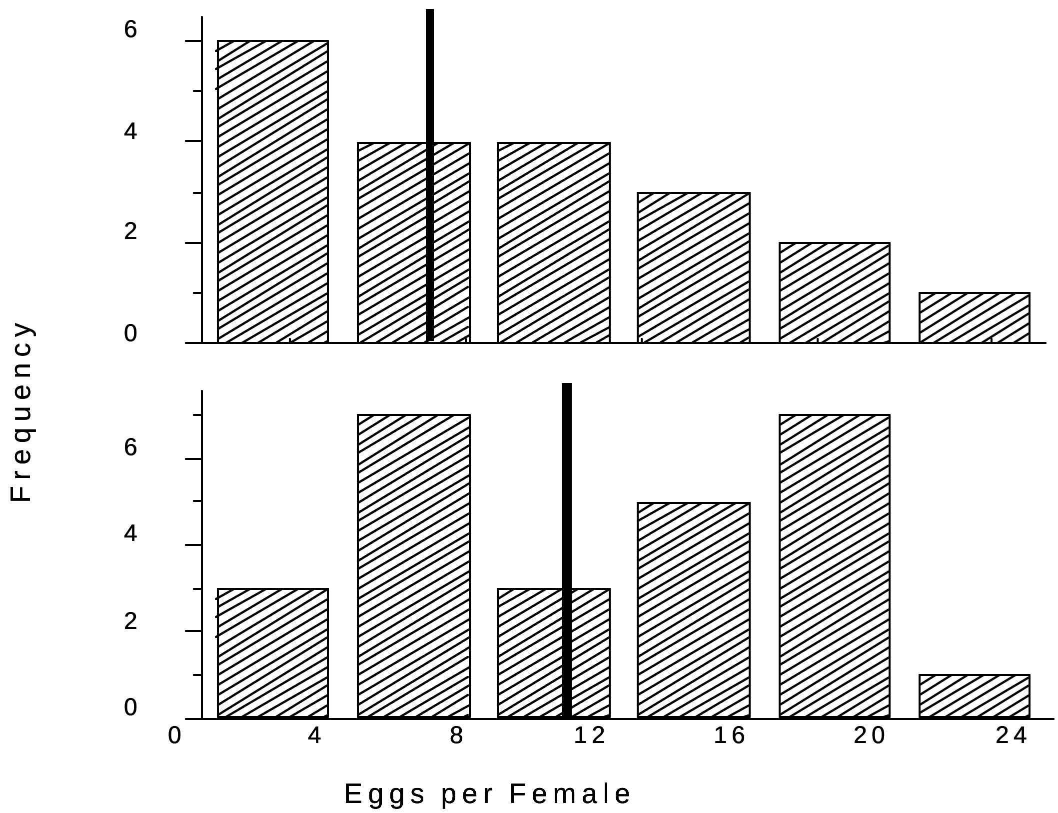

Figure 1. Histograms of egg production rates from copepods (*Acartia*) at Stations 2 (top) and 3 (bottom) during the first cruise. A different species, not shown here, was most abundant at Station 1. Vertical lines indicate medians. Differences in egg production rates such as these will be analyzed for relationships with salinity, temperature, chlorophyll, and other environmental variables.

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

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Coastal eutrophication that results from increases in nutrient supply is a critical problem worldwide, causing major changes in marine populations and com- munities. The overall goal of this project is to develop an indicator that quantifies nutrient supply to tropical and temperate marine ecosystems using the tissue nitrogen (N) and phosphorus (P) content of macroalgae. To com- plement the present suite of indicators used to measure eutrophication, this indicator is targeted to be especially useful in systems where nutrients are supplied in pulses or those where nonpoint sources of nutrients such as groundwater or fluxes from the benthos are important.

The five specific objectives are to: (1) continue to identify and test potential species for use as indicators; (2) establish relationships between timing and magnitude of nutrient supply and accumulation of N and P in algal tissue; (3) establish quantitative relationships between environmental conditions, N and P supply, and tissue N and P; (4) develop a numerical simulation model based on experimental results that may be used as a "standard curve" for the indicator to hindcast nutrient supply in the field; and (5) field test the indicator.

Macroalgae from two regions in the Caribbean (Southwest Puerto Rico and Caribbean Panamá) and two in the Eastern Tropical Pacific (Gulfs of Panamá and Chiriquí, Panamá) were collected to determine if the nitrogen (N) and phosphorus (P) content of their tissues reflected differences in nutrient availability in these systems, making them good indicator species. Large differences were found in the tissue P content of *Hypnea musciformis*, an exotic red alga that has invaded most tropical areas. *H. musciformis* from the Caribbean had one-half the amount of P than in the ETP. There also were significant differences in N content, but these were of a lower magnitude. Similarly, *Dictyota spp.* in the Carribean had only one-half the tissue P content found in the ETP; however, tissue N of *Dictyota spp.* was not different among these ocean areas, suggest ting it may not be useful as an indicator of P (see Figure 1).

The differences in P content in algal tissue between the Caribbean and ETP may reflect the greater percentage of P-adsorbing carbonates in Caribbean sediments. Tissue P content also was significantly different among regions: Gulf of Panamá > Gulf of Chiriquí > Caribbean Panamá > Puerto Rico. This contrasts with tissue N where the pattern was Gulf of Panamá > Gulf of Chiriquí = Puerto Rico > Caribbean Panamá. While greater N and P in the Gulf of Panamá may be attributed to seasonal upwelling, the larger N content in Puerto Rico compared to Caribbean Panamá may be due to anthropogenic inputs.

The tissue N and P content of macroalgae may provide useful insight into nutrient availability in tropical systems. Furthermore, cosmopolitan species such as *H. musciformis* may be especially good indicators of nutrient regime.

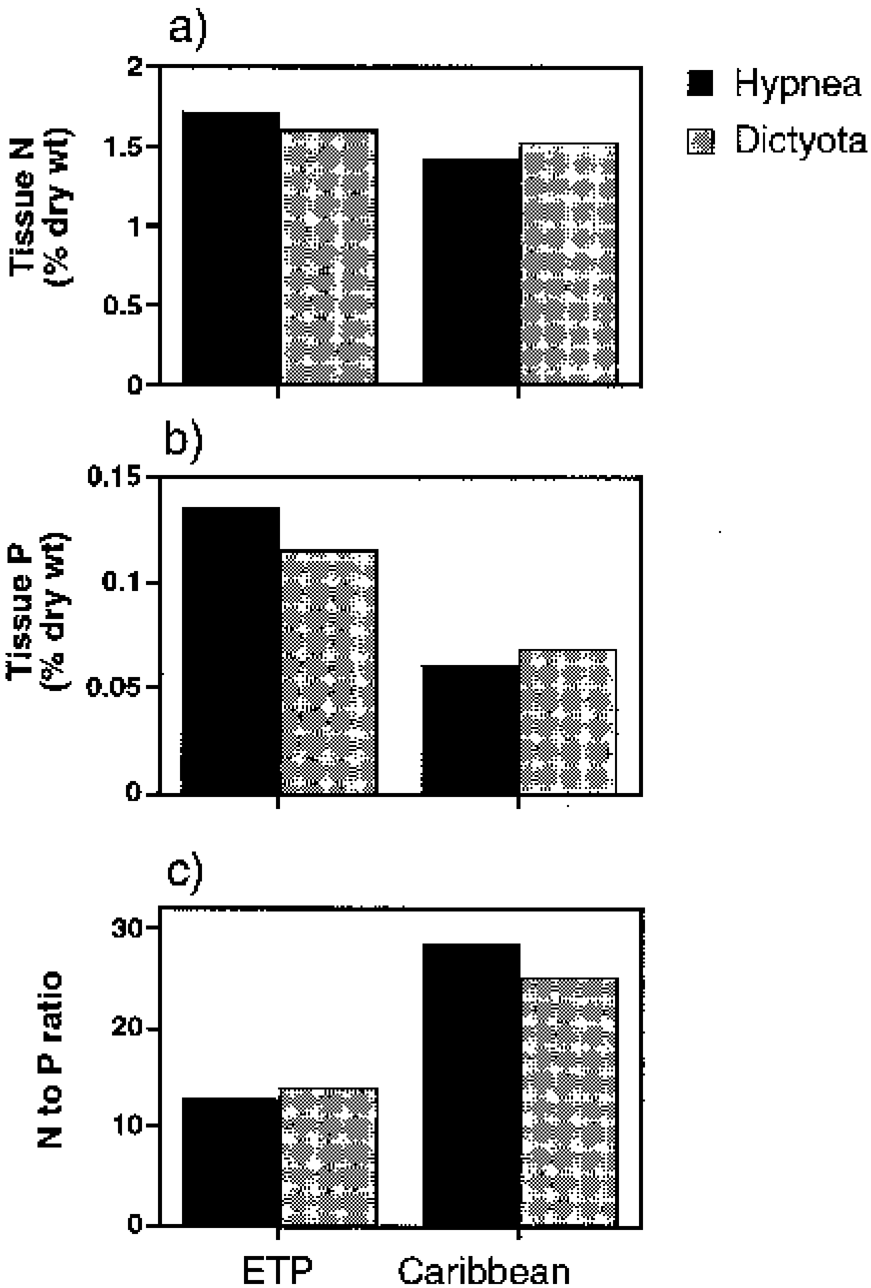

Figure 1. Differences were found between *Hypnea musciformis* and *Dictyota spp.* sampled from the Eastern Tropical Pacific (ETP) and the Caribbean in terms of: a) tissue N, b) tissue P, and c) N to P ratio.

Microbial Indicators of Biological Integrity and Nutrient Stress for Aquatic Systems

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This project explores connections between biological integrity and nutrient supply and limitation, focusing on the microbial component of lakes and reservoirs. Several hypotheses are being tested concerning patterns and correlations among indicators of biological integrity that are based on nutrients (i.e., carbon, nitrogen, phosphorus, and trace minerals) and microbial communities. The temporal and interregional variability of these indicators also are being assessed.

Two reservoirs in north Texas are being examined year-round, and two lakes in the Experimental Lakes Area (Ontario, Canada) are being studied during the ice-free growing season (see Figure 1). One of the latter lakes is pristine, while the other has been experimentally eutrophied with phosphorus additions. Much of this work involves measures of seston element composition and measures of changes in microbial communities following experimental manipulation of nutrients (bioassays).

Seasonal patterns have been analyzed and comparisons made among lakes for the first year of sampling. It was hypothesized that indicators based on the C:N:P composition of seston would agree with growth responses in bioassay experiments.

In Texas lakes, temporal correlations between indicators of P-limitation and seston C:P and N:P ratios were all in the hypothesized directions. Correlations between indicators of N-limitation often were opposite to predictions. Indicators of algal and bacterial nutrient limitation were positively correlated in Texas lakes, as hypothesized. Bioassay experiments in Canadian lakes were generally uninformative, largely due to high variability and a number of unexpected negative effects from nutrient enrichment.

Physiological profiles of bacterial communities based on the use of 95 carbon substrates support a hypothesis that bacterial community structure shifts in response to seasonal changes in nutrient limitation. Principal components analyses reveal relatively high response to amino and carboxylic acids in cool seasons, and to carbohydrates in warm seasons. The amplitude of this pattern was stronger in the experimentally eutrophied Canadian lake than in the oligotrophic reference lake.

These results address the relative utility of bioassay experiments and measures of seston C:N:P composition as indicators of nutrient limitation. The two approaches perform more consistently when P-limitation is diagnosed than when N-limitation is diagnosed. Indicators based on seston C:N:P ratios appear to be more widely applicable than those based on bioassay experiments, because the latter performed poorly in Canadian lakes. On the other hand, where applicable (in Texas lakes), bioassay experiments yield insights to microbial populations that may be useful in modeling and lake management. Physiological profiling of bacteria suggests seasonal shifts in use of carbon substrates, which may be linked to seasonal changes known for other limnetic organisms. These seasonal patterns appear to be more pronounced in eutrophic than in oligotrophic lakes, and may help characterize lakes of different trophic status.

As the additional data from Years 2 and 3 are analyzed, interannual variations in the indicators and the patterns of correlation summarized above will be assessed. To obtain greater insight from indicators based on seston composition, size-fractionated sampling of seston in Canada during 1999 was instituted, though data are not yet completely analyzed.

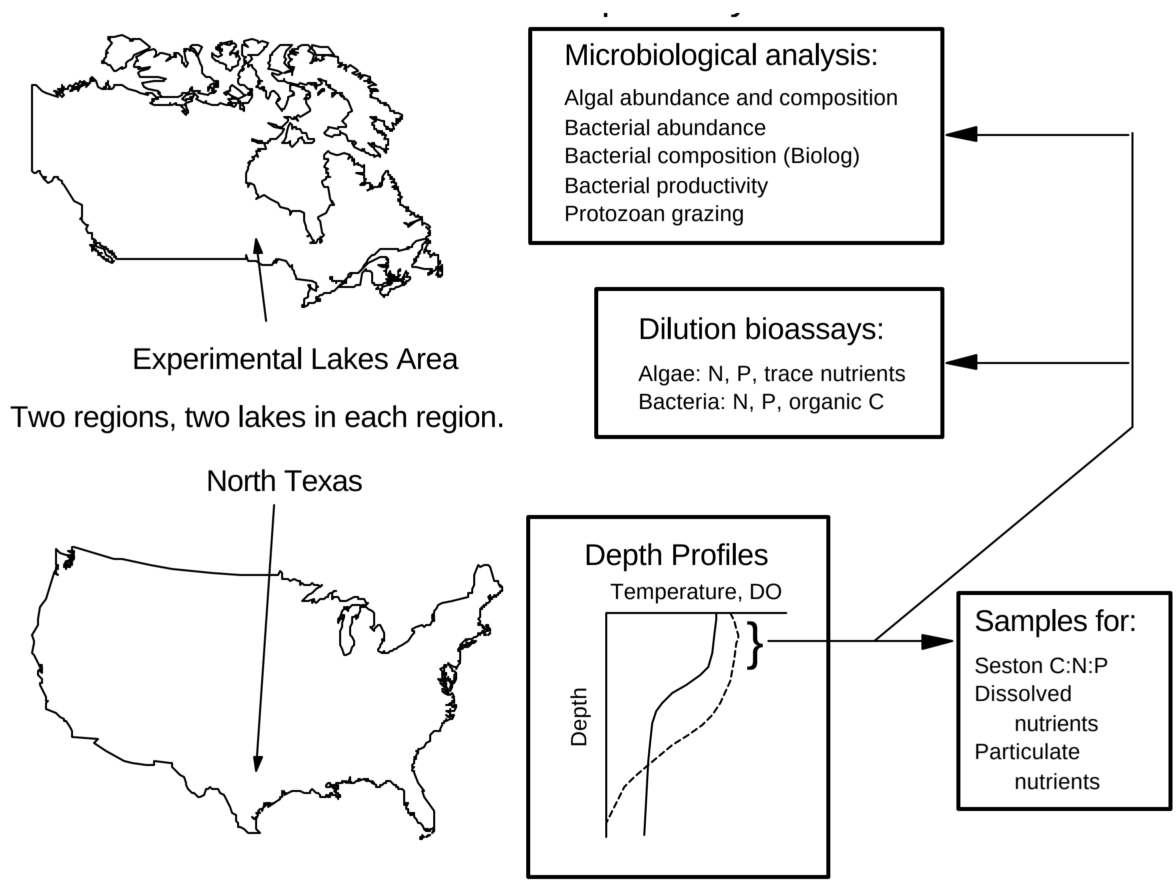

Figure 1. Microbial indicators of biological integrity and nutrient stress for aquatic systems.

Foraminifera as Ecosystem Indicators: Phase 1. A Marine Benthic Perturbation Index; Phase 2. Bioassay Protocols

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The objectives of this project are to develop procedures for the routine use of foraminifera as indicators of biological integrity in field and laboratory applications. In Phase 1, sediment cores are being used to test an index for assessing perturbations of marine ben- thic ecosystems based on changes in key taxa of foraminifera. In Phase 2, protocols for assessing stress responses in foraminifera induced by elevated temperature and visible and ultraviolet radiation are being developed.

A prototype index to quantify change in benthic ecosystems that can be applied to historical, sediment-core, and surface-sediment datasets has been developed. The model ranks relative abundances of key foraminiferal taxa and morphogroups, and total abundances, generating an index value when foraminiferal assemblages are compared temporally or spatially. Tests of the index using sediment cores from Florida Keys reefs and Tampa Bay are in progress.

Bleaching has been observed in populations of *Amphistegina* in all subtropical oceans since 1991. Field sampling in the Florida Keys in 1997B1999 revealed continued stress, with a significant increase in the seasonal bleaching peak in 1998. Stress symptoms in *Amphistegina* can be documented using visual, physiological, and cytological techniques. Visual rankings, which can be verified by cytological analyses using electron microscopy and image analysis, remain the most cost-effective method of documenting stress responses. Protocols for measuring ATP to determine viability and metabolic activities were adapted and results compared with visual and cytological assessments. High variability and standardization limitations indicate that ATP analysis is not cost-effective.

Exposure to elevated intensities of photosynthetically active radiation (400B700 nm), alone or in combination with added UVB (280B320 nm), induced symbiont loss in *A. gibbosa* similar to that seen in field populations. Comparison of responses to two spectrally different visible light sources revealed that, although experimental intensities of blue and white light were the same in photons (7.76 $\mu E/m^2s$), the higher-energy blue light (7.21 x 10^{-5} W/m^2) both induced faster growth and more symbiont loss than the lower-energy white light (5.69 x 10^{-5} W/m^2) (see Figure 1).

Mass bleaching devastated corals nearly worldwide in 1998. Bleaching in *A. gibbosa* in the Florida Keys also peaked in the summer of 1998. A key difference between bleaching in corals and foraminifers is that coral bleaching correlates most consistently with elevated sea surface temperatures, which can induce photoinhibitory stress, while bleaching in *Amphistegina* appears to be directly associated with photoinhibitory stress. Other researchers using molecular biomarkers have found evidence of stress in corals 3 months prior to visible bleaching, indicating that corals may be responding to the same stresses that are bleaching the foraminifera. Recognizing similarities and differences between these two physiologically similar, though taxonomically different symbiotic systems, should facilitate understanding the global decline of coral reefs. Visual techniques remain the most dependable and cost-effective methods of documenting and analyzing bleaching symptoms and shell breakage in *Amphistegina spp.*

Goals for the year 2000 are to standardize visual protocols using digital photography and image analysis, while continuing to document responses to photic and temperature stress. Contacts also have been established with scientists applying molecular biomarker protocols to a variety of reef organisms, with the goal of determining whether these techniques can be applied to *Amphistegina*. The results of the core analyses and stess experiments will be prepared for publication.

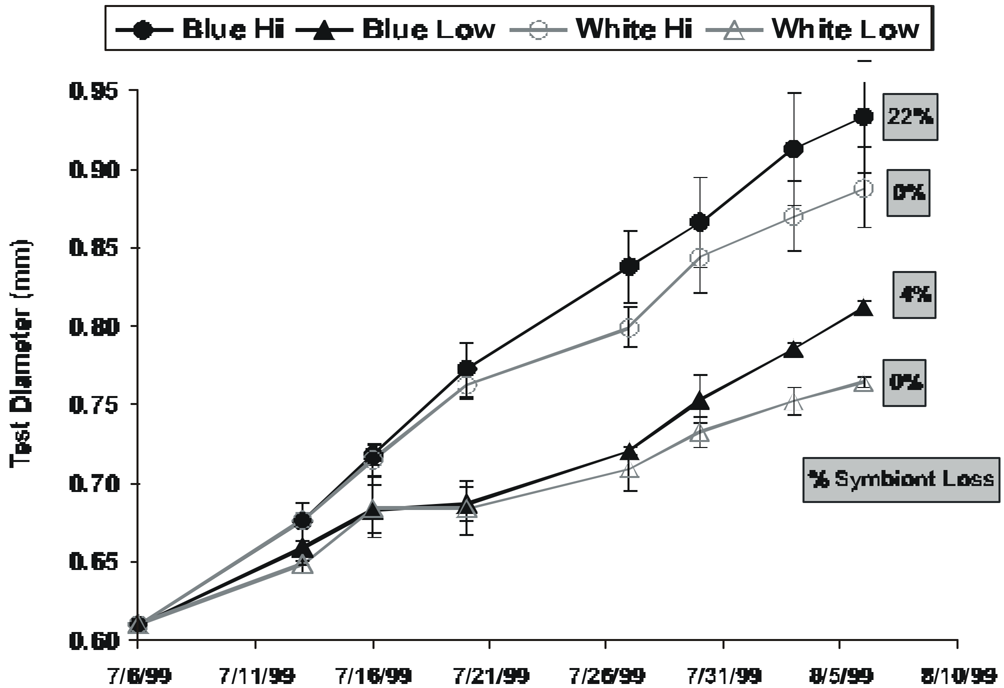

Figure 1. Influence of spectral quality on growth and symbiont loss. Mean diameter of 45 *A. gibbosa* individuals exposed to two intensities (7.76 and 2.81 μE/m^2) of two spectrally different light sources (blue lamp-photons are concentrated in the higher energy portion of the electromagnetic spectrum, white lamp-photons are more concentrated in the lower energy portion). Shaded boxes show the percentages of individuals showing visible symbiont loss after 4 weeks (grown in 10 cm petri dishes at 25^o C in nutrient-enriched Erdschreiber medium).

Biogeochemical Indicators of Watershed Integrity and Wetland Eutrophication

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The central hypothesis of this research is that rates of biogeochemical cycling of C, N, and P in wetlands can be used to indicate the ecological integrity of wet- lands, and that the concentrations of certain forms of these elements can accurately predict the rates of ecolog- ically important processes.

The objectives of this research are to: (1) identify the key biogeochemical processes impacted by nutrient loading and measure the rates of these processes along the nutrient gradient; (2) develop relationships between a "process" and its related, easily measurable "indicator"; (3) determine the spatial and temporal distribution of easily measurable indicators for a test wetland ecosystem; (4) determine the spatial variations in biogeochemical processes, and develop spatial maps for various processes to determine the extent of impact and risk assessment; and (5) validate the predictability of empirical relationships by making independent measurements of biogeochemical processes in different wetland ecosystems. It is planned to test the hypotheses presented above in the Blue Cypress Marsh Conservation Area (BCMCA), located within Upper St. Johns River Basin, FL. Some areas of the BCMCA have been impacted over the years as a result of nutrient loading from adja- cent uplands, resulting in distinct nutrient and vegetation gradients. The BCMCA provides the benefit of established gradients of high-nutrient (impacted) to low-nutrient systems (unimpacted), to test the hypotheses of this proposal.

The research will focus on key biogeochemical processes and microbial communities regulating the fate of nutrients in the soil and overlying litter layer at various spatial and temporal scales. This will be accomplished by conducting a series of laboratory and field experiments.

Statistical and modeling tools will be used for analysis and synthesis of the biogeochemical process and indicator data, including: (1) descriptive statistics related to frequency distribution and central tendency of indicator values to make comparisons between areas of low and high anthropogenic impact; (2) multivariate analyses to evaluate the relationship between biogeochemical indicators, biogeochemical processes, and ecological integrity; (3) geostatistical analysis to compare the characteristic spatial patterns and structure of biogeochemical indicators; and (4) temporal analysis of indicators in low- and high-impact areas.

The first stage of this research is expected to result in the confirmation of the utility of simple chemical measures as reliable predictors of rates of biogeochemical processes. The study also will produce statistical models describing these relationships.

Such models would provide valuable research and management tools. Because these measures would be rapid and inexpensive, investigations of biogeochemical processes could be expanded providing a better understanding of these fundamental mechanisms of ecosystems.

Development and Evaluation of Ecosystem Indicators for Urbanizing Midwestern Watersheds

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Urbanization is thought to have negative impacts on stream ecosystems, and yet the actual causal relationships between land use change and stream community response have not been well studied. This project focuses on the development of predictive indicators of urbanization that are applicable to Midwestern watersheds and stream ecosystems.

The objectives of this project are to: (1) quantify the impacts of urbanization on hydrologic regimes, water quality, and habitat structure of stream ecosystems using paired experimental watersheds, and to develop linked models that accurately predict these impacts; (2) use the linked models as a virtual laboratory within which to generate and test indicators of urbanization and hydrologic change in terms of responses of fish and macroinvertebrate communities; and (3) use these models and indicators to assess the response of stream communities to alternative urbanization scenarios with extension to larger watersheds in the region.

The research examines seven watersheds in central Indiana that are in transition from rural to urban. Linkages between increased urban runoff, altered channel morphology, water quality effects, and reduced biotic integrity are being evaluated for three sites on each stream. Percent urbanization of the watershed, derived from 1997 SPOT satellite imagery, is used in a runoff model (L-THIA) to predict flow regime at each site. Intensive water quality sampling at selected sites provides a dataset for the development and testing of a physically-based water quality model. Stream cross-section measurements are used to determine the critical discharge (Q_{crit}) required to mobilize substrate.

Statistics describing the long-term exceedance of Q_{crit} characterize the level of habitat disturbance at the site. Physical habitat at each site has been measured using standard procedures, including a qualitative habitat evaluation index (QHEI). Macroinvertebrate, periphy- ton, and fish collections at each site are used to char- acterize stream community structure.

A dynamic hydrology model has been developed that can simulate cross-sectional averaged velocities, shear stress velocities, and water depth variability during storm peaks. Water temperature dynamics and nutrient transport also have been modeled with satisfactory results. Stream habitat measurements (QHEI) and biological collections have shown significant differences across the range of urbanization.

A suite of functional biological metrics currently are being evaluated. This work will provide a sound basis for the use of specific indicators as tools in regional planning of watershed development. The risk analysis portion of the work will provide a probabilistic measure as to whether a potential urbanization scenario can achieve stream water quality and biological targets. The hydrologic and water quality models developed thus far will be extended for use with dissolved oxygen and nutrient transport, and will be linked to the L-THIA runoff model. Stream biota and habitat quality, which already have been correlated to general land use (see Figures 1B3), will be further correlated to channel morphometry and flow variability.

Once this is accomplished, a risk analysis can be conducted on the effects of various runoff patterns on the stream community metrics.

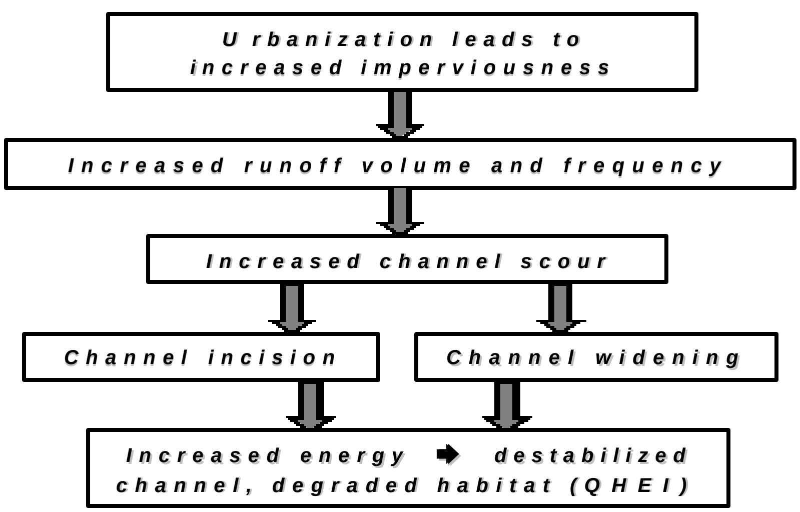

Figure 1. A conceptual model of stream channel changes associated with urban watersheds.

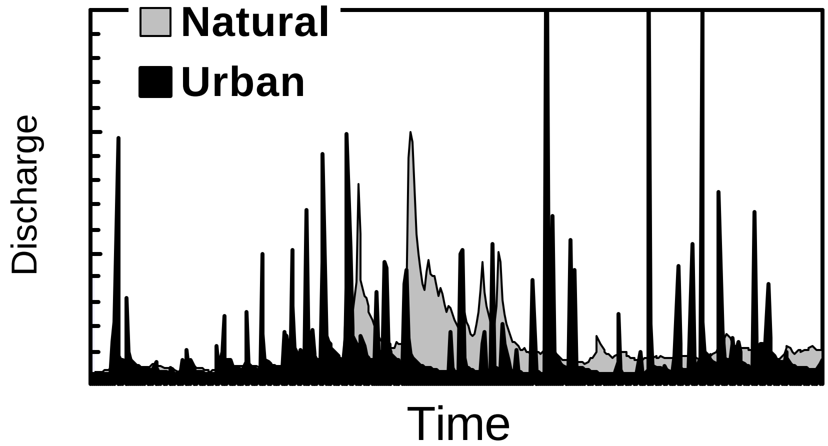

Figure 2. Hydrographs in urban streams are expected to show increased mean flow as well as increased frequency of storm peak flow.

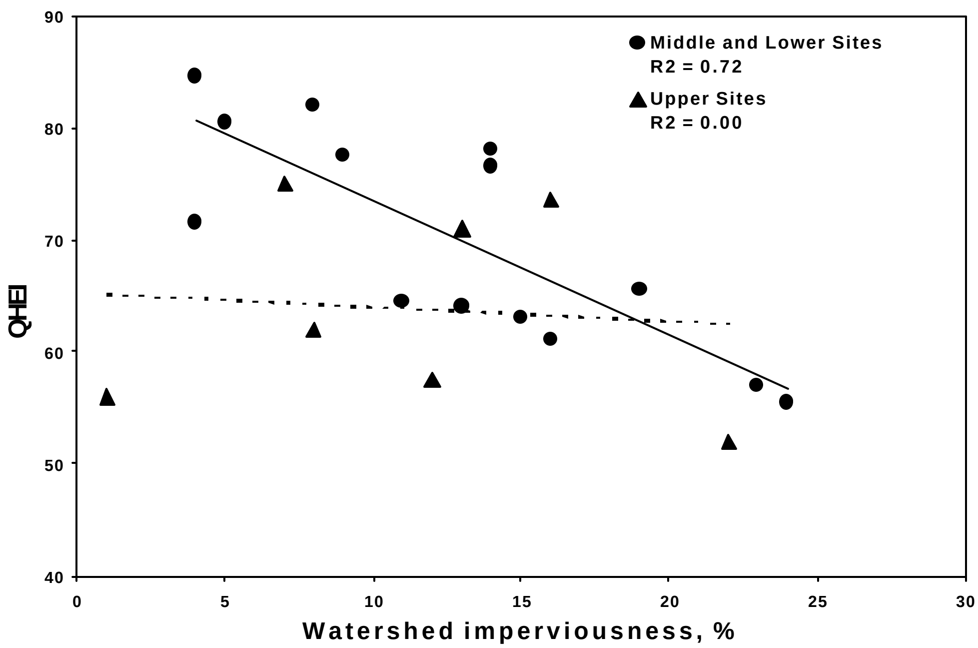

Figure 3. Significant degredation of fish habitat quality, as measured by QHEI, has been observed in streams undergoing urbanization in the region near Indianapolis, IN.

An Integrative Aquatic Ecosystem Indicator

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This project uses measures of the relative supply of carbon (C), nitrogen (N), and phosphorus (P) to integrate watershed, lake, and pelagic zooplankton assemblages in a multitier ecological indicator for monitoring lake integrity. Complex species assemblages are aggregated into simple consumer guilds that reflect relative elemental N and P intracellular requirements of species. Element supply ratios relate a variety of potential risks to lake ecosystem function such as loss of the cool-water refuge, bioaccumulation of toxins, algal turbidity, acidification, and UV-B toxicity. These risks are associated with zooplankton assemblage structure along the N:P ratio gradient.

Objectives of the project include the following:

1. Evaluate metrics based on the C, N, and P supply of the total dissolved plus seston fraction of lake water as indicators of the character of lake zooplankton assemblage.

2. Establish the strength of the linkage between the relative C, N, and P supply from the watershed to the C, N, and P supply to the lake. The hypothesis that N:P, C:N, and C:P ratios in lake water and watershed C, N, and P supply to lakes are significantly influenced by landscape factors related to human land use activity, forest ecosystem composition, and regional air pollution gradients will be tested (see Figure 1 for conceptual model).

3. Conduct a landscape characterization analysis of features in the lake-watershed basins which, in conjunction with concurrent watershed-associated stream and groundwater measurements, will be used to develop proxy measures for expected relative C, N, and P supply conditions for lake water.

4. Establish zooplankton assemblage-derived variables that reflect risks to ecosystem function, struct- ure, or human health as a function of the elemental sup- ply gradient.

5. Conduct a sensitivity analysis of the zooplankton metrics using existing and extensive temporal and spatial datasets. These analyses will establish statistical confidence and power to detect change for measures of lake integrity.

In the first field season, a total of 36 visits to 26 lakes in the Adirondack Mountain and Saint Lawrence Valley regions of New York and Vermont were conducted. Fifteen of 75 selected tributary riparian zone locations were sampled for field characterizations that included vegetation survey, leaf-litter sampling, and soil characterization and sampling.

The foundation data layers (topographic factors, land cover/land use, soils, hydrography, climate, and atmospheric deposition) of a regional GIS that will be used for the characterization of the study watersheds have been acquired, converted to grid data, reprojected, and resampled as necessary. The generation of riparian zone overlays is in progress.

The EMAP dataset was analyzed for sensitivity of various zooplankton metrics to distinguish differences among lakes on both regionwide and subregional scales. Sensitivity of metrices increased in one or more subregions relative to regionwide estimates for all metrics tested. Richness and population metrics of calanoid copepods and large cladocerans had 80**B**90 percent of their variance associated with the lake component relative to variance from temporal and interaction sources. Metric performance improved at spatial scales, reflecting underlying historical biogeographic associations.

Patterns in the structure of zooplankton assemblages of the EMAP lakes in relation to effects of predators and nutrient treatments in experimental mesocosms also were evaluated. Fish predation reduced the body sizes of comprising taxa in the mesocosms, but N and P addition determined whether the assemblages were calanoid (high N:P ratio), or cladoceran and rotifer (low N:P) dominant. Nutrient effects were immediate and substantial.

The factors tested in the experiments are identical to those identified in principal component analysis (PCA) of natural lakes**C**total N, total P, N:P ratio, and young fish. The results from these independent but mutually supporting studies greatly strengthens the underlying importance of nutrient ratios in controlling structure of zooplankton assemblages and further strengthens the foundations for nutrient-based zooplankton indicators.

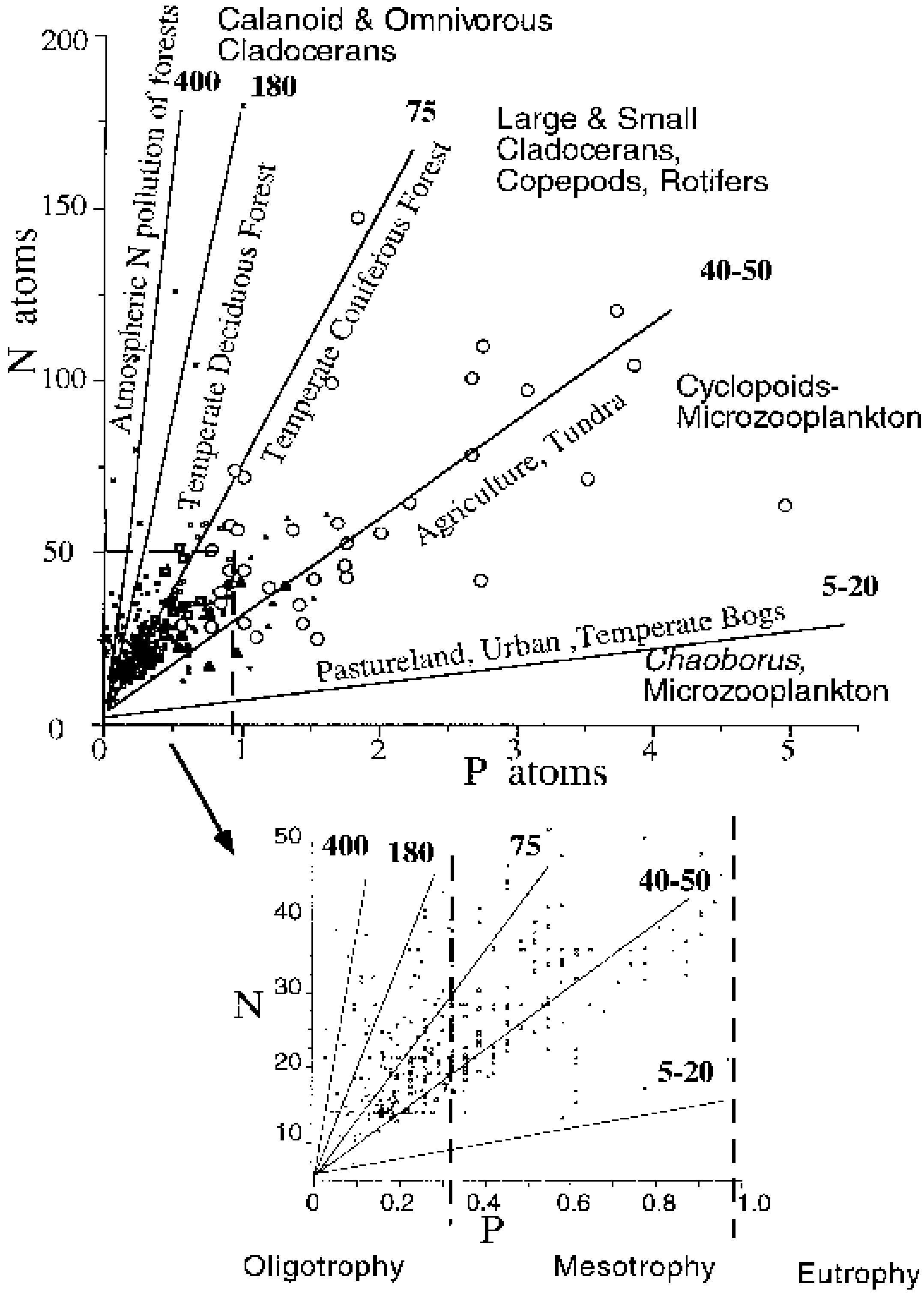

Figure 1. Plot of N and P space showing how the zooplankton-NP ratio indicator is interpreted within different classes of terrestrial watershed vegetation cover and land use. Open circles indicate lakes having Chl a values > 12 µg/L . These systems are associated with P values > than 1 µmole/L and reflect riparian, urban, or agricultural P pollution. Dashed line in upper right panel delimits the N, P space (50 µmoles L-1 of N and 1 mole/L P) that characterizes the majority of Northeastern lakes. Broken vertical lines in lower panel delimit lake trophic state based on total-P criteria: O=oligotrophic lakes (<10 µg/L total P), M=mesotrophic lakes (>10 < 30 µg/L total P), and E=eutrophic lakes (> 30 µg/L total P). Note all zooplankton groups may occur within each trophic state category and that total biomass will increase with increasing P.

Effects of Interacting Stressors in Agricultural Ecosystems: Mesocosm and Field Evaluation of Multilevel Indicators of Wetland Responses

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The primary objective is to evaluate indicators of molecular, cellular, population, community, and ecosystem responses to multiple, potentially interacting, natural and anthropogenic stressors at different spatial and temporal scales in agricultural wetlands.

The indicators are chosen to represent a selection of mechanism-based and system-level integrative characteristics that might be amenable to cost-effective routine monitoring. The null hypothesis is that indicators that effectively characterize ecosystem responses to single stressors are also scale- and interaction-independent (i.e., useful even when there are multiple, interacting stressors with diverse operational scales). In the project's first year, attention was focused on the development of a Geographic Information Systems (GIS) database for the watershed of the Little Tallahatchie River in northern Mississippi. Physical features, agricultural land use practices, and potential pesticide loads in the basin have been characterized.

These datasets were incorporated into the GIS database that will be used to randomly select approximately 150 field sites from about 18,000 potential sampling areas (ponds, oxbow lakes, impoundments, and intermittent stream segments) that have been identified. Physical features have been mapped from 1:100,000 and 1:24,000 from U.S. Geological Survey data. Features not visible on 1:100,000 data (already in digital form) were digitized from 1:24,000 maps (e.g., intermittent streams, small ponds).

Land use practices have been characterized from data obtained through county Farm Services Agency offices that maintain separate records for each farm in their jurisdiction. For each square mile of the study area, crop data has been gathered for all major and minor crops planted in 1998.

Potential pesticide loads were calculated for the following chemicals: chlorpyrifos, atrazine, methyl parathion, and monosodium methane arsonate**C**which comprise the majority of pesticides applied to major crops in the region (soybeans, cotton, corn, broadcast grains)**C**by multiplying the chemical application rate (amount of chemical per acre) by the number of acres in a square mile planted in a crop for which a particular chemical is used.

Application rates were obtained from resource information at the Lafayette County (Mississippi) Extension Office. This information incorporates data from companies that produce the chemicals as well as data pertinent to Mississippi that may affect application (e.g., weather patterns, soil types). From these chemical distributions, watershed areas have been identified that are most likely to have wetlands with agrichemical loadings typical of the single or multiple stressor treatments planned for the mesocosm experiment (see Figure 1), which is to commence in May 2000.

Many of the chemical combinations to be used in the mesocosm experiment (even the four chemical combinations) are represented in the GIS by multiple sections with numerous ponds that can be used for field evaluation. In the first year, the analytical and sampling protocols to be used in the upcoming mesocosm experiment have been further developed.

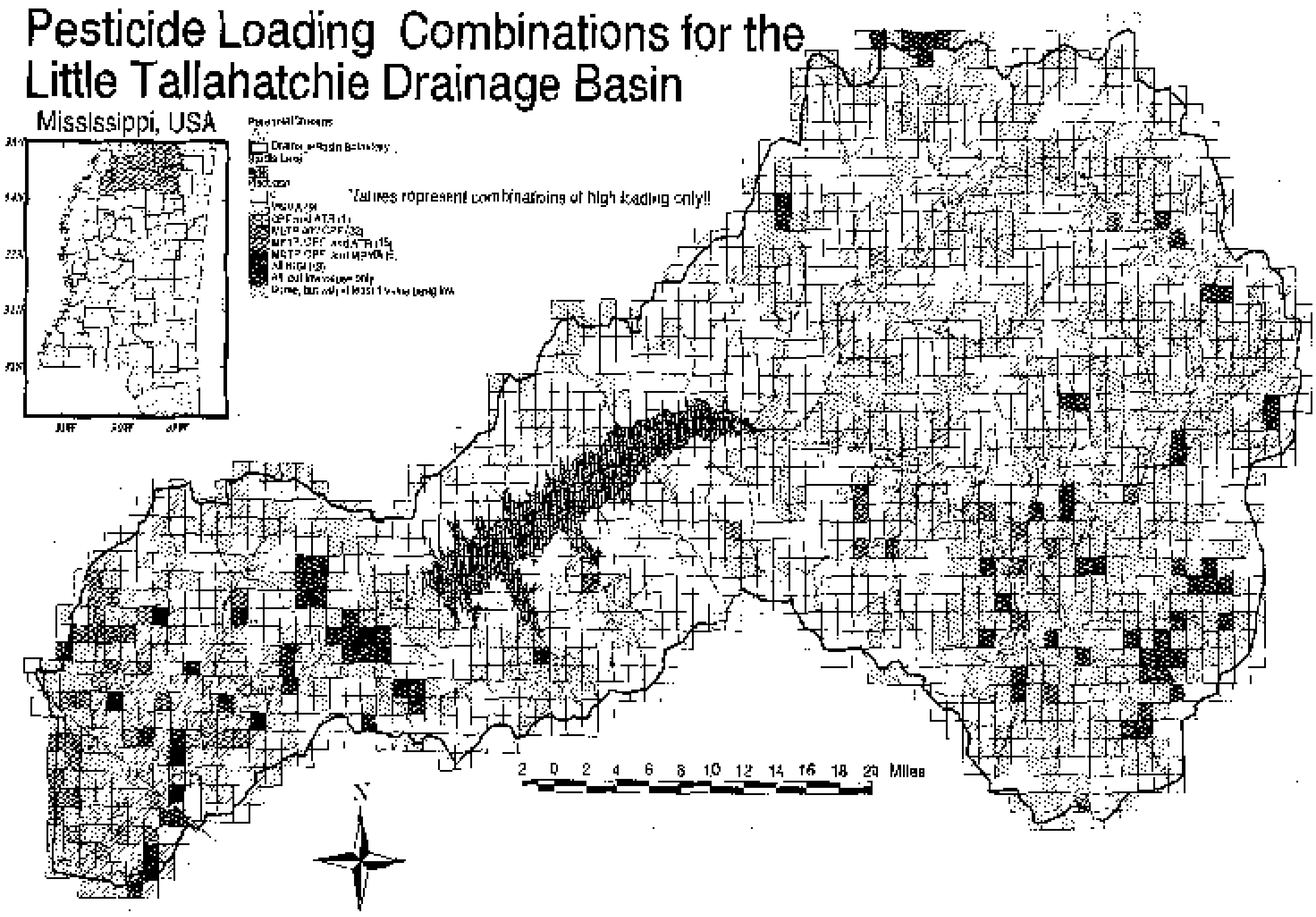

Figure 1. Pesticide loading combinations for the Little Tallahatchie River basin. Different shading patterns identify square mile sections of the drainage basin that are characterized by chemical loadings (single and multiple pesticide combinations) that also will be used in the mesocosm experiment.