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

Deliverable 2A: Compendium Report 1997 grants

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

31 January 2005

Submitted to

Perot Systems Government Services
31 Schoosett Street
Pembroke, MA 02359

Submitted by

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

1. INTRODUCTION

1.1 Background

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

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

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

1.2 Objectives

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

1.3 Summary

Eight 1997 Ecosystem Indicator EPA STAR grants were reviewed in detail to determine how results of this body of research can or are being used by the larger community. The eight grants fell into one or more of the following categories and themes (Table 1, Appendix A):

Table 1: Themes and Categories for the 1997 EPA STAR Ecosystem Indicator Grants

Theme	Category	Number of Grants
Study System	Forest	4
	Wetlands	1
	Grassland	1
	Freshwater	1
	Marine	1
Water Quality		6
Focus of Study	Landscape	2
	Forest	1
	Birds	1
	Fish	1
	Amphibians	1
	Freshwater mussels	1
	Macroinvertebrates	2
	Protists	1
	Bacteria	1
	Algae	1
Stressor	Anthropogenic	4
	Grazing	1
	Nutrients	2
Genetics		0
GIS/Remote Sensing		5

Overall the eight grants produced a total of one new method (a new bioassay that tracks coral reef habitat suitability by using foraminifera microorganisms, which are closely related to corals), nine tools (including models and GIS coverages), 39 peer-reviewed papers, and 168 oral presentations (Appendix B). The study areas for the eight grants were spread across the United States (Appendix C).

2. SUCCESSES, RESULTS, AND FINDINGS

2.1 STAR Grant R82-5865

Foliar Chemistry as an Indicator of Forest Ecosystem Status, Primary Production and Stream Water Chemistry
Principal Investigators: John Aber, R. Hallett, M. Martin, S. Bailey, S. Ollinger, M. Smith

Impacts:

Nitrogen saturation of forested watersheds in the northeastern United States, due in part to acid rain, can result in increased nitrogen export to groundwater, streams, and lakes. It is hypothesized that large areas of the forested northeastern United States are susceptible to becoming saturated with nitrogen, which could increase delivery of nitrogen to aquatic ecosystems and result in negative impacts to these systems.

Successes and Lessons Learned:

- *Nutrient (nitrogen and calcium) levels in leaves are directly related to soil nutrient levels.*
- *Nutrient levels in leaves can be predicted using advanced remote sensing instruments.*
- *A single calibration equation can be used to predict nitrogen levels across multiple remote sensing images.*
- *Calcium levels in leaves are directly related to stream levels of calcium in the White Mountain National Forest, but a similar relationship for nitrogen was not apparent.*

Indicators developed/tested:

- *Leaf Nitrogen: The level of nitrogen in leaves can be used to quickly and efficiently determine the level of nitrogen in the soil, which makes it a good indicator of whether a soil is nitrogen saturated.*
- *Remote Sensing: The power of airborne remote sensing lies in its ability to collect data over large areas quickly. If this technology is proven to accurately measure parameters such as leaf nitrogen, biomass, or stream water quality, it could result in an increase in data while decreasing overall costs of monitoring.*
- *Stream Water Quality: Ultimately excess nitrogen found in forested regions should be transported to streams which may also be impacted by this stressor.*

Products:

- *Digital: Website: http://www.mapbgc.sr.unh.edu/*
- *Publications: 3 peer reviewed articles*
- *Presentations: 11 oral presentations*

User Groups/Community:

- *Forest managers (non-government, state/local, federal)*
- *Global change scientists*
- *Federal, state, and local regulatory agencies*

Keywords/Themes:

forest, water quality, nutrients, GIS/RS

Background

Temperate forested areas in the northeastern United States are an important global sink for elements such as carbon, and estimating forest biomass production is a critical component of global-change forecasting. Large areas of the forested northeastern United States are hypothesized to be saturated with nitrogen, and there is a strong, established relationship between carbon (an indicator of forest productivity) and nitrogen in temperate forest ecosystems. If the Northeast forests are saturated with nitrogen, any excess nitrogen delivered to these forests by acid rain will be transported directly to aquatic ecosystems, which could have a negative impact on the aquatic ecosystems. Plot level data collection is not a cost-effective method for determining ecosystem health over large areas. Scaling field studies to a regional level can be done using

remote sensing; however, remote sensing instruments only access forest canopies, making it difficult to directly measure soil nitrogen from aircraft.

This study was conducted in the White Mountain National Forest (New Hampshire and western Maine), and was designed to provide a method to link nitrogen levels in soils to leaf chemistry in order to predict large forested areas which are close to nitrogen saturation. Aquatic ecosystems in nitrogen saturated forests are suspected to be more likely to exhibit negative effects related to increased nitrogen inputs.

Goals

- Map leaf levels of nitrogen and calcium over landscape areas using hyperspectral remote sensing imagery.
- Use soil and leaf nutrient data to develop relationships with measured stream water chemistry and forest productivity.

Findings

Leaf Nitrogen – This study showed that levels of leaf nitrogen are strong predictors of two measures of forest productivity: wood production and aboveground net primary production (the amount of biomass that results from photosynthesis). Canopy nitrogen concentrations also have a strong relationship with soil carbon-nitrogen ratios. These relationships suggest that leaf chemistry is a good indicator of soil processes. Relationships between leaf and soil nitrogen levels were significantly different for disturbed (by fire or logging) and undisturbed sites. The data from disturbed forest stands show slower nitrogen cycling rates.

Remote Sensing of Leaf Nitrogen – This study showed that leaf nitrogen levels can be accurately predicted using airborne remote sensing imagery. The NASA AVIRIS sensor was used to collect 56 scenes (each being 38 mi^2) over the White Mountain region of New Hampshire at a medium ground resolution (3000 ft^2). This was the first study to develop a single calibrated equation to predict nitrogen across multiple (56) image scenes. The calibrated equation accounted for 82% of the variation in leaf nitrogen, and was applied to the entire study area to produce a map of predicted leaf nitrogen concentrations. The match between field-measured leaf nitrogen and AVIRIS-predicted leaf nitrogen was generally good, but a tendency to under-predict leaf nitrogen at the upper end of the range was noted.

Remote Sensing of Biomass – The statistical relationship of leaf nitrogen to wood production (described earlier) was applied to the entire study area to produce a map of predicted wood production (biomass) from predicted (from remote sensing) leaf nitrogen concentrations. The comparison between field measured wood production and AVIRIS predicted wood production was even more accurate than for leaf nitrogen, and accounted for 86% of the variation found in field measurements.

Stream Water Quality Relationships – Streams in 46 watersheds were sampled for nitrate, calcium, pH, and magnesium quarterly for three years. The AVIRIS-predicted leaf nitrogen was averaged for all hardwoods and conifers in each watershed and compared to streamwater nitrate averaged yearly for each watershed. There was no relationship between predicted leaf nitrogen levels of hardwoods or conifers and streamwater nitrate. Preliminary data suggest that individual species levels of leaf nitrogen (for example Paper Birch or Red Spruce) may be able to predict streamwater nitrate better than lumping all species together. A weak relationship between AVIRIS-predicted leaf calcium concentrations (averaged for hardwoods and conifers) and streamwater calcium was detected. The relationship between predicted leaf calcium and streamwater calcium was strengthened when predicted leaf calcium levels for only Beech, Spruce, and Sugar Maple were used.

Implications

A strong four-way relationship between measured leaf nitrogen concentration, wood production, and leaf production, and remote sensing signals has been established. However, the relationship between nutrient levels in streams and these parameters has not been clearly established.

Next Steps

This study has laid the groundwork for advanced monitoring and detection of forested areas that are at risk of becoming nitrogen saturated. Much work remains; the sample sizes that were used to develop the statistical relationships between leaf chemistry, remote sensing, and stream water quality are relatively small and thus the results should be considered preliminary. Further research could conclusively link remote sensing technologies to stream water quality. Finally, it would be helpful to test relationships and findings from this study in the Catskill Mountains (New York), the source of much of New York City's drinking water, using AVIRIS imagery recently acquired for the area.

2.2 STAR Grant R82-5866

Using Bioindicators to Develop a Calibrated Index of Regional Ecological Integrity for Forested Headwater Ecosystems
Principal Investigators: Robert Brooks, R. Mulvihill, T. Master, T. O'Connell, S. Laubscher

Impacts:

Forested headwater streams in the Mid-Atlantic Highlands are impacted by a variety of environmental stressors. These systems are important to the ecological integrity, recreational quality, and food production of riparian and estuarine ecosystems throughout the region. The objective of this project was to develop an index to easily and confidently target headwater protection and restoration efforts.

Successes and Lessons Learned:

- *A simple indicator such as percent forest cover can be used to determine how much an area has changed from a reference point.*

Indicators Developed/Tested:

- *Percent Forest Cover: Percent forest cover was used to assess landscape level degradation.*
- *Stream Condition: Streams were ranked based on measurements of sedimentation, eutrophication, and physical modification. Analyses of stream aquatic insects were used to rank sites on a scale of ecological integrity*
- *Acidification: Measurements of pH in streams were used to rank streams in terms of acidification and determine if degradation is occurring.*
- *Louisiana Waterthrush and Other Riparian Songbirds: Louisiana Waterthrush is a bird species that requires large patches of mature forest and clear flowing water. The density of Louisiana Waterthrush territories, other riparian songbird territories, and the number of riparian bird species, are three indicators developed to assess landscape level degradation.*
- *Headwater Stream Assessment Index: The average of ranks for percent forest cover, stream condition, and acidification was used to prioritize headwaters for protection and restoration.*

Products:

- *Publications: 1 peer reviewed journal*
- *Presentations: 26 oral presentations*

User Community:

- *Non-Government organizations*
- *Local and state government*
- *U.S. Environmental Protection Agency Mid-Atlantic Integrated Assessment*

Keywords/Themes:

forest, birds, macroinvertebrates, anthropogenic disturbance

Background

Forested headwater streams comprise 60-75 percent of the total stream length and watershed area in the Mid-Atlantic Highlands (Pennsylvania, Virginia, and West Virginia) and are impacted by a variety of environmental stressors. It is not practical to collect data on all of the organisms in the region and how they respond to these stresses. The objective of this project was to develop an index that managers and decision-makers can use easily to target headwaters protection and restoration efforts.

Three indicators and one synthesis index were developed to assess environmental response to three different stressors: percent forest cover was used to assess terrestrial habitat fragmentation; stream condition was used to assess stream habitat; and pH was used to assess acidification of streams caused by deposition or coal mine drainage. For those sites with contradictory indicators (e.g., percent forest high, pH low) the utility of using data on songbirds and aquatic insects to assist in degradation discrimination

was evaluated. The three indicators (or four if needed) were averaged to calculate a Headwater Stream Assessment (HSA) index which allowed easy comparisons among headwater streams within the region.

Goals

- Develop a simple approach to assess forested headwater stream condition based on easily measured variables.
- Calibrate the newly established approach against a set of biological parameters which vary according to ecological stress.

Findings

Percent Forest Cover – This study found that measuring the single variable of percent forest cover was sufficient to measure deviation in habitat condition from a reference condition. Percent forest cover was measured in 0.6 mile radius circles and was found to have a significant and relatively strong correlation with a multiple species bird index termed Bird Community Index (BCI). The BCI reflects the biological integrity of the Mid-Atlantic Highland region and was developed by previously funded EPA research. The BCI uses species specific life history traits (e.g., food source, nesting habitat) to rank species which are summed for a study site to calculate the calibrated index.

Stream Condition – This study found that stream condition, as measured by sedimentation, eutrophication, and physical modification, does not seem to be correlated with aquatic insect communities. A possible threshold was determined to help build the HSA.

Acidification – A possible threshold of impacted streams was determined to be water with pH greater than 6.5. If a particular stressor is suspected, or if a stream has contradictory results from the three previous indicators (forest cover, stream condition and pH) this study found that collecting data on macroinvertebrate or riparian birds may be necessary.

Louisiana Waterthrush and Other Riparian Songbirds – This study found that the density of Louisiana Waterthrush territories is relatively strongly related to the number of young Louisiana Waterthrush fledged, making density a good indicator of breeding success. However, the density of Louisiana Waterthrush territories is not related to the percent forest cover of a study area, nor stream condition. Additionally, the number of young Louisiana Waterthrush fledglings is not related to acidification of streams.

Headwater Stream Assessment Index – Three bioindicators (percent forest, stream condition, acidification) were averaged to calculate a regional index which facilitated comparisons among streams in the entire region.

Implications

The HSA was useful for comparing streams, and could be used in other areas with similar ecologic issues (i.e., stream acidification, channel modification, and eutrophication). Despite the presence of weak relationships with most of the other factors examined in this study, the use of bird species as indicators holds promise. The BCI is intended to function as a landscape-scale indicator of biotic integrity, integrating conditions across large sample sites containing diverse ecological resources and intensities of human use.

Next Steps

The BCI has been calibrated for the Mid-Atlantic Highlands. In order to prove its utility in other regions, additional pilot studies must be conducted.

2.3 STAR Grant R82-5867

Environmental Factors That Influence Amphibian Community Structure and Health as Indicators of Ecosystems
Principal Investigators: Val Beasley, L. Johnson, R. Cole, P. Schoff, M. Piwoni, C. Richards

Impacts:

Wetlands have been severely impacted by human activities, degrading habitat for many species which are intimately linked to these small waterbodies. Amphibians, especially frogs, are dependent on wetland areas for much of their lives and may be useful indicators of ecosystem health.

Successes and Lessons Learned:

- *Numbers of frog species were highest where suitable wetland and upland habitats, including forest, were more closely interconnected.*
- *Increasing agricultural land was associated with fewer species of amphibians and macroinvertebrates in wetlands.*
- *Malformation rates in randomly selected sites were about four times higher than historic rates. Most malformations involved missing limbs or limbs of reduced size.*
- *High levels of agriculture were associated with high levels of cattails and chloride. Parasite infections in frogs increased as cattails and chloride increased in the wetlands.*
- *Mink frogs appear to be more readily infected with chytrid fungi than leopard frogs.*
- *High arsenic and zinc in frogs from study sites in Minnesota were associated with poor body condition.*

Indicators developed/tested:

- *Landscape: Landscape variables exert strong influences on water quality. The presence and type of agricultural activity is a key variable.*
- *Water Quality: Amphibian life history traits make them especially susceptible to degraded water quality.*
- *Parasites: Parasite types and numbers in amphibians are linked to habitat quality.*
- *Malformations: Malformations have been reported throughout the midwestern United States and have been linked to environmental variables and human activities.*

Products:

- *Digital: Website http://www.nrri.umn.edu/cwe/hostedpages/amphweb.html*
- *Publications: 1 book chapter, 3 peer-reviewed articles, 1 PhD Thesis, other works in progress*
- *Presentations: 36 oral or poster presentations*

User Groups/Community:

- *Natural resource and agricultural land managers*
- *State/local monitoring agencies*

Keywords/Themes:

wetlands, water quality, frogs, anthropogenic disturbance, GIS/RS

Background

Declines in amphibian populations have been noticed by scientists since the late 1980s. Field research has produced compelling evidence that habitat loss, climate change, pollutants, infectious diseases, and predation by invasive species are all factors contributing to this decline. No single stressor is likely to account for all amphibian declines. It has been suggested that land cover patterns, fragmentation patterns, connectivity of landscape elements, and spatial position of roads, water bodies, and other landscape elements in the region influence amphibian community structure and health at regional scales. At local scales, biotic interactions with other species, water quality, parasite burdens, and contaminant levels in the environment are believed to exert strong influences on amphibian communities.

The unique life history and physiology of amphibians make these animals sensitive to their surroundings, and thus they can be considered early indicators of changes in the health of the environment. The overall goal of this research is to assess the relative influence of landscape patterns, biotic interactions, water quality, and contaminants on the community structure and health of amphibians. This study surveyed 64 wetlands in Illinois, Minnesota, and Wisconsin, representing a range of land use patterns and environmental conditions known to impact amphibians. In addition, detailed assessments on 37 wetlands in Minnesota were conducted that focused on water quality, contaminants, and amphibian body condition, parasite burdens, and lesions.

Goals

- Determine the influence of wetland- and watershed-scale factors on amphibian community structure and health.
- Determine whether amphibian community structure and health are indicative of ecological integrity.

Findings

Landscape – For each of the 64 wetlands, aerial photographs were analyzed to determine land use/land cover, habitat fragmentation, and connectivity of landscape elements at three spatial scales: the local wetland level, 1.25 miles out from the wetland, and 6 mile out from the wetland. Vegetation surveys along transects were conducted, aquatic invertebrates were collected, and soil and sediment samples were analyzed. A total of 200 variables were analyzed. No single environmental variable was found that had an overriding influence on amphibian distributions, and relationships among the measured variables varied across species and scales. The number of aquatic invertebrate species was negatively correlated with agricultural land use, and the percent of organic matter in soil was positively correlated with agricultural land use. The number of frog species was correlated with only the broadest of landscape variables. Additional results indicated that the presence of forested woodlands was likely to be an important predictor of the presence of certain species of frogs. Number of frogs captured was also positively related to the amount of coontail (a submerged wetland associated plant) but not duckweed (a floating wetland plant, which forms dense growths that can reduce sunlight to the water column).

Water Quality – Almost 1000 individual water samples were collected from the 37 Minnesota wetlands in this study. Analysis found that the number of frogs captured was related to dissolved oxygen. A number of elements known to be toxic to amphibians were found at levels above EPA recommendations: aluminum (84% of sites), copper (30%), iron (86%), lead (35%), and zinc (8%). High arsenic and zinc in frog tissues were associated with poor body condition (a health assessment measurement which is the ratio of body mass to length).

This study concluded that pesticides and other organic contaminants were present at comparatively low concentrations in the wetlands of south-central Minnesota. Although a number of pesticides, including atrazine, were tested for the low sensitivity of the assay in the second year of field studies, conclusions as to their significance as disrupters of frog reproduction and development were precluded.

Parasites – Over 128,000 individual parasites were counted in this research. In total, 26 parasite taxa were found across all sites. A high parasite load can be lethal to frogs and recently parasites have been shown to cause limb abnormalities. All wetland sites had frogs with some parasite loading, and 40-100% of frogs at wetlands tested positive for echinostome infections. The echinostome parasites reproduce asexually in snails, and then infect kidneys of tadpoles. These infections were found to be highly lethal to early stage tadpoles. The environmental variables collected and summarized above (water quality and ecological assessment) were analyzed to determine relationships with frog parasite loads. Subsequent analysis showed percentages of forest and wet forest were important predictor variables of parasite presence.

Malformations – No obvious clusters or "hot spots" of malformations, mostly missing limbs or smaller than normal limbs, were found. Over the three-year study, 5,975 frogs were examined for malformations, and an overall malformation rate of 1.8% was found. Rates were not consistent within sites across years, and no site had consistently high malformation rates. The recorded historic malformation rate in frogs of the study region, 0.5%, is almost four times lower than the rate observed in this study. The pattern of malformations contrasted with other studies which found "hot spot" outbreaks, where large numbers of malformed frogs have been found, and where "extra limbs" was the most prevalent malformation.

Implications

Worldwide declines of amphibians have concerned scientists for over twenty years and this research is part of a growing body that shows that answers to questions about the causes of these declines are complex and require further analysis. However, the current study illustrates negative impacts of agriculture on amphibian communities even in an area of limited pesticide pollution (i.e. increased parasite load), and suggests that maintaining linked habitats that offer ecological features such as buffers between wetlands and row crops or pastures, forested areas, and oxygenating plants in the water, are important to the sustainability of amphibian communities in the upper Midwest.

Next Steps

Data related to the detection of certain pesticides need to be analyzed. The large quantity of data resulting from the entire study will be used to construct a theoretical dynamic model on habitat quality and amphibian health.

2.4 STAR Grant R82-5868

Microbial Indicators of Biological Integrity and Nutrient Stress for Aquatic Ecosystems
Principal Investigators: James Grover, T. Chrzanowski

Impacts:

Lake water quality is a concern from an ecological and a human use perspective as many communities use lakes as a source for drinking water. Increases in nutrients can in turn increase organic matter that can form cancer causing compounds after the water has been treated for human consumption.

Successes and Lessons Learned:

- *Algae and bacteria can be useful indicators of nutrient stress between different lakes and seasonally within the same lake.*
- *Based on data from two lakes, algae and bacteria cannot be used to test the strength of nutrient stress in Canadian lakes.*

Indicators Developed/Tested:

- *Ratios of Elements in Lake Biomass: Ratios of carbon, phosphorus, and nitrogen in floating living matter should reflect nutrient limitation.*
- *Lake Biodiversity: The number of different species of plankton and other living organisms should be related to whether a lake ecosystem is limited by one or more nutrients.*
- *Bacteria Resource Use: Bacteria communities in nutrient stressed lakes will shift. If bacteria populations become depressed from low nutrient levels, populations of organisms feeding on them will also decrease.*
- *Algae/Bacteria Productivity: Growth rates and biomass production of algae and bacteria should be similar in nutrient limited lakes.*

Products:

- *Publications: 5 peer reviewed journal articles*
- *Presentations: 17 oral presentations*

User Community:

- *Drinking water utilities*
- *Water resource managers*
- *Watershed groups*

Keywords/Themes:

freshwater, water quality, algae, bacteria, nutrients

Background

Nutrient enrichment of lakes and streams caused by humans is a serious issue. Nutrient enrichment can cause conspicuous events such as summer algal blooms that lead to chronic low levels of dissolved oxygen, fish kills, murky water, and loss of biodiversity, which may affect desirable recreational fisheries. Increases in algae and turbidity lead to increases in chlorination of drinking water, this, in turn, leads to higher levels of disinfection by-products that have been shown to increase the risk of cancer.

Changes in nitrogen and phosphorus in lakes can dramatically alter ecosystem functions. These changes usually begin at the microbial level because these organisms extract nutrients to make biomass. Every organism has certain requirements for nutrients that must be met if they are to persist, and organisms differ in their competitive ability to use these nutrients. Thus, when the nutrient conditions of environments change, there will be changes in community composition. This study focused on how the ratios of key nutrients in two lakes in Texas and two lakes in Canada may influence competition, nutrient recycling, and community integrity under the theory that bacteria and algae are indicators that respond rapidly to increased nutrient supply and alterations in supply ratios of different nutrients.

Goals

- Demonstrate that ecosystem integrity is reflected in nutrient supply to, and nutrient limitation of, the microbial component of lakes and reservoirs.
- Develop indicators emphasizing connections between processing of nutrients and the population and community dynamics of microorganisms.

Findings

Ratios of Elements in Lake Biomass – Experiments which used algae and bacteria to diagnose nutrient limitation in two Canadian lakes did not perform well. Data collected seem to indicate that at least one experimental assumption may have been violated by samples from Canadian lakes: that the algae suffer negligible loss during experiments. This violation could have occurred if organisms grazing on algae were not filtered from samples prior to experimentation. Experiments in Texas lakes showed that the ratio of C:N:P in microbial biomass can be used to show that phosphorus limitation does occur, and to a lesser extent this ratio shows nitrogen limitation. These results agreed with classical indicators such as chlorophyll *a* and total phosphorus concentrations in water. This indicator would be more useful to document seasonal nutrient limitation than limitation between lakes because there was a wide overlap of values between lakes.

Lake Biodiversity – This study developed computationally intensive statistical analyses to examine whether the number of species of algae and/or bacteria was a reflection of the limiting nutrient. The lakes in this study gave conflicting results for the lakes studied and given that implementing this indicator is very labor intensive (determining each species of algae and bacteria) the utility of this indicator is questionable.

Bacteria Resource Use – This study quantified the degree that bacteria utilized 95 unique carbon sources. Results were used to develop a community level physiological profile. This technique is promising because of the ease of implementing this indicator with appropriate equipment, but is sufficiently controversial that preliminary results should be confirmed with other methods.

Algal/Bacteria Productivity – Attempts to develop relationships between bacteria population and nutrients in two Canadian lakes were inconclusive, in part because of the failure to remove bacteria predators from the samples. Experiments in Texas lakes showed that bacteria populations did not have a simple relationship with nutrients and predators. Additionally, algal/bacterial productivity (the amount of biomass created) was not related to the productivity of the entire lake, making it a poor indicator of within lake, seasonal variations. Results showed that this indicator might be useful for determining differences between lakes. However, this indicator is moderately time intensive and should be cautiously applied.

Implications

This study tested seven hypotheses related to nutrient limitation and microbial dynamics. Some of the indicators developed seem to have the potential to determine differences either within a season or between different lakes. Current theory predicts that microbes will respond rapidly to increase nutrient supply, but this study documented mixed results.

Next Steps

Several techniques explored by this study are controversial and/or in a preliminary stage of development and more research is needed before these indicators can be proven to be useful. New statistical techniques are needed to analyze large amounts of data within a season to determine if a relationship between nutrient limitation and microbial indicators can be constructed.

2.5 STAR Grant R82-5869

Foraminifera as Ecosystem Indicators: Phase 1. A Marine Benthic Perturbation Index; Phase 2. Bioassay Protocols
Principal Investigator: Pamela Hallock Muller

Impacts:

Impacts to coral reef ecosystems can have significant biodiversity, economic, and environmental effects. To date, differentiating human-influenced effects from natural events has been difficult. This study developed an independent measurement of water quality as it relates to coral reefs.

Successes and Lessons Learned:

- *Marine protists can be used to indicate past and present water quality in tropical ecosystems.*

Indicators Developed/Tested:

- *Population Structure: An index that shows general reef condition by sampling shelled marine single celled organisms (protists). This indicator measures water quality conditions on a time scale of weeks to months.*
- *FORAM Index: A single-metric index of water quality condition based on field sampling of sands which contain shells of many species of protists. Species relative abundance is the basis of this index and characterizes water quality conditions on a time scale of months to years.*
- *Reef Sediment: A single-metric index of water quality condition based on relative abundance of protists in sediment cores. This indicator's time scale is decades to centuries.*
- *Foraminifera Bioassay: This protocol describes how to test environmental stresses to protists.*

Products:

- *Protocol: Bioassay for foraminifera with symbiotic algae*
- *Digital: Website http://www.marine.usf.edu/reefslab/*
  CD-Rom describing indicators, taxonomic information, and bibliography
- *Publications: 11 in peer reviewed journals or books, 4 in proceedings, 4 in preparation, 7 theses and dissertations*
- *Presentations: 30 oral presentations*

User Groups/Community:

- *state/local environmental monitoring agencies*
- *coral reef manager*
- *water quality specialists*

Keywords/Themes:

marine, water quality, protists

Background

Slow growth rates, low recruitment rates, and long life spans make coral reefs especially susceptible to chronic human induced stresses such as nutrient enrichment, sedimentation, and over-fishing practices. Coral reefs are also under stress due to natural events such as hurricanes, bleaching, and disease outbreaks.

Corals have evolved a complex relationship with algae in order to thrive in nutrient poor tropical waters. Corals provide protection and in return, symbiotic algae provide sugar and nutrients by way of photosynthesis. Corals are thus dependent upon light reaching them. As a result, it is critical that an indicator of suitable water quality for corals also respond to parameters that may affect light transmission. Foraminifera are small, single celled organisms (protists) which live in the same types of environments as coral reefs. They are abundant, can reach sizes of over 0.75 inches, and also have symbiotic algae within them (although some have different species than corals). These similarities make foraminifera a likely independent indicator of the suitability of habitat for coral reefs and can to be used to determine whether

water quality is sufficient to support reefs. This study was conducted at coral reefs in Florida and Puerto Rico.

Goals

- Develop an indicator for assessing marine ecosystems based on changes in key species of protists. This indicator would provide a mechanism for assessing how benthic communities have changed over time.
- Evaluate the protist indicator using EPA Evaluation Guidelines for Ecological Indicators and produce a CD-ROM based report to promote and support its routine use.
- Develop methods to use protists in laboratory studies of the effects of key stressors.

Findings

Population Structure of Marine Protists – This study described a technique to census living foraminifera found in habitats suitable for coral reefs. It was found that the abundance of foraminifera at depths of 16-65 feet was a sensitive indicator of the suitable water quality for coral reefs. Additionally, the abundance, presence/absence of bleaching, and type of foraminifera shell breakage indicated the suitability of the water quality for coral reefs over the preceding weeks to months.

FORAM Index – The basic premise upon which this index is based is that when foraminifera shells are found in sand, the environment is also suitable for proliferation of corals. This study described a technique to collect and analyze sand samples for the presence of foraminifera shells. The researcher determined that a sample size of 150-200 shells per sample provided a useful compromise between the needed precision and cost of processing. Results showed that changes in dominant foraminifer species reflected changes in the suitability of water quality for corals, and that the foraminifera index was independent of sand grain size and sample depth.

Reef Sediment – This study also described a second technique, in which FORAM Indices were calculated for sediment core samples. Using sediment cores yielded results that characterized water quality over a much longer time scale than the FORAM Index.

Foraminifera Bioassay – Experiments using foraminifera as test subjects have significant benefits over the use of corals or coral larvae. Foraminifera experiments require much smaller containers; collection of statistically significant numbers of individuals is easier; and foraminifera are typically available year round. A method for culturing foraminifera was described and the study found that experimentally bleached specimens were statistically identical to field collected bleached specimens. This study also demonstrated that broken shells were not related to pollution stressed environments (contradicting earlier studies).

Implications

A critical implication of this research is that the foraminifer based index differentiated coral decline caused by nutrification from responses to episodic events that are independent of water quality. While the immediate cause of coral population decline is often a mortality event (e.g., temperature extremes or hurricanes), if chronic nutrification has also occurred, coral populations continue to decline rather than recover from that event. Because the foraminifera index is based on functional groups rather than specific taxa, it can be used for reefs worldwide, as well as to interpret changes on geologic time scales. In addition, field and experimental work with the foraminifera *Amphistegina spp.*, which is abundant on reefs worldwide, verified that light stress, very likely associated with stratospheric ozone depletion, plays a critical role in the well documented increase in bleaching and susceptibility to diseases in reef-dwelling organisms, which has occurred over the past 25 years.

Next Steps

Population Structure of Marine Protists – Because the relationships among *Amphistegina spp.* abundance, recruitment, bleaching, and predation are complex and non-linear, further research is needed to develop a single-metric index that can synthesize several parameters.

FORAM Index – Testing the index in a wide variety of reef systems is required. The index is currently being applied in Biscayne Bay, Florida, and requests have been made to apply the procedures in Hawaii and the Red Sea. A researcher from Canada is trying to develop a North American coastal monitoring program that would include foraminifera.

Foraminifer Bioassay – The bioassay should be tested on a wide variety of environmental conditions and stresses. The results of these studies could help determine if water quality and other environmental characteristics are suitable for reef recovery after catastrophic mortality events (i.e. hurricanes).

Multiscale Approaches to Reef Monitoring and Risk Assessment – Based on research from this project, a multiscale approach to improve coral-reef monitoring and risk assessment has been proposed. A key challenge is to develop ways to encourage reef managers to implement the recommendations of the multiscale approach.

2.6 STAR Grant R82-5870

Development and Evaluation of Multi-Scale Mechanistic Indicators of Regional Landscapes
Principal Investigators: Carl Richards, G. Host, L. Johnson

Impacts:

Significant portions of the natural landscapes of Minnesota and Michigan have been converted to agriculture, with notable changes in water quality. Indicators that identify ecosystem health trends using readily available data at the field, watershed, and regional levels are necessary to understand the effects of these changes.

Successes and Lessons Learned:

- *The accuracy of using satellite imagery to map agriculture near stream zones was quantified using aerial photographs and is consistent with other published results.*
- *Regional similarities and differences exist in the relationships between stream ecosystem functions and land use and/or geology.*
- *Extent and distribution of row crop agriculture is an important feature that influences stream ecosystem function.*

Indicators Developed/Tested:

- *Field: Aquatic insects, physical, chemical, and ecological characteristics were sampled to identify a local indicator of ecosystem health.*
- *Watershed: Existing data on land use, soils, elevation, roads and population density were compiled to determine how these characteristics influenced field level indicators of ecosystem health.*
- *Regional: Watershed scale data from Michigan and Minnesota were compared to determine if indicators were consistent between similar regions.*

Products:

- *Digital: Geographic Information System for 72 Minnesota and Michigan watersheds*
- *Publications: 1 peer reviewed journal article, 3 in review, 1 masters thesis, 1 proceedings*
- *Presentations: 22 oral presentations*

User Community:

- *State/local monitoring agencies*
- *Watershed groups*

Keywords/Themes:

forest, water quality, landscape, anthropogenic disturbance, GIS/RS

Background

This project attempted to develop watershed-scale ecological indicators that strongly and consistently characterized stream biological components at the regional, local, and stream scale. Hierarchy theory predicts that relationships between biological and physical attributes of the environment have influence at local, watershed, regional, continental, and global scales. Streams in 72 watersheds (36 in Minnesota and 36 in Michigan) were sampled to first determine the strongest relationships between stream scale environmental factors, fish, algae, and aquatic insects. The study then used the selected relationships to identify important watershed scale indicators that characterized those relationships and regional differences between these agriculturally dominated landscapes.

A watershed scale model developed by the U.S. Department of Agriculture named SWAT (Soil and Water Assessment Tool) was used to determine the effect of land use and geology on the hydrologic and nutrient output of 72 stream watersheds. The SWAT model combines land use, geology, rainfall, and elevation data to predict stream flow, sedimentation, nutrient transport and a host of other variables.

Goals

- Develop models that integrate landscape-scale factors with reach-scale physical and chemical stream attributes.
- Quantify key compositional and structural attributes of stream integrity.
- Derive ecosystem indicators at multiple spatial scales (field, watershed, region).
- Distinguish and quantify natural variation in indicators from human influence

Findings

Field – A substantial portion of the diversity of aquatic insects can be attributed to just the presence of downed wood in the stream. The most important stream variable related to aquatic insect communities was a simple measure of habitat diversity (number of habitats). The ratio of stream width to depth, and percent fine sediment were also important. There was no nutrient limitation in the 72 streams, less than 3 percent of the 172 samples showed limitation by a single or combination of several nutrients. Finally, land use data in close proximity to streams derived from satellite imagery should be used cautiously, because percent agriculture (crops) identified in the field was different from aerial photographs.

Watershed – Vegetation type, percent urban land use, and elevation parameters in a watershed had the strongest relationships with the total amount of wood in a stream (shown above to be a useful predictor of aquatic insect communities). Algal growth in streams was influenced by width of stream; the amount of vegetation, open water, and fine sediment; water temperature; and residential land uses. SWAT model simulations indicated that geology and land use were important in predicting water flow and nutrient transport and that at least nine stream variables responded to land use, two to geology, and three to both. Percent commercial land use, percent wetland, soil erosivity, and the standard deviation of elevation were the most important watershed variables driving in-stream physical variables.

Region – The proportion of fine sediment in streams, habitat diversity, and width to depth ratio were the most important stream indicators for stream invertebrate communities in both Michigan and Minnesota. The common watershed variables affecting these variables were percent commercial land use and the standard deviation of elevation. Other commonalities between Michigan and Minnesota were lacking, for example, in Michigan the watershed factor that influenced sedimentation was residential land use, but in Minnesota it was soil characteristics. Strong regional differences in the magnitude and relative importance of geology and land use (as simulated by the SWAT model) were found and the relationship between agriculture and geology was different between the two regions.

Implications

The use of hierarchy theory to develop indicators that step down from the regional, to watershed, to field scales has several time saving implications. Providing that these indicators have strong relationships among scales, regional or watershed variables are relatively easy to collect and could save time and expense if they accurately predict aquatic insect community parameters at smaller scales. Geologic and land use characteristics of regions are important for understanding the relationship between watersheds and streams.

Next Steps

Relationships to predict watershed and stream characteristics that are similar across different regions have shown promise. Further analysis of this large dataset may uncover more relationships and the utility of these relationships should be proven in other regions within the U.S. Simulation modeling of landscapes with different characteristics from the watersheds in this study may lead to better understanding and analysis of effective indicators.

2.7 STAR Grant R82-5871

Development and Evaluation of Ecosystem Indicators for Urbanizing Midwestern Watersheds
Principal Investigators: Anne Spacie, J.M. Harbor, M. Hondzo, B.A. Engel

Impacts:

Urbanization is thought to have negative long-term effects on stream ecosystems. In midwestern watersheds, sediment and nutrient loads decrease as land shifts from agriculture to urban uses due to the high non-point source pollution problems associated with agriculture. This relationship is the opposite of that found in most other systems studied in the United States.

Successes and Lessons Learned:

- *A highly successful model of percent imperviousness based on remote sensing data was created.*
- *The Extreme Value Method can be used to successfully predict daily dissolved oxygen levels in Midwestern watersheds.*

Indicators developed/tested:

- *Stream Stability: Stream channel shape is a critical link between land use and aquatic habitat quality, as urbanization affects channel stability.*
- *Periphyton: The effect of increased water velocity on periphyton (organisms, such as algae and small crustaceans, that live attached to surfaces on stream bottoms) communities was investigated using artificial stream channels.*
- *Freshwater Mussels: Presence and absence of freshwater mussels is thought to be sensitive to habitat parameters which are altered by increased urbanization.*
- *Aquatic Insects: Eleven indicators were evaluated for sensitivity to the urban environment.*
- *Fish Community: Qualitative Evaluation Habitat Index and Index of Biotic Integrity were assessed to determine if they could discriminate urbanization levels.*

Products:

- *Digital: A 1997 and 1999 Land Use/Land Cover map of Indianapolis area. L-THIA – A Long-Term Hydrologic Impact Assessment model designed to quantify the impact of land-use change on water quantity and quality.*
- *Publications: 12 peer reviewed journals, 5 proceedings, 4 theses/dissertations.*
- *Presentations: 13 oral presentations.*

User Groups/Community:

- *L-THIA is used by Planning with POWER, a statewide educational program coordinated by Illinois-Indiana Sea Grant College Program and the Purdue University Cooperative Extension Service which links land-use planning with watershed planning at the local level.*

Keywords/Themes:

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

Background

The focus of this project was to develop predictive indicators of urbanization for Midwestern watersheds and stream ecosystems. Unlike other parts of the country, watersheds in this region actually experience a decline in sediment and nutrient loadings as land use shifts from agricultural to urban uses. Thus it is unlikely that Midwestern streams will respond to urbanization in the same way as streams in more forested regions of the United States.

The project examined eight watersheds in central Indiana that ranged from rural to urban classification. Land use was determined using standard remote sensing techniques on recently acquired (1997 and 1999) medium resolution satellite imagery. The percentage of a watershed that was impervious to water was

calculated from this land use data and was used as an indication of urbanization, the response variable for all subsequent analysis.

Goals

- Quantify the impacts of urbanization on stream water cycles, water quality, and habitat structure of stream ecosystems using paired watersheds.
- Generate and test indicators of urbanization and hydrologic change in terms of responses of fish and aquatic insect communities.
- Assess the response of stream communities to alternative urbanization scenarios with extension to larger watersheds in the region.

Findings

Stream Stability – The model built by this study accounted for stream geometry, variable slopes, variable flow regimes, and unsteady boundary conditions. The final model was successful in predicting temperature fluctuations every 15 min over the 25 day measurement period and thus can be used to predict temperature limits for fish that may be stressed during summertime, low flow conditions.

Periphyton – Periphyton are sensitive indicators of environmental change in streams in part because they integrate physical and chemical disturbances within a stream over their short lifetime. This study showed effects of increased nutrient levels resulting from urbanization on periphyton biomass (periphyton biomass is expected to increase with increased nutrient levels) were counteracted by effects of increased flow due to urbanization (periphyton mortality increases with higher flows because high flows cause periphyton to detach and float downstream).

Freshwater Mussels – The study found that urban streams have reduced mussel populations compared to rural streams (present in 28 percent of urban streams compared to 46 percent of rural streams). Additionally, measurements of presence/absence of freshwater mussels showed that unstable channels and high shear stress (whether in urban or rural areas) were negatively correlated with the presence of mussels.

Aquatic Insects – Eleven unique indices and/or metrics of aquatic insects were investigated to determine their relationships with urbanization. Increased imperviousness was most strongly correlated to decreases in clinging species, and increases in the ratio of Baetis (Caddis Flies) to Ephemeroptera (Mayflies). However, these relationships held true for only one of the two years of data collection.

Fish Communities – Fish communities have been shown to respond to variations in stream water levels. This study showed a clear degradation of the Qualitative Habitat Evaluation Index (QHEI, a qualitative assessment of fish habitat) in relation to urbanization. This study also showed a relationship, but less strong, between a fish based Index of Biotic Integrity (IBI, an index that measures the health of a stream based on multiple attributes of the resident fish assemblage) and urbanization.

Response of Streams to Alternate Scenarios

A long-term hydrologic impact assessment (L-THIA) model was created to predict annual runoff and non-point source pollutant loading. The model combines long-term rainfall records, soil types, and land use within a Geographic Information Systems environment. The model is accessible through the internet for use by communities, planners, and others.

Next Steps

The utility of the L-THIA model and the other indicators should be tested in other urban environments to determine how applicable the results of this study may be to other regions.

2.8 STAR Grant R82-6112

Characterization of Ecological Integrity of Commercially Grazed Rangelands Using Remote Sensing-based Ecological Indicators
Principal Investigators: Neil E. West, R. D. Ramsey, R. A. Washington-Allen

Impacts:

The goal for sustainable rangeland management is to provide financial returns to the livestock owner while maintaining native flora and fauna, high-quality water, and other ecosystem services. Traditional ecological integrity monitoring approaches have generally failed because obtaining timely, repeatable, and statistically adequate time series data was too demanding of technical expertise, data collection timing, and budgets to complete adequate analyses and reporting.

Successes and Lessons Learned:

- *Historic satellite imagery can be used to gauge the degree of land degradation.*

Indicators Developed/Tested:

- *Soil-Adjusted Vegetation Index (SAVI): Satellite imagery was used to derive SAVI, a measurement of vegetation greenness which was specifically developed for arid environments and is a surrogate measure of vegetation cover*
- *Soil Stability Index (SSI): Satellite imagery was used to derive SSI, a measurement which discriminates between erosive, stable and depositional soil types.*
- *Change in Landscape Composition and Patterns: Changes in land cover over time were assessed to determine if grazing, fire or climate had effects on vegetation dynamics.*
- *Grazing Gradients: Large mammals require regular access to drinking water, and in arid environments water availability determines grazing patterns. Water sources become foci of grazing activity, resulting in a zone of decreasing grazing impact (piosphere) as distance to the water source increases.*

Products:

- *Digital:* *Website http://cc.usu.edu/~new369/deseret/intro.htm*
  Complete Geographic Information System for Deseret Land and Livestock Ranch
  GIS-Based Piosphere Generation Tool - http://www.gis.usu.edu/~doug/SERDP/Tutorial/Piosphere/piosphere.html
  GIS-based Relative Atmospheric Correction Tool- http://www.gis.usu.edu/~doug/SERDP/Tutorial/Atmos/tutorial.html
- *Publications: 3 peer-reviewed papers, 2 peer-reviewed abstracts, 11 proceedings of meetings*
- *Presentations: 12 oral presentations*

User Community:

- *Range managers (both government and non-government).*

Keywords/Themes:

grassland, landscape, grazing, GIS/RS

Background

This research project assessed the ecological integrity of a semi-arid landscape grazed by domestic and wild animals. Effective monitoring of vegetation changes in Utah's semi-arid rangelands must separate the influence of climate from animal use and be representative of the entire landscape. Most past assessments were done at the field level and were both spatially and temporally limited. This study used satellite imagery from 1972 to 1998 (27 years) for the privately owned and managed Deseret Land and Livestock Ranch (133,000 acres in northeast Utah) where livestock have been raised for the past 109 years.

A 57 layer Geographic Information System (GIS) was developed and included animal use data (both cattle and wild herbivores), records of prescribed and wild fires, herbicide and reseeding activities, and

locations of water, ecological sites, pastures, and physical, biological, and cultural features. This study examined the relationship between these 57 layers and the time series of satellite imagery to determine if ecological thresholds existed during the change from grassland to predominately woody vegetation.

Goals

- Develop software to process and standardize large multi-temporal satellite datasets.
- Describe the behavior and trend of remote sensing-based ecological metrics.
- Determine the relationship of these metrics to land management (grazing, prescribed fire, etc.), wild fire, and climatic constraints.
- Develop software to analyze the effects of grazing along grazing gradients.

Findings

Soil-Adjusted Vegetation Index – The SAVI was significantly linearly correlated with water availability and non-linearly correlated with grazing. The SAVI detected the effects of both El Niño-Southern Oscillation (ENSO) and la Niña climatic episodes. Validity of the SAVI is dependent on scaling of field data.

Soil Stability Index – Strong relationships were found between the satellite derived SSI and both a soil's field organic matter, and a soil's wind erosion index. The wind erosion index is the theoretical, long-term amount of soil lost per year through wind erosion. The SSI was also significantly correlated with site water-availability and grazing. Over the 27 years of data examined in this study, the SSI exhibited a slightly increasing trend, tending toward stable conditions.

Landscape Patterns – A six category vegetation map was made for each of the 27 years. Landscape metrics showed that fire had little effect on the vegetation distribution in the study area, and that grasslands were declining over time. Though shrub cover remained fairly consistent over the 27 year period, analyses detected an abrupt increase in fragmentation of shrub cover in 1990, a pattern that has persisted since that time. The number of days grazed had the most significant relationship with changes in vegetation.

Grazing Gradients – The typical landscape pattern resulting from grazing arid environments is permanent gradients of vegetation degradation surrounding water sources. There is a decrease in degradation during wet periods as compared to dry periods, and an increase in the extent of degradation as time passes. "Permanent" grazing gradients, with evidence of degradation, were detected at five water sources that were examined in a single test area. However, no evidence of degradation was found in the riparian corridor.

Implications

This study showed that it was possible to reconstruct the history of vegetation changes that have occurred since 1972 on an arid ranch in Utah. Many changes in vegetation occurred coincident with ENSO and La Niña climactic events. Because these events can be predicted with a six month to one year lead time, range managers can opportunistically reduce land degradation by altering grazing patterns, and the timing of burning, seeding and other restoration projects.

Next Steps

The ability to use the satellite derived SAVI to determine vegetation impacts has not been conclusively proven and further analysis is warranted. There is a need to address scaling issues of high frequency remote sensing protocols such as MODIS, which are becoming more readily available and popular.

APPENDIX A – COMMON THEMES

Grant	Study System	Water Quality	Focus of Study	Focus of Study	Focus of Study	Primary Stressor	Genetics	GIS/RS
R82-5865	Forest	Water Quality	Forest			Nutrients		GIS/RS
R82-5866	Forest		Birds	Macro-invertebrates		Anthropogenic disturbance		
R82-5867	Wetlands	Water Quality	Amphibians			Anthropogenic disturbance		GIS/RS
R82-5868	Freshwater	Water Quality	Algae	Bacteria		Nutrients		
R82-5869	Marine	Water Quality	Protists					
R82-5870	Forest	Water Quality	Landscape			Anthropogenic disturbance		GIS/RS
R82-5871	Forest	Water Quality	Macro-invertebrates	Fish	Freshwater mussels	Anthropogenic disturbance		GIS/RS
R82-6112	Grassland		Landscape			Grazing		GIS/RS

APPENDIX B – PRODUCTS

Grant	New Method	Articles	Presentations	Tools/Models/GIS
R82-5865		3	11	1
R82-5866		1	26	
R82-5867		3	36	1
R82-5868		5	17	
R82-5869	1	11	30	1
R82-5870		1	23	1
R82-5871		12	13	2
R82-6112		3	12	3

APPENDIX C – GEOGRAPHIC DISTRIBUTION