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The Influence of Land Use on Fish Health and Fish Communities in Wadeable Steams in South Carolina

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THE INFLUENCE OF LAND USE ON FISH HEALTH AND FISH
COMMUNITIES IN WADEABLE STEAMS IN SOUTH CAROLINA

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A Dissertation
Presented to
the Graduate School of
Clemson University

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of the Requirements for the Degree
Doctor of Philosophy
Environmental Toxicology

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by
Andrew Nicholas Sayer
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Accepted by:
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ABSTRACT

In 2011 the State of South Carolina population was over 4.6 million people and has increased at a rate of around 15% (1.5% per year) throughout the last decade. While the population density per square mile is relatively low compared to other US states, it seems certain the population will further increase in years to come. Increasing population density leads to urbanization resulting in an increase in impervious surfaces such as roadways, parking lots, and building roofs. This changing land use can have dramatic effects on smaller streams and creeks which form the upper reaches of watersheds. Although water systems can become fragmented, watersheds encompass the entire drainage area of a region. A changing landscape upstream can have dramatic effects many miles downstream from the initial source of disturbance.

Freshwater species worldwide face accelerated extinction rates relative to most other wildlife taxa. The southeastern U.S. in particular is of high concern due to long term declines in native fish and aquatic species. Consequences of poor land management practices (i.e. siltation, excessive nutrients, flow disruption) can negatively impact flora and fauna that depend on these water sources for survival, reproduction, and/or development. Due to the interconnected nature of water systems, water flow will end up in larger rivers, reservoirs, and coastal areas. Because of the importance and uniqueness of these habitats, local flora and fauna could be at high risk if the wadeable streams in the upper reaches are developed. Currently there is not enough information on how the surrounding landscape influences the quality of water and aquatic ecosystems to make

informed decisions regarding aquatic conservation and restoration. Because the human population is ever increasing, a better understanding of anthropogenic influences would allow us to make better, more informed land management decisions.

The goal of this dissertation was to study effects of a changing land use on fish health and fish assemblage by measuring a set of biochemical biomarkers in an abundant fish species, *Lepomis sp.* (sunfish), commonly found in wadeable streams in South Carolina. The data set incorporates information on land use, fish species assemblage, abiotic habitat characteristics, and biomarker responses from over a hundred random wadeable stream sites throughout the state. Biomarker responses, which are changes at the biochemical and cellular level, were correlated with changes throughout multiple levels of organization (i.e. tissue, individual, population, and community).

Results of this work indicate *Lepomis sp.* are a widely distributed fish type that can be used as a model to represent all fish species in an assemblage at a sampling location, the magnitude of chemical contamination detected in *Lepomis sp.* via biomarker response can be used to determine changes in overall fish assemblage structure, and 10% urban surface and greater is a threat pathway leading to deleterious effects on aquatic ecosystems (both in disruption of fish assemblage integrity and increasing biomarker response). Within a watershed, fish health at the organism, population, and community scales declines concomitantly in response to increasing urban surfaces.

As the human population continues to increase, there will be an increased burden of aquatic contaminants resulting in a decline in the diversity of fish and other aquatic life. Sensitive species will be the first to disappear. Eventually, a watershed may become so badly deteriorated that only a few tolerant or specialized species will remain. Results of this research revealed threat pathways to fish health and aquatic resources, identify the magnitude of the anthropogenic impact on watersheds at a statewide level, and provide a scientific basis for sustainability from the scientific community to stakeholders, land developers, and policy creators.

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Lastly, I'm not an overly spiritual or religious person or anything, but I'd like to thank whatever it is we're inherently given as humans that allows us to persevere. That thing that picks us up after our failures, that drives us to move forward when everything seems to have fallen apart. Finishing this dissertation is more than finishing at Clemson, its closure to all my time spent in grad school, when I honestly didn't think I could do it but refused to believe I couldn't. Who do you thank for that part of you that refuses to allow

you to give up? The Almighty? Good genes? Completing this dissertation had nothing

to do with proving people wrong who thought I couldn't do it. It was about proving to

myself that I could.

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CHAPTER 1: INTRODUCTION

Introduction

This project is a component of a larger, long-term assessment of South Carolina's wadeable streams. The disciplines of landscape ecology, aquatic systems ecology, environmental toxicology, and watershed management are encompassed within the scope of the overall objectives. The central theme presented within this work characterizes threats to aquatic resources from a variety of potential sources focusing on landscape alteration. While the classification of ecological disturbance will be heavily emphasized, the ultimate intent is to provide evidence promoting a need for aquatic system conservation, sustainability, and restoration.

This dissertation is divided into four interconnected sections. The first section (Chapter two) presents a comparison of *Lepomis sp.* as the model fish species used to compare total fish species abundance and diversity at sample sites. In order to characterize presence/absence of fish species found at sample sites an association must be made between molecular, individual, and population responses within *Lepomis sp.* prior to extrapolation to other fish species. The second section (Chapter three) builds on findings in Chapter two to compare biomarker response in *Lepomis sp.* with total fish species assemblage structure at sample sites. Once associations are made in chapters 2 and 3, the third section (Chapter four) examines influences of urbanization on fish health at statewide and river basin levels. Finally, the fourth section (Chapter five) examines changes throughout multiple levels of biological organization.

Conceptual Background

In 2011 the State of South Carolina, USA population was over 4.6 million people and has increased at a rate of around 15% (1.5% per year) throughout the last decade. While the population density per sq. mile is relatively low compared to other US states, it seems certain the population will further increase in years to come. Increasing population density leads to urbanization resulting in an increase in impervious surfaces like roadways, parking lots, and building roofs. This changing land use can have dramatic effects on smaller streams and creeks which form the upper reaches of watersheds. Although water systems can become fragmented, watersheds encompass the entire drainage area of a region. A changing landscape upstream can have dramatic effects many miles downstream from the initial source of disturbance (Scott, 2006).

As the percentage of urban and impervious surfaces increases as a result from development, there is growing concern over the environmental impact (SARP, 2007; SCDNR, 2006). In eastern North America, the development of once forested land causes habitat loss and interferes with natural processes and cycles especially in aquatic systems (Dudgeon et al., 2006; Vitousek et al., 1997). For example, during rainfall events metals, polycyclic aromatic hydrocarbons (PAHs), and other contaminants are washed into lakes, rivers, and streams instead of being infiltrated by the ground or taken up by plants. Contributing to the issue are structures necessary for urban sustainability such as wastewater treatment and sanitation facilities. It has been discovered that wastewater treatment facilities are not completely effective in eliminating contaminants and

chemicals (such as endocrine disrupting compounds) from the water supply (Auriol et al., 2006; Kolpin et al., 2002; Truman et al., 2009). As a result many pharmaceuticals and other personal care products are accumulating downstream of these facilities with unknown consequences on fish and aquatic organism health. Furthermore, these chemicals are being found in drinking water which is often prepared from surface water.

Strategies for sustainability can only be developed if scientific data exist to support decisions. However, currently there is not enough information on how the surrounding landscape influences the quality of water and aquatic ecosystems to make informed decisions regarding aquatic conservation and restoration (Palmer, 2009). Because the human population is ever increasing, a better understanding of anthropogenic influences would allow us to make better, more informed land management decisions.

Watershed Management and Land Use

Successful aquatic conservation includes the surrounding landscape when analyzing watershed impacts (Allan, 2004; Scott, 2006). During storm and rainfall events, runoff introduces metals, nutrients, and other contaminants from the watershed to the water system (nonpoint source pollution). Fish and other organisms living in streams are among the first animals to become exposed. Fish sensitivity and resiliency to contaminants determines which species remain or leave once a toxic threshold is exceeded. Yet, which species are more or less sensitive than others during times of contaminant stress is largely unknown. Specific landscape characteristics which

contribute to contamination are also uncertain. Land use information, fish abundance and diversity data, water quality parameters, and fish health measurements would be the data required to address this question.

Watershed Studies

Studies examining watershed health are not uncommon albeit at different scales and endpoints. The impact of landscape alteration on tidal estuarine ecosystems has been examined by the National Oceanic and Atmospheric Administration's (NOAA) National Centers for Coastal Ocean Science organization in studies lasting several years. Their findings revealed impervious cover greater than 10% resulted in conditions unfavorable for macrobenthic taxa. Conditions became detrimental to organisms when impervious cover was greater than 30% (Holland et al., 2004). Subsequent analysis revealed food web degradation was due to water quality deterioration as a result of increased urbanization of the surrounding landscape (Lerberg et al., 2000). Another tidal estuarine study examining watershed land use and grass shrimp health found increased PAH levels in close proximity to urban and impervious cover (Garner et al., 2009). Chapman found an increase in rates of detoxification transcription enzymes in oysters as a result of increasing population growth in coastal areas (Chapman et al., 2009). He attributed deterioration of the watershed from urbanization as the major factor in the increase in detoxification system expression.

While estuarine ecosystems are highly sensitive, similar relationships have been found in freshwater systems. Allan found strong decreases in habitat and biological indices of integrity in freshwater ecosystems as agricultural land cover increased greater than 20% (Allan et al., 1997). Another study reported freshwater biodiversity declining from a number of anthropogenic sources including water pollution, flow modification, and habitat degradation (Dudgeon et al., 2006). A comprehensive report by Schueler revealed several negative associations between urban and impervious cover and watershed degradation. Watershed runoff coefficients, nutrient inputs, and water temperature increased as a result of percent increase cover. Best management practices presented some stability but were ineffective as percent impervious cover increased. The study by Schueler revealed freshwater fish, insect, habitat quality, and other aspects of biological integrity all declined when urbanization was greater than 10% (Schueler et al., 1994).

While smaller scales studies are important, larger scale watershed research can be conducted successfully. Some current studies over a large geographic scale encompassing multiple watersheds have been ongoing for decades. One example of this is the International Boundary and Water Commission (IBWC) involving the United States and Mexico. In addition to water regulation, the IBWC focuses on sustainability and threats to conservation by conducting environmental assessments, water quality reports, and environmental impact investigations. Studies examining metal introduction to the water system, nutrient loading, pesticides, aquatic life, and biological control

organism have been undertaken by the IBWC. They continue to work to improve water quality and find threats to aquatic resources internationally (IBWC, 2011). The Hudson River Watershed Alliance (HRWA) centered in New York is another large scale study of human influences on the Hudson River watershed. Like the IBWC, the HRWA identifies threats to the Hudson River, determines best management strategies, and conveys findings to the public. This group conducts community outreach and information sharing with shareholders within the watershed (HRWA, 2011).

The research presented in this dissertation attempts to quantify changes in fish assemblage and biomarker response due to watershed deterioration from anthropogenic development at the statewide level. Similar to previous watershed studies, the large scale and types of responses were measured and assessed for the magnitude of change. While the endpoints are different, biomarker responses can be used as an indication of a changing aquatic ecosystem. Changes in biological integrity, as indicated by changes in a fish assemblage, can also be indicative of an altered surrounding landscape. However, due to the interconnected range of watersheds, disturbance at the upper reaches may affect a fish assemblage many miles from the initial source of disturbance. Responses were seen in both biomarker response and fish assemblage, both of which were negatively associated with increasing urban surface in a watershed. Our results suggest >10% urban surface results in a significant increase in biomarker response and other health and fitness indicators. Reducing the scale from the state to river basin, relationships were even more pronounced (which are possibly attributed to the size of the

project and spread of sample locations). The study conducted here are similar in purpose to watersheds conducted by the previously mentioned groups/organizations. The impact of human development has altered ecosystem dynamics and will continue to do so until a sufficient dataset is conveyed to those with the authority to instill policy change.

Sedimentation and Siltation

Sedimentation is the settling out of suspended particles in an aquatic environment. During a storm event, particulate matter is flushed into water systems and carried downstream. Over time these particles will settle to the bottom or substrate level. However, this process occurs slowly, is size-dependent based on the physical properties of the suspended particles, and is water dependent. Related to sedimentation, siltation occurs when erosion or other terrestrial inputs enter the aquatic system and remain suspended (ARC, 2003).

During a storm event, nutrients, garbage, terrestrial fuel leaks, breakdown materials from cars, guardrails, and anthropogenic structures as well as other contaminants are washed into channels. Erosion processes occur through the deterioration of the sides of banks. Other times storm drains incorporate pipes inputting water directly into natural channels and streams. Inevitably, smaller fragmented water channels connect to form bigger, more centralized water systems such as creeks or streams.

During a storm event water is carried into these channels at high velocities thereby increasing the energy of the water flow (Hewlett et al., 1984). Upon reaching the channel, water at high velocity stirs up the sediment, creates a turbid aquatic system, and causes bank erosion. Siltation and sedimentation are particularly problematic wherever there is curvature of the stream channel. Suspended particles hit the curved surfaces washing up disturbed sediment alongside the bank. Deposits alongside these areas contain soils impacted on the side of the channel. Vegetation in the riparian zone (and prior to the riparian zone) is capable of extracting/removing nutrients, minerals, contaminants, and other constituents from water entering the aquatic system. Removal of flora in the riparian zone is of particular concern because this vegetation helps stabilize the bank from further erosion (ARC, 2003).

Effects of Sedimentation and Siltation on Fish

Sedimentation and siltation create a turbid aquatic environment. This has several consequences for fish and other aquatic organisms. In a highly turbid environment predation is greatly reduced as a direct result of decreased visibility. Suspended particles provide breeding sites for bacteria, viruses, and parasites which can become ingested by fish. Additionally, metals, PAHs, contaminants, and other toxicants bind with suspended sediment and become ingested. The suspended particles clog gills of fish which can impede oxygen exchange. As particulate matter settles it can smother and suffocate eggs and larvae on the benthos (Jones, 2011).

A decrease in light penetration is another effect of a turbid body of water. As light is attenuated there is a decrease in photosynthetic production from aquatic vegetation. Aquatic vegetation is a valuable food source for aquatic invertebrates and other organisms. As a result, there is disruption throughout the food web and food web dynamics. A second drawback to a reduction in aquatic plants is a decrease in oxygenation of the surrounding water which can ultimately lead to anoxic zones (Davies-Colley et al., 2001; Jones, 2011).

In some circumstances gills are able to adapt as the aquatic environment changes. Because gills come into contact with chemicals in the immediate aquatic environment they are vulnerable to contaminant stress. Coping mechanisms have evolved as adaptive responses to certain stressors. One example involves gill epithelial thickening (hypertrophy) and increasing the number of cells (hyperplasia) upon excessive mercury exposure making it more difficult for chloride to pass into the fish. A thickening of the gill epithelium would hinder toxicant progression and allow the fish to detoxify or excrete mercury more rapidly (Newman et al., 2008). This coping mechanism would also decrease the water exchange rate and increase the distance between water and blood. These types of cellular and physiological processes protect the fish from acute contaminant exposure. Toxicants which induce these types of responses in fish gills are the metals copper, nickel, and zinc. If fish are unable to tolerate a turbid environment then they must adapt, migrate to a new environment, or die.

Total dissolved solids (TDS) are another stressor to fish in freshwater systems. High concentrations of dissolved ions can greatly disrupt ionoregulation. An imbalance of Na^+, K^+, Ca^+, Cl^-, and additional ions results in complications associated with cellular homeostasis. Additionally, toxic ions including Cu, Ar, Co, and others can displace and interfere with uptake pathways even in small quantities. If ions are elevated in the water column as a result of runoff, sedimentation, siltation, then the consequences on fish and other aquatic organisms will be much more severe (Newman et al., 2008).

Best Management Practices

There is a growing body of evidence suggesting land alteration leads to watershed deterioration. Even minor alterations in the landscape such as removal of trees from a forest result in increased nutrient runoff into freshwater systems (Hur et al., 2008). Development of once undisturbed land by removal of trees, shrubs, and other vegetation disrupts or eliminates groundwater infiltration. The removal of flora prevents the uptake of nutrients and other constituents through the roots. This is especially problematic at the riparian zone along streams or creeks where plant roots solidify the bank before the water body. Removal of plants from these areas leads to an increase in soil erosion causing instability. In addition, without riparian flora excessive amounts of nutrients and contaminants are allowed to enter the water system at high velocities during storm events (Casey et al., 2001).

Due to increasing awareness in contaminant runoff from landscape development, creation of best management practices (BMPs) has resulted. BMPs are designed to limit and/or control point and non-point sources of pollution (Lynch et al., 1985). There are several design types of non-structural and structural BMPs. Non-structural BMPs include open vegetated conveyance systems, natural stream buffers, disconnected rooftop to pervious area, grass or alternative paving surfaces, and natural infiltration. Structural BMPs include dry detention basins, wet detention ponds, storm water wetlands, biofilters, media filters, and manufactured hydrodynamic devices (GCSC SWMDM, 2010; SCDHEC, 2011).

BMPs are designed to mitigate stormwater runoff into water systems especially at high velocities. The effectiveness of BMPs is dictated by site-specific characteristics, influent concentrations (total suspended solids, phosphorus, nitrogen, nitrate, copper, lead, zinc, fecal coliforms, PAHs, etc.), flow rates, and flow volumes (GCSC SWMDM, 2010). BMPs include strategies to reduce high velocity runoff into creeks, streams, and rivers thereby reducing turbidity.

The higher the volume of water during a storm event, the higher the burden placed on the BMP to remain effective. This can be problematic when a high amount of contaminants have quickly accumulated in the storm water. This can also be problematic as the percentage of impervious surface increases. A study by Schueler, 1994, demonstrated a 0.95 Pearson correlation between percent impervious surface and water runoff (expressed

as a runoff coefficient). This is compared to a meadow with a 0.06 runoff coefficient during a storm event. Therefore, as the percent impervious surface increases, the more water will runoff from the landscape into a collecting body of water (Schueler, 1994). Peak discharge rate, runoff volume, runoff velocity, and phosphorus, nitrogen, and zinc load were shown to increase when the surface was impervious (parking lot) compared to non-impervious (meadow).

Land Use Classifications

The United States Geological Survey (USGS) Department of the Interior functions as an agency that evaluates land use and how land has changed over time. This information is provided on their website (www.usgs.gov) to the general public. As computer software has evolved throughout the years, data presented by the USGS can be examined to determine land classifications, land alteration, elevation, water flow and hydrology, vegetation, roads, animal movement, and many other types of information (US Geological Survey, 2011).

The USGS provides a dynamic online map interface used to view datasets called the Multi-Resolution Land Characteristics Consortium (MRLC) generated from data by the Environmental Protection Agency (EPA). The MRLC incorporates data from the Earth Resources Observation and Science (EROS) center satellite imagery to map the earth and provide a composite of land classes called the Seamless Data Distribution System. The USGS seamless digital elevation models and land cover dataset are used in ESRI's Arc

GIS v. 9.0 spatial analyst extension and flow direction and flow accumulation to delineate watersheds upstream of stream sample locations. USGS models can also be used to categorize 100 m riparian buffer zones and compare changes over time based on the available land use dataset available. Using what is known as the Anderson Classification system created in 2006, land has been categorized into the following designations: open water, barren, forest, glassland/shrubland, agricultural, wetlands, and urban (reduced from over 21 land cover class definitions for 1992). Within each category subdivisions exists further differentiating each subgroup. For example, the urban classification can be subdivided into low, medium, and high intensity to more accurately depict residential areas with lawns and gardens from such things as parking lots and roadways (US Geological Survey; MRLC.gov).

How land is used greatly influences the surrounding watershed (Scott, 2008). Not only is runoff from impervious surface greater in developed areas, contaminant input into the water system is increased. Additionally, different land classifications may result in different contaminant inputs into the water system. Runoff from heavily urbanized settings includes PAHs, metals, personal care products, pharmaceuticals, etc. compared to agricultural settings that would introduce nutrients into a water system, nitrogen and phosphorus in particular. Pesticide runoff is also a problem in agricultural settings which can cause fish and other aquatic organism mortality. The effects of pharmaceuticals and personal care products on fish and wildlife are largely unknown. Recent studies suggest

behavioral abnormalities and amphibian feminization may occur (Solomon et al., 1996; Sowers et al., 2009).

Biomarkers

Biomarkers are changes in cellular or biochemical structure, function, or behavior measurable in bodily fluids, tissues, or organs. They are used to gauge physical, chemical, or biological stressors which can be attributed to certain environmental conditions. Biomarkers have been demonstrated on multiple occasions to be useful indicators of health status within an organism (Gomez-Martinez et al., 2006; Schreiber et al., 2006; Van der Oost et al., 2003). As opposed to designing new biomarkers for measuring contaminants in the environment our objective is to utilize existing, validated biomarkers to assess fish health.

Biomarkers provide valuable information at the molecular level of biological organization. Cellular alterations as a result of an environmental change form the basis of a cascade of subsequent effects within higher up levels of organization. Measuring these changes enables an investigator to make associations with cause-and-effect endpoints. However, because a cellular change can be measured does not necessarily imply negative outcomes will occur within that organism. Biomarkers of exposure are defined as cellular alterations leading to a measurable cellular change but do not indicate biological harm is occurring. Conversely, biomarkers of effect are measurable cellular alterations associated with potential health impairment due to an exposure event. Finally,

biomarkers of susceptibility are measurable organism responses to an external contaminant, chemical, or other agent that may predispose that individual to a future negative adverse affect (Keaton, 2007).

On the large scale, biomarkers have been proposed to be valuable tools to assess changes throughout multiple levels of biological organization (van der Oost et al., 2003). They have been used as important tools in ecological risk assessment and used as bioindicators by the World Health Organization, the Organization for Economic Co-operation and Development, and the European Centre for Ecotoxicology and Toxicology of Chemicals. Other studies examined more specific impacts of biomarker responses on fish health. One study examined contaminant exposure and liver and skin tumor prevalence in brown bullhead fish in the tidal Potomac River watershed. Evaluations were made for CYP 450 activity, PAH metabolites, organochlorine pesticides, and PCB concentrations. Significant differences were observed in liver tumor prevalence related to exposure to contaminants in the water system (Pinkney et al., 2001). Another study by Mathieu et al., 1996 found higher levels of EROD activity and a decrease in acetylcholinesterase activity in a St. John's, Newfoundland watershed. The trout used in the study were taken from the Virginia and Rennie's Mill Rivers and compared with the nearby South Brook reference river (Payne et al., 1996). A third study evaluated indicators at the population level using allozymes, CYP 450 activity, and DNA strand break formation using chub from the surrounding Rhone River watershed. The study authors noticed an increase in allele 90 at two contaminated sites compared to the reference site, high EROD activity,

and significant DNA damage at one of the two contaminated sites. A high HSI ratio was associated with chronic exposure of the fish to pollutants which the authors associated with high DNA damage repair. The authors associate fish with low DNA damage and/or high EROD activity as being tolerant to pollutants. Lastly, the study authors associated heterozygous fish in the contaminated systems as a necessity to survive in such a deteriorated aquatic ecosystem (Larno et al., 2001).

While studies have been conducted evaluating specific pollution threats on fish health and fish populations, few studies have been conducted integrating land use, water quality measurements, and the application of biomarkers as indicators of exposure to contaminants. An understanding of how these parameters influence each other will allow us to elucidate mechanisms associated with fish fitness and the disappearance of fish species. Therefore a comprehensive study linking landscape characteristics with aquatic endpoints is needed.

Biological Levels of Organization

Expanding the confines of biomarkers at the cellular level, it is possible to compare how changes at the molecular level influence parameters at the tissue/organ, individual, population, community, and ecosystem levels of organization. For example, chronic exposure to a hepatotoxin will result in liver impairment. When the liver is assessed it can be quantified via the hepatosomatic index (HSI). Measuring a dysfunctional liver using the HSI will not provide information regarding changes at the molecular level.

Likewise, molecular alterations at the cellular level do not necessarily imply a harmful effect on the liver. However, if measurements at the molecular level can be correlated with alterations at the tissue/organ level then there would appear to be a progression up the biological levels of organization providing evidence for causation.

Indices exist for measuring tissues/organs, organisms fitness, population composition, and community structure, each of which is another level of biological organization. These metrics are designed to ascertain a given parameter at a given point in time. Inferring relationships at any one of these levels is inappropriate without understanding the contributing factors to that level of organization and the ability of a level to tolerate changes. Furthermore, individual and species variation exist which provide an additional source of uncertainty. However, utilizing an approach capable of linking changes at the molecular level throughout all levels of organization would be a novel way to assess ecosystem change.

Fish Assemblage

Biological integrity can be measured several ways, however, community or assemblage levels are considered to be the most robust and representative (Hughes et al., 1998). A fish assemblage is best defined as groups of fish populations overlapping at the same place at the same time. A fish assemblage differs from a fish community in that a community incorporates the surrounding ecosystem and ecosystem dynamics which are constantly changing.

Incorporating a fish assemblage into a species diversity and abundance analysis is beneficial for a number of reasons. Individual species richness, habitat requirements, guild composition, health, and other metrics can be determined and scored by a common indicator. Likewise, disparities such as ecoregions, river basins, location, range, and other parameters can also be determined. Factors that influence a fish assemblage will likely reflect outside stressors once internal covariation is accounted for. In this way, a fish species gradient can be made identifying species with specific habitat requirements (i.e. abiotic parameters) and other responses to environmental stressors.

With natural variation such as geographic location accounted for it is possible to focus on fish species sensitivity. This is especially important during times of contaminant influx when fish are exposed to a number of external stressors, both physical and chemical in nature. While contaminant influx might occur at point sources in the aquatic environment, another input occurs during storm events. During these events contaminant

runoff enters the aquatic ecosystem directly contacting fish and other organisms that reside there. If a fish species is able to tolerate contaminant fluctuations by upregulating detoxification mechanisms, then that species will likely remain at that site. If, however, a fish species is not able to tolerate sudden contaminant increases they will likely migrate away from that site or die.

Lepomis species

From the family *Centrarchidae*, the *Lepomis* genus is a freshwater fish species common to the southeastern United States. The complex St. Lawrence, Great Lakes, and Mississippi River systems all contribute to the migratory routes this genus has taken within the continental United States. It can also be found in Hawaii, Africa, South America, and Europe (Sims-Parr, 2002). Due to the geographic range of *Lepomis sp.* it is worthy to note this fish can accommodate to different environmental conditions. Despite the wide geographic dispersion, the home range of *Lepomis sp.* is generally less than 50 meters (Delinsky et al., 2009).

The species of *Lepomis sp.* commonly found in wadeable streams in South Carolina include *Lepomis macrochirus* (bluegill sunfish), *Lepomis auritus* (redbreast sunfish), *Lepomis gulosus* (warmouth sunfish), *Lepomis microlophus* (redear sunfish), *Lepomis gibbosus* (pumpkinseed sunfish), *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish). These species prefer slow moving, rocky streams but can also be found in deep water pools or smaller pools where vegetation is present. The main

diet of *Lepomis sp.* consists largely of invertebrates, aquatic insects, zooplankton, algae, and other smaller fish. While other species of the genus *Lepomis sp.* exist, this study will focus on these seven species due to the high prevalence in wadeable streams throughout South Carolina. Of the seven species, *Lepomis marginatus* and *Lepomis punctatus* are considered to be least tolerant to habitat alterations and therefore the most sensitive of *Lepomis* species.

Lepomis sp. are found in a wide geographic range because they are a more tolerant type of fish. During times of contaminant input these fish are more resilient than other fish species making them a good indicator for a changing habitat. Therefore, *Lepomis sp.* can be found in aquatic systems where contaminants are present while other fish species have left the area. The US Environmental Protection Agency (EPA) has identified members of the *Centrarchidae* family as biological indicators of watershed health (EPA, 2010). The EPA uses *Lepomis sp.* and other *Centrarchidae* family members in indices to determine levels of contamination within a water system. Usually measurements can provide information regarding the surround landscape upstream from the sample site. The goal of the EPA is to evaluate a water system and determine if aquatic life is negatively influenced. In addition to measuring water quality characteristics, assessing biological data as an indicator of water quality is one way this goal can be met.

In this study *Lepomis* are used as a model species for contaminant input. Taking into account water systems incorporate the drainage of everything upstream, an examination

of a sentinel fish species can provide valuable information regarding land alteration, contaminant input, fish assemblage and diversity, and biomarkers as indicators of environmental contamination. The high prevalence of *Lepomis sp.* in wadeable streams throughout South Carolina provides this information at a statewide level. Utilizing *Lepomis sp.*, contaminant measurements can be made in the aquatic ecosystem which can then be related to the surrounding landscape at the watershed level. Additionally, a fish assemblage gradient can be constructed indicating which fish species are more sensitive to contaminant stress compared to those less sensitive to contaminant stress. The resiliency and tolerance of *Lepomis sp.* to contaminant fluctuations makes this fish and ideal species for comparative purposes.

Statement of Rationale and Environmental Need

Freshwater species worldwide face accelerated extinction rates relative to most other wildlife taxa (Ricciardi et al., 1999; Sala et al., 2000). The southeastern U.S. in particular is of high concern due to long term declines in native fish and aquatic species. The Southeast Aquatic Resource Partnership (SARP) and SC Department of Natural Resources (SCDNR) are two agencies which share this concern and are looking for ways to identify problems and create solutions. In 2006 the SCDNR submitted a Comprehensive Wildlife Conservation Plan to the U.S. Fish and Wildlife Service which included descriptions of 125 species of fish, herpetofauna, crayfish, and snails that are dependent on aquatic systems for most or all of their life-stages (SCDNR, 2006).

Aquatic systems are highly complex, interconnected, and encompass the entire drainage area of the watershed. Drought and flooding can complicate issues by fragmenting or merging streams, rivers, and lakes (Pringle, 2001). Consequences of poor land management practices (i.e. siltation, excessive nutrients, flow disruption) can negatively impact flora and fauna that depend on these water sources for survival, reproduction, and/or development. Due to the interconnected nature of water systems, water flow will end up in larger rivers, reservoirs, and coastal areas. Because of the importance and uniqueness of these habitats, local flora and fauna could be at high risk if the wadeable streams in the upper reaches are developed (Marion, 2008). Human dependence on aquatic resources (i.e. drinking water) could be in jeopardy as well.

Results of this research will provide the basis upon which to quantitatively predict and monitor changes in ecosystem health as a consequence of urbanization. Randomly selected wadeable streams throughout South Carolina serve as the sample sites to connect the condition of uplands drained by the stream to the quality of water and aquatic habitat. A study of this size employing biomarkers has not yet been attempted. It could serve as a model for future studies evaluating additional parameters of human influence on the aquatic ecosystem. As the human population continues to increase, it will be essential to have a scientific-based understanding of the anthropogenic influence on the environment. Land management strategies and risk assessments need to have scientific basis before recommendations and policies can be made. Often risk assessments are made with a best

estimate based on available information. This study will be one of the first to provide scientific evidence of a large-scale need for conservation efforts in South Carolina.

The large data set we are generating incorporates information on land use, fish species assemblage, abiotic habitat characteristics, and biomarker responses from dozens of wadeable stream sites throughout South Carolina. Biomarker responses, which are changes at the biochemical and cellular level, are correlated with changes throughout multiple levels of organization (i.e. tissue, individual, population, and community). The primary goal of this dissertation is to study effects of a changing land use on fish health and fish assemblage by measuring a set of biochemical biomarkers in an abundant fish species, *Lepomis sp.* (sunfish) commonly found in wadeable streams in South Carolina.

Objectives

The following objectives were explored in an effort to describe sources of environmental contaminants as well as determine mechanisms of response observed in levels of biological organization.

Chapter Three

Objective: Does the occurrence of *Lepomis sp.* correlate with total fish species abundance and diversity?

Null Hypothesis: There is no correlation between the presence of *Lepomis sp.* and total fish species abundance and diversity.

Fish populations depend on several aspects of the aquatic environment for survival such as water chemistry, habitat requirements, predation/prey dynamics, and survival aspects. However not all fish species are alike and general indicators of fish health may be unable to associate environmental stressors with the disappearance of a species. Some fish species are more resilient than others making them more tolerant in times of stress. Yet it is unclear which fish species are more tolerant when levels of contaminants increase and which species are more sensitive to changes and disappear from the assemblage.

Lepomis is a more tolerant fish genus found in freshwater systems. They are generally one of the first fish species to migrate to a previously contaminated area and become an established population. Because of this *Lepomis sp.* will likely be present at sample sites regardless of the conditions of the immediate aquatic environment (unless the aquatic body is severely degraded) (EPA, 2010). Those fish species that require more selective living requirements will not be found at sample sites unsuited to their specific living needs.

The specific life history characteristics of fish differ among species. In order to determine associations between contaminant responses in fish, a relationship between *Lepomis sp.* and other fish species needs to be established. The presence of *Lepomis sp.*

at sample sites will serve as an indicator of fish species and diversity and form the basis upon which subsequent comparisons between fish species sensitivity can be made. Chapter two will explore these relationships and determine if *Lepomis sp.* can be a representative fish genus used to represent the entire assemblage.

Chapter Four

Objective: Can *Lepomis sp.* biomarker response be used to indicate total fish species assemblage structure?

Null Hypothesis: There is no correlation between *Lepomis sp.* biomarker response and total fish species assemblage structure.

Biomarkers are changes in cellular or biochemical structure, function, or behavior measurable in bodily fluids, tissues, or organs. They are used to gauge physical, chemical, or biological stressors which can be attributed to certain environmental conditions. Biomarkers have been demonstrated on multiple occasions to be useful indicators of health status within an organism (Gomez-Martinez et al., 2006; Schreiber et al., 2006; Van der Oost et al., 2003).

Chapter three will assess fish health and ascertain whether biomarker measurements can predict which fish species in an assemblage are more sensitive during times of contaminant stress. Fish assemblage is defined as groups of fish populations living in the

same areas at the same time. *Lepomis sp.* are used as the model species to make predictions regarding levels of aquatic contamination. Since *Lepomis sp.* are considered a sentinel species when contaminant levels are high in aquatic systems, their biomarker response will indicate a proximate level of contaminant exposure in all fish in the assemblage. Due in part to their tolerance, the *Lepomis* genus will represent levels of biomarker response for the fish assemblage present at each site.

Chapter Five

Objective: Does urban surface significantly influence the health of fish?

Null Hypothesis: There is no relationship between urban surface and fish biomarker response in *Lepomis sp.*, fish health, fish population, and fish assemblage.

The development of land as a result of an increasing human population contributes to increasing percentages of urban and impervious surfaces. As more undisturbed land is converted to developed land natural processes will be altered and/or disrupted. During storm events less water will be infiltrated by the ground but instead run off into streams, rivers, and other bodies of water. Nutrient export, for example, has been reported to increase in rivers even after minimal human development (Hur et al., 2008). The high water velocity of runoff into drainage systems leading into channels causes erosion, sedimentation, and siltation (Scott, 2008). For areas where there is a great amount of

human development, increased levels of contaminants, sediment, and other substances entering the water system during storm events.

In an effort to quantify relationships between land development and fish health, Chapter four will evaluate biomarker responses measured in *Lepomis sp.* from wadeable streams in urbanized areas compared to undeveloped reference areas. Activation of metabolizing enzymes, PAH concentrations, estrogenicity, oxidative stress levels, mercury concentrations, and organism level fitness indicators will be assessed. These relationships will be continued through the population and community levels of organization for assemblage comparison at site locations. Biomarker and fish assemblage assessments will be compared to percent urban surface.

Chapter Six

Objective: Are there changes in *Lepomis sp.* molecular, organ, organism, population, and total fish assemblage levels of organization as a result of contaminant exposure?

Null Hypothesis: There are no changes between multiple levels of biological organization in *Lepomis sp.*

At higher levels of biological organization it can be difficult to determine if groups of organisms are being exposed to a stressor at the molecular level for several reasons. Detoxification mechanisms, behavior modification, and gene activation are examples of

how organisms adapt to a changing environment. The ability to leave or migrate away from a stressful ecosystem is another strategy employed (Schueler, 1994). While research may indicate a contaminant is harmful to cells in laboratory experiments it may not accurately reflect detoxification processes and coping strategies utilized by a living organism. Therefore toxic effects at the molecular level do not necessarily result in increased mortality at population or community levels. However, energy needs to be expended to cope with the harmful agent (Siligato et al., 2001).

Although it can be difficult to link changes at the assemblage level to a molecular level stressor, exposure to a contaminant at a high enough concentration for a long enough duration could result in a harmful effect. Chapter five will examine relationships between the molecular, tissue/organ, individual, population, and assemblage levels of organization. This chapter will examine the magnitude of biomarker response in *Lepomis sp.* and carry it through multiple levels of organization.

Environmental Benefits of Analysis

As the human population continues to grow the need to carefully monitor aquatic resources becomes imperative. Pollution of aquatic resources could harmfully affect exposed flora and fauna. Not only will fish and other aquatic organisms be at risk for negative health outcomes, but there is a real possibility terrestrial wildlife and humans could be exposed to contaminants and experience negative health consequences. Humans may be dependent on water more than any other resource for a variety of reasons. In an

article published in Science by Viousek et al. (1997) regarding human population growth, "Of all of the environmental security issues facing nations, an adequate supply of clean water will be the most important." Relating land use to fish health through the application of biomarkers appears to be a promising way to assess aquatic integrity.

In 2008 Hur et al. reported even minimal changes in land use leading to development caused an increase in nutrient runoff as well as an increase in storm hydrograph. Hwang el al. (2006) reported that during storm events an increase in urban runoff resulted in elevated exposure of metals and PAHs to fish and other aquatic organisms. These studies indicate that 1) the presence of urban/impervious surfaces leads to an increase in contaminant runoff that will enter the aquatic environment (i.e. stream, river, lake), and 2) fish and other organisms living in the watershed will be exposed to these contaminants.

The results of this investigation will reveal threat pathways to fish health and aquatic resources. They will elucidate which fish species are most resilient during times of stress, and which species are the first to disappear from a site. Since there will be a progression from least-developed to highly-developed land, the spectrum of fish disappearing first (most sensitive) to those that disappear later (less sensitive) will be clarified. Our data will help better understand the link between land use and aquatic contamination. The data will allow us to better ascertain the magnitude of the anthropogenic influence at a statewide level. In addition to the science gained, our results

can be used to communicate preservation and low-impact land management strategies from the scientific community to stakeholders, land developers, and policy creators.

Expected Outcomes

Elevated biomarker levels in developed areas are one expected outcome. Streams located in rural settings, expected to be least-affected by human influence, will be viewed as reference sites for comparative purposes. Developed or developing sites would be those most likely to demonstrate elevated biomarker responses in fish. Upon exposure to some contaminant(s), responses in *Lepomis sp.* will be increased at those sites. Due to the suite of biomarker indicators proposed in this study there is a high probability a response will be detected for a contaminant(s) if present (Keaton, 2007; Van der Oost et al., 2003).

Somatic indices offer insight to general health of fish. A fatty liver may be a sign of an environmental stressor triggering dysfunctional lipid metabolism, which can be detected from the HSI. Likewise, exposure to toxicants could be related to an enlarged spleen, which would be detected by the SSI. An abnormal GSI value would be indicative of improper gonadal development. These fitness indicators could be linked with other biomarker indicators to strengthen a relationship between the impacts of urbanization on fish health and population viability (Mayon et al., 2006).

Given that wastewater treatment facilities are not completely capable of removing compounds with estrogenic properties, pharmaceuticals, and other personal care products,

it would be plausible sites located nearby or downstream of these facilities will evoke a response detected via molecular, tissue, or organism levels assessments (Keaton, 2007; Sowers et al., 2009). While there may be instances substances enter the environment naturally, we postulate the highest number of sites that elicit a detectable response will be near these urban centers or wastewater treatment facilities (Truman et al., 2009).

Early results of our research have reinforced the need to continue the investigation. An initial investigation conducted by Keaton (2007) revealed urbanization resulted in a significant negative relationship with the hepatosomatic index (HSI) of *Lepomis sp.* Her study also revealed a positive relationship between bile fluorescence, a bioindicator for PAH exposure, and urbanization. A decreased HSI as well as elevated levels of PAHs indicated fish were undergoing physiological stress, the source of which has yet to be determined. The conclusions from her analysis warrant the need for further investigation.

The data generated in this dissertation expands upon this preliminary study and further demonstrates outcomes of human growth and land development on fish health and fish assemblage composition. This study will link biomarker responses in *Lepomis sp.* throughout the molecular level to assemblage levels of organization. By doing so, changes in fish assemblage composition can be related through biomarker response. Land alteration and watershed disruption will reveal pathways deteriorating fish health. Consequences of different land use types (i.e. urban, agricultural, forested) will demonstrate and predict threats to aquatic resources. An overall quantitative assessment

of ecosystem health will provide support for more informed land management strategies

and risk assessments.

CHAPTER 2: METHODS

Sampling Protocol

Sampling was conducted following a strict protocol developed by the South Carolina Department of Natural Resources (SC DNR) (Scott 2008). Stream segments were classified based on watersheds of wadeable size (4 km^2 to 150 km^2), ecoregions, and river basins. For simplicity, the geographic features of ecoregions and river basins were combined and reclassified as "ecobasin". Sampling sites were selected based on ecobasin and stream size using a multistage design that selected 127 random sites in well-known stream segments within the state. River basins in this study included the Ashepoo-Combahee-Edisto (ACE), Saluda, Catawba/Wateree, Pee Dee, and Savannah. Stream segments were generated using ArcGIS spatial analyst with flow direction and flow accumulation data incorporating 30 x 30 m digital elevation map models with less than 15 meters slope. A 100 meter stream segment that drained a 4 km^2 to 150 km^2 area was designated a random site number and incorporated into the site selection database. Sampling occurred during summer months between June 2006 and May 2010. Abiotic parameters were measured at each site (Appendix, Table A-1).

South Carolina is divided in half (geologically) by a fall line that runs through the middle of the State. Fish collections upslope of the fall line were conducted along a stream reach that was 30x the wetted stream width using one pass backpack electrofishing and dipnets. Lowlands sample sites utilized the same equipment but with three passes through a stream reach that was 20x the wetted stream width. All fish were collected, identified, counted, and released other than those needed for specimen collection. Fish populations

and assemblage were documented for each site. Stream class size was determined by watershed area. Stream class 1 ranged 4-24.99 km^2, class 2 ranged 25-74.99 km^2, and size 3 ranged 75-150 km^2.

Fish Metrics and Water Quality

Lepomis sp. were sacrificed on site (approx. 5-10 fish per site) with the liver, spleen, gall bladder, and gonads removed and flash frozen in liquid nitrogen for subsequent laboratory biomarker analysis. Weights, lengths, and fish gender were recorded for condition factor (K) and overall fitness comparisons. One inch by one inch sections of the dorsal side *Lepomis sp.* fish tissue (fillet) was removed and flash-frozen for mercury analysis. Total fish relative abundance was calculated by dividing the total number of total fish found at each site by the total number of fish species at each site. Species richness was assessed by adding total fish species numbers identified at each sample location. Similar relationships were assessed incorporating *Lepomis* for a genus-only population assessment.

Water quality parameters were measured and recorded for each sampling. A YSI 556 multimeter was used to record dissolved oxygen, water temperature, and conductivity. Turbidity was measured with an HF Scientific MicroTPW meter. Stream depth and water velocity were recorded using a Marsh-McBirney Flo-Mate 2000. Landscape slope was assessed by measuring stream rise over segment reach.

Lepomis sp. Somatic Indices and Fish Fitness

Liver mass was divided by whole fish body mass (g) to obtain the hepatosomatic index (HSI). Spleen mass was divided by whole fish body mass and multiplied by 100 to get the spleen-somatic index (SSI). Male fish gonad index was divided by whole fish body mass and multiplied by 100 to get the gonadosomatic index (GSI). Condition factor (K) was determined by fish length (cm) divided by weight x 100.

Lepomis sp. Population Fish Metrics

Total fish relative abundance was calculated by dividing the total number of total fish found at each site by the total number of fish species at each site. Species richness was assessed by adding total fish species numbers identified at each sample location. Similar relationships were assessed incorporating *Lepomis* for a genus-only population assessment. Tolerant *Lepomis* species included *Lepomis macrochirus* (bluegill sunfish), *Lepomis auritus* (redbreast sunfish), *Lepomis gulosus* (warmouth sunfish), *Lepomis microlophus* (redear sunfish), and *Lepomis gibbosus* (pumpkinseed sunfish). *Lepomis marginatus* (dollar sunfish) and *Lepomis punctatus* (spotted sunfish) are not considered tolerant in comparison to the other species.

Ethoxyresorufin-O-Deethylase Assay

The ethoxyresorufin-O-deethylase (EROD) assay was used to measure cytochrome P450-1A (CYP1A) induction in fish livers. The post-mitochondrial (S9) fraction was obtained by grinding up fish livers in a standard homogenization buffer. Samples were centrifuged

for twenty minutes at 10,000 x g, and frozen at -80°C until analysis. Upon initiation of the EROD assay, samples were thawed but kept on ice. A working buffer containing BSA, $MgCl_2$, water, and ethoxyresorufin was made to carry out the reaction (deethylation of ethoxyresorufin to a fluorescent product resorufin). The reaction was catalyzed by the addition of NADPH and read over a thirty minute period at ten minute intervals using a fluorescent spectrophotometer. Results were normalized against post-mitochondrial (S9 fraction) protein content and recorded in pmol/mg/min.

Glutathione-S-Transferase Assay

S9 fractions from fish livers were obtained in similar fashion as the EROD assay. 50 µl S9 subsamples were put into wells on a 96-well microplate in triplicate. A premix solution containing 100 mM HEPES buffer (25µl), 1mM glutathione (GSH)(5µl), and water (95ul) was added. 5µl of 1-chloro-2,4-dinitrobenzene (CDNB) was mixed with 25µl DDI water for each well. Immediately after the addition of (CDNB) the microplate was put into a UV plate reader at 344 nm. The plate was read every nine seconds over a two minute period. Results were normalized against post-mitochondrial (S9 fraction) protein content and measurements reported in nm/mg/min.

Estrogen Binding Assay

A 50 ml aliquot of bile/DI H_2O (1:15 bile:DI H2O) was pipetted into a 12x75 borosilicate glass culture tube. Samples were evaporated under a gentle stream of N2 and then reconstituted in 30 µl Na-acetate buffer and 15 ul of glucuronidase/ sulfotransferase (at

least 100,000 activity per unit). Samples were incubated in a water bath at 37°C for two hours. The reaction was stopped with 1 ml MeOH. Samples were centrifuged at 2000 x g for ten minutes. Aliquots of 50, 100, and 200 µl were made in duplicate and stored at -20°C for analysis.

For estrogenicity analysis, samples were evaporated under a gentle stream of air. 50 ul Binding buffer consisting of 1 mg/ml BSA, 10 mM tris-neutral, 10% glycerol, 2mM dithiothreitol, and DI H_2O was added. β-Estrogen receptor was added next to each tube to obtain 0.30 pmol receptor per 50 µl binding buffer. 100 ul radiolabled 3H-17-β-estradiol was added to each tube (~100,000 cpm, > 65% machine efficiency), followed by centrifugation at 1000 x g for five minutes and left overnight.

The following day all sample tubes were kept on ice. 1 ml of a charcoal solution (3g charcoal/ml binding buffer) was added for five minutes. Sample tubes were then centrifuged at 1800 x g for ten minutes. The supernatant was poured into 7 ml scintillation vials. 4 ml scintillation fluid was then added. Sample vials were capped and shaken, and counted using a Beckman LS 1800 liquid scintillation counter. Samples were compared against a 17β-estradiol standard curve. Total and nonspecific binding were also measured.

Mercury Analysis

Mercury content from fish fillets was analyzed at the National Institute of Standards and Technology (NIST) at the Hollings Marine Laboratory in Charleston, SC. Samples were analyzed using a DMA-80 Direct Mercury Analyzer to undergo thermal decomposition, catalytic reduction, amalgamation, desorption, atomic absorption spectroscopy for analysis of mercury. Samples were compared against an external calibration curve.

The mass fraction of Hg was determined with a direct mercury analyzer DMA 80 (Milestone Scientific, Shelton, CT) by external calibration utilizing Standard Reference Material (SRM) 1946 Lake Superior Fish Tissue and Pygmy Sperm Whale Liver Homogenate Control Material (QC03LH3) was performed once prior to performing sample analysis of standards and unknown samples. The external calibration curve was created by aliquoting different masses of the fresh frozen CRMs into nickel sample boats. Bracketing standards of QC97LH2 and SRM 1946 were used in between blocks of 7-10 unknown samples in order to verify the instrument calibration and monitor for drift. The samples were then dried at 300 ℃ for 60 s, thermally decomposed at 850 ℃ for 180 s, catalytically reduced to Hg0, and trapped on a gold amalgamation trap.

The slope and intercept from the calibration curve based on the measured response of the Hg atomic absorption of the SRM 1946 and QC03LH3, were used to calculate the concentration of Hg in the liver samples. QC97LH2 and SRM 1946 were used as bracketing standards to verify instrument calibration and monitor for potential instrument

drift. The ordinate is defined as [y * g of Hg per g of sample], where y is the blank corrected Hg atomic absorption signal and the abscissa is defined as the mass of added Hg spike per gram of sample.

x=(y-b)/m

where: m is the slope of the calibration curve

y is the measured Hg atomic absorption signal

b is the Y-intercept of the calibration curve

x is the concentration

Bile Fluorescence Assay

The bile fluorescence assay was use to measure fish exposure to 2, 4, and 5-ring polycyclic aromatic hydrocarbons (PAHs). Fish gall bladders were thawed and bile was carefully drained and mixed with DI H_2O to a total volume of approximately 150 µl. A subsample was aliquoted for a protein analysis. Another 30 µl was diluted with MeOH:H_2O to make 1:250, 1:500, 1:1000, and 1:1500 dilutions. Fluorescence was measured at three excitation/emission wavelengths, 290/335, 341/383, and 380/430 nm florescence (FAC) corresponding to 2, 4, and 5-ring aromatic structures. Fluorescence was normalized to bile protein content. The dilution which compensated for the inner filter effect (dilution which had the highest point of the downward linear range) was the dilution used for analysis (Hanson et al., 2008).

Simpson's Diversity Index

The Simpson's Diversity Index (D) is defined as the sum of the total number of individuals of a particular species divided by the total number of individuals of all species (Hunter et al., 1988):

$$D = \sum n(n-1)/N(N-1)$$

n=total number of individuals of a particular species

N=total number of individuals of all species

As D decreases there is a greater number and diversity of total fish abundance and species in the assemblage. In this study the inverse (1-D) was used as an indicator of overall fish assemblage integrity.

Land Use Analysis

Land use analysis was conducted using data generated by Marion (2008). Terrain and land cover data originated from the U.S. Geological Survey (USGS) in conjunction with Earth Resources Observation and Science (EROS) from 2006. EROS incorporated data from the Multi-Resolution Land Characteristics Consortium (MRLC) used to map the earth and provide a composite of land classes termed the Seamless Data Distribution System. The USGS Seamless Digital Elevation Models (DEM) were used in ESRI's ArcGIS version 9.0 Spatial Analyst extension with Flow Direction and Flow Accumulation data, comprising 30 x 30 m spatial resolution with a vertical accuracy of 15 meters or less, to delineate watersheds upstream of sample locations. The

classification and percentage of urban was determined through this technique. Data shown have not been converted to a percent.

Statistical Analysis

All calculations were performed using the statistical software JMP 9.0.0 (SAS Institute, Inc., Cary, NC). Fish and abiotic variables were used to adjust and/or modify the relationships (Appendix, Table A-1). When multiple fish were measured at a sampling location the median values of the variables were used in the analysis. Multiple linear regression methods were used to measure the relationships of total fish variables with other location variables. A model was created with total fish variables as the dependent (Y) variable and the *Lepomis sp.* and abiotic variables as the array of independent (Xi) variables. Stepwise procedures were used when necessary to choose a subset of the independent variables that had the best overall relationship with the dependent variables $(Y = \beta 0 + \beta 1 X1 + \cdot \cdot \cdot + \beta p X p)$.

Three adjustments were used during the multiple regression analysis. First, since all variables were not measured at all sites, an imputation procedure was conducted to predict the missing values (Yuan 2000). Basically a series of multiple regression models were created, one for each variable measured. Each variable measured served as the dependent variable in a model and all other variables served as the independent variables. This allowed estimation of the missing values of each dependent variable. Second, since the model residuals for the total fish variables did not all meet the assumptions of

normality and/or homogeneous variance; some were transformed either by the logarithm or square root to meet the assumptions. Third, some sites had values of the variables that were clearly outliers. However removing outlier values did not significantly alter the model results and therefore the values were left in the data set.

Changes in multiple levels of biological organization were assessed using linear regression and Pearson's correlation analysis. All comparisons were measured in *Lepomis sp.* except for assemblage level metrics which utilized total fish abundance, total fish species richness, and total fish diversity (as indicated by the Simpson's Diversity Index). Molecular level comparisons were conducted through EROD activity, GST activity, estrogenicity, 5-ring aryl hydrocarbon exposure, and mercury concentrations. Tissue/organ comparisons were performed through hepatosomatic index (HSI), spleen-somatic index (SSI), and gonadosomatic index (GSI). Individual level comparisons were achieved using condition factor (K). Population level comparisons were accomplished via total *Lepomis sp.* abundance and *Lepomis sp.* richness. Assemblage level analyses were carried out via total fish abundance, total fish species richness, and total fish diversity.

CHAPTER 3:THE OCCURRENCE OF *LEPOMIS SP.* AND TOTAL FISH SPECIES, ABUNDANCE, AND DIVERSITY IN WADEABLE STREAMS IN SOUTH CAROLINA, USA

Abstract

Lepomis (sunfish) are a genus of freshwater fish commonly found in water bodies throughout the United States, southern Canada, and northern Mexico. They are especially plentiful in the southeastern region of the United States. In South Carolina, *Lepomis sp.* are present in approximately 80% of wadeable streams and are considered biological indicators of watershed health. Throughout 2006-2010 an investigation was conducted to evaluate diversity and abundance of freshwater fish species in wadeable rivers, streams, and creeks throughout South Carolina. The objective of this study was to determine if *Lepomis sp.* could serve as a model species to represent total fish assemblage structure. Fish populations were assessed at approximately one-hundred twenty randomly selected wadeable stream sample sites located throughout South Carolina and fish abundance and species richness were assessed. Models that incorporated *Lepomis sp.* abundance and richness along with various abiotic geographic parameters were used to predict total fish abundance, richness, and assemblage composition. Total *Lepomis sp.* resulted in a significant relationships in both abundance and richness with all other fish in the assemblage ($p<0.01$; $r^2=0.13$ and $p<0.01$; $r^2=0.07$, respectively). Adding abiotic variables strengthened the relationship in both abundance and richness ($p<0.01$, $r^2=0.23$ and $p<0.01$; $r^2=0.22$, respectively). Separating the state by five major river basins resulted in stronger, significant relationships ($p<0.01$) with total fish abundance with r^2 values ranging from 0.30-0.99. Results indicated that *Lepomis sp.* could serve as a representative for total fish assemblage composition. These results support the use of *Lepomis sp.* as a sentinel organism in South Carolina streams.

Introduction

An assemblage of fish, defined as a group of fish populations overlapping at the same place at the same time, is an important component of an ecosystem consisting of a large number of fish species (Fauth et al., 1996). Fish assemblages can be indicators of environmental contamination and overall habitat health. Knowing historical patterns of native fish species over their geographic range is an important component to understanding an ecosystem. In any given assemblage physiological differences exist, such as responses to contaminant stress, making it difficult to compare individual species in the same assemblage within the same body of water (Hughes et al., 1998). Specific life history requirements, habitat preference, food sources, competition, and other biotic and abiotic parameters affect the diversity and abundance of fish found in rivers, streams, and creeks. Over great distances fish assemblages can differ due to geographic separation. These conditions and factors dictate the types of fish species found in a given body of water (Jackson et al., 1989; Imhof et al., 1995; Power et al., 1988).

Incorporating a fish assemblage into a species abundance and diversity analysis is beneficial for a number of reasons. Individual species richness, habitat requirements, guild composition, fish health, and other metrics can be determined and scored using a common indicator (Hughes et al., 1998). Likewise, variables such as ecoregion, river basin, geographic location, home range, and other parameters can be incorporated. Factors influencing fish species in an assemblage will likely reflect external stressors once confounding variables and covariation is accounted. In this way a fish species

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gradient can be made identifying species with specific living requirements and how those species respond to external environmental stressors. This gradient can indicate a range from sensitive to tolerant fish species (Allan, 2004).

Aquatic systems are highly complex and include inputs from everything in the surrounding watershed upstream of an area. The environment in which fish and other aquatic organisms live in is dynamic and constantly changing. One example of external pressure on an aquatic system is changing land use. In many parts of North America, the urbanization of once forested land results in habitat loss, interferes biogeochemical cycle, and exports significant amounts of eroded soil into adjacent surface waters. Further, during rainfall events metals, polycyclic aromatic hydrocarbons (PAHs), and other contaminants are washed into lakes, rivers, and streams instead of being infiltrated into the ground or taken up by flora (Dudgeon et al., 2006; Vitousek et al., 1997). Scott (2006) reported that surface waters in urban areas had less fish abundance, diversity, and integrity than those in developed rural watersheds.

Biomarkers are changes in cellular or biochemical structure, function, or behavior measurable in bodily fluids, tissues, or organs, used to gauge the presence of physical, chemical, or biological stressors (Gomez-Martinez et al., 2006; Schreiber et al., 2006; Van der Oost et al., 2003). Biomarker measurements are being increasingly incorporated in environmental studies because they are quick, efficient, and accurate ways to indicate the health status of organisms. Information from these measurements can be extrapolated

to gauge habitat conditions of all organisms at a location. For example, fish biomarker response measurements can represent the occurrence and presence of contaminants in the aquatic environment to which all fish are being exposed. However, it is can be difficult (and unfeasible) to take specimens from every fish species in a sampling locale and measure it for contaminant response. An ideal fish type is one that would be present throughout a range of sites, pristine through contaminated, and exhibit measureable contaminant detoxification responses. Therefore a model fish type is needed to represent the assemblage (Sayer, Chapter one).

The *Lepomis* genera, in the family *Centrarchidae*, are common to rivers throughout the US especially in the southeast. In South Carolina these species are prevalent in over 80% of the streams (Keaton, 2007). The seven *Lepomis sp.* commonly encountered are *Lepomis macrochirus* (bluegill sunfish), *Lepomis auritus* (redbreast sunfish), *Lepomis gulosus* (warmouth sunfish), *Lepomis microlophus* (redear sunfish), *Lepomis gibbosus* (pumpkinseed sunfish), *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish). The goal of this research was to determine if the prevalence of the *Lepomis sp.* could be used to represent total fish species abundance, richness, and assemblage in South Carolina surface waters.

Results

Fish Abundance and Species Richness

During the four summers of sampling, fish abundance at individual sampling locations ranged from 1 to 1908 fishes (Appendix, Table A-6). Total fish species at individual sites ranged from 1 to 29 species. The abundance of *Lepomis sp.* found at sites ranged from 1 to 329 fishes. The number of individual *Lepomis sp.* ranged from 1-7 species. The number of fish from tolerant *Lepomis sp.*, which excludes *Lepomis marginatus* (dollar sunfish) and *Lepomis punctatus* (spotted sunfish), ranged 0-329 fish and 0-5 species at sample sites. *Lepomis macrochirus* (bluegill sunfish) and *Lepomis auritus* (redbreast sunfish), were the two most commonly encountered *Lepomis* species and ranged from 0-282 total fish. The number of priority fish species, which are those of South Carolina concern due to their threatened or endangered status, ranged from 0-207 fish and 0-8 species at individual sample sites (Appendix, Table A-7). Priority species were found at eighty of the one-hundred twenty-seven sites (63%). However, there was 10 or less total priority fish found at forty of the eighty sites.

Regression Analysis

A comparison of the abundance of total fish to the abundance of *Lepomis sp.* at all sample locations resulted in a significant, positive correlation (Figure 3-1a). When abiotic variables (average stream width, water velocity, and water temperature) were included with *Lepomis sp.* abundance the model the relationship improved significantly (Figure 3-1b). Singling out the contribution of only *Lepomis sp.* abundance reveals a

large value of the partial coefficient of determination ($r^2=0.129$) in the overall model (Table 3-1).

a)

b)
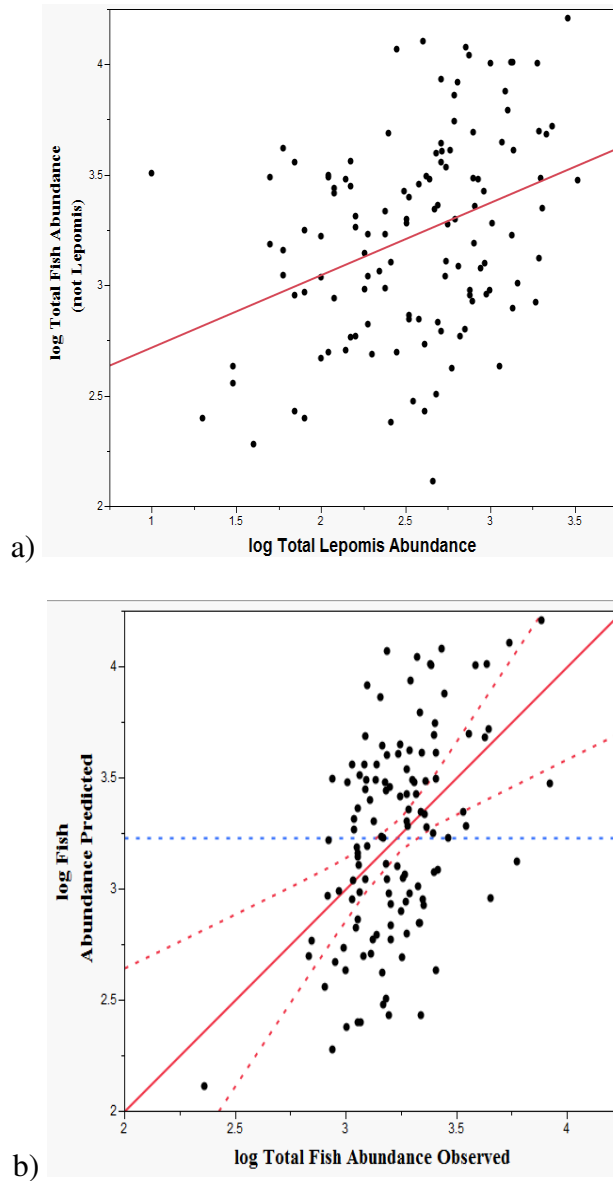
Figure 3-1. (a) The log *Lepomis sp.* abundance vs. total fish abundance (excluding *Lepomis sp.*) in a xy linear regression analysis (p<0.01; $r^2=0.13$). (b) Predicted fish abundance vs. observed fish abundance (excluding *Lepomis sp.*) increases in a stepwise

multiple linear regression analysis including average stream width, water velocity, water temperature, and total *Lepomis sp.* individuals as covariates (p<0.01; r^2=0.23). Regression equations are in Appendix Table A-12.

Removing *Lepomis sp.* from total fish abundance reveals a significant positive relationship between remaining fish abundance in the assemblage when covariates are taken into account. However, the inclusion of *Lepomis sp.* in the total fish assemblage as a covariate resulted in a stronger relationship (r^2=0.23) compared to total fish abundance without *Lepomis sp.* (r^2=0.10). Because of the high prevalence of *Lepomis sp.* in streams throughout South Carolina their absence weakens relationships pertaining to the total numbers of fish found at sites (Table 3-1).

Table 3-1. Partial coefficient of determination values from the stepwise multiple linear regression analysis comparing total fish abundance to predicted fish abundance exclusive of *Lepomis sp.* (p<0.01; r^2=0.23).

Model variables	Total Fish Abundance	
	p value	partial r2
Total *Lepomis* Abundance	**<0.0001**	0.129
Width	**0.0098**	0.006
Velocity	**0.0005**	0.074
Temperature	**0.027**	0.025
R^2 of Multiple Regression	0.23	

Bold p-values were considered statistically significant (p < 0.05).

There was a good correlation between total fish species richness and *Lepomis sp.* at all sample locations (Figure 3-2a). The abiotic variables together resulted in a better prediction of total species richness than did *Lepomis sp.* alone (Figure 3-2b). The variance for the two models, as described by the partial coefficients of determination, shows inclusion of *Lepomis sp.* results in a stronger association. Singling out the contribution of only *Lepomis sp.* richness in the model reveals the value of the partial coefficient of determination (r^2=0.05) in the overall model (Table 3-2).

Table 3-2. Partial coefficient of determination values from the stepwise multiple linear regression analysis comparing species richness exclusive of *Lepomis sp.* (p<0.01; r^2=0.22).

Model variables	Total Fish Richness	
	p value	partial r2
Depth	**0.023**	0.055
Temperature	**<0.0002**	0.11
Total *Lepomis* Richness	**0.0092**	0.05
R^2 of Multiple Regression	0.22	

Bold p-values were considered statistically significant ($p < 0.05$).

a)

b)
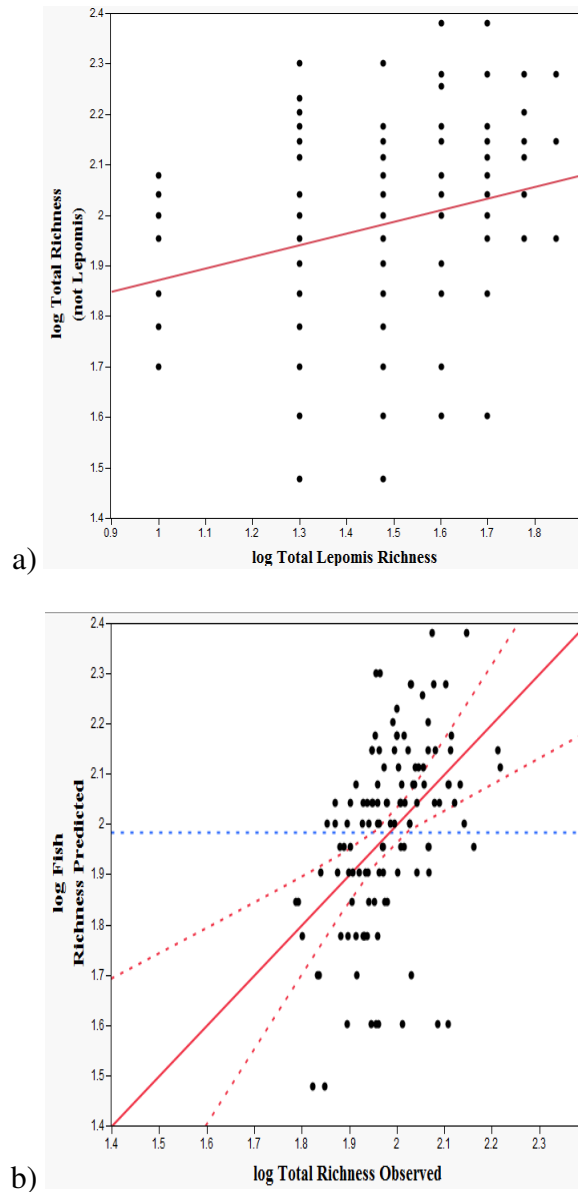
Figure 3-2. (a) The log *Lepomis sp.* richness vs. total fish species richness (excluding *Lepomis sp.*) in a xy linear regression analysis (p<0.01; r^2=0.07). (b) Predicted total species richness vs. the number of observed fish species increases in a stepwise multiple linear regression analysis (p<0.01, r^2=0.22). Covariates included in the models were

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stream depth, water temperature, and total *Lepomis sp.* richness. Regression equations

are in Appendix Table A-12.

A significant positive relationship existed in both cases when comparing priority fish

abundance and richness with *Lepomis sp.* abundance and richness ($p<0.01$). In both cases

Lepomis sp. had a minor contribution to the model even though the multiple linear

regressions were significant (partial r^2 values for *Lepomis sp.* abundance and richness

were 0.01 and 0.05, respectively; Figure 3-3). The ability to predict Simpson's diversity

index (D) was also explored and a significant, positive relationship was found with

covariates stream width, depth, turbidity, total *Lepomis sp.* abundance and richness

($p<0.01$; $r^2=0.18$). Note that D incorporates both total fish abundance as well as species

diversity. At the statewide level, fish diversity and abundance vary considerably, which

is illustrated by the low contribution of *Lepomis sp.* to the model (partial r^2 values for

Lepomis sp. abundance and richness were 0.001 and 0.01, respectively).

a)

b)
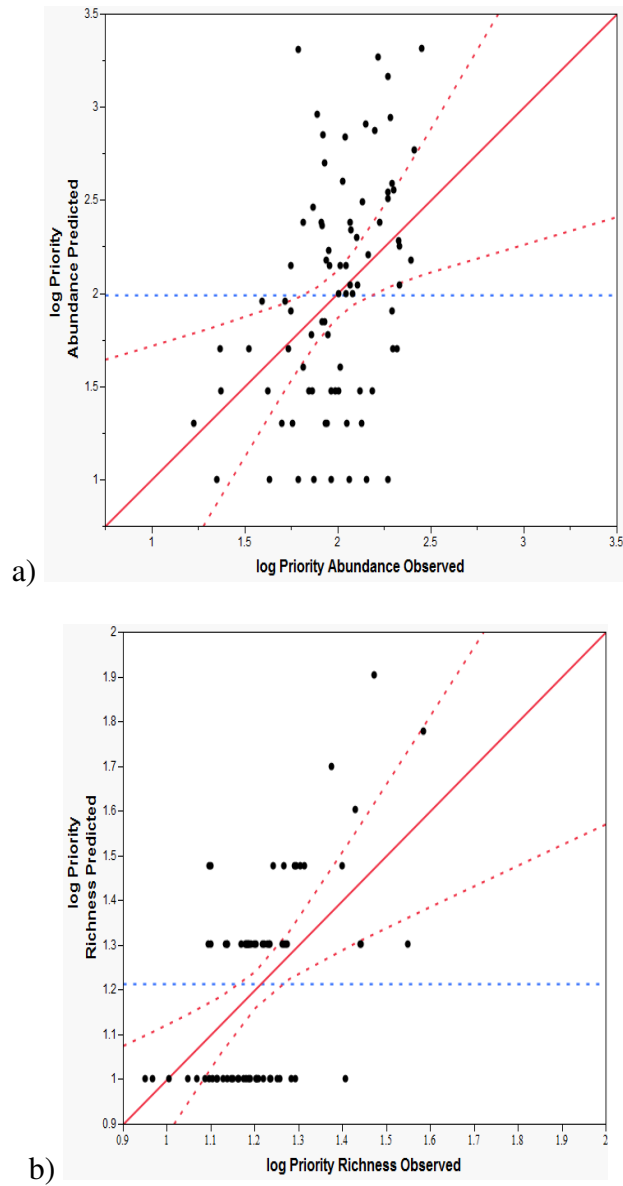
Figure 3-3. (a) Predicted priority abundance vs. observed priority abundance increases in a stepwise multiple linear regression analysis (p<0.01; r^2=0.16). Covariates included in the model were stream slope, dissolved oxygen and total *Lepomis sp.* abundance. (b) Predicted priority species richness vs. observed priority richness in a stepwise multiple linear regression analysis (p<0.01; r^2=0.27). Covariates included in the model were

stream width, stream velocity, stream slope, and total *Lepomis sp.* richness. Regression

equations are in Appendix Table A-12.

Fish abundance was broken up based on river basin. The five major river basins explored

in this study were the Ashepoo-Combahee-Edisto (ACE), Catawba/Wateree, Pee Dee,

Saluda, and Savannah basins. Reducing the scale of the assessment resulted in stronger

relationships between total fish abundance at the river basin level. Each analysis at the

river basin level was significant ($p<0.01$) with r^2 values ranging from 0.30-0.99 (Table

3-3).

Table 3-3. Summary of statistical findings (p-values) examining the influences of abiotic

parameters and fish parameters on the five South Carolina River Basins.

River Basin	ACE	Catawba/Wateree	Pee Dee	Saluda	Savannah
Model variables	**n=8**	**n=16**	**n=33**	**n=28**	**n=37**
Width	> 0.100	> 0.100	> 0.100	**0.0434**	> 0.100
Depth	> 0.100	> 0.100	> 0.100	> 0.100	**0.0045**
Velocity	**0.010**	> 0.100	> 0.100	**0.0054**	> 0.100
Slope	**0.0171**	> 0.100	> 0.100	**0.0003**	> 0.100
pH	**0.0152**	> 0.100	> 0.100	**0.0009**	> 0.100
Dissolved Oxygen	> 0.100	> 0.100	> 0.100	**0.003**	> 0.100
Turbidity	**0.0068**	> 0.100	> 0.100	> 0.100	> 0.100
Bluegill/Redbreast Abundance	**0.0035**	> 0.100	> 0.100	> 0.100	> 0.100
Tolerant *Lepomis* Richness	**0.0032**	> 0.100	> 0.100	> 0.100	**0.0042**
Tolerant *Lepomis* Abundance	> 0.100	> 0.100	> 0.100	> 0.100	> 0.100
Total *Lepomis* Richness	> 0.100	> 0.100	> 0.100	> 0.100	0.062
Total *Lepomis* Abundance	>0.100	**0.0295**	**0.001**	**0.0083**	> 0.100
R^2 of Multiple Regression	0.99	0.32	0.30	0.71	0.40

Bold p-values were considered statistically significant ($p < 0.05$).

Values shown as > 0.100 indicate that the stepwise multiple regression model eliminated the model effect and that trait was removed from the total model.

Discussion

The objective of this study was to determine if *Lepomis sp.* could be used as a representative species for all fish species in South Carolina surface water. Results of this work indicate that *Lepomis sp.* are good predictors of total fish abundance, richness, and assemblage at sample sites in South Carolina. With natural variation such as water quality characteristics, stream attributes, and abiotic factors accounted for it is possible to more selectively focus on differences in fish species sensitivity. This is especially important during times of rapid contaminant influx when fish are exposed to a number of external stressors both physical and chemical in nature. While contaminant influx might occur at point sources (i.e. industrial waste discharge) in the aquatic environment another input occurs during storm events. During these events contaminant runoff enters the aquatic ecosystem coming into direct contact with fish, invertebrates, and other organisms residing there. If a fish species is able to tolerate contaminant fluctuations by upregulating detoxification mechanisms then that species will likely remain at that site. However, if a fish species is not able to tolerate contaminant increases or fluctuations they will likely migrate away from that site or die (Gido et al., 2000; Siligato et al., 2001).

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An indicator species can provide valuable information regarding fish assemblage changes as a result of natural and anthropogenic stressor in the ecosystem. The US EPA (2010) considers members of the *Lepomis* genus as biological indicators of watershed health. These fish typically do not migrate far from their home and they are one of the last fish species to leave a newly polluted site. They are also one of the first fish to migrate and reestablish a site that has begun recovery from previous stress. These attributes make *Lepomis* an excellent sentinel genus to monitor aquatic systems (Delinsky et al., 2009; Theodorakis et al., 2007).

Sentinel species like *Lepomis sp.* continue to be commonly used in environmental studies to determine if contaminants are present in the aquatic ecosystem (Fitzgerald et al., 1999; Gibbons et al., 1998; van der Schalie et al., 1999). Identifying and examining one species can provide information for the environment in which all organisms reside. Not only can information from a sentinel species provide information for other fish and aquatic organisms, but harmful contaminants may biomagnify throughout the food web. Through this way birds and terrestrial predators or opportunists are put at risk (Black et al., 2009). The potential for negative human health endpoints is also plausible.

One reason *Lepomis sp.* are found in such a wide geographic range is because they are capable of surviving a diverse variety of aquatic habitats. They typically favor slow moving streams with deep pools or vegetation (Sims-Parr, 2002). They can often be the dominant species in mountainous headwater streams great distances away from major

water bodies. These fish can be found in aquatic systems where chemical contaminants are present and other fish species have left the area. Streams in urbanized areas can have an abundance of *Lepomis sp.* (adults and juveniles). During times of contaminant influx *Lepomis sp.* tend to be more resilient than other fish because they are tolerant of chemical contaminants (Grabarkiewicz et al., 2008; TPWD, 2012; US EPA, 2010).   Despite their large geographic dispersion, their home range is generally less than 50 meters (Delinsky et al., 2009; Keaton, 2007). The US Environmental Protection Agency (EPA) has classified members of the *Centrarchidae* family, including *Lepomis sp.*, as biological indicators of watershed health and therefore, an important part of the Index of Biotic Integrity (US EPA, 2010). Almost all states use some form of the EPA's Index of Biotic Integrity to understand the health of water bodies within their State, which includes a metric for the number and identity of *Lepomis sp*.

In the South Carolina, significant positive relationships existed between *Lepomis sp.* abundance and richness, and total fish abundance, total fish richness, and total fish assemblage diversity, as indicated by D, at the statewide level. Incorporating abiotic parameters into this model as covariates significantly strengthened these relationships. Segregating the data by South Carolina river basins resulted in stronger correlations between *Lepomis sp.* and fish assemblage parameters in nearly all circumstances (Table 3-3). Habitat requirements, food preference, and life history characteristics are all potential explanations for stronger relationships at the smaller scale. Similar fish species are more likely to live in adjacent rivers compared to non-neighboring aquatic systems

(Jackson et al., 1989). Analogous living conditions and short migration could account for similarities. Habitat diversity, competition, and predation were associated with local aspects while climate, dispersal barriers, and historical biogeography are associated with regional factors. A separate study determined community diversity reflective of local ecosystem dynamics including competition, predation, disease, and long-term natural disturbance (Ricklefs, 1987). It is plausible similar relationships can be found upon subsequent examination of different South Carolina geographic land designations. South Carolina ecoregion and ecobasin (where a river basin crosses a river basin) are two potential study areas for future exploration for assemblage comparison with *Lepomis sp.*

Priority fish species and total assemblage (D) both displayed significant relationships. Priority fish species are those of greatest conservation concern, are endangered, threatened, or rare. Their population status, factors limiting their success, and exploitation potential are largely unknown. Because of this, they are not nearly as abundant compared to *Lepomis sp*. The few priority species found were in random, scattered sites where specific living conditions were favorable for survival. While *Lepomis sp.* are tolerant to changing conditions, species of priority concern are much less ecologically robust. Priority fish revealed weak relationships with *Lepomis sp.* Although relationships were significant it was possible priority species were influenced by parameters not examined in this study. For example, aquatic contaminant exposure, land development, and other anthropogenic disturbances may play a role in how a fish

assemblage changes in both numbers and diversity. Further investigation is needed to identify additional influences to priority species change in an assemblage.

Conclusions

Lepomis sp. can be used to represent the occurrence of other fish species in South Carolina surface waters. This was particularly true when the data were segregated by watershed. The ability to use *Lepomis sp.* to represent other fish assemblages is ideal for a variety of reasons: 1. A genus like *Lepomis* is widely distributed throughout the state of South Carolina; 2. It is found consistently despite the geographic diversity and presence of different fish species at sites; and, 3. *Lepomis sp.* are found in high enough numbers to be representative of a population within an assemblage. Future research will quantify the levels of contaminants and other biomarkers in *Lepomis sp.* and test the hypothesis that their variability can be explained by differences in land uses among watersheds.

CHAPTER 4: CHANGES IN FISH ASSEMBLAGE COMPOSITION INDICATED BY *LEPOMIS SP.* BIOMARKER, HEALTH AND FITNESS RESPONSES

Abstract

Assessing fish assemblage is one reliable method to gauge biological integrity in freshwater ecosystems. Freshwater ecosystems are affected in large part by the surrounding landscape. They are highly complex, integrated, and encompass the entire drainage of everything upstream in a watershed. As a result, consequences of land alteration to accommodate human population growth can be observed in water systems many miles downstream from the source of disturbance. Biomarkers are being increasingly incorporated into environmental studies to determine the physical, chemical, and biological health effects of fish and other aquatic species. If water conditions are unfavorable for fish to live they must adapt, migrate away from the area, or die. Fish species that remain at sites will exhibit compensatory mechanisms at the molecular level to cope with a changing environment. An investigation was conducted from 2006 to 2010 to determine how fish assemblages change as a result of aquatic system contaminant variability in freshwater streams throughout South Carolina. Using *Lepomis sp.* as a freshwater fish model, responses for a suite of biomarkers were measured at approximately one-hundred twenty sample sites. Besides biomarkers, *Lepomis sp.* fitness indicators and abiotic parameters were measured at each site. These measurements were compared with *Lepomis sp.* abundance and richness as well as the total fish abundance, richness, and assemblage. Using multiple linear regression analyses, this research demonstrated significant relationships ($p < 0.01$) among biomarkers and fish health and fitness indicators. Total fish and total *Lepomis sp.* abundance r^2 values were 0.23 and 0.33, respectively, with the inclusion of biomarkers and abiotic factors. A significant

relationship was found with total fish species diversity and biomarker responses (p<0.01; r^2=0.52). Evaluating the state by major river basin resulted in significant relationships (p<0.01) between total fish species diversity and biomarker response with r^2 values ranging from 0.50-0.89. Isolating contributing covariates from the model revealed an increase in harmful contaminant response measurements led to negative fish assemblage parameters. This study was one of the first to assess sequential changes throughout multiple levels of biological organization (molecular, individual, population, and community) at a statewide level.

Introduction

At higher levels of biological organization it can be difficult to determine if groups of organisms are being exposed to a stressor for several reasons. Detoxification mechanisms, behavior modification, and gene activation are examples of how organisms adapt to a changing environment. The ability to leave or migrate away from a stressful ecosystem is another strategy employed (Schueler, 1994). While research may indicate a contaminant is harmful to cells in laboratory experiments it may not accurately reflect detoxification processes and coping strategies utilized by a living organism. Therefore toxic effects at the molecular level do not necessarily result in increased mortality at population or community levels; however, energy from individual organisms needs to be expended to cope with the contaminant (Siligato et al., 2001). As new strategies for evaluating chemicals emerge it will be critical that quantitative connections are made

between cellular/molecular levels of effects and organism/population levels (Villeneuve et al., 2011).

Several different techniques exist to measure and assess biological integrity. One method to measure integrity in freshwater systems is to evaluate fish assemblages (Karr, 1991). An assemblage, defined as groups of fish populations overlapping at the same place at the same time, differs from a community in that the latter incorporates the surrounding ecosystem and ecosystem dynamics which are constantly changing (Hughes et al., 1998). Changes in a fish assemblage, as a result of contaminant input, can indicate modifications in the surrounding aquatic environment unfavorable for survival. Sensitive species will leave the assemblage while more tolerant ones will remain. As a result, the integrity of the assemblage declines. For this reason a fish assemblage can be considered a high quality, robust metric to gauge biological integrity (Adams et al., 2000; Moore et al., 1997).

A genus of fish that resides in many freshwater bodies throughout the United States (the southeast in particular) is *Lepomis sp*. This genus of fish is found in roughly 80% of streams in South Carolina (Keaton, 2007). In South Carolina *Lepomis sp.* are commonly found in abundance, the main species being *Lepomis macrochirus* (bluegill sunfish), *Lepomis auritus* (redbreast sunfish), *Lepomis gulosus* (warmouth sunfish), *Lepomis microlophus* (redear sunfish), *Lepomis gibbosus* (pumpkinseed sunfish), *Lepomis*

marginatus (dollar sunfish), and *Lepomis punctatus* (spotted sunfish). This study will focuses on these seven *Lepomis* species (Sayer, Chapter one).

Members of the *Lepomis* genus are considered by the US EPA to be biological indicators of watershed health (EPA, 2010). One reason *Lepomis sp.* are found in such a wide geographic range is because they are capable of surviving a diverse array of aquatic habitats. These fish can be found in aquatic systems where contaminants are present in high concentrations when other fish have left the area (Grabarkiewicz et al., 2008; TPWD, 2012; US EPA, 2010). During times of contaminant influx these fish species are more resilient than other fish making them good indicators for a changing habitat. When other fish species have left a polluted site *Lepomis sp.* remain. The high prevalence of *Lepomis sp.*, tolerance to contaminant input, and resiliency make them a good sentinel species to monitor aquatic systems. Furthermore, despite their wide geographic dispersion, their home range is generally less than 50 meters. A model fish species like *Lepomis* can indicate aquatic contamination through measurements of detoxification response (Keaton, 2007). These responses provide information that can be extrapolated to assess changes in fish assemblage composition as well as watershed health.

Throughout 2006-2010 an investigation was conducted to evaluate how fish assemblages change as a result of aquatic system contaminant stress in one-hundred twenty-seven wadeable freshwater streams through South Carolina. Biomarker responses, which are measurable changes in cellular or biochemical structure, function, or behavior measurable

in bodily fluids, tissues, or organs, were employed to assess aquatic contaminants in *Lepomis sp*. Biomarkers are being increasingly used in environmental studies because they are quick and efficient ways to indicate the health status of an organism. They are utilized to gauge the physical, chemical, and biological stressors attributed to environmental conditions (Gomez-Martinez et al., 2006; Schreiber et al., 2006; Van der Oost et al., 2003). Previously, we have reported on the appropriateness of using *Lepomis sp*. as an indicator of total fish assemblage in these streams (Sayer Chapter two). The objective of this study was to investigate the relationship between biomarkers of contaminant exposure in *Lepomis sp* and total fish assemblage integrity. In addition to biomarker measurements, indicators of overall *Lepomis sp.* health and fitness were assessed.

Results

Stream parameters were measured and recorded. Average stream width ranged from 1.2-45 m. Average stream depth ranged from 0.07-0.58 m (Appendix, Table A-3). Average stream velocity ranged from -0.0022-0.45 m/sec. Average landscape slope ranged from -0.228-1.05 m. Water pH ranged from 4.85-8.25. Average water temperature ranged from 14.46-31.54 °C. Dissolved oxygen in water ranged from 0.2-10.45 mg/L. Water turbidity ranged from 0.84-69.4 NTU. Water conductivity ranged from 11-868 microsiemens/cm.

Molecular level biomarker responses of fish from sample sites had wide ranges. EROD activity ranged from 0.03-294 pmol/mg/min (Appendix, Table A-11). GST activity ranged from 58.9-792 nm/mg/min. Bile fluorescence ranged from 283-1900000 FAC for two-ringed congeners, 118-838000 FAC for four-ringed congeners, and 17.7-173000 FAC for five-ringed congeners. Estrogenic content ranged from 93.0- 41800 estrogen binding equivalents. Individual organism biomarkers were assessed. Condition factor (K) ranged from 1.17-3.16. The hepatosomatic index (HSI) ranged from 0.48-2.43. The spleen-somatic index (SSI) ranged from 0.0037-0.52. The gonadosomatic index (GSI) ranged from 0.003-9.7. Mercury concentrations in fish tissue ranged from 2.82-379 ng/g Hg.

The range of total fish found at sample locations was 1-1908 fish (mean = 340; median = 232) (Appendix, Table A-6). The range of total fish species found at sites was 1-29 species (mean = 14; median = 14). The range of total *Lepomis sp.* found at sites was 1-329 fish (mean = 60; median = 42). The range of total *Lepomis sp.* found at sites ranged from 1-7 species. Tolerant *Lepomis sp.*, which excludes *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish), ranged from 0-329 fish and from 0-5 species at sample sites. *Lepomis macrochirus* (bluegill sunfish) and *Lepomis auritus* (redbreast sunfish), two commonly encountered *Lepomis sp.,* ranged from 0-282 total fish. Simpson's Diversity Index (D) calculations ranged from 0.01—1 (Appendix, Table A-9).

Using all the independent variables in a stepwise linear regression resulted in a model that predicted 23% of the variability in fish abundance (Figure 4-1a). Covariates included in the model were EROD activity, GST activity, condition factor, and abiotic measurements average stream width, stream temperature, and dissolved oxygen. Similar results were obtained when only *Lepomis sp.* abundance was predicted (Figure 4-1b). A comparison between total fish and *Lepomis sp.* can be seen in Table 4-1.

Table 4-1. Partial coefficient of determination of variables from the stepwise multiple linear regression analysis between predicted and actual total fish abundance to ($p<0.01$; $r^2=0.23$) and between *Lepomis sp.* and actual *Lepomis sp.* abundance ($p<0.01$, $r^2=0.33$).

	Total Fish		*Lepomis*	
Model variables	p value	partial r2	p value	partial r2
EROD	0.0005	0.03	0.072	0.05
GST	0.006	0.007	<0.0001	0.2
K	0.01	0.043	0.33	0.006
Ave Stream Width	0.016	0.006	0.22	0.03
Temperature	0.005	0.017	0.91	0.007
DO	<0.0001	0.12	0.005	0.05
R^2 of Multiple Regression	**0.23**		**0.33**	

(a)   (b)
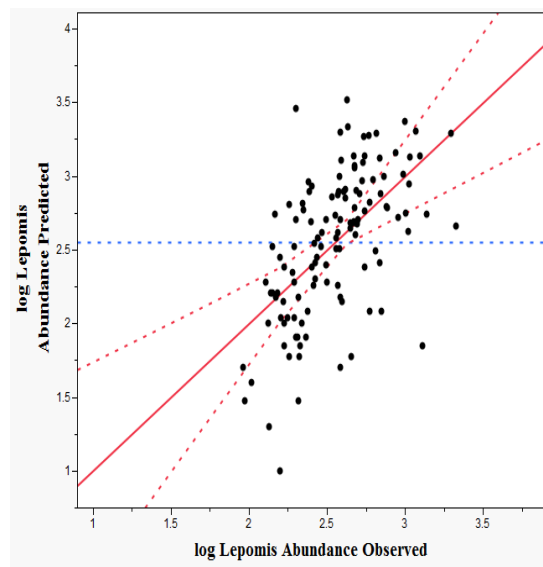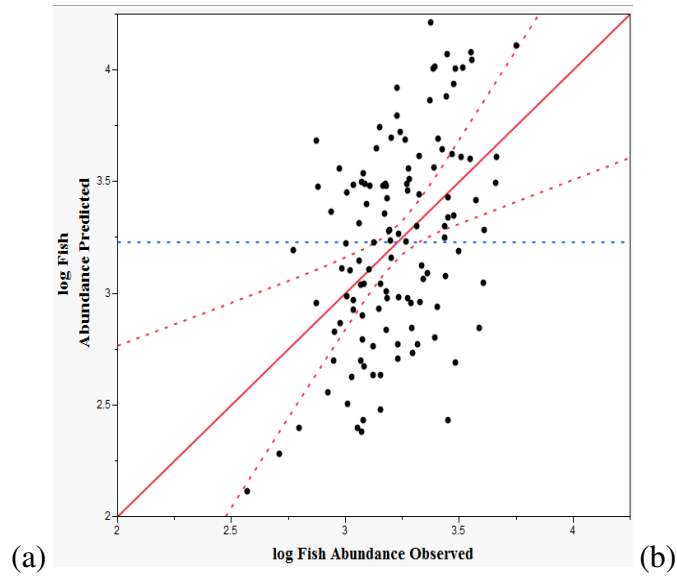
Figure 4-1. (a) The multiple linear regression analysis depicting the relationship between predicted and actual total fish abundance incorporating all influencing biomarker, fitness, and abiotic parameters (p<0.01, r^2=0.23). (b) The multiple linear regression analysis depicting the relationship between *Lepomis sp.* predicted and actual abundance

incorporating all influencing biomarker, fitness, and abiotic parameters ($p<0.01$, $r^2=0.33$). Regression equations are in Appendix Table A-12.

Fish abundance and richness in an assemblage was evaluated using the Simpson's diversity index (D). A plot of actual D values versus predicted values based on a model that included EROD activity, mercury concentration, condition factor, average stream width, and stream slope resulted in a cluster of points around the ideal 1:1 line (Figure 4-2). EROD activity, mercury concentrations, condition factor, average stream width, and stream slope were included in the model (Table 4-2). Although significant, condition factor does not have a strong contribution to the overall model (partial $r^2=0.0674$); however, the positive relationship shows an increase in fish assemblage integrity as condition factor (a measurement of fish health) increases. Table 4-2 illustrates the significance of highly contributing biomarker and fish health and fitness indicators in D. When the abiotic stream parameters have been excluded from this model, there was a significant contribution of independent variables included in this model ($p<0.01$, $r^2=0.50$). Biotic variables were much more important than abiotic variables in the model.
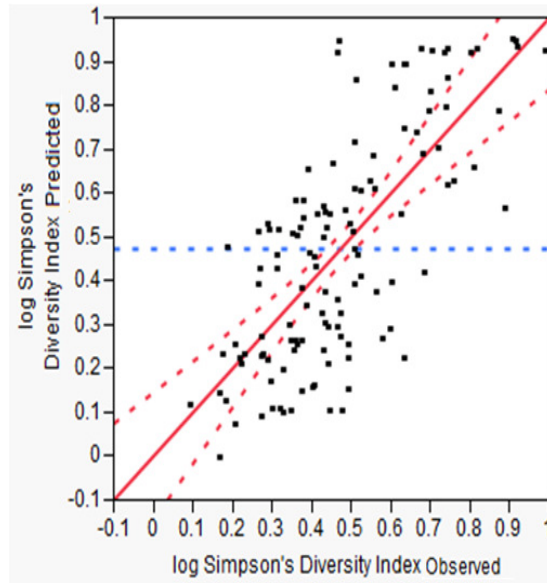
Figure 4-2. A multiple linear regression analysis exemplifying the relationship between predicted and actual species diversity as influenced by biomarker response, fitness indicators, and abiotic parameters ($p<0.01$, $r^2=0.52$). Regression equations are in Appendix Table A-12.

Table 4-2. Partial coefficients of determination of variables from the stepwise multiple linear regression analysis between predicted and actual Simpson's diversity index (D) ($p<0.01$, $r^2=0.52$).

Model variables	p value	partial r2
EROD Activity	<.0001	0.0361
Hg	<.0001	0.3947
K	<.0001	0.0674
Average Stream Width	0.0424	0.0109
Stream Slope	0.0761	0.0131

Similar to results shown in Chapter Two, dividing the data set up among watersheds provided good models for the prediction of D. These models differed among watersheds (Table 4-3). In each case dividing fish assemblage by river basin resulted in stronger coefficients of determination than grouping fish together at a statewide level with one exception. The Savannah River Basin had equal coefficients of determination for D compared to total fish D at the statewide level.

Table 4-3. Summary of statistical findings (p-values) excluding the influences of abiotic parameters and fish parameters on the five South Carolina River Basins for the determination of Simpson's diversity index (D).

River Basin	ACE	Catawba/Wateree	Pee Dee	Saluda	Savannah
Model variables	n=8	n=16	n=33	n=28	n=37
EROD Activity	**0.0322**	> 0.100	**0.002**	> 0.100	**0.0003**
Hg (ng/g)	> 0.100	**0.013**	**<0.0001**	**0.0031**	**0.0401**
Bile Fluorescence (5-Ring)	0.083	> 0.100	> 0.100	> 0.100	> 0.100
K	**0.0428**	**0.0044**	**0.0065**	> 0.100	**0.0038**
SSI	0.0611	**0.0499**	> 0.100	> 0.100	> 0.100
Estrogenicity	> 0.100	> 0.100	> 0.100	**0.0041**	> 0.100
GST	> 0.100	> 0.100	> 0.100	**0.0045**	**0.005**
R^2 of Multiple Regression	0.89	0.65	0.75	0.54	0.50

Bold p-values were considered statistically significant ($p < 0.05$).

Values shown as > 0.100 indicate that the forward stepwise multiple regression model eliminated the model effect and that trait was removed from the total model.

Biomarkers measured in this study can also be used to determine the relationship between predicted and actual South Carolina fish of priority concern abundance. Included in the

model were biomarkers EROD and GST activity, fish health and fitness measurements mercury burden and GSI, and abiotic parameters stream slope and dissolved oxygen. A significant relationship was observed (p<0.01, r^2=0.33). Increasing GST activity, an indicator of contaminants inducing oxidative stress, had an inverse relationship with priority species abundance. Another model that can be determined from the biomarkers measured in this study is the prediction of the priority species richness. Included in the model are the biomarker EROD activity, the fish health indicator mercury burden, and abiotic parameters stream water velocity and stream slope. There is a significant relationship between these covariates and priority species richness (p<0.01, r^2=0.38). For prediction of priority species richness, increasing EROD activity (an indicator of increasing aryl hydrocarbon exposure) is negatively correlated.

Discussion

Our previous work demonstrated that *Lepomis* genus could be used to predict fish assemblage in South Carolina watersheds (Chapter two). Using a sentinel species such as *Lepomis sp.* is a commonly employed technique to assess fish health in an aquatic ecosystem (Fitzgerald et al., 1999; Gibbons et al., 1998; van der Schalie et al., 1999). *Lepomis sp.* are considered indicators of watershed health (EPA, 2010). This genus of fish is tolerant to contaminant input when other fish species are not. They are one of the last fish species to leave a newly polluted site. They are also one of the first fish to migrate and settle at a site that has been previously polluted and uninhabited. With a

home range of approximately 50 meters, *Lepomis sp.* make ideal candidates for a sentinel species for contaminant exposure (Delinsky et al., 2009; Theodorakis et al., 2007).

In this study incorporating biomarkers, fish health and fitness indicators, and abiotic measurements into a model resulted in significant predictions of fish assemblage. At a statewide level, incorporating abiotic parameters into models increased predictability when relationships between *Lepomis sp.* and fish assemblage were assessed. However, while biomarker responses contributed to the model overall, they did not highly influence the regression. In most cases weaker coefficients of determination resulted when abiotic variables were removed. Other studies have identified the abiotic ecosystem heavily influencing fish assemblages. Studies suggest assessing neighboring fish communities is meaningless since the combination of biotic and abiotic variables will always differ (Jackson et al., 2001). The effect of "chemical" contamination is only one part of a bigger ecosystem framework. Abiotic variables including dissolved oxygen, conductivity, turbidity, and water temperature were shown to influence the diversity of floodplain fish in the Kaskaskia River in Illinois (Shoup et al., 2009). In the Shoup et al. (2009) study, lake depth was positively related to *Lepomis macrochirus* (Bluegill) and vegetated area was related to *Lepomis gulosus* (Warmouth) when sampling for catch per unit effort. The distance from the river to the oxbow lake was also positively related to diversity. While that study did not examine biomarker measurements, their results suggest abiotic variables can play a significant role in assemblage diversity in addition to chemical contaminants.

The combination of variables contributing to significant regressions differed between total fish species and *Lepomis sp.* EROD activity measures induction of the CYP 450 1A enzyme system in response to aryl hydrocarbon exposure. A comprehensive study by Whyte et al. (2000) listed multiple chemicals capable of EROD induction in fish. These included polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls, polybrominated biphenyls, pesticides, metals, and many other substances. Steatosis, cholestasis, free radical injury, and cirrhosis all result in a higher HSI indicative of liver impairment. Parasites found in the liver of some *Lepomis sp.* suggest fish are immunocompromised, stressed, and unable to fight infection. These outcomes are harmful to fish which would lower total fish species in an assemblage. Our results suggest increasing EROD activity and HSI is negatively associated with numbers of total fish species at sample locations. Chapter two showed little relationships between D and *Lepomis sp.*, total fish abundance, richness, and abiotic parameters. The combination of biomarker, fish health and fitness indicators, and abiotic parameters showed a significant strong coefficients of determination ($p<0.01$, $r^2=0.52$). Moreover, removing abiotic parameters from the model resulted in a similar coefficients of determination with D ($r^2=0.50$). Data presented here suggests D is more influenced by biotic factors as opposed to abiotic characteristics.

Studies suggest changes in diversity are dependent on the scale in which they are measured even after habitat disturbance (Dumbrell et al., 2008). Habitat requirements,

food availability, competition, predator/prey dynamics, and life history characteristics are all potential rationalizations for stronger relationships in neighboring systems. In this study, relationships between biomarker and fish health and fitness indicators were greater when grouped by river basin compared to the statewide level. When looking at a parameter like HIS, all river basins in South Carolina elicit similar responses (Figure 4-3a). However, differences are seen in other biomarker responses, such as 2-ring aromatic structures, based on river basins (Figure 4-3b). Grouping the river basins helps us to understand potential changes in habitats from one system to another. Grouping sites at a smaller scale would be difficult in this study since the density of sites is not high at any one area.

Exposure to contaminants does not necessarily imply a harmful endpoint will occur. Exposure to aryl hydrocarbons, for example, will induce activation of the CYP 450 1A detoxification pathway. Harmful endpoints may be avoided if the detoxification response is rapid enough. However, should a toxic threshold be reached and contaminants exceed detoxification systems, insult or injury to that organism is possible (Facey et al., 2005; Van der Oost et al., 2003).

a)

b)
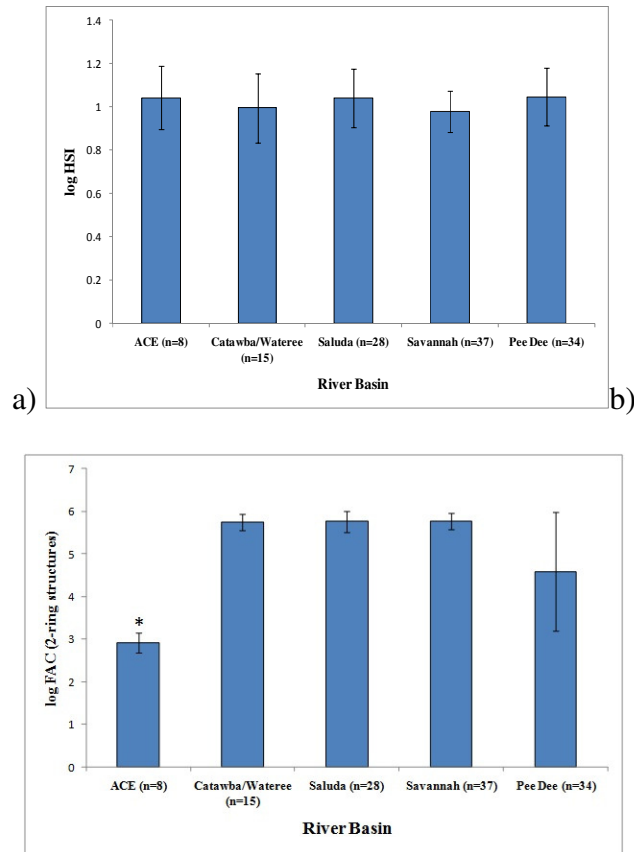
Figure 4-3. (a) Average HSI responses in *Lepomis* grouped by river basins. (b) Average FAC responses in *Lepomis* grouped by river basins. Error bars represent the standard deviation. Asterisks (*) indicates a significant difference ($p<0.05$) compared to the other river basins.

Conclusions

The objective of this study was to investigate relationships between contaminant exposure and fish assemblage integrity based on biomarker responses measured in *Lepomis sp*. Significant relationships existed between biomarker response

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measurements, fish health and fitness indicators, and fish assemblage. Abiotic variables were not as strong contributors to the model compared to biotic variables, but need to be taken into consideration. Grouping sites by river basin demonstrated better relationships compared to the statewide level. Changes in biomarker response may drive alterations at higher levels of biological organization. These results suggest chemical contamination at the molecular level may significantly influence fish assemblage integrity. Chemical inputs from anthropogenic origin are the likely sources for the introduction of contaminants into the water system. As human population densities rise, watershed deterioration will increase thereby placing an additional burden on fish and aquatic organisms exposed to these chemicals. Assemblage alterations will continue to occur with sensitive species leaving via migration or death. This process will continue until few tolerant or specialized species remain.

CHAPTER 5: THE EFFECTS OF CHANGING LAND ON FISH HEALTH AND FISH ASSEMBLAGE IN WADEABLE STREAMS IN SOUTH CAROLINA

Abstract

The human population of South Carolina has increased 15% (1.5% per year) over the last decade to reach 4.6 million people. Despite the relatively small population size compared to other states, this rapid influx of people is causing a strain on the ecosystem through habitat alteration and land development. During and after development the surrounding watershed is a collecting body for contaminants due to the interconnected nature of water systems. Therefore, in an aquatic system, consequences of poor landscape management decisions can be seen many miles from the initial source of disturbance. It is important to be able to measure how much a watershed has been disturbed as a result of the changing land development. One way to measure the magnitude of watershed disturbance is to assess contaminant response in fish from the watershed. The objective of this study was to determine if urban surface results in increased biomarker response measurements in the model fish genus *Lepomis*. A second objective was to determine if increasing urban development altered the native fish assemblage. With the inclusion of abiotic factors in the multiple regression model, total fish richness was significantly related to increasing urban surface at the statewide level ($p<0.01$, $r^2=0.26$). The hepatosomatic index (HSI) was also significantly related to increasing urban surface ($p<0.01$; $r^2=0.25$). In the Saluda River Basin, with the inclusion of abiotic factors, there was a significant relationship between urban surface and total fish species richness ($p<0.01$; $r^2=0.62$), total fish species excluding *Lepomis* sp. ($p<0.01$; $r^2=0.56$), and total *Lepomis sp*. abundance ($p<0.01$; $r^2=0.37$). Also in the Saluda River Basin, 5-ring aromatic hydrocarbon exposure and the HSI were both significantly

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influenced by increasing percent urban surface ($p<0.01$; $r^2=0.54$ and $p<0.01$; $r^2=0.73$, respectively). Results indicate increases in urban development resulted in increased biomarker response and altered native fish assemblage characteristics. This study demonstrates how land development and watershed alteration can negatively influence aquatic life at multiple levels of biological organization.

Introduction

Throughout the past decade the population of the State of South Carolina, USA increased at a rate close to 15% (1.5% per year; US Census, 2010) to reach 4.6 million people. While the population density per square mile is relatively low compared to other US states, the rate of growth is high. As population density increases the resulting urban development results in an increase in impervious surfaces such as roadways, parking lots, and building roofs. This changing land use can have dramatic effects on smaller streams and creeks which form the upper reaches of watersheds. Further, changing landscape in the upper reaches of a stream can have dramatic effects many miles downstream from the original land disturbance (Scott, 2008).

As the percentage of urban and impervious surfaces increases there is also an increase in the potential for environmental impact. Roughly 40% of priority species in South Carolina, which includes fish, herpetofauna, mussels, crayfish, and snails, depend on freshwater systems for some or all stages of life (SARP, 2007; SCDNR, 2006). In eastern North America the development of once forested land causes habitat loss and

interferes with natural processes and cycles especially in aquatic systems (Dudgeon et al., 2006; Vitousek et al., 1997). In urban settings, for example, during rainfall events metals, nutrients, spilled petroleum products, combustion by-products, garbage, and other non-point source contaminants are washed into lakes, rivers, and streams instead of being infiltrated into the ground or taken up by flora. Contributing to the issue are structures necessary for urban sustainability including wastewater treatment and sanitation facilities. Wastewater treatment facilities are not completely effective in eliminating contaminants and chemicals (such as endocrine disrupting compounds) from the water supply (Sowers et al., 2009; Truman et al., 2009). As a result many pharmaceuticals and other personal care products are accumulating downstream of these facilities in an active formulation with unknown consequences on fish and aquatic organism health. Priority species with specific life history requirements could be in jeopardy if freshwater systems become altered.

Strategies for sustainability can only be developed if scientific data exist to support decisions. However, currently there is paucity of information on the impact of changing landscape on water quality and ecosystem health; this makes it difficult to make informed decisions regarding aquatic conservation and restoration (Palmer, 2009). Because human population is ever increasing, a better understanding of these anthropogenic influences would facilitate informed and prudent land management decisions.

Understanding watershed processes is essential for quantifying the connection between changes in land use and aquatic ecosystem health (Allan, 2004; Scott, 2006). Fish and other organisms living in streams are among the first animals to become exposed. Fish sensitivity and resiliency to contaminants determines which species remain or leave once a toxic threshold is exceeded. Yet which species are more or less sensitive than others during times of contaminant stress is not known. Landscape characteristics that contribute to contamination are also largely unknown. One method to assess watershed health at the ecosystem level is to use a biological indicator of ecosystem integrity. An indicator fish species can assess the magnitude of contamination in the aquatic environment as a result of land transformation regardless of the initial source of disturbance.

A genus of fish that resides in many freshwater bodies throughout the United States (the southeast in particular) is *Lepomis* (sunfish). One reason *Lepomis sp.* is found in such a wide geographic range is because they are capable of surviving a variety of freshwater habitat conditions. These fish can be found in aquatic systems where contaminants are present in high concentrations when other fish species have left the area (Grabarkiewicz et al., 2008; TPWD, 2012; US EPA, 2010). During times of contaminant influx these species are more resilient than other fish species making them good indicators for a changing habitat. In South Carolina *Lepomis sp.* is found in roughly 80% of streams. The main species found in large numbers are *Lepomis macrochirus* (bluegill sunfish), *Lepomis auritus* (redbreast sunfish), *Lepomis gulosus* (warmouth sunfish), *Lepomis*

microlophus (redear sunfish), *Lepomis gibbosus* (pumpkinseed sunfish), *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish) (Keaton, 2007; Sayer, Chapter one).

Members of the *Lepomis* genera are considered by the US EPA to be biological indicators of watershed health (EPA, 2010). The high prevalence and tolerance to contaminant input make *Lepomis sp.* good candidates to monitor aquatic systems. Despite their vast geographic dispersion their home range is generally less than 50 meters. Evaluating *Lepomis sp.* health and fitness allows for an assessment of aquatic system contaminants in the vicinity (Keaton, 2007).

One way members of the *Lepomis* genera indicate aquatic contamination is through biomarker response measurements. Biomarker responses, which are measurable changes in cellular or biochemical structure, function, or behavior measurable in bodily fluids, tissues, or organs, are employed to assess aquatic contaminants in *Lepomis sp.* Biomarkers are being increasingly utilized in environmental studies because they are quick, efficient, and accurate ways to indicate the health status of an organism. They are utilized to gauge the physical, chemical, and biological stressors attributed to environmental conditions (Gomez-Martinez et al., 2006; Schreiber et al., 2006; Van der Oost et al., 2003). Biomarker measurements in a model fish species can be used to evaluate alterations in fish assemblage composition in addition to water quality characteristics and watershed health (Sayer, Chapter one).

Lepomis sp. biomarker measurements depict the magnitude of contaminants present in the surrounding aquatic environment being exposed to organisms. The greater biomarker responses at a site, the more likely fish at that location are being exposed to a contaminant compared to a site where response measurements are low. Using *Lepomis sp.* as a model fish, a species sensitivity gradient can be determined, identifying those fish species first to leave a site when contaminants are introduced. Remaining fish will be exposed to those contaminants and demonstrate elevated levels of detoxification enzymes in response. Biomarker responses can provide valuable information regarding how contaminants change fish assemblage, diversity, and overall watershed health (Allen et al., 2004). Examining how land is being used in a watershed can provide information correlating the introduction of contaminants in the water system with the disappearance of fish species.

While studies have been conducted examining specific pollution threats on fish health and fish populations, few studies have been conducted integrating land use, stream characteristics, fish species abundance and diversity, and the application of biomarkers as indicators of contaminant exposure. Therefore a comprehensive study linking landscape characteristics with aquatic endpoints is needed. An understanding of how these parameters influence each other will allow us to elucidate mechanisms associated with changes to fish health and fish assemblage. Furthermore, biomarker responses are changes at the molecular level of biological organization. Few studies analyze data

spanning the molecular through assemblage levels of organization. The goals of this study were two-fold in an effort to assess watershed health. The first objective was to evaluate if increasing percent urban surface results in increased biomarker response measurement in *Lepomis sp.* The second objective was to evaluate if increasing percent urban surface alters fish assemblage composition.

Results

Molecular level biomarker responses of fish from sample sites had wide ranges. EROD activity ranged from 0.03-294 pmol/mg/min (Appendix, Table A-11). GST activity ranged from 58.9-792 nm/mg/min. Bile fluorescence ranged from 283-1900000 FAC for two-ringed congeners, 118-838000 FAC for four-ringed congeners, and 17.7-173000 FAC for five-ringed congeners. The hepatosomatic index (HSI) ranged from 0.48-2.43.

The range of total fish found at sample locations was 1-1908 fish (mean = 340; median = 232) (Appendix, Table A-6). The range of total fish species found at sites was 1-29 species (mean = 14; median = 14). The range of total *Lepomis sp.* found at sites was 1-329 fish (mean = 60; median = 42). The range of total *Lepomis sp.* found at sites ranged from 1-7 species.

Stream parameters were measured and recorded. Average stream width ranged from 1.2-45 m. Average stream depth ranged from 0.07-0.58 m (Appendix, Table A-3). Average stream velocity ranged from -0.0022-0.45 m/sec. Average landscape slope ranged from -

0.228-1.05 m. Water pH ranged from 4.85-8.25. Average water temperature ranged from 14.46-31.54 °C. Dissolved oxygen in water ranged from 0.2-10.45 mg/L. Water turbidity ranged from 0.84-69.4 NTU. Water conductivity ranged from 11-868 microsiemens/cm.

There was a significant relationship between percent urban surface and ethoxyresorufin-O-deethylase (EROD) activity ($p<0.01$, $r^2=0.10$). EROD activity is often used as an indicator of aryl hydrocarbon exposure. A similar significant relationship was found between percent urban surface and glutathione-s-transferase (GST) activity ($p<0.01$, $r^2=0.13$). GST activity is often used as an indicator of exposure to compounds that generate free radicals and cause oxidative stress. Relationships were also found between percent urban surface and 5-ring aromatic compounds ($p<0.01$, $r^2=0.04$), and hepatosomatic index ($p<0.01$, $r^2=0.06$), an indicator of liver health.

Dividing the dataset into river basins improved some of the relationships. For example, the relationships between percent urban surface and EROD activity ($p<0.51$, $r^2=0.14$), GST activity ($p<0.01$, $r^2=0.39$), 5-ring aromatic hydrocarbon exposure ($p<0.03$, $r^2=0.17$), and hepatosomatic index ($p<0.03$, $r^2=0.17$) improved when only data from the Saluda River watershed were considered. Most relationships had low coefficients of determination.

In an attempt to strengthen these relationships and explain more of the variation in the data, multiple linear regression (MLR) analysis was used to incorporate abiotic parameters and account for natural site variability at the statewide scale. In general, the addition of abiotic independent variables strengthened relationships. Adding water temperature and dissolved oxygen as additional independent variables significantly improved the relationship between hepatosomatic index (HSI) and percent urban surface (Figure 5-1).
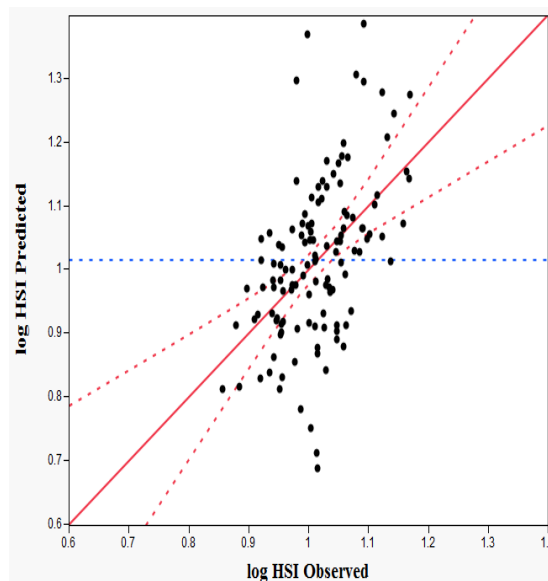
Figure 5-1. Linear regression between the HSI and the percent urbanized surface: coefficient of determination (r^2) equal to 0.25. Data presented as the predicted value of HSI based on the regression model taking into account significant factors including water temperature and dissolved oxygen. Regression equations are in Appendix Table A-12.

MRL analysis was conducted by river basin. For example, in the Saluda River Basin, there was a strong relationship between 5-ring aromatic hydrocarbon exposure and the independent covariates of percent urban surface, average slope, water temperature, and water turbidity ($p<0.01$, $r^2=0.54$) (Table 5-1). There was a strong relationships between the hepatosomatic index and covariates percent urban surface, average stream depth, average water velocity, water temperature, dissolved oxygen, water turbidity, and water conductivity (Table 5-1; $p<0.01$, $r^2=0.73$).

Table 5-1. Partial correlation coefficient of variables from the stepwise multiple linear regression analysis comparing percent urbanization to 5-ring aromatic hydrocarbons and HSI in the Saluda River Basin ($p<0.01$; $r^2=0.54$ and $p<0.01$; $r^2=0.73$).

Model variables	5-ring aromatic hydrocarbons		HSI	
	p value	partial r^2	p value	partial r^2
Percent Urban Surface	0.077	0.17	**0.018**	0.17
Stream Slope	**0.001**	0.23	> 0.100	-
Water Temperature	0.099	0.08	0.234	0.08
Turbidity	0.078	0.07	0.123	0.01
Ave Stream Depth	> 0.100	-	**0.0095**	0.10
Ave Water Velocity	> 0.100	-	**0.0016**	0.07
Conductivity	> 0.100	-	> 0.100	0.04
Dissolved Oxygen	> 0.100	-	**0.0064**	0.27
R^2 of Multiple Regression	0.54		0.73	

Bold p-values were considered statistically significant ($p < 0.05$).Values shown as > 0.100 indicate that the stepwise multiple regression model eliminated the model effect and that trait was removed from the total model.

Fish population and assemblage metrics were compared in the Saluda River basin using xy linear regression analysis. A negative correlation was found between increasing percent urban surface and total fish species richness in a linear regression analysis ($p<0.03$, $r^2=0.16$). While a positive correlation was found between increasing percent urban surface and total *Lepomis sp.* abundance in a linear regression analysis ($p<0.04$, $r^2=0.15$), there was no significant relationship between urban surface and total fish species richness, excluding *Lepomis sp.* ($p<0.05$).

As with the lower levels of biological organization, MLR was used to strengthen relationships at the population/assemblage levels and explain variation in the data at the statewide scale. The addition of abiotic independent variables strengthened relationships. Total fish richness was best explained using MLR with percent urban surface, average stream depth, water temperature, and dissolved oxygen as independent variables ($p<0.01$, $r^2=0.26$) (Figure 5-2).

Using MLR analysis in the Saluda River basin, total fish species richness was compared with percent urban surface and covariates average stream width, water pH, and water turbidity, resulting in a much stronger relationship ($p<0.01$, $r^2=0.62$). There was a significant relationship between total *Lepomis sp.* abundance and percent urban surface and average water depth ($p<0.01$, $r^2=0.37$). Total fish species richness other than *Lepomis sp.* and percent urban surface, average stream width, and water pH ($p<0.01$, $r^2=0.56$) also displayed a significant relationship.
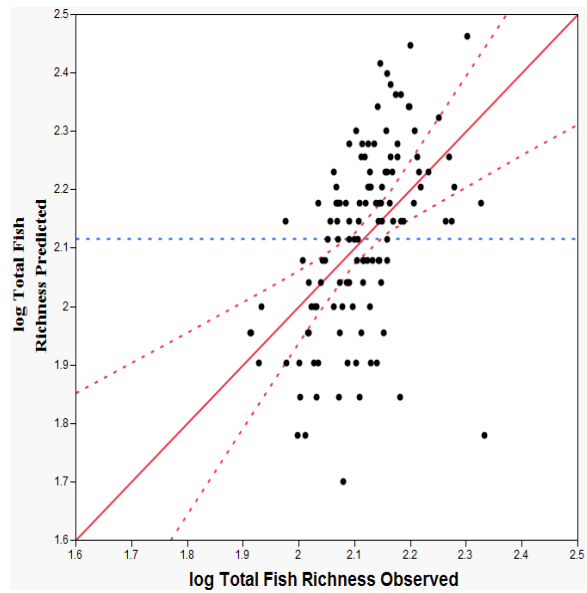
Figure 5-2. Linear regression between total fish richness and the percent urbanized surface: coefficient of determination (r^2) equal to 0.26. Data presented as the predicted value total fish richness based on the regression model taking into account significant factors including percent urban surface, average stream depth, water temperature, and dissolved oxygen. Regression equations are in Appendix Table A-12.

Table 5-2. Partial correlation coefficient of variables from the stepwise multiple linear regression analysis comparing urban surface with total fish richness, inclusive and exclusive of *Lepomis sp.*, and total *Lepomis sp.* abundance, in the Saluda River basin.

Model variables	Total Fish Richness		Total *Lepomis* Abundance		Total Richness (not *Lepomis*)	
	p value	partial r2	p value	partial r2	p value	partial r2
Percent Urban Surface	**0.0008**	0.16	**0.011**	0.15	**0.0079**	0.12
Ave Stream Width	**0.002**	0.30	> 0.100	-	**0.0004**	0.29
pH	**0.040**	0.11	> 0.100	-	**0.009**	0.15
Turbidity	0.097	0.05	> 0.100	-	> 0.100	-
Ave Stream Depth	> 0.100	-	**0.0071**	0.22	> 0.100	-
R^2 of Multiple Regression	0.62		0.37		0.56	

Bold p-values were considered statistically significant ($p < 0.05$).Values shown as > 0.100 indicate that the stepwise multiple regression model eliminated the model effect and that trait was removed from the total model.

Discussion

Studies examining watershed health are not rare although they are performed at different scales and endpoints. The impact of landscape alteration on tidal estuarine ecosystems has been examined by the National Oceanic and Atmospheric Administration's (NOAA) National Centers for Coastal Ocean Science organization in studies lasting several years. Their findings revealed impervious cover greater than 10% resulted in conditions unfavorable for macrobenthic taxa. Conditions became detrimental to organisms when impervious cover was greater than 30% (Holland et al., 2004). Subsequent analysis revealed food web degradation was due to water quality deterioration as a result of increased urbanization of the surrounding landscape (Lerberg et al., 2000). Another tidal estuarine study examining watershed land use and grass shrimp health found increased

PAH levels in close proximity to urban and impervious cover (Garner et al., 2009). Chapman et al. (2009) found an increase in transcription rates of detoxification enzymes in oysters as a result of increasing population growth in coastal areas. He attributed deterioration of the watershed from urbanization as the major factor in the increase in detoxification system expression.

Similar relationships have also been seen in freshwater systems. Allan et al. (1997) found strong decreases in habitat and biological indices of integrity in freshwater ecosystems as agricultural land cover increased greater than 20%. Another study reported freshwater biodiversity was declining from a number of anthropogenic sources including water pollution, flow modification, and habitat degradation (Dudgeon et al., 2006). In this study, biodiversity, as determined by total fish richness, decreased as a result of urbanization.

Current watershed studies over a large geographic scale encompassing multiple watersheds have been ongoing for decades. One example of this is the International Boundary and Water Commission (IBWC) involving the United States and Mexico. In addition to water regulation, the IBWC focuses on sustainability and threats to conservation by conducting environmental risk assessments, water quality reports, and environmental impact investigations. Studies examining metal introduction to the water system, nutrient loading, pesticides, aquatic life, and biological control organism have been undertaken by the IBWC (IBWC, 2011). The Hudson River Watershed Alliance

(HRWA) centered in New York is another large scale study of human influences on the Hudson River watershed. Like the IBWC, the HRWA identifies threats to the Hudson River, determines best management strategies, and conveys findings to the public (HRWA, 2011).

The Center for Watershed Protection (CWP) is an organization that works to protect, sustain, conserve, and restore watersheds and water bodies (i.e. streams, rivers). The CWP is a major contributor to the scientific understanding of healthy watershed characteristics and factors leading to the degradation of water quality. One of their focuses is urban wetland protection and conveyance of scientific findings to watershed professionals. Their continued study of negative watershed impacts has lead to innovated ideas such as permeable pavement (Virginia DCR, 2010). The CWP has reported impervious cover can result in drastic declines in biological integrity. One of the biggest contributors of impervious cover is urban surface. An increasing human population will result in an increase in urban/impervious structures necessary for human sustainability. Using a general watershed planning model called the impervious cover model by the CWP, stream quality indicators show a decline when impervious cover is greater than 10%. Severe degradation occurs when impervious cover exceeds 25% (CWP, 2003).

Another comprehensive report by Schueler et al. (1994) revealed several negative associations between urban and impervious cover with watershed degradation. Schueler demonstrated a 0.95 Person correlation between percent impervious surface and water

runoff (expressed as a runoff coefficient assuming the parking lot is 100% impervious with a 3% slope). This is compared to a meadow with a 0.06 runoff coefficient during a storm event (assuming a 3% slope). The runoff volume for the impervious surface was 3450 cubic feet compared to 218 cubic feet for the meadow. As the percent impervious surface increases, more water will run via overland flow into a collecting body of water. Peak discharge rate, runoff volume, runoff velocity, and phosphorus, nitrogen, and zinc load were shown to increase when the surface was impervious (parking lot) compared to non-impervious (meadow). Watershed runoff coefficients, nutrient inputs, and water temperature all increased as a result of percent increase cover. Best management practices provided some stability, but were ineffective at higher percent impervious cover. The study by Schueler et al. (1994) also revealed freshwater fish, insect, habitat quality, and other aspects of biological integrity all declined when urbanization was greater than 10%.

This study showed that with increases in the percent urban surfaces, there was an increase in the EROD activity, GST activity, 5-ring aromatic hydrocarbon exposure, and decrease in the HSI. The increases in urban surfaces potentially increase urban runoff resulting in elevated aquatic exposure of chemical contaminants. Schreiber et al. (2006) reported that during storm events, there was an increase in urban runoff resulted in elevated exposure of metals and PAHs to fish and other aquatic organisms. Development of once undisturbed land by removal of trees, shrubs, and other vegetation disrupts or eliminates groundwater infiltration. Hur et al. (2008) reported even minor alterations in the

landscape such as removal of trees from a forest result in increased nutrient runoff into freshwater systems. The removal of flora prevents the uptake of nutrients and other constituents through the roots. This is especially problematic at the riparian zone along streams or creeks where plant roots stabilize the bank before the water body. Removal of plants from these areas leads to an increase in soil erosion. In addition, without riparian flora excessive amounts of contaminants are allowed to enter the water system at high velocities during storm events (Casey et al., 2001).

In this study an evaluation of results at the statewide level further identified potential negative impacts on fish health and fish species richness. With covariates accounted for, decreases in fish health indices are observed as indicated by the hepatosomatic index as well as total number of fish species. Urban watersheds contain a larger percentage of impervious surfaces compared to more rural areas. Contaminant sources are typically greater in these areas. Information in this study indicated fish sensitive to aquatic contaminants in urban watersheds are forced to leave the area (via migration or death). At some point a contaminant threshold will become exceeded and additional species will disappear. While a contaminant "threshold" has not been determined, a general consensus is around 10% urban surface there starts to be significant declines in biological diversity (Allan et al., 1997; Schueler, 1994; Wang et al., 2000). Our study is in accordance with this estimated value. Both biomarker responses increased while fish assemblage integrity measurements decreased around 10-15% urban surface. There was considerable variation and randomness in assemblage and biomarker measurements less

than this 10% threshold percentage. For other types of land use classifications, such as agricultural, that percentage may be different before declines are observed.

Focusing on the Saluda River Basin enhanced relationships compared to the statewide level. It is probable fish living in a river basin are exposed to similar contaminants compared to non-neighboring systems. Saluda River impacts of increasing urbanization resulted in increases in biomarker response and alterations in total fish species richness. Simple linear regression analysis models for EROD activity, GST activity, 5-ring aryl hydrocarbon exposure, and hepatosomatic index increased as a consequence to increases in percent urban surface. The liver, a major detoxification organ in fish function in *Lepomis sp.*, was measured with these assays. With abnormal liver function and size expected after prolonged exposure to contaminants, it is interesting to note the increase in HSI with increased urbanized surfaces.

There is a decrease in the total fish species as urban surface increases, possibly due to the reduction of sensitive fish species or those fish with specific life history requirements. Interestingly, the abundance of *Lepomis sp.* increased as percent urban surface increased. *Lepomis sp.* are a more resilient fish species know for their tolerance to a changing environment. It is possible the opportunistic *Lepomis sp.* migrated and occupied new available areas when sensitive fish species have left. While this is an interesting observation, other studies report no effect of increased urbanization on fish abundance. A study by Scott et al. (1986) reported no decreases in salmonids after habitat alteration

and nutrient loading in a 30 month investigation. That study did note, however, a difference in species diversity between salmonid and nonsalmonid species. A study by Daniels et al. (2005) spanning decades of recorded data reported changes in species in a fish assemblage in the Hudson River due to dredging, industrial and domestic waste discharge, urban development, watershed deforestation, an increase in agriculture, and water removal for commercial, industrial, and agricultural purposes. Populations of several native species declined (i.e. rainbow smelt and Atlantic tomcod) while other threatened species increased (i.e. striped bass). Other species in the study remained stable throughout the habitat alteration (i.e. spottail shiner). The authors observed a shift from species associated with open water to those associated with vegetation including the *Centrarchidae* family (which includes *Lepomis sp.*). Furthermore, elevated water temperature, the introduction of contaminants, and the introduction of zebra mussels were used to explain assemblage alterations over time.

In this study, an incorporation of abiotic parameters greatly strengthened relationships within models. Five-ring aryl hydrocarbon exposure as well as HSI exemplified much stronger relationships when covariates were included. An investigation conducted by Keaton (2007) revealed urbanization resulted in a significant negative relationship with the hepatosomatic index of *Lepomis sp.* Her study also revealed a positive relationship between bile fluorescence, a bioindicator for PAH exposure, and urbanization.

Total fish richness, *Lepomis sp.* abundance, and total fish richness other than *Lepomis sp.* displayed much stronger correlations. This suggests the importance of accounting for natural variability and abiotic factors. Other studies have examined the importance of abiotic variables incorporated in fish assemblage analyses. Dissolved oxygen, conductivity, turbidity, and water temperature can greatly influence the presence or absence of fish species (Shoup et al., 2009). Other studies have evaluated several aspects that alter fish communities of which chemical contamination is only one part. Like abiotic factors and the physical environment, predator/prey dynamics, competition, scale, habitat preference, and stream size all play a role when determining if a fish species will be found (Jackson et al., 2001). Further characterization of effects of percent urban surface with abiotic parameters (water temperature, dissolved oxygen, and turbidity) would be beneficial.

While xy linear relationships were weak, most were significant at the p<0.01 level. The wide geographic range of sites at the statewide and even Saluda River Basin scale may be too large to identify strong trends using this technique. In some instances considerable distance was traversed even when sampling adjacent sites. The influence of distance or scale between sample sites is not new (river basin compared to statewide scales for example). The diversity of fish species may be dependent on scale regardless of habitat alteration (Dumbrell et al., 2008). A study by Soimasuo et al. (1995) examined juvenile whitefish from five stations downstream from a bleached pulp and paper mill for one month. The five sample locations were placed at different locations within a 16 km

stretch of Lake Saimaa, Finland. Endpoints in their investigation included CYP 450 1A, GST activity, bleached kraft pulp mill effluences, and other conjugation enzymes. Significant CYP 450 1A induction occurred at the two sites closest to the mill compared to reference sites, 3.3 and 5.8 km downstream. Chlorinated organics (chlorophenolics, organic halogens, and chlorophenolics) were all significantly increased at the first sample location. There was no significant induction in GST or conjugation enzymes. Distance from the discharge site seemed to play a role in CYP 450 1A induction with sites further downstream unaffected. Even over a large area, the low numbers of sample sites used in the study prevents more definitive relationships between urbanization, biomarker response, and fish assemblage. A majority of sites were isolated in more rural, low-developed areas as opposed to urban, high-developed locations. This could explain the lack of significant biomarker relationships. Even after chemical contamination a response may not be detected because of the low statistical power.

For most other biomarker measurements, random patterns of values were observed when examining measurements less than 15% urbanization. Greater than 10-15% urban surface was consistent with the decline of fish assemblage integrity. This is consistent with other studying findings in the literature where no discernable pattern of fish assemblage alteration typically occurs until around 10-15% urbanization (Allan et al., 1997; CWP, 2003; Schueler, 1994). As the percent urban surface exceeds 15% a steadily increasing response occurred in most instances.

Conclusions

Indicators of fish health at the organism, population, and community scales decline concurrent with an increase in urban surface area within a watershed. Biomarkers and fitness indicators suggest these declines may be due to exposure to anthropogenic contaminants increasing with urbanization. Fish assemblage alterations at the population and community levels indicate these declines might also be due to changes in habitat as a result of urbanization. With the inclusion of abiotic factors in the multiple regression model, biomarker, fish health and fitness indicators, and fish assemblage integrity were significantly related to increasing urban surface at the statewide level. Combination of contaminant exposure and habitat alteration will result in loss of those species which are unable to tolerate a changing aquatic ecosystem. Those species which are most sensitive will be the first to leave. Additional species will be forced to leave once the surrounding ecosystem has deteriorated beyond their tolerance. These results facilitate a quantitative prediction of aquatic ecosystem health as a function of land use changes within a watershed. This relationship may assist urban planners avoid excessive aquatic habitat deterioration while meeting the needs of a growing urban population.

CHAPTER 6: CHANGES IN *LEPOMIS SP.* MOLECULAR, ORGAN, INDIVIDUAL, POPULATION AND FISH ASSEMBLAGE LEVELS OF BIOLOGICAL ORGANIZATION AS A RESULT OF CONTAMINANT EXPOSURE

Abstract

Environmental contaminants impact organisms in a variety of different ways. While these stressors may be harmful to an organism's health it may not be detrimental for a number of reasons. Detoxification mechanisms and other coping strategies exist which protect individuals from harm. At lower levels of biological organization these molecular-type interactions happen frequently although they are not always associated with negative endpoints. At higher levels of organization, such as the community level, changes do not occur until alterations throughout the biological organization cascade have already occurred. For this reason changes at higher levels of organization are considered to be most severe since it implies all the organisms in the group may have already been detrimentally exposed to a harmful agent(s). By the time the community displays abnormal signs it is usually in the later stages of disease, distress, or contaminant exposure. As a consequence many organisms in the population or community are unlikely to survive. In order to assess changes at multiple levels of biological organization a vast dataset is required. From 2006-10 a large scale project was initiated in order to determine how changes at the molecular level of biological organization affect the tissue, organ, individual, population, and assemblage levels of organization. *Lepomis sp.* (sunfish) were collected from approximately one-hundred twenty wadeable stream sites throughout South Carolina. *Lepomis sp.* were assessed for contaminant exposure at the molecular level using biomarker response measurements. The molecular changes were compared with changes at the tissue/organ, individual, and population levels in *Lepomis sp.* as well as with the entire fish assemblage. Using xy linear regression

analysis, the results of this investigation reveal significant relationships between individual levels of biological organization. Stream size class 2 showed a significant positive relationships between tissue mercury concentration and the hepatosomatic index (HSI) ($p<0.05$; $r^2=0.14$) and condition factor with HSI ($p<0.05$; $r^2=0.10$). At the statewide level, significant positive relationships were observed between total *Lepomis sp*. abundance and total fish abundance ($p<0.05$; $r^2=0.13$) and total *Lepomis sp*. richness and total fish richness ($p<0.05$; $r^2=0.08$). Relationships between lower levels (i.e. molecular and tissue/organ) were more poorly related than those at higher levels (i.e. population and assemblage). The vast majority of relationships at all levels showed very poor correlation coefficients regardless of scale (statewide, stream size class, or river basin). The hepatosomatic index (HSI) at the tissue/organ level was the most identifiable metric in the analysis.

Introduction

As the human population continues to escalate there is growing concern over the environmental consequences. Structures required for anthropogenic growth and sustainability have a poorly understood impact on the surrounding ecosystem. Commercial, industrial, and residential buildings are constructed altering the native habitat and natural conditions. Increasing population density results in an increase in impervious surfaces like roadways, parking lots, and building roofs. This changing land use can have dramatic effects on smaller streams and creeks which form the upper reaches of watersheds (Allan, 2004).

Aquatic systems are highly complex, interconnected, and encompass the entire drainage area of a watershed. Drought and flooding can complicate issues by fragmenting or merging streams, rivers, and lakes (Pringle, 2001). Although water systems can become fragmented, watersheds encompass the entire drainage area of the region. Consequences of poor land management practices (i.e. siltation, excessive nutrients, flow disruption) can negatively impact flora and fauna that depend on these water sources for survival, reproduction, and/or development. Due to the interconnected nature of water systems, water flow will end up in larger rivers, reservoirs, and coastal areas. As a consequence, a changing landscape upstream can have dramatic effects many miles downstream from the initial source of disturbance (Scott, 2008).

If the wadeable streams in the upper reaches of a watershed are developed, the uniqueness of local flora and fauna could be at high risk (Marion, 2008). Sensitive species will be forced to leave the area if their habitat is altered or degraded. Non-sensitive species can also be put at risk. Removal of course woody habitat for developmental purposes, for example, caused largemouth bass to consume less fish and grow more slowly compared to a non-altered reference site (Sass et al., 2006). In the same study yellow perch greatly declined compared to the reference site as a result of increased predation (Sass et al., 2006). The problem becomes difficult to address as more variables are introduced. In regards to separating natural and anthropogenic sources of disturbance, Clements states, "Distinguishing natural variation in structure and function

from variation due to anthropogenic stress is one of the most challenging aspects of interpreting results of biomonitoring studies. Estimating natural background variation is especially important where effects of disturbance are moderate" (Clements, 1997).

Roughly 40% of priority species in South Carolina, which includes fish, herpetofauna, mussels, crayfish, and snails, depend on freshwater systems for some or all stages of life (SARP, 2007; SCDNR, 2006). Freshwater species worldwide face accelerated extinction rates relative to most other wildlife taxa (Ricciardi et al., 1999; Sala et al., 2000). The southeastern U.S. in particular is of high concern due to long term declines in native fish and aquatic species. The Southeast Aquatic Resource Partnership (SARP) and SC Department of Natural Resources (SCDNR) are two agencies which share this concern and are looking for ways to identify problems and create solutions. In 2006 the SCDNR submitted a Comprehensive Wildlife Conservation Plan to the U.S. Fish and Wildlife Service which included descriptions of 125 species of fish, herpetofauna, crayfish, and snails that are dependent on aquatic systems for most or all of their life-stages (SCDNR, 2006).

An introduction of contaminants into the aquatic ecosystem from urban sources impacts organisms at the molecular level of biological organization. However, molecular changes as a result of contaminant exposure do not always result in unfavorable outcomes. While research may indicate a contaminant is harmful to cells in laboratory experiments it may not accurately reflect detoxification processes and coping strategies utilized by a living

organism. Therefore toxic effects at the molecular level do not necessarily result in decreased fitness or increased mortality at population or community levels. However, energy needs to be expended to cope with the harmful agent (Siligato et al., 2001). If detoxification mechanisms are not able to overcome the harmful stressor(s) then the health and fitness of that organism will deteriorate. Still, if one individual is exposed, the rest of the population may remain unharmed.

A fish assemblage or community is considered a highly robust and representative measure of biological integrity (Hughes et al., 1998). A fish assemblage is defined as groups of fish populations overlapping at the same place at the same time. A fish assemblage differs from a fish community in that the latter incorporates the surrounding ecosystem and ecosystem dynamics which are constantly changing. A change at these levels only occurs if changes in lower levels have already occurred. For this reason, negative effects at upper levels of biological organization are considered more severe. Organisms in the community have been exposed to a harmful agent in the environment for long enough duration to induce negative health outcomes in all exposed organisms. Detecting a negative change at this level usually occurs when it is too late for organisms to leave or adapt.

It can be difficult to measure changes between levels of biological organization for a number of reasons. Detoxification mechanisms, behavior modification, and gene activation are examples of how organisms adapt to a changing environment. The ability

to leave or migrate away from a stressful ecosystem is another strategy employed (Schueler, 1994). The CYP450 enzyme system, for example, is used to metabolize aryl hydrocarbons to non-toxic substances. Antioxidant systems are in place to prevent free radicals from exerting a harmful effect on membranes, proteins, and other organelles. However, should any detoxification systems become overwhelmed then a harmful injury may result. If an organism does not succumb to the contaminant directly it may become impaired. Behavior alteration, predation, parasitism, disease, and other forms of secondary distress may occur if individual fitness is reduced (Kramer et al., 2010; Neuman-Lee et al., 2011). Additional energy must be expended to cope with the stressor. Spatial and temporal scales impact the biotic and abiotic environment where fish reside influencing their presence or absence (Adams et al., 2000). One study found lake depth and distance from a river to a lake positively related to fish diversity (more diversity was found at the river/lake interface). Abiotic parameters including dissolved oxygen, conductivity, turbidity, and water temperature were influencing factors as well (Shoup et al., 2009). Dunson and Travis (1991) allege abiotic factors are the most important group of variables in a community assessment and must be incorporated into a study design. Accounting for mixtures can be problematic and extrapolation across multiple levels of organization is often needed but can be difficult and inconclusive (Munns, 2006).

One technique to measure changes at the molecular level is through biomarker response. Biomarkers are changes in cellular or biochemical structure, function, or behavior measurable in bodily fluids, tissues, or organs. They are used to gauge physical,

chemical, or biological stressors which can be attributed to certain environmental conditions. Biomarkers have been demonstrated on multiple occasions to be useful indicators of health status within an organism (Gomez-Martinez et al., 2006; Schreiber et al., 2006; Van der Oost et al., 2003). Biomarkers provide valuable information at the molecular level of biological organization (Sayer, Chapter one). Cellular alterations as a result of an environmental change form the basis of a cascade of subsequent effects for higher up levels of organization. One drawback to measuring biomarkers is the molecular interactions occur rapidly and are stressor-specific. There are uncertainties demonstrating causality at the molecular level to population and community level endpoints (Clements, 2000).

Fish and other organisms living in aquatic systems are among the first animals to become exposed to contaminants that enter the water. If a fish species is able to tolerate contaminant fluctuations by upregulating detoxification mechanisms, then that species will likely remain at that site. However, if a fish species is not able to tolerate contaminant increases they will likely migrate away from that site or die. Remaining fish will demonstrate elevated levels of detoxification enzymes in response. Measuring biomarker responses allows an investigator to determine the magnitude of contaminant exposure that that site. Through this way a gradient from least to most impacted sites can be determined. Changes in a fish assemblage as a result of contaminant input can indicate modifications in the surrounding aquatic environment unfavorable for survival.

Sensitive species will leave the assemblage while more tolerant ones will remain. As a result, the integrity of the assemblage declines.

One genera of fish that resides in many freshwater bodies throughout the United States (the southeast in particular) is *Lepomis*. This genus of fish is found in roughly 80% of streams in South Carolina (Keaton, 2007). In South Carolina *Lepomis sp.* are commonly found in abundance, the main species being *Lepomis macrochirus* (bluegill sunfish), *Lepomis auritus* (redbreast sunfish), *Lepomis gulosus* (warmouth sunfish), *Lepomis microlophus* (redear sunfish), *Lepomis gibbosus* (pumpkinseed sunfish), *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish) (Sayer, Chapter one).

Members of the *Lepomis* genera are considered by the US EPA to be biological indicators of watershed health (EPA, 2010). One reason *Lepomis sp.* are found in such a wide geographic range is because they are capable of surviving a diverse array of aquatic habitats. These fish can be found in aquatic systems where contaminants are present in high concentrations when other fish have left the area (Grabarkiewicz et al., 2008; TPWD, 2012; US EPA, 2010). During times of contaminant influx these fish species are more resilient than other fish making them good indicators for a changing habitat. When other fish species have left a polluted site *Lepomis sp.* remain. The high prevalence of *Lepomis sp.*, tolerance to contaminant input, and resiliency make them a good sentinel species to monitor aquatic systems. Furthermore, despite their wide geographic

dispersion, their home range is generally less than 50 meters (Keaton 2007). A model fish like *Lepomis sp.* can indicate aquatic contamination through measurements of biomarker response. These responses provide information that can be used to determine contaminant exposure and how the assemblage becomes altered.

The purpose of this study was to characterize the biological impacts of contaminant exposure at the molecular/biochemical, tissue/organ, individual, population, and community levels of organization. *Lepomis sp.* were used as a fish model to assess changes throughout the levels. Different measurements, including biomarkers and somatic indices, were used to describe contaminant endpoints at the molecular and tissue/organ levels of organization.

Results

Sampling occurred during summer months from June 2006 through May 2010 at one-hundred twenty-seven random sample sites throughout South Carolina. Molecular level biomarker responses at sample sites had wide ranges (Appendix, Table A-11). EROD activity ranged from 0.03-294 pmol/mg/min. GST activity ranged from 58.9-792 nm/mg/min. Bile fluorescence ranged from 283-1900000 FAC for two-ringed congeners, 118-838000 FAC for four-ringed congeners, and 17.7-173000 FAC for five-ringed congeners. Estrogenic content ranged from 93.0- 41800 estrogen binding equivalents. Individual organism biomarkers were assessed. Condition factor (K) ranged from 1.17-3.16. The hepatosomatic index (HSI) ranged from 0.48-2.43. The spleen-somatic index

(SSI) ranged from 0.0037-0.52. The gonadosomatic index (GSI) ranged from 0.003-9.7. Mercury concentrations in fish tissue ranged from 2.82-379 ng/g Hg.

The range of total fish found at sample locations was 1-1908 fish (mean = 340; median = 232; Appendix, Table A-6). The range of total fish species found at sites was 1-29 species (mean = 14; median = 14). The range of total *Lepomis sp.* found at sites was 1-329 fish (mean = 60; median = 42). The range of total *Lepomis sp.* found at sites ranged from 1-7 species. Tolerant *Lepomis sp.*, which excludes *Lepomis marginatus* (dollar sunfish), and *Lepomis punctatus* (spotted sunfish), ranged from 0-329 fish and from 0-5 species at sample sites. *Lepomis macrochirus* (bluegill sunfish) and *Lepomis auritus* (redbreast sunfish), two commonly encountered *Lepomis sp.*, ranged from 0-282 total fish. Simpson's Diversity Index (D) calculations ranged from 0.01—1 (Appendix, Table A-11).

Statewide comparisons between biological levels of organization were conducted using xy linear regression. Analysis of levels showed minimal relationships at the statewide scale (Table 6-1). Significant relationships occurred more between higher levels of organization (i.e. individual and assemblage) compared to lower levels (i.e. molecular and tissue/organ).

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Table 6-1. Coefficients of determination (r^2) of different levels of biological organization at the statewide level. Bold values indicate significance ($p<0.05$).

		Tissue/Organ			
		HSI	SSI	GSI	K
Molecular	EROD Activity	0.001	0.01	0.0001	0.01
	GST Activity	0.02	0.001	0.01	0.03
	Estrogenicity	0.018	0.02	0.02	0.004
	5-Ring Aromatics	0.03	0.08	**0.08**	0.001
	Mercury (Hg)	**0.06**	0.01	0.001	0.007

		Tissue/Organ		
		HSI	SSI	GSI
Individual	K	0.002	0.001	**0.04**

		Population				
		Total *Lepomis* Abundance	Total *Lepomis* Richness	Tolerant *Lepomis* Abundance	Tolerant *Lepomis* Richness	Bluegill/Redbreast Abundance
Individual	K	**0.04**	0.001	0.019	0.001	0.01

		Population				
		Total *Lepomis* Abundance	Total *Lepomis* Richness	Tolerant *Lepomis* Abundance	Tolerant *Lepomis* Richness	Bluegill/Redbreast Abundance
Assemblage	Total Fish Abundance	**0.13**	0.008	**0.07**	0.02	**0.13**
	Total Fish Richness	**0.16**	**0.08**	**0.09**	**0.05**	**0.17**
	Simpson's Diversity Index	0.017	0.01	0.03	0.007	0.02

Stream classes were assessed for molecular and tissue/organ relationships. When assessing class 1 streams a significant relationship at the molecular and tissue/organ levels for estrogenicity and HSI ($p<0.05$, $r^2=0.18$). This was the only significant relationship seen for class 1 streams. The assessment of class 2 streams showed a significant relationship for Hg concentration and HSI (Figure 6-1a; $p<0.05$, $r^2=0.14$) and also a significant relationship for K and HSI (Figure 6-1b; $p<0.05$, $r^2=0.10$). Poor

relationships and correlation coefficients were seen at the molecular and tissue/organ levels when analyzed by stream scale.

a)

b)
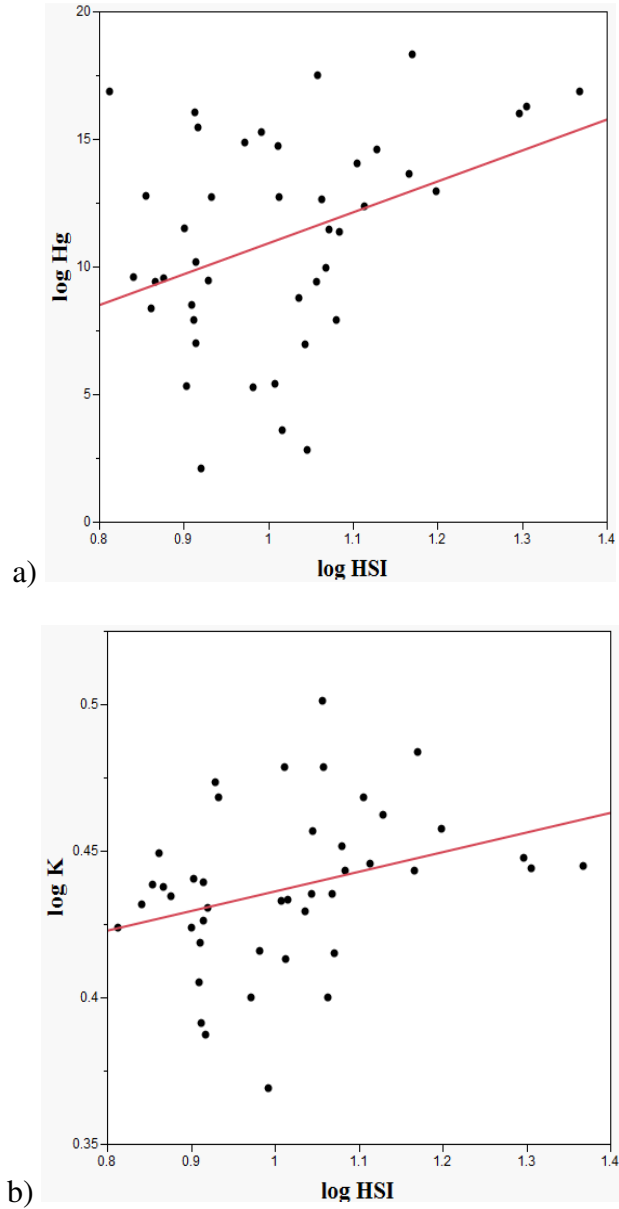
Figure 6-1. A statewide stream size class 2 comparison of (a) molecular and tissue/organ levels of organization between *Lepomis sp.* mercury concentration and the hepatosomatic index ($p<0.05$, $r^2=0.14$) and (b) of tissue/organ and individual levels of organization

between *Lepomis sp.* condition factor (K) and hepatosomatic index (p<0.05, r^2=0.10). Regression equations are in Appendix Table A-12.

The ACE, Pee Dee, and Savannah River basins were separated from the statewide level to assess molecular and tissue/organ relationships within river basins. During the assessment few significant relationships between the different levels of organization were found. The ACE River basin showed significant, positive relationship between EROD activity and spleen-somatic index (p<0.05, r^2=0.90). The Pee Dee River basin showed a significant relationship at the molecular and tissue/organ levels for GST activity and HSI (p<0.05, r^2=0.18). The Savannah River basin demonstrated a significant relationship between estrogenicity and GSI (p<0.05, r^2=0.13). There was also a significant relationship between GST activity and SSI (p<0.05, r^2=0.20). Insignificant relationships and poor correlation coefficients were seen at the vast majority of the biological organization levels when analyzed by river basin.

A statewide comparison between population and assemblage levels of organization was analyzed. There was a significant relationship between *Lepomis sp.* abundance and total fish abundance (Figure 6-2a; p<0.05, r^2=0.13) and a significant relationship between *Lepomis sp.* richness and total fish species richness (Figure 6-2b; p<0.05, r^2=0.08). Poor or uncorrelated relationships were seen at the statewide level for remaining levels of organization. In the Pee Dee River basin, a significant moderate relationship at the population and assemblage levels for total *Lepomis sp.* abundance and total fish

abundance ($p<0.05$, $r^2=0.25$), and also a significant, moderate relationship at the population and assemblage levels for tolerant *Lepomis sp.* richness and total fish species richness ($p<0.05$, $r^2=0.18$).   Other river basins showed poor or weakly correlated relationships.
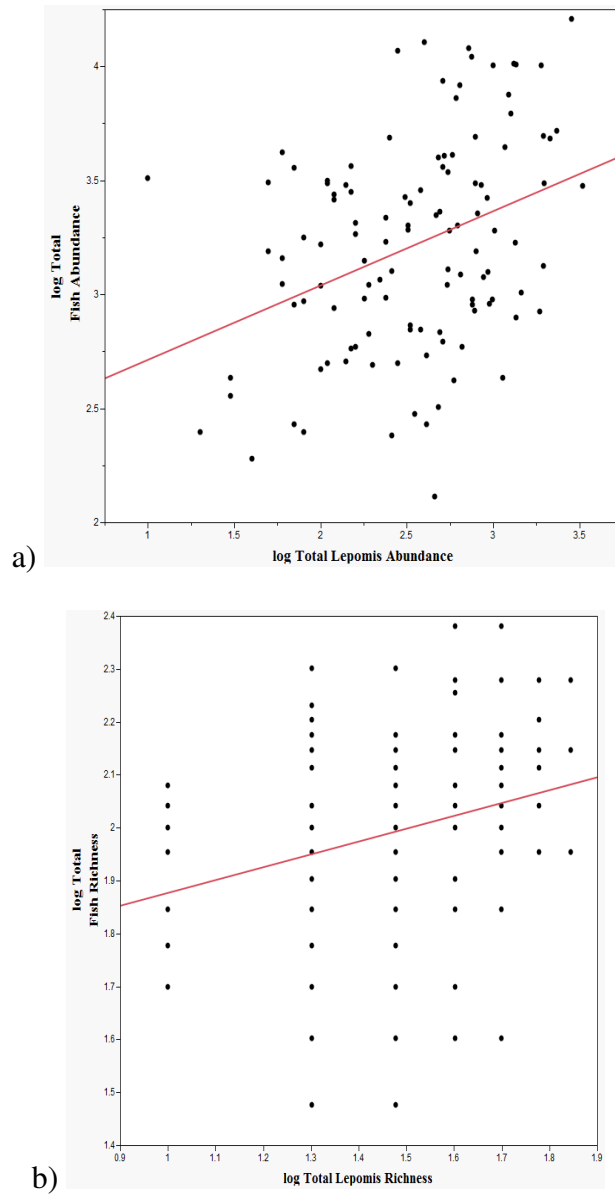
Figure 6-2. Linear regression between (a) total *Lepomis sp.* abundance and total fish abundance ($p<0.05$, $r^2=0.13$) and (b) total *Lepomis sp.* richness and total fish richness at the statewide level ($p<0.05$, $r^2=0.08$). Regression equations are in Appendix Table A-12.

Discussion

With the ever growing number of environmental issues, an understanding of how changes at the molecular level affect the multiple levels of biological organization is important. Still, an assessment of changes in a fish population, assemblage, or community stemming from a molecular cause may be difficult for a number of reasons. Detoxification mechanisms, changes in behavior, and protective gene induction are all means implored by fish to prevent injury from chemical contaminants (Adams et al., 2000; Van der Oost et al., 2003). Water temperature, pH, turbidity, conductivity, and other water characteristics influence the presence of fish at a location (Shoup et al., 2009). Fish can migrate away from an area that is becoming unfavorable for survival. A short duration of exposure to a contaminant may not be enough to cause harm. Alternatively, should a fish be exposed to a toxic or harmful substance for an extended duration then the likelihood of an unfavorable health outcome increases. The progression of distress due to a molecular interaction will be carried to another level of biological organization.

Previous attempts to link molecular endpoints with community level effects investigated differences between the Pigeon and Little Rivers originating in North Carolina and Tennessee, respectively (Adams et al., 1992). The Pigeon River is highly contaminated with bleached kraft mill effluents (BKME) while the Little River is not. In comparison to Little River, the study authors noted elevated EROD activity, DNA integrity abnormalities, microsomal proteins, spleen-somatic index, and a decrease in the HSI in Pigeon River. These results were related to several metrics of fish abundance and

richness. Pigeon River displayed lower fish abundance, richness, native species, specialized species, and fish per catch rate. There was an increase in tolerant species, omnivores, and fish with disease, tumors, or fin damage. According to the study authors the overall Index of Biological Integrity (IBI) was 54 for Little River and 20 for Pigeon River. Some parameters in our study coincide with parameters measured in the Adams et al. (1992) study (fish abundance, EROD activity, spleen-somatic index, hepatosomatic index). In comparison to Adams et al. (1992), our study shows there are few significant relationships in isolated stream classes from South Carolina. However, we did see a significant relationship involving the hepatosomatic index (HSI).

The HSI is used as a general indicator of liver health. Certain substances, such as polycyclic aromatic hydrocarbons, can lower condition factor, induce hepatic lesions, and initiate cancer (Logan, 2007; Myers et al., 1991). In this investigation estrogenic compounds and mercury concentration were linked with an increase in the HSI. The relationship between estrogenic compounds and HSI is unclear. In a study by Diniz et al. (2005) crucian carp were exposed to high concentrations of municipal sewage effluent with estrogenic activity. The effluent induced vitellogenin in males and caused a reduction in the gonadosomatic index (GSI) in all fish. The HSI, also examined in the study, remained unchanged. Adams et al. (1989) found a distance gradient decrease (from closest to farthest) in CYP 450 1A activity and HSI compared to controls in an East Tennessee stream receiving point source industrial discharge of mixed contaminants. The four treatment sites exhibited low species richness at sites closest to the source

discharge which steadily increased as distance increased (compared to controls). In this study condition factor (K) was related to an increase in HSI. A healthy, functioning liver is related to overall fish fitness while a decrease in K is indicative of a diversion of resources to combat a stressor (Lambert et al., 1997).

The Pee Dee River basin demonstrated a decrease in HSI as a result of oxidative stress exposure, as indicated by the GST assay. Compounds inducing oxidative stress would be harmful to liver cells and liver function. In a study by Monteiro et al. (2006) freshwater characid fish were exposed to an oxidative stress-inducing agent, methyl parathion, in an attempt to characterize antioxidant response in different tissues/organs. That study observed a decrease in HSI after exposure yet the overall fish body weight remained unchanged.

The ACE, Pee Dee, and Savannah River basins were investigated to further characterize relationships. In the ACE River basin, EROD activity was strongly positively correlated to the spleen-somatic index (SSI). The function of the spleen is largely in response to an infection or a pathogen (Cesta, 2006). The strong correlation between EROD activity at the molecular level and SSI suggests exposure to aryl hydrocarbons leaves fish susceptible to secondary infection. However, other rationalizations are possible. In a study by van Ginneken et al. (2009) the SSI was shown to be induced in a group of European eel over 27 days exposed to PCBs. The study author's hypothesized biotransformation of PCBs was a potential explanation for the elevated SSI since the

spleen is also an active site of xenobiotics transformation. Spleen hypertrophy or enlargement was observed to be greatest in the largest PCB-exposed group.

The Savannah River basin depicted a positive relationship between estrogenicity and GSI. This suggests estrogenic compounds in Savannah River sites are inducing gonadal development in *Lepomis sp*. The majority of *Lepomis sp.* used in this study were male fish implying they were exposed to a female sex hormone in higher than normal concentrations. This finding contradicts several studies in the literature. One study showed an inverse relationship between increasing estrogenicity and GSI in crucian carp. In that study, fish were exposed to treated sewage effluent for 28 days. There was a significant decrease in GSI of both male and female fish in the highest effluent concentration tested. Vitellogenin was significantly induced at every concentration. The HSI remained unchanged throughout (Diniz et al., 2005). Another study exposed male and female freshwater roach to sewage effluent at increasing concentrations over a four month period showing an increase in GSI in male fish. However, the study authors attributed the increased GSI to seasonal development as opposed to effluent exposure. They acknowledged other studies in which the GSI had decreased as a result of exposure to xenobiotic estrogens (Rodgers-Gray et al., 2000). A third study examined GSI in male carp in a river contaminated with estrogenic chemicals. Compared to the reference river, the GSI and testis were significantly lower in fish. However, no histological abnormalities were discovered in exposed male testes although spermatogenesis was delayed (Hassanin et al., 2002).

GST activity was shown to be positively related to spleen size in Savannah River sites. One study examining oxidative stress indicated a lower SSI in male and female fish in close proximity to a copper mine. The study authors indicated fish at the site were stressed and the release of red blood cells from the spleen was seen as an adaptive response (Almroth et al., 2008). The adaptive response was a result of spleen contraction to generate higher energy production. In this process erythrocytes become released into circulation, a process that can be induced by free radical causing agents (i.e. metals) (Witeska, 2005). In our study, it is possible moderate oxidative stress caused the spleen to swell prior to release of erythrocytes. Alternatively, oxidative stress exposure may have left fish susceptible to secondary infection causing spleen hypertrophy.

While xy linear relationships were weak, most were significant ($p<0.01$). Stronger correlation coefficients were seen between population and assemblage levels compared to lower levels (Table 6-1). This could be because remaining fish at sample sites were already capable of overcoming molecular, tissue/organ, and individual level stressors. Alternatively, contaminants may not have been present in the water. Those species sensitive to contaminant stress would not be forced to leave the assemblage. More strongly correlated results were observed in other studies. Adams et al. (2000) reported an alteration in fish populations and communities in a gradient-dependent fashion in East Fork Poplar Creek located in Tennessee. In that study fish were sampled immediately below a point source discharge, and 4, 9, and 17 km downstream. Molecular and

individual endpoints (in *Lepomis auritus*) were found to be significantly different in fish populations and communities based on distance from discharge source. For example EROD induction was up to 473% more induced compared to reference sites. In a separate investigation at the same site, Theodorakis et al. (2000) examined industrial effluent and incidence of genetic aberrations (chromosome damage and single strand DNA breaks) at four downstream sample sites. They found an increase in tolerant species at sites closest to the effluent source but decreased diversity compared to more distant sites. Strong correlations with genetic damage and assemblage alteration were observed as sites became closer to the source. Another study compared changes in fish assemblage in the Halawakee, Wacoochee, and Little Uchee creeks in Alabama from data in the 1970s and 1995. The study authors found a high species turnover and different types of assemblages over time. These changes were associated with an increased anthropogenic presence and less water availability with a degradation of the aquatic environment (Johnston et al., 2009).

In our study, there could be several possible explanations for few significant or strongly correlated relationships. The wide geographic range of sites at statewide, stream size class, and river basin scale may be too large to identify trends using the methodology employed here. In some instances considerable distance was traversed even when sampling adjacent sites. Over such a large area low numbers of sample sites in close proximity prevented more definitive relationships between levels of biological organization. Furthermore, most of the sites in this study were located in rural areas

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compared to urban centers. It is possible these sites are unimpacted by anthropogenic influence and relatively pristine. Fish at these locations would be expected to be minimally exposed to contaminants and remain healthy. Also, while the suite of biomarker and fitness indicators was diverse it is possible they were not selective or sensitive enough to measure a contaminant in exposed fish.

A newer methodology was developed in an attempt to incorporate molecular and organ level endpoints to the community level using an aquatic ecosystem health index (AEHI) (Yeom et al., 2007). AEHI's are one level above IBIs (like the Simpson or Shannon Diversity Index). IBIs do not always contain enough ecological information, use subjective data reporting at times (for different contaminant endpoints), are not suitable for communities with few species, and are region dependent. Conversely, AEHIs relate changes in fish communities based on sub-organism, individual, and population data. They appear to be a promising tool for assessing higher level changes based on a combination of lower level variables (Yeom et al., 2007). Thus far strong relationships at the community level have been identified using this technique. In future studies of SC wadeable streams, an assessment using AEHI may be incorporated to understand changes at different organization levels.

Conclusions

The research presented here suggests there were significant relationships ($p<0.05$) between multiple levels of organization. Fish sampled in this analysis were likely not

exposed to contaminants at high enough concentrations to elicit dramatic shifts throughout multiple levels. While correlations were poor, multiple significant relationships existed between the population level and species diversity at the assemblage level. Sensitive species will be the first to leave an increasingly contaminated habitat thereby altering assemblage composition. Changes in multiple levels of organization are likely to become more strongly correlated as the aquatic ecosystem becomes increasingly deteriorated. While there were few significant or strongly correlated molecular and tissue/organ relationships, they could be the precursor to harmful affects at upper levels. Detection of changes at the molecular and tissue/organ levels are important steps to determine the level of contamination in the aquatic environment. At the assemblage level species diversity and abundance will be poor. This type of biomonitoring can help assist in the quantitative prediction of habitat decline during urban planning and in stressed aquatic ecosystems. Preventive measures or remediation efforts can be developed to mitigate impacts within a watershed.

CHAPTER 7: SUMMARY AND CONCLUSIONS

The findings presented in this dissertation integrate comparisons of total fish assemblage with *Lepomis sp.*, biomarker response measurements within *Lepomis sp.* relative to the assemblage, the effects of urbanization on fish health and fish assemblage, and changes throughout multiple levels of biological organization. This section will serve to summarize the findings, discuss implications, and provide study limitations. Potential further work will also be discussed.

Immediate Outcomes

Lepomis sp. abundance and richness were compared with total fish abundance, richness, and diversity. The results reveal *Lepomis sp.* correlate well with total fish assemblage structure. While *Lepomis* are considered a tolerant fish genus, it was necessary to determine if they correlate with total fish assemblage members. If no relationship was found then it would make further biomarker and land use analysis more difficult. However, significant relationships with the assemblage were discovered, both in abundance and richness. These results indicate *Lepomis sp.* can be used as a representative or model species for the assemblage. One drawback was the lack of a strong relationship with overall fish diversity. This could be because there was a lack of diversity over a statewide level, or there was a lack of species uniformity between sample sites at a large scale. Because the Simpson's Diversity Index was an inherently low metric, strong correlations were not found with *Lepomis sp*.

Once the relationship between *Lepomis sp.* abundance and richness with fish assemblage was determined the biomarker responses with fish assemblage parameters were examined. In some instances, such as EROD induction, the result strongly correlated with fish assemblage declines. In other analyses, the relationship was significant but less well correlated. The inclusion and contribution of abiotic parameters in the analysis was very beneficial in strengthening relationships, the correlation coefficient in particular. However, in some instances the abiotic parameters were a more heavily contributing parameter than actual biomarker response. Furthermore, the biomarker response was not always significant without the abiotic covariate. Health and fitness indicators revealed similar relationships to biomarker analysis. A further characterization of abiotic influences on the biomarker dataset could shed light on their contribution. Conducting analysis between temperature, pH, dissolved oxygen, etc. and fish assemblage parameters would be one way to evaluate the abiotic contribution.

The relationship of urbanization with fish health and fish assemblage was also examined. While the results of this chapter were largely nominal, several relationships existed between increasing percent urbanization, biomarker response, and divisions from the native fish structure. This is indicative of a negative effect of a growing human population on fish health. Simple xy linear regression analysis indicated significant relationships in nearly every comparison. However, while these relationships were significant the correlation coefficients were not always strong. This suggests either there

is a poor relationship between increasing urbanization on fish or the fish did not accumulate contaminants in order to elicit a dramatic biomarker response. A majority of sites were located in relatively pristine, undeveloped locations. It is likely these low-impact sites did not contain contaminants compared to more heavily developed areas. These locations were spread out over a large scale area (throughout the State of South Carolina) which might be too great a distance to elucidate differences in fish assemblage structure even if sites were in close proximity (i.e. less than 5 miles apart).

One way to determine if *Lepomis sp.* was being exposed to higher levels contaminants would be to compare heavily impacted and low impacted sites within the same region. Heavily impacted sites are those likely to induce the highest enzyme induction in *Lepomis sp.* as a result of contaminant exposure. These sites could then be compared to low-impact sites for reference. One drawback of this study was too many sites almost served as pristine sites due to the lack of biomarker response detected.

Finally the changes throughout multiple levels of biological organization were examined. Few associations could be made between any of the different levels. The most strongly correlated were those at the population and assemblage levels. Many of the molecular and issue/organ relationships were not significant and had poor correlation coefficients. As in chapter four, a lack of contaminated sites could be one explanation for the lack of significance. It is possible relationships were missed due to the specificity of the test. A

more robust, comprehensive suite of biomarker tests could be implemented in order to identify contaminants not detected by the assortment in this study. For example, a cholinesterase assay next to an agricultural facility might reveal high acetylcholinesterase activity (from runoff) leading to fish death and an alteration in native fish assemblage structure. In addition, different analytical methods may reveal the beginnings of causal pathways not originally identified via index-based methodologies. Histological or pathological analytical techniques could be more sensitive and better applied. Continued work on additional biomarker analysis could lead to significant findings.

This dissertation provides information which opens up the need for additional areas of investigation. The relationship between fish structure, priority species, or sensitive species may be revealed upon subsequent evaluation of the dataset. The distribution of species at the statewide level is not uniform. Analyzing sites by ecobasin or ecoregion was not explored here. It may be possible to re-analyze the data and discover significant relationships if sites are group by a different classification. As mentioned in the body of the dissertation, there is a large disparity between the uplands and lowlands of South Carolina. Once fish species have been confined to a more specific river basin, ecoregion, or ecobasin it may be possible to re-evaluate the data to provide more significant relationships.

The notion of scale is objective and may be a factor when analyzing biomarker responses in future studies. In this investigation, one hundred twenty-seven sample sites were scattered randomly throughout South Carolina. A subsequent investigation may reveal better relationships if sample locations were located in closer proximity. Ideally these sample locations would traverse a gradient of low to high impact sites. It is possible the lack of relationships with biomarker response may have been from a lack of contaminant exposure. Examining a stream segment in an urban watershed is one way this could be accomplished. Multiple sites before and after an urban center would allow for the best determination of a species sensitivity gradient as well as a biomarker response comparison. Sites upstream of urban centers would be expected to elicit greater responses than pre-urban sites.

In addition to the smaller sample scale, an investigation of additional land use categories may provide insight to relationships not revealed by using urbanization as the independent variable. Agricultural, forested, open water and barren land designations could be explored for comparison for biomarker response measurements. These relationships may further characterize patterns not seen when using the urbanization land use designation.

Dissertation Implications

Impacts of this dissertation reveal threats to fish health and fish assemblage integrity through the alteration of native land, particularly urban development. From this work investigators will be able to use *Lepomis sp*. as a model fish to determine if a freshwater system has been degraded. If possible, the magnitude of degradation can be ascertained through biomarker response. An assessment of fish abundance and species richness can be used for comparative purposes as an indicator of biological integrity. That is, the higher the abundance and richness of fish at a location, the more ecologically fit that site.

These factors may be especially important for policy creators and stakeholders who may be obligated to minimize damage to watershed health due to litigation during urban development. In order to prevent watershed deterioration laws need to be instilled and enforced in order to promote efforts reducing stress on water systems. Consequences of failing to act are illustrated throughout this dissertation.

Based on the findings an increase in urban surface will increase biomarker response and alter native fish assemblage structure. Data presented here can be used to promote change in current regulations. Best management practices is one type of litigation remediation effort, however, clearly more stringent regulations are needed.

This dataset identifies urban surface as being a major contributor to the decline of fish health. The watershed resources being protected are not only beneficial for fish and other aquatic life, but for humans as well. A disregard for current policies and litigation is a contributing factor to the decline of aquatic system health. The strain on the ecosystem through watershed deterioration should not and cannot be tolerated. This dissertation provides the justification for the creation of new legislation. This dataset can easily be used in cohesion with the many other scientific studies dedicated to the preservation of watershed integrity and the aquatic ecosystem.

Watershed protection needs to be a central theme throughout the litigation process as the majority of aquatic contaminants originate from terrestrial sources. This dissertation presents potential pathways and highlights resultant effects on fish in the aquatic ecosystem. An extrapolation from fish to all aquatic life may be practical (or required) especially considering other sensitive organisms (i.e. amphibians, reptiles, invertebrates) require the aquatic system for some or all of their life stages. Without additional regulation and enforcement the deterioration of the water system will persist and may become degraded to the point where the aquatic environment is able to sustain specialized organisms, if any.

While negative relationships were seen between urban surface, biomarker response, and fish assemblage integrity, the remarkable concept was at how low changes occurred. For

example, the majority of sites in this study were located in areas that were relatively untouched by anthropogenic influence. However, because aquatic systems are interconnected, nothing downstream is free of harm. Most relationships between biological levels of organization were poor yet even under these reasonably pristine conditions there were significant relationships which were undesirable and clearly negatively associated. If outcomes like the ones found in the study are significant, it should be a warning to what may come to be if policy remains unchanged and nothing is done to rectify the problem. In rural and sub-urban areas continued anthropogenic growth and development will continue to further degrade the aquatic ecosystem in such a way that may be irreparable. This fact should be cause for extreme concern. In highly urbanized areas this should be alarming and viewed as a watershed deterioration epidemic.

Major themes and conclusions of this dissertation can be summarized as follows:

Lepomis sp. are a widely distributed fish type that can be used as a model to represent all fish species in an assemblage at a sampling location.

The magnitude of chemical contamination detected in *Lepomis sp.* via biomarker response can be used to determine changes in overall fish assemblage structure at sampling locations.

10% urban surface and greater is a threat pathway leading to deleterious effects on aquatic ecosystems.

Anthropogenic development leads to watershed deterioration resulting in contaminant exposure to fish and other aquatic organisms. As the human population continues to increase, there will be an increased burden of aquatic contaminants resulting in a decline in the diversity of fish and other aquatic life. Sensitive species will be the first to disappear. Eventually, a watershed may become so badly deteriorated that only a few tolerant or specialized species will remain.

Fish health at the organism, population, and community scales decline concurrently with an increase in urban surface area within a watershed.

Detection of changes at the molecular level of biological organization in *Lepomis sp.* can be used to infer changes in fish assemblage integrity. Based on this relationship, it will be possible to determine the progression or level of watershed deterioration.

A quantitative prediction of aquatic ecosystem health, as a function of land use changes within a watershed, was developed as a result of the work done in this dissertation. Urban planners can use this information to understand aquatic habitat deterioration and predict threats to aquatic ecosystems.

Data from this research can be used by policy makers and stakeholders to drive development of low-impact development strategies on aquatic ecosystems. Additionally,

urban planners can use this work to develop sustainable long-term low-impact strategies on aquatic ecosystems.

Failure to create or alter current watershed policies and regulations will result in the deterioration of a watershed in which remediation may not be possible.

Final Remarks

The deterioration of watershed health will need to be more heavily scrutinized in years to come. As the human population escalates, water quality will continue to decline. Not only will fish and other aquatic organism health decline, but freshwater resources will no longer be usable for human purposes. The research presented here is a culmination of a multiple year investigation providing a scientific basis for consequences of poor landscape management. Stakeholders, policy makers, and other authority figures can use this information to support implementation of protective watershed legislation. Sustainability is currently not occurring and will not be without awareness of awareness of stressors and complexity of an aquatic ecosystem. This research was focused with that idea in mind. This research demonstrates the need for continued study and monitoring of watershed health. Without more scientifically-based studies, there may come a point when remediation efforts are not possible due to the severity of watershed degradation.

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APPENDIX

Table A-1. Summary of assemblage and abiotic variables used in stepwise linear regression models

Fish Assemblage Parameters	*Lepomis* Parameters	Abiotic Variables
Total Fish Abundance	Total *Lepomis* Abundance	Temperature
Total Fish Richness	Total *Lepomis* Richness	pH
Total Fish Abundance (not *Lepomis*)	Bluegill/Redbreast Abundance	Dissolved Oxygen
Total Fish Richness (not *Lepomis*)	Tolerant *Lepomis* Abundance	Conductivity
Simpson's Diversity Index (D)	Tolerant *Lepomis* Richness	Turbidity
		Stream Slope
		Stream Velocity
		Stream Width

Table A-2. Site and stream information for samples taken in South Carolina

Site Number	Date Sampled	River Basin	Ecoregion	Ecobasin	Size Class	Elevation	Stream Name	Sample Longitude	Sample Latitude
207511	5.24.2006	Saluda	Sandhills	SALSAND	1	382	Long Branch	-81.3	34.0
205370	5.24.2006	Saluda	Sandhills	SALSAND	1	180	Double Branch	-81.1	34.0
216167	5.24.2006	Saluda	Sandhills	SALSAND	2	343	Twelvemile Creek	-81.3	33.9
287580	6.6.2006	SavanNAh	Sandhills	SAVSAND	2	210	Little Horse Creek	-81.9	33.6
346456	6.13.2006	SavanNAh	Sandhills	SAVSAND	1	137	Gantts Mill Creek	-81.5	33.2
258489	8.01.2006	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	124	Home Branch	-80.3	33.7
231143	8.01.2006	Pee Dee	Atlantic Southern Loam Plains	PDASLP	3	127	Cane SavanNAh Creek	-80.4	33.9
132724	8.02.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	2	104	Catfish CaNAl	-79.5	34.4
236192	8.03.2006	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	38	Caney Branch	-79.4	33.7
153122	8.8.2006	Pee Dee	Atlantic Southern Loam Plains	PDASLP	3	85	High Hill Creek	-79.8	34.2
178408	8.9.2006	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	115	Tributary to Alligator Branch	-79.8	34.1
177553	8.9.2006	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	82	Cane Branch	-79.7	34.1
100467	8.15.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	2	65	Muddy Creek	-79.7	34.5
159553	8.16.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	2	60	Tributary caNAl to Tobys Creek	-79.5	34.2
145731	8.16.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	59	Gum Swamp	-79.5	34.3
155269	8.17.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	160	High Hill Creek	-79.9	34.3
215668	8.22.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	2	155	Mush Branch	-80.4	33.9
142478	8.23.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	71	Tributary to Little Pee Dee River	-79.2	34.3
87719	9.6.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	135	Tributary to Muddy Creek?	-79.7	34.6
145650	9.27.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	3	53	Back Swamp	-79.7	34.3
98871	9.27.06	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	137	Tributary to Little Pee Dee River	-79.4	34.5
236403	5.1.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	87	Trestles Branch	-80.0	33.9
215410	5.2.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	50	Tributary to Big Swamp	-79.6	33.9
221551	5.3.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	2	86	Camp Branch	-79.8	33.9
269220	5.7.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	50	Tributary to Kingstree Swamp CaNAl	-79.8	33.7
328809	5.8.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	19	White Oak Bay	-79.3	33.3
256489	5.9.07	Pee Dee	Atlantic Southern Loam Plains	PDASLP	1	124	Home Branch	-80.3	33.7
320217	5.14.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	38	CaNAan Branch	-79.6	33.4
219819	5.15.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	59	Palmetto Swamp	-79.2	33.9
234542	5.16.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	2	19	Crab Tree Swamp	-79.1	33.9
245228	5.29.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	3	84	Tearcoat Branch	-80.1	33.8
198174	5.30.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	2	32	Buck Creek	-78.8	34.0
219094	5.30.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	3	13	Simpson Creek	-78.8	33.9
212220	5.31.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	2	22	Simpson Creek	-78.8	33.9
202676	6.12.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	48	SavanNAh Creek	-79.3	34.0
204277	6.12.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	2	39	Cypress Creek	-79.4	34.0
265577	6.13.07	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	13	Withers Swash	-78.9	33.7
362289	6.19.07	ACE	CaroliNA Flatwoods	ACEFLATW	2	62	Little Swamp	-80.9	33.1
380145	6.20.07	ACE	CaroliNA Flatwoods	ACEFLATW	3	41	Ireland Creek	-80.6	32.9
307367	6.21.07	ACE	CaroliNA Flatwoods	ACEFLATW	1	146	Tributary to Middle Pen Swamp	-80.7	33.4
318064	6.26.07	Lower Santee	CaroliNA Flatwoods	LSFLATW	2	80	Tributary to Lake Marion	-80.2	33.4

NA = not available

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Table A-2 (cont). Site and stream information for samples taken in South Carolina

Site Number	Date Sampled	River Basin	Ecoregion	Ecobasin	Size Class	Elevation	Stream Name	Sample Longitude	Sample Latitude
342523	6.26.07	ACE	CaroliNA Flatwoods	ACEFLATW	2	16	Bull Head Run	-79.9	33.2
341665	6.27.07	ACE	CaroliNA Flatwoods	ACEFLATW	3	19	CaNAdy Branch/Broad Ax Branch	-80.0	33.2
346136	6.27.07	ACE	CaroliNA Flatwoods	ACEFLATW	2	29	Nicholson Creek	-79.8	33.2
305009	7.10.07	ACE	CaroliNA Flatwoods	ACEFLATW	1	167	Cow Castle Creek	-80.8	33.4
319073	7.11.07	ACE	CaroliNA Flatwoods	ACEFLATW	3	117	Cow Castle Creek	-80.7	33.4
39891	5.7.08	SavanNAh	Inner Piedmont	SAVIPIED	2	890	Rices Creek	-82.7	34.8
93949	5.8.08	SavanNAh	Outer Piedmont	SAVOPIED	1	676	Salem Creek	-82.7	34.5
103468	5.8.08	SavanNAh	Outer Piedmont	SAVOPIED	1	692	Dye Creek	-82.7	34.5
24932	5.13.08	SavanNAh	Inner Piedmont	SAVIPIED	1	987	Mill Shoals Creek	-82.8	34.9
86207	5.14.08	SavanNAh	Outer Piedmont	SAVOPIED	1	755	Little Beaverdam Creek	-82.6	34.6
93236	5.14.08	SavanNAh	Outer Piedmont	SAVOPIED	2	741	Broadway Creek	-82.5	34.5
90631	5.14.08	Saluda	Outer Piedmont	SALOPIED	1	705	Tributary to Horse Creek	-82.3	34.6
36620	5.15.08	Saluda	Inner Piedmont	SALIPIED	1	956	Georges Creek	-82.6	34.8
27771	5.15.08	Saluda	Inner Piedmont	SALIPIED	2	956	Reedy River	-82.5	34.9
45813	5.21.08	SavanNAh	Inner Piedmont	SAVIPIED	1	959	Tributary to Cane Creek	-83.1	34.8
26150	5.21.08	SavanNAh	Inner Piedmont	SAVIPIED	1	874	Boones Creek	-82.9	34.9
109522	5.22.08	SavanNAh	Outer Piedmont	SAVOPIED	2	640	Big Generostee Creek	-82.7	34.5
56287	5.29.08	Saluda	Outer Piedmont	SALOPIED	3	815	Big Brushy Creek	-82.5	34.7
72426	5.29.08	Saluda	Outer Piedmont	SALOPIED	2	764	Huff Creek	-82.3	34.7
15377	6.4.08	Saluda	Inner Piedmont	SALIPIED	3	940	Oolenoy River	-82.6	35.0
145961	6.5.08	Saluda	Outer Piedmont	SALOPIED	3	439	Bush River	-81.7	34.3
181037	6.10.08	SavanNAh	Outer Piedmont	SAVOPIED	3	404	Big Curltail Creek	-82.3	34.1
171515	6.10.08	SavanNAh	Outer Piedmont	SAVOPIED	2	473	Big Curltail Creek	-82.3	34.2
157067	6.11.08	SavanNAh	Outer Piedmont	SAVOPIED	3	462	Long Cane Creek	-82.3	34.2
145150	6.11.08	SavanNAh	Outer Piedmont	SAVOPIED	2	530	Long Cane Creek	-82.3	34.3
152376	6.11.08	SavanNAh	Outer Piedmont	SAVOPIED	2	481	Park Creek	-82.4	34.3
218747	6.12.08	SavanNAh	Outer Piedmont	SAVOPIED	2	431	Rocky Creek	-82.3	33.9
203358	6.12.08	SavanNAh	Slate Belt	SAVSLATE	2	416	Beaverdam Creek	-82.1	34.0
358497	6.18.08	SavanNAh	Atlantic Southern Loam Plains	SAVASLP	2	136	Bentleys Branch	-81.4	33.1
363121	6.18.08	SavanNAh	Atlantic Southern Loam Plains	SAVASLP	1	127	Tributary to Miller Creek	-81.4	33.1
295697	6.24.08	SavanNAh	Outer Piedmont	SAVOPIED	1	140	Tributary to SavanNAh River	-82.0	33.5
304020	6.25.08	SavanNAh	Sandhills	SAVSAND	2	254	Upper Three Runs	-81.6	33.5
311961	6.25.08	SavanNAh	Sandhills	SAVSAND	3	186	Hollow Creek	-81.8	33.4
313573	6.26.08	SavanNAh	Sandhills	SAVSAND	1	218	Boggy Gut	-81.6	33.4
102026	7.15.08	Saluda	Outer Piedmont	SALOPIED	2	567	Little River	-82.0	34.5
92979	7.15.08	Saluda	Outer Piedmont	SALOPIED	2	662	North Rabon Creek	-82.2	34.6
101334	7.15.08	Saluda	Outer Piedmont	SALOPIED	3	582	South Rabon Creek	-82.2	34.5
136855	7.16.08	Saluda	Outer Piedmont	SALOPIED	2	486	Mulberry Creek	-82.2	34.3
111855	7.16.08	Saluda	Outer Piedmont	SALOPIED	3	570	Broadmouth Creek	-82.3	34.5
13515	7.17.08	SavanNAh	Outer Piedmont	SAVOPIED	2	641	Broadway Creek	-82.6	34.5
31263	7.29.08	SavanNAh	Inner Piedmont	SAVIPIED	3	920	Twelvemile Creek	-82.7	34.9

NA = not available

Table A-2 (cont). Site and stream information for samples taken in South Carolina

Site Number	Date Sampled	River Basin	Ecoregion	Ecobasin	Size Class	Elevation	Stream Name	Sample Longitude	Sample Latitude
222764	7.30.08	SavanNAh	Slate Belt	SAVSLATE	2	357	Sleepy Creek	-82.0	33.9
225891	7.30.08	SavanNAh	Slate Belt	SAVSLATE	3	333	Little Stevens Creek	-82.0	33.9
232326	7.30.08	SavanNAh	Slate Belt	SAVSLATE	3	344	Turkey Creek	-82.0	33.9
220793	7.31.08	SavanNAh	Slate Belt	SAVSLATE	2	369	Little Stevens Creek	-81.9	33.9
14464	8.7.08	Saluda	Blue Ridge	SALBLUER	3	956	South Saluda River	-82.6	35.0
75961	8.12.08	SavanNAh	Outer Piedmont	SAVOPIED	3	680	Eighteenmile Creek	-82.8	34.6
69184	8.12.08	SavanNAh	Outer Piedmont	SAVOPIED	3	758	Coneross Creek	-83.0	34.7
203483	8.19.08	Saluda	Slate Belt	SALSLATE	1	467	Red Bank Creek	-81.9	34.0
164521	8.19.08	Saluda	Outer Piedmont	SALOPIED	1	403	Tributary to Sharps Branch	-81.8	34.2
152737	8.19.08	Saluda	Outer Piedmont	SALOPIED	3	411	Mud Lick Creek	-81.8	34.3
180206	8.20.08	Saluda	Outer Piedmont	SALOPIED	1	457	Tolbert Branch	-82.0	34.1
181437	8.20.08	SavanNAh	Outer Piedmont	SAVOPIED	3	418	McKenley Creek	-82.5	34.1
133573	8.20.08	SavanNAh	Outer Piedmont	SAVOPIED	2	571	Hogskin Creek	-82.4	34.4
120740	9.3.08	SavanNAh	Outer Piedmont	SAVOPIED	3	561	Big Generostee Creek	-82.8	34.4
37901	9.3.08	Saluda	Outer Piedmont	SALOPIED	3	882	Reedy River	-82.4	34.8
86384	5.20.09	Catawba/Wateree	Outer Piedmont	CWOPIED	3	376	Little Rocky Creek	-81.0	34.6
23159	5.20.09	Catawba/Wateree	Outer Piedmont	CWOPIED	2	526	Manchester Creek	-81.0	35.0
205019	6.02.09	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	59	Mill Branch	-79.2	34.0
216018	6.04.09	Pee Dee	CaroliNA Flatwoods	PDFLATW	1	96	Tributary caNAl to Camp Branch	-79.9	33.9
112131	6.09.09	Catawba/Wateree	Outer Piedmont	CWOPIED	3	239	Beaver Creek	-80.8	34.5
123998	6.09.09	Catawba/Wateree	Slate Belt	CWSLATE	3	230	Flat Rock Creek	-80.6	34.4
58433	6.10.09	Catawba/Wateree	Slate Belt	CWSLATE	2	498	Gills Creek	-80.8	34.7
84562	6.10.09	Catawba/Wateree	Outer Piedmont	CWOPIED	2	350	Camp Creek	-80.8	34.6
60751	6.23.09	Catawba/Wateree	Slate Belt	CWSLATE	1	573	Tributary to Gills Creek	-80.7	34.7
72211	6.24.09	Catawba/Wateree	Slate Belt	CWSLATE	1	511	Rum Creek	-80.8	34.7
45921	6.30.09	Catawba/Wateree	Outer Piedmont	CWOPIED	3	501	South Fork Fishing Creek	-81.1	34.8
25398	6.30.09	Catawba/Wateree	Outer Piedmont	CWOPIED	2	561	Six Mile Creek	-80.8	35.0
51137	7.2.09	Catawba/Wateree	Slate Belt	CWSLATE	2	517	Camp Creek	-80.7	34.8
156577	7.14.09	Catawba/Wateree	Atlantic Southern Loam Plains	CWASLP	2	131	Little Pine Tree Creek	-80.6	34.2
156581	7.15.09	Catawba/Wateree	Atlantic Southern Loam Plains	CWASLP	3	131	Big Pine Tree Creek	-80.6	34.2
180390	7.28.09	Catawba/Wateree	Sandhills	CWSAND	2	150	Spears Creek	-80.7	34.1
147751	7.29.09	Catawba/Wateree	Sandhills	CWSAND	3	190	Big Pine Tree Creek	-80.5	34.3
208930	7.29.09	Catawba/Wateree	Sandhills	CWSAND	3	140	Colonels Creek	-80.7	34.0
262461	8.11.09	Pee Dee	Atlantic Southern Loam Plains	PDASLP	2	90	Big Branch	-80.3	33.7
301204	8.12.09	Pee Dee	CaroliNA Flatwoods	PDFLATW	2	38	Murray Swamp	-79.6	33.5
12 Mile-Low	8.19.09	SavanNAh	Inner Piedmont	SAVIPIED	3	NA	Twelvemile	-82.8	34.8
12 Mile-Above	8.19.09	SavanNAh	Inner Piedmont	SAVIPIED	3	NA	Twelvemile	-82.8	34.8
RHC	5.5.10	Saluda	Outer Piedmont	SALOPIED	NA	NA	Richland	-82.4	34.9
RH-1	5.5.10	Saluda	Inner Piedmont	SALIPIED	NA	NA	Reedy HW	-82.5	34.9
LC	5.5.10	Saluda	Inner Piedmont	SALIPIED	NA	NA	Langston	-82.4	34.9
Huff	5.6.10	Saluda	Outer Piedmont	SALOPIED	NA	NA	Huff	-82.4	34.7
Rocky	5.6.10	Saluda	Outer Piedmont	SALOPIED	NA	NA	Rocky	-82.3	34.7
Bush	5.11.10	Saluda	Outer Piedmont	SALOPIED	NA	NA	Brushy	-82.4	34.8
Bald	5.11.10	Saluda	Outer Piedmont	SALOPIED	NA	NA	Baldwin	-82.3	34.7
L-C	5.11.10	Saluda	Outer Piedmont	SALOPIED	NA	NA	Laurel	-82.3	34.8

NA = not available

Table A-3. Site information including width, depth, velocity, slope, pH, temperature, dissolved oxygen (DO), conductivity, and turbidity

Site	Avg Width	Avg Depth	Avg Velocity	Avg Slope	pH	Temperature	DO	Conductivity	Turbidity
207511	2.04	0.18	0.0371	0.900	6.95	27.7	6.32	85.0	3.68
205370	1.56	0.09	0.0280	0.428	6.81	21.4	5.65	69.0	3.13
216167	6.1	0.37	0.0711	0.598	7.07	21.6	5.67	70.0	2.99
287580	5.66	0.45	0.1670	0.549	6.45	24.6	7.63	21.0	8.17
346456	3.26	0.18	0.1594	0.830	6.95	23.2	8.66	11.0	4.16
258489	1.25	0.09	0.0024	0.850	6.48	23.9	1.38	93.0	6.95
231143	4.48	0.29	0.0806	0.640	5.92	28.9	6.82	34.0	2.79
132724	2.46	0.18	0.0534	0.580	6.34	28.8	6.22	86.0	13.60
236192	NA	NA	NA	NA	NA	NA	NA	NA	NA
153122	5.96	0.49	0.1208	0.410	6.73	28.0	3.94	149.0	7.97
178408	2.36	0.27	0.0024	0.270	6.26	27.0	1.13	111.0	52.25
177553	1.38	0.12	0.0052	0.850	6.39	25.1	1.47	76.0	9.46
100467	4.31	0.19	0.0296	0.240	6.37	24.0	4.82	49.0	3.74
159553	3.08	0.53	0.0000	0.210	6.44	23.0	1.13	68.0	27.59
145731	2.48	0.21	0.0000	0.090	5.02	24.9	4.13	72.0	15.66
155269	4.64	0.36	-0.0022	0.180	6.39	22.6	2.88	141.0	4.01
215668	4.1	0.33	-0.0022	0.460	6.07	25.5	0.20	63.0	11.28
142478	1.8	0.14	0.1138	0.730	6.11	23.6	4.82	78.0	59.29
87719	2.31	0.21	0.0470	0.240	6.56	23.8	4.72	39.0	16.63
145650	6.58	0.43	0.0322	0.197	6.93	18.0	6.46	84.0	13.27
98871	2.56	0.08	0.0240	0.383	6.51	18.5	5.94	99.0	6.41
236403	1.99	0.12	0.0466	0.210	7.46	17.0	6.10	144.0	16.28
215410	2.03	0.18	0.0016	0.580	7.53	18.5	2.16	106.0	17.65
221551	3.42	0.12	0.1388	0.460	6.93	20.1	7.31	123.0	18.96
269220	2.79	0.3	0.0146	0.422	7.37	15.0	7.19	67.0	1.45
328809	1.87	0.15	0.0000	0.440	7.17	14.9	1.16	115.0	9.81
256489	NA	NA	NA	NA	NA	NA	NA	NA	NA
320217	2.91	0.4	0.0006	0.350	6.66	18.7	3.10	89.0	4.70
219819	2.6	0.4	0.0026	0.150	7.04	18.8	1.76	195.0	7.04
234542	3.88	0.09	0.1088	0.520	8.06	19.9	9.72	267.0	29.39
245228	7.94	0.58	0.0006	0.180	7.15	22.9	5.51	112.0	5.10
198174	4.73	0.15	0.1782	0.490	7.98	24.7	10.02	646.0	2.34
219094	5.1	0.12	0.1018	0.176	7.64	20.3	8.32	232.0	8.55
212220	3.96	0.12	0.2182	0.320	7.76	19.9	8.34	208.0	5.32
202676	3.64	0.45	0.0020	0.270	6.86	23.3	2.75	135.0	4.37
204277	3.68	0.21	0.0056	0.210	5.60	23.1	1.55	54.0	9.40
265577	3.26	0.17	0.0434	0.200	7.19	22.0	3.66	868.0	7.87
362289	3.7	0.28	0.0072	0.283	7.04	23.5	5.07	74.0	2.50
380145	5.61	0.31	0.1072	0.384	6.05	22.1	6.86	59.0	5.58
307367	2.5	0.31	0.1192	0.640	6.76	22.8	6.59	135.0	5.26
318064	3.21	0.26	0.0412	0.564	7.78	20.3	6.27	223.0	5.04

NA = not available

Table A-3 (cont). Site information including width, depth, velocity, slope, pH, temperature, dissolved oxygen (DO), conductivity, and turbidity

Site	Avg Width	Avg Depth	Avg Velocity	Avg Slope	pH	Temperature	DO	Conductivity	Turbidity
342523	2.46	0.12	0.0000	0.210	6.99	24.7	1.63	135.0	11.62
341665	3.76	0.35	0.0624	0.580	7.50	24.2	7.20	141.0	5.35
346136	3.00	0.19	0.0000	0.260	7.29	25.1	1.88	370.0	11.45
305009	3.10	0.09	0.0386	0.520	7.18	26.6	6.24	134.0	3.91
319073	6.10	0.14	0.1372	0.475	7.60	23.0	7.46	150.0	1.80
39891	7.44	0.45	0.1170	NA	7.23	17.8	8.64	45.0	8.17
93949	4.00	0.2	0.2288	NA	7.14	18.5	9.07	49.0	14.68
103468	2.46	0.16	0.2231	NA	7.30	14.5	10.33	116.0	10.20
24932	2.16	0.2	0.1828	NA	7.22	18.4	10.45	40.0	5.09
86207	4.98	0.39	0.1671	NA	7.20	16.4	8.25	62.0	10.50
93236	5.62	0.35	0.2200	NA	7.03	16.9	8.48	56.0	23.70
90631	2.59	0.18	0.1448	NA	6.80	16.1	9.43	44.0	16.64
36620	2.78	0.38	0.0484	NA	6.93	15.4	8.31	61.0	4.41
27771	4.70	0.24	0.2066	NA	6.82	17.0	8.06	53.0	11.11
45813	3.70	0.38	0.0632	NA	6.90	15.8	7.84	36.0	11.87
26150	2.76	0.22	0.3146	NA	7.12	15.7	10.34	24.0	3.52
109522	6.88	0.28	0.4311	NA	6.87	17.4	8.83	167.0	11.18
56287	7.38	0.25	0.3154	NA	6.88	17.3	8.29	119.0	11.96
72426	5.78	0.25	0.1908	NA	6.75	21.9	7.52	53.0	4.65
15377	10.06	0.4	0.2928	NA	6.89	20.1	8.57	35.0	10.98
145961	5.10	0.31	0.0810	NA	7.43	23.7	5.92	211.0	9.16
181037	4.14	0.11	0.1843	NA	7.60	22.9	8.05	129.0	7.90
171515	3.93	0.23	0.0778	NA	7.31	23.8	5.39	140.0	6.88
157067	4.96	0.19	0.1977	NA	7.53	22.7	7.96	109.0	12.21
145150	5.94	0.21	0.0788	NA	7.27	24.1	6.82	98.0	9.77
152376	3.76	0.26	0.0577	NA	7.34	24.9	6.92	109.0	15.86
218747	3.78	0.18	0.0036	NA	7.50	23.7	3.02	298.0	3.33
203358	3.58	0.21	0.0306	NA	7.33	23.2	2.50	209.0	15.45
358497	2.81	0.2	0.1064	NA	7.44	22.5	7.68	95.0	3.44
363121	1.31	0.13	0.0030	NA	6.49	21.8	1.01	82.0	10.67
295697	2.06	0.17	0.0283	NA	7.67	31.5	7.18	230.0	0.84
304020	4.50	0.35	0.2427	NA	5.84	19.0	9.32	16.0	1.51
311961	5.48	0.35	0.2988	NA	5.44	22.5	9.18	17.0	1.60
313573	2.74	0.27	0.0388	NA	5.75	23.7	3.33	27.0	2.14
102026	3.36	0.19	0.0557	NA	7.36	22.0	5.17	104.0	11.59
92979	3.92	0.23	0.1030	NA	7.43	22.7	7.59	88.0	8.11
101334	3.28	0.18	0.1245	NA	7.27	23.4	7.24	84.0	15.58
136855	5.36	0.26	0.0350	NA	7.11	20.4	3.71	133.0	28.38
111855	4.88	0.19	0.0971	NA	7.20	25.1	7.39	75.0	11.16
13515	5.26	0.28	0.0936	NA	6.99	21.1	6.55	77.0	14.47
31263	9.40	0.38	0.4504	NA	7.20	27.1	10.21	58.0	5.42

NA = not available

Table A-3 (cont). Site information including width, depth, velocity, slope, pH, temperature, dissolved oxygen (DO), conductivity, and turbidity

Site	Avg Width	Avg Depth	Avg Velocity	Avg Slope	pH	Temperature	DO	Conductivity	Turbidity
222764	4.04	0.15	0.0000	NA	7.05	24.5	3.98	150.0	NA
225891	5.98	0.27	0.0000	NA	6.95	25.4	5.93	132.0	2.28
232326	7.80	0.13	0.0000	NA	7.35	26.8	7.24	154.0	1.55
220793	3.22	0.07	0.0000	NA	7.05	25.1	2.04	137.0	4.57
14464	11.46	0.41	0.1672	NA	6.89	25.4	9.31	35.0	3.57
75961	6.28	0.1	0.1647	NA	7.68	20.4	8.27	442.0	40.31
69184	6.22	0.22	0.1122	NA	7.08	20.4	9.98	62.0	9.17
203483	3.04	0.23	0.0000	NA	6.77	19.8	0.37	237.0	56.23
164521	1.20	0.15	0.0000	NA	7.05	22.0	0.51	122.0	16.46
152737	1.42	0.21	0.0000	NA	6.98	21.7	0.38	245.0	69.40
180206	2.20	0.14	0.0495	NA	7.26	20.6	6.19	135.0	9.42
181437	1.86	0.13	0.0156	NA	6.62	23.7	2.63	331.0	4.41
133573	2.22	0.12	0.0000	NA	4.85	22.8	3.82	67.0	8.15
120740	6.54	0.23	0.2559	NA	7.12	22.0	7.26	198.0	3.94
37901	5.73	0.45	0.2020	NA	7.43	23.4	8.05	92.0	4.70
86384	NA	NA	NA	NA	NA	NA	NA	NA	NA
23159	NA	NA	NA	NA	NA	NA	NA	NA	NA
205019	NA	NA	NA	NA	NA	NA	NA	NA	NA
216018	NA	NA	NA	NA	NA	NA	NA	NA	NA
112131	NA	NA	NA	NA	NA	NA	NA	NA	NA
123998	NA	NA	NA	NA	NA	NA	NA	NA	NA
58433	NA	NA	NA	NA	NA	NA	NA	NA	NA
84562	NA	NA	NA	NA	NA	NA	NA	NA	NA
60751	NA	NA	NA	NA	NA	NA	NA	NA	NA
72211	NA	NA	NA	NA	NA	NA	NA	NA	NA
45921	NA	NA	NA	NA	NA	NA	NA	NA	NA
25398	NA	NA	NA	NA	NA	NA	NA	NA	NA
51137	NA	NA	NA	NA	NA	NA	NA	NA	NA
156577	NA	NA	NA	NA	NA	NA	NA	NA	NA
156581	NA	NA	NA	NA	NA	NA	NA	NA	NA
180390	NA	NA	NA	NA	NA	NA	NA	NA	NA
147751	NA	NA	NA	NA	NA	NA	NA	NA	NA
208930	NA	NA	NA	NA	NA	NA	NA	NA	NA
262461	NA	NA	NA	NA	NA	NA	NA	NA	NA
301204	NA	NA	NA	NA	NA	NA	NA	NA	NA
12 Mile-Low	24.13	0.471	0.3010	NA	6.50	23.7	8.37	51.0	10.92
12 Mile-Above	45.00	0.524	0.0705	NA	6.83	25.9	8.50	54.0	10.69
RHC	4.78	0.170	0.2739	NA	NA	20.5	8.24	97.0	2.86
RH-1	5.28	0.308	0.2103	NA	6.43	16.5	8.95	50.0	9.61
LC	4.86	0.224	0.1988	NA	NA	17.8	8.23	47.0	4.39
Huff	7.80	0.253	0.1852	NA	7.24	18.4	8.28	51.0	6.01
Rocky	5.88	0.189	0.2661	NA	8.25	19.8	9.29	70.0	2.86
Bush	6.93	0.272	0.1889	NA	6.40	15.4	9.41	79.0	4.53
Bald	2.92	0.128	0.1548	NA	7.05	15.6	9.98	46.0	3.76
L-C	6.06	0.226	0.2341	NA	NA	15.0700	9.78	44.0	4.4

NA = not available

Table A-4. Watershed information for sample locations from South Carolina

Site	Watershed Area (km2)	Watershed Water	Watershed Urban	Watershed For.	Watershed Pasture/Scrub	Watershed Agriculture	Watershed Wet
207511	5.3	0.0240	0.252	0.366	0.341	0.000	0.017
205370	6.2	0.0009	0.801	0.105	0.045	0.011	0.038
216167	59.7	0.0198	0.108	0.345	0.250	0.203	0.074
287580	63.0	0.0177	0.084	0.476	0.298	0.078	0.047
346456	5.07	0.0011	0.012	0.641	0.192	0.094	0.059
258489	4.11	0.0000	0.072	0.199	0.337	0.225	0.167
231143	130	0.0059	0.040	0.287	0.383	0.123	0.161
132724	26.7	0.0000	0.027	0.121	0.289	0.330	0.232
236192	7.48	0.0030	0.053	0.187	0.275	0.155	0.327
153122	92.9	0.0051	0.101	0.126	0.147	0.419	0.201
178408	6.52	0.0000	0.057	0.208	0.233	0.196	0.306
177553	5.35	0.0000	0.053	0.384	0.228	0.095	0.240
100467	72.1	0.0079	0.094	0.230	0.166	0.233	0.269
159553	30.8	0.0000	0.016	0.358	0.125	0.150	0.350
145731	24.5	0.0055	0.037	0.261	0.147	0.208	0.343
155269	16.8	0.0000	0.037	0.172	0.182	0.397	0.212
215668	32.8	0.0147	0.463	0.160	0.216	0.056	0.092
142478	5.17	0.0000	0.079	0.126	0.138	0.548	0.108
87719	5.12	0.0000	0.269	0.129	0.127	0.411	0.065
145650	78.6	0.0112	0.034	0.282	0.126	0.337	0.210
98871	4.62	0.0000	0.048	0.073	0.172	0.546	0.161
236403	7.53	0.0000	0.098	0.114	0.193	0.327	0.269
215410	5.96	0.0022	0.068	0.212	0.188	0.387	0.143
221551	25.8	0.0000	0.055	0.227	0.174	0.330	0.213
269220	9.51	0.0000	0.093	0.082	0.446	0.346	0.033
328809	4.31	0.0000	0.013	0.433	0.186	0.003	0.366
256489	4.11	0.0000	0.072	0.199	0.337	0.225	0.167
320217	4.88	0.0000	0.008	0.600	0.332	0.000	0.060
219819	4.54	0.0004	0.132	0.170	0.204	0.327	0.167
234542	25.1	0.0003	0.106	0.226	0.282	0.109	0.277
245228	80.1	0.0006	0.046	0.219	0.189	0.308	0.237
198174	34.4	0.0047	0.070	0.180	0.183	0.193	0.369
219094	101	0.0011	0.044	0.213	0.185	0.289	0.267
212220	49.8	0.0007	0.050	0.131	0.195	0.342	0.281
202676	11.2	0.0006	0.138	0.149	0.209	0.294	0.209
204277	39.2	0.0065	0.022	0.250	0.112	0.086	0.524
265577	7.49	0.0000	0.805	0.090	0.042	0.000	0.062
362289	26.2	0.0014	0.042	0.422	0.297	0.114	0.124
380145	84.5	0.0031	0.080	0.382	0.272	0.077	0.186
307367	8.15	0.0000	0.046	0.328	0.270	0.300	0.056
318064	37.6	0.0000	0.024	0.398	0.341	0.073	0.164

Table A-4 (cont). Watershed information for sample locations from South Carolina

Site	Watershed Area (km2)	Watershed Water	Watershed Urban	Watershed For.	Watershed Pasture/Scrub	Watershed Agriculture	Watershed Wet
342523	26.9	0.0002	0.027	0.462	0.185	0.010	0.316
341665	81.2	0.2671	0.061	0.294	0.090	0.012	0.276
346136	25.2	0.0001	0.008	0.362	0.032	0.000	0.598
305009	5.09	0.0000	0.246	0.218	0.273	0.210	0.054
319073	84.9	0.0001	0.0818	0.2924	0.2643	0.2416	0.1198
39891	33.9	0.0095	0.2093	0.4047	0.0969	0.2648	0.0049
93949	10.6	0.0038	0.4494	0.2163	0.0662	0.2362	0.0238
103468	7.49	0.0000	0.8020	0.1360	0.0186	0.0271	0.0130
24932	4.75	0.0088	0.0384	0.7669	0.1132	0.0664	0.0000
86207	22.2	0.0033	0.1312	0.3293	0.1182	0.3849	0.0200
93236	26.3	0.0062	0.1218	0.2893	0.1129	0.4494	0.0122
90631	7.56	0.0030	0.0601	0.5274	0.1907	0.1801	0.0158
36620	7.99	0.0000	0.6862	0.2243	0.0361	0.0520	0.0000
27771	25.9	0.0057	0.2944	0.3739	0.0912	0.1709	0.0566
45813	4.08	0.0000	0.1083	0.6568	0.0954	0.1382	0.0000
26150	5.00	0.0028	0.0251	0.7480	0.1056	0.1186	0.0000
109522	60.0	0.0015	0.5112	0.1971	0.0788	0.1949	0.0112
56287	84.1	0.0069	0.3076	0.3646	0.0865	0.2181	0.0117
72426	30.5	0.0087	0.3658	0.2498	0.0798	0.2533	0.0392
15377	111	0.0037	0.0665	0.8271	0.0589	0.0356	0.0047
145961	122	0.0049	0.0862	0.4667	0.0693	0.3184	0.0492
181037	78.9	0.0084	0.1242	0.6437	0.0752	0.1381	0.0084
171515	26.8	0.0188	0.1586	0.5062	0.0745	0.2371	0.0034
157067	121	0.0029	0.0478	0.4701	0.1630	0.2922	0.0180
145150	39.2	0.0046	0.0454	0.4551	0.1271	0.3423	0.0211
152376	31.8	0.0045	0.0523	0.6359	0.1079	0.1656	0.0250
218747	29.4	0.0042	0.0627	0.6679	0.1565	0.0801	0.0267
203358	30.4	0.0036	0.0440	0.6876	0.1513	0.1017	0.0090
358497	25.4	0.0057	0.0271	0.4623	0.2953	0.0668	0.1429
363121	7.77	0.0023	0.0090	0.8013	0.0577	0.0052	0.1245
295697	7.08	0.0056	0.5431	0.4073	0.0176	0.0201	0.0045
304020	30.3	0.0033	0.0287	0.2844	0.4051	0.2037	0.0719
311961	101	0.0052	0.1490	0.3300	0.2148	0.1807	0.1111
313573	21.7	0.0096	0.0257	0.3960	0.2596	0.2057	0.1035
102026	31.8	0.0107	0.0624	0.5135	0.1116	0.2756	0.0253
92979	57.9	0.0075	0.0638	0.4275	0.0996	0.3839	0.0122
101334	75.2	0.0011	0.0504	0.5234	0.0797	0.3188	0.0237
136855	27.0	0.0009	0.0728	0.5937	0.1841	0.1343	0.0061
111855	82.1	0.0061	0.1242	0.4148	0.1433	0.2896	0.0163
13515	68.2	0.0052	0.1502	0.3238	0.1077	0.3942	0.0133
31263	92.1	0.0074	0.0632	0.7117	0.1256	0.0849	0.0028

Table A-4 (cont). Watershed information for sample locations from South Carolina

Site	Watershed Area (km2)	Watershed Water	Watershed Urban	Watershed For.	Watershed Pasture/Scrub	Watershed Agriculture	Watershed Wet
222764	65.5	0.0004	0.0354	0.7367	0.1257	0.0616	0.0160
225891	113	0.0036	0.0387	0.7148	0.1297	0.0821	0.0169
232326	98.6	0.0090	0.0425	0.6951	0.1128	0.1079	0.0291
220793	55.4	0.0028	0.0324	0.7516	0.1089	0.0721	0.0183
14464	116	0.0171	0.0300	0.9035	0.0182	0.0277	0.0012
75961	132	0.0019	0.2340	0.4765	0.1215	0.1432	0.0172
69184	109	0.0057	0.1204	0.5434	0.1357	0.1860	0.0007
203483	17.3	0.0036	0.0688	0.5717	0.0805	0.2562	0.0093
164521	4.39	0.0045	0.0419	0.7911	0.0727	0.0854	0.0043
152737	78.1	0.0045	0.0523	0.6359	0.1079	0.1656	0.0250
180206	4.97	0.0076	0.0424	0.4728	0.0750	0.3957	0.0029
181437	88.1	0.0044	0.0568	0.4773	0.1629	0.2692	0.0221
133573	27.9	0.0050	0.0516	0.4393	0.1419	0.3467	0.0112
120740	125	0.0028	0.3683	0.2545	0.1019	0.2504	0.0165
37901	84.0	0.0054	0.6097	0.2316	0.0533	0.0673	0.0305
86384	131	0.0021	0.0328	0.7744	0.1127	0.0529	0.0157
23159	31.1	0.0000	0.0412	0.8525	0.0390	0.0672	0.0000
205019	4.36	0.0006	0.0259	0.2364	0.1144	0.4100	0.2117
216018	8.82	0.0000	0.0521	0.1585	0.1337	0.4072	0.2485
112131	105	0.0030	0.0153	0.8623	0.0703	0.0209	0.0199
123998	75.7	0.0021	0.0330	0.7986	0.0900	0.0522	0.0171
58433	52.1	0.0088	0.0865	0.5595	0.0428	0.2972	0.0027
84562	71.0	0.0021	0.0328	0.7744	0.1127	0.0529	0.0157
60751	4.27	0.0057	0.1196	0.4332	0.0287	0.4128	0.0000
72211	13.0	0.0020	0.3374	0.4230	0.0679	0.1513	0.0127
45921	125.2	0.0005	0.0690	0.4312	0.1592	0.3382	0.0018
25398	62.1	0.0034	0.1917	0.6014	0.0189	0.1734	0.0054
51137	35.0	0.0099	0.0509	0.6719	0.0615	0.2018	0.0038
156577	28.8	0.0033	0.0287	0.2844	0.4051	0.2037	0.0719
156581	132	0.0360	0.1434	0.3878	0.1996	0.0857	0.1475
180390	69.0	0.0150	0.2482	0.2682	0.2186	0.1065	0.1337
147751	77.2	0.0069	0.0582	0.4293	0.2722	0.1114	0.1195
208930	136	0.0077	0.0612	0.5521	0.2410	0.0502	0.0877
262461	28.4	0.0067	0.0452	0.1182	0.0615	0.5347	0.2338
301204	33.1	0.0004	0.0444	0.4465	0.2974	0.0999	0.1115
12 Mile-Low	NA	0.0033	0.0287	0.2844	0.4051	0.2037	0.0719
12 Mile-Above	NA	0.0033	0.0287	0.2844	0.4051	0.2037	0.0719
RHC	14.5	0.0025	0.7945	0.1749	0.0071	0.0157	0.0016
RH-1	18.1	0.0042	0.2880	0.4618	0.0855	0.1258	0.0225
LC	13.4	0.0036	0.5509	0.3428	0.0284	0.0470	0.0218
Huff	15.4	0.0015	0.5675	0.1533	0.0642	0.1788	0.0325
Rocky	21.5	0.0017	0.5725	0.2378	0.0436	0.1067	0.0211
Bush	23.3	0.0000	0.8865	0.0922	0.0054	0.0117	0.0030
Bald	5.02	0.0023	0.5012	0.3176	0.0355	0.1264	0.0161
L-C	28.5	0.0093	0.4453	0.4281	0.0448	0.0671	0.0046

NA = not available

Table A-5. Total fish abundance and richness, and total *Lepomis sp.* individuals and richness at each site

Site	Total Fish Abundance	Total Fish Species	Total *Lepomis* Individuals	Tolerant *Lepomis* Individuals	Total *Lepomis* Richness	Tolerant *Lepomis* Richness
207511	69	8	20	20	4	4
205370	125	8	66	66	3	3
216167	163	14	78	73	5	4
287580	68	14	41	21	3	2
346456	108	14	38	6	3	1
258489	27	8	2	1	2	1
231143	103	14	33	18	4	2
132724	117	17	6	5	5	4
236192	0	0	0	0	0	0
153122	187	18	65	36	5	3
178408	314	15	5	5	2	2
177553	379	10	15	15	2	2
100467	150	17	6	1	3	1
159553	222	18	16	15	4	3
145731	324	12	1	0	1	0
155269	412	19	51	25	5	4
215668	184	17	55	53	3	2
142478	138	12	22	20	3	2
87719	194	11	24	13	2	1
145650	504	26	198	108	7	5
98871	280	7	49	1	2	1
236403	23	6	4	4	3	3
215410	39	6	3	3	1	1
221551	1908	14	286	227	3	1
269220	73	14	15	13	3	2
328809	61	9	11	11	2	2
256489	0	0	0	0	0	0
320217	235	10	80	2	2	1
219819	176	12	10	10	2	2
234542	445	9	48	31	5	4
245228	215	21	136	78	7	5
198174	293	17	102	83	6	4
219094	1159	19	132	115	5	3
212220	246	14	56	49	4	3
202676	200	13	16	16	1	1
204277	46	7	3	3	1	1
265577	0	0	0	0	0	0
362289	158	12	18	17	4	3
380145	117	14	49	39	4	3
307367	34	7	7	5	3	2
318064	219	16	93	45	5	3

Table A-5 (cont). Total fish abundance and richness, and total *Lepomis* individuals and richness at each site

Site	Total Fish Abundance	Total Fish Species	Total *Lepomis* Individuals	Tolerant *Lepomis* Individuals	Total *Lepomis* Richness	Tolerant *Lepomis* Richness
342523	80	15	48	29	6	4
341665	303	16	134	93	7	5
346136	696	15	214	56	4	2
305009	287	8	12	12	1	1
319073	399	15	55	49	3	1
39891	561	14	117	117	4	4
93949	299	11	31	31	3	3
103468	262	9	62	62	2	2
24932	207	12	88	88	2	2
86207	194	12	99	99	5	5
93236	324	18	38	38	4	4
90631	159	5	5	5	2	2
36620	1	1	1	1	1	1
27771	157	12	114	114	5	5
45813	95	8	41	41	2	2
26150	425	7	6	6	1	1
109522	186	10	95	95	3	3
56287	269	13	47	45	3	2
72426	466	13	58	58	2	2
15377	224	22	32	32	2	2
145961	386	19	79	79	2	2
181037	99	10	12	12	2	2
171515	1197	16	28	28	3	3
157067	1272	24	72	72	5	5
145150	1199	23	189	189	4	4
152376	878	15	123	123	3	3
218747	113	13	51	51	3	3
203358	33	8	8	8	2	2
358497	166	15	76	35	5	3
363121	101	10	59	0	1	0
295697	241	14	24	21	3	2
304020	272	23	12	4	3	1
311961	185	18	8	1	2	1
313573	75	12	16	5	3	1
102026	164	20	54	54	5	5
92979	1112	17	100	100	2	2
101334	571	17	79	79	5	5
136855	891	15	64	64	4	4
111855	912	18	51	51	3	3
13515	692	19	195	195	6	6
31263	1319	15	40	40	3	3

Table A-5 (cont). Total fish abundance and richness, and total *Lepomis* individuals and richness at each site

Site	Total Fish Abundance	Total Fish Species	Total *Lepomis* Individuals	Tolerant *Lepomis* Individuals	Total *Lepomis* Richness	Tolerant *Lepomis* Richness
222764	114	12	18	18	2	2
225891	750	28	127	127	4	4
232326	787	22	61	61	4	4
220793	296	16	15	15	2	2
14464	1177	29	75	75	5	5
75961	458	15	52	52	4	4
69184	757	20	233	231	6	5
203483	325	8	11	11	4	4
164521	97	11	7	7	4	4
152737	86	8	19	19	2	2
180206	512	11	25	25	2	2
181437	319	13	11	11	1	1
133573	367	11	7	7	1	1
120740	1156	19	136	136	4	4
37901	328	11	195	195	3	3
86384	106	10	33	10	2	2
23159	171	13	76	76	3	3
205019	65	15	35	23	5	3
216018	386	22	85	37	6	4
123998	129	16	19	19	3	3
58433	78	15	28	27	6	5
84562	119	11	10	10	2	2
60751	65	9	14	14	4	4
72211	121	9	24	24	3	3
45921	627	20	329	329	5	5
25398	358	17	92	92	2	2
51137	284	11	33	33	3	3
156577	247	16	145	135	5	3
156581	270	25	186	167	6	4
180390	57	10	10	10	2	2
147751	101	18	8	6	5	3
208930	50	12	26	13	4	3
262461	315	14	14	1	3	1
301204	232	17	32	20	5	4
12 Mile-Low	153	16	26	26	4	4
12 Mile-Above	59	6	46	46	2	2
RHC	354	8	42	42	2	2
RH-1	134	9	71	71	5	5
LC	190	7	19	19	3	3
Huff	546	9	137	137	3	3
Rocky	614	15	61	61	4	4
Bush	426	10	203	203	2	2
Bald	489	14	51	51	4	4
L-C	346	12	44	44	4	4

Table A-6. Total and relative priority species abundance and richness at each site

Site	Priority Richness	Priority Total Abundance	Priority % Relative Abundance	Priority Relative Richness
207511	0	0	0.00%	0.00%
205370	0	0	0.00%	0.00%
216167	1	1	0.61%	7.14%
287580	1	4	5.88%	7.14%
346456	3	23	21.30%	21.43%
258489	0	0	0.00%	0.00%
231143	1	1	0.97%	7.14%
132724	1	3	2.56%	5.88%
236192	0	0	0.00%	0.00%
153122	1	2	1.07%	5.56%
178408	2	9	2.87%	13.33%
177553	1	2	0.53%	10.00%
100467	2	3	2.00%	11.76%
159553	1	1	0.45%	5.56%
145731	1	3	0.93%	8.33%
155269	2	24	5.83%	10.53%
215668	2	5	2.72%	11.76%
142478	0	0	0.00%	0.00%
87719	1	17	8.76%	9.09%
145650	1	3	0.60%	3.85%
98871	1	1	0.36%	14.29%
236403	0	0	0.00%	0.00%
215410	0	0	0.00%	0.00%
221551	2	10	0.52%	14.29%
269220	1	1	1.37%	7.14%
328809	0	0	0.00%	0.00%
256489	NA	NA	NA	NA
320217	0	0	0.00%	0.00%
219819	2	5	2.84%	16.67%
234542	1	24	5.39%	11.11%
245228	2	2	0.93%	9.52%
198174	1	1	0.34%	5.88%
219094	2	5	0.43%	10.53%
212220	0	0	0.00%	0.00%
202676	2	8	4.00%	15.38%
204277	0	0	0.00%	0.00%
265577	0	0	0.00%	0.00%
362289	0	0	0.00%	0.00%
380145	0	0	0.00%	0.00%
307367	0	0	0.00%	0.00%
318064	0	0	0.00%	0.00%

NA = not available

Table A-6 (cont). Total and relative priority species abundance and richness at each site

Site	Priority Richness	Priority Total Abundance	Priority % Relative Abundance	Priority Relative Richness
342523	0	0	0.00%	0.00%
341665	0	0	0.00%	0.00%
346136	0	0	0.00%	0.00%
305009	1	0	0.00%	12.50%
319073	2	14	3.51%	13.33%
39891	1	185	32.98%	7.14%
93949	0	0	0.00%	0.00%
103468	0	0	0.00%	0.00%
24932	2	19	9.18%	16.67%
86207	0	0	0.00%	0.00%
93236	3	20	6.17%	16.67%
90631	0	0	0.00%	0.00%
36620	0	0	0.00%	0.00%
27771	0	0	0.00%	0.00%
45813	1	3	3.16%	12.50%
26150	0	0	0.00%	0.00%
109522	0	0	0.00%	0.00%
56287	2	15	5.58%	15.38%
72426	2	11	2.36%	15.38%
15377	6	39	17.41%	27.27%
145961	2	40	10.36%	10.53%
181037	1	1	1.01%	10.00%
171515	2	71	5.93%	12.50%
157067	3	36	2.83%	12.50%
145150	1	81	6.76%	4.35%
152376	2	75	8.54%	13.33%
218747	2	6	5.31%	15.38%
203358	0	0	0.00%	0.00%
358497	0	0	0.00%	0.00%
363121	1	2	1.98%	10.00%
295697	2	11	4.56%	14.29%
304020	5	59	21.69%	21.74%
311961	4	88	47.57%	22.22%
313573	0	0	0.00%	0.00%
102026	1	3	1.83%	5.00%
92979	3	146	13.13%	17.65%
101334	2	10	1.75%	11.76%
136855	1	6	0.67%	6.67%
111855	2	31	3.40%	11.11%
13515	2	22	3.18%	10.53%
31263	2	24	1.82%	13.33%

Table A-6 (cont). Total and relative priority species abundance and richness at each site

Site	Priority Richness	Priority Total Abundance	Priority % Relative Abundance	Priority Relative Richness
225891	2	69	9.20%	7.14%
232326	2	32	4.07%	9.09%
220793	2	14	4.73%	12.50%
14464	8	207	17.59%	27.59%
75961	0	0	0.00%	0.00%
69184	2	18	2.38%	10.00%
203483	1	5	1.54%	12.50%
164521	1	1	1.03%	9.09%
152737	1	3	3.49%	12.50%
180206	3	91	17.77%	27.27%
181437	2	204	63.95%	15.38%
133573	2	29	7.90%	18.18%
120740	2	35	3.03%	10.53%
37901	0	0	0.00%	0.00%
86384	1	3	2.83%	10.00%
23159	1	8	4.68%	7.69%
205019	1	2	3.08%	6.67%
216018	2	16	4.15%	9.09%
112131	NA	NA	NA	NA
123998	3	15	11.63%	18.75%
58433	1	3	3.85%	6.67%
84562	2	7	5.88%	18.18%
60751	0	0	0.00%	0.00%
72211	0	0	0.00%	0.00%
45921	3	10	1.59%	15.00%
25398	3	11	3.07%	17.65%
51137	1	9	3.17%	9.09%
156577	0	0	0.00%	0.00%
156581	3	5	1.85%	12.00%
180390	2	7	12.28%	20.00%
147751	2	50	49.50%	11.11%
208930	1	2	4.00%	8.33%
262461	0	0	0.00%	0.00%
301204	1	2	0.86%	5.88%
12 Mile-Low	0	0	0.00%	0.00%
12 Mile-Above	0	0	0.00%	0.00%
RHC	0	0	0.00%	0.00%
RH-1	0	0	0.00%	0.00%
LC	0	0	0.00%	0.00%
Huff	0	0	0.00%	0.00%
Rocky	2	24	0.04%	0.13%
Bush	0	0	0.00%	0.00%
Bald	0	0	0.00%	0.00%
L-C	1	14	0.04%	0.08%

NA = not available

Table A-7. Total bluegill and redbreast sunfish at each site

Site	Total Bluegill	Total Redbreast	Bluegill/Redbreast Richness	Bluegill/Redbreast Abundance	Relative Bluegill/Redbreast Richness	Relative Bluegill/Redbreast Abundance
207511	17	1	2	18	25.00	26.09
205370	55	2	2	57	25.00	45.60
216167	25	40	2	65	14.29	39.88
287580	4	17	2	21	14.29	30.88
346456	0	6	1	6	7.14	5.56
258489	0	0	0	0	0.00	0.00
231143	1	17	2	18	14.29	17.48
132724	2	1	2	3	11.76	2.56
236192	0	0	0	0	0.00	0.00
153122	9	18	2	27	11.11	14.44
178408	0	0	0	0	0.00	0.00
177553	1	0	1	1	10.00	0.26
100467	0	0	0	0	0.00	0.00
159553	0	0	0	0	0.00	0.00
145731	0	0	0	0	0.00	0.00
155269	0	10	1	10	5.26	2.43
215668	39	0	1	39	5.88	21.20
142478	0	19	1	19	8.33	13.77
87719	0	0	0	0	0.00	0.00
145650	22	75	2	97	7.69	19.25
98871	0	0	0	0	0.00	0.00
236403	1	1	1	2	16.67	8.70
215410	0	0	0	0	0.00	0.00
221551	0	227	1	227	7.14	11.90
269220	10	0	1	10	7.14	13.70
328809	10	0	1	10	11.11	16.39
256489	NA	NA	NA	NA	NA	NA
320217	0	0	0	0	0.00	0.00
219819	5	0	1	5	8.33	2.84
234542	18	7	2	25	22.22	5.62
245228	28	31	2	59	9.52	27.44
198174	26	55	2	81	11.76	27.65
219094	1	109	2	110	10.53	9.49
212220	2	46	2	48	14.29	19.51
202676	0	0	0	0	0.00	0.00
204277	0	0	0	0	0.00	0.00
265577	0	0	0	0	0.00	0.00
362289	7	6	2	13	16.67	8.23
380145	5	31	2	36	14.29	30.77
307367	0	4	1	4	14.29	11.76
318064	1	42	2	43	12.50	19.63

Table A-7 (cont). Total bluegill and redbreast sunfish at each site

Site	Total Bluegill	Total Redbreast	Bluegill/Redbreast Richness	Bluegill/Redbreast Abundance	Relative Bluegill/Redbreast Richness	Relative Bluegill/Redbreast Abundance
342523	1	22	2	23	13.33	28.75
341665	2	77	2	79	12.50	26.07
346136	0	51	1	51	6.67	7.33
305009	0	12	1	12	12.50	4.18
319073	0	49	1	49	6.67	12.28
39891	50	44	2	94	14.29	16.76
93949	4	8	2	12	18.18	4.01
103468	0	44	1	44	11.11	16.79
24932	85	3	2	88	16.67	42.51
86207	6	83	2	89	16.67	45.88
93236	13	20	2	33	11.11	10.19
90631	2	3	2	5	40.00	3.14
36620	0	1	1	1	100.00	100.00
27771	45	29	2	74	16.67	47.13
45813	0	40	1	40	12.50	42.11
26150	0	6	1	6	14.29	1.41
109522	21	68	2	89	20.00	47.85
56287	10	35	2	45	15.38	16.73
72426	7	51	2	58	15.38	12.45
15377	20	12	2	32	9.09	14.29
145961	3	76	2	79	10.53	20.47
181037	6	6	2	12	20.00	12.12
171515	16	11	2	27	12.50	2.26
157067	17	46	2	63	8.33	4.95
145150	72	83	2	155	8.70	12.93
152376	2	120	2	122	13.33	13.90
218747	0	21	1	21	7.69	18.58
203358	0	6	1	6	12.50	18.18
358497	2	32	2	34	13.33	20.48
363121	0	0	0	0	0.00	0.00
295697	10	11	2	21	14.29	8.71
304020	0	0	0	0	0.00	0.00
311961	1	0	1	1	5.56	0.54
313573	0	5	1	5	8.33	6.67
102026	20	30	2	50	10.00	30.49
92979	8	92	2	100	11.76	8.99
101334	48	18	2	66	11.76	11.56
136855	8	46	2	54	13.33	6.06
111855	2	48	2	50	11.11	5.48
13515	108	42	2	150	10.53	21.68
31263	20	7	2	27	13.33	2.05

Table A-7 (cont). Total bluegill and redbreast sunfish at each site

Site	Total Bluegill	Total Redbreast	Bluegill/Redbreast Richness	Bluegill/Redbreast Abundance	Relative Bluegill/Redbreast Richness	Relative Bluegill/Redbreast Abundance
225891	5	100	2	105	7.14	14.00
232326	0	48	1	48	4.55	6.10
220793	0	13	1	13	6.25	4.39
14464	23	48	2	71	6.90	6.03
75961	3	16	2	19	13.33	4.15
69184	7	78	2	85	10.00	11.23
203483	1	1	2	2	25.00	0.62
164521	1	1	2	2	18.18	2.06
152737	0	14	1	14	12.50	16.28
180206	1	24	2	25	18.18	4.88
181437	0	11	1	11	7.69	3.45
133573	0	7	1	7	9.09	1.91
120740	13	115	2	128	10.53	11.07
37901	7	175	2	182	18.18	55.49
86384	20	13	2	33	20.00	31.13
23159	19	56	2	75	15.38	43.86
205019	10	0	1	10	6.67	15.38
216018	3	1	2	4	9.09	1.04
112131	NA	NA	NA	NA	NA	NA
123998	4	13	2	17	12.50	13.18
58433	16	4	2	20	13.33	25.64
84562	0	9	1	9	9.09	7.56
60751	6	3	2	9	22.22	13.85
72211	20	3	2	23	22.22	19.01
45921	89	193	2	282	10.00	44.98
25398	18	74	2	92	11.76	25.70
51137	17	0	1	17	9.09	5.99
156577	1	132	2	133	12.50	53.85
156581	22	134	2	156	8.00	57.78
180390	3	7	2	10	20.00	17.54
147751	4	1	2	5	11.11	4.95
208930	1	8	2	9	16.67	18.00
262461	0	0	0	0	0.00	0.00
301204	16	0	1	16	5.88	6.90
12 Mile-Low	13	8	2	21	12.50	13.73
12 Mile-Above	6	40	2	46	33.33	77.97
RHC	0	35	1	35	12.50	9.89
RH-1	18	17	2	35	22.22	26.12
LC	10	6	2	16	28.57	8.42
Huff	20	116	2	136	22.22	24.91
Rocky	0	49	1	49	6.67	7.98
Bush	22	181	2	203	20.00	47.65
Bald	0	45	1	45	7.14	9.20
L-C	2	17	2	19	16.67	5.49

NA = not available

Table A-8. Relative *Lepomis sp.* abundance, richness, and total diversity at each site

Site	Relative *Lepomis* sp. Abundance	Relative *Lepomis* sp. Richness	Relative Tolerant *Lepomis* Abundance	Relative Tolerant *Lepomis* Richness	Simpson Diversity Index (D)	1-D (Simpson's Diversity Index)
207511	29	50	29	50	0.787	0.213
205370	53	38	53	38	0.733	0.267
216167	48	36	45	29	0.826	0.174
287580	60	21	31	14	0.829	0.171
346456	35	21	6	7	0.873	0.127
258489	7	25	4	13	0.535	0.465
231143	32	29	17	14	0.819	0.181
132724	5	29	4	24	0.798	0.202
236192	0	0	0	0	0.000	1.000
153122	35	28	19	17	0.817	0.183
178408	2	13	2	13	0.547	0.453
177553	4	20	4	20	0.442	0.558
100467	4	18	1	6	0.837	0.163
159553	7	22	7	17	0.859	0.141
145731	0	8	0	0	0.831	0.169
155269	12	26	6	21	0.869	0.131
215668	30	18	29	12	0.817	0.183
142478	16	25	14	17	0.871	0.129
87719	12	18	7	9	0.833	0.167
145650	39	27	21	19	0.861	0.139
98871	18	29	0	14	0.390	0.610
236403	17	50	17	50	0.631	0.369
215410	8	17	8	17	0.715	0.285
221551	15	21	12	7	0.372	0.628
269220	21	21	18	14	0.867	0.133
328809	18	22	18	22	0.802	0.198
256489	NA	NA	NA	NA	0.000	1.000
320217	34	20	1	10	0.732	0.268
219819	6	17	6	17	0.672	0.328
234542	11	56	7	44	0.324	0.676
245228	63	33	36	24	0.877	0.123
198174	35	35	28	24	0.737	0.263
219094	11	26	10	16	0.387	0.613
212220	23	29	20	21	0.593	0.407
202676	8	8	8	8	0.800	0.200
204277	7	14	7	14	0.498	0.502
265577	0	0	0	0	0.000	1.000
362289	11	33	11	25	0.583	0.417
380145	42	29	33	21	0.871	0.129
307367	21	43	15	29	0.713	0.287
318064	42	31	21	19	0.758	0.242

NA = not available

Table A-8 (cont). Relative *Lepomis sp.* abundance, richness, and total diversity at each site

Site	Relative *Lepomis* sp. Abundance	Relative *Lepomis* sp. Richness	Relative Tolerant *Lepomis* Abundance	Relative Tolerant *Lepomis* Richness	Simpson Diversity Index (D)	1-D (Simpson's Diversity Index)
342523	60	40	36	27	0.858	0.142
341665	44	44	31	31	0.829	0.171
346136	31	27	8	13	0.833	0.167
305009	4	13	4	13	0.577	0.423
319073	14	20	12	7	0.762	0.238
39891	21	29	21	29	0.812	0.188
93949	10	27	10	27	0.676	0.324
103468	24	22	24	22	0.645	0.355
24932	43	17	43	17	0.764	0.236
86207	51	42	51	42	0.702	0.298
93236	12	22	12	22	0.672	0.328
90631	3	40	3	40	0.619	0.381
36620	100	100	100	100	0.000	1.000
27771	73	42	73	42	0.842	0.158
45813	43	25	43	25	0.676	0.324
26150	1	14	1	14	0.594	0.406
109522	51	30	51	30	0.743	0.257
56287	17	23	17	15	0.833	0.167
72426	12	15	12	15	0.686	0.314
15377	14	9	14	9	0.874	0.126
145961	20	11	20	11	0.826	0.174
181037	12	20	12	20	0.804	0.196
171515	2	19	2	19	0.682	0.318
157067	6	21	6	21	0.772	0.228
145150	16	17	16	17	0.820	0.180
152376	14	20	14	20	0.754	0.246
218747	45	23	45	23	0.851	0.149
203358	24	25	24	25	0.751	0.249
358497	46	33	21	20	0.882	0.118
363121	58	10	0	0	0.628	0.372
295697	10	21	9	14	0.813	0.187
304020	4	13	1	4	0.667	0.333
311961	4	11	1	6	0.873	0.127
313573	21	25	7	8	0.817	0.183
102026	33	25	33	25	0.874	0.126
92979	9	12	9	12	0.788	0.212
101334	14	29	14	29	0.669	0.331
136855	7	27	7	27	0.754	0.246
111855	6	17	6	17	0.856	0.144
13515	28	32	28	32	0.834	0.166
31263	3	20	3	20	0.678	0.322

Table A-8 (cont). Relative *Lepomis sp.* abundance, richness, and total diversity at each site

Site	Relative *Lepomis* sp. Abundance	Relative *Lepomis* sp. Richness	Relative Tolerant *Lepomis* Abundance	Relative Tolerant *Lepomis* Richness	Simpson Diversity Index (D)	1-D (Simpson's Diversity Index)
222764	16	17	16	17	0.820	0.180
225891	17	14	17	14	0.901	0.099
232326	8	18	8	18	0.855	0.145
220793	5	13	5	13	0.713	0.287
14464	6	17	6	17	0.829	0.171
75961	11	27	11	27	0.575	0.425
69184	31	30	31	25	0.837	0.163
203483	3	50	3	50	0.147	0.853
164521	7	36	7	36	0.650	0.350
152737	22	25	22	25	0.618	0.382
180206	5	18	5	18	0.663	0.337
181437	3	8	3	8	0.639	0.361
133573	2	9	2	9	0.595	0.405
120740	12	21	12	21	0.660	0.340
37901	59	27	59	27	0.643	0.357
86384	31	20	9	20	0.151	0.849
23159	44	23	44	23	0.165	0.835
205019	54	33	35	20	0.117	0.883
216018	22	27	10	18	0.107	0.893
112131	NA	NA	NA	NA	NA	NA
123998	15	19	15	19	0.169	0.831
58433	36	40	35	33	0.138	0.862
84562	8	18	8	18	0.156	0.844
60751	22	44	22	44	0.516	0.484
72211	20	33	20	33	0.218	0.782
45921	52	25	52	25	0.166	0.834
25398	26	12	26	12	0.112	0.888
51137	12	27	12	27	0.452	0.548
156577	59	31	55	19	0.311	0.689
156581	69	24	62	16	0.276	0.724
180390	18	20	18	20	0.221	0.779
147751	8	28	6	17	0.214	0.786
208930	52	33	26	25	0.154	0.846
262461	4	21	0	7	0.159	0.841
301204	14	29	9	24	0.270	0.730
12 Mile-Low	17	25	17	25	0.803	0.197
12 Mile-Above	78	33	78	33	0.512	0.488
RHC	12	25	12	25	0.546	0.454
RH-1	53	56	53	56	0.815	0.185
LC	10	43	10	43	0.637	0.363
Huff	25	33	25	33	0.477	0.523
Rocky	10	27	10	27	0.779	0.221
Bush	48	20	48	20	0.643	0.357
Bald	10	29	10	29	0.705	0.295
L-C	13	33	13	33	0.709	0.291

Table A-9. Fish information for each site of collection

Site	Fish #	Sex	Weight (g)	Length (cm)	Liver	Spleen	Gonad	Fish Types/Comments
207511	3	M	26.6	11.5	0.2132	0.015	0.012	Bluegill
205370	3	J	2.762	6	0.0383	0.002	NA	Bluegill
216167	5	M	47.35	14.45	0.40285	0.0585	1.5885	Red Breast
287580	3.5	M	32.9415	12.65	0.3255	0.0195	2.35	Red Breast
346456	1	M	33.861	11.8	0.287	0.02	0.04	Red Breast
258489	1	M	20	9.5	NA	NA	NA	Dollar-Parasites
231143	4.5	M	26.5	12	0.1507	0.0148	0.0493	Red Breast
132724	2.5	M	9	8.35	0.0916	0.01095	1.1636	Bluegill Juvenile
236192	6.5	M	17.5	10.1	0.2046	0.03795	0.477	Dollar
153122	5.5	M	50	15.45	0.3711	0.03355	0.9868	Red Breast
178408	2.5	M	84	14.7	0.86365	0.0799	0.29755	Warmouth
177553	5.5	J	5.5	7.4	0.04965	0.00625	0.00925	Warmouth Juvenile
100467	5	M	21	10.35	0.3014	0.0177	0.2318	Dollar
159553	4	M	15	10.2	0.1796	0.0268	0.0989	Pumpkinseed-Parasites
145731	2	M	NA	13	0.2424	0.0257	0.3121	Warmouth
155269	5.5	M	17	9.95	0.189	0.02265	0.068	Red Breast
215668	5.5	M	21.5	11.15	0.26415	0.0478	0.0396	Bluegill
142478	6.5	M	17	10.1	0.1297	0.0231	0.05095	Red Breast
87719	6	M	6	7	0.0866	0.024	0.0283	Dollar, Parasites
145650	7	M	74	16.4	0.3549	0.0615	0.0803	Red Breast
98871	5.5	M	18	9.65	0.2596	0.0911	0.07525	Dollar
236403	1	M	42	11	0.325	0.0173	NA	Bluegill
215410	2	J	NA	NA	NA	NA	NA	Warmouth
221551	5	F	4	6.5	0.095	0.0095	2.4233	Redbreast
269220	4.5	M	14	9.5	0.1225	0.016	0.04705	Warmouth
328809	5.5	M	7	8.1	0.1215	0.0163	0.0088	Bluegill with Parasites
256489	1	J	4	7	0.085	0.0061	NA	Redbreast
320217	5.5	M	17	9.9	0.188	0.05035	0.06785	Dollar with Parasites
219819	3	M	8	7.5	0.078	0.0079	0.19335	Warmouth
234542	5.5	M	17.5	9.25	0.201	0.0193	0.68715	Spotted
245228	3	M	18	10.4	0.156	0.0131	4.3951	Redbreast
198174	5.5	M	24.5	11.2	0.362	0.024	0.5859	Redbreast
219094	4	M	36	13.6	0.257	0.0202	0.449	Redbreast
212220	5.5	M	7	8.3	0.0655	0.0092	0.3517	Redbreast
202676	3.5	M	16.5	9.5	0.299	0.0333	0.5735	Pumpkinseed with Parasites
204277	2	M	25	11.2	0.635	0.0227	1.1955	Pumpkinseed
265577	4	M	13	9.6	0.146	0.0107	0.15505	Bluegill with Parasites
362289	3.5	M	52.5	14.45	1.293	0.0355	5.12015	Redbreast
380145	4.5	M	36.5	12.5	0.8725	0.05855	0.9454	Redbreast
307367	3	M	NA	11.4	0.554	0.0431	0.22765	Redbreast
318064	4.5	M	24.5	11.45	0.281	0.02325	0.07165	Redbreast with Parasites

Table A-9 (cont). Fish information for each site of collection

Site	Fish #	Sex	Weight (g)	Length (cm)	Liver	Spleen	Gonad	Fish Types/Comments
342523	4	M	12	9.3	0.11	0.012	0.0424	Redbreast with Parasites
341665	5.5	M	50.5	15.2	0.603	0.0689	1.0483	Redbreast with Parasites
346136	5.5	M	60	15	0.4625	0.0696	2.3579	Redbreast
305009	5	M	9	8.5	0.095	0.013	0.0355	Redbreast
319073	5	M	18	10.5	0.1805	0.023	0.064	Redbreast
39891	4	M	58	17	0.6442	0.067	0.43775	Redbreast
93949	4.5	M	9.5	8.75	0.0852	0.0125	0.00315	Redbreast
103468	3.5	M	15.5	10	0.24615	0.03525	0.3933	Redbreast
24932	2.5	M	8.5	8.75	0.0712	0.01265	0.0395	Bluegill/Parasites
86207	6.5	M	20	11.1	0.1993	0.0222	0.21725	Redbreast
93236	5.5	M	58.5	14.75	0.413	0.04555	0.3171	Bluegill
90631	2.5	F	13	9.1	0.1368	0.02325	0.67715	Redbreast
36620	1	M	46	12.8	0.6749	0.0017	NA	Redbreast
27771	5.5	M	66.5	14.95	0.5872	0.07335	0.2713	Warmouth/Parasites
45813	2.5	M	16	9.85	0.23735	0.0207	0.8628	Redbreast
26150	2	M	27	12	0.2262	0.0215	0.0333	NA
109522	5.5	M	39	12.85	0.48235	0.0699	0.2447	Redbreast
56287	5.5	M	69.5	14.8	1.10405	0.0899	0.49485	Redbreast
72426	5.5	M	67.5	14.3	0.5295	0.04455	0.4432	Redbreast/Parasites
15377	4	M	48	13.4	0.4623	0.0344	0.3356	Redbreast
145961	3.5	M	39	12.55	0.24885	0.0315	0.078	Redbreast
181037	4	M	15	9.8	0.1039	0.011	0.0497	Redbreast
171515	5.5	F	26	11.1	0.2177	0.0181	1.11195	Redbreast
157067	4	M	34	12.6	0.2694	0.0312	0.4708	Redbreast
145150	5.5	M	34	12.6	0.2318	0.02235	0.208	Redbreast
152376	4.5	M	57.5	15	0.6071	0.0585	0.56485	Redbreast
218747	2.5	M	30.5	12.8	0.2202	0.07725	0.05305	Redbreast
203358	3	M	30	11.6	0.5955	0.0262	1.756	Redbreast/Parasites
358497	6	M	36	12.1	0.303	0.0591	0.1742	Redbreast
363121	2	J	15	9.3	0.1716	0.0183	0.1319	Dollar
295697	2	M	25	11.4	0.3001	0.0188	0.121	Redbreast
304020	3	M	45	13.4	0.3759	0.0257	0.169	Warmouth
311961	3.5	F	45	13.05	0.3812	0.0405	0.8201	Spotted
313573	5.5	M	40.5	12.55	0.4756	0.054	0.9477	Redbreast
102026	5.5	M	35.5	12.8	0.2803	0.0409	0.2197	Redear
92979	4	M	46	13.6	0.3685	0.03465	0.3261	Redbreast
101334	2.5	F	NA	12.8	0.3563	0.0295	2.22365	Redbreast/Parasites
136855	4	M	36	13	0.5376	0.0291	0.4222	Redbreast
111855	4	M	49	14.2	0.5359	0.0647	0.209	Redbreast
13515	4	M	28	12.2	0.266	0.0288	0.09315	Redbreast
31263	2.5	M	30	12.75	0.20775	0.02275	0.2258	Bluegill

Table A-9 (cont). Fish information for each site of collection

Site	Fish #	Sex	Weight (g)	Length (cm)	Liver	Spleen	Gonad	Fish Types/Comments
222764	3.5	M	38	13.05	0.3936	0.03895	0.07285	Redbreast
225891	4	M	43	13.7	0.4623	0.0587	0.1525	Redbreast
232326	2	M	36	13.1	0.3445	0.0494	0.2051	Redbreast
220793	3	M	25	11.9	0.367	0.0424	0.2773	Redbreast/Parasites
14464	7	M	60	15.1	0.4787	0.0335	0.19355	Redbreast
75961	4	M	27	11.6	0.2873	0.0402	0.07775	Redbreast
69184	3.5	M	52	14.3	0.30565	0.09005	0.14585	Redbreast
203483	3	M	19	10.6	0.32	0.0223	0.1126	Bluegill/Parasites
164521	2.5	M	39.5	12.9	0.55025	0.06765	0.09525	War
152737	4	M	31	12.4	0.3598	0.0281	0.0924	War
180206	3.5	M	31	12.75	0.282	0.0515	0.1186	Redbreast
181437	3	M	22	11.2	0.2356	0.0275	0.0212	Redbreast
133573	2	M	24	11.8	0.2726	0.0413	0.1265	Redbreast
120740	5.5	M	33.5	12.8	0.36245	0.03635	0.07535	Redbreast
37901	5.5	M	51	14.6	0.3773	0.0482	0.1969	Bluegill
86384	2	M	28	11.5	0.7243	0.0343	0.5484	Redbreast
23159	3.5	M	64	14.1	0.63235	0.07065	0.4218	Redbreast
205019	2	M	43	13.3	0.6656	0.0507	0.2035	Bluegill
216018	4	M	58	13.5	0.5715	0.1312	0.6338	War
112131	2	M	46	13.8	0.3514	0.0546	0.38925	Redbreast
123998	2	F	39	12.9	0.4338	0.0408	NA	Redbreast
58433	5.5	M	85.5	16.4	0.9199	0.0809	0.36735	Bluegill
84562	2	M	29	11.3	0.2774	0.034	0.1635	Redbreast
60751	3	M	NA	NA	0.3784	0.05595	0.262	NA
72211	3	M	NA	NA	0.6374	0.0383	0.053	NA
45921	5.5	M	84	15.7	0.63485	0.0929	0.56535	Redbreast
25398	5.5	M	72.5	15.4	0.65285	0.05955	0.376	Redbreast
51137	2	F	51	13.8	0.5607	0.0802	0.1446	War
156577	5.5	M	53	14.55	0.4106	0.0357	0.3415	Redbreast
156581	4	M	63	14.9	0.3152	0.0446	0.2944	Redbreast
180390	2.5	M	88.5	16.55	0.65315	0.0493	0.55275	Redbreast
147751	2	M	70	15.4	0.6663	0.0621	0.0668	Bluegill
208930	1.5	M	171.5	20.45	0.92335	0.06655	0.77675	Redbreast
262461	2	M	27	10.7	0.3462	0.04785	0.3398	Spotted
301204	2.5	M	23.5	11.25	0.2007	0.0327	0.1756	Bluegill
12 Mile-Low	5	M	46	14.4	0.2827	0.0434	0.0797	Bluegill
12 Mile-Above	3.5	M	31.5	12.75	0.3113	0.03475	0.1851	Redbreast
RHC	5	M	34	11.8	0.6532	0.0849	0.22295	Redbreast
RH-1	4	M	43	13.5	0.48445	0.0315	0.274	Warmouth
LC	3	F	16	9.5	0.4476	0.0164	NA	Redbreast
Huff	5.5	M	47	14.05	0.49475	0.07405	0.184	Redbreast
Rocky	5.5	M	43.5	13.9	0.72665	0.07435	0.2442	Redbreast
Bush	5.5	M	71	15.55	0.9381	0.092	0.4527	Redbreast
Bald	3.5	M	73	16.15	0.515	0.0623	0.4609	Redbreast
L-C	3.5	M	50.5	14.1	0.6258	0.1096	0.18215	Redbreast

Table A-10. Measurements of mercury (Hg), bile protein, fluorescence 1, 2, and 3, estrogen binding assay, EROD, GST, K, HSI, SSI, and GSI.

Site	Hg (ng/g)	Bile protein	Fluor1	Fluor2	Fluor3	ER Binding Assay (EBE)	EROD	GST	K	HSI	SSI	GSI
207511	NA	2.23	201536	50195	10932	NA	11.8	164.1	1.7	0.8	0.1	0.0
205370	NA	0.63	1900143	838483	173147	NA	NA	NA	1.3	1.4	0.1	NA
216167	NA	3.22	125427	48064	12805	NA	2.6	119.3	1.7	0.7	0.1	4.9
287580	NA	3.17	145665	16494	4061	NA	1.1	107.5	1.6	1.0	0.1	6.9
346456	NA	NA	NA	NA	NA	NA	-0.2	123.0	2.1	0.8	0.1	0.1
258489	NA	NA	NA	NA	NA	NA	NA	NA	2.3	NA	NA	NA
231143	NA	0.59	1148763	1110639	222665	NA	2.3	129.8	1.5	0.8	0.1	0.1
132724	NA	0.95	543730	39737	11798	NA	0.3	85.9	1.6	1.0	0.1	9.7
236192	NA	1.13	811729	786913	63827	NA	0.0	58.9	1.7	1.1	0.2	2.5
153122	NA	1.56	647410	262413	56149	NA	4.6	127.2	1.5	1.0	0.1	1.0
178408	NA	3.90	467465	20991	154785	NA	66.9	167.5	2.3	1.1	0.1	0.3
177553	NA	0.58	154530	246652	1346146	NA	NA	NA	1.3	1.2	0.1	0.3
100467	NA	1.20	690231	79827	41598	NA	0.5	89.1	1.8	1.3	0.1	1.1
159553	NA	1.23	882914	148605	70584	NA	12.7	136.0	1.5	1.2	0.2	0.8
145731	NA	0.89	196190	37725	14693	NA	NA	NA	NA	NA	NA	NA
155269	NA	1.29	760600	218117	43121	NA	1.7	150.5	1.7	1.1	0.1	0.3
215668	NA	20.8	19905	4746	625	NA	5.8	145.7	1.6	1.2	0.3	0.2
142478	NA	1.48	920967	338557	47817	NA	3.9	99.3	1.6	1.0	0.2	0.5
87719	NA	0.50	809709	452482	86400	NA	9.8	97.7	1.8	1.4	0.4	0.4
145650	NA	3.28	197811	33164	8862	NA	5.1	191.6	1.6	0.5	0.1	0.1
98871	NA	0.94	524536	36569	17735	NA	2.3	165.1	2.0	1.4	0.5	0.4
236403	NA	1.47	806	394	114	114	67.8	151.6	3.2	0.8	0.0	NA
215410	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
221551	NA	0.81	1034	1089	123	4631	30.2	94.3	1.8	2.0	0.1	6.4
269220	NA	0.95	891	716	85	648	31.4	228.5	1.6	0.9	0.1	0.3
328809	NA	0.84	727	634	89	2055	40.2	169.2	1.4	1.9	0.2	0.1
256489	NA	-0.02	NA	NA	NA	1336	36.0	221.5	1.2	2.1	0.2	NA
320217	NA	1.83	650	318	57	5801	33.0	386.6	1.6	1.1	0.3	0.4
219819	NA	1.86	628	372	46	1253	47.3	153.8	1.7	1.0	0.1	1.3
234542	NA	0.32	1761	1541	220	1330	151.7	400.5	2.2	1.1	0.1	4.8
245228	NA	1.23	1213	782	111	NA	36.2	303.7	1.7	1.1	0.1	7.0
198174	NA	1.53	774	404	63	3044	52.8	379.2	1.5	0.8	0.1	0.9
219094	NA	2.23	621	506	47	1332	72.3	367.4	1.5	0.8	0.1	0.8
212220	NA	1.03	695	456	47	NA	21.7	386.5	1.4	0.8	0.1	2.4
202676	NA	1.93	961	289	42	10708	72.4	180.1	1.9	1.5	0.2	3.6
204277	NA	1.20	929	695	98	213	34.6	63.7	1.8	2.0	0.1	4.8
265577	NA	1.50	1126	1103	131	2788	148.6	494.4	1.5	1.0	0.1	1.1
362289	NA	2.79	659	183	29	NA	14.4	267.4	1.8	2.3	0.1	9.7
380145	NA	2.36	802	287	48	NA	49.9	313.3	1.8	1.4	0.2	0.9
307367	NA	1.03	596	311	67	1781	30.0	510.2	NA	NA	NA	NA
318064	NA	1.38	1012	564	88	13232	17.5	316.3	1.7	1.2	0.1	0.2

NA = not available

Table A-10 (cont). Measurements of mercury (Hg), bile protein, fluorescence 1, 2, and 3, estrogen binding assay, EROD, GST, K, HSI, SSI, and GSI.

Site	Hg (ng/g)	Bile protein	Fluor1	Fluor2	Fluor3	ER Binding Assay (EBE)	EROD	GST	K	HSI	SSI	GSI
342523	NA	0.69	1420	1019	147	258	27.1	385.6	1.3	1.0	0.1	0.5
341665	NA	5.03	283	118	18	4180	24.5	452.3	1.6	1.0	0.1	2.6
346136	NA	4.62	1184	178	33	NA	39.2	408.4	1.8	0.8	0.1	5.2
305009	NA	0.56	1112	1059	151	1781	27.5	347.4	1.6	1.0	0.1	0.5
319073	NA	0.29	1362	1533	247	1759	31.7	488.0	1.5	0.9	0.1	0.4
39891	NA	4.36	286174	120299	19089	372	45.1	NA	1.9	1.1	0.1	0.4
93949	NA	1.35	403892	78175	16621	372	57.8	NA	1.4	1.1	0.1	0.0
103468	NA	1.67	788414	329563	40601	12747	97.3	NA	1.6	1.6	0.2	2.6
24932	NA	1.33	323904	323373	23063	522	16.7	NA	1.3	0.9	0.1	0.5
86207	NA	2.57	588738	155209	23352	715	50.2	NA	1.5	1.0	0.1	1.3
93236	NA	3.12	565712	90049	12734	1629	35.9	NA	1.7	0.8	0.1	0.6
90631	NA	0.93	1613517	465789	76559	484	NA	NA	1.7	1.2	0.2	2.6
36620	NA	1.95	617363	201054	25435	4787	287.1	NA	2.2	1.5	0.0	NA
27771	NA	4.28	327160	130608	27743	588	30.4	NA	1.7	1.1	0.1	0.5
45813	NA	2.18	884979	232852	29356	604	72.8	NA	1.7	1.3	0.1	5.2
26150	NA	1.37	559058	287253	40958	526	21.1	NA	1.5	0.8	0.1	0.1
109522	NA	5.64	540614	109530	14857	973	120.1	NA	1.8	1.2	0.1	0.5
56287	NA	5.40	539294	102182	16536	1611	201.4	NA	2.0	1.4	0.1	0.7
72426	NA	1.43	1637040	340495	52802	1038	125.7	NA	1.7	1.1	0.1	0.9
15377	NA	2.91	667868	190602	28613	944	23.6	NA	1.8	1.1	0.1	0.6
145961	NA	3.55	513365	167620	23691	725	63.6	NA	2.0	0.6	0.1	0.2
181037	NA	2.10	392093	227826	29206	1979	62.9	NA	1.6	0.7	0.1	0.2
171515	NA	1.04	1381026	521711	73094	4024	37.0	NA	1.7	0.8	0.1	3.6
157067	NA	2.40	785128	197556	26190	220	79.8	NA	1.7	0.9	0.1	0.8
145150	NA	3.38	521320	98262	30363	NA	83.7	NA	1.7	0.8	0.1	0.4
152376	NA	3.43	448807	124347	20117	531	83.4	NA	1.7	1.0	0.1	0.8
218747	NA	3.51	699137	165419	28926	407	77.6	NA	1.6	0.8	0.3	0.2
203358	NA	2.62	592652	186495	25548	8328	56.9	NA	1.9	1.6	0.1	5.9
358497	NA	1.69	513573	205818	34493	372	52.5	NA	1.7	0.8	0.2	0.8
363121	NA	2.98	750749	156425	24193	331	NA	NA	1.8	1.1	0.1	0.9
295697	NA	3.29	745676	169637	25745	6001	NA	NA	1.8	1.2	0.1	0.6
304020	NA	3.58	702164	143930	18073	249	NA	NA	1.8	0.7	0.1	0.3
311961	NA	2.80	500735	173397	28640	753	NA	NA	2.1	0.9	0.1	1.7
313573	NA	2.80	448442	123578	25393	1177	NA	NA	1.7	1.0	0.2	1.9
102026	NA	2.89	503833	154602	25761	7501	NA	NA	1.7	0.7	0.1	0.5
92979	NA	3.81	641812	159433	25797	6788	76.9	NA	1.7	0.8	0.1	0.7
101334	NA	3.25	456942	149418	23160	341	56.8	NA	NA	NA	NA	NA
136855	NA	2.81	603887	210901	26350	316	87.8	NA	1.8	1.2	0.1	1.1
111855	NA	3.07	866032	188419	33859	6242	34.5	NA	1.7	1.1	0.1	0.4
13515	NA	3.15	1024314	797371	108928	1065	54.6	NA	1.5	0.8	0.1	0.2
31263	NA	NA	NA	NA	NA	13596	22.0	NA	1.4	0.6	0.1	0.6

NA = not available

Table A-10 (cont). Measurements of mercury (Hg), bile protein, fluorescence 1, 2, and 3, estrogen binding assay, EROD, GST, K, HSI, SSI, and GSI.

Site	Hg (ng/g)	Bile protein	Fluor1	Fluor2	Fluor3	ER Binding Assay (EBE)	EROD	GST	K	HSI	SSI	GSI
222764	NA	3.40	912947	260428	37984	186	48.0	NA	1.7	1.0	0.1	0.2
225891	NA	3.98	704471	134364	23592	93	20.2	NA	1.7	1.0	0.1	0.4
232326	NA	3.51	408878	144857	23752	2325	24.3	NA	1.7	1.1	0.2	0.5
220793	NA	3.70	575205	198869	28885	215	17.4	NA	1.8	1.5	0.2	0.7
14464	NA	3.17	370575	128690	22178	4169	46.7	NA	1.7	0.7	0.0	0.3
75961	NA	2.21	864799	313452	51946	347	203.9	NA	1.6	1.0	0.1	0.2
69184	NA	2.55	913023	249800	34338	589	29.4	NA	1.7	0.7	0.2	0.2
203483	NA	1.87	659735	314939	41361	1617	113.0	NA	1.6	1.8	0.1	0.6
164521	NA	3.89	657321	181664	36009	742	25.0	NA	1.7	1.3	0.1	0.3
152737	NA	2.62	880985	349798	42718	320	13.2	NA	1.6	1.1	0.1	0.3
180206	NA	3.22	896715	196094	24897	359	9.1	NA	1.5	0.9	0.2	0.4
181437	NA	3.05	524086	241922	23607	477	47.0	NA	1.5	1.0	0.1	0.1
133573	NA	2.31	NA	NA	NA	540	24.2	NA	1.5	0.9	0.1	0.3
120740	NA	2.71	1010581	295290	53678	1290	130.0	NA	1.6	0.9	0.1	0.2
37901	NA	4.39	626284	311547	43525	855	153.8	NA	1.7	0.8	0.1	0.4
86384	NA	3.26	445977	139785	23774	1070	16.8	NA	1.7	2.4	0.1	1.4
23159	NA	1.79	661194	161871	24123	360	163.8	NA	1.9	1.3	0.1	0.7
205019	NA	2.16	653237	225874	29270	768	19.6	NA	1.8	1.1	0.2	0.7
216018	NA	4.23	693388	208356	30025	1132	34.8	NA	2.0	0.9	0.2	0.6
112131	NA	1.59	452204	263801	41413	480	16.9	NA	1.6	0.9	0.1	0.8
123998	NA	2.88	588641	152510	21925	5007	5.9	NA	1.8	1.1	0.1	NA
58433	NA	3.19	748634	164429	30071	2011	28.5	NA	2.0	1.1	0.1	0.6
84562	NA	2.12	1139783	238524	36334	669	12.3	NA	2.0	1.0	0.1	0.6
60751	NA	2.46	713661	334300	78137	13613	36.3	NA	NA	NA	NA	NA
72211	NA	2.89	905977	210240	32221	1686	28.2	NA	NA	NA	NA	NA
45921	84.4	5.67	252013	70548	11345	1374	21.6	NA	2.2	0.8	0.1	0.7
25398	89.0	5.97	351462	115674	18884	1712	54.2	NA	2.0	0.9	0.1	0.6
51137	197	5.67	464412	104162	17768	12376	16.1	NA	1.9	1.3	0.2	0.3
156577	163	2.62	320388	187776	22181	1122	107.4	NA	1.7	0.7	0.1	0.7
156581	169	2.62	564997	270832	39821	395	43.6	NA	1.7	0.5	0.1	0.5
180390	161	2.57	758519	228129	35215	487	47.6	NA	1.9	0.9	0.1	0.6
147751	196	4.74	928627	118005	18496	707	25.5	NA	1.9	0.9	0.1	0.1
208930	231	4.64	408748	136805	26004	2828	30.0	NA	2.0	0.6	0.0	0.4
262461	335	3.12	577615	226739	34193	4072	67.2	NA	2.0	1.5	0.1	0.8
301204	285	2.35	1682698	641563	74742	3247	24.2	NA	1.7	0.6	0.1	0.3
12 Mile-Low	60.3	2.33	589876	202019	31285	1488	43.2	NA	1.6	0.7	0.1	0.1
12 Mile-Above	131	1.68	756429	293384	38784	1588	37.7	NA	1.6	0.9	0.1	0.3
RHC	70.6	3.00	364246	143105	66845	1146	293.8	NA	2.1	1.9	0.2	0.4
RH-1	120	1.44	584382	472899	63293	2838	96.0	NA	1.8	1.3	0.1	0.6
LC	57.0	2.23	671290	300574	35513	1348	58.9	NA	2.0	2.0	0.1	NA
Huff	66.5	1.57	367751	197186	31794	1405	168.7	NA	1.7	1.2	0.1	0.4
Rocky	85.8	3.21	643565	230754	28187	672	17.1	NA	1.7	1.5	0.2	0.4
Bush	60.4	3.87	556610	196338	67241	1022	235.5	NA	1.9	1.4	0.1	0.7
Bald	123	3.43	552692	181347	23900	1188	67.3	NA	1.8	0.8	0.1	0.7
L-C	69.6	1.72	392602	238840	30433	1044	10.4	NA	1.8	1.2	0.2	0.4

NA = not available