Barry
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Factors Influencing the Demise of Native Unionids in the Clinch River Watershed, Virginia

Research Prospectus

By

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Table of Contents

GENERAL OVERVIEW OF PROPOSED PROJECT

Statement of Purpose

The primary focus of this research project is to determine through analysis of current and historical ecotoxicological data, the factors influencing the demise upon native unionid mussels in the Clinch River Watershed, Virginia. This goal will be accomplished by pinpointing all the major stream/tributary inputs into the Clinch River and by determining land development, water uses and influences of each input. We will target community responses of macroinvertebrates, native mussels, and Asian clams. We will also develop a rapid hazard assessment (RHA) of the Clinch River at concerned sites of selected stream/tributary confluences with the mainstem. After we have performed these rapid hazard assessments of concerned sites, we will then conduct in-depth ecotoxicological evaluations at 12-15 riverine sites by developing an Ecotoxicological Rating (ETR) of each site incorporating at least 10 parameters.

The American Electric Power Company (AEP) at Carbo, Virginia has expressed an interest in the determination of compounding impairment factors to native unionid mussel populations within the Clinch River Watershed. Therefore, we will attempt to incorporate an inclusive analysis of the entire Clinch River Watershed to determine likely causes of native unionid impairment and toxicity.

For the purposes of this study, we will incorporate field and laboratory techniques and protocols that have been previously used by Cherry et al. (1996) and others in this riverine system. In addition, we will attempt to develop new protocols with *in situ* mussel toxicity testing. We will also rely on data previously recorded to determine a baseline for which to compare recent data using Analysis of Variance (ANOVA) and other forms of comparative and descriptive statistics.

Both point and non-point pollutant sources will be evaluated to determine impairment. This will be accomplished through the use of *in situ* Asian clam (*Corbicula fluminia*) density, *in situ* Asian clam growth and survival, *in situ* mussel growth and survival, modified EPT analysis, and laboratory toxicity studies with *Ceriodaphnia dubia*, and a native mussel species. There are a variety of synergistic, additive, and antagonistic causes of impairment with multiple point/non-point source discharges that we will evaluate throughout the course of this project.

The Clinch River Section of Interest

This project will focus on the Clinch River from the headwaters located in Tazwell, Virginia downstream to the Virginia-Tennessee border. This effort will encompass approximately 611.6 river kilometers through southwestern Virginia (Goudreau et al. 1993). Within this broad range, the Clinch River is subjected to a variety of compounding factors that are contributing to the demise of native unionids. Some of these factors include but are not limited to wastewater treatment plants, agricultural runoff, agricultural runoff from livestock grazing on pasture land, and natural bank erosion. Decades of coal mining have also been sources of impairment stemming from active coal mine processing plant runoff, acid mine drainage (AMD) from some forgotten sites and hollow fills that leach AMD, recently reclaimed abandoned mine processing, active coal mining and AMD inputs from some major watersheds with no previous data, reclaimed mining sites, industrial effluents, urban effluents, nutrient enrichment from septic tank/straight pipe runoff/discharge, tributary influenced with a concoction of all of the above and some combination of two or three mixtures of the above.

Chapter One: A Literature Review of Compounding Sources of Impairment to the Tributaries of the Clinch River, Virginia.

INTRODUCTION

There are three distinct groups of fauna in the Mississippi River system of central North America that contain the most diverse freshwater mussel communities in the world. The Ohio River system contains two of these groups, (1) the general Ohio basin and (2) the Ohio River proper tributaries in the Central lowlands and the Interior Low Plateau. The third group is found only in streams of the Tennessee and Cumberland River systems flowing out of the Southern Appalachians and the Cumberland Plateau (Ortmann, 1924; Ortmann, 1925). Most of the species of this "high country," Cumberlandian Fauna still live in those few tributaries of the Cumberland and Tennessee Rivers that remain relatively unmodified. The fauna of the Clinch River above Norris Reservoir seems to be one of the best preserved of the entire system (Stansbery, 1973).

The Clinch River is a major tributary of the Tennessee River, and supports an assemblage of freshwater mussels that is among the most diverse in the world. This system supports somewhere between 45 and 60 species of freshwater mussels (Stansbery, 1973; Sheehan et al., 1989). Unfortunately for more than 80 years this river has seen development and environmental stressors that have eradicated mussels from sections of this river where the fauna was once diverse and abundant (Ortmann, 1918). This river still retains most of the species recorded by Ortmann (1918), but at least five are now believed to be extinct (Stansbery, 1973).

METHODS

➢Native unionid density/distribution
➢Asian clam *in situ* tests
➢Benthic macroinvertebrate community assessment
➢U.S. EPA RBP
➢Laboratory toxicity testing
➢Acute and chronic water column
➢Sediments
➢Physical/ chemical parameter assessment
➢Water chemistry
➢Habitat assessment

Chapter Two: *In Situ* Asian Clam Testing in Tributaries Near the Confluence of the Mainstem Influencing the Demise of Native Unionids in the Clinch River, Virginia.

METHODS

In situ test organisms, *Corbicula fluminea* [Müller], will be collected from a consistently thriving population of Asian clams inhabiting the New River, near Ripplemead, Virginia. Clam rakes will be used to collect organisms which will be immediately transported to the Virginia Tech Ecosystem Simulation Laboratory (ESL), Blacksburg, VA in water-filled coolers. Clams measuring between 9.0 mm and 12.0 mm will be selected as test organisms. For each site, five clams will be checked for mortality, measured for length to the nearest 0.01 mm using Max-Cal ® digital calipers, uniquely marked, and placed into each of five replicate cages representing the two enclosure procedures (a total of 10 total cages per site). During May 2002, clam bags will be transported in ice-filled coolers to the 12 sampling locations. The arrangements of field

sediment ?
source

cages will proceed as follows. At each site, within an area approximately 2 m in radius and comprised of uniform substrate and flow, five stakes will be driven into the river substratum. One biobox cage will be fastened to each stake and oriented so that it will be in contact with the natural substrate and could receive adequate flow. Nearby cobbles will be used to secure cages to the streambed, and care will be taken to prevent crushing of organisms during placement. After monthly survival and growth examinations, cages will be randomly returned to stakes so that no single cage will be exposed to the same microhabitat conditions for the duration of the experiment. Tests organisms will remain at sites for three months with growth and survival determination in the field at ~31-day intervals. At the end of the testing period, clams will be retrieved so that final survival and growth values can be determined. Clams will be counted as 'dead' if valves are separated or if they are easily teased apart. Mean values for percent survival and growth will be calculated for each site.

Water samples will be collected from all study sites during surveys and *in situ* testing in 1-L Nalgene® bottles. Measures of pH, conductivity, temperature, and dissolved oxygen (DO_2) will obtained in the field; alkalinity and hardness values will be determined in the laboratory. We will use an Accumet® (Fisher Scientific, Pittsburgh, PA, USA) pH meter with an Accumet gel-filled combination electrode (accuracy $< \pm$ 0.05 pH at 25°C) to measure pH in standard units. Dissolved oxygen will be measured using a Yellow Springs model 54A meter calibrated for elevation (RDP, Dayton, OH, USA). A Hach conductivity/TDS meter® (Hach, Loveland, CO, USA) will be used to measure specific conductivity. Alkalinity and hardness will be measured by titration of 50 ml samples according to the methods in APHA et al. (1998).

Three qualitative assessments of benthic macroinvertebrate communities will be conducted during June 2002, September 2002, and June 2003 using the U.S. Environmental Protection Agency's (US EPA) Rapid Bioassessment Protocols (RBP) (Barbour et al. 1999). Four dip net (800-μm mesh) samples will be collected at each site by surveying riffle, run, pool, and shoreline (root exposed) habitats. Field samples will be preserved in 70% ethanol, and organisms will be identified to the lowest practical taxon (usually genus) using standard keys (Pennak 1989; Merritt and Cummins 1996).

Chapter Three: *In Situ* Freshwater Mussel Testing in Tributaries Influencing the Demise of Native Unionids in the Clinch River, Virginia.

INTRODUCTION

Since a variety of compounding sources of impairment threaten the native Unionids of the Clinch River, it seems only logical to incorporate *in situ* testing of organisms that are the source of the concern in this riverine system. Freshwater mussels have been declining in this river for at least 100 years. This decline has been poorly documented at best, and needs an aggressive approach at a solution to this problem.

According to the Virginia Department of Game and Inland Fisheries, there are 32 species of threatened and endangered species of mussels found in this system. To this point, it has been difficult to determine whether habitat and river reach are suitable for artificial reintroduction or natural recolonization. Neves et al. have mounted a campaign to reintroduce some of these species back to their native range within this riverine system.

In situ toxicity tests with field-caged Asian clams have been employed to detect various sources of ecotoxicological impairment ranging from dilute nutrient inputs in low order streams (Soucek et al. 2001) to different sources of contamination in drinking water supplies (Belanger et al. ????check out with matt). There are several endpoints that can be considered in a modification to *in situ* mussel testing. Growth and survival (ASTM 2001) and various other sub-lethal effects may be evaluated. Since survival to many chemicals is often insensitive for many chemicals (ASTM 2001), other endpoints must be considered.

Another important consideration with *in situ* testing of freshwater mussels is age of test organism. Sheehan et al. (1989) employed the use of adult mussels ranging in size from 104-152 mm. Virtually no mortality was observed in this experiment with adult mussels suggesting the use of a more sensitive age class. We will employ the use of approximately two-month old juveniles for the purposes of our research. This decision was based on two factors; ease of organism culturing, and availability of test organisms.

The simplest alternative to *in situ* studies is testing water samples in standard toxicity and genotoxicity assays and using the results obtained to predict effects at the organism, population, or ecosystem level (Blaise 1991; Godet et al. 1993; Stewart 1996). Because concentrations of pollutants in water are usually too low for most short-term bioassays, chemicals in the samples are often pre-concentrated using different extraction techniques (Sabaliunas et al. 1998). This approach has several shortcomings. First, it provides information on only a moment in time. Second, it involves significant technical difficulties such as transporting and processing large volumes of water. Third, it often fails to discriminate between differences in compound bioavailability, which determine composition and concentrations of pollutants in the organism tissue, i.e., at the site of their action (Sabaliunas et al. 1998). Therefore, due to the vast complexities of multiple specific chemical extractions and concentrations, we have decided to employ *in situ* juvenile mussel chambers capable of exposing organisms for varying lengths of time.

Due to the vast number of stream tributary inputs associated with this river, we will only incorporate tributaries that have shown significant impairment through a previous rapid bioassessment and previous research done (i.e. Cherry et al., 1996) within this system. For the purposes of this study, we will be incorporating 15 different tributaries for *in situ* freshwater mussel testing.

SITE SELECTION

Site	Directions	Conditions
1. Copper Creek	Right off Rt 23 onto Rt 627	Very limited sedimentation and other adverse impairment at the confluence. Exceptionally large drainage area with possible anthropogenic impairment.
2. Little Stock Creek	Right off Rt 23 onto Rt 65	Limited sedimentation and impairment, but it confluences in the Clinch River (CR) just above the lowest in river site from the Cherry et al (1996) report.
3. Cove Creek	On Rt 65 between Stant and Kerns	Heavily silted from active livestock grazing pasture upstream and from the Rt 65 bridge.
4. Big Stony Creek	On Rt 65 at Junction of Rt 65/72	Benchmark reference stream that drains ~12 tributaries of the national forest. This is the epitome of stream ecological integrity in this watershed. Large Jefferson National Forest drainage area.
5. Guest River	On Rt 72 near Coeburn, the sampling site in Cherry et al (1996).	Severely impaired by coal fines, acid mine drainage (AMD), active mining, coal storage piles, etc., from as far upstream as Norton, VA and above where it springs from underground. This river has the largest flow discharge into the Clinch River.

6. Russell Creek	On Rt 58 Alternate toward Saint Paul at Virginia City.	Adversely impacted by active mining and has a holding pond at toe of hollow fill. Cherry et al. have a recent study on this creek at the confluence with the CR.
7. Big Spring	Just above Saint Paul on left side of CR as one travels upstream. Take Rt 640 upstream a few miles.	Impaired by nutrient enrichment from a number of trailer park homes lined up along this creek at Rt 640 and continue at Junction of Rt 615 and further thereafter. We need to enumerate the total number of trailer homes in this stretch of the creek.
8. Dumps Creek	Take Rt 640 after Big Spring to Rt 614 to Carterton, over CR and onward to Clinch River Plant (CRP).	Impaired by active mining with conductivity of ~1600 μmhos/cm. Asian clams abound but no unionids. Hull et al. has benthic macroinvertebrate assemblage data.
9. Big Cedar Creek	Take Main St. east of Lebanon to outer edge of town.	Unknown about stream condition except occasional pasture/livestock use in the past. Where is the WWTP there? Need to recon further about county road accessibility and creek confluence with CR.
10. Little River	Rt 19E and left on Rt 80 N at Rosedale; go a few miles and rite on Rt 640 for 1-2 miles.	Little River is probably unimpaired and has some agricultural/grazing land along the river, but mussels are there.
11. Swords Creek	Rt 80 N toward Honeker and rite at Rt 80/67 intersection onto Rt 67 for a few miles.	Vastly impaired by mining activity and numerous small hamlets along the way to its headwaters. Also, how do 14 stormwater portal runoffs from the local masonry plant influence CR during a rapid rain event?
12. Coal Creek	Junction at Rt 67/460 at Raven	May be most vastly impaired stream from coal mining (active or abandoned), straight-pipe septic systems, and more. This is a severely impaired stream and confluence with CR must be ascertained.
13. Big Creek	In center of Richlands where Rt 67/460 separate and Rt 67 goes to rite. We did not sample far enough up Rt 67 to find it and sampled in CR by mistake.	Unknown status but we need to find this creek and also evaluate non-point runoff sources from Richlands into the creek and CR. Also, where is the WWTP around Richlands?
14. Middle Creek	Onto Rt 460 E Business and first exit at Cedar Bluff off Rt 460 E, go ~1 mile and rite onto private drive to CR.	Impaired by some hollow fill seeps and recovering from an abandoned coal-processing site that is being removed. No active mining in this creek. Merricks et al. have data on this stream.
15. Indian Creek	Continue furthur into town a few hundred feet.	Perhaps impaired by much active coal mining in upper reaches of watershed. It covers a large area of landscape. This

		creek supports rare and or endangered mussels. We need to pursue an upstream recon effort here.
16 North Fork CR	Continue on Rt 19/460 E to North Tazewell, take rite on Rt 61E and go over North Fork of CR.	Seems to have enrichment as it heads upstream paralleling that section of town. We need to document point/nonpoint entries here.
17. South Fork CR	Continue further on Rt 61 E less than 1 mile and South Fork joins CR at two sections, one of which has active highway construction in it. As you turn around on Rt 61 W and backtrack, you can see the initial mainstem of the CR just before the Rt 19/460 overpass.	Siltation from active highway construction but there are two forks here. We need to document point/nonpoint entries to decide if the upper reach of CR starts out pristine, polluted, or somewhere in between.
18. Cavitts Creek	Before Rt 61 to N. Tazewell, there is Rt 16. We reviewed it during the previous trip and it should be sampled now that we know where the mainstem of the CR starts along Rt 61 a few miles east of here.	Unknown about potential impairment, but there were some unusual dams and backwater areas along the stream that deserves another look. Hopefully, we may be able to eliminate this sampling area but not until we take some preliminary data.

METHODS

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- Asian clam *in situ* tests
- Benthic macroinvertebrate community assessment
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- Laboratory toxicity testing
- Acute and chronic water column
- Sediments
- Physical/ chemical parameter assessment
- Water chemistry
- Habitat assessment

Chapter Four: Development of a Modified Ecotoxicological Rating System for Tributary Sources of Unionid Impairment in the Clinch River, Virginia.

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- ➢Laboratory toxicity testing
    - ➢Acute and chronic water column
    - ➢Sediments
- ➢*In situ* toxicity testing
    - ➢Metals analysis of test organisms
- ➢Incorporation of EPT
- ➢Habitat assessment
    - ➢Assessment of localized non-point source contaminant potential
- ➢Rain event sampling
    - ➢Toxicity testing
    - ➢Metals analysis
- ➢Physical/ chemical parameter assessment
    - ➢Water column metals analysis
    - ➢Habitat variability

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Initial Research Prospectus

Factors Influencing the Demise of Native Unionids in the Clinch River Watershed, Virginia

By:

Patrick M. Barry
April 21, 2002

Research Goals:

1. Pinpoint all the major stream/tributary inputs into the Clinch River and determine land development, water uses and influences of each input

2. Rapid hazard assessment (RHA) of Clinch River at concerned sites of selected stream/tributary confluences

3. Conduct in-depth ecotoxicological evaluations at 15-20 riverine sites by developing an Ecotoxicological Rating (ETR) of each site

Research Hypotheses and Objectives:

Chapter One: A Literature Review of Compounding Sources of Impairment to the Tributaries of the Clinch River, Virginia.

Hypothesis: The decline in native Unionids within the Clinch River Watershed has occurred due to compounding factors from a variety of stream/tributary, land development, and water use influences.

- Compile literature/report database for as many sites as available from AEP report literature (e.g., Cherry et al. 1996)

- Compile a listing of all substantial streams influencing the river mainstem

- Classify stream types by agriculture, active mining outfalls, abandoned mine lands, municipal townships, rural undeveloped landscape, wastewater treatment plant outfalls and other industrial influences (power plants, rock quarry, building materials, etc)

Chapter Two: *In Situ* Asian Clam Testing in Tributaries Influencing the Demise of Native Unionids in the Clinch River, Virginia.

Research Hypotheses and Objectives:

Asian clams have proven themselves worthy *in situ* test organisms. The determination of growth and survival will give us insight on degree of impairment from tributary sources.

- Determine growth and survival of test organisms over at least a four month span

Methods

- Asian clam *in situ* tests
  - Metals analysis of test organisms
- Benthic community assessments
- Laboratory toxicity testing
  - Acute and chronic water column
  - Sediments
- Physical/ chemical parameter assessment
  - Water column metals analysis
  - Habitat assessment

Chapter Three: *In Situ* Freshwater Mussel Testing in Tributaries Influencing the Demise of Native Unionids in the Clinch River, Virginia.

Research Hypotheses and Objectives:

A variety of sources of impairment have different effects on different organisms. We are proposing use of an endemic species to determine toxicity.

Find a more sensitive test species

- Collect ecotoxicological data at all field sites
- Determine if aquatic biota are significantly impaired

Methods

- Freshwater mussel *in situ* tests
  - Metals analysis of test organisms
- Benthic community assessments
- Laboratory toxicity testing
  - Acute and chronic water column
  - Sediments
- Physical/ chemical parameter assessment
  - Water column metals analysis
  - Habitat assessment

Chapter Four: Development of a Modified Ecotoxicological Rating System for Tributary Sources of Unionid Impairment in the Clinch River, Virginia.

Hypothesis: The development of a modified ecotoxicological rating (ETR) will increase understanding of impairment and designate areas of greatest concern for remediation efforts.

- Parameters comprising the ETR (to be determined) may include 10 parameters: invertebrate taxon richness; % Ephemeroptera abundance; *in situ* Corbicula density; *in situ* clam growth in cages; habitat assessment score; acute *Ceriodaphnia dubia* survival in sediment pore water; Daphnia magna survival in chronic sediment tests; *in situ* juvenile mussel survival in cages held in sediment; and sediment pore water concentrations of iron and aluminum

- Review ETR parameters used by Cherry et al (2001), Soucek et al. (2000), Schmidt et al. (2002), and Cherry et al. (in review)

- Develop an ETR for each riverine site based upon 100 maximum points with 90-100 as excellent, 80-89 as acceptable, 70-79 as marginal, 60-69 as stressed, and <60 as severely stressed

- Conduct a priority pollutant scan of sediment pore water to determine if some other types of inorganic or organic constituents are elevated and compare concentrations to New York State and Canadian sediment quality guidelines

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