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Mussel Survivorship, Growth Rate and Shell Decay Rate in the New River Basin of Tennessee: an Experimental Approach Using Corbicula fluminea

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To the Graduate Council:

I am submitting herewith a thesis written by Grant Andrew Mincy entitled "Mussel Survivorship, Growth Rate and Shell Decay Rate in the New River Basin of Tennessee: an Experimental Approach Using Corbicula fluminea." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Geology.

Michael L. McKinney, Major Professor

We have read this thesis and recommend its acceptance:

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Accepted for the Council:
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(Original signatures are on file with official student records.)

Mussel Survivorship, Growth Rate and Shell Decay Rate in the New River Basin of Tennessee: an Experimental Approach Using *Corbicula fluminea*

A Thesis Presented for the
Master of Science
Degree
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Grant Andrew Mincy
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ABSTRACT

The New River Basin (NRB) of Tennessee is home to a number of rare endemic aquatic communities. One such community of particular importance to the area, experiencing a precipitous population decline due to the fouling and pollution of their freshwater systems, is that of freshwater mussels (Bogan 2006). This study in the NRB involves measuring the mortality rates of live Asian clams (*Corbicula fluminea*) assemblages and the shell decay rates of their death assemblages. This study also examines the decay rates of the native *Villosa iris* to gather information on molluscan health and the ability of their shells to be incorporated into the freshwater record, or to be used by other organisms for ecological engineering. A common property of aquatic systems influenced by anthropogenic activity is increased conductivity (a proxy correlated to the ability of water to pass an electric current because of increased metal and dissolved solids concentrations) which studies show may impair clam health and enhance shell decay rates. Our study of five impacted streams within the NRB and a control stream of similar geology tests this correlation. Silos containing live *Corbicula fluminea* were placed in several localities in streams of the NRB that receive mining drainage as mine drainage is commonly associated with elevated conductivity. To measure the effects of this anthropogenic activity on shell decay rates, mesh bags containing shells of *Corbicula fluminea* and *Villosa iris* were placed in several localities in creeks of the NRB that receive varying degrees of mine drainage. The weight of these shells were periodically measured, over the course of 120 days, to determine the rate of decay. I found that growth and mortality rates of the life assemblages are correlated with the conductivity and water temperature levels that the living clams are exposed to, and that shell decay rates did not correlate with conductivity but were influenced by calcium levels and water velocity.

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CHAPTER I
INTRODUCTION

Margaritiferidae and Unionidae are families of freshwater mussels that have a world wide distribution, but reach their greatest diversity in North America. The North American fauna consists of 297 recognized taxa with 281 species and 16 subspecies (Williams et al. 1992). Adult specimens can range in size from 40 to 300 mm and occupy a wide range of aquatic habitats. The most common habitats for freshwater mussels are lotic ecosystems (Williams et al.1992). Mussels are filter feeders and most species primarily consume plankton. Mussels themselves serve as a source of nutrient for vertebrate predators (Williams et al. 1992). Freshwater bivalves are among the largest families of aquatic invertebrates in the United States and make up a major portion of invertebrate biomass in lakes rivers and streams (Bogan 2006).

As freshwater mollusks are one of the most diverse specie assemblages of North America, they are also one of the most endangered (Lydeard et al. 2004), and are declining worldwide in number of species per area and abundance (Vaughn et al. 2004). Recent studies suggest that 44% of all known freshwater molluscs are threatened with extinction (IUCN 2008). Numbers of individuals and species diversity of native mussels have been on the decline throughout North America as well. Freshwater mussels, and aquatic species in general, are imperiled at a higher rate than terrestrial species (Rooney 2010). Conserving mollusk diversity, therefore, is of great importance and a challenge for aquatic diversity management (Rooney 2010). The main threat to mussel populations is anthropogenic activities (Lyons et al. 2007) such as habitat destruction and degradation (Williams et al. 1992). The decline is precipitous and went unrecognized until the past few decades (Williams et al. 1992).

Habitat destruction for the bivalve species comes from a number of different sources. Erosion from deforestation, poor agricultural practices and riparian zone degradation has led to an increase in silt loading of streams. The modification of, or shifting of unstable stream bottoms (Williams et al. 1992), sewage effluent, sedimentation from a number of factors, sand and gravel dredging and other activities all contribute to the destruction of habitat (Bogan 2006). Siltation with contamination from heavy metals, pesticides, acid/alkaline mine drainage, among others, are all threats to native mussel populations. These activities can directly affect the mussels themselves, or indirectly by harming obligate host fishes due to changes in water quality or habitat loss (Bogan 2006). A unique problem to the Southeast, acid and alkaline mine drainage from coal surface mining, and various point and non-point pollution sources, continues to contribute to the decline of local populations (Bogan 2006). Mussels have very limited mobility which makes them highly susceptible to contaminants and pollution in their water column (Rooney 2010). The only line of defense the molluscs have to pollutants is the closure of their valves. However, closure is difficult to maintain for extended periods of time due to the need for oxygen, food and waste excrement (Rooney 2010).

Mussels pump large volumes of water, while feeding and respiring, so when pollution enters their ecosystem the organisms will bio-concentrate metals and organic contaminates in their tissues. For this reason, mussels are used as a biological indicator of pollution (Viarengo and Canesi 1991). As mussels filter feed pollutants, these introduced "stessors" can alter a mussels physiology (Viarengo and Canesi 1991). Studies concerning how mussels respond to the stressors have focused on growth. Growth provides a sensitive measure of stress to an organism as growth represents an integration of major physiological responses, specifically between feeding and digestion (energy acquisition) to energy expenditure (metabolism and excretion). Studies have shown (Widdows et al. 1996;Williams et al. 1992) that growth rate can range from a maximum positive value in pristine/optimal conditions and decline to even negative values when the mollusc is severely stressed and utilizing body reserves. Experimental studies have also shown that a reduction in growth rate of the organism results in reduced fecundity, declines in gamete variability and overall reduction of larval growth and development (Widdows et al. 1996). All of these factors have negative impacts on the growth and maintenance of mussel populations.

As destruction of freshwater mussel habitat continues, expansion of the distributions and populations of invasive mollusc species has occurred. The Asian clam *Corbicula fluminea*, and the zebra mussel *Dreissena polymorpha*, are both occupying ecosystems where native populations have become extinct and competing where the invasive and native species share habitat (Williams et al. 1992). The Asian clam is the most widespread non-native bivalve mollusk in North America and was first discovered on the west coast in the 1930s. The Asian clam lives in dense populations (up to thousands per square meter) and this dense population has been attributed to native unionid decline in some cases. Harm to native populations from the Asian clam varies as they can also coexist together (Williams et al. 1992).

Like native unionids, *Corbicula* burrows into the stream bottom and is a filter feeding organism, however, *Corbicula* differs from the native populations in a number of significant ways (Vaughn and Hakenkamp 2001). The Asian clam is less sedentary, has a shorter life span (one to five years) grows very rapidly reaching maturity faster, reproduces two to three times a year and disperses both actively and passively throughout its life style. *Corbicula* frequently occur in dense aggregations that are either solely *Corbicula,* or mixed with other unionids (Vaughn and Hakenkamp 2001). The Asiatic is typically smaller than other freshwater molluscs yet has an incredible reproductive rate which allows for the higher abundance of the clam and dominance in lotic systems. The abundance of the Asian clam allows for a high community infiltration rate that exceeds native bivalve assemblages (Vaughn and Hakenkamp 2001).

The expansion of *Corbicula* across North America has impacted native bivalve abundance and diversity. The Asian clam may potentially affect natives in several ways. With such high densities, burrowing activity of the *Corbicula* may uproot native assemblages in sandy sediments (Vaughn and Hakenkamp 2001). Strayer (1999) noted that *Corbicula* compete for benthic food resources with other freshwater clams such as sphaeriids and juvenile unionids. Strayer (1999) also noted that bioturbation from the Asian clam could reduce available habitat for sphaeriids. *Corbicula* have greater filtration rates on a biomass basis than native assemblages as well. This gives the invasive potential to limit the availability of nutrients to native species. Furthermore, *Corbicula* has the ability to deposit feed small organic particles, so it has a broader diet when there is little food available in the water column (Vaughn and Hakenkamp 2001). In rivers where *Corbicula* have become established, the clams biomass may replace or compensate for lost native unionid biomass. Native populations and *Corbicula* share many functional roles because *Corbicula* functions in a manner similar to unionids (Vaughn and Hakenkamp 2001). The decline, therefore, in freshwater molluscan diversity may have little impact on the functional role of molluscs in these lotic systems (Vaughn and Hakenkamp 2001).

A key function that freshwater mollusks play in their ecosystem is the filtering of phytoplankton, bacteria and particulate organic matter from the water column. *Corbicula* and sphaeriids both remove organic matter from sediment by deposit feeding as well (Vaughn and Hakenkamp 2001). Water filtration rates vary with bivalve species, size, abundance and concentration of food particles. Bivalves also affect nutrient dynamics in lotic systems through excretion and bio-deposition of faeces. Excretion rates are size and species dependent and vary greatly with temperature and food availability (Vaughn and Hakenkamp 2001). Furthermore, burrowing into the sediment increases sediment and water oxygen content and releases nutrients from the sediment into the water column. Filter feeding and bio-deposition allow bivalves to naturally improve water quality which is one of the reasons they are used as a bio-indicator (Vaughn and Hakenkamp 2001).

Bivalves are not only particularly important to aquatic systems because they graze phytoplankton and help regulate water quality, but they also serve as ecological engineers by creating large amounts of shell (Strayer and Malcom 2007). Shell material can persist for a long time after the mollusc dies and can provide habitat for other organisms. The importance of shell production is widely discussed in scientific literature, but accumulation of shell material in the ecosystem also depends on the rates of shell dissolution or decay (Strayer and Malcom 2007). Dissolution is rarely studied by freshwater ecologists, as shell dissolution rates are highly variable in freshwater systems. Differences in shell dissolution can depend on a number of parameters including shell surface area, dissolved solids, temperature and stream flow (Strayer and Malcom 2007).

In our study, we are concerned with mussel habitat in the New River Basin (NRB) of Tennessee. The greatest number of freshwater mussels species on the continent is centralized in the Southeast, particularly, between the NRB of Tennessee and the Warrior Basin of Alabama (Bogan 2006). The southeast is home to over 100 species of mussels (Bogan 2006), including several endemics within the NRB, five of which (*Alasmidonta atropurpurea, Epioblasma brevidens, E. florentina walkeri, Pegias fabula*, and *Villosa trabalis)* are federally endangered species (Peeples 2006). Mussels are experiencing a population decline throughout the region because of fouling and pollution of freshwater systems (Bogan 2006).

Of importance to this study are the effects of anthropogenic land use to freshwater mussel mortality, growth rate and shell decay rate. There are a number of industrial activities occurring in the NRB including gas exploration, logging, road building and coal surface mining. The largest agent of land use change in the NRB is coal surface mining. A noted environmental implication of mining is the disturbance of freshwater resources and long-term detrimental effects to watersheds (Pile 1984). Coal production in Tennessee is centralized to the NRB of the Cumberland Mountains and the southern Cumberland Plateau. Surface mining can impair freshwater resources and aquatic communities (Ferrari 2009). Streams throughout the Appalachian coalfields are being chemically monitored and unusual pH measurements (acid and alkaline mine drainage) and dissolved metals are found in streams and groundwater (Blackburn 1996). Increases in conductivity, hardness and concentrations of arsenic, sulfate, mercury, lead and selenium occur downstream from surface operations (Soucek et al. 2003). Rare mussel populations are particularly declining due to land use change in the region (Soucek et al. 2003; Bernhardt and Palmer 2011). Furthermore, aquatic habitat is also impacted by unsuccessful reclamation practices. Though trees and shrubs are being planted on reclamation sites, habitats are being affected by mass erosional events such as landslides at reclaimed sites resulting from the absence of mature, second or third growth forests (Bullock 2007).

To regulate the effects of mining to freshwater resources and habitats, the United States Environmental Protection Agency (EPA) released a new water quality guidance program in 2010. Under the new EPA program, enforced by the Clean Water Act, coal mining companies will be required to keep conductivity at a safe level (EPA 2010). The new program defines conductivity as the ability of water to pass an electric current, which is affected by the presence of pollutants such as heavy metals (EPA 2010). The program states that the higher the conductivity, the higher the pollutants in a given stream.

The EPA's new guidance program uses conductivity as this proxy for contamination. Conductivity is easy to test and easy to interpret. The EPA also suggests that conductivity does effectively gauge pollution from mine sites (EPA 2010). For Southern Appalachian coalfields the EPA regulates water conductivity as follows: streams with conductivities between 0 – 300 µS/cm (microsiemens per centimeter) are considered healthy, 300 – 500 µS/cm are considered moderately polluted and detrimental to aquatic organisms, and 500 + µS/cm indicates high water pollution and ecological impairment (EPA 2010).

My study in the NRB utilized field and laboratory equipment to test the effectiveness of using conductivity and shell decay rates as a proxy of ecological health of streams exposed to anthropogenic land use. My hypothesis (Table1-1) was that increased conductivity would lead to increased mortality, slowed growth and higher rates of dissolution. Conductivity and shell dissolution both are proxies for poor water quality therefore they should correlate. Shell growth and clam survivorship both indicate good conditions so they should correlate. Conductivity should negatively correlate with growth and survivorship. Shell dissolution should also negatively correlate with growth and survivorship. To gather this information I tested the conductivities of study streams and gauged the health of live field-caged *Corbicula fluminea* and the dissolution of their shells coupled with *Villosa iris.*

The major objective of this research was to investigate the proposed correlation between mussel survivorship, growth rates and shell dissolution with conductivity to help assist the sustainability of the unique mussel populations of the NRB. This approach required field measurements of conductivity of the waterways throughout the basin coupled with the measurements of growth/survivorship of living clams and the dissolution of their death assemblages. Most importantly, I studied how anthropogenic land use is affecting habitat for freshwater mussels. I assessed the conductivity of water and shell dissolution as proxies for mussel habitat within the NRB.

Table 1-1. The proposed hypothesis. High growth rates should not correlate with high mortality. High conductivity should correlate with high mortality. High conductivity should correlate with low growth rates. Dissolution rates will be high in streams with high mortality. Dissolution rates will be high in streams with low growth rates. Enhanced dissolution rates will be found in field localities with high conductivity.

Hypothesis	**Mortality**	**Growth**	**Conductivity**	**Dissolution**
Mortality	X	X	X	X
Growth	Fail to reject	X	X	X
Conductivity	Reject	Reject	X	X
Dissolution	Reject	Reject	Fail to reject	X

CHAPTER II
REGIONAL SETTING

New River Basin: Geologic Setting

The study area is part of the New River Basin of Tennessee (NRB), a 615 square kilometer watershed across Anderson, Campbell, Morgan and Scott counties in the Cumberland Mountains of East Tennessee. The NRB has moderate temperatures and high annual precipitation (134 cm), typical of the humid Appalachian region (Pile 1984). The highest mountains of the Cumberland Plateau are located within the upper reaches of the NRB. The NRB contains rugged terrain with elevations ranging from 332 to over 914 meters above sea level (Pile 1984). The topography has an average slope of 13%. Elevation change can be very pronounced across the watershed as slopes can reach up to 50% along the mountainsides. Because of the humid climate and moderate temperatures across the NRB, the watershed is naturally heavily forested (Pile 1984).

The NRB (Figure 2-1) is a physio-graphic sub-province of the Cumberland Plateau in East Tennessee. The study area also lies within the Wartburg Basin (Pile 1984). The rocks within the study area represent a transition from lower Pennsylvanian to upper Pennsylvanian delta plain depositional environments. The Cumberland Plateau is a long narrow arch between the coal basins of the central Appalachians and the Black Warrior Basin in Alabama (Blackburn 1996).

The New River hydraulic system drains the Northern Cumberland Plateau and is contained within the Wartburg Basin (Minear and Tshantz 1976). Rock exposed within the NRB is of Pennsylvanian age consisting of conglomerates, calcareous sandstone, limestone, siltstone, shale, and trace amounts of coal (Thompson 1977). The middle Pennsylvanian section is approximately 1,219 meters thick. The lower unit of this section is dominated by sandstone and lesser amounts of coal (Pile 1984). The upper part of the section consists of increased coal with thick shale layers relative to sandstone (Pile 1984). Of the multiple coal seams present throughout the exposed section, there are two of primary interest to the coal industry: the Big Mary and the Pewee seams (Minear and Tshantz 1976). The Big Mary is located at approximately 686 meters in elevation and the Pewee at about 792 meters in elevation (Minear and Tshantz 1976). Infrequently, seams higher and lower than these two main sources are mined throughout the NRB, as well (Minear and Tshantz 1976).

In general, the Southern Cumberland Plateau can be characterized as a broad asymmetrical syncline. The axis of this syncline is parallel to the western side of the Sequatchie Valley (Blackburn 1996). Throughout the western portion of the escarpment of the Sequatchie Valley, the rocks dip to the northwest, they then flatten out and begin to rise to the northwest as they respond to the syncline (Blackburn 1996). The region is capped by Pennsylvanian age sandstone (Pile 1984).

The NRB is the principal watershed for the Cumberland River and Tennessee River drainage basins. The Tennessee Valley Divide crosses the NRB in a northeast to southwest direction (Blackburn 1996). Streams south of the divide flow to the southeast into Big Brush Creek. Big Brush Creek is a tributary of the Sequatchie River, which itself, is a tributary to the Tennessee River. Streams to the north of the divide flow in a northerly direction (Blackburn 1996). These streams flow into Rocky River which is a tributary of Caney Fork which is a tributary to the Cumberland River (Blackburn 1996).Average annual precipitation throughout the NRB is approximately 134 cm (Blackburn 1996). This can change from 88.9 cm in dry years to more than 177.8 cm in wetter years. Climate of the study area is described as humid continental as it lacks a definitive “wet” and “dry” season (Blackburn 1996).

New River Basin: Status of Freshwater Mussels

Records of historical mussel fauna from the NRB date back to the early 1920's. The University of Michigan Museum of Zoology at Ann Arbor has records of freshwater mussels (Table 2) from the New River collected in 1924 (collected by A.E. Ortmann) and 1939 (collected by C.S. Shoup). Five different mussel species are held in the museums records (Ahlstedt et al. 2008).

In 1940, Shoup and Peyton reported eight mussel species from the New River during sampling in 1938 and 1939. They documented seven species from New River Station in Scott County (*Elliptio dilatata, Lampsilis cardium, Lasmigona costata, Ligumia recta, Quadrula pustulosa, Tritogonia verrucosa,* and *Villosa taeniata)* and an eroded individual (*Actinonias ligamentia)* from Cordell in Scott County (Ahlstedt et al. 2008). The researchers did not give an account of the abundance of the mussels they found.

Lokey (1979) studied benthic macro-invertebrate populations in the New River drainage. This study sampled eight New River sites and 10 tributary streams: Indian Fork Creek, Cage Creek, Ligias Fork, Beech Fork Creek, Smokey Creek, Montgomery Fork, upper and lower Buffalo Creek, Straight Fork, Paint Rock Creek, and upper and lower Brimstone Creek (Ahlstedt et al. 2008). In five of the New River sites, Lokey (1979) found native mussels, Asian clams and pond and pleurocerid river snails. Native freshwater mussels were found at two sites: *Elliptio dilatata* was found in the upper New River upstream from Cage Creek and *Lasmigona costata* (listed as a fragment) was located downstream from Montgomery Fork. *Elliptio dilatata* was not indicated as live, dead or relict. Of the 10 tributary streams sampled by Lokey, fingernail clams and snails occurred in only Cage Creek, upper Buffalo Creek and Paint Rock Creek (Ahlstedt et al. 2008).

In September of 2008, the most recent report of the status of freshwater mussels in the NRB was published for the U.S. Department of the Interior and the Office of Surface Mining Reclamation and Enforcement. This report sampled the New River, Buffalo Creek, Brimstone Creek and Smoky Creek. For the 2008 study, a total of 78 sites were sampled for freshwater mussels (Figure 2-2). In the headwaters of the New River six species were found: *Lampsilis cardium, Lampsilis fasciola, Lasmigona costata, Elliptio dilatata,* and *Quadrula pustulosa.* The *E. dilatata,* and *Q. pustulosa* species were found as relics and the latter is of questionable identification because of shell erosion.

The largest concentration of mussels found in the New River exist downstream of the confluence of Bull Creek (Site 39) where 35 live individuals of three different species were found (Ahlstedt et al. 2008). At this location female *Lampsilis fasciola* were observed spawning and attracting host fish with their artificial fish-like lure. This location is also home to one live federally protected freshwater mussel, the Cumberland elk toe (*Alasmidonta atropurpurea*) and is one of only two locations in the New River where it was found other than the tributary Buffalo Creek (Ahlstedt et al 2008).

Some of the best mussel habitat observed in the NRB exists upstream from the confluence of Buffalo Creek (Sites 42 and 35). Site 42 contained nine live mussels of two species, *Lampsilis cardium* and *L. fasciola* and site 35 was home to one live *L. cardium.* The report states that sites 42 and 35 should have had greater diversity and number of mussels, but acid mine drainage originating in sources from Straight Fork draining into lower Buffalo Creek present a water quality barrier in the New River for upstream mussel colonization via host fish (Ahlstedt et al. 2008).

In Buffalo Creek, seven live mussels and one relic were reported and one live mussel at the confluence of Smith Creek was also noted. At Site 51 in Buffalo Creek, six species, including five live individuals of *Alasmidonta atropurpurea* were reported. The rare species *Anodontoides denigrada* was reported live from three sites in Buffalo Creek (46 – 48). *Anodontoides denigrada* is an endemic species to the Cumberland River system (Ahlstedt et al. 2008). Prior to this report the mussel had never been reported in Tennessee or the Big South Fork Cumberland River (Ahlstedt et al. 2008). All mussels found in Buffalo Creek only exist upstream from the confluence of Straight Fork. Straight Fork was reported as biologically dead from acid mine wastes which are affecting lower Buffalo Creek as well (Ahlstedt et al. 2008).

The upper New River may never have had a great diversity of mussel fauna prior to anthropogenic activity based upon naturally occurring environmental conditions (Ahlstedt et al. 2008). The lower reach of the New River, the largest portion, has access to the Big South Fork where 27 mussel species, as of 2008, are extant. Historically, this portion of the river contained up to 55 species (Ahlstedt et al. 2008). Mussel fauna within the NRB is limited and is comprised of relatively older individuals that exist in isolated pockets separated by long deep pools, gradient change or coal effluent. Mussel reproduction in the NRB is limited from their small population size. Their reproductive life cycle may also be continually disrupted by poor water quality conditions, habitat destruction or loss of host fish during spawning periods (Ahlstedt et al. 2008).

Unlike the invasive *Corbicula*, native unionoid bivalves have an obligate parasitic life stage on the gills or fins of host fish in their ecosystems (Bogan 2006). These freshwater mussels release a cloud, known as glochidia, as host fishes swim by. This method of dispersal involves such remarkable strategies as mimicry, where the bivalve develops mantle extensions in the shape of a small fish and are, in fact, pigmented to look like fish, including distinct eye spots and dorsal and caudal fins completed with characteristic movements (Bogan 2006). These mantle extensions lure in host fish and then the glochidia cloud is released, attaching the mussel parasite to the obligate host fish (Bogan 2006). For this reason, the status of fish fauna in freshwater ecosystems are directly linked to mussel fecundity.

The fish fauna is exhibiting a slow recovery in the NRB despite an increase in anthropogenic activity, notably mining, in the past few years and is beginning to colonize previously degraded aquatic habitat (Ahlstedt et al. 2008). The life cycle of freshwater mussels and their host fish is complex and complicated by natural factors that exist to the NRB, even before anthropogenic activity. These conditions still persist but were exacerbated by unregulated mining activity and logging within in the watershed (Ahlstedt et al. 2008). The extent of the affected area pre-law, with ongoing regulated resource extraction, may take many years of major reclamation projects to restore and stabilize the drainage system. Water flowing from the New River into the Big South Fork is critical for maintaining rare, endemic species and for providing clean drinking water for wildlife and human consumption (Ahlstedt et al. 2008). Mussels are indicators of water quality and only survive in clean water with the availability of their host fish. With water quality degradation to the NRB and habitat destruction of their host fish from coal surface mining in the area, the native mussel populations, the most endangered faunal group in North America, are declining in the watershed (Ahlstedt et al. 2008).

Table 2 – 1. The University of Michigan Museum of Zoology at Ann Arbor record of five different freshwater mussel species from the New River, Tennessee collected in 1924 by A.E. Ortmann and 1939 collected by C.S. Shoup.

Species	Museum Catalog Ascension Numbers and Locations
Alasmidonta atropurpurea	(#62174): New River at New River Station, Ortmann, 1924.
Elliptio dilatata	(#134856): New River at New River Station, C.S. Shoup, 1939.
Elliptio dilatata	(#134861): New River at Cordell, C.S. Shoup, 1939.
Lampsilis cardium	(#134859): New River at New River Station, C.S. Shoup, 1939.
Lasmigona costata	(#134853): New River at New River Station, C.S. Shoup, 1939.
Ligumia recta	(#133128): New River at New River Station, C.S. Shoup, 1939.
Quadrula pustulosa	(#133129): New River, Scott County, C.S. Shoup, 1939.
Quadrula pustulosa	(#134858): New River at New River Station, C.S. Shoup, 1939.
Tritogonia verrucosa	(#134854): New River at New River Station, C.S. Shoup, 1939.

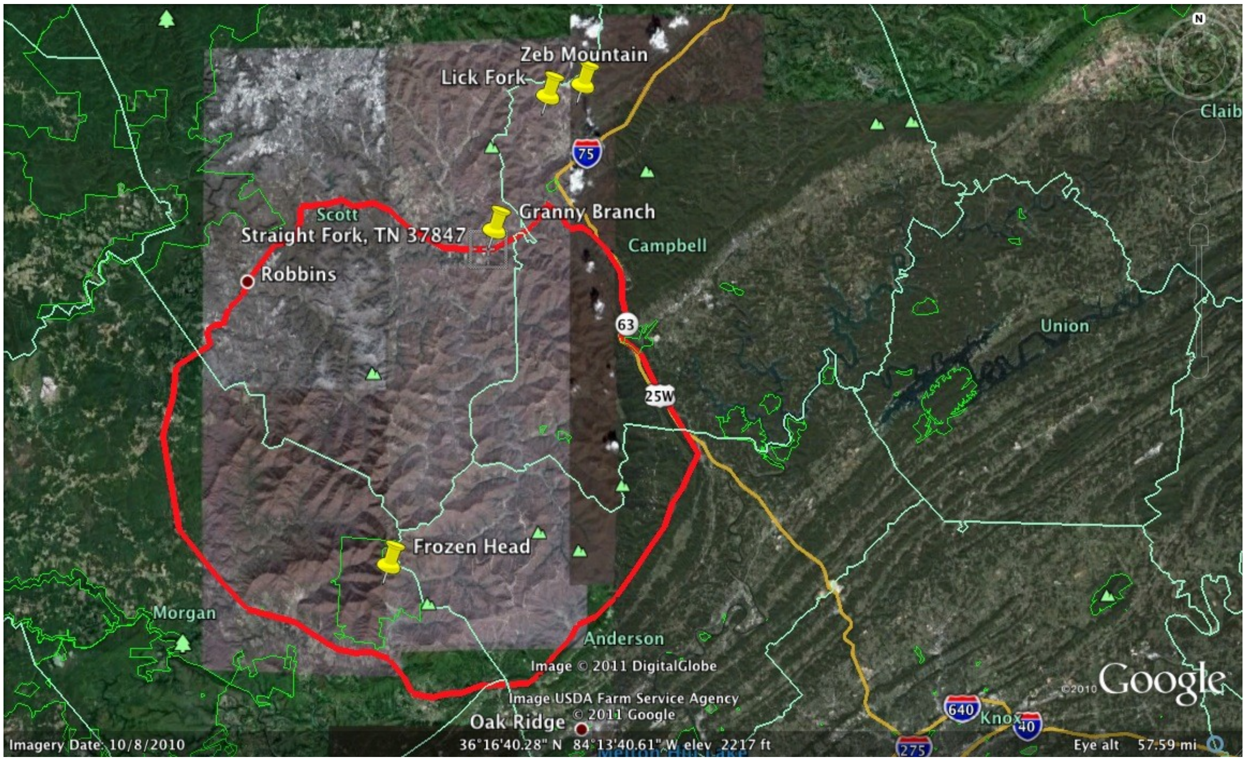


FIGURE 2-1. The New River Basin of Tennessee is outlined in red. Ligias Fork, sites I and II, and Charleys Branch are located near Frozen Head State and Natural Park in the southern end of the watershed. Buffalo Creek I and II and Straight Fork are located near Straight Fork TN in the northern section of the watershed.

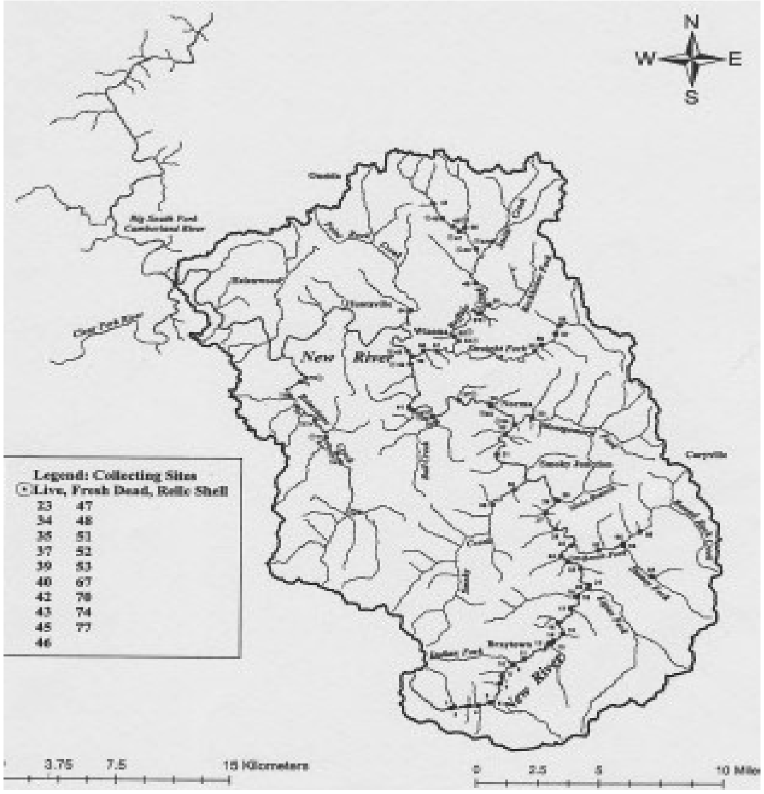


FIGURE 2 – 2. Freshwater mussel collecting sites in the New River, Big South Fork Cumberland Drainage, Tennessee, for the status of freshwater mussels report for the U.S. Department of the Interior and and the Office of Surface Mining Reclamation and Enforcement.

CHAPTER III
METHODS

Methods Overview

Silos containing live *Corbicula fluminea* were placed in several localities in streams of the New River Basin (NRB) and a control stream outside of the basin. Each stream represents unique aquatic habitat and varying levels of ecological stressors (Table 3-1). The streams were also chosen because they either currently contain, or historically provided, habitat for the invasive clam. The effects of these stressors were measured with field and laboratory equipment and correlated with clam growth rates and mortality. To measure the effects of these individual stressors on shell decay rates, mesh bags containing shells of *Corbicula fluminea* and *Villosa iris* were placed in several localities in streams of the NRB. Decay was measured by physical abrasion, weight loss and half life calculations.

Testing Growth and Mortality Rates of the Asian Clam.

Live samples of *Corbicula fluminea,* taken from the abundant population of the Ocoee river below dam site 1, were placed in a cooler partially filled with river water and ice and aerated as they were transported back to the lab. Once at the lab, the clams were placed in an aerated holding tank that was lined with mud and rounded pebbles to simulate their habitat. Each Asian clam was then measured to the nearest 0.01 mm. Measurements were taken from the anterior to the posterior, the dorsal to the ventral and point of greatest thickness of each shell with digital calipers to gauge the length, height and width of each sample. After measurement, the clams were specifically numbered on their eroded area (Figure 3-1) with a permanent marker for later identification.

In situ mortality and growth analysis followed the methods described by Hull et al. (2006), who found that such tests with field caged clams effectively detect various sources of ecotoxicological impairment. They outlined how to measure mortality and sub-lethal end points such as growth. I "caged" our clams in mussel silos (Figure 3-2) constructed by, and purchased from, Ogden College of Science and Engineering at Western Kentucky University. Ten clams, randomly sampled, were placed in each silo (four in Ligias Fork, two in Charleys Branch, two in Straight Fork, four in Buffalo Creek and two in the Ocoee River). The mussel silos were composed of a 10-kg concrete dome and a PVC inner chamber with standard 1.6-mm mesh size fiberglass screen ends to house the mussels. Silos were designed so that, as water flows over the silo, it creates a Bernoulli effect which draws water up through the mussel enclosure, thus providing a continuous supply of fresh water and nutrients, while keeping mussels enclosed and easily retrieved for data collection. Mussel silos allow for containment of the mussels in the river, while avoiding cage design features which may collect debris.

During the first week of February, 2012, the live clam samples were transported to the field sites in coolers filled with Ocoee River water and ice. Field sites were chosen for environmental variability, presence of stressors and habitat viability (Table 3-1). At the field site, the clams were gathered into PVC piping enclosed by mesh screening and then placed into the silos. The silos were deployed to the desired field locality. Silos were placed in areas comprised of uniform substrate flow and in areas suitable for freshwater bivalve habitat, determined by water velocity, water depth and stream gradient. Silos were fastened and oriented to be in contact with the natural substrate so the clams could naturally filter feed. At each field locality, two silos containing 10 *Corbicula* each were deployed for a total of 20 specimens at each locality. Nearby cobble was used to secure silos to the stream bed. The organisms were assesed for survivorship and growth at every 30 days, the study lasted 150 days. Clams were classified as dead if valves were separated or easily teased apart. Care was taken to ensure that no silo was exposed to the same micro-habitat conditions for the duration of the experiment. Statistically, mean values of survivorship and growth of clams were calculated for each site.

At the Ocoee River, Charleys Branch and Straight Fork, an additional silo containing three individuals was also deployed for comparison to the more densely packed silos. The Ocoee, a stream with good water quality, Charleys Branch impaired by mining and Straight Fork with moderate/fair water quality due to an abandoned pit mine, were chosen for their differences in environmental health. In these additional silos, three *Corbicula*, including a juvenile, mid-sized clam and an adult were placed to measure their growth rates each week for a comparison to the 30-day data.

Growth rates were compared to those in the study by Welch and Joy (1984) who tested the growth rates of the Asian clam. The Welch and Joy clams were assigned to classes based on length: Class I (<10 mm); Class II (10 to 11.9 mm); and Class III (12 to 14 mm). They where placed in separate cages in the Kanawha River at Marmet, West Virginia, for a 12-week period (16 July - 7 October, 1983) when mean water temperature was 26.6 °C (Welch and Joy 1984).

“Warm water” growth rates (length/weight) were: Class I, 0.95 mm/week; Class II, 0.86 mm/week; Class III, 0.80 mm/week. An additional 78 clams were assigned to the same shell length classes and maintained in the Kanawha River for a 12-week period (II October 1983 through 3 January 1984) when mean water temperature was 10.37 °C (Welch and Joy 1984). “Cold water” growth rates were: Class I, 0.09 mm/ week; Class II, 0.08 mm/week. Class III clams were destroyed by a predator (Welch and Joy 1984). Summer growth rates were approximately 10.7 times higher than winter growth rates based on length (Welch and Joy 1984). Growth rate was calculated as: **Growth = (Recent Width/Initial Width) -1.**

Testing Decay Rates of Death Assemblages.

The Ocoee River near Chattanooga, Tennessee contains a great abundance of both life and death assemblages of *Corbicula fluminea.* The death assemblages of the Asian clam were taken from this locality. *Villosa iris* shells used in decay study were donated by the McClung Museum at the University of Tennessee, Knoxville (UTK) campus. To measure shell dissolution rates, I followed the methods of Strayer and Malcom (2007). I deployed “litter bags” composed of 1.6-mm fiberglass window screening into our six streams within the NRB and our control stream. Each bag contained 10 *Corbicula* specimens split into 20 valves and 5 *Villosa iris* shells. These shells were weighed to the nearest 0.01mg. Their length width and height was measured to the nearest 0.01 mm. Measurements were taken from the anterior to the posterior, the dorsal to the ventral, and at the point of greatest thickness of each shell with digital calipers to gauge the length, height and width of each sample (Figure 3-1). The sample was then numbered with permanent marker to allow for later identification. Both *Corbicula* and *Villosa* shells were used because the Strayer and Malcom study (2007) noted that smaller shells would decay faster than larger assemblages. The *Villosa* shells were larger than *Corbicula*, thus allowing me to estimate how long the death assemblages of the native and the invasive would be available in the freshwater record.

The litter bags were placed in the field areas, cabled with 29-kg fishing line to 30 cm steel spikes which were hammered into the sediments of the testing locations. Bags were placed in all of the study streams in February 2012 and brought back to the lab for analysis every 30 days until July 2012. Upon retrieval, bags were transported to the lab where they were rinsed to remove all sediment and debris, dried by baking in an oven at 49 °C for one hour to remove moisture from the samples, reweighed and measured to gauge rates of decay. They were transported back to the field and redeployed for analysis to continue. Shell decay calculated as follows: **Decay = 1 - (Recent Weight/Initial Weight).**

Identifying Sampling Points

Data was collected across seven different localities (Table 3-1): Four streams across six different localities within the New River Basin (NRB) impacted by various degrees/types of pollution and a control stream. The control stream is the Ocoee River whose geologic setting is similar to the study streams within the NRB. Due to the various types of pollution to which the streams have been subjected, there was a decent range of different conductivities across the field sites.

The study sites were chosen because of their variety of environments suitable for clam survival and variety of stressors that would possibly affect the specimens. Streams were also chosen because they are either currently habitat for *Corbicula fluminea*, or have historically provided habitat to the Asian clam. We did not introduce the specimens to streams that have always been absent of the invasive. For this reason, silos at Charleys Branch were placed at the tributaries confluence with the New River. Litter bags containing death assemblages of *Corbicula fluminea* and *Villosa iris* were placed next to the silos. All study areas were closely monitored by the guidelines of the EPA's stream guidance program to determine if an ecological or public health risk exists in the area.

Equipment and Sampling Methods

During the course of this study, I looked at a number of different water quality parameters for a number of different reasons (Table 3-2). The HI 9828 Multi-Parameter Water Quality Portable Meter from Hanna Instruments was used for site visits and field testing. The water quality probe was equipped with a conductivity meter, pH probe, and dissolved oxygen (DO) probe. The meter can also test for total dissolved solids (TDS) and water temperature (°C). The meter was standardized with appropriate buffer solutions according to the HI 9828 instruction manual and calibrated for each field visit. Probes were washed with distilled water with excess drops removed (Hanna 2006). Finally the probe was placed directly into the water source. This allowed the meter to provide immediate measurements that were recorded (Hanna 2006). The probe was then removed from the water source and rinsed thoroughly with distilled water. Measurements were taken every week until completion of the study. The location of sample points were logged using GPS coordinates so the location could be re-visited for continual testing.

I also used a HACH 2800 spectrophotometer and a Pin Point II, American Marine Incorporated, calcium monitor for laboratory testing. The spectrophotometer was used to measure the amount of sulphate from my sampling points. As three of our study areas are streams impacted by mining activity, this instrument was used to measure SO_4^{2-} in accordance to the EPA's Water Quality Guidance Program which states that streams with an SO_4^{2-} reading over 70 mg/L are indicative of impairment from mining activity. The calcium monitor was used to measure the amount of Ca^{2+} available in the water supply to be utilized by, or dissolved from, the field caged clams.

Sampling procedures for the spectrophotometer were conducted with a 250-ml clean Nalgene bottle. The bottle was filled with water from the field site and gently swirled to rinse the entire bottle (HACH 2009). This sample was either discharged downstream or on the stream bank to not contaminate testing point. The bottle was again gently placed in the water and filled completely, leaving as little airspace as possible, and capped under water (HACH 2009). The bottle was labeled for later identification, placed on ice, and transported back to the laboratory. Sulfate measurements were taken within six hours of sampling as suggested by the HACH instrumentation guide (HACH 2009). Measurements were taken every week until completion of the study. Samples for calcium monitoring were conducted using the same methodology. The probe was washed with distilled water with excess drops removed. In the laboratory, samples were allowed to warm to room temperature, then the probe was inserted into the sample and the calcium reading was recorded. Measurements were taken every week until completion of study.

Water velocity was measured in meters/second using a Geopaks digital flow meter. The flow meter was placed into the water with the impeller placed into stream flow (Figure 3-2). The propeller was rested above the mussel silo to record velocity flowing over the silos. Once in position, the meter was turned on and held in position for 60-sec at which time the meter counted the revolutions of the impeller (Geopaks). At the 60-sec mark, the number of revolutions was fitted to an equation to determine flow in m/sec. Water velocity was determined as follows (in which C is revolutions per minute): **Water Velocity (V) m/sec = (0.000854C) + 0.05** (Geopaks).

Data Analysis

For the living specimens in this study, I was concerned with both the growth/mortality rate of the field caged clams through time and their average growth/mortality rates over the seasons. I was also concerned with which of the multiple variables we measured most affected growth and survivorship in the living clams. To understand this, I looked at the overall time trend of mean growth in all our streams, compared them to one another, and ran a Multiple Regression Analysis (MRA) to analyze these data. MRA is a statistical tool that allows the examination of how multiple autonomous variables (physical and chemical stream parameters) are related to a dependent variable (such as clam health as a function of growth and mortality)(Higgins 2005). I analyzed how individual stressors correlate the growth and survivorship of *Corbicula*, gathered the information from the different parameters and, through multiple regression, was able to more accurately predict how these individual factors affected growth and survivorship.

For the shell decay of the death assemblage data set, I was also concerned with decay through time and the average decay rates across the seasons. I then determined which of the multiple variables most correlated to decay rates. Following the MRA described above, I examined the all over time trend of mean decay and used regression analysis to best predict which variables had the most impact on shell decay during the course of the study.

For both the life and death assemblages after completing the MRA, a Partial Regression Coefficient (PRC) was looked at to explain leftover residuals and the r^2 values of the analysis. Since an r^2 value reflects the ability of our results to predict a trend (if $r^2 = 1$, probability of the model is 100%), the PRC helped statistically predict the probability of all the independent variables at play. The PRC is able to do this because the analysis gives the amount by which the dependent variable (growth rate) increases or decreases when an independent variable (such as conductivity) is increased by a single unit and all of the other independent variables are held constant (Adbi 2003). The coefficient is partial because its value depends on the other independent variables (Adbi 2003). More specifically, the value of the PRC for one independent variable will vary depending on the other independent variables included in the regression equation (Adbi 2003).

Final decay measurements followed the method developed by Strayer and Malcom (2007). I calculated the "k" instantaneous decay rate of our death assemblages where instantaneous decay rate (k) was calculated as : **k = (1/t) [ln(final mass/initial mass)]** (Strayer and Malcom 2007). Time (t) was determined as the amount of time the death assemblages where in the field streams as percent loss in years.

TABLE 3- 1. Field localities by stream name, their drainage basin, if affected by industry activities and GPS location of mussel silos. Asteracts indicate streams receiving mine drainage.

Stream Name	**Drainage Basin**	**Mining Activity**	**GPS Location**
Charleys Branch*	New River	Reclaimed mine land as of May 2011.	N 36° 11.5865 W 084° 20.0808
Ligias Fork I*	New River	Headwaters originate on active surface mine atop Wind Rock Mountain.	N 36° 10.5315 W 084° 17.1866
Ligias Fork II*	New River	Headwaters originate on active surface mine atop Wind Rock Mountain.	N 36° 12.1285 W 084° 18.2721
Buffalo Creek I	New River	Not impaired by mining. Subjected to rural agriculture and cattle farming.	N 36° 23.2688 W 84° 25.2171
Buffalo Creek II*	New River	Southern portion of the stream impacted from deep mining from Straight Fork.	N 36° 22.4800 W 84° 27.0639
Straight Fork*	New River	Occasional mine drainage from an abandoned pit mine that was operated during the 1920's.	N 36° 25.8419 W 084° 28.0684
Ocoee River	Hiwassee River	Control stream in an area of similar geology not impacted by any mining.	N 35° 05.8990 W 084° 39.3635

TABLE 3 – 2. Water quality parameters measured during the course and why they are of importance.

Table 3 – 2. continued Parameters	Significance to Study
Conductivity (µS/cm)	According to the new EPA water quality guidance program, conductivity is the cumulative impact of elevated concentrations of stressors that leads to biological impairment of freshwater systems (EPA 2010). As conductivity is a measure of cumulative ionic strength, the parameter is an effective predictor of biological impairment (Bernhardt and Palmer 2011). Currently, it is believed that conductivities above 300 µS/cm represent a water quality threshold for sensitive taxa (Bernhardt and Palmer 2011);(EPA 2010). We wished to test conductivity as a proxy for clam health and shell decay rates of dead populations.
DO (ppm and %)	Most organisms in freshwater systems require a suitable, maintained supply of free oxygen for survival (Shoup 1950). Dissolved oxygen (DO) is recorded to determine if systems have received pollution from organic wastes or if there is enough free oxygen to support aquatic organisms (Shoup and Peyton 1940). Most aquatic systems require a minimum of 4 ppm DO (Shoup 1950).
pH	The NRB, with low limestone and abundance of calcareous sandstone and shales in conjunction with extensive mining in the area, is a relatively unstable system with fluctuating pH (Lokey 1979). We looked at how pH correlated with both the growth rates of our field-caged clams, and the decay or dissolution rates of their shells.
Temp (°C)	Welch and Joy (1984) found that growth rates of live *Corbicula fluminea* specimens were 10.7 times faster in warm water as compared to cold water growth rates. As we compared growth of the field caged clams across the seasons, we wanted to compare growth rates to water temperature across our seven field sites.
TDS (ppm)	Total dissolved solids (TDS) are the amount of all organic and inorganic materials within a freshwater system (Bernhardt and Palmer 2011). We measured TDS to see if there was a strong correlation with conductivity, coupled with mortality of living specimens and shell decay.
SO_4^{2-} (mg/L)	Charleys Branch, Ligias Fork and Straight Fork are all impacted by coal mining. Exposure of coal seams during coal mining allows for the leaching of sulfate (SO_4^{2-}) which allows for a localized point source of SO_4^{2-} to the drainage network (Bernhardt and Palmer 2011). The relationship between mining activities and high SO_4^{2-} concentrations is very well established and regulatory agencies suggest SO_4^{2-} concentrations greater than 50 mg/L can be used as an indicator of water quality impairment from mining activity (Bernhardt and Palmer 2011).
	Ca^{2+} is a co-occurring contaminant of SO_4^{2-} (Bernhardt and Palmer 2011). As SO_4^{2-} reacts with carbonate rocks in the Appalachian mountain

Table 3 – 2. continued Parameters	Significance to Study
Ca^{2+} (ppm)	regions, increases in Ca^{2+} may be expected (Bernhardt and Palmer 2011). This can lead to an increase of pH in receiving streams (unlike the more understood acid mine drainage). The release of Ca^{2+} ions can lead to increases in electrical conductivity and total solids in receiving streams (Bernhardt and Palmer 2011). It is also expected that, in streams containing high concentrations of Ca^{2+}, decay rates of mussel shells will be lower (Strayer and Malcom 2007).
Flow (m/sec)	Strayer and Malcom (2007) noted that current velocity was a key variable in shell decay rates of death assemblages. We measured the velocity of our streams to examine the relationship of shell decay between the physical properties of our streams against the water quality parameters measured.

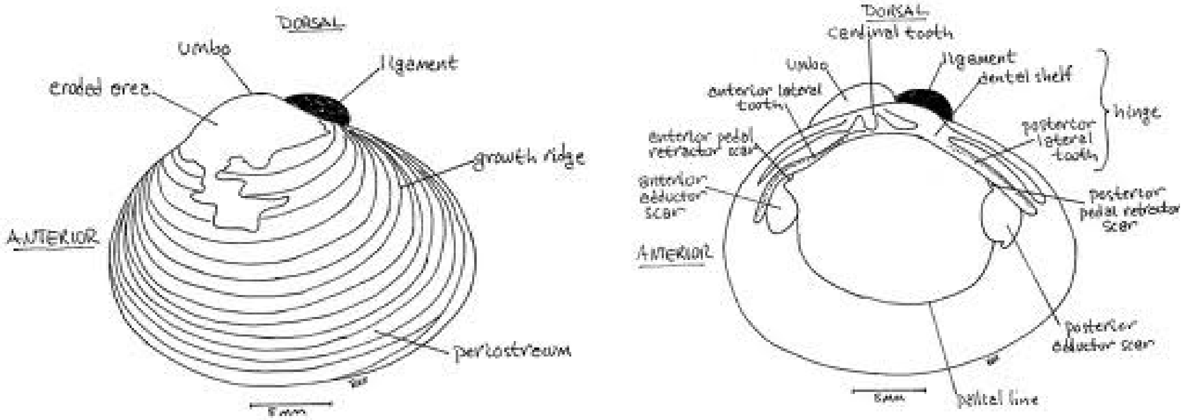


FIGURE 3-1. Anatomy of *Corbicula fluminea.* From north to south, the dorsal to the ventral, and west to east is the anterior to the posterior.

FIGURE 3 – 2. Photo of mussel silos deployed for this study. The silos are composed of a 10-kg concrete dome and a PVC inner chamber with standard 1.6-mm mesh size fiberglass screen to house the mussels. The silos were designed so that as water flows over the silo, it creates a Bernoulli effect, which draws water up through the mussel enclosure providing a continuous supply of fresh water and nutrients, while keeping mussels enclosed and easily retrieved for data collection.

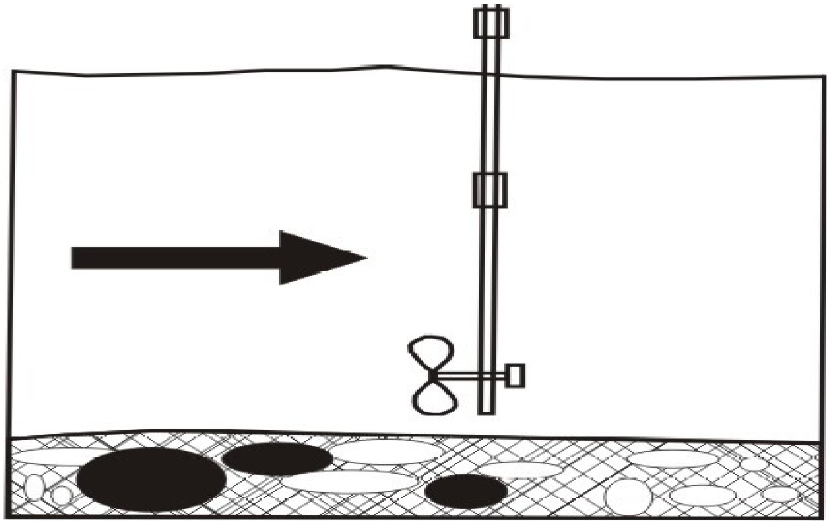


FIGURE 3 – 3. Image displaying the proper use of our Geopaks digital flow meter. The flow meter was placed into the water with the impeller placed into stream flow. Velocity was measured in m/sec.

CHAPTER IV
RESULTS

Survivorship and Growth Rate of the Asian Clam

The seven field sites allowed for a wide range of mean conductivities during the course of the 150-day study as well as a good degree of variation among other measured parameters (Table 4 -1). As defined by the US EPA water quality guidance program, I found that two of the study streams, the Ocoee my control, and Buffalo Creek in the NRB, never exhibited conductivities that suggested impairment. This was to be expected as the Ocoee has an abundance of *Corbicula fluminea* and the greatest abundance of freshwater mussels species in the NRB, according to Ahlstedt (2008), occurs in Buffalo Creek. Straight Fork, a stream periodically receiving mine drainage from an old pit mine, was documented as having a significant rise in conductivity with every 30-day interval. Charleys Branch, below reclaimed mine land, had varying degrees of conductivity yet, for most of the study, could be considered impaired by the water quality guidance program. The Ligias Fork experienced the greatest elevated conductivities during the entirety of the study and consistently scored above impairment as defined by the EPA, except for the lower reaches of the stream (Ligias II) in the last 90 days of the study.

As the study continued, a clear relationship between time and growth rate was established (Figure 4-1). Charleys Branch, Ligias Fork and even the portion of Buffalo Creek that receives drainage from Straight Fork experienced a period of negative or regressed growth. The *Corbicula* generally began growing more rapidly by day 60. All of the clams experienced positive growth by day 100. The Multiple Regression Analysis (MRA) documented that of all the parameters measured, water temperature and conductivity were most correlated with growth rate. Temperature was the most important variable, explaining roughly 33% of the variation in growth rate. As temperatures began to rise we saw a significant increase in growth (Figure 4-2). We also found that as conductivity decreased, clam growth rate also increased (Figure 4-3 and Figure 4-5). Correlation analysis shows that conductivity is independent of temperature (Table 4-2). Total dissolved solids were found to be important as well, as conductivity is the measure of ionic strength, it follows that the more dissolved solids found in a system, the higher the conductivity of that system would be. Our streams with high conductivities also correlated very well with high averages of TDS (Figure 4-4). In short, the analysis shows that the relationship between conductivity and growth is highly significant and independent from temperature.

The greatest growth rates were experienced within the Ocoee River, the control stream (Figure 4-1). Mean conductivity never exceeded 48 µS/cm and the amount of TDS in the stream never exceeded 25 ppm. Both the conductivity and TDS measurements were significantly lower than the other field sites in this study. I also found strong growth at Buffalo Creek I. At this site, mean conductivity never exceeded 149 µS/cm, which was well below the EPA pollution guideline, and TDS never exceeded 72 ppm, a measurement that was also lower than the other streams within the NRB. The lower Buffalo Creek site, Buffalo Creek II, however, experienced lower conductivities than other streams in the NRB, on the order of 117 – 237 µS/cm, but experienced regressed growth during the first 30 days of the study and was the slowest growth of all of the streams. Growth rates remained low even as water temperature exceeded 13 °C.

Of the 140 clams used during this study, there was only a single mortality, during the first 120 days, which occurred in Charleys Branch at the confluence of the New River beneath reclaimed mine land. The death occurred within the first 30 days of silo deployment into the stream and was not considered to be of significant influence to the results. What was significant was the 30-day period of regressed growth found in the stream. Average conductivity fluctuated in this stream between 196 and 391 µS/cm. The majority of conductivity measurements taken on this stream during the course of the study, however, exceeded the EPA limit of 300 µS/cm, including the first 30 days of the experiment (331 µS/cm) which also had an elevated average TDS reading of 153 ppm. As the seasons began to change, conductivity remained relatively constant, and the growth rate began to increase as waters warmed above 13 °C.

When the 150 day data was recovered, water level and stream flow was greatly reduced. The summer weather experienced record breaking temperatures in the region and a period of low precipitation. At this time, the Ligias Fork I had measured conductivity of 953 µS/cm. At this point, an additional four moralities were discovered at this site. Unfortunately, due to weather conditions, Charleys Branch and the Buffalo Creek II localities had dried up, resulting in the mortality of all field caged clams at these locations. At the Buffalo Creek I and Ligias Fork II localities, the silos were not found during the 150 day data collection.

Ligias Fork I and Ligias Fork II experienced the highest conductivities of all the study streams during the course of the study. Ligias Fork I experienced a range of conductivity averages between 366 and 390 µS/cm and exceeded the EPA guidelines. TDS measurements ranged from 155 to 192 ppm, which were significantly high in comparison to other streams.

Growth remained slow in the Ligias Fork I until the water temperature exceeded 14 °C, this stream was warmer than streams with lower conductivities. Ligias Fork II experienced the greatest amount of negative growth than all of the other study streams. Both the 30 and 60 day measurements revealed negative growth and it was not until the 100-day data was gathered that positive growth was seen. Though the Ligias Fork II had a wider range of conductivities than its counterpart (208 – 345 µS/cm), both episodes of regressed growth occurred when the average conductivity exceeded the 300 µS/cm limit enacted under the water quality guidance program. TDS measurements were also elevated during the 30 and 60 day intervals (172 and 129, respectively). Growth did not occur in Ligias II until conductivity fell below 300 µS/cm and average temperature exceed 14 °C. The clams in Straight Fork experienced steady growth over the 150-day study period. Conductivity increased with each measured 30-day period, from 173 – 320 µS/cm; respectively. TDS values have also increased with every 30-day interval from 88 – 161 ppm. Temperature during this time has also steadily increased from 6.5 to 17.8 °C. Mean temperatures from the 60-day data exceeded 13 °C. There was no period of regressed growth documented at this field site.

Decay Rates of Death Assemblages

Though influences on growth were statistically significant, especially in regards to temperature and conductivity, decay rates of both the *Corbicula fluminea* and *Villosa iris* death assemblages did not show trends as significant as the growth rates of the living field caged clams. All measured parameters are statistically insignificant. The measured parameter that showed the best correlation with decay rate was flow (m/sec). I was primarily concerned with conductivity but thought that Ca^{2+} (ppm) and pH would also be significant to shell decay. Results show that these variable have no measurable trend, however, and show no relevant correlation statistically. Though the decay rates showed great fluctuation across all the field sites for the duration of the study, half life calculations (Figures 4-6 and 4-7; Table 4-3) exhibited a strong measured trend across the course of the study.

Shell decay was highest in the control stream, the Ocoee River, and lowest in one of the most polluted streams, Ligias Fork II. This was unexpected and disproves my original hypothesis of shell decay being greater in the streams of elevated conductivity. Half life calculations best correlate with stream pH (Figure 4-6). The trends in our study indicate that elevated pH measurements in streams will result in a longer half life for our death assemblages. pH remained relatively constant in the NRB for most of the study. Extreme ranges of pH are apparent in Charleys Branch (4.56 to 8.5) and Ligias Fork I (8.2 to 9.00). Across the study sites, however, ranges of pH averaged between 7.5 – 8.5, exhibiting streams that are fairly neutral to basic. The results also exhibit a correlation between half life and Ca^{2+}. Trends for this parameter indicate that the more Ca^{2+} available in a given stream the higher the half life (Figure 4-7).

The half life measurements are also significant because they exhibit a short time frame for shells to be used for ecological engineering by other organisms. In the control stream, results indicated that half life of the death assemblages will be in as little as 253.7 days for *Corbicula*. The longest half life was in the Ligias Fork at 2061.7 days. The results indicated that the death assemblages could disappear from all streams in as few as 8 – 10 half lives, meaning within a few thousand days, or up to an approximate 11 years. The decay rates also exhibited a high correlation between the *Corbicula* and *Villosa* shells (Figure 4-8). The low slope may imply that *Villosa* is decaying faster than the invasive clam but this relationship is not statistically significant. This may, in part, be explained by the low half life of 392.44 at Buffalo Creek II, the same site that was affected by individuals not associated with the study. Shells at this site were noted to be fractured when the 120 day data were collected. Since the confidence interval was so low, it is reasonable to assume that decay rates between the two species were relatively similar.

Shell decay rates also correlated with flow and Ca^{2+}. The Ca^{2+} measurements among specimens showed a strong correlation between the species (Figure 4-9) . The decay rate of *Corbicula* as a function of Ca^{2+} saw a decrease in decay with high levels of the calcium ion. Also, increased levels of the calcium ion showed a decrease of decay for the *Villosa iris* death assemblages. Decay, as a function of the calcium ion was calculated at an instantaneous rate because of extreme fluctuations in data from month to month values across the field sites. Figure 4-8 illustrates the relationship of shell decay between *Corbicula* and *Villosa* over the course of the study. The results documented a general trend among both species, but that the Asian clam may decay at a higher rate in waters with elevated Ca^{2+} readings. In order to linearized the data, a semi log plot on the y-axis was made to produce Figure 4-10. This documented a more linear trend among the decay rates of the two species as a function of the calcium ion. An increase in the ion led to a decrease in over all shell decay for both species. The data suggests that there is a probable correlation with shell size, calcium concentrations and shell decay.

As stream velocity increased (m/sec), rates of decay were also increased (Figure 4-11). This was a significant find as the highest decay rates were found in the Ocoee River. The Ocoee field site is below TVA dam Site 1 at the Parksville reservoir in Parksville, Tennessee. Flow varies significantly at this field site based on dam release schedules. There are two generators operating at this dam site. All flow measurements were taken when no generators were running. No measurements were recorded when one or two generators were releasing water into the reservoir area due to safety issues. The observed data from the TVA (Table 4-4) demonstrates the range of velocity discharged from the dam recorded on 6/24/2012. Discharge in cubic ft/cm ranged from 0 to 2036, throughout the course of the day (TVA 2012) Discharge is dependent on the release of water from the generators and is obviously a very important variable to this stream.

Final decay measurements followed the method developed by Strayer and Malcom (2007). Here, I calculated the k instantaneous decay rate of our death assemblages where instantaneous decay rate (k) was calculated as : **k = (1/t) [ln(final mass/initial mass)]** (Strayer and Malcom 2007). Time (t) was determined as the amount of time our death assemblages where in the field streams as percent loss in years. Figure 4-12 documented the instantaneous decay rate as a function of Ca^{2+} for our six sites based on change from 0 to 120 days as one interval. Furthermore, Table (4-5) documented the instantaneous decay rate per year for all of our study streams. Both confirm the half life data and that shell decay will be greatest in the Ocoee (5.47%/yr of original shell material for *Villosa iris* and 10.8%/yr of original shell material for *Corbicula fluminea*).

The data suggested rapid decay of the death assemblages. This decay, however, was not strongly correlated with conductivity and water temperature. Decay was weakly correlated with stream flow (m/s) . The results were not statistically significant. Bias may have been introduced to the decay study, both from outside influences and human error. To gather information on decay, the death assemblages were transported back to the lab where they were rinsed to remove all sediment and debris, dried by baking in an oven at 120 °F for one hour to remove moisture from the samples, then reweighed and measured to gather results. Specimens were dried (see Methods), but with each bake protein chipped off the shell, which would have affected our measurements. For future work, before the study is conducted, the protein should be removed from the shells to avoid this potential bias. Though decay rates may have been compromised by our method, a relationship exists between the rates of decay of both species and their half lives.

TABLE 4 – 1. Averages of measured water quality parameters at each field site over 30-day intervals during the course of the study.

Stream Name	**Number of Days**	**Mean Growth Rate**	**Mean µS/cm**	**Mean DO ppm**	**Mean pH**	**Mean Temp °C**	**Mean TDS ppm**	**Mean DO%**	**Mean SO_4^{2-}**	**Mean Flow m/s**	**Mean Ca^{2+}**
Ocoee River	30	0.00337	44.8	3.6	7.97	9.65	22.2	33.5	41.5	0.41	63.2
	60	0.000300	45	21.2	8.07	12.7	22.2	82.2	27.2	0.41	63.2
	100	0.008200	44.8	18.8	7.3	19.5	23	23	30.5	0.41	60.7
	120	0.004900	47.8	10.95	7.13	21.6	25.5	21.8	22.2	0.41	59.9
	150										
Charleys Branch	30	-0.000017	331	8.74	4.56	12.9	153	91	79	0.47	200.1
	60	0.000000	196	10.6	7.6	12.2	110	97.8	83	0.46	216
	100	0.003000	318	31	7.8	13.8	170	30.2	145	0.46	179
	120	0.001500	391	39.7	8.5	16.3	200	43	80	0.26	190.3
	150										
Ligias Fork I	30	0.000083	379	13	8.2	8.3	165	149	70	0.58	85
	60	0.000066	370	11.5	9	13.5	192	12.3	69	0.56	87.3
	100	0.003120	366	25.3	8.56	14.9	155	26.7	74	0.56	72
	120	0.001220	390	27.4	8.4	17.4	178	19.8	75	0.43	56.7
	150										
Ligias Fork II	30	-0.000260	345	15.9	8.3	5.36	172	201	41.8	0.58	85.5
	60	-0.000083	307	10.8	8.7	13.9	129	108	46.8	0.59	85.5
	100	0.002400	208	19	8.6	14.8	105	74.3	52	0.58	85.2
	120	0.001400	296	13	8.7	20.5	137	27.2	70	0.49	81.2
	150	N/A									
Straight Fork	30	0.000260	173	8.8	7.6	6.5	88	68	47.3	0.6	22.1
	60	0.000330	187	10.2	8.6	13.6	119	101	66	0.62	23.5
	100	0.002000	251	37.6	7.1	16.3	148	21	70	0.63	20.3
	120	0.002300	320	21	6	17.8	161	29.2	78	0.47	28.2
	150										
Buffalo Creek I	30	0.000500	114	6.5	7.4	6.5	59	53.6	25.3	0	22.2
	60	0.000330	125	9	8.5	14.1	66	95.2	23.3	0	22.5
	100	0.004900	128	13.5	7.5	16.7	65	13.8	21	0	22.7
	120	0.003800	149	10.7	7.3	20.1	72	17.7	31	0	22.3

Table 4 – 1. continued

Stream Name	Number of Days	Mean Growth Rate	Mean µS/cm	Mean DO ppm	Mean pH	Mean Temp °C	Mean TDS ppm	Mean DO%	Mean SO_4^{2-}	Mean Flow m/s	Mean Ca^{2+}
	150										
Buffalo Creek II	30	-0.002000	117	5.4	7.7	6.3	58	36.7	30	0.41	18.6
	60	0.000600	144	9	8.3	13.2	68.3	93	42.2	0.44	21
	100	0.000620	215	39.4	8	16.8	108	41	53	0.46	21.5
	120	0.000550	237	22.2	8	19.8	129	29.2	60.3	0.39	20.2
	150	N/A									

TABLE 4 – 2. Correlation analysis among all independent parameters and how they relate to each other. The analysis shows conductivity (**µS/cm)** to be independent of temperature.

Variables	**µS/cm**	**DO ppm**	**pH**	**°C**	**tds ppm**	**DO%**	**SO_4^{2-}**	**Flow (m/s)**	**Ca^{2+}**
µS/cm	**1.000**	0.416	0.118	-0.001	0.977	0.161	0.696	0.401	0.425
DO ppm	0.416	**1.000**	0.088	0.386	0.469	-0.269	0.467	0.174	0.150
pH	0.118	0.088	**1.000**	-0.670	0.111	0.121	-0.043	0.127	-0.155
°C	-0.001	0.386	-0.067	**1.000**	0.011	-0.589	0.078	-0.150	-0.052
TDS ppm	0.977	0.469	0.111	0.011	**1.000**	0.135	0.747	0.407	0.428
DO%	0.161	-0.269	0.121	-0.589	0.135	**1.000**	-0.065	0.271	0.200
SO_4^{2-}	0.696	0.467	-0.043	0.078	0.747	-0.065	**1.000**	0.441	0.615
Flow (m/s)	0.401	0.174	0.127	-0.150	0.407	0.271	0.441	**1.000**	0.193
Ca^{2+}	0.425	0.150	-0.155	-0.052	0.428	0.200	0.615	0.193	**1**

TABLE 4 – 3. Beginning and end weights coupled with calculated half lives in days for *Corbicula fluminea* and *Villosa iris* over the course of the 150 day study.

Stream	**Species**	**Beginning Weight (mg)**	**End Weight (mg)**	**Half Life in Days**
Ocoee River	*Corbicula fluminea*	16875	12157.36	253.7
Charleys Branch	*Corbicula fluminea*	18117.29	16715.11	1032.8
Ligias Fork I	*Corbicula fluminea*	19543.32	18896.87	2061.7
Ligias Fork II	*Corbicula fluminea*	16858.84	15481.35	976.4
Straight Fork	*Corbicula fluminea*	18262.83	16956.23	1121.2
Buffalo Creek I	*Corbicula fluminea*	18782.62	N/A	N/A
Buffalo Creek II	*Corbicula fluminea*	20077.4	18101	802.8
Ocoee River	*Villosa iris*	9755.16	8264.21	501.49
Charleys Branch	*Villosa iris*	13424.46	12757.02	1631
Ligias Fork I	*Villosa iris*	11136.09	10565.12	1580.3
Ligias Fork II	*Villosa iris*	16432.46	16132.45	4514.2
Straight Fork	*Villosa iris*	11202.77	10140.3	834.8
Buffalo Creek I	*Villosa iris*	14051.38	N/A	N/A
Buffalo Creek II	*Villosa iris*	15002.3	12136.97	392.44

TABLE 4 – 4. Recorded data from TVA operated dam Site 1 along the Ocoee River. Data represents day and hour, elevation above dam, elevation below dam and discharge in cubic ft/cm from 12:00am 6/24/2012 to 12:00am 6/25/2012. Elevations are recorded in feet above sea level.

Day and Hour	**Reservoir Elevation Behind Dam (ft above sea level)**	**Tailwater Elevation Below Dam (ft above sea level)**	**Average Hourly Discharge (cubic ft/sec)**
6/24/201212 AM EDT	828.5	713.11	0
6/24/2012 1 AM EDT	828.5	713.1	0
6/24/2012 2 AM EDT	828.5	713.57	13
6/24/2012 3 AM EDT	828.48	713.95	596
6/24/2012 4 AM EDT	828.48	713.11	0
6/24/2012 5 AM EDT	828.48	713.1	0
6/24/2012 6 AM EDT	828.48	713.55	13
6/24/2012 7 AM EDT	828.46	713.95	596
6/24/2012 8 AM EDT	828.46	713.11	0
6/24/2012 9 AM EDT	828.46	713.1	0
6/24/2012 10 AM EDT	828.45	713.6	15
6/24/2012 11 AM EDT	828.49	713.95	601
6/24/2012 12 PM EDT	828.52	713.11	0
6/24/2012 1 PM EDT	828.59	713.1	0
6/24/2012 2 PM EDT	828.64	713.96	26
6/24/2012 3 PM EDT	828.65	716.14	1365
6/24/2012 4 PM EDT	828.64	716.92	2036
6/24/2012 5 PM EDT	828.64	716.93	2034
6/24/2012 6 PM EDT	828.64	715.94	1357
6/24/2012 7 PM EDT	828.71	713.13	9
6/24/2012 8 PM EDT	828.77	713.11	0
6/24/2012 9 PM EDT	828.81	713.46	8
6/24/2012 10 PM EDT	828.81	713.95	599
6/24/2012 11 PM EDT	828.81	713.11	0
6/25/2012 12 AM EDT	828.82	713.1	0

TABLE 4 – 5. Instantaneous decay (k) rate and calculated % decay per year of *Villosa iris* and *Corbicula fluminea* across six study sites.

Locality	*Villosa* k Rate	*Villosa* % Decay/yr	*Corbicula k Rate*	*Corbicula* % Decay/yr
Ocoee River	0.054734537	5.47	0.108206501	10.82
Charleys Branch	0.016828914	1.68	0.265826980	26.58
Ligias Fork I	0.011180369	1.11	0.024844436	2.48
Ligias Fork II	0.002930111	0.29	0.024496735	2.81
Straight Fork	0.032879421	3.28	0.024496735	2.44
Buffalo Creek I	N/A	N/A	N/A	N/A
Buffalo Creek II	0.047177506	4.71	0.034197114	3.41

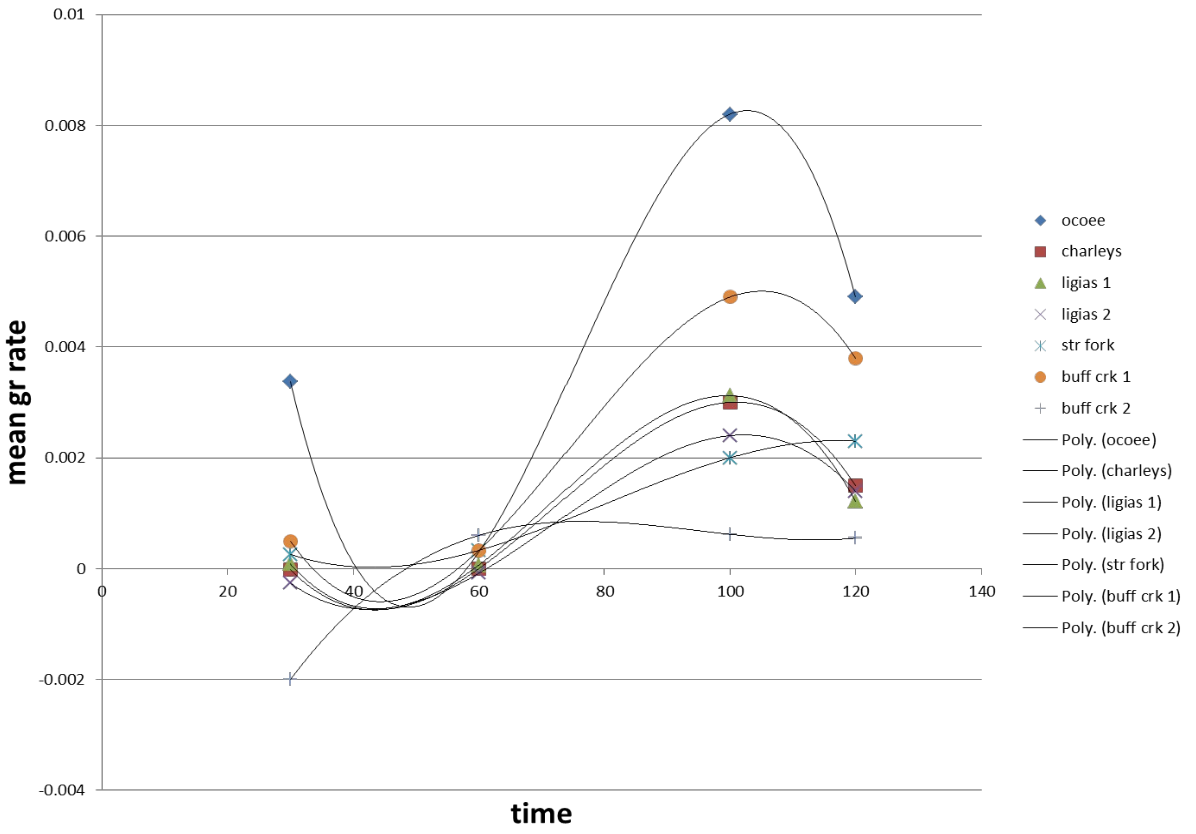


Figure 4-1. Mean growth rates of field caged *Corbicula fluminea* across all seven field localities as a function of time.

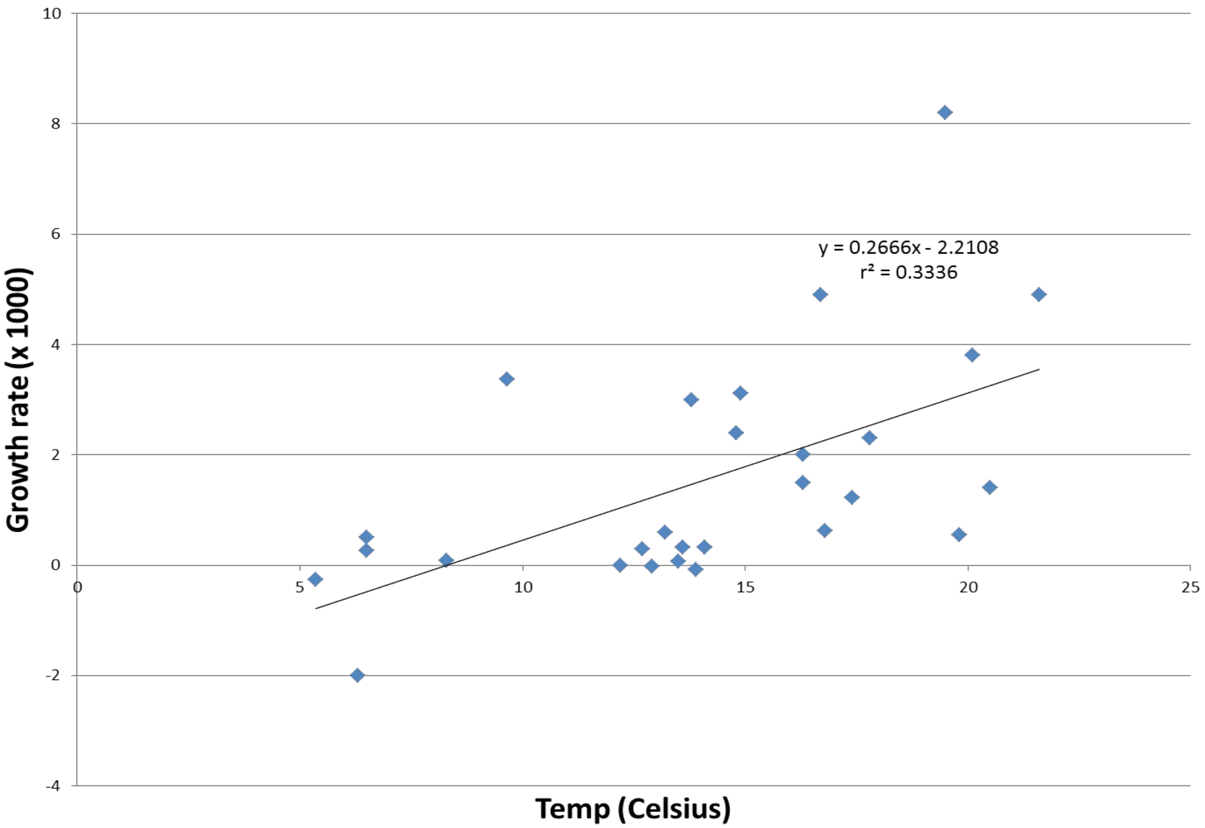


Figure 4-2. Growth rate of field caged *Corbicula fluminea* as a function of temperature (°C). Results show that as temperature increases, there is an acceleration of growth.

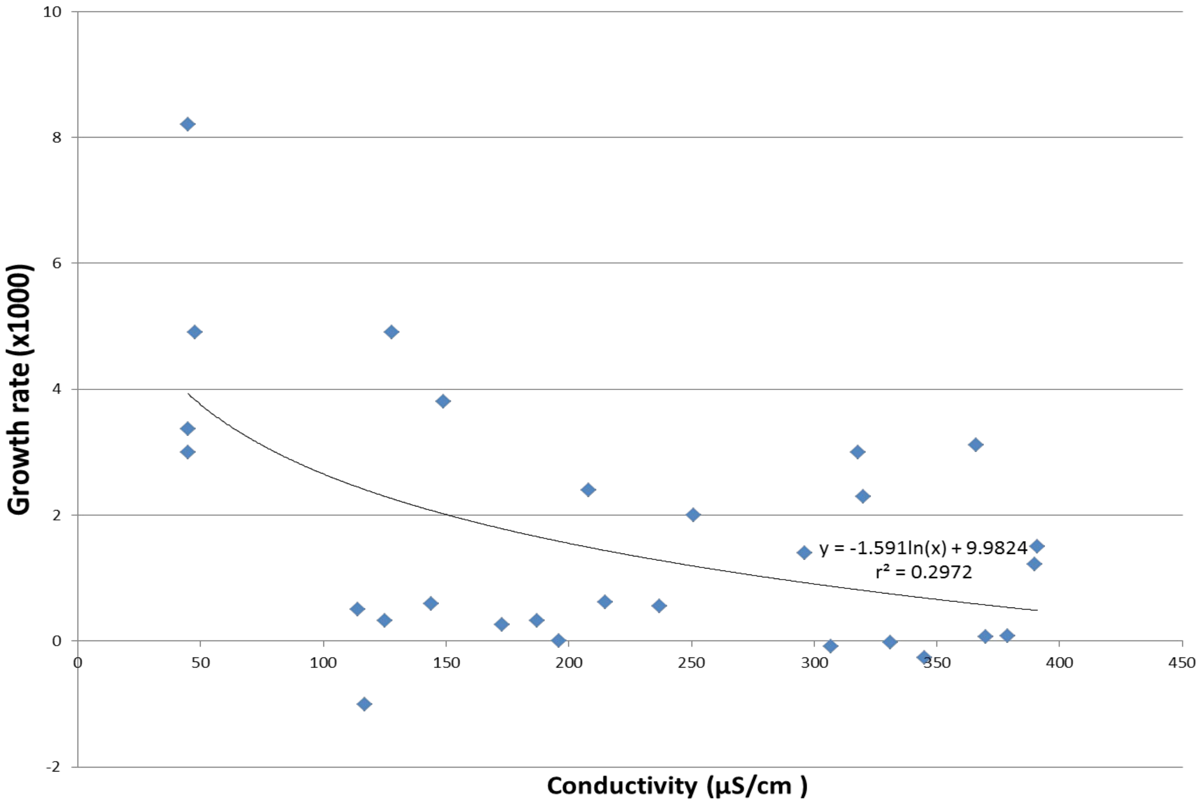


Figure 4-3. Growth rate as a function of conductivity across all study sites. As conductivities increased, growth rate decreased.

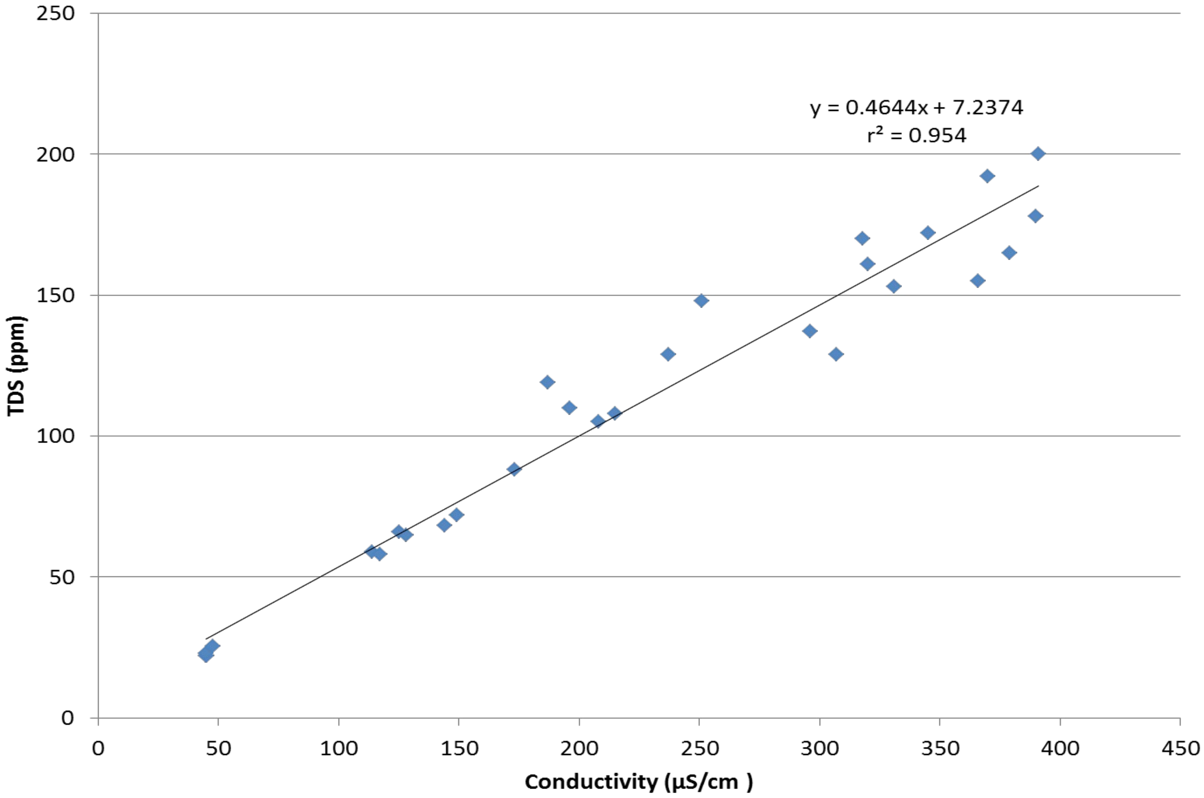


FIGURE 4 – 4. Relationship of total dissolved solids with conductivity. As conductivities increased there was a higher concentration of dissolved solids.

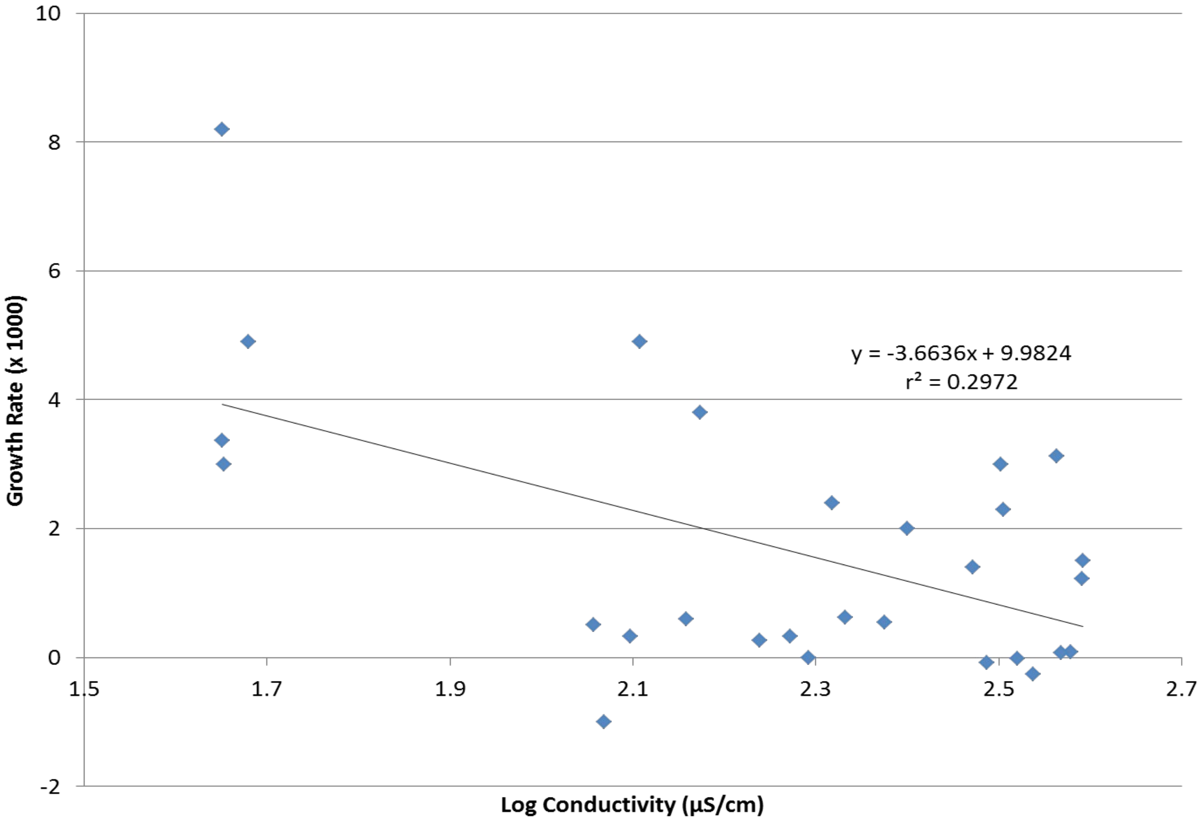


Figure 4-5. Growth rate as a function of log transform of conductivity. Conductivities were log transformed to help linearize the data.

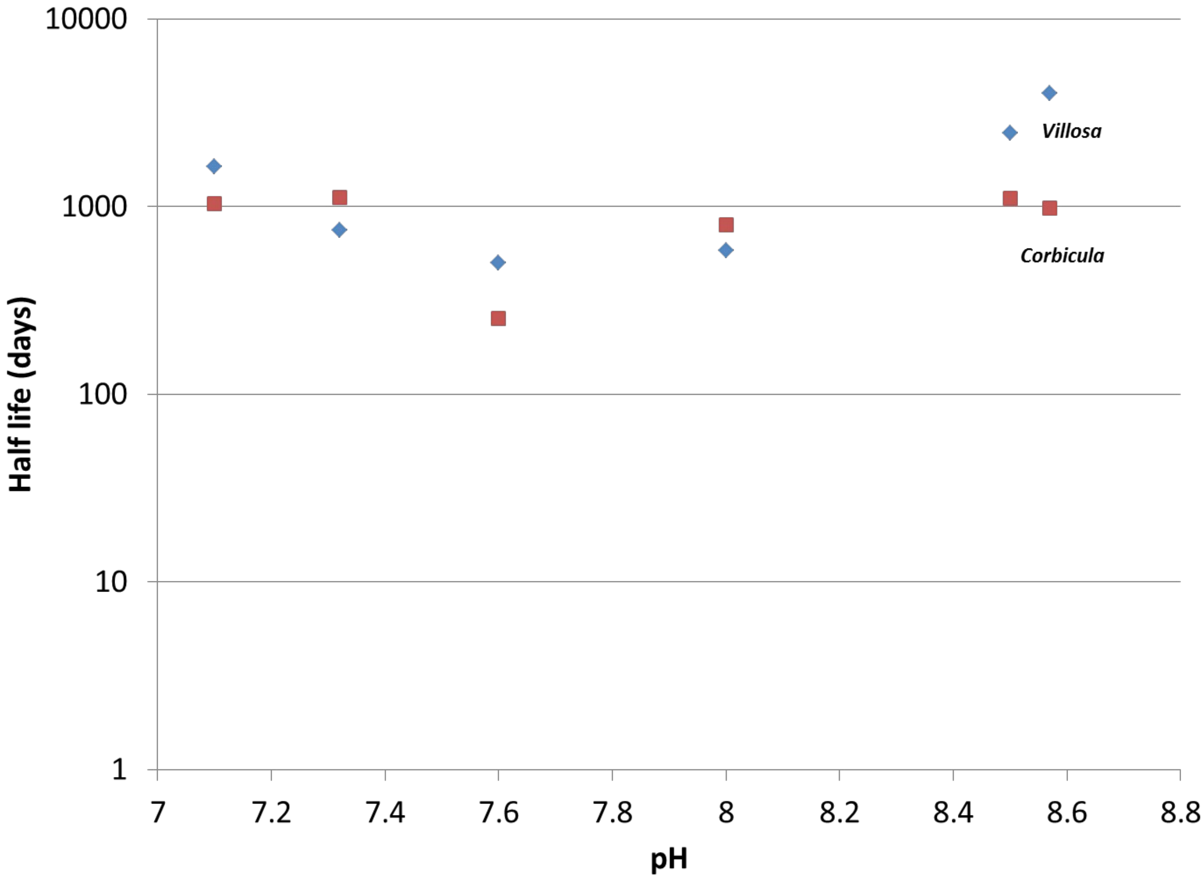
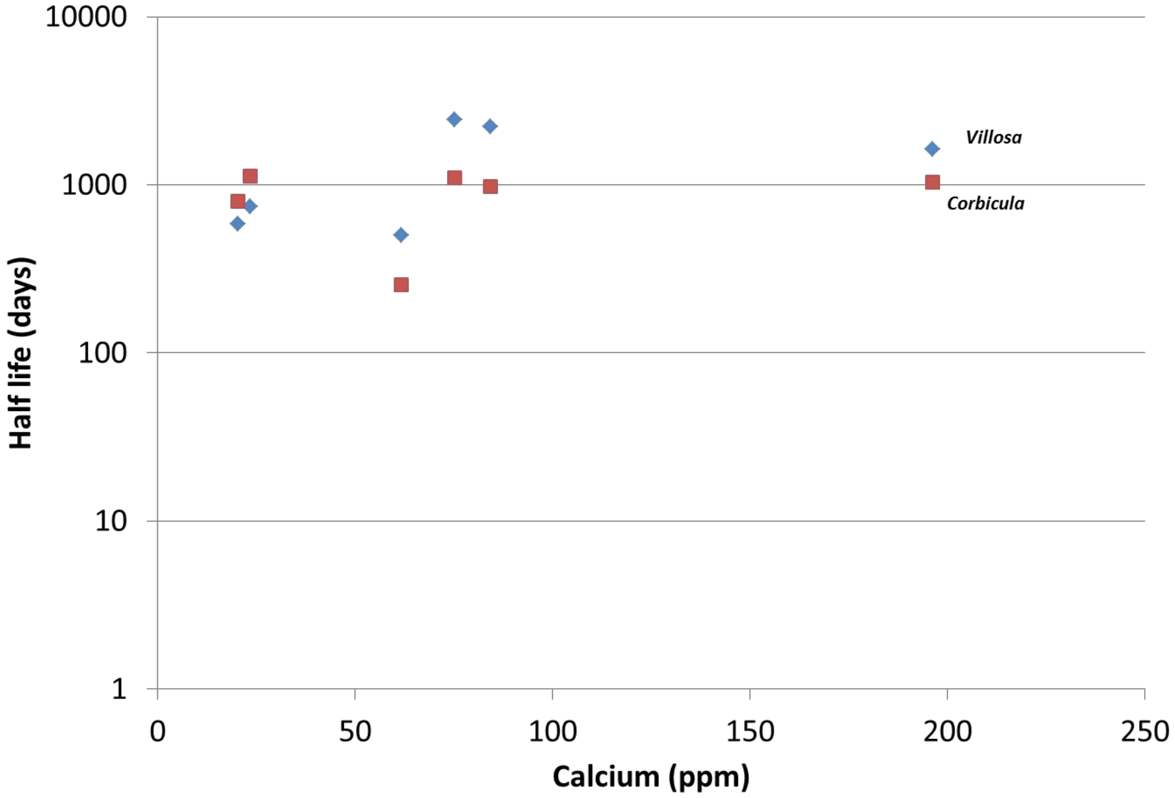


FIGURE 4 – 6. The half life relationship between *Villosa iris* and *Corbicula fluminea* death assemblages with pH.



Corbicula fluminea.

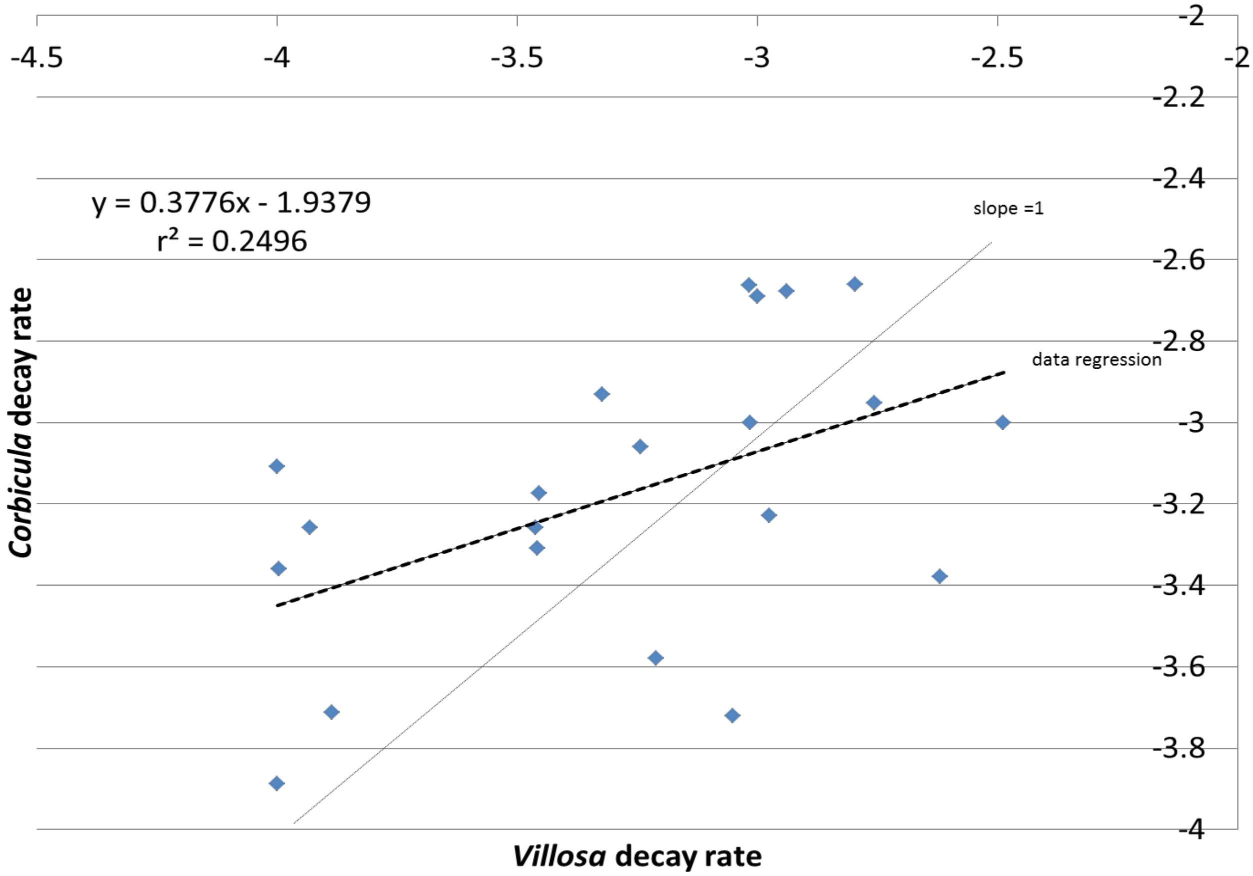


FIGURE 4 – 8. Correlation between the decay rate of *Corbicula fluminea* and *Villosa iris* death assemblages across the course of the study.

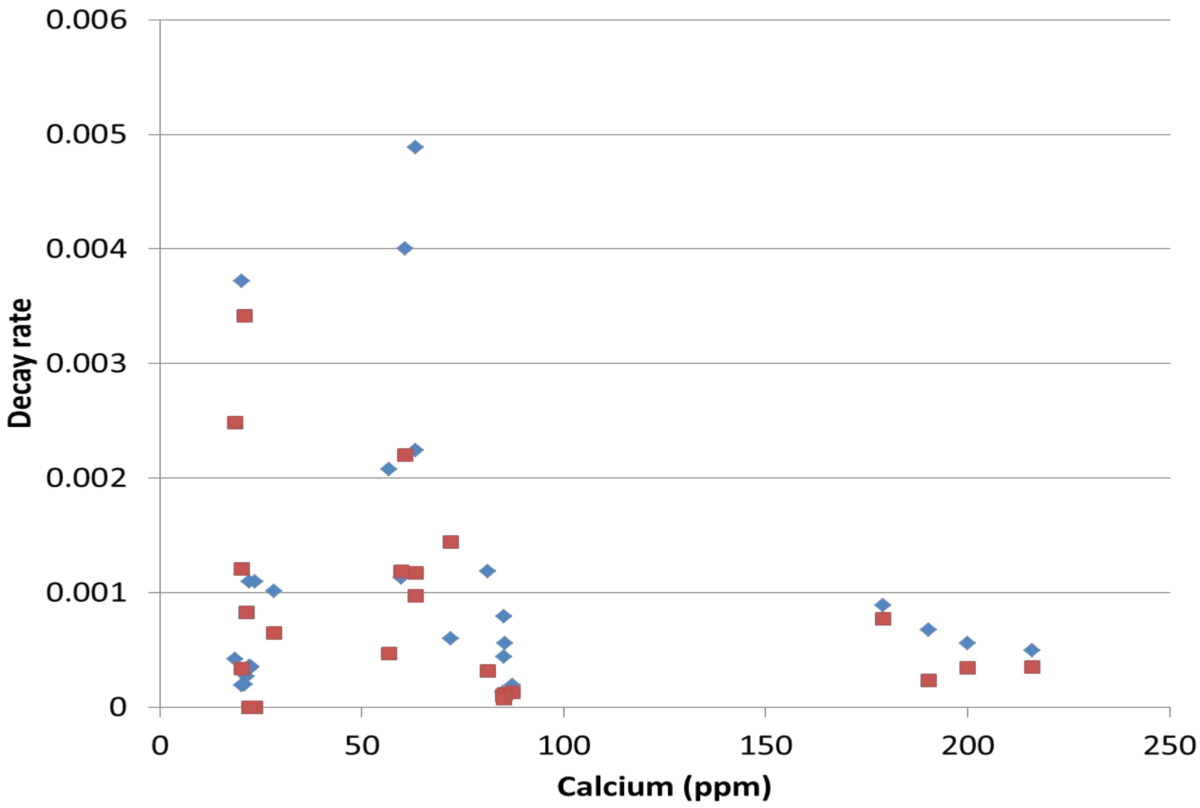


FIGURE 4 – 9. Shell decay of *Corbicula* and *Villosa* assemblages as a function of calcium across the study period. *Corbicula* may decay faster than *Villosa* with regards to calcium.

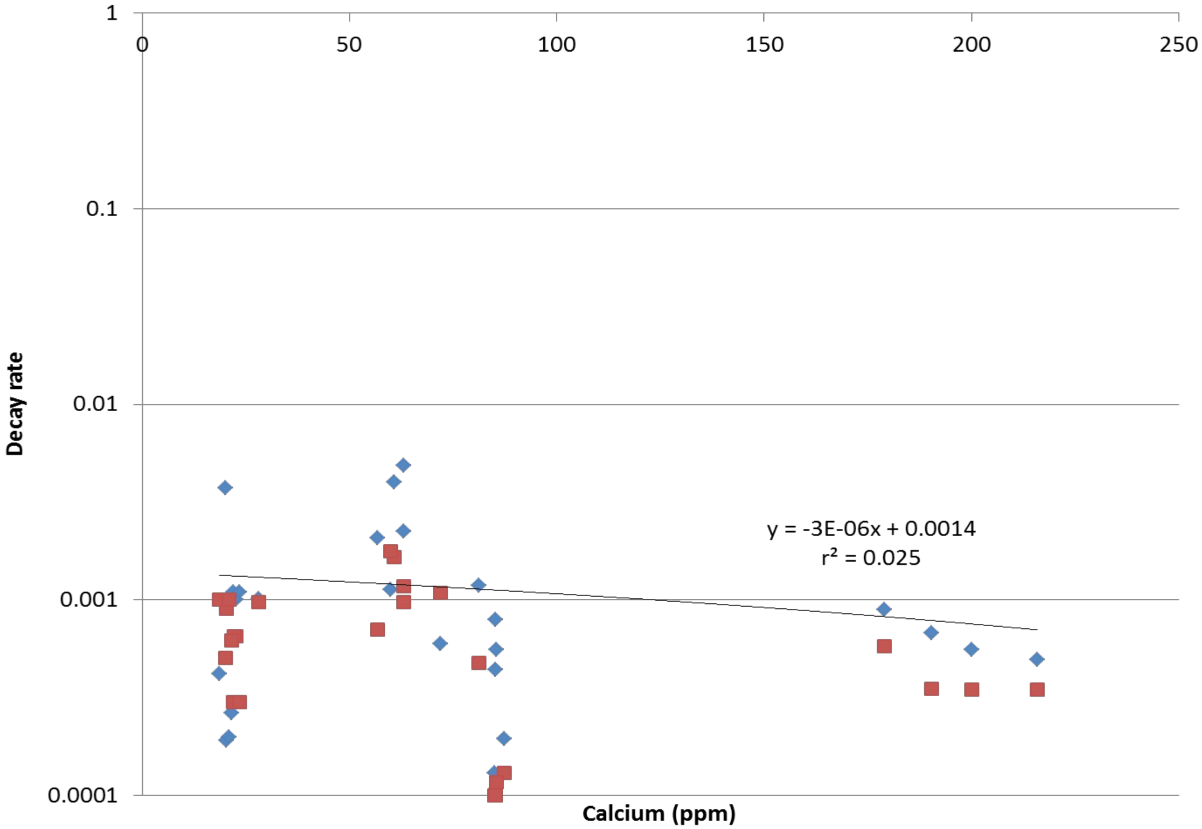


FIGURE 4 – 10. Decay rate of both species as a function of calcium linearized to better see trend.

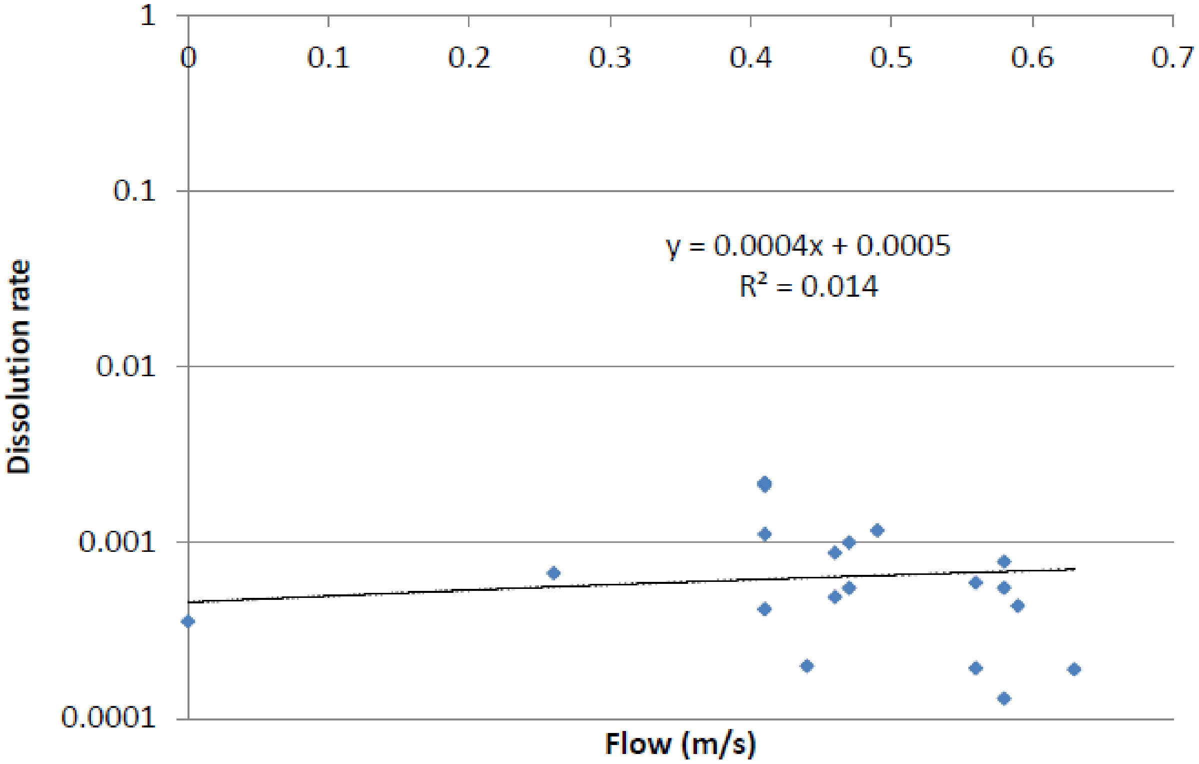


FIGURE 4 – 11. Relationship of flow (m/s) to shell decay across the duration of the study.

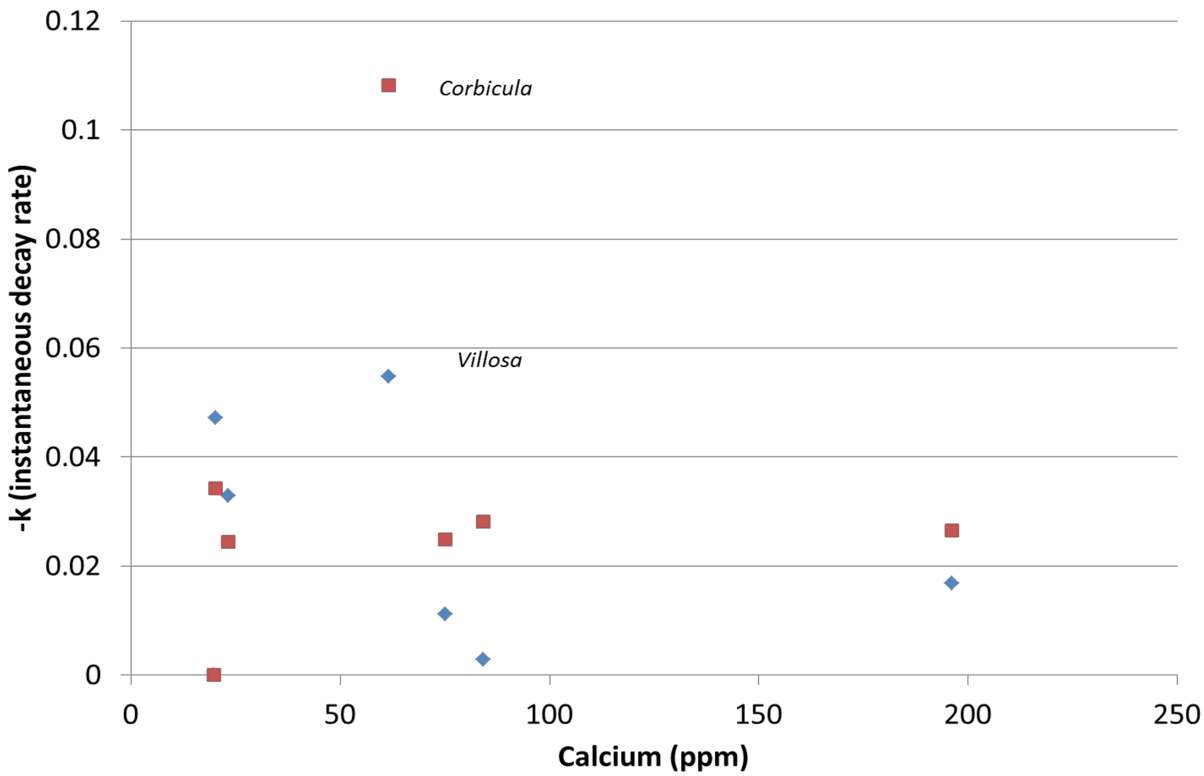


FIGURE 4 – 12. Instantaneous decay rate of *Corbicula* and *Villosa* as a function of calcium from 0 to 120 days.

CHAPTER V
DISCUSSION

Survivorship and Growth Rate of the Asian Clam

During the course of this study, a very clear relationship between the growth rate of the field caged clams and time developed. As the seasons changed and water temperature rose, growth rate rapidly accelerated (Figure 4-3). There was also a clear relationship between growth and conductivity. Although streams varied with respect to conductivity, the individual field sites maintained consistent conductivity levels during the course of the study. As water temperature rose, mean measurements of conductivity either remained relatively the same, or in some cases along the Ligias Fork for example, declined. In streams with cooler waters and elevated conductivity the clams either experienced regressed growth or stagnant growth; during the warmer seasons higher conductivities resulted in slower growth of individuals. In streams where conductivities remained low, however, greater growth rates were recorded.

Contrary to what was expected, the field caged *Corbicula fluminea* did not have significant mortality in any of the field sites. The one death that did occur was in Charleys Branch within the first 30 days of the study, a stream considered impaired for the majority of the study. Studies, though, have used field caged *Corbicula* to detect ecotoxicological impairment of streams (Hull et al. 2004). The 2004 study have measured mortality and sub-lethal end points such as growth rates. What has been discovered is that growth is more important than overall mortality because it integrates all physiological processes that occur in the organism and has been directly linked to ecotoxilogical impairment of bivalve populations (Hull et al. 2004). Even more significant for this study though, is that it has been noted that growth rate of field caged *Corbicula* is more sensitive than mortality (Hull et al 2004) as the suspension feeder can close their valves to regulate the ingestion of contaminants (Rooney 2010).

A significant result of this study was the regressed growth found in Charleys Branch, the Ligias Fork I and II and Buffalo Creek II. Negative growth was measured in all of these field sites at the 30-day interval. Ligias Fork II had negative growth measured until the 60-day interval. The Ligias Fork and Charleys Branch had significantly higher mean averages of conductivity, a proxy for stream impairment, than any other stream. Buffalo Creek II, however, never produced conductivities considered problematic during the course of this study. The stream does receive drainage from Straight Fork, which is impaired from an old pit mine, but this does not explain the regressed or overall slow growth rate of this field site. There was observed anthropogenic activity in this stream, such as ATV use, fishing and swimming, that was absent from the others. The mussel silos housing our field caged clams used for this study were also believed to have been removed from this site as well, before completion of the experiment, by someone not affiliated with the study. This outside activity may account for regressed/slow growth in the field area (fuel or motor oil as point source pollution, or physical abrasion of habitat) and introduced a bias, in general, to this locality.

The regressed growth exhibited by these clams is arguably a product of valve closure and regulation of the clams feeding mechanisms. The negative growth was small (approximately 0.02 mm) and we believe this may resemble the tightening of the clam shells as the specimens regulated their nutrient intake. The elevated conductivities of Charleys Branch and the Ligias Fork sites I and II indicate that, under the EPA water quality guidance program, the streams were polluted and possible impairment of aquatic communities is likely. As *Corbicula* are able to pump large volumes of water, when the pollution enters their system, the clams react to the contamination. *Corbicula* was actively filter feeding the pollutants and studies have shown (Viarengo and Canesi 1991; Rooney 2010) that this can lead to bivalves altering their physiology (Viarengo and Canesi 1991). Growth provides a sensitive measure of stress to an organism as growth represents an integration of major physiological responses, specifically between feeding and digestion (energy acquisition) to energy expenditure (metabolism and excretion). Studies have shown (Williams et al 1992; Widdows et al 1996) that growth can range from a maximum positive value in pristine/optimal conditions and decline to even negative values when the mollusc is severely stressed and utilizing body reserves. We believe that the negative growth recorded in these streams was the result of the *Corbicula* regulating its nutrient uptake by closing its shell and utilizing their body reserves until feeding could resume again in warmer, more nutrient rich waters.

In all field localities, conductivity played a significant role in the growth rate of our clams. The Ocoee River and Buffalo Creek I exhibited the lowest conductivities of all the fields sites and also the greatest growth. Mean conductivities of the Ocoee never breached 50 µS/cm and the greatest growth rates were found at this locality. Straight Fork had moderate conductivity levels, ranging from 173 – 320 µS/cm, and also moderate growth rates. Ligias Fork I and II, coupled with Charleys Branch, exhibited conductivities exceeding 300 µS/cm for the majority of the study and had similar low growth rates. This was a significant find as it supports our hypothesis that conductivity can be used as a proxy to examine freshwater bivalve habitat. As conductivity is a cumulative measure of ionic strength, our findings indicate that it is an effective indicator of molluscan impairment.

For many streams, the cumulative impact of elevated concentration of multiple stressors have led to biological impairment (Bernhardt and Palmer 2011). Ionic stress associated with elevated conductivities can prove toxic to aquatic communities as well as provide an indication of the additive impacts of solutes in the system (Bernhardt and Palmer 2011). The impact of these solutes can cause bivalve mollusks, such as clams, to close their shells when in contact with water that has unfavorable solute concentrations (McClary and Duffy 2008). This closing of the shell when exposed to elevated solutes further supports our measurements of regressed growth.

Conductivity has proven to be a valuable proxy for gauging stream health. It is widely supported that individual pollutants rarely exist alone in stream settings (Bernhardt and Palmer 2011). Conductivity allows for a measure of combined toxicity of individual stressors in field settings. My data documented that as conductivities increased, the growth rate of *Corbicula fluminea* was slowed. My results suggested that it will be unlikely to find unimpaired bivalve communities in areas of elevated conductivities.

The largest variable affecting growth of our caged clams outside of conductivity was water temperature. As the seasons changed from winter to summer, growth increased rapidly in every stream. A number of studies (Welch and Joy 1984; Dauble et al 1985; French and Schloesser 1991) have linked *Corbicula* survival and growth rates to water temperatures and seasonal variability of nutrients. Welch and Joy (1984) assigned three classes to their clams based on shell length: Class I (<10 mm); Class II (10 to 11.9 mm); and Class III (12 to 14 mm). The Asian clams where placed in separate cages in the Kanawha River at Marmet, West Virginia, for a 12-week period (16 July thru 7 October 1983) when mean water temperature was 26.6 °C (Welch and Joy 1984). “Warm water” growth rates (length/weight) were: Class I, 0.95 mm/week Class II, 0.86 mm/week; Class III, 0.80 mm/week. An additional 78 clams were assigned to the same shell length classes and maintained in the Kanawha River for a 12-week period (II October 1983 through 3 January 1984) when mean water temperature was 10.37 °C (Welch and Joy 1984). “Cold water” growth rates were: Class I, 0.09 mm/ week; Class II, 0.08 mm/week. Class

III clams were destroyed by a predator (Welch and Joy 1984). The study found that optimum growth rate occurred when streams had mean temperatures in the mid-20 °C range.

Coupled with warm water is the availability of more nutrients in a given system. Seasonality shifts from winter to summer months see a rise in photosynthesis and availability of nutrients, such as algae or plankton. Dauble et al (1985) found that *Corbicula* growth was directly dependent upon food supply and warm water temperatures. In cooler water temperatures, low phytoplankton densities, and nutrient garnishment resulted in low growth rates. French and Schloesser (1991) found that *Corbicula* growth occurred when water temperatures were above 9 °C. Maximum growth (0.78 mm/week) was measured between the months of August and September. During this period temperatures were between 16 – 23 °C (French and Schloesser 1991), the same temperatures that maximum growth occurred during my study. Conversely, in water temperatures below 9 °C, growth was found to be very low and sometimes immeasurable (French and Schloesser 1991). Higher mortality rates of the *Corbicula* were noted in the winter months as well. Over all, the study found that cold water reduced growth rates, delayed sexual maturity, delayed reproduction and increased mortality (French and Schloesser 1991).

These studies and others support the findings of accelerated growth rate over time as water temperatures increased. What is interesting is the independent relationship the regression analysis showed between conductivity and temperature. Though growth was seen to increase with time overall, even in the warmer waters we recorded slower growth rates in streams with higher conductivities. The findings indicated that even in seasons where *Corbicula* is reported to have maximum growth and nutrient availability, elevated conductivity, as an indicator of pollution and fouling of aquatic habitat, still negatively impacted the health of the Asian clam. The contamination of streams introduces multiple individual stressors to a given aquatic system. Whether or not individual contaminants will have a negative impact on aquatic organisms will be hard to measure, but the cumulative effect of elevated concentrations of multiple stressors is very clearly associated with the fouling of water quality and biological integrity (Bernhardt and Palmer 2011). All research to date suggests that conductivity is a very useful measure of the cumulative or additive impacts of the elevated concentrations of multiple contaminants that lead to biological impairment of streams (Bernhardt and Palmer 2011). Individual pollutants that enter a system raise conductivity and may have additive or multiplicative ecological impacts (Bernhardt and Palmer 2011). Our study supports this past research and supports our hypothesis stating that conductivity can be used as a measure of clam health.

Decay Rates of Death Assemblages

The decay of bivalve shells is ecologically significant because the spent shells are utilized by other organisms to engineer habitat. The shell itself provides habitat for smaller organisms, especially in soft sediments (Strayer and Malcom 2007). Shells also fill an important role in CO_2 and Ca^{2+} cycling in both lentic and lotic ecosystems. Though significant to freshwater systems, shell decay is rarely studied. $CaCO_3$ concentrations vary widely in freshwater environments and, as a result, shell decay of bivalve death assemblages is highly variable (Strayer and Malcom 2007). In freshwater ecosystems, rates of shell decay have been reported to depend highly on water chemistry, flow and among species of different size and composition (Strayer and Malcom 2007). My study further supports these data, though we used only two species of bivalves, which demonstrated a significant correlation in rates of decay.

Shell decay is related to stream water chemistry (notable to our study Ca^{2+} and pH) and stream velocity. These are the same parameters highlighted by Strayer and Malcom (2007) who evaluated the decay rates of four different freshwater bivalve species, one of which being *Corbicula fluminea*. Strayer and Malcom (2007) also found that the relationships recorded between shell decay rates and these parameters suggested that the decomposition rate of the shells, may be best controlled by extrinsic (water chemistry, stream current, physical abrasion) and intrinsic (shell size, mineralogy, chemical composition) factors. My study suggested that extrinsic factors play a more critical role in the decay of our death assemblages.

My analysis of shell decay followed the methods of Strayer and Malcom (2007) who recorded Ca^{2+} and pH as well as calculating (k) instantaneous decay rate. Their study found that elevated instantaneous decay rates correlated well with presence of a current and low Ca^{2+} (Strayer and Malcom 2007). The instantaneous decay rates, across their seven study sites, varied from 1.1% to greater than 99% a year (Strayer and Malcom 2007). The (k) decay rates correspond strongly with the presence of currents and low calcium. The study also found that the smaller the shell, the higher the decay (Strayer and Malcom 2007).

My study supports the findings of the 2007 study rather well, though our data points have a great deal of variance. Our results also suggests that decay is enhanced by stream velocity and waters under saturated with calcium. The instantaneous decay rates, however, varied only from

2.44 to 26.58%, much lower than the maximum 99% recorded from the Strayer study. My calcium measurements varied as well. Strayer and Malcom (2007) recorded a range of Ca^{2+} measurements from 56.6 to 396 ppm with and average across all field sites of 170.23 ppm. My study recorded a range of Ca measurements from 61.75 to 196.35 ppm with an average across all viable study sites of 87.09 ppm. Interestingly, the streams with low Ca^{2+} measurements, Buffalo Creek II (81.3 ppm) and the Ocoee River (61.75), both exhibited high decay rates, though Straight Fork, which boasted the lowest recorded calcium (23.53 ppm), and had very low decay rates. My study in the NRB found that higher pH resulted in longer half life, Strayer and Malcom (2007) found that increased pH resulted in decreased shell decay as well. The pH measurements between our two studies are very well correlated. Strayer and Malcom (2007) recorded a range of pH across their sites between 6.63 and 8.03, with a mean pH of 7.42. We recorded a range of pH between 4.56-9.00, with a mean across study sites of 7.84. There was also a significant correlation of decay among our *Villosa iris* and *Corbicula fluminea* death assemblages. Strayer and Malcom (2007) reported that smaller shells decay at higher rates than larger shells. *Villosa* is a larger organism than *Corbicula,* yet our study may suggest that *Villosa* and *Corbicula* are degrading at approximately the same rate.

My data rejected my original hypothesis as decay rate does not correlate with increased conductivity. The decay measurements were also not as statistically significant as our growth rate analysis. The data does support the Strayer and Malcom (2007) study, suggesting that flow and water chemistry are important when gauging shell decay, though our study places emphasis on stream current. The streams of the NRB also move a lot of sediment and rock fragments due to the anthropogenic activity occurring in the basin suggesting that mechanical abrasion is of high importance to the field area as well. Of particular importance to the study was the half life of our death assemblages. My data suggests that in as little as 10 years, evidence of more ancient populations may be gone in our streams, most notably, the Ocoee River. As shells are used for habitat engineering by other organisms, the abundance of native to invasive species, and their shells, needs to be further investigated to gain further information on the functional role of these shells in freshwater habitat.

CHAPTER VI
CONCLUSION

The field caged clams had very high survivability (only five mortalities) over the course of the 150 day study. Though growth was regressed, stagnant or slow during the winter months, when water temperature began to rise with seasonality our life assemblages all experienced enhanced growth rates. This change in growth indicated that water temperature was a very significant variable influencing the growth of *Corbicula fluminea*. In streams with impaired water quality, conductivity proved to be independent of temperature in its importance to clam health and growth rate. Streams with elevated conductivities, even in the warmer months, experienced slower growth rates than our control stream and streams considered to not be impaired in the New River watershed. My study provides supporting evidence that conductivity is an effective measurement of the cumulative or additive impacts of elevated concentrations of individual stressors and can be used to predict water quality and aquatic biology impairment.

Data collected from the decay rates of the death assemblages were not as statistically significant as the living field caged clam study. The decay experiment also rejected our original hypothesis that streams with higher conductivities would result in higher rates of shell degradation. My data does support the findings of Strayer and Malcom (2007) who reported that high decay rates correlated with elevated stream current, low pH and low Ca^{2+} concentrations. My study supports these results and places emphasis on stream current and mechanical abrasion from sediment flow. My study found that, in as little as a decade, shells from deceased populations may disappear from our field areas. A potential bias was introduced to the decay experiment. Protein from the shells was lost during each laboratory analysis. Future experiments should scrape off all of the periostracum from the death assemblages to obtain better data from weight measurements.

As I only used the invasive Asian clam, future studies may include using an abundant, native mussel population of the NRB to compare growth and mortality rates between the two species. As *Corbicula* tends to respond better to pollutants than natives, I believe that endemic populations may be more sensitive to elevated conductivities. Using different numbers of individuals in the silos may also be employed so that growth rates among less and more dense populations can be determined. This would help determine if habitat density also affects growth. Finally, noting the amount of juvenile, mid-sized and adult individuals could be useful as well.

Juveniles grow at a faster rate than adults, and adult specimens can reach a maximum growth limit. Keeping track of individual growth rates among different aged individuals can add statistical significance to future studies.

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Vita

Grant Mincy was born April 16, 1984, in Michigan. After a number of moves, his family settled in Maryville, Tennessee, in 1997 where he graduated from William Blount High School in 2002. He graduated from the University of Tennessee, Knoxville, with a BS in Geology in 2007. The 2008 - 2009 service year Grant worked for the Washington Conservation Corps, a national civil service program of Americorps. During this time he became very interested in stream health, aquatic habitat and restoration ecology. He is currently finishing his MS in geology with a concentration in environmental geology and conservation.