

DEMOGRAPHY OF FRESHWATER MUSSELS WITHIN THE LOWER FLINT RIVER

BASIN, SOUTHWEST GEORGIA

by

JUSTIN C. DYCUS

(Under the Direction of Robert Bringolf)

ABSTRACT

Environmental and spatial variation can potentially influence mussel populations through acute and chronic mechanisms. The objectives of this study were to identify and quantify the chronic factors affecting freshwater mussel growth. Live mussels were collected within the lower Flint River Basin, sacrificed, and their shells were thin-sectioned. Thin sections revealed the production of internal annuli, which were used to determine individual ages and estimate annual growth. I evaluated the relation between annual growth and presumed variables responsible for altering growth using mixed linear models. Growth was indicated to vary in relation to seasonal streamflow, species, age, tagging, channel confinement, and physiographic province. The effect of tagging should be accounted for in subsequent mark-recapture studies, and species- and site-specific characteristics should be considered when implementing management decisions to prevent future harm to freshwater mussel populations.

INDEX WORDS: Thin Section, Annuli, Freshwater Mussel, Streamflow, Umbo, *Villosa*

*lienosa*, *Villosa vibex*, *Elliptio crassidens*

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A.S., Sandhills Community College, 2006  
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## DEDICATION

I dedicate this thesis to my parents, Charles and Amy Dycus, who first instilled my love for all things outdoors and raised me to be who I am today. Though both may not fully know and understand all that I have done during my enrollment at the University of Georgia, they have loved and supported me every step of the way.

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Upon graduation from North Carolina State University, I quickly realized if I wanted to pursue a career in fisheries, then I would have to obtain a Master of Science degree. With guidance and support from my former professor, mentor and friend, Dr. Tom Kwak, in three months I managed to obtain an interview with Dr. Jim Peterson, at the University of Georgia. During my interview, I realized Dr. Peterson wanted me to conduct research on freshwater mussels; a species I formerly had very little knowledge or interest in. After some consideration, I realized that I wanted to conduct stream research regardless the species of interest, Dr. Peterson's reputation put him top of the list of professors I had inspired to work with, and so I decided to accept the assistantship. Before my enrollment at the University of Georgia, the only time I had spent in Georgia was at gas stations off Interstate 95 as I traveled south to Florida. Each place I visited and person I met were brand new to me, but I am thankful that individuals within Warnell quickly made Georgia feel like home.

First, I would like to acknowledge my major professor, Dr. James T. Peterson, for his patience and guidance on this work. Dr. Peterson has been a great mentor and has helped further my abilities as a biologist. Whether allowing me to attend workshops, conferences, or volunteer opportunities, Dr. Peterson has always been supportive of any activities to further my education and work experience in fisheries. Dr. Peterson is one of the most brilliant minds I have had the honor to work with and I can only aspire to one day obtain similar knowledge and understanding of the ecological processes driving the world in which we live.

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## CHAPTER 1

### INTRODUCTION

North America stands out as having the world's largest variety of freshwater mussels that also are among the most imperiled animal communities (Stansbery 1979, Williams et al. 1993). Of the nearly 300 species of North American fresh water mussels, 70% are extinct, endangered, or listed as a species of special concern (Williams et al. 1993). The highest level of freshwater mussel diversity occurs within southeastern United States (i.e., Alabama 178 species, Tennessee 129 species, and Georgia 123 species; Williams et al. 2008). Throughout the Southeast, mussel populations are declining at a substantial rate (McMahon 1991, Haag and Warren 2007). To develop strategies for reversing the decline of freshwater mussel populations, natural resource managers need information on mussel population responses to anthropogenic and environmental changes.

Several anthropogenic factors reportedly affect aquatic biota in the Southeast. Human populations are steadily increasing throughout the region (U.S. Census Bureau 2000). With an increase in population numbers, there is an increased demand for water that is often accommodated by construction of reservoirs, via impounding streams and rivers. Impoundments alter the downstream flow regime and can influence the abundance and distribution of mussel populations (Baxter 1977, Poff et al. 1997, Allan and Castillo 2007). Human population growth in the region also is responsible, in part, for the increased demand for agriculture goods for consumption and biofuel production. Southeastern farmers generally produce 2-4 crops a year

within the fertile soils in the Coastal Plain physiographic province and many of these fields are irrigated (Cummings et al. 2001). Increased agricultural water demand too can negatively affect the flow regime downstream of irrigation systems and can alter freshwater mussel persistence (Vaughn 2010). The recent increased human population within the Southeast also has been accompanied by increased land development with substantial increases in urban and exurban construction. Land development leads to various scales of land clearing and increases in impervious surfaces, which increases the amount of sedimentation and pollutants that reach adjacent streams and decreases water quality (Dunne and Leopold 1978, Williams et al. 2008). The United States Environmental Protection Agency (EPA) classified sedimentation as the most common form of pollutant within United States streams and rivers (U.S. EPA 1990, Williams et al. 2008). These alterations of water quality within streams can also affect mussel growth (Williams et al. 2008, Vaughn 2010).

Climate change has recently influenced temperature and precipitation patterns in the southeastern United States. The annual average temperature has been slowly increasing in the past century leading to increased water temperatures (USGCRP 2009). In addition, the time between rainfall intervals has increased resulting in a decrease in annual precipitation (USGCRP 2009). The Southeast also has been experiencing drought during the majority of the past decade, leaving some streams with very low or no flow (USGS 2010). Decreased streamflows can cause increased stream temperatures, decreased dissolved oxygen, and can reduce the potential amount of suspended food particles for mussel consumption (Spooner and Vaughn 2008). All of which are potentially detrimental to mussel populations.

The potential threats to freshwater mussels posed by anthropogenic activities in the Southeast are typified in the Flint River Basin, Georgia. The Flint River begins at Hartsfield-

Jackson Atlanta International Airport and drains southwest until it connects with the Chattahoochee River forming Lake Seminole in the far southwestern corner of Georgia. The upper portion of the basin is contained in the Piedmont physiographic province and is significantly altered by urbanization and impoundment for water supply for the adjacent metropolitan Atlanta area. The lower portion of the basin is located in the Coastal Plain physiographic province and is primarily irrigated agricultural land use, producing around 80 percent of the state's row crops (McDowell 2005). The lower portion of the Flint River (henceforth, LFRB) also contains among the most diverse and unique assemblage of aquatic species in the United States (Couch et al. 1996). The LFRB was historically occupied by approximately 30 species of freshwater mussels, but recent research suggests that only 22 species are present in the drainage today (Table 1; Brim Box and Williams 2000). A wide range of factors are reportedly responsible for the decline of freshwater mussel populations in the LFRB including: land use changes, human population increases, irrigation, channelization of streams, pollution, stream impoundment and stream degradation (Strayer et al. 2004, Haag and Warren 2007, Cope et al. 2008). Of these, water development and use along with other anthropogenic activities associated with agricultural practices (e.g., center-pivot irrigation, clear-cutting, and stream impoundment) are believed to be the primary factors affecting mussels in the LFRB. However, the mechanisms that cause the population declines (e.g., reproductive failure, low survival) are poorly understood for many species (Haag and Warren 2008). Incomplete knowledge of these factors makes it difficult to develop and implement conservation and recovery plans for freshwater mussel populations in the LFRB. Therefore, I propose to study freshwater mussels in the LFRB with the following objectives.

- 1) I will estimate the age and growth of three species of freshwater mussels in the lower Flint River Basin.
- 2) I will evaluate the influence of stream and watershed characteristics and streamflows on mussel growth.
- 3) I will evaluate mussel year class strength.

## CHAPTER 2

### LITERATURE REVIEW

The unusual life history characteristics and ecological requirements of freshwater mussels are believed to strongly influence their distribution patterns (Vaughn 1997). Unionidae, a family of Unioniformes (freshwater mussels) are comprised of approximately 120 genera and have a multifaceted life history in which the larvae (glochidia) are obligate parasites on the gills or fins of fish (Williams et al. 2008). Most freshwater mussels are dioecious, in which male mussels release their gametes into the water column with the anticipation that a fertile female will siphon in the gametes to produce viable glochidia. Studies have shown that some populations of male mussels are often located upstream of females to assist in the fertilization process (Williams et al. 2008). It has been documented that Unionidae species produce between 9,000 and 750,000 glochidia annually, but there is no clear relationship between mussel size and fecundity (Williams et al. 2008). Many glochidia can survive and transform to juveniles on a narrow range of fish species as hosts (Way 1988, Watters 1994). Once glochidia transform into juveniles and shed from their host, they burrow into the substrate below. Juveniles remain buried and pedal feed with their ciliated foot to prevent being swept downstream (Yeager et al. 1994, Williams et al. 2008). Since only juveniles that shed or drift into suitable habitat will survive, few individuals survive to adulthood (Neves and Widlak 1987).

Mussels are capable of obtaining food and nutrition needed for growth and survival by one of two methods. First, by siphoning water from within the lower portion of the water column

using their apertures. Water enters the mussel within the incurrent aperture, which removes the nutrients and bacteria desired for consumption and releases non-consumptive water through another aperture used for excretion purposes. This method is described as the filter- or suspension-feeding method (Williams et al. 2008, Vaughn 2010). Some mussels also feed using a method described as deposit or pedal feeding, in which the mussel uses its ciliated foot to sweep through the substrate releasing desired bacterium and nutrients for consumption (William et al. 2008, Vaughn 2010). The rate at which mussels are capable of filtering water/feeding is related to water temperature, streamflow, and varies among mussel species (Spooner and Vaughn 2008).

Streamflow reportedly affects mussel survival and reproduction through both acute and chronic mechanisms. Acute mechanisms are defined here as those factors causing immediate death or preventing reproduction, whereas chronic mechanisms are those factors that cause long-term stress that can result in reduced fecundity and slower growth. Of these, the acute mechanisms are the best understood. Low streamflows affect stream temperatures, dissolved oxygen, and the availability of nutrients and bacteria within a stream that are required for mussel growth (Galbraith and Vaughn 2009). Extended periods of drought can cause high temperatures and low stream flows, including complete drying of the streambed, resulting in desiccation and direct mass mussel mortality (Johnson 2001, Haag and Warren 2008). During drought periods, mussel populations can become isolated in pools or shallow stream stretches, which can increase predation by terrestrial vertebrates (i.e., muskrats and raccoons; Johnson 2001). Low flows also can negatively affect obligate host fish (McCargo and Peterson 2010), potentially preventing or reducing reproductive success (Galbraith and Vaughn 2009). In contrast to low flows, mussels can become susceptible to the effect of river scour during high flows and can become flushed

downstream causing direct mortality (Vaughn 1997, Strayer et al. 2004). After extended periods of high flushing flows, extensive stretches of river bottom can be left with sparse mussel distribution (Strayer et al. 2004). Mussels also become susceptible to suffocation during high flows in response to increased movement of fine sediments along the stream bottom (Strayer et al. 2004).

Although the acute effects of streamflows on mussels are well documented, much less is known about the chronic effects of streamflows on mussel populations. Mussels reportedly have optimal feeding temperatures and streamflows (Vaughn and Hakenkamp 2001). Therefore, changes to the flow regime could affect mussel growth. For example, low flows decrease the overlying volume of water capable of distributing nutrients and dissolved oxygen to filter-feeding, limited mobility, freshwater mussels. Stream temperatures generally increase during low flow periods in the summer months and can limit the filtration rate of mussel species and further induce stress (Vaughn and Hakenkamp 2001). During periods of high flows, mussels may burrow within the substrate to prevent being washed downstream, thus preventing filter feeding (Strayer et al. 2004). High flows increase the overlying volume of water subsequently decreasing the filtration efficiency of freshwater mussels (Vaughn and Hakenkamp 2001). Suspended sediments are also generally higher and visibility lower during periods of high streamflow, which can limit the reproductive success of mussels that use lures to attract specific host fish (Haag and Warren 1998, Haag et al. 1999). High flows also decrease reproductive success of broadcast spawners by increasing the present volume of water and decreasing the likelihood that mussel glochidia will encounter a proper host fish. Upon the observation of annuli to reference growth with historical flow and water quality data, this study plans to increase the knowledge of chronic stressors on mussel populations.

Freshwater mussels demonstrate indeterminate growth, defined as growth that continues after maturation allowing species to grow throughout their lifespan (Sebens 1987, Heino and Kaitala 1999, Karkach 2006). Freshwater mussels grow seasonally, secreting shell material during a portion of the year followed by a dormant season that mussels cease growing and lay down a corresponding annulus. Mussels can be aged by the cyclic production of annual growth rings (i.e. annuli), which denote periods of varied growth throughout their life history. Because freshwater mussel annuli represent periods of varied growth, they also can reflect environmental conditions during an individual's life. For example, dissolved oxygen concentrations, water temperature and the availability and concentration of suspended food particles vary with streamflows (Rypel et al. 2008). Consequently, growth can vary in response to alterations in flow regimes (Vaughn and Taylor 1999, Galbraith and Vaughn 2009). Alteration of the flow regimes can occur naturally (e.g., during droughts) and may be, in part, due to effects of impoundments, irrigation, and municipal withdrawal (Watters 1994, Vaughn and Taylor 1999). Thus, mussel growth can vary from stream to stream and through time.

Mussel's growth may slow or cease when mussels experience stress. Freshwater mussels experiencing periods of stress deposit pseudo-annuli, which can have negative effects on mussel aging techniques (Johnson 2001, Rypel et al. 2008). Types of induced stress may include; predation attempts, increased levels of sedimentation, poor water quality, low nutrient availability (Richter et al. 1997). Observing the mechanisms responsible for inducing chronic stress and altering mussel growth could lead to better future conservation strategies of remnant mussel populations.

## CHAPTER 3

### METHODS

#### *Study Area*

I studied the age and growth of freshwater mussels in the lower Flint River Basin (LFRB), located in southwest Georgia (Figure 1). The basin contains the Fall Line Hills and Dougherty Plain districts of the Coastal Plain physiographic province. Streams located within the Fall Line Hills district receive most of their water contribution from surface water runoff during base flows and are characterized by sandy-mud substrate with higher turbidity. Streams within the Dougherty Plain district receive substantial amounts of water input from the underlying Floridan aquifer and tend to have greater amounts of coarse substrates and lower turbidity (Mosner 2002, Peterson et al. 2009). Sample sites were selected based on focal species (defined below) presence determined by post-2000 and post-2007 research conducted throughout the LFRB, of which 130 and 32 sample sites, respectively were sampled for species presence (Shea 2011). In total, post-2000 and post-2007 mussel surveys found *V. lienosa* and *V. vibex* to be the 2<sup>nd</sup> and 3<sup>rd</sup> most abundant species in the LFRB; found at 40 and 38% of sample sites, respectively (Shea 2011). To potentially minimize sampling effort and increase sample size of focal species; I selected sites stratified throughout the LFRB with the highest abundance (i.e., greatest probability of capture) of the three focal species (C. Shea, University of Georgia, personnel communication). Several streams had multiple sample sites to account for possible

variation of growth in response to longitudinal gradients. Mussels were sampled from a minimum 9 sites within each stratum and 20 sites total (Figure 1).

### *Focal Species*

I studied age and growth of three mussel species *Villosa vibex* (southern rainbow), *Villosa lienosa* (little spectaclecase), and *Elliptio crassidens* (elephantear). These three freshwater mussel species were chosen because their populations are relatively stable (Table 1), they are distributed throughout the LFRB, and are relatively easy to identify (Williams et al. 2008, Shea 2011), which would minimize any potential biases because of species misidentification (Shea et al. 2011). The *Villosa* species appeared to be the most similar in this study, but there are distinct differences. *Villosa vibex* differs from *V. lienosa* by their more broadly rounded posterior margin, prominent green rays, thinner shell, bluish white nacre, and the dark pigmentation that extends the ventral length of the mantle (Williams et al. 2008). The nacre of *V. lienosa* differs from *V. vibex* in that it is typically a shade of purple or salmon, and the pigmentation along the ventral mantel margin ends anteriorly to the mantle fold (Williams et al. 2008). *Elliptio crassidens* is a more robust species, which lacks any rays as an adult, and has a light to dark brown colored periostracum. Unlike other mainstem LFRB long-lived, thick-shelled, freshwater mussels, *E. crassidens* lack any form of shell plication (i.e., *Elliptoideus sloatianus*, *Megalonaia nervosa*, *Amblema neislerii*; Williams et al. 2008).

*Villosa vibex* is a short-lived, thin-shelled, unionid species. Their distribution ranges from Atlantic and Gulf Coast drainages as far north as North Carolina, south as Florida, and west as Louisiana. *Villosa vibex* can be found in a variety of stream sizes from small creeks to large rivers, and occur in substrates of mixtures of sand, clay, and gravel. This species is a long-term brooder that is gravid from late summer or autumn until the next summer (Williams et al. 2008).

*Villosa lienosa* is a short-lived, thin-shelled, unionid species. Their distribution ranges as far north as the Ohio and Cumberland River drainages to the Gulf Coast drainages in Florida and as far west as San Jacinto River drainage in Texas. *Villosa lienosa* can persist and reproduce in a variety of stream sizes and substrate types, ranging from small headwater streams to large rivers (Williams et al. 2008). The species persist in such a wide variety of habitats presumably, because of its reproductive strategy of attracting host fish with a visual lure (i.e., mantle flaps; Haag and Warren 1998). *Villosa lienosa* is a long-term brooder found gravid from late summer to autumn through the following summer (Williams et al. 2008).

*Elliptio crassidens* is a long-lived, thick-shelled, unionid species. They are a reportedly widespread species found predominantly in riverine systems. This species has been collected as far north as Minnesota, as far south as Louisiana, and as far east as the Gulf Coast drainages of Georgia. *Elliptio crassidens* inhabit substrates composed of sand, mud, gravel, and cobble. The only known host fish of *E. crassidens* glochidia is *Alosa chrysocloris* (skipjack herring), which may explain why few individuals are found outside of larger river systems. *Elliptio crassidens* are short-term brooders and are gravid from April to August, depending on geographic location.

#### *Mussel Sampling and Aging*

Specimens were collected from each site by hand from November 2009 through August 2010. Mussels within several streams were previously tagged with 8mm x 4mm oval Hallprint shellfish tags (Hallprint Pty Ltd, Victor Harbor, South Australia) affixed with cyanoacrylate glue (Peterson et al. 2011). We recorded the tag number for recaptured mussels to account for the potential effects of handling and marking on growth during the analysis.

Validation of annulus formation is imperative when deriving information on age and growth from annuli production (Beamish and McFarlane 1983). To confirm that presumed annuli

corresponded to annual growth checks, individual mussels of two focal species *V. lienosa* and *V. vibex*, collected during sampling conducted between June and July 2009, were notched using the triangular edge of a rectangular file. A notch produced a small triangular indentation in the margin of the mussel's valves directly below the umbo. Notched mussels were tagged or had been previously tagged, returned by hand orientated anteriorly into the stream reach in which they were collected, and left for a minimum of 1 year before recapture. Tagged and recaptured mussels provided known age and growth since first capture, while notching illustrated both visual signs of external and internal growth. Previously notched recaptured individuals provided visual proof of external annual growth, while thin sectioning through the notch (detailed below) documented the production of internal annuli.

During sampling conducted post notching (November 2009 through August 2010), field crews sampled each stream for approximately 1 hour, and then examined the collected specimens. In sites where mussel numbers appeared plentiful, 20-30 individuals of each focal species, representing all size classes, were collected. In sites that insufficient numbers of focal species were collected after minimum standard effort, additional sampling was conducted to increase the sample size. If population numbers appeared to be very low in a sample site (i.e., number of mussels collected were <10), mussel specimens were returned to the stream to prevent harm to the freshwater mussel populations. While in the field, all collected mussels were identified to species by field personnel, measured (i.e., length, width, height) to the nearest millimeter with 144 mm plastic dial calipers, and sacrificed by separating the left and right valves along the umbo. Both valves were assigned unique identifiers to ensure that matching shells remain paired. After returning to the lab, each specimen was placed in an individual plastic bag labeled with the following information: the scientific name, stream name, georeferenced

location, sample date, individual reference number, and sampling personnel. Labeled bags were then stored in the lab.

Accurately estimating age of freshwater mussels is essential when studying their population demographics (Neves and Moyer 1988). Thin sectioning is reportedly the most reliable freshwater mussel aging technique (Neves and Moyer 1988, Haag and Commens-Carson 2008), and is therefore the method that I used to evaluate the age and growth of mussels. All thin sections were produced using the right valve of each mussel. Mussel shells were sectioned using a Buehler Isomet low speed saw (Buehler Ltd., Evanston, Illinois), with a Series 15HC diamond impregnated blade (Buehler Ltd., Evanston, Illinois). The first cut was made from the umbo down to the ventral margin. Once the first cut was made both halves were visually inspected, and the best half of the valve (i.e., smooth flat surface without chips and burrs) was polished sequentially using four grades of sandpaper: 320, 400, 600 and 2000 grit, respectively. Remaining imperfections were removed by polishing the section with a 203 mm Buehler MicroCloth polishing pad (Buehler Ltd., Evanston, Illinois). I used a repetitive figure eight motion for all sanding and polishing. After polishing, the valve was cleaned with water to remove dust and debris, wiped dry with a paper towel, and mounted to a Fisherfinest Premium fully frosted 25.4 mm x 76.2 mm x 1 mm microscope slide (Fisher Scientific, Pittsburgh, Pennsylvania) with Loctite 5 Minute Instant Epoxy (Henkel Corporation, Westlake, Ohio). After the epoxy dried, the slide with the mounted half mussel shell was affixed to a wafering chuck with paraffin wax, attached to the lowering arm of the low speed Isomet saw, and cut to produce the thin section. The thin section then was sanded and polished using the five step polishing process, outlined above. To prevent misidentification, each microscope slide with attached thin section was labeled with the following information: scientific name of specimen, stream name,

georeferenced location, sample date, individual reference number, and placed into a labeled microscope slide box.

Mounted thin shell sections were photographed using a Leica MZ6 modular stereomicroscope with 6.3:1 zoom equipped with a Leica DFC295 digital microscope color camera. Photographs were taken at 0.63x magnification and analyzed using Image-Pro Plus (version 7.0, Media Cybernetics, Inc., Bethesda, Maryland). Mussel thin sections that were too large to be captured in a single image were photographed using Image Pro's tiling procedure (version 7.0, Media Cybernetics, Inc., Bethesda, Maryland) that consisted of taking a series of photographs along a common axis and combining them into a single image based on spatially autocorrelated similarities (R. Bunn, Vashaw Scientific Inc., personnel communication). All electronic images were labeled and organized by species and sample site.

Freshwater mussels have been documented to produce internal annuli (Neves and Moyer 1988, Haag and Commens-Carson 2008, Rypel et al. 2008), but it is important to validate annuli production in focal species to increase the validity of one's research (Haag and Commens-Carson 2008). Mussels were aged by identifying and counting the number of annuli present in each thin section image using a multiple-observer method. Before any freshwater mussels were aged in this study, all three observers met and discussed the features denoting internal annuli for the three focal species of freshwater mussels. Distinction between true versus pseudo-annuli was illustrated by observing previously photographed *E. crassidens*, *V. lienosa* and *V. vibex* thin sections. True annuli originated from the umbo of the thin section and were traceable to the margin of the shell (Neves and Moyer 1988, Haag and Commens-Carson 2008). Some annuli exhibited faint lines, which at times were difficult to trace, but other contributing characteristics added to the classification of true annuli versus pseudo-annuli. True annuli often were

accompanied by a corresponding halo, which would surround the annulus as it joined the ventral margin of the thin section (Haag and Commens-Carson 2008, Rypel et al. 2008). In addition, annuli commonly corresponded with the cyclic production of a light band of growth (initial annual growth), then a dark band of growth (decreasing growth), and finally followed by an annulus (i.e., traceable dark line marking ceased winter growth; Figure 2).

The multiple-observer method included 3 observers that independently identified annuli and marked their presumed location on the thin section image. The age of a mussel was estimated as the sum of the number of marked annuli. Observers also recorded the species, sample site, reference number, and estimated age in a separate database. The observer-specific estimated annuli and ages of the individual mussels then were compared and used to evaluate the initial observer agreement. The initial observer agreement represented the percent of time observers independently agreed on a mussel's age. In addition, the average difference in estimated ages among observers was estimated by calculating the absolute difference between observer-specific estimates for each mussel. The differences for each mussel then were averaged. Because ageing error was likely related to the age of a mussel, I also calculated the relative difference in estimated ages by dividing the absolute difference by the ages determined during a concert reading session (described below).

The final annuli used to estimate mussel age and growth were determined during a concert read in which mussel shell images were viewed simultaneously by all 3 observers. Annuli and reference points (detailed below) were identified by consensus. Thin section images that had been previously marked during the independent observer readings were compared to distinguish the agreed annuli. If there was 100% initial observer agreement, then those annuli were kept constant during the concert read. If there were discrepancies between independent

observer readings, then agreed upon annuli (i.e., annuli marked in all three independently marked images) were marked first, then observers discussed the placement of further annuli. Consensus annuli were identified and marked on the thin section images, and the mussel ages were estimated as the total number of consensus annuli. If observers failed to reach a consensus on the location of annuli, then the mussel was removed from the study. During the concert read, observers also collectively identified and marked a reference point used to measure annual height-at-age for each thin section. The reference point was placed at the highest arch of the umbo (Figure 2) that corresponded to the longest distance capable of being measured in the field with the 144 mm plastic dial calipers to measure mussel height (i.e., umbo to ventral margin measurement). To account for the potential effects of shell wear (i.e., erosion) on growth measurements, the amount of shell wear from the umbo to the margin was visually estimated by consensus as the percentage of external shell of each thin section that was eroded (Figure 2). For each mussel, the height-at-age was estimated by measuring the distance from the reference point to each annulus on the shell margin (Figure 3) to the nearest 0.00001 mm. To ensure accurate measurements, Image-Pro was calibrated using a DR-867 two millimeter in 200 divisions stage micrometer (Klarmani Ruling, Inc., Litchfield, New Hampshire) that was certified by the National Institute of Standards and Technology (NIST; April 5, 2010). Annual growth was estimated as the difference between the linear measurements of height-at-age minus the previous year's height-at-age (Figure 3); with an exception of the 1<sup>st</sup> year's growth that was estimated as the amount of growth from the reference point to the 1<sup>st</sup> annulus.

#### *Verification of Annuli*

*Villosa lienosa* and *V. vibex* that had previously been notched aided in the distinction between true and pseudo-annuli production. Validation of true annuli versus pseudo-annuli

production increased the accuracy of observer age verification (Beamish and McFarlane 1983, Campana 2001). Accurate verification of annuli production required observers to agree that notched thin sections only had one true annulus post notching. Notched thin section photographs were mixed in with non-notched thin sections, and randomly placed in the aging sets. Observers blindly aged all individuals, while following the above criteria to distinguish true annuli. During the concert read, notched thin sections were identified and closely observed to compare age and annuli placement. If all 3 observers marked 1 annulus in the same location post notching for a specimen, then annuli production was considered validated for that specimen. If observers marked multiple annuli or 1 annulus in multiple locations post notching, then observers discussed the discrepancy and attempted to reach a consensus. If observers failed to reach a consensus or agreed on multiple annuli post notching, then annuli production lacked validation for that specimen. If more than 50% of notched specimens for a species had validated annuli production, then I assumed that the species of freshwater mussel had valid annuli production.

#### *Definitions and Analysis*

Presumably, streamflows influence freshwater mussel life history, yet little is known about this relationship (Rypel et al. 2008). To identify the streamflow regime components that had the greatest influence on mussel growth required daily discharge data for each study site. Daily discharge data were obtained for sample sites that were located immediately up or down stream (< 5 km away) of a USGS stream gage (USGS 2011). For ungaged streams, I estimated discharge using published discharge models (McCargo and Peterson 2010) when available. For the remaining sites, I used linear regression (Sokal and Rohlf 1995) to develop site-specific models relating discharge measured during sampling at the ungaged sites to average daily discharge data from 8 long-term U.S. Geological Survey (USGS) gaging stations located in the

lower Flint River Basin (Figure 1). The best-approximating model for each study site was selected as that with the largest coefficient of determination ( $r^2$ ). For each model, linear regression residuals were checked to ensure that regression assumptions regarding normality and homogeneity of variance were met. When necessary, discharge data were natural log transformed. The best-approximating models were used to estimate daily discharge at the ungaged study sites for the mussel growth record. The observed and estimated daily discharges at the gaged and ungaged sites, respectively, then were used to calculate seasonal stream flow statistics.

One of my primary objectives was to evaluate the relative influence of streamflows on freshwater mussel growth. Previous studies suggest that various components of the flow regime influence physical and ecological processes in streams that could vary seasonally (Craven et al. 2010, McCargo and Peterson 2010, and Peterson et al. 2011). Thus, I characterized the flow regime for each season using three components: short-term low flows, short-term high flows, and long-term average flow conditions. Short-term low flows were characterized by the 10-day minimum discharge that was calculated as the lowest average discharge for 10 consecutive days during a season. Short-term high flows were characterized by the 10-day maximum discharge that was calculated as the highest average discharge for 10 consecutive days during a season. Long-term average flow conditions were characterized as the median discharge for each season of recorded mussel growth. I only considered the effect of streamflows during growing seasons that were defined as spring, March-June, and summer, July-October, following Peterson et al. (2011). The remaining months were excluded because previous studies report that freshwater mussels become dormant in the winter months with little or no growth (Haag and Commens-Carson 2008, Rypel et al. 2008). Additionally, my annulus verification (discussed below)

indicated that winter was the period of slow growth and annuli deposition. All flow regime components were adjusted prior to analysis by dividing each by the contributing watershed area upstream of each site to allow for comparison of the effects of stream flows on very different sized streams. To calculate contributing watershed area, I delineated the watershed boundaries using the Georgia Land-use Trend Program (GLUT 2005) 30 m DEM and the ArcGIS watershed tool to create watershed boundaries upstream of each study site (Esri 2011).

To evaluate the relative influence of stream size and hydro-geomorphology on mussel growth, sample sites were characterized based on physiographic province, gross stream channel morphology, stream size, and land cover in the contributing watershed. Sites were classified as within either the Dougherty Plain or Fall Line Hills physiographic province based on their location within the LFRB using the physiographic map of Georgia (USGS 1996) in ArcGIS (Esri 2011). Stream size was characterized at each study site using link magnitude, which was defined as the number of first-order stream segments located upstream from a given stream reach, which each site was located within (Shreve 1966). Link magnitude was calculated by manually counting the number of first order tributaries contributing to each sampled stream reach based on 1:24,000 NHD stream network layers (USGS 2001b). Gross channel morphology was defined as either a confined or an unconfined channel following Peterson et al. (2009). Confined channels were single-threaded with high, well-defined banks and infrequent overbank flow, while unconfined channels featured low and indistinct banks, braided channels, and seasonal direct connections between channel flows and floodplain wetlands (Peterson et al. 2009). Land cover was classified as urban, agricultural, and other and was estimated using the 2001 National Land Cover Dataset (USGS 2001a). The original land cover data set consisted of 15 classification schemes (USGS 2001a) that I grouped into urban, agriculture, and other. Urban land cover was a

combination of classification schemes 21, 22, 23, and 24 that represented developed open space, developed low, medium, and high intensity, respectively. Agriculture land cover was a combination of classification schemes 71, 81, and 82 that represented grasslands/herbaceous, pasture/hay, and cultivated crops, respectively. The other category consisted of classification schemes 11, 31, 41, 42, 43, 52, 90, and 95 that represented open water, barren land, deciduous forest, evergreen forest, mixed forest, scrub/shrub, woody wetlands, and emergent herbaceous wetlands, respectively. Percent land cover represented the percentage of urban and agricultural land cover in each watershed containing a sample site.

I evaluated the influence of seasonal stream flow regime components and site-specific stream characteristics on freshwater mussel growth by fitting a linear regression model (Sokal and Rohlf 1995). The annual growth of individual mussel specimens was potentially dependent upon seasonal stream flow regime components and site-specific stream characteristics. In addition, there was potentially spatial and temporal autocorrelation in the data, which would preclude the use of traditional regression models (Sokal and Rohlf 1995). Thus, I initially fit a global (i.e., model containing all of the predictors) linear regression model that related annual mussel growth to sample site characteristics and seasonal stream flow regime components. An analysis of variance (ANOVA) of the residuals from the global model indicated significant dependence for mussel growth between the sample site ( $F = 2.68$ ;  $df = 19, 5783$ ;  $P < 0.001$ ) and individual mussels ( $F = 1.16$ ;  $df = 776, 5783$ ;  $P < 0.0034$ ) but not among years ( $F = 1.15$ ;  $df = 45$ ;  $P = 0.230$ ) regression model. To account for the dependence, I employed a hierarchical linear model to examine the relations between seasonal stream flows, site-specific stream characteristics, and annual mussel growth. Hierarchical linear models differ from more familiar regression techniques in that dependence among measurements taken on a single mussel, defined

as lower-level units (individual mussels) within upper-level units (sample sites), is incorporated by including random effects (Bryk and Raudenbush 2002). For my study, I include random effects corresponding to individual mussels and individual sites that were assumed to vary normally with a mean of zero and random effect-specific variance. The random components represented unique effects associated with each individual mussel and site, respectively that were unexplained by the predictors in the model. All models were fit using SAS PROC MIXED (9.1, SAS Institute, Inc., Cary, North Carolina). An evaluation of the residuals from the global hierarchical model indicated that the dependence among mussels and sites had been accounted for by the random effects.

Prior to constructing my candidate models, Pearson correlations were run on all pairs of predictor variables and only uncorrelated predictor variables ( $r^2 < 0.45$ ) were included in candidate models to avoid multicollinearity. I also created binary indicator variables (i.e., 0 or 1) for categorical predictors species; with *V. vibex* coded as 1 when the species was *V. vibex* and 0 otherwise, *E. crassidens* coded as 1 when the species was *E. crassidens* and 0 otherwise (i.e., *V. lienosa* was the baseline species); channel confinement with unconfined channels coded as 1 and 0 otherwise; tagged with tagged individuals coded as 1 and 0 otherwise; and physiographic province with Dougherty Plain sites coded as 1 and 0 otherwise.

I used the information-theoretic approach, described by Burnham and Anderson (2002), to evaluate the relative plausibility of models relating site-specific stream characteristics, and species characteristics to the annual growth of the three focal mussel species. I began by developing hypotheses to explain the relative influence of individual mussel characteristic and site-specific stream characteristic components on mussel growth. For clarity, I grouped the hypotheses into themes (Table 2). The 1<sup>st</sup> theme characterized the effects of streamflows on

mussel growth, and consisted of 5 hypotheses that were represented by 4 seasonal flow regime components (Table 2). The 2<sup>nd</sup> theme characterized the effects of stream size and hydro-geomorphology on mussel growth, and consisted of 7 hypotheses that are represented by 4 stream size and hydro-geomorphology components as well as their interaction with streamflow (Table 2). The 3<sup>rd</sup> theme denoted the effects of anthropogenic land use on mussel growth, and consisted of 3 hypotheses represented by 3 land cover components (Table 2). The 4<sup>th</sup> theme characterized the effects of an individual mussel's age and shell morphology on growth, and consisted of 8 hypotheses represented by 4 biological and individual components as well as their interaction with streamflow (Table 2).

To evaluate the relative support for hypotheses regarding the effect of factors affecting mussel growth, I created candidate models that contained all combinations of predictors in the 4 themes identified above resulting in 64 candidate models. Theme 4 predictor variables were included in every candidate model, because these predictors had been previously documented to affect freshwater mussel growth (Haag and Commens-Carson 2008, Rypel et al. 2008, Williams et al. 2008) and were not the primary focus of my study. Candidate models only contained predictor variables that were not strongly correlated ( $r^2 < 0.45$ ). Link magnitude, age, and species by flow interactions were only included in models that the main effects were present. Quadratic terms for each flow regime component were always included when a flow regime component was in a candidate model because growth was assumed to be nonlinearly related to flow regime. To assess the relative fit of each candidate model, I calculated Akaike's Information Criterion (AIC; Akaike 1973) with the small-sample bias adjustment (AICc; Hurvich and Tsai 1989). AIC is an entropy-based measure used to compare candidate models for the same data (Burnham and Anderson 2002), with the best approximating model having the lowest AICc. The number of

parameters used to estimate AICc included the fixed and random effects (Burnham and Anderson 2002). The relative plausibility of each candidate model (i.e., hypothesis) was assessed by calculating Akaike weights as described in Burnham and Anderson (2002). These weights can range from 0 to 1, with the most plausible candidate model having the highest weight.

Model averaging, as a means of incorporating model selection uncertainty into parameter estimates, is not appropriate for models consisting of fixed and random effects (Grueber et al. 2011). Therefore, instead of basing my inferences and predictions on a single best model, I report estimates of fixed and random effects for my confidence set of mixed linear models, as determined by the Akaike weights. A confidence set of models is analogous to a confidence interval for a parameter estimate and is a useful means of assessing model selection uncertainty (Burnham and Anderson 2002). Because the ratio of Akaike weights for two candidate models can be used to assess the degree of evidence for one model over another (Anderson et al. 2000), the confidence set of models included only those candidate models with Akaike weights that were within 10% of the largest weight, which is similar to the general rule of thumb (i.e., 1/8 or 12%) suggested by Royall (1997) for evaluating strength of evidence.

The precision of parameter estimates was estimated by calculating 95% confidence intervals based on a *t*-statistic with *n*-1 degrees of freedom (Littell et al. 1996). Confidence intervals that contained 0 indicated inconclusive results because I could not determine the nature of the relationship (i.e., whether positive or negative) because of imprecision in parameter estimates. To facilitate comparisons among parameter estimates on very different scales (e.g., age vs. link magnitude), I also created standardized parameter estimates by refitting models in the confidence set using predictor data standardized to a mean of zero and standard deviation of one. The relative importance of individual predictor variables was estimated as the sum of

Akaike weights for candidate models in which each predictor occurred (Burnham and Anderson 2002). Goodness-of-fit for the candidate set of models was evaluated by examining the normal probability plot and the residual plot (following Bryk and Raudenbush 2002).

## CHAPTER 4

### RESULTS

In total, 830 mussels were thin-sectioned. Fifty-three thin sections had either large amounts of shell wear, were poor quality thin sections (e.g., extreme light or dark mineral compositions), or the observers failed to reach a consensus on age of the specimen. The remaining 777 mussel thin sections were included in the analysis, though not all species were found at all 20 sites. In total, there were 402 *Villosa lienosa* collected at 18 sites, 282 *Villosa vibex* at 17 sites, and 93 *Elliptio crassidens* at 3 sites (Table 3). *Elliptio crassidens* were only found at the 2 sites on the mainstem Flint River and Chickasawhatchee Creek, a large tributary in close proximity to the mainstem Flint River. *Villosa lienosa*, *V. vibex* and *E. crassidens* represented 51.7, 36.3, and 12.0%, respectively of the total number of freshwater mussels analyzed. Fifty-six of the mussel specimens (i.e., 7%) were tagged and 16 *V. lienosa* and 5 *V. vibex* were notched for annuli validation and age verification (Table 3).

#### *Annuli Validation and Observer Agreement*

Annulus production was present in the majority of notched *Villosa* species. Thin section photographs of 3 notched mussels (i.e., 14% of total) were of poor quality or failed to capture the notch due to thin-sectioning precision (Figure 4a). Four additional notched mussels (i.e., 19% of total) had little growth post notching. All four of these specimens displayed some shell growth, but the mussels were older and larger with less annual growth than smaller specimens and the amount of growth was insufficient to detect an annulus beyond the notch (Figure 4b). All 3

observers viewed and agreed that 14 notched *V. lienosa* and *V. vibex* (i.e., 67% of total) displayed a disturbance ring (i.e., response to notching) at the bottom of the notch followed by one true annulus post notching (Figure 5). No thin section had multiple annuli post notching, and annulus placement on legible thin sections was consistent between observers. Therefore, I assumed that observed annuli represented annual growth checks of *V. lienosa* and *V. vibex* within the lower Flint River Basin. I did not recover any notched *E. crassidens* and was unable to verify annulus formation. The observed growth checks for *V. lienosa* and *V. vibex* suggested that other unionid species in the LFRB likely exhibit the same cyclic production of annuli. Thus, I assumed that the annuli represented annual growth checks for *E. crassidens*.

Initial observer agreement was calculated as the percent of the total number of agreed upon aged mussels per species that zero, two, or three observers initially agreed upon. Comparing the initial observer agreements revealed that at least 2 observers initially agreed more than 50% of the time for each of the focal species ages (Table 5). For both *E. crassidens* and *V. vibex*, the average differences in ages among observers was relatively low (i.e., <20%) compared to the agreed upon ages. In comparison to *V. vibex* and *E. crassidens*, the relative difference in estimated ages among observers was higher for *V. lienosa* (Table 5).

*Elliptio crassidens* were the oldest individuals aged in this study with 12% of specimens estimated ages  $\geq 25$  years and an oldest specimen estimated age of 46 years (Table 3, Figure 6). The average age and size of an *E. crassidens* was 15 years old with a shell height of 43 mm (Table 3 and 4). *Villosa lienosa* and *V. vibex* had maximum estimated ages of 16 and 18, respectively (Table 3). Both *V. lienosa* and *V. vibex* represented very similar age and size distributions with average ages of 6 years old and average shell heights of 23 and 25 mm, respectively (Table 3 and 4). Species-specific shell height-at-age revealed substantial overlap in

mussel size at varying age classes (Table 4). Of the mussels sectioned in this study, *E. crassidens* appeared to have a bimodal distribution of ages with highest year classes of 7 year olds recruited in 2003 and 20 to 22 year olds recruited between 1990-1988 (Figure 6). The smallest year classes of *E. crassidens* were 12 to 16 year old individuals, which were recruited between 1998 and 1994 (Figure 6). *Villosa lienosa* and *V. vibex* had highest year classes for 5-6 year old and 7 year old individuals, respectively, which were recruited between 2005-2003, and had the lowest year class of individuals  $\geq$ 13 years of age recruited before 1998 (Figure 6). The single highest year class of all 3 species of mussels was 7 year old individuals that were recruited in 2003 (Figure 6). There were lack of any 1-year-old *E. crassidens* and only few 1-year-old *Villosa* species collected in this study.

#### *Site-Specific Stream Characteristics*

Five of the sampled sites were located close to a USGS gage and published models were available for estimating discharge at 7 sites (Table 6). Discharge models created to estimate discharge at the remaining 8 sites fit very well with  $r^2$  values  $>0.95$ . The seasonal discharges in the LFRB varied substantially during the lifespan of mussel specimens used in this study. During an average 6 year lifespan of *V. lienosa* and *V. vibex*, the flows that individual mussels experienced included 3 wet years (2005, 2009-10) and 3 drought years (2006-08; Figure 7). Sample site seasonal stream discharges included observed 0 (i.e., no discharge) for the record of mussel growth at all 20 sites (Table 7). On average, summer 10-day low flows were less than 57% of spring 10-day low flows (Table 7). Summer 10-day high flows also were on average 55% lower than spring 10-day high flows, while the maximum-recorded 10-day high flow occurred during the summer of 1994.

### *Mussel Growth Models*

An examination of the residuals from the global hierarchical linear model relating mussel growth to individual and site-specific characteristics indicated heterogeneity of variance. I natural log transformed growth, refit the model, and the residuals indicated that the model adequately fitted the data. Therefore, I conducted the model selection using the natural log transformed growth data.

The best approximating candidate model for predicting mussel growth contained summer 10-day low flow, Dougherty, unconfined, link magnitude, age, *E. crassidens*, *V. vibex*, percent shell wear, tagged, two quadratic terms: summer 10-day low flow<sup>2</sup> and age<sup>2</sup>, and six 2-way interactions: link magnitude by unconfined, age by summer 10-day low flow, age by *E. crassidens*, age by *V. vibex*, *E. crassidens* by summer 10-day low flow, *V. vibex* by summer 10-day low flow (Table 8). The Akaike weights ( $w_i$ ) indicated that the best approximating model was 2.7 times more likely than the next best-approximating model, which was similar in that it included all the same predictor variables with the addition of urban and agriculture land cover, but differed in that spring 10-day high flow was included in place of summer 10-day low flow (Table 8). The 3 best-approximating models represented the confidence set of models (% $w_{max}$   $\geq 10\%$  of the best approximating model; Table 8).

Akaike importance weights of the predictor variables indicated strong support that annual freshwater mussel growth was related to seasonal stream flow regime components with an AIC importance weight of 0.998 (Table 9). Of the seasonal stream flow regime components, summer 10-day low flow had the greatest relative importance on mussel growth with an importance weight of 0.781, whereas spring 10-day high flow was the next best supported flow with an importance weight of 0.210 (Table 9). Akaike importance weights of all other flow regime

components suggested very little to no support with weights  $\leq 0.002$  (Table 9). The importance weight for interactions between species and age with flow regime components was 0.996 (Table 9) suggesting strong support that the effect of streamflows on growth varied with species and age. The importance weight for the effects of stream size and hydro-geomorphology and their interaction was 0.906 (Table 9) suggesting strong support that freshwater mussel growth varied with stream size, confinement and underlying geomorphology.

The annual growth of freshwater mussels varied among focal species and decreased as mussels became older. Parameter estimates indicated that annual growth of *E. crassidens* was, on average, 3.3 and 4.2 times greater than *V. vibex* and *V. lienosa*, respectively (Table 10; Figure 8). However, the parameter estimates for *V. vibex* were small and imprecise in all 3 models in the confidence set suggesting that, on average, the growth of *V. vibex* was not biologically different than *V. lienosa*. The best-approximating model estimated that as mussels became older annual growth decreased most for *V. lienosa* and *V. vibex* and least for *E. crassidens* (Figure 8). Annual mussel growth also was slower for the year following capture and tagging. I estimate that annual growth of tagged mussels was, on average, 25% lower for the year following tagging, across species (Figure 8).

All streamflow components were positively and non-linearly related to mussel growth, and the effect of flows varied with age and among species (Table 10). Parameter estimates for the effect of flow regime components from the confidence set of models were precise and indicated that annual mussel growth was positively related to summer 10-day low flows and spring 10-day high flow at low flow components, but growth decreased as the magnitude of these flow components increased. The effect of the flow regime components on annual growth also decreased as mussels became older. I estimate that the effect of summer 10-day low flow

conditions on the annual growth of the focal mussel species decreased, on average, over 60% with each 4-year increase in age (Figure 9). The effect of summer 10-day low flows also differed among the focal species and was lowest for *E. crassidens* and greatest for *V. lienosa* (Table 10). However, the parameter estimates for the interaction between summer 10-day low flows and *V. vibex* were relatively imprecise suggesting that the effect of 10-day low flow on growth was similar between the *Villosa* species (Table 10). In contrast, all of the parameter estimates for the interaction between spring 10-day high flows and species were imprecise. The nonlinear relation between flow regime components and annual growth also suggested the presence of an optimal value for growth. On average, both 2 and 6 year old *Villosa* species had estimated optimum annual growth at higher average summer 10-day low flows ranging from 0.006 to 0.009 (e.g., 4-6 times the 7Q10 at reference gage numbers 02350600 and 02352500; McCargo and Peterson 2010), whereas the optimum for *E. crassidens* was at lower values (Figure 9).

Annual growth of mussels was related to stream size and geomorphic channel features and land use in the catchment. Parameter estimates from the confidence set of models indicated that the growth of mussels in streams in the Fall Line Hills physiographic province was on average, 17% greater than in the Dougherty Plain (Figure 10). The effect of channel confinement on mussel growth was more complex and varied with stream size. On average, annual mussel growth in small streams was lower in unconfined stream channels relative to confined stream channels, whereas the pattern was reversed in larger streams (Table 10; Figure 10). The parameter estimates from the confidence set of models also suggested that mussel growth was negatively related to link magnitude (stream size) and urban and agricultural land use but the parameter estimates for these effects were imprecise (Table 10).

## CHAPTER 5

### DISCUSSION

Validation of annulus formation is essential when acquiring information on a species age and growth from the production of annuli (Beamish and McFarlane 1983). A substantial number of age and growth studies lack validation of annual growth increments (Beamish and McFarlane 1983). Campana (2001) published a literature review containing 372 papers reporting age validation for fish species, of which only 15% validated the absolute age of wild fish, though more than 50% did validate growth increment periodicity. Unlike fisheries, the practice of validating the absolute age using known age specimens (i.e., hatchery individuals) to correct estimates from field samples is not widely applicable in freshwater mussel research (Haag and Commens-Carson 2008). However, validation of annuli production is a common practice in freshwater mussel research. Shell notching of the ventral margin has been documented the most successful annuli validation method of freshwater mussels (Neves and Moyer 1988), and was therefore my method of choice. Although annuli validation was not the primary objective of this research, I validated 1 annulus post notching in 67% of notched *V. lienosa* and *V. vibex*. The lack of multiple annuli post notching in any thin section verified annuli formation and assisted in distinguishing true verse pseudo-annuli. Observation of annuli production was most prevalent in younger individuals who had greater annual growth, and a majority of the *V. lienosa* and *V. vibex* specimens were younger aged (i.e.,  $\leq 7$  years-of-age). Previous freshwater mussel age and growth studies acknowledged the limitations when notching older individuals and generally attributed the inability to distinguish annuli post notching to poor quality thin sections and

minimum annual growth (Negus 1966, Neves and Moyer 1988, Haag and Commens-Carson 2008, Rypel et al. 2008). Lack of validated annuli production in 33% of notched thin sections in this study also was primarily due to poor quality thin sections and slow growth of larger notched specimens. This combined with the observed annuli production in 67% of notched specimens suggested that freshwater mussels in the LFRB produce annuli. Therefore, I believe that thin sectioning was appropriate for determining the age and annual growth of freshwater mussels in the LFRB.

The accuracy of the estimated age and growth of freshwater mussels depends on the ability of observers to identify annuli. Because identification of annuli is subjective and depends on the skill of the observers, previous studies have used multiple observers to evaluate the consistency among observers and relative accuracy of annuli determinations with high reader agreement indicating relatively accurate determinations (Boehlert 1985, Campana and Moksness 1991, Eklund et al. 2000). Unfortunately, I know of no published freshwater mussel studies that have reported observer agreement, but there are several age and growth studies published on freshwater fish that reported observer agreement. Hurley et al. (2004) reported 46% observer agreement between two experienced observers who identified annuli in pectoral ray sections, and concluded that their observer agreements were comparable to similar published studies that reported observer agreements from 17-37%. Butler et al. (1996) used a 5-person observer method to identify annuli in Pacific sardine (*Sardinops sagax*) otoliths and reported a mean 5-person reader agreement of 31%. Butler et al (1996) concluded that low observer agreement was due to variability in the clarity of annuli among specimens and reader agreement increased with fewer observers. My research found two or more observers initially agreed on mussel ages for more than 50% of specimens and initial agreement rates for 3 observers were, on average, 13%

for a species. In addition, there was 100% consensus reader agreement for the annuli used to estimate the age and growth of all specimens in this study. Thus, I believe that annuli were relatively accurate and that the estimates represent the true age and annual growth of freshwater mussels in the LFRB.

Freshwater mussel growth in the LFRB was found to vary in relation to multiple individual and site-specific characteristics over a large spatial extent. Empirical data collected on species- and stream-specific characteristics allowed me to evaluate the effects of potential growth-altering variables on freshwater mussel growth. In this study, growth was measured as the linear increase in a specimen's height-at-age (i.e., distance between umbo to ventral margin) between deposited annuli. Annual increases in a specimens shell size were presumably the result of nutrient intake, and nutrients are often products of local environmental conditions (Sebens 1982, Sebens 1987). For example, individuals growing under low food availability (i.e., nutrients) reach smaller maximum size than do those under a similar physical regime but with greater food availability (Sebens 1987). A freshwater mussel's ability to obtain nutrients can depend on environmental conditions and their life history stage. The environment acting through on individuals life history stage determines the allocation of nutrients (i.e., reproduction, mass, size; Sebens 1982). Environmental conditions are site-specific and allocation of growth is individual-specific. To understand the factors affecting freshwater mussel growth requires an understanding of the effect of the local (stream reach) and large (basin) factors on nutrient availability and how ontogeny affects the allocation of those nutrients.

Annual growth of freshwater mussels in the LFRB varied among species, was greatest for *E. crassidens*, and was similar for *V. lienosa* and *V. vibex*. Environmental factors acting through ontogeny aid in determining size and growth rate for a species, while size affects freshwater

mussel energetics, life history, competition, and susceptibility to predation (Sebens 1987). Of the 3 mussel species analyzed, *E. crassidens* were a long-lived species that primarily inhabited large streams, while the *Villosa* species were a short-lived species that occurred in all stream sizes. Mussels that inhabit larger rivers experience greater risk of predation due to meso-predators (Neves and Odom 1989) and a greater risk of mortality due to the greater shear stresses during high flow events (Morris and Corkum 1999). To minimize these risks, individual mussels in larger streams should, presumably have greater growth rates (Morris and Corkum 1999). The greater growth rate of *E. crassidens* is consistent with this supposition. However, the observed effect of stream size on growth rate of mussels was weak and inconsistent suggesting that the growth differences between species were not primarily environmental. Mussel growth also is influenced by life history stage (Sebens 1987). Because mussel survival is positively related to shell size (Morris and Corkum 1999), young mussels should devote greater resources toward growth to increase their chances for survival. Mussel fitness, however, also is positively related to fecundity, so a mussel should devote more energy to reproduction and less to growth once it reaches maturity. The observed decrease in the growth rate with increased age for the 3 species of mussels in the LFRB was consistent with this hypothesis. *Villosa lienosa* and *V. vibex* are short-lived species that have similar life history strategies and become mature at a younger age compared to the long-lived *E. crassidens* (Williams et al. 2008). Visual inspection of marsupial gills of LFRB female *V. lienosa* and *V. vibex* indicated gravid specimens with similar minimum shell lengths of 34 and 36 mm, respectively (Wizniewski and Shea unpublished). However, the minimum age or size at maturity is not known for these species (Thorp and Covich 2010). Therefore, I hypothesize that differences in growth among species was primarily due to differences in life histories strategies.

Tagging and shell wear were negatively related to annual growth of LFRB freshwater mussel species. Of the specimens collected, less than 10% were tagged and recaptured individuals, but the growth of tagged mussels was on average 25% lower post tagging compared to untagged mussels. Previous research suggested that freshwater mussels are extremely sensitive to handling (Haag and Commens-Carson 2008), and handling between sampling intervals caused a disruption in the growing process and reduced annual growth (Negus 1966, Haag and Commens-Carson 2008, Williams et al. 2008, Haag 2009). Even temporary removal of mussels from the streambed can cause a disruption in growth due to an individual's mantel (i.e., organ responsible for secreting shell material) retraction from the shell margin, and thus potentially bias age estimates (Haag and Commens-Carson 2008). Thin-shelled species of freshwater mussels may be more sensitive to tagging in comparison with thick-shelled species (Haag and Commens-Carson). Annual growth also was negatively related to percent shell wear across species. During our consensus-observer-reading, mussel thin sections were estimated to have 0 to 75% shell wear from their umbo to their ventral margin. The periostracum and subsequent internal layers of mussel shells have been used to document historic environmental stressors (Carell et al. 1987), and could be used as visual indicators of induced stress. Periods of high flows increase the erosive capacity of streams (Peterson et al. 2011) that can increase shell wear on suspension-feeding mussels. Shell wear has been found to increase with mussel age (Kesher and Bailey 1993) and in streams with coarser substrates (Green et al. 1989). There were no observed relation between amount of shell wear and age, but visual inspections of specimens collected in turbulent streams with coarser substrates had greater amounts of shell wear. For example, one preliminary site (i.e., Ichawaynochaway at Highway 200) was dominated by coarse substrate and 100% of collected mussels had too much wear to thin section, whereas site 7 (i.e.,

Ichawaynochaway 2) was dominated by fine substrate and 80% of collected mussels had  $\leq 10\%$  wear. Therefore, I hypothesize that reduction in growth among tagged specimens and severely eroded specimens was primarily due to stress experienced during the growing season.

Mussel growth was positively and nonlinearly related to streamflows during the spring and summer seasons. During the summer months, dissolved oxygen levels decrease and water temperatures generally increase as streamflows decrease resulting in sub-lethal low DO and high temperatures that potentially stressed mussels, decreasing growth. Summer streamflows at 2 sample sites also ceased (i.e., 0 flow) during the years that spanned the recorded growth of mussel specimens. Ceased flow events can lead to desiccation or exposure forcing mussels to burrow within the substrate, thus preventing feeding and presumably growth (Strayer et al. 2004). This suggests the relations between growth and streamflow may have been due, in part, to the effect of low flows on mussel stress. However, the model results suggest that effect of growth was distinctly nonlinear and was negative and greater at high flow values. Instead, I hypothesize that the nonlinear relation between streamflow and growth was due the effect of flow on mussel feeding efficiency. The primary feeding mechanism of freshwater mussels is suspension-feeding where mussels filter the overlying volume of water through an incurrent aperture and excrete non-palatable sediment through an excurrent aperture (Williams et al. 2008, Vaughn 2010). The ability of a stream to transport fine particulate organic matter (henceforth, FPOM) and sediment is positively related to streamflows. At low flows, the transport capacity is at a minimum and particulate matter can precipitate out of the water column (Allen and Castillo 2007) and become unobtainable to suspension-feeding mussels (Vaughn and Hakenkamp 2001, Spooner and Vaughn 2008). At high flows, the transport capacity of streams increases and the streams are able to carry greater amounts of suspended sediment. These high concentrations of suspended

sediment can clog the gills of suspension-feeding mussels and impair their ability to respire and feed (Williams et al. 2008). Thus, at low flows mussel growth was affected by the availability of FPOM that increased as flow increased until the flows were high enough to transport greater concentrations of sediment that decreased mussel feeding efficiency.

Short-term low flows during the summer had the greatest influence on freshwater mussel growth. The metabolic rate of ectothermic mussels increase with warmer stream temperatures, allowing specimens to more efficiently feed and process obtained nutrients (Jørgensen 1990, Vaughn 2010). Thus, the stronger relation between summer flow and growth was likely due the greater growth of mussels associated with increased stream temperatures. The importance of short-term flow conditions suggests that most of the annual growth of freshwater mussels takes place over relatively short periods during the summer. Allen (1914) suggested while at the surface of the streambed, mussels continuously filtered water and obtained FPOM regardless of appetite, due to processed and unprocessed particles found throughout the digestive system during dissections. Allen (1914) hypothesized that due to unknown food availability and ability to suspension feed; freshwater mussels continuously consumed FPOM (i.e., suspension fed), while regulating the secretion of digestive juices to satisfy their appetite. On average, 70-90% of mussels in streams in the LFRB are at the streambed surface during the summer (Peterson et al. 2010). Freshwater mussels seasonally burrow to avoid adverse stream conditions, but reasons for temporary mid-summer burrowing remain obscure (Amyot and Downing 1997). My analysis estimated short-term summer low flows as the best predictor of freshwater mussel growth, thus indicating there may be an optimum short duration at which mussels can efficiently process nutrients to obtain the most substantial growth.

The effect of streamflow on freshwater mussel growth in the LFRB varied with age and was greatest on older ages. Freshwater mussels typically feed by one of two methods that depend on life history stage (i.e., juvenile or adult). Juvenile mussels are assumed to pedal feed, which mussels burrow in the substrate and obtain nutrients by using their ciliated foot to sweep through the substrate (Yeager et al. 1994, Gatenby et al. 1996), while adult mussels suspension feed using their incurrent aperture to filter the overlying water column for nutrients (Brim Box and Mossa 1999, Strayer 1999). Juvenile mussels are capable of obtaining nutrients both methods (Yeager et al. 1994), but are believed to more commonly pedal feed because they are more susceptible to scour and displacement due to increased stream flows (Howard and Cuffey 2006). Streamflows can affect the ability of suspension-feeding mussels to obtain nutrients (Vaughn and Hakenkamp 2001) and presumably should have a smaller effect on pedal feeding juvenile mussels. Thus, I hypothesize that the smaller effect of streamflows on juvenile growth was primarily due to their feeding mode.

Freshwater mussel growth varied in relation to stream geomorphology characteristics. Several studies evaluated the relation between stream geomorphology and freshwater mussel distribution and population status (Brim Box and Mossa 1999, Arbuckle and Downing 2002), but I know of no studies that have evaluated the relation between stream geomorphology and freshwater mussel growth. This study found evidence that mussel growth varied in relation to stream channel morphology (i.e., confinement) and parent geology (i.e., Dougherty). Freshwater mussel growth was slower for individuals reared in the Dougherty Plain physiographic province and in unconfined stream channels. Streams within the Dougherty Plain often have an alluvial connection to the Floridan aquifer (Mosner 2002). Previous studies suggested that aquatic organisms within streams with baseflows supplied by an underlying aquifer are less affected by

low flow conditions compared to those supplied by surfical aquifers (Arbuckle and Downing 2002). Freshwater mussel growth in Dougherty Plain streams was lower than streams in the Fall Line Hills, which was not consistent with the expected effects. I believe that water quality of streams within the Fall Line Hills were more suitable for mussel growth compared to water quality of streams in the Dougherty Plain province. Streamflow contribution from alluvial aquifer can act as thermal refugia for aquatic organisms (Peterson et al. 2009) providing warmer winter and potentially cooler summer flows compared to streams with surfical aquifer contributions. Cooler water temperatures could have decreased the metabolic rate of mussels within Dougherty Plain streams, thus causing decreased growth. Streams within the Fall Line Hills are more turbid then streams within the Dougherty Plain (Peterson et al. 2009). Increased turbidity could be due to increased suspended FPOM that provide more nutrients for mussels inhabiting Fall Line Hills streams and therefore increasing growth. Unconfined stream channels in the LFRB tended to be wider and shallower then similar sized confined stream channels (Li 2006), therefore having a decreased ability to transport suspended sediments. Decreased ability to transport sediment can allow particles to settle out of the water column and induce stress on filter-feeding mussels (Brim Box and Mossa 1999); thus limiting growth. Peterson et al. (2009) found unconfined stream channels had lower dissolved oxygen levels and increased stream temperatures compared to confined stream channels. Low dissolved oxygen and increased temperatures (i.e., reaching species thermal tolerance level) can restrict the ability of mussel to obtain nutrients and reduce growth. Therefore, I hypothesize that growth varied in relation to geomorphology due to varied water quality associated with differing channel morphology and parent geology.

### *Management Implications*

Researchers have used mark-recapture methods for years to estimate aspects of freshwater mussel growth and recruitment, providing essential information for understanding the factors that affect the population dynamics of this group (e.g., Neves and Moyer 1988, Howard and Cuffey 2006, Haag and Commens-Carson 2008). However, because handling and marking may alter subsequent measures of growth, the utility of mark-recapture may be limited if such biases are not accounted for. Similar to the results of Negus (1966) and Haag and Commens-Carson (2008), we found that tagging and handling negatively impacted the growth of marked specimens. Disruption in annual growth caused by handling can lead to the production of pseudo-annuli, which may ultimately lead to overestimated mussel ages and underestimated annual growth (Haag and Commens-Carson 2008). Thus, if resource managers use mark-recapture methods to estimate freshwater mussel growth, they should acknowledge and attempt to quantify the effects of such biases before they draw conclusions regarding the impacts of specific environmental factors on mussel growth.

Resource managers often implement low flow standards to ensure adequate in-stream flows for aquatic biota (Tharme 2003). The results of my analysis, which found that freshwater mussel growth in the LFRB was positively and non-linearly related to short-term summer low flow, suggest a level of optimum discharge that may be capable of maximizing the growth of each focal mussel species. The two *Villosa* species were biologically similar being short-lived and had a younger age at maturation compared to long-lived, late age at maturation *E. crassidens*, and the effect of increased short-term summer low flows was greater on a long-lived, late maturing species. The regulation of flows to maximize growth of more sensitive species (e.g., late maturing) will benefit growth of all species due to close optimum flows and ability of

young age at maturation species to grow during increased flows. Although my research estimated growth of non-endangered species of mussels, endangered species inhabit similar areas and have similar life history strategies as focal species. Given the similarities between groups, these methods are likely to result in positive effects on the growth of endangered species. In this aspect, managers can regulate water use to balance the concerns of two late maturing, federally endangered, LFRB species (i.e., *Elliptoideus sloatianus* and *Amblema neislerii*; Williams et al. 2008), and those of resource users in the area.

To prevent further extirpation of native aquatic organisms, resource managers can implement managed relocation (Olden et al. 2011). Because of today's ever-changing environment and the limited mobility of freshwater mussels, relocation may be one of only a few valid options for ensuring continued species persistence. To increase the success of relocation, resource managers must take into consideration both the habitat where the species currently occurs and habitat of the purposed area of relocation (Cope and Waller 1995). In particular, my research indicated that aspects of channel morphology and parent geology should be considered when contemplating relocation of a mussel species. Mussels within unconfined channels with high link magnitudes and confined channels with low link magnitudes were estimated to have faster growth, while growth was estimated almost 20% faster depending on parent geology. Resource managers should conduct habitat assessments and relocate mussels to sites associated with optimum habitat estimated for fastest growth, thus increasing the effectiveness of managed relocation. Further, my models also predicted mussel species with varied life history strategies (e.g. age at maturity) had differing responses of growth to flow intensity. Because increased flows had a greater effect on the growth of late maturing species, long-term flows at the relocation site should be considered. Therefore, managers contemplating managed relocation

should consider site-specific characteristic at both local (reach) and large (basin) spatial scale before implementing a relocation program.

As the demand for water increases in the continuously growing southeastern United States, resource managers will need to implement strategies to prevent further imperilment of freshwater mussels, while utilizing maximum potential water use. Therefore, future studies should continue to research species- and site-specific characteristics that effect freshwater mussel growth and persistence.

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Table 1. Freshwater mussel species found in the lower Flint River Basin along with their present population status (from Williams et al. 2008).

Species	Common Name	Status
<i>Alasmidonta triangulata</i>	southern elktoe	Special Concern
<i>Amblema neislerii</i>	fat threeridge	Federally Endangered
<i>Anodonta heardi</i>	Apalachicole floater	Threatened
<i>Anodontoides radiatus</i>	rayed creekshell	Endangered
<i>Elliptio arctata</i>	delicate spike	Special Concern
<i>Elliptio crassidens</i>	elephantear	Currently Stable
<i>Elliptio fraterna</i>	brother spike	Presumed Extirpated
<i>Elliptio fumata</i>	variable spike	Currently Stable
<i>Elliptio nigella</i>	winged spike	Recently Rediscovered
<i>Elliptio pullata</i>	Gulf spike	Currently Stable
<i>Elliptio purpurella</i>	inflated spike	Currently Stable
<i>Elliptoideus sloatianus</i>	purple bankclimber	Federally Threatened
<i>Hamiota subangulata</i>	shinyrayed pocketbook	Federally Endangered
<i>Lampsilis binominata</i>	lined pocketbook	Presumed Extinct
<i>Lampsilis floridensis</i>	Florida sandshell	Currently Stable
<i>Lampsilis straminea</i>	southern fatmucket	Special Concern
<i>Lasmigona subviridis</i>	green floater	Presumed Extirpated
<i>Medionidus penicillatus</i>	Gulf moccasinshell	Federally Endangered
<i>Megalonaia nervosa</i>	washboard	Currently Stable
<i>Pleurobema pyriforme</i>	oval pigtoe	Federally Endangered
<i>Pyganodon cataracta</i>	Eastern floater	Special Concern
<i>Pyganodon grandis</i>	giant floater	Currently Stable
<i>Quadrula infucata</i>	sculptured pigtoe	Special Concern
<i>Toxolasma paulum</i>	iridescent lilliput	Currently Stable
<i>Unionerius columbensis</i>	Apalachicola pondhorn	Currently Stable
<i>Utterbackia imbecillis</i>	paper pondshell	Currently Stable

Table 1. (continued)

<b>Species</b>	<b>Common Name</b>	<b>Status</b>
<i>Utterbackia peggiae</i>	Florida floater	Currently Stable
<i>Villosa lienosa</i>	little spectaclecase	Currently Stable
<i>Villosa vibex</i>	southern rainbow	Currently Stable
<i>Villosa villosa</i>	downy rainbow	Special Concern

Table 2. Hypotheses and corresponding predictors used to explain the variation in mussel growth among species and sample sites in the lower Flint River Basin, Georgia.

Predictor variables	Biological interpretation (hypothesis)
<b>Theme 1: Seasonal (spring and summer) stream flow regime components affect freshwater mussel growth</b>	
10-day low flow	Short-term low flows have a greater effect on freshwater mussel growth because of the decreased the amount of fine particulate matter within the water column and increased stream temperatures.
10-day high flow	Short-term high flows have a greater effect on freshwater mussel growth because of the increased sediment load and water volume, which inhibited freshwater mussel siphon feeding.
Long term average flow	Long-term average seasonal stream flows have a greater effect on freshwater mussel growth because they reflected season long delivery of fine particulate matter that influenced food consumption.
Flow <sup>2</sup>	Freshwater mussel growth was non-linearly related to streamflows.
None	Freshwater mussel growth was unrelated to seasonal streamflow conditions.

Table 2. (continued)

<b>Predictor variables</b>	<b>Biological interpretation (hypothesis)</b>
<b>Theme 2: Stream size and geomorphology affect freshwater mussel growth</b>	
Stream size (Link magnitude)	Freshwater mussel growth was greater in larger streams because the availability of fine particulate organic matter (freshwater mussel primary food source) increased with stream size.
Stream channel confinement	The transport and delivery of sediment and fine particulate organic matter was lower in unconfined stream channels, which decreased food availability and decreased annual freshwater mussel growth.
Physiographic Province (Fall Line Hills ~ Surface Water) (Dougherty Plains ~ Floridian Aquifer)	The characteristics of the primary aquifer feeding a stream affected the chemical composition and dissolved oxygen of the stream, which affected freshwater mussel growth. Ground water fed streams were more productive than surface water fed streams, and often have more mineral rich water. With increased dissolved oxygen and productivity, and an enhanced chemical composition there was an increase in freshwater mussel growth in ground water fed streams.
Link magnitude X channel confinement	The effect of channel morphology on transport and delivery of sediment and fine particulate organic matter differed with stream size.

Table 2. (continued)

Predictor variables	Biological interpretation (hypothesis)
<b>Stream size and geomorphology mediate the effect of streamflows on freshwater mussel growth</b>	
Link magnitude X flow	The effects of seasonal streamflows on freshwater mussel growth varied with stream size.
Channel confinement X flow	The effects of seasonal streamflows on freshwater mussel growth were mediated by gross channel morphology.
No effect	Freshwater mussel growth did not vary in relation to stream size or geomorphology.
<b>Theme 3: Land use influences freshwater mussel growth</b>	
Percent urban land cover	Stream water quality was negatively related to increasing urban land use; thus mussel growth decreased with an increase in urban land use within the watershed.
Percent agriculture land cover	Agricultural fields require a lot of water for irrigation from nearby streams, rivers, or underlying aquifers. With an increase in agriculture land use there was an increase in consumptive water usage, an increase in stream sedimentation, and a decrease in riparian buffers. All of which decreased freshwater mussel growth.
No agriculture or urban land cover	Both agriculture and urban land coverage decreased freshwater mussel growth. Freshwater mussel growth increased in a watershed with no agricultural or urban land coverage.

Table 2. (continued)

Predictor variables	Biological interpretations (hypothesis)
<b>Theme 4: Freshwater mussel growth is affected by age and shell morphology</b>	
Age	Freshwater mussel growth varied with age.
Species	Freshwater mussel growth varied by species.
Age X species	As freshwater mussels increase in age the way they add shell growth varied with shell morphology and hence, species.
Tagging	Handling induced stress on freshwater mussels and decreased their potential growth.
Percent shell wear	Shell wear affected the observer's ability to accurately mark a reference point and identify internal annuli affecting growth estimates.
Age <sup>2</sup>	The growth of freshwater mussels was nonlinearly related to age.
<b>Species and age mediate the effect of streamflows on freshwater mussel growth</b>	
Flow X species	The effect of streamflow on freshwater mussel growth varied with mussel species.
Flow X age	The effect of streamflow on freshwater mussel growth varied with mussel age.

Table 3. A summary of mussels that were used to evaluate annual growth in 20 stream reaches in the lower Flint River Basin, Georgia.

<b>Species</b>	Number of Sites Collected	Individuals Collected	<b><u>Shell height (mm)</u></b>				<b><u>Mussel age (yr)</u></b>			
			Tagged	Notched	Mean	Minimum	Maximum	Mean	Minimum	Maximum
<i>Villosa lienosa</i>	18	402	23	16	27.13	15	44	6.15	1	16
<i>Elliptio crassidens</i>	3	93	0	0	45.18	19	63	15.39	2	46
<i>Villosa vibex</i>	17	282	33	5	29.84	15	44	6.75	1	18

Table 4. Percent agreement of estimated mussel age for three observers (top) and the average and standard deviation (in parenthesis) of absolute and relative differences in estimated age among observers, by species.

Number observers agreed		Elliptio crassidens (N=81)	Villosa lienosa (N= 381)	Villosa vibex (N=233)
0		46.9	46.5	36.5
2		40.7	43.8	47.6
3		12.3	9.7	15.9
<b><u>Difference in estimated ages among observers</u></b>				
Absolute		2.59 (2.64)	2.07 (1.86)	1.37 (1.30)
Relative		0.15 (0.11)	0.34 (0.26)	0.19 (0.18)

Table 5. Mean shell height at age (SD in parentheses) of each of the three focal species of freshwater mussel studied in the lower Flint River Basin, Georgia.

<b>Age</b>	<b><u>Shell height (mm)</u></b>		
	<i>Villosa lienosa</i>	<i>Villosa vibex</i>	<i>Elliptio crassidens</i>
1	13 (3.0)	15 (4.7)	15 (4.4)
2	17 (3.0)	20 (5.1)	24 (5.8)
3	20 (2.9)	22 (5.5)	30 (6.8)
4	21 (2.9)	24 (5.8)	33 (7.0)
5	22 (2.9)	25 (5.8)	35 (7.1)
6	23 (2.9)	25 (6.0)	38 (7.3)
7	23 (2.8)	26 (5.9)	39 (7.2)
8	23 (2.8)	26 (5.9)	38 (5.7)
9	24 (2.8)	27 (5.9)	39 (5.6)
10	24 (2.9)	27 (5.6)	40 (5.2)
11	24 (2.9)	28 (5.4)	40 (5.0)
12	24 (3.3)	28 (7.6)	41 (4.8)
13	24 (3.8)	28 (6.0)	42 (4.7)
14	25 (4.6)	30 (5.2)	42 (4.6)
15	27 (2.1)	30 (3.6)	43 (4.6)
16	27 (2.8)	30 (3.5)	44 (4.6)

Table 6. The characteristics of the 20 freshwater mussel sample sites are located in the lower Flint River Basin, Georgia.

Sample Site	Contributing Watershed Area (km <sup>2</sup> )	Link Magnitude	Channel geomorphology <sup>a</sup>	USGS Reference Gage	Percent Agriculture	Percent Urban	Discharge Equation <sup>b</sup>
Chickasaw	857.2	286	DP, C	2354500	3.1	40	—
Chokee	147.6	75	DP, UC	2350080	2.9	45.1	$\exp[-0.003 + \log_e(Q)*1.022]$
Cooleewahee	164.2	23	DP, C	2530600	49.5	4.9	$\exp[-9.874 + \log_e(Q)*1.902]$
Flint River 1	7988.6	8104	DP, C	2353000	55.9	6.1	—
Flint River 2	7988.6	8104	DP, C	2353000	55.9	6.1	—
Ichaway 1	230.3	52	FLH, UC	2353265	36.4	2.4	$\exp[-3.324 + \log_e(Q)*1.360]$
Ichaway 2	39.1	11	FLH, C	2353265	36.4	2.4	$\exp[-3.324 + \log_e(Q)*1.360]$
Kinch 1	356.3	306	FLH, C	2350600	26.2	2.3	$\exp[-0.600 + \log_e(Q)*0.961]$
Kinch 2	839.8	545	FLH, C	2350600	23	2.5	$\exp[-0.515 + \log_e(Q)*1.310]$
Lanahassee 1	52.1	72	FLH, C	2350600	17.3	2.2	$\exp[0.175 + \log_e(Q)*0.296]$
Lanahassee 2	132.5	72	FLH, UC	2350600	17.3	2.2	$\exp[-0.531 + \log_e(Q)*0.298]$
Lime	97.7	133	FLH, C	2350080	49.8	5	—
Limestone	50.6	17	FLH, C	2350080	58	3.6	$\exp[0.449 + \log_e(Q)*-0.403]$
Mercer Mill	116.3	106	DP, C	2351890	44	5	$\exp[-1.834 + \log_e(Q)*0.536]$
Muckalee 1	101.6	61	FLH, C	2351500	26.1	1.9	$\exp[-0.529 + \log_e(Q)*0.872]$

Table 6. (continued)

Sample Site	Contributing Watershed Area (km <sup>2</sup> )	Link Magnitude	Channel geomorphology <sup>a</sup>	USGS Reference Gage	Percent Agriculture	Percent Urban	Discharge Equation <sup>b</sup>
Muckalee 2	494.4	291	FLH, C	2351500	38.9	4.9	$\exp[0.318 + \log_e(Q)*0.966]$
Muckalee 3	1021.2	564	DP, C	2351890	57.6	5.7	$\exp[0.318 + \log_e(Q)*0.966]$ $\exp[0.5487 +$ $\log_e(Q)*0.0735]$
Muckaloochee Spring	142.7	76	FLH, UC	2351890	43.1	6.6	—
Swift	732.1	328	DP, UC	2357000	48.8	13.3	—
	110.5	55	DP, C	2350080	48.7	6.8	$\exp[0.817 + \log_e(Q)*0.011]$

<sup>a</sup>Abbreviations represents Fall Line Hills (FLH) and Dougherty Plains (DP) physiographic provinces and confined (C) and unconfined (UC) stream channels.

<sup>b</sup>Q is discharge at the USGS reference gage; “exp” represents the exponential function.

Table 7. A summary of streamflows experienced by mussels specimens used to evaluate growth at the 20 sites in the lower Flint River Basin, Georgia. Site-specific stream flow regime components were standardized (divided) by watershed area.

<b>Flow regime component</b>	<b>Mean (SD)</b>	<b>Minimum</b>	<b>Maximum</b>
<b>Spring</b>			
Long-term average flow	0.010 (0.014)	0	0.12
10-day low flow	0.007 (0.007)	0	0.04
10-day high flow	0.071 (0.112)	0.01	0.98
<b>Summer</b>			
Long-term average flow	0.007 (0.007)	0	0.04
10-day low flow	0.004 (0.004)	0	0.02
10-day high flow	0.039 (0.113)	0	1.84

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Table 8. Predictor variables, number of parameters ( $K$ ), Akaike's Information Criterion with the small-sample bias adjustment (AICc),  $\Delta$ AICc, and Akaike weights ( $w$ ) for the 10 best-approximating candidate models ( $i$ ) for predicting annual growth of three species of freshwater mussels in lower Flint River Basin, Georgia.

Model	<b>K</b>	<b>AICc</b>	<b><math>\Delta</math>AICc</b>	$w_i$	$\%w_{max}$
summer 10-day low flow, Dougherty, unconfined, link, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , percent shell wear, tagged, summer 10-day low flow <sup>2</sup> , age <sup>2</sup> , link					
magnitude*unconfined, age*summer 10-day low flow, <i>Villosa vibex</i> *summer 10-day low flow, <i>Elliptio crassidens</i> *summer 10-day low flow, age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i>	21	16110.5	0.00	0.56	100
spring 10-day high flow, Dougherty, unconfined, link, percent agriculture, percent urban, age, <i>Villosa vibex</i> , <i>Elliptio crassidens</i> , percent shell wear, tagged, spring 10-day high flow <sup>2</sup> , age <sup>2</sup> , link*unconfined, age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , age*spring 10-day high flow, <i>Villosa vibex</i> *spring 10-day high flow, <i>Elliptio crassidens</i> *spring 10-day high flow	23	16112.5	1.98	0.21	37
summer10-day low flow, percent agriculture, percent urban, Dougherty, unconfined, link, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , summer10-day low flow <sup>2</sup> , age <sup>2</sup> , link*unconfined, age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , age*summer 10-day low flow, <i>Villosa vibex</i> *summer 10-day low flow, <i>Elliptio crassidens</i> *summer 10-day low flow	24	16114.2	3.71	0.09	16

Table. 8 (continued)

Model	K	AICc	ΔAICc	$w_i$	% $w_{max}$
summer 10-day low flow, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , summer 10-day low flow <sup>2</sup> , age <sup>2</sup> , age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , age*summer 10-day low flow, <i>Villosa vibex</i> *summer 10-day low flow, <i>Elliptio crassidens</i> *summer 10-day low flow	17	16115.8	5.29	0.04	7
summer 10-day low flow, percent urban, percent agriculture, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , summer 10-day low flow <sup>2</sup> , age <sup>2</sup> , age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , age*summer 10-day low flow, <i>Villosa vibex</i> *summer 10-day low flow, <i>Elliptio crassidens</i> *summer 10-day low flow	19	16115.9	5.41	0.04	7
summer 10-day low flow, percent urban, percent agriculture, Dougherty, unconfined, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , summer 10-day low flow <sup>2</sup> , age <sup>2</sup> , link*unconfined, age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , unconfined*summer 10-day low flow, link, link*summer 10-day low flow, age*summer 10-day low flow, <i>Villosa vibex</i> *summer 10-day low flow, <i>Elliptio crassidens</i> *summer 10-day low flow	25	16116.1	5.61	0.03	6

Table 8. (continued)

Model	K	AICc	$\Delta\text{AICc}$	$w_i$	% $w_{max}$
summer 10-day low flow, percent urban, percent agriculture, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , summer 10-day low flow <sup>2</sup> , age <sup>2</sup> , age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , age*summer 10-day low flow, <i>Villosa vibex</i> *summer 10-day low flow, <i>Elliptio crassidens</i> *summer 10-day low flow	19	16117.7	7.19	0.02	3
summer 10-day low flow, Dougherty, unconfined, link, percent urban, percent agriculture, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , summer 10-day low flow <sup>2</sup> , age <sup>2</sup> , link*unconfined, age* <i>Villosa vibex</i> , age* <i>Elliptio crassidens</i>	20	16121	10.51	0.00	1
summer long-term average flow, Dougherty, unconfined, Link, percent Urban, percent agriculture, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , summer long-term average flow <sup>2</sup> , age <sup>2</sup> , link*unconfined, age*summer long-term average flow, <i>Villosa vibex</i> *summer long-term average flow, <i>Elliptio crassidens</i> *summer long-term average flow	23	16122.1	11.63	0.00	0
spring long-term average flow, link, Dougherty, unconfined, percent Urban, percent agriculture, percent shell wear, tagged, age, <i>Elliptio crassidens</i> , <i>Villosa vibex</i> , spring long-term average flow <sup>2</sup> , age <sup>2</sup> , link*unconfined age* <i>Elliptio crassidens</i> , age* <i>Villosa vibex</i> , unconfined*spring long-term average flow, link*spring long-term average flow, age*spring long-term average flow, <i>Villosa vibex</i> *spring long-term average flow, <i>Elliptio crassidens</i> *spring long-term average flow	25	16122.3	11.82	0.00	0

Table 9. Akaike importance weights for predictor variables included in the candidate set of freshwater mussel growth models.

<b>Theme/ Predictor variables</b>	<b>Importance weights</b>
Seasonal (spring and summer) stream flow regime components affect freshwater mussel growth	0.998
summer 10 day low flow	0.781
spring 10 day high flow	0.210
spring long-term average flow	0.002
summer long-term average flow	0.002
spring 10 day low flow	0.002
summer 10 day high flow	0.001
no flow component in model	<0.001
Stream size and geomorphology affect freshwater mussel growth	0.906
link	0.705
unconfined	0.705
Dougherty	0.906
link magnitude * unconfined	0.906
Stream size and hydro-geomorphology mediate the effect of streamflows on freshwater mussel growth	0.262
Land coverage (agriculture and urban) influences freshwater mussel growth	0.389
Species and age mediate the effect of streamflows on freshwater mussel growth	0.996

Table 10. Parameter estimates, standard errors (SE), standardized parameter estimates (STD), and 95% confidence intervals of fixed and random effects for confidence set of linear mixed models of the growth of three species of freshwater mussels in the lower Flint River Basin, Georgia. Note that the species-specific estimates should be interpreted relative to the baseline species, *Villosa lienosa*.

Parameter	Estimate (SE)	STD Estimate	Lower CL	Upper CL
<b>Best-approximating model</b>				
Fixed effects				
Intercept	2.53900 (0.06193)	2.486	2.4151	2.6629
Age	-0.46350 (0.00878)	-0.454	-0.4807	-0.4463
<i>Elliptio crassidens</i>	0.46340 (0.10810)	0.301	0.2515	0.6753
<i>Villosa vibex</i>	-0.02421 (0.06168)	-0.066	-0.1451	0.0967
Percent shell wear	-0.81130 (0.11400)	-0.106	-1.0348	-0.5878
Tagged	-0.28590 (0.07282)	-0.286	-0.4287	-0.1432
Dougherty	-0.16300 (0.05316)	-0.163	-0.2672	-0.0588
Unconfined	-0.15600 (0.06319)	-0.555	-0.2799	-0.0321
Link magnitude	-0.00001 (0.00001)	-0.038	<-0.0001	<0.0001
Summer 10-day low flow	53.13900 (14.92260)	0.094	23.8841	82.3938
Age * <i>Villosa vibex</i>	0.04835 (0.01012)	0.048	0.0285	0.0682
Age * <i>Elliptio crassidens</i>	0.12670 (0.00973)	0.127	0.1077	0.1458
Summer 10-day low flow <sup>2</sup>	-3745.25000 (838.76000)	-0.042	-5389.59	-2100.900
Age <sup>2</sup>	0.00693 (0.00028)	0.007	0.0064	0.0075
Summer 10-day low flow *	2.80260 (0.89520)	0.009	1.0477	4.5576
Age				
Summer 10-day low flow *	-13.28800 (9.35970)	-0.045	-31.6371	5.0612
<i>Villosa vibex</i>				
Summer 10-day low flow *	-48.73010 (13.99980)	-0.163	-76.1759	-21.2840
<i>Elliptio crassidens</i>				

Table 10. (continued)

<b>Parameter</b>	<b>Estimate (SE)</b>	<b>STD Estimate</b>	<b>Lower CL</b>	<b>Upper CL</b>
Unconfined * Link magnitude	0.00037 (0.00002)	1.207	-0.0001	0.0008
Random effects				
Individual Mussel	0.01630 (0.00568)	0.016	0.0049	0.0277
Study Site	0.00403 (0.00296)	0.004	-0.0019	0.0099
Residuals	0.92460 (0.01785)	0.925	0.8889	0.9603

Table 10. (continued)

Parameter	Estimate (SE)	STD Estimate	Lower CL	Upper CL
<b>Second-best-approximating model</b>				
Fixed effects				
Intercept	2.53380 (0.09462)	2.495	2.3446	2.7230
Age	-0.45460 (0.00815)	-0.450	-0.4706	-0.4386
<i>Elliptio crassidens</i>	0.16380 (0.11040)	0.195	-0.0528	0.3803
<i>Villosa vibex</i>	-0.07156 (0.05945)	-0.053	-0.1881	0.0450
Percent shell wear	-0.78270 (0.11400)	-0.102	-1.0061	-0.5592
Tagged	-0.26830 (0.07371)	-0.268	-0.4128	-0.1238
Percent Urban	0.00231 (0.00342)	0.031	-0.0044	0.0090
Percent Agriculture	-0.00091 (0.00211)	0.017	-0.0032	0.0050
Dougherty	-0.23180 (0.07525)	-0.232	-0.3794	-0.0843
Unconfined	-0.15570 (0.07016)	0.645	-0.2933	-0.0182
Link magnitude	0.00003 (0.00001)	-0.009	<-0.0001	0.0001
Spring 10-day high flow	1.06790 (0.40370)	-0.012	0.2764	1.8594
Spring 10-day high flow <sup>2</sup>	-1.38070 (0.43120)	-0.016	-2.2261	-0.5353
Age <sup>2</sup>	0.00665 (0.00027)	0.007	0.0061	0.0072
Age * <i>Elliptio crassidens</i>	0.13460 (0.00954)	0.135	0.1159	0.1533
Age * <i>Villosa vibex</i>	0.04304 (0.01016)	0.043	0.0231	0.0630
Unconfined * Spring 10-day high flow	-0.55430 (0.31980)	-0.060	-1.1812	0.07253
Spring 10-day high flow *	-0.00051 (0.00013)	-0.179	-0.0008	-0.0003
Link magnitude				
Spring 10-day high flow *				
Age	0.06401 (0.03857)	0.007	-0.0116	0.1396
Spring 10-day high flow *				
<i>Villosa vibex</i>	0.26240 (0.26040)	0.028	-0.2481	0.7729

Table 10. (continued)

Parameter	Estimate (SE)	STD Estimate	Lower CL	Upper CL
Spring 10-day high flow *				
<i>Elliptio crassidens</i>	0.42820 (1.08070)	0.046	-1.6905	2.5469
Unconfined * Link magnitude	0.00044 (0.00023)	1.427	<-0.0001	0.0009
Random effects				
Individual Mussel	0.01782 (0.00572)	0.018	0.0064	0.0293
Study Site	0.00341 (0.00279)	0.003	-0.0022	0.0090
Residuals	0.92260 (0.01781)	0.923	0.8870	0.9582

Table 10. (continued)

Parameter	Estimate (SE)	STD Estimate	Lower CL	Upper CL
<b>Third-best-approximating model</b>				
Fixed effects				
Intercept	2.50490 (0.10370)	2.506	2.2975	2.7123
Age	-0.46340 (0.00879)	-0.454	-0.4806	-0.4462
<i>Elliptio crassidens</i>	0.45890 (0.11010)	0.297	0.2431	0.6748
<i>Villosa vibex</i>	-0.02537 (0.06174)	-0.069	-0.1464	0.0957
Percent shell wear	-0.81140 (0.11400)	-0.106	-1.0348	-0.5879
Tagged	-0.29240 (0.07367)	-0.292	-0.4369	-0.1480
Percent Urban	0.00198 (0.00349)	0.026	-0.0049	0.0088
Percent Agriculture	0.00089 (0.00218)	0.017	-0.0034	0.0052
Dougherty	-0.19460 (0.07735)	-0.195	-0.3462	-0.0429
Unconfined	-0.16740 (0.06839)	-0.586	-0.3015	-0.0333
Link magnitude	-0.00001 (0.00001)	0.036	<-0.0001	<0.0001
Summer 10-day low flow	53.02240 (14.89430)	0.094	23.8231	82.2218
Summer 10-day low flow <sup>2</sup>	-3726.01000 (839.65000)	-0.042	-5372.1	-2079.9
Age <sup>2</sup>	0.00693 (0.00028)	0.007	0.0064	0.0075
Summer 10-day low flow	2.81820 (0.89590)	0.009	1.0618	4.5746
* Age				
Summer 10-day low flow				
* <i>Villosa vibex</i>	-13.16090 (9.36500)	-0.044	-31.5204	5.1985
Summer 10-day low flow				
* <i>Elliptio crassidens</i>	-48.48510 (14.14530)	-0.163	-76.2160	-20.7540
Age * <i>Elliptio crassidens</i>	0.12660 (0.00974)	0.127	0.1075	0.1456
Age * <i>Villosa vibex</i>	0.04861 (0.01014)	0.049	0.0287	0.0685
Unconfined * Link magnitude	0.00040 (0.00024)	1.279	-0.0001	0.0009

Table 10. (continued)

<b>Parameter</b>	<b>Estimate (SE)</b>	<b>STD</b>	<b>Lower</b>	<b>Upper</b>
		<b>Estimate</b>	<b>CL</b>	<b>CL</b>
Random effects				
Individual Mussel	0.01637 (0.00570)	0.016	0.0050	0.0278
Study Site	0.00515 (0.00322)	0.004	-0.0019	0.0097
Residuals	0.92310 (0.01781)	0.925	0.8889	0.9409

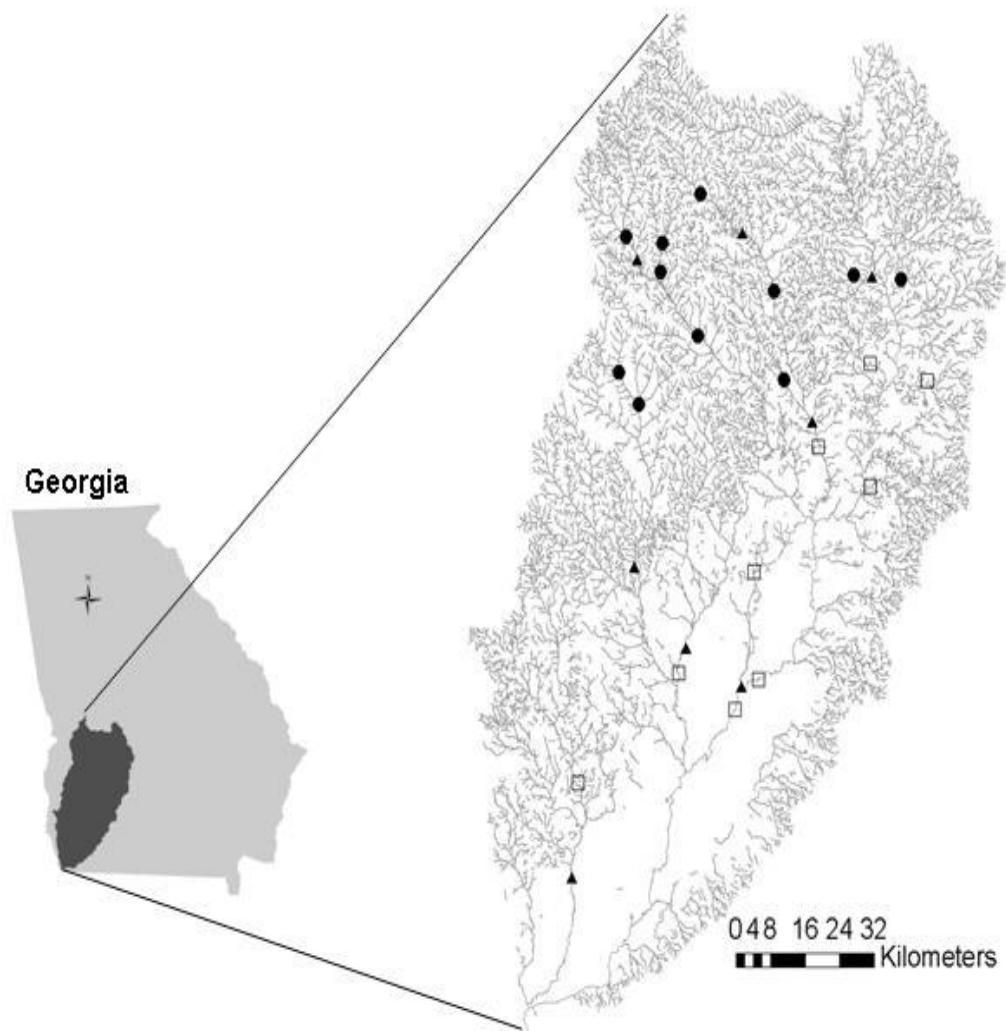


Figure 1. Locations of 20 study sites sampled in the lower Flint River Basin, Georgia. Triangles represent the location of the eight U.S. Geological Survey gages used to model site-specific seasonal discharges. Circles represent the location of 11 sites within the Fall Line Hills and squares represent the location of 9 sites within the Dougherty Plains.

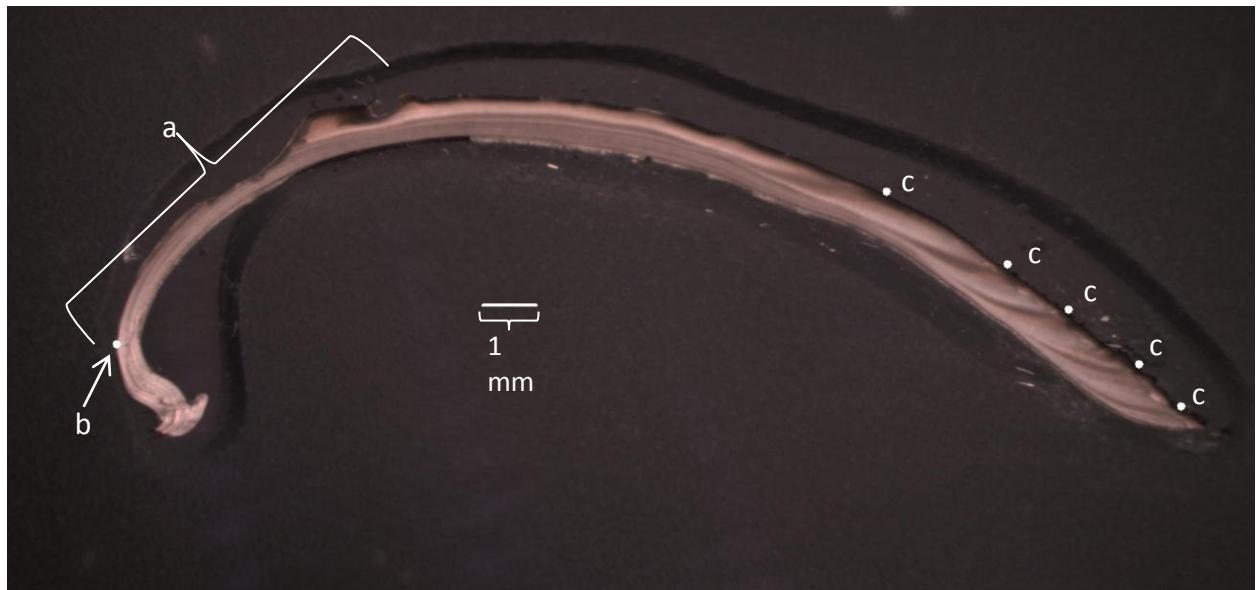


Figure 2. Thin section of an estimated five-year-old *Villosa lienosa* collected from Kinchafoonee Creek at Highway 45 showing the (a) shell wear present on the thin section estimated to be 20%, (b) the reference point used to measure annual growth, and the (c) marks for each of the five annuli's on the ventral margin of the shell.

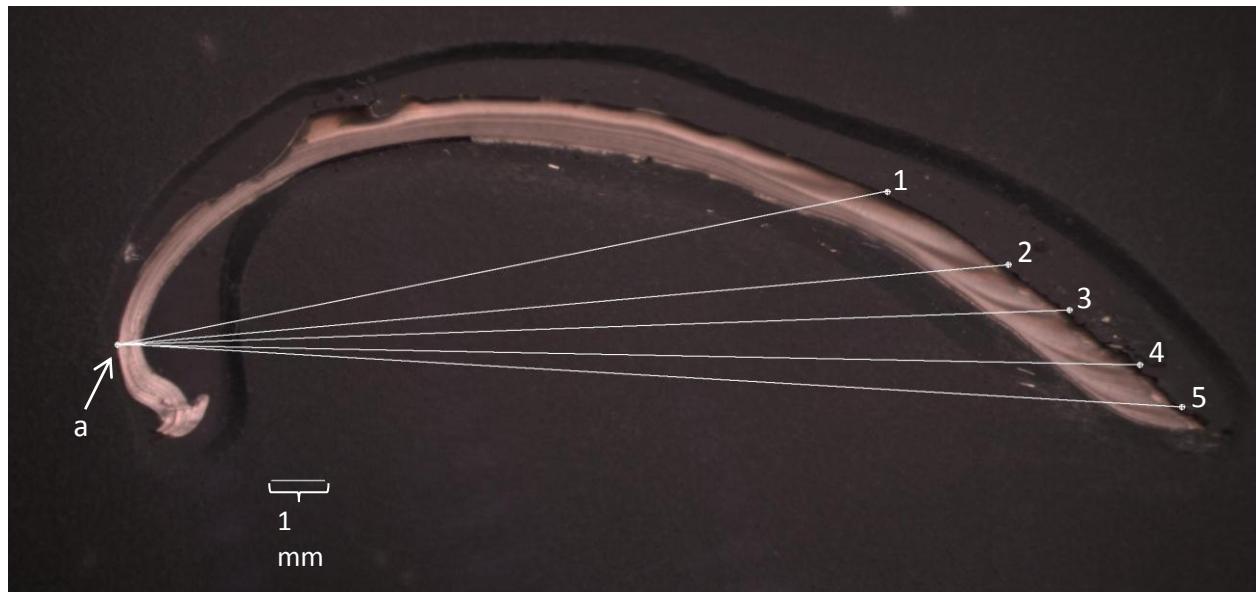


Figure 3. Annual growth increments denoting height-at-age from the reference point (a.) to each annulus (1-5), for a five-year-old *Villosa lienosa*.

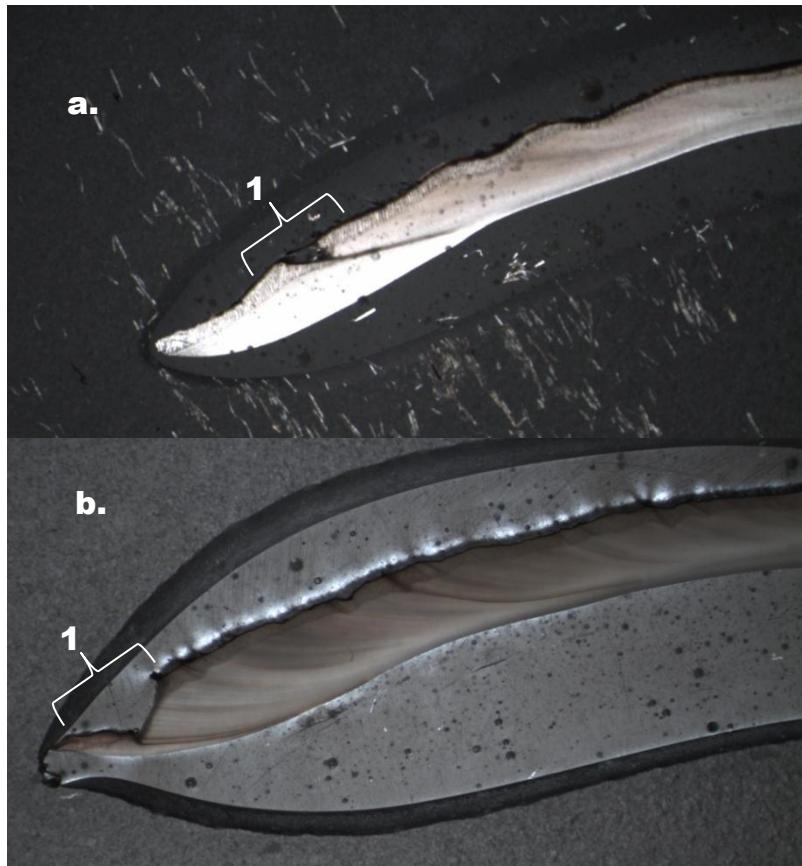


Figure 4. Thin section photographs (a. and b.) of two notched *Villosa lienosa*. Point (1) on both photos marks the notch produced by the rectangular file. Photograph (a) represents a poor quality thin section that is difficult to distinguish an annuli post-notching, due to the thinly polished tip. Photograph (b) represents an older mussel with little growth post-notching.

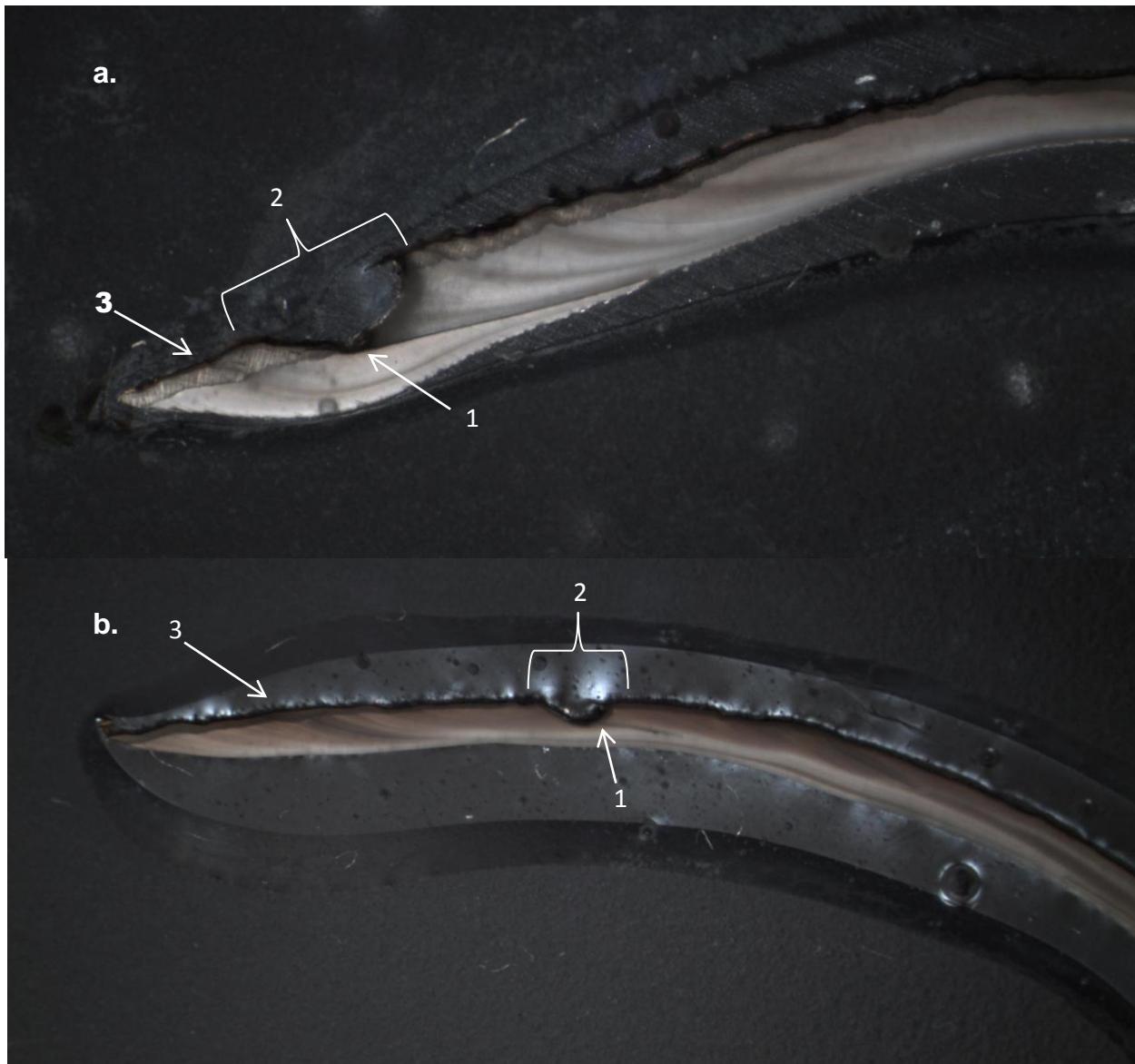


Figure 5. Thin section photographs of a notched *Villosa vibex* (a.) and *Villosa lienosa* (b.). Point (1.) is marking the disturbance ring in response to notching, (2.) is marking the notch created by the rectangular file, and (3.) is marking the first true annulus post notching.

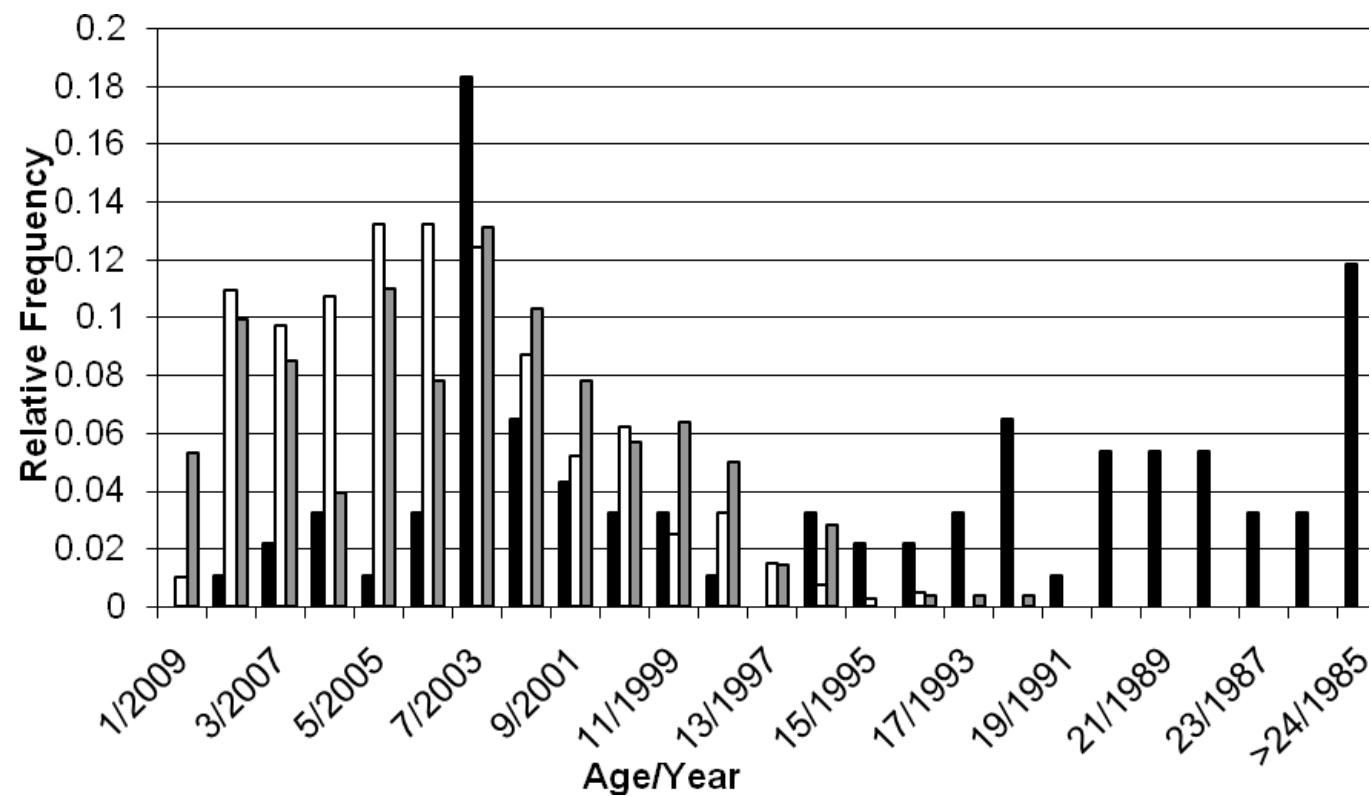


Figure 6. Relative frequency of freshwater mussel ages and year produced for *Elliptio crassidens* (black bar), *Villosa lienosa* (white bar), and *Villosa vibex* (gray bar) collected and thin sectioned for estimating species age and growth. Histogram is not believed to be a true representation of species demographics due to easier detection of larger individuals and sampling methods inability to collect young specimens (i.e., <2 years of age).

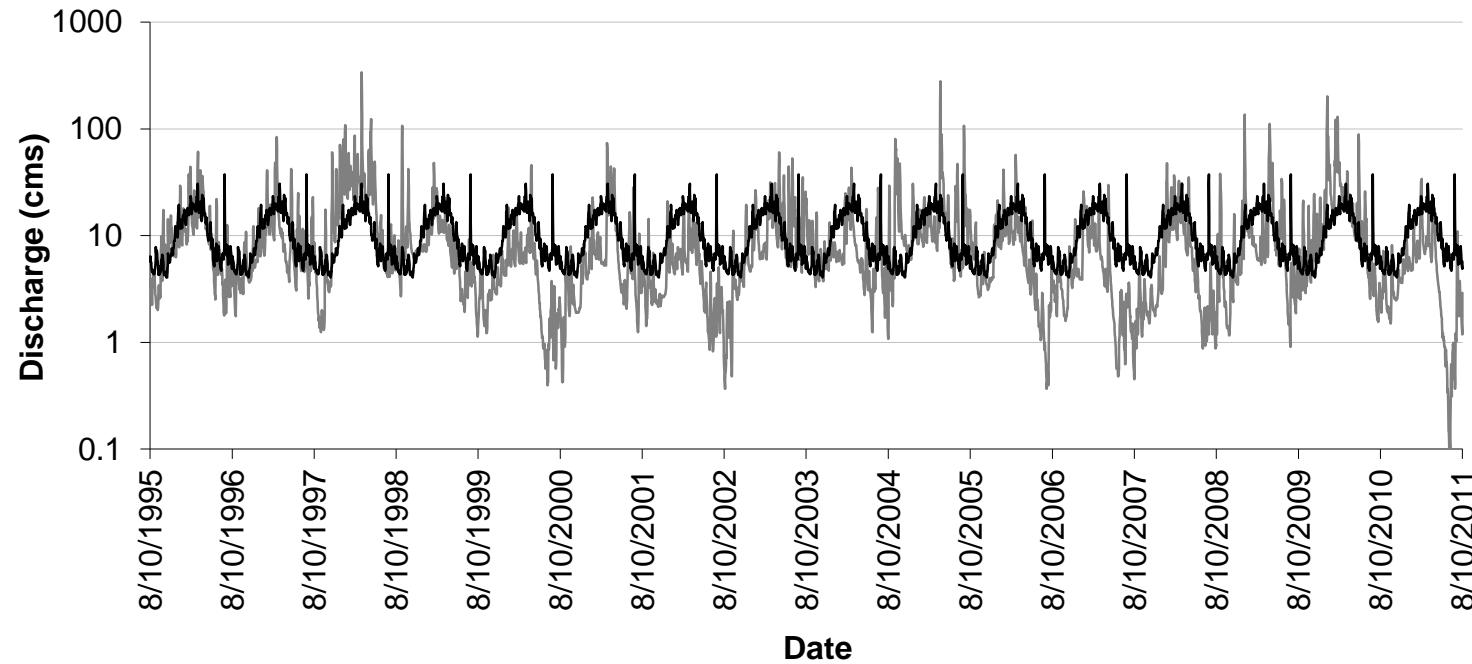


Figure 7. Log scaled average daily discharge from 1995 through 2011 (*gray line*) and log scaled daily average for the period of record (*black line*) at USGS stream gage 02351890 located on Muckalee Creek, near Leesburg, Georgia.

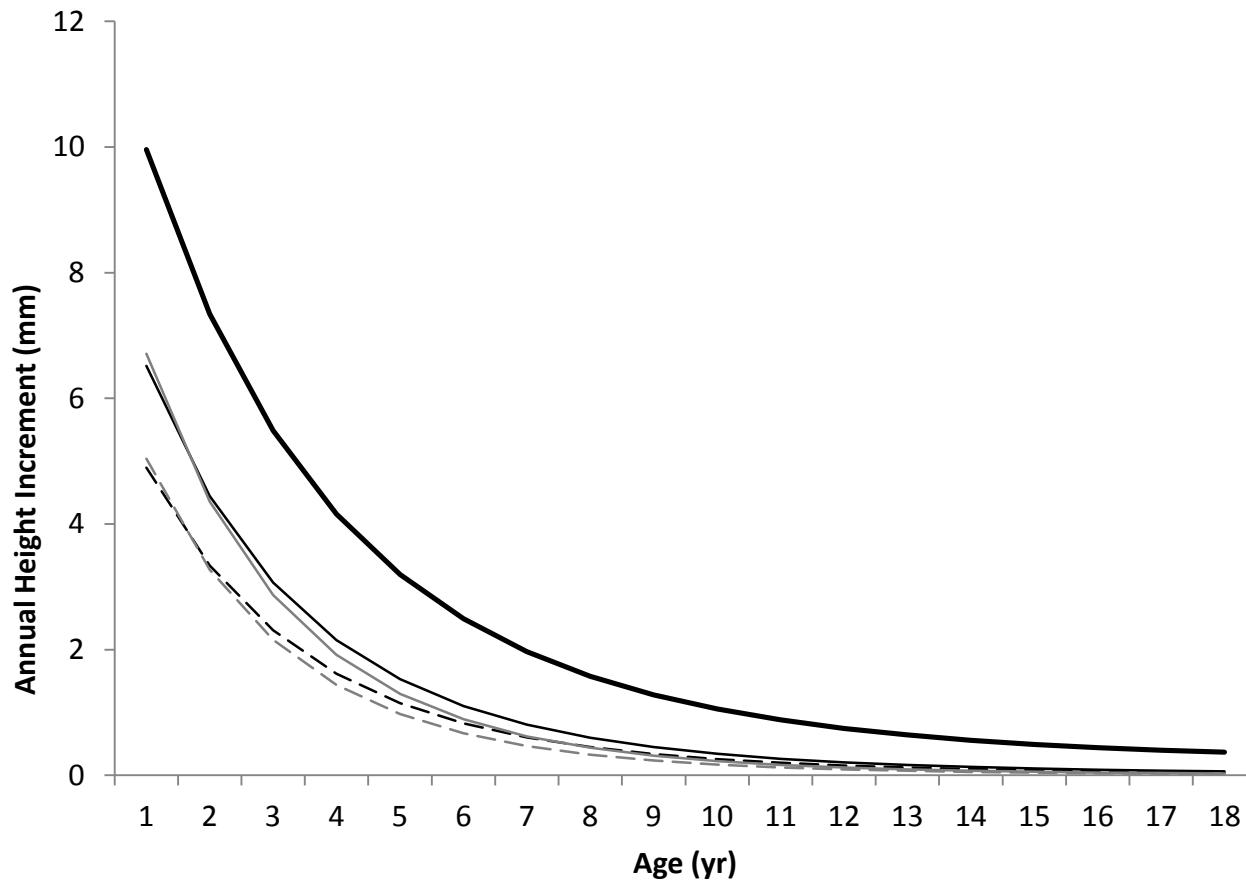


Figure 8. Estimated relation between age and annual growth for tagged (broken lines) and non-tagged (solid lines) *V. lienosa* (thin gray lines) and *V. vibex* (thin black lines), as well as non-tagged *E. crassidens* (thick black line). Annual growth was estimated using best approximating model and assuming a confined stream channel and average observed values for size stream and 10-day summer low flow in the lower Flint River Basin, Georgia.

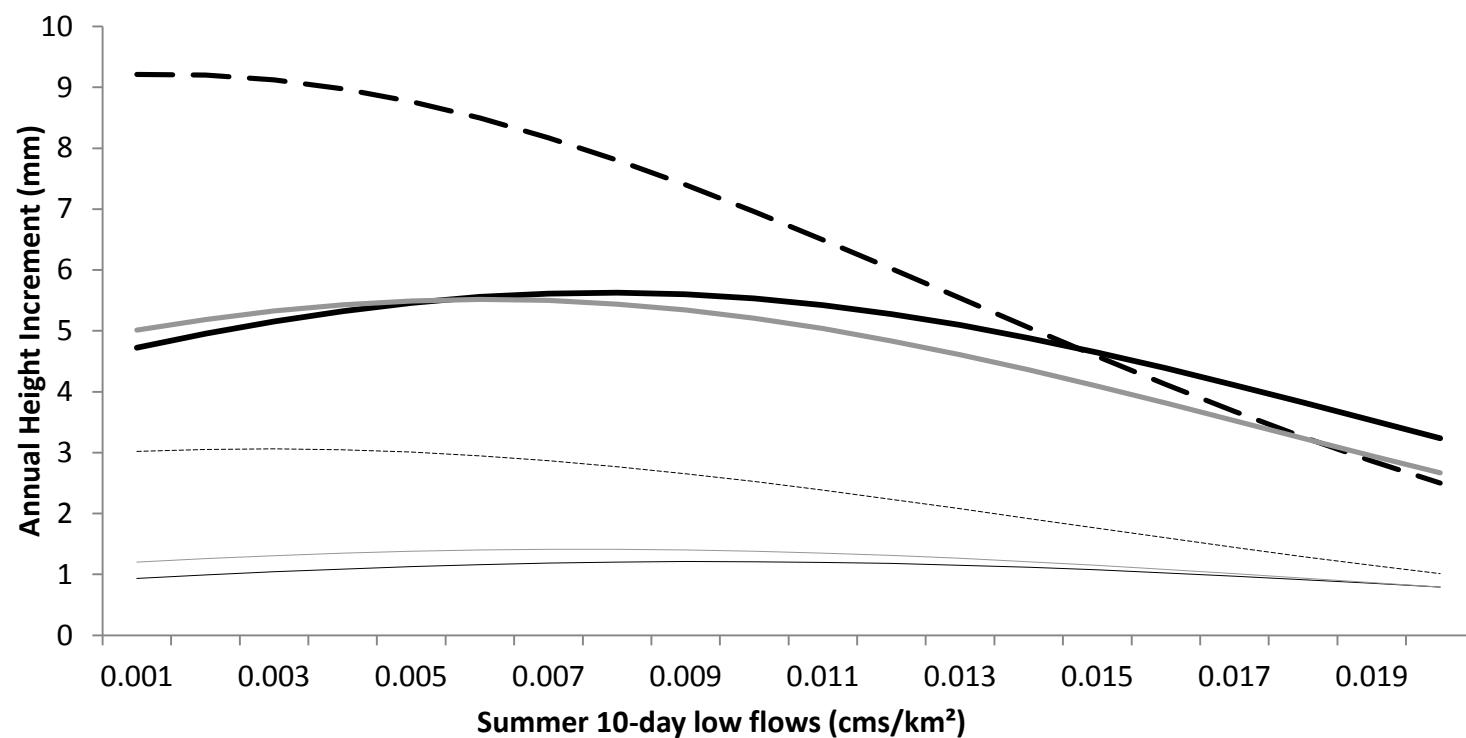


Figure 9. Estimated effect of summer 10-day low flows standardized by contributing watershed area on the growth of non-tagged *Villosa vibex* (gray lines), *Villosa lienosa* (black solid lines), and *Elliptio crassidens* (black broken lines) freshwater mussel species, at 2 (thick lines) and 6 (thin lines) years of age. Annual growth was estimated using best approximating model and assuming an unconfined stream channel and average observed value for size stream located within the Dougherty Plains physiographic province.

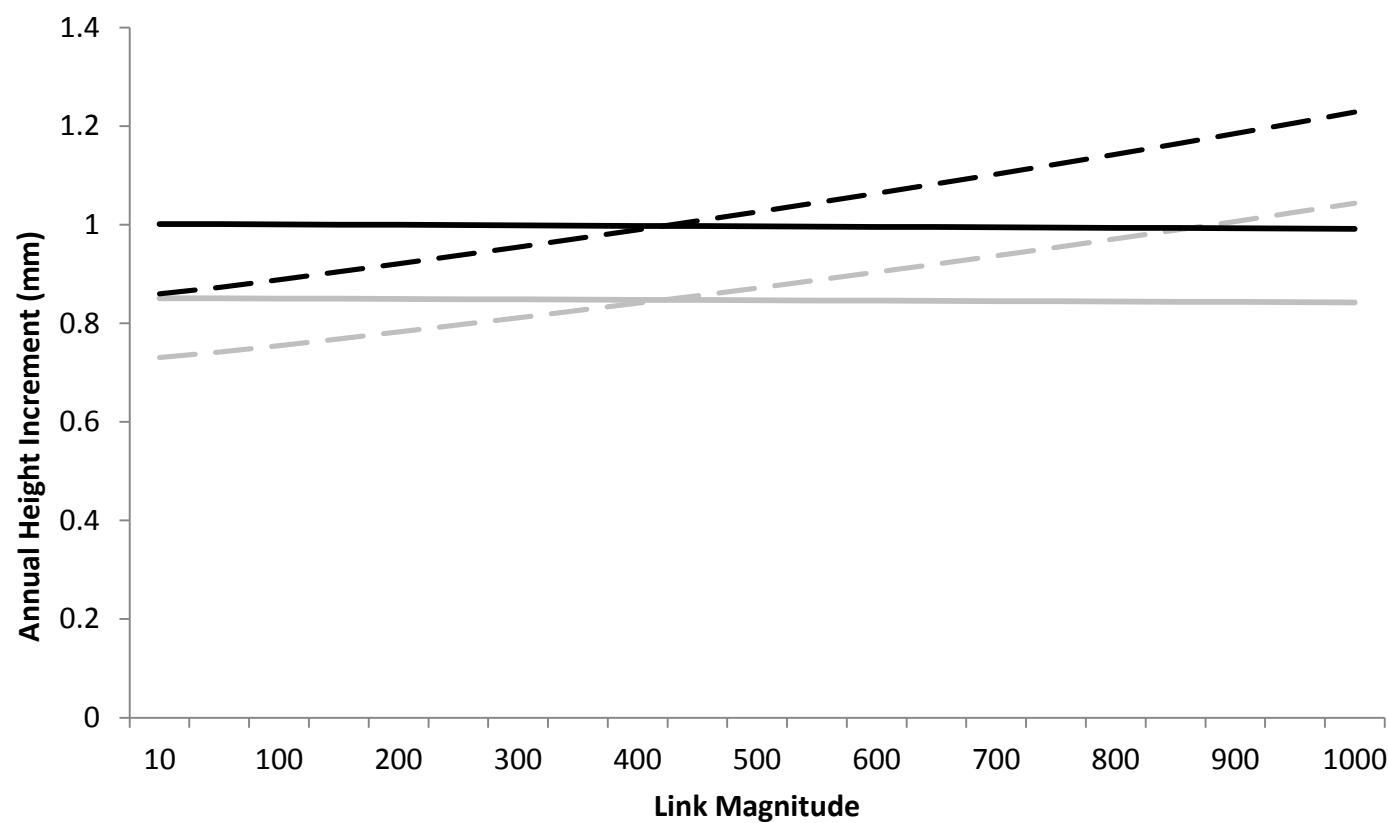


Figure 10. Estimated effects of different sized stream on the annual growth of an average aged *V. lienosa* in both an unconfined (broken lines) and confined stream (solid lines) within the Dougherty Plain (gray lines) and Fall Line Hills (black lines) physiographic province, during the average summer 10-day low flow. Annual growth was estimated using the best approximating model and assuming an untagged, average aged *Villosa lienosa*.