

EFFECTS OF CLIMATE, LAND USE AND IN-STREAM HABITAT ON
APPALACHIAN ELKTOE (*ALASMIDONTA RAVENELIANA*) IN THE NOLICHUCKY
RIVER DRAINAGE, NORTH CAROLINA

A Thesis
by
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Range-wide effects of climate change and increasing water temperature on the federally endangered Appalachian elktoe (*Alasmidonta raveneliana*)

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Abstract

The Appalachian elktoe is endemic to cool, high-elevation streams draining the Blue Ridge Physiographical Province in western North Carolina and eastern Tennessee. Populations are small and geographically isolated in the headwaters of 10 streams in western North Carolina. Appalachian elktoe populations face a range of threats including land use change, population fragmentation and modification of in-stream habitat by dams. Additionally climate change-related factors, including stream warming and altered flow regimes, may affect Appalachian elktoe populations as this species appears to occur near the upstream dissolved ion and temperature tolerance limits of freshwater bivalves. I examined long term water chemistry and temperature data obtained for 19 streams that historically supported Appalachian elktoe populations. Data were obtained from the US EPA STORage and RETrival (STORET) dataset and I computed land use statistics using 1992 and 2011 coverages. I grouped streams into two categories; those with apparently stable Appalachian elktoe populations and those with extirpated or severely declining populations. Land use in all streams was predominantly forest with some catchments having >90% forest in both 1992 and 2011. Forest cover in streams with stable Appalachian elktoe populations changed little compared to streams with declining or extirpated populations. Stream temperatures were

negatively correlated with both 1992 and 2011 forest cover. Moreover, I found that streams with stable Appalachian elktoe populations were cooler with higher dissolved oxygen (DO) and lower specific conductance levels compared to streams with declining or extirpated populations. Although the mechanisms responsible for Appalachian elktoe population declines remain poorly understood, my data provide compelling evidence that increased water temperatures, likely in response to forest loss, may be an important underlying factor. Future work should experimentally test the hypothesis that Appalachian elktoe growth, survival or reproductive output is affected by changing water temperature.

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Foreword

Chapters 1 and 2 will be submitted for publication and are formatted according to specific journal formatting requirements: Global Change Biology (Chapter 1), and Freshwater Science (Chapter 2).

CHAPTER 1

Range-wide effects of climate change and increasing water temperature on the federally endangered Appalachian elktoe (*Alasmidonta raveneliana*)

Introduction

Although Earth's climate varies substantially over seasonal and millennial time scales, the current rate of climate change is unprecedented (Intergovernmental Panel on Climate Change, 2007; Visser, 2008). Changes to climatic conditions are regarded by many scientists as the greatest known threat to biodiversity and global ecosystems (IPCC, 2007; Woodward *et al.*, 2010). Anthropogenically-derived increases in atmospheric carbon dioxide gas (CO₂) levels are associated with increased temperatures, increased temperature variability and altered precipitation regimes worldwide (Siegenthaler & Oeschger, 1978; Easterling *et al.*, 2000). Elevated CO₂ levels in the atmosphere are driving warming of polar latitudes and montane systems and leading to cooling and desertification of more tropical latitudes (Hassan *et al.*, 2005; Woodward *et al.*, 2010). Climate change is affecting the seasonality and severity of flooding and drought in many river systems (Easterling *et al.*, 2000; Hastie *et al.*, 2003; Woodward *et al.*, 2010). Analyses by the Intergovernmental Panel on Climate Change (IPCC) of global surface temperature data conclude that over the past 30 years (yr), the earth's mean temperature has risen ~0.2° C per decade (Hastie *et al.*, 2003). Climate records also indicate that Earth's warming trend over the past 50 years is nearly double that of the last 100 yr (IPCC, 2007).

Stream temperature changes associated with forest clearing in rural regions can be substantial and may mirror changes seen in more urbanized catchments (Lo *et al.*, 1997; Kaushal *et al.*, 2010). Most models suggest that the largest changes to stream temperature are expected in high latitude and high altitude catchments. Changes to riverine thermal regimes may

dramatically alter the growth, survival and functional efficiency of lotic species while altering the thermal environment and also the concentration of dissolved oxygen and other ions (Vannote & Sweeney, 1980; Chadwick & Feminella, 2001; Durance & Ormerod, 2007). Although climate-change effects are predicted to be most evident in developed catchments, recent data suggest that climate change effects are also evident in streams draining largely forested watersheds (Kaushal *et al.*, 2010).

Climate and other changes have already begun to affect water quality, ecosystem function, and biodiversity across the globe. (Hastie *et al.*, 2003; Bernhardt & Palmer, 2011). Although freshwater systems make up <0.8 % of Earth's surface, they support ~6% of global species diversity (Dudgeon *et al.*, 2006; Woodward *et al.*, 2010). Despite the fact that the increasing scarcity of freshwater resources has been identified as a current and future driver of geo-political unrest, the direct effects of climate change on streams remain under-studied (Meyer *et al.*, 1999; Daufresne *et al.*, 2004; Durance & Ormerod, 2007). Understanding the implications of this unprecedented rate of climate change for stream biodiversity and ecosystem function will be among the great challenges for 21st century conservation efforts (Kaushal *et al.*, 2010). Forecasting future stream responses to long-term fluctuation in water temperature will be critical in regulation of eutrophication, nutrient cycling and other ecosystem processes (Caissie, 2006). Many freshwater species are restricted to discrete thermal regimes and their ability to disperse and move to other habitats may be impeded by an ever-changing and increasingly fragmented hydroscape (Urban *et al.*, 2013).

Freshwater mussels are among the world's most-imperiled groups of freshwater taxa. In North America >74% of freshwater mussel taxa are considered imperiled or extinct (Williams *et al.*, 1993; Parmalee & Bogan, 1998; Strayer *et al.*, 2004; Cope *et al.*, 2008). These bivalves are

acutely sensitive to changes in water temperature and other stream physicochemical parameters. Freshwater mussel larvae (glochidia) are obligate fish parasites and reside on hosts for a few days to a few weeks, presumably as a dispersal mechanism (Williams *et al.*, 1993; Strayer *et al.*, 2004; Bey & Sullivan, 2015). Increasing stream temperatures are predicted to negatively affect mussel populations by potentially altering reproductive timing, fecundity, juvenile survival, adult longevity, and fish host availability or behavior (Vaughn & Taylor, 2000; Hastie *et al.*, 2003; Bey & Sullivan, 2015).

In this study, I examine changes to stream ecosystems across the range of Appalachian elktoe, an endangered freshwater mussel. I hypothesize that Appalachian elktoe populations are sensitive to changes in stream temperature and water chemistry and predict that streams that have lost populations will exhibit evidence of changes to habitat conditions, whereas streams with stable populations will exhibit little change in water chemistry parameters. By examining these changes in association with changes to forest cover over the past two decades, I will attempt to describe whether observed changes to stream physicochemical conditions are related to land use and climate change.

Methods

Stream Physicochemical Parameters

The United States Environmental Protection Agency (EPA) archives physical and chemical data obtained from atmospheric, sediment and water samples from multiple agencies. This data set is publicly available from the STORET web portal operated by the US EPA (EPA STORET website: <https://www.epa.gov/waterdata/storage-and-retrieval-and-water-quality-exchange>). I

obtained data from four commonly measured parameters: water temperature ($^{\circ}\text{C}$), specific conductance ($\mu\text{s}/\text{cm}$), dissolved oxygen (mg/L), and pH (as H^+ ion concentration) from the STORET portal. I obtained data for streams across the known range of Appalachian elktoe as of 17 February 2016. These data were largely collected by the North Carolina Department of Environment and Natural Resources (NCDENR) during routine stream water-quality monitoring studies.

I separated streams into those with extant and presumably viable Appalachian elktoe populations and those exhibiting population declines or from which Appalachian elktoe are believed to have been extirpated. Streams were separated into stable vs declining/extirpated populations by analyzing previous US Fish and Wildlife Service (USFWS) and North Carolina Wildlife Resource Commission (NCWRC) survey data. I removed sites that did not have monthly sampling events near continuously over the period of interest (1970-2009) from the dataset. This resulted in a total $n = 18$ sites, 5 sites were classified as stable and 13 were classified as extirpated/declining. Data were nearly continuous for all sites and across all decades (Table 1).

North Carolina Wildlife Resource Commission and US Fish and Wildlife Service data were used to assess trends in Appalachian elktoe populations (USFWS, 1994; USFWS, 2002; NC Wildlife Action Plan, 2005; USFWS, 2009). The South Toe, Mills, Little, Tuckasegee, and Cane Rivers were characterized as stable populations whereas the French Broad, Pigeon, Little Tennessee, Nolichucky, North Toe, and Cheoah Rivers were categorized as declining or extirpated populations. The Cane River was affected by a recent (2008) sewage treatment plant spill that resulted in the extirpation of much of that stream's Appalachian elktoe populations. However, the majority of the water quality data used in this study were from pre-2008 when

Appalachian elktoe populations were much larger, for the purpose of this analysis, the Cane River is considered to be a stable population.

Land use and Land Cover

I determined land use and land cover (LULC) cover for 1992 and 2011. To accomplish this I used ArcGIS v10.3 (ESRI, Redlands, CA 2015) and the ArcHydro toolset to delineate drainages for each of the 18 sites. Drainages were delineated using amended protocol for Merwade (2012). In my analysis, I amended the protocol by changing the stream initiation threshold. ArcHydro toolset > Flow Accumulation tool uses the slope of each digital elevation model (DEM) cell to calculate the number of cell's flowing in a down-hill direction. Initially every cell is assigned a value of 1, and as cells flow downhill they combine cell value, more commonly referred to as cell weight. I chose to set the Flow Accumulation tool to initiate a stream catchment where one cell obtained a weight of 1000. This differed from Merwade (2012) as they allowed the default Flow Accumulation threshold to automatically select the cell weight based on overall number of cells in the entire map. The default setting for the ArcHydro Toolset was to use the number of cells contained in 1% of the overall map area. I amended this cell weight threshold after visually assessing stream catchments and determining that default auto-selection under represented 1st and 2nd order streams thereby altering watershed size.

Drainages were delineated from the Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) Digital Elevation Models (DEM) downloaded from the US Geological Survey (USGS) Earth Explorer (30-m cell resolution) operated by USGS. The ASTER Global Digital Elevation Models were initial developed by the National Aeronautics and Space Administration (NASA) and Japan's Ministry of Economy, Trade and Industry (METI). I

obtained land use and land cover (LULC) data also from the USGS Earth Explorer data portal. Land use data was obtained as 30m x 30m cells, where each cell represented a unique LULC value. Watershed drainages calculated from the DEM file were used to extract the corresponding LULC data.

To simplify statistical analysis, I combined several categories of USGS LULC characteristics. Deciduous, evergreen and mixed forest land use characteristics were combined into a single category called forest cover. I also combined the USGS categories of low, moderate, and highly disturbed areas to one category, called disturbed. Finally, USGS LULC coverage data for row crop and pasture/hay LULC were combined to form the category of agriculture (Chapter 2).

Statistical Analysis

I implemented a commonly-used method for analyzing temperature, cumulative degree days (GDD), to assess temperature change at the monthly scale (McMaster & Wilhelm, 1997; Cayton *et al.* 2015). The following equation was used to derive the cumulative degree months (CDM) in my study:

$$CDM = \left[\frac{(\text{Max}^T + \text{Min}^T)}{2} \right] - \text{Base}^T$$

Where CDM is cumulative degree months, Max^T is the monthly highest temperature, Min^T is the monthly low temperature, and Base^T is a standardized temperature threshold used commonly in other studies, in this case 10°C. If the CDM calculation resulted in a negative value (i.e. $CDM < 10^\circ\text{C}$) the CDM for that month was set to zero (McMaster & Wilhelm, 1997; Cayton *et al.*, 2015). This equation yielded the cumulative temperature per month for each Appalachian elktoe

population. I determined CDM for each month of each decade as data were divided into the decadal scale to graphically illustrate temperature change over the given time frame (~1970-2009). Further, CDM was calculated for each river individually, then averaged among stable and declining/extirpated sites.

I used Spearman correlations to assess whether water temperature, specific conductance, dissolved oxygen (DO), and pH for the decades of 1990-2000 and 2000-2010 were correlated with 1992 or 2011 forest cover. I used *T*-tests to assess whether physicochemical parameters differed within each decade (1970s, 1980s, 1990s, 2000s) between sites with stable and declining or extirpated Appalachian elktoe populations. Because it was unknown whether many of the sites were occupied during each decade, I used the current distribution of Appalachian elktoe to assign population status classes. However it is important to note that some sites considered declining or extirpated (e.g., Little Tennessee River, North Toe River) had stable (at the time) Appalachian elktoe populations up until the mid-2000s (Fraley & Simmons, 2006; USFWS, 2009).

Results

Cumulative Degree Months

Analysis of cumulative long-term temperature data as degree months indicates that there has been considerable warming of all streams that historically supported Appalachian elktoe populations. Although cumulative water temperatures are much greater at sites with declining or extirpated Appalachian elktoe populations, streams with stable populations have become substantially warmer over the last 40 y (Figures 1 and 2). Additionally, cumulative temperatures in streams with stable populations have only increased during the late summer and fall months

whereas temperatures in streams with declining populations have increased during all portions of the growing season.

Water Temperature

T-tests indicate that water temperatures were statistically different between sites with and without stable Appalachian elktoe populations during the 1980s ($p=0.05$, $n=18$), 1990s ($p=0.036$, $n=18$) and 2000s ($p=0.038$, $n=18$). Differences between sites during the 1970s were not significant ($p=0.583$, $n=13$). However, I had a lower overall sample size and lacked 1970s data for 2 of the 5 extant Appalachian elktoe populations (Table 2).

Mean water temperatures were significantly negatively related to forest cover during both the 1990s ($p=0.002$, $r=-0.668$, $n=18$) and 2000s ($p=0.006$, $r=-0.617$, $n=18$) (Figure 3 and 4). Streams with more forested watersheds had generally cooler waters. Models suggest that for each 30x30 m area of forest cover lost, water temperatures increase by 0.08 (1990s) to 0.10 (2000s) °C (Table 3).

Dissolved Oxygen

I found no significant differences in DO concentration at the decadal scale between sites with and without stable Appalachian elktoe populations (Table 2). However, there was a significant positive relationship between DO levels and the percent of forest cover from 1992 ($p=0.001$, $r=0.705$, $n=18$) and 2011 ($p=0.021$, $r=0.538$, $n=18$). Models suggest that DO increases by 0.02 and 0.03 mg/L with every corresponding increase in forest cover for the 1992 and 2011 datasets (Table 3, Figure 3 and 4).

Specific Conductance

My data revealed no differences between specific conductance between sites with and without stable Appalachian elktoe populations for all decades (Table 2). However, I found a significant negative relationship between forest cover and specific conductance using both the 1992 ($p=0.002$, $r=-0.678$, $n=18$) and 2011 ($p=0.005$, $r=-0.628$, $n=18$) land cover data sets (Table 2). Models suggest that for every corresponding unit of forest cover loss, specific conductance increased by 3.72 (1992) and 3.71 (2011) units (Table 3, Figure 3 and 4).

pH

I observed differences in pH between sites with and without stable Appalachian elktoe populations during the 1980s ($p=0.029$, $n=18$) but pH was not different during the 1970s, 1990s and 2000s (Table 2). Additionally, I found no significant interactions between 1992 and 2011 forest cover and pH values (Table 3, Figure 3 and 4). While not significant, pH decreased by 0.01 (1992) and 0.02 (2011) with each increase in percentage of forest cover (Table 3).

Discussion

Although there has been considerable speculation about the potential for long-term changes in stream water quality and other habitat conditions to affect freshwater mussels and other sensitive riverine biota (e.g., Scott *et al.*, 2002; Hastie *et al.*, 2003; Daraio *et al.*, 2014), surprisingly few studies have used publically available datasets like EPA Storet to examine whether evidence exists for hypothesized changes to water or habitat quality (but see Daufresne *et al.*, 2004; Durance & Ormerod, 2007). These data provide some of the first evidence that changes in the

distribution and status of an imperiled freshwater mussel are associated with long-term (multi-decadal) changes to stream physicochemical parameters. Moreover, they suggest that these changes appear to be attributable to both relatively subtle shifts in land use and long term climate change. It is worrisome that relatively small losses to forest cover may trigger biologically meaningful changes in stream habitat conditions and while this is not likely unique to streams draining the southwestern end of the Blue Ridge Escarpment, it may reflect interactions between topography, geology and hydrology that are likely important to the conservation and management of sensitive freshwater taxa in this region (Chapter 2). Further, it appears that long term climate variation has been increasing temperatures over the past 4 decades while more recent land use and land cover degradation are exacerbating these changes by further altering water physicochemistry and in-stream habitat.

The limited amount of Appalachian elktoe population and distributional data available prior to the 1990s made it challenging to assess where populations historically occurred. In 1994 when this species was added to the Endangered Species List only three streams were known to contain Appalachian elktoe (The Little Tennessee, Nolichucky-North Toe and Cane rivers). Between 1996 and 2006 researchers discovered Appalachian elktoe populations in 7 additional streams (Tuckasegee [1996], South Toe [1998], Pigeon and West Fork Pigeon [1999], Cheoah [2000], Little [2000], and Mills [2003] Rivers). The Appalachian elktoe holotype was described by Isaac Lea from the Swannanoa River in Buncombe Co. NC in 1834. The species is also historically recorded from Tennessee in Tulula Creek near Robbinsville in Graham County in the Tennessee River drainage (Clarke, 1981). However, it appears populations in Tulula Creek and Swannanoa River were eliminated prior to the species listing in 1994 (Clarke, 1981; USFWS 2002, 2009). The limited data available suggest that they certainly occupied much larger areas

within their current watersheds (e.g., there are pre-1990 records of the Appalachian elktoe from the lower Nolichucky Drainage) and it seems reasonable to assume that many upper Tennessee River Drainage streams supported populations historically but there are few collections from this region prior to 1990.

Stream Physicochemistry and Mussels

The mechanisms by which changes to stream temperature, DO, conductivity, and pH may be affecting Appalachian elktoe populations in these streams are unclear. However, changes to thermal profiles can dramatically affect the local and longitudinal distribution of organisms in stream community structure in a number of ways (Vannote *et al.*, 1980; Strayer *et al.*, 2004; Hill *et al.*, 2013). Appalachian elktoe glochidia are obligate fish parasites, so thermally-mediated changes to fish distributions or behaviors may affect mussel reproductive success (Hastie *et al.*, 2003, Strayer *et al.*, 2004; Lydeard *et al.*, 2004; Cope *et al.*, 2008; Daraio *et al.*, 2014; Bey & Sullivan, 2015). Appalachian elktoe parasitize *Cottus bairdii* and *Cottus carolinae*, two sculpins that are closely-associated with cold or cool-water streams (USFWS, 2009). Therefore, it is possible that Appalachian elktoe could be undergoing recruitment failure due to thermally-mediated shifts in host fish abundance (Edwards & Cunjak, 2007). Water temperature may also influence mussel metabolic rates, reduce survivorship of glochidia, and alter other life history parameters (Zimmerman & Neves, 2002; Hastie *et al.*, 2003; Williams *et al.*, 2008; Pandolfo *et al.*, 2010). Further it has been shown that several species of freshwater mussels are at their upper thermal limits in many southeastern US streams (Pandolfo *et al.*, 2010).

The solubility of dissolved oxygen DO is inversely related to water temperature (Downing & Truesdale, 1955; Caissie, 2006). Low DO and hypoxic events are episodic stressors

in human-impacted freshwater systems but are relatively rare in cool-water streams (Bailey & Ahmadi, 2014). Adult mussels are relatively tolerant of low DO episodes but respiratory and thermal stress are both linked to spontaneous abortion of under-developed glochidia (Tankersley & Dimock, 1993; Martin *et al.*, 2004). Presumably premature glochidia abortion facilitates more efficient oxygen uptake and reduces stress on adults (Lomte & Nagabhushanam, 1971). Further, adult mussels living at the substrate/water interphase are exposed to much greater DO concentrations than juvenile mussels buried in the substratum. Interstitial DO levels are typically lower compared to levels in the water column (Sparks & Strayer, 1998). Declines in overall DO may stress both adult and juvenile mussels but most studies reveal that hypoxia is more stressful to juveniles compared to adult mussels.

Aqueous pH levels in Appalachian stream drainages are linked to DO concentrations as well as underlying geology and organic matter level inputs (Scott *et al.*, 2002; Haag, 2012). Tolerance levels of Appalachian elktoe are unknown but site-scale mean pH values across streams with stable populations ranged from 6.48 to 7.73 across the period of study (1970-2009). Makela and Oikari (1992) found that decreases in stream pH may dramatically alter the ion concentrations in mussel hemolymph. Further, changes to pH coupled with the availability of aqueous Ca^{2+} may influence the distribution and abundance of freshwater mollusks (Okland & Okland, 1986). Changes in mollusk species composition associated with increased acidity have been documented by previous studies (Haines, 1981; Havas & Hutchinson, 1983) and some blackwater streams are too acidic or have too little free calcium to support mollusks (Williams *et al.*, 2008). However, in comparison to other physicochemical parameters, relatively little is known about interactions between environmental pH and freshwater mussels (Makela & Oikari, 1992).

Cumulative degree day models are commonly used to model the growing season of plants and ectothermic animals. Implicit in the use of these models is that growth only occurs within a certain thermal regime (Cayton *et al.*, 2015; McMaster & Wihelm, 1997). I used a modified version of this approach to illustrate how thermal conditions have changed over time in streams with and without stable Appalachian elktoe populations. My results demonstrate that temperature has increased during the past ~40 y in all streams historically and currently occupied by Appalachian elktoe (Figures 1 and 2). Additionally, temperature shifts appear much smaller and are not evident until later in the year (August-September) in streams with stable populations. However, in streams with declining or extirpated populations these shifts occur much sooner (March-April) and remain pronounced throughout the growing season (Figures 1 and 2). Appalachian elktoe exchange gametes during late summer, brood larvae throughout the fall and winter months and release glochidia in early spring (April-May, USFWS 1994, 2009). Therefore, it is possible that increased temperatures may directly affect the timing and duration of Appalachian elktoe gamete exchange or larval brooding. Changes to the timing of mussel reproductive and developmental traits may exacerbate the potential for phenology-based mussel-host fish mismatches. Future research should investigate the thermal range which is most optimal for Appalachian elktoe. However, because the Appalachian elktoe is federally protected, research determining lethal temperature levels would require the use of propagated individuals.

Land use and Land cover

A large number of studies have demonstrated links between land use/land cover (LULC) and stream habitat parameters including temperature (e.g. Burton & Likens, 1973; Scott, 2006; Pandolfo *et al.*, 2010; Daraio *et al.*, 2014) and hydrogeology (Hopkins & Roush, 2013). Loss of

forest cover has been implemented as the causative agent behind the decline and extirpation of multiple freshwater taxa including hellbenders (Pugh *et al.*, 2015), fishes (Hopkins & Roush, 2013) and freshwater mussels (Hopkins, 2009). These data suggest that watershed land use is correlated with all physicochemical parameters analyzed in this study (Table 3) and Appalachian elktoe presence during both 1992 and 2011 (Table 2). More comprehensive analysis of habitat and land use parameters across the Nolichucky Drainage suggest that riparian forest cover is an important predictor of Appalachian elktoe presence (Chapter 2).

Management Implications

These data suggest that there is some evidence that changes to stream thermal regimes across the range of Appalachian elktoe are due to extrinsic forces like climate change. Additionally, I found evidence that land use change may be responsible for the recent extirpation of many populations. These findings underscore the basic understanding that conservation of forest cover is critical to the long-term persistence of sensitive freshwater taxa (Scott *et al.*, 2002, Helms *et al.*, 2009) specifically, retention of forest cover may play a critical role in mitigating the potential effects of both extrinsic and intrinsic threats to the persistence of these now small and highly-fragmented populations. All of the streams included in this study except the Mills River were listed as critical habitat for Appalachian elktoe by the USFWS in 2002. However, apart from parts of the upper Cheoah River and a short reach of the Little Tennessee River, all of the stream reaches currently or recently occupied by Appalachian elktoe are in private ownership but the headwaters of several key populations (Cane, South Toe, Tuckasegee, West Fork Pigeon rivers) drain lands managed by the US Forest Service (USFWS, 2009).

Protected forest in the headwaters of many streams with existing Appalachian elktoe populations may help mitigate threats in the lower reaches of these watersheds. Chapter 2 found that stream substrate composition was a key factor associated with the presence of Appalachian elktoe populations in the Nolichucky Drainage and these findings mirror those of several natural resource agencies. For example, the lower Cane River and the entire formerly-occupied reach of the North Toe were recently added to the NC Department of Environmental Quality's 303(d) impaired streams list for excessive turbidity levels (USFWS, 1994, 2009). Critical habitat designation only provides protection from modification resulting from federally-funded projects and does not prevent private landowners from developing or clearing streamside or upland habitats. Although protection of forested lands can be challenging at a drainage scale, managing riparian forest cover is more practical and may be equally or more important in these watersheds (Chapter 2). Because the overall trend has been for elktoe populations to shift from larger streams to increasingly small and isolated sub-catchments, management of riparian corridors and forest cover at small scales becomes increasingly important. This is supported by my findings that readily observable and biologically meaningful changes to stream physicochemical parameters are associated with seemingly low levels of forest cover disturbance (<15%) and indicates an acute need for more active watershed protection within the increasingly small range of the Endangered Appalachian elktoe mussel.

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Tables and Figures

Site	GPS Coordinate	P/A	Temperature (°C)				Dis. Oxygen (mg/L)				Spc. Con. (µS/cm)				pH			
			1970	1980	1990	2000	1970	1980	1990	2000	1970	1980	1990	2000	1970	1980	1990	2000
CR1	36.014, -82.328	P	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
CH1	35.329, -83.809	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
FB1	35.299, -82.623	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
FB2	35.609, -82.578	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
FB3	35.708, -82.662	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
FB4	35.786, -82.661	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
LR1	35.192, -82.615	P	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
LTR1	35.122, -83.378	A	—	x	x	x	—	x	x	x	—	x	x	x	—	x	x	x
LTR2	35.234, -83.395	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MR1	35.399, -82.596	P	—	x	x	x	—	x	x	x	—	x	x	x	—	x	x	x
NT1	35.981, -82.016	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
NT2	35.929, -82.115	A	x	x	x	x	—	x	x	x	x	x	x	x	x	x	x	x
Noli1	36.075, 82.345	A	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PR1	35.525, -82.841	A	—	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PR2	35.535, -82.911	A	x	x	x	x	—	x	x	x	x	x	x	x	x	x	x	x
PR3	35.667, -82.990	A	—	x	x	x	—	x	x	x	—	x	x	x	—	x	x	x
ST1	35.831, -82.184	P	—	x	x	x	—	x	x	x	—	x	x	x	—	x	x	x
TR1	35.428, -83.446	P	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Table 1. Locality information and availability of decadal water physicochemical parameters. Streams with multiple sites were numbered ascending order in a downstream direction. CR=Cane, CH=Cheoah, FB=French Broad, LR=Little River, LTR=Little Tennessee River, MR=Mills River, NT=North Toe, Noli=Nolichucky, PR=Pigeon River, ST=South Toe, TR=Tuckasegee River. Bolded dashes (—) indicated decades in which water physicochemical parameters were not available from STORET Spc. Cond. = Specific Conductance and Dis. Oxygen = Dissolved Oxygen and P/A = Present or Absent.

Decade		Forest 1992	Forest 2011	Temp (°C)	DO (mg/L)	Spc. Con. (µS/cm)	pH
1970	p	-	-	0.583	0.877	0.952	0.726
	df	-	-	11	12	12	13
	n	-	-	13	14	10	15
1980	p	-	-	0.050	0.397	0.304	0.029
	df	-	-	16	16	16	16
	n	-	-	18	18	18	18
1990	p	0.014	-	0.036	0.213	0.248	0.061
	df	16	-	16	16	16	16
	n	18	-	18	18	18	18
2000	p	-	0.018	0.038	0.162	0.191	0.188
	df	-	16	16	16	16	16
	n	-	18	18	18	18	18

Table 2. Results of Students *T*-Tests comparing stream physicochemical and land use parameters for streams with extant and declining/extirpated Appalachian elktoe populations. Bold values indicate that differences were statistically significant ($p < 0.05$). DO = Dissolved Oxygen, Spc. Con. = Specific Conductance.

LULC Year	Decade		Temperature (°C)	DO (mg/L)	Sp. Con. (µS/cm)	pH
Forest 1992	1990	p	0.002	0.003	0.002	0.192
		Δ	-0.08	0.02	-3.72	-0.01
		r	-0.668	0.705	-0.678	-0.322
		n	18	18	18	18
Forest 2011	2000	p	0.006	0.021	0.005	0.063
		Δ	-0.1	0.03	-3.71	-0.02
		r	-0.617	0.538	-0.628	-0.447
		n	18	18	18	18

Table 3. Results of Spearman correlations between 1992 and 2011 % forest cover and decade-scale stream physicochemical parameters. Bold values indicate that differences were statistically significant ($p < 0.05$). DO = Dissolved Oxygen, Sp. Con.= Specific Conductance.

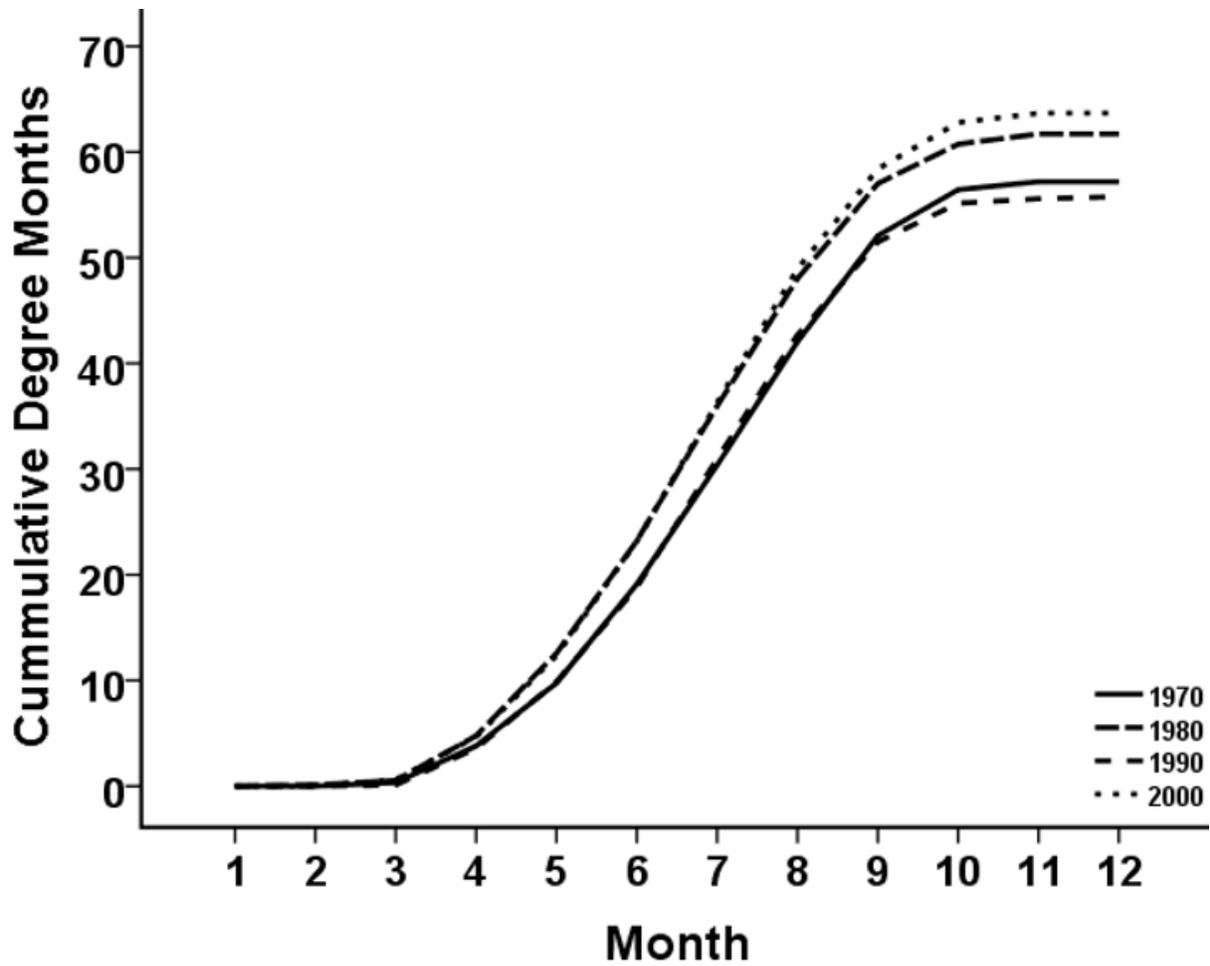


Figure 1. Cumulative Degree Months line graph of sites with declining Appalachian elktoe populations, each line represents a decade (1970-2000). Notice early separation of temperature in March-April (Appalachian elktoe spawning season) continuing throughout the year and into study species breeding season (August-September).

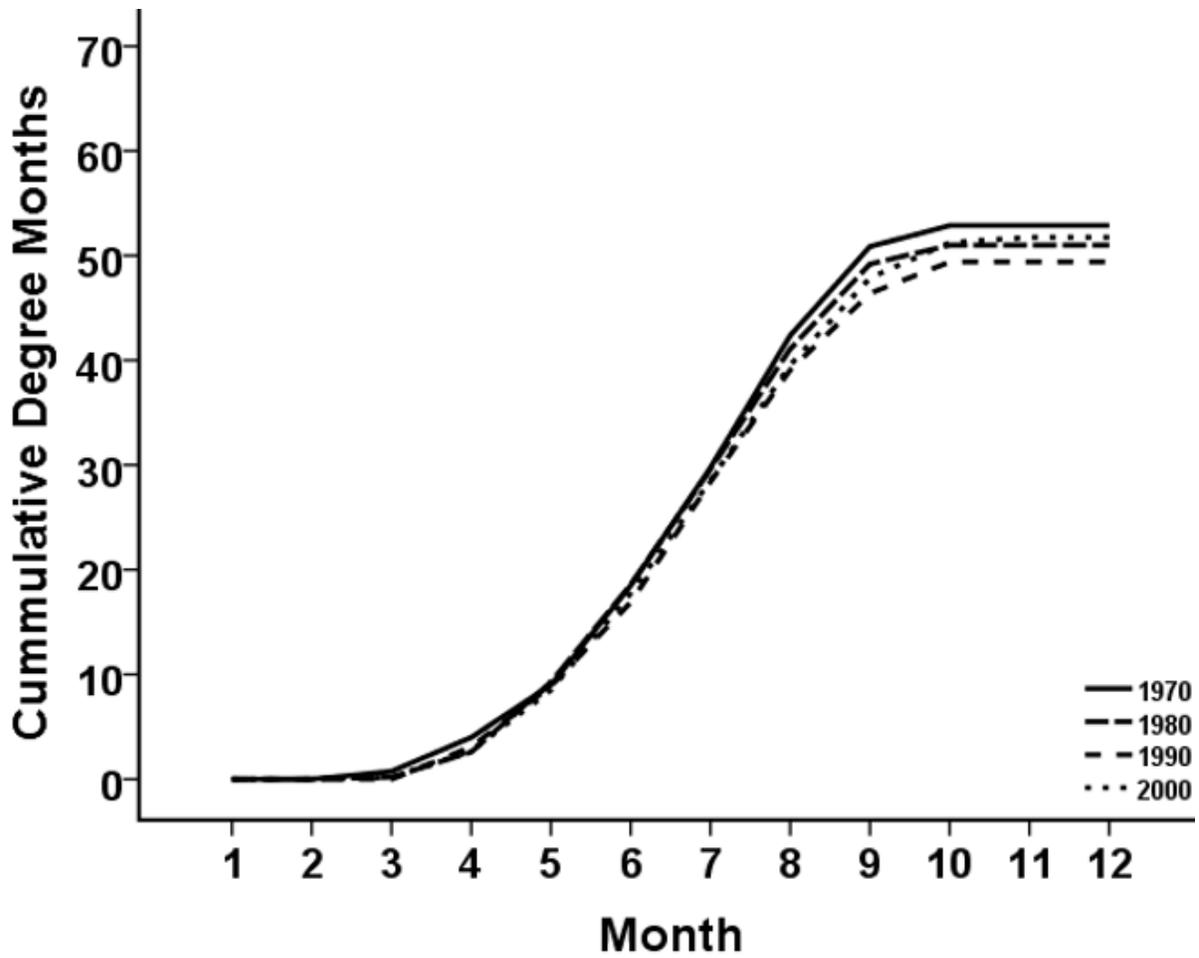


Figure 2. Cumulative Degree Months line graph of sites with stable Appalachian elktoe populations, each line represents a specific decade (1970-2000). Notice little variation in monthly temperature April-May (Appalachian elktoe breeding season) until July-August (prior to Appalachian elktoe breeding season) slight increase in temperature.

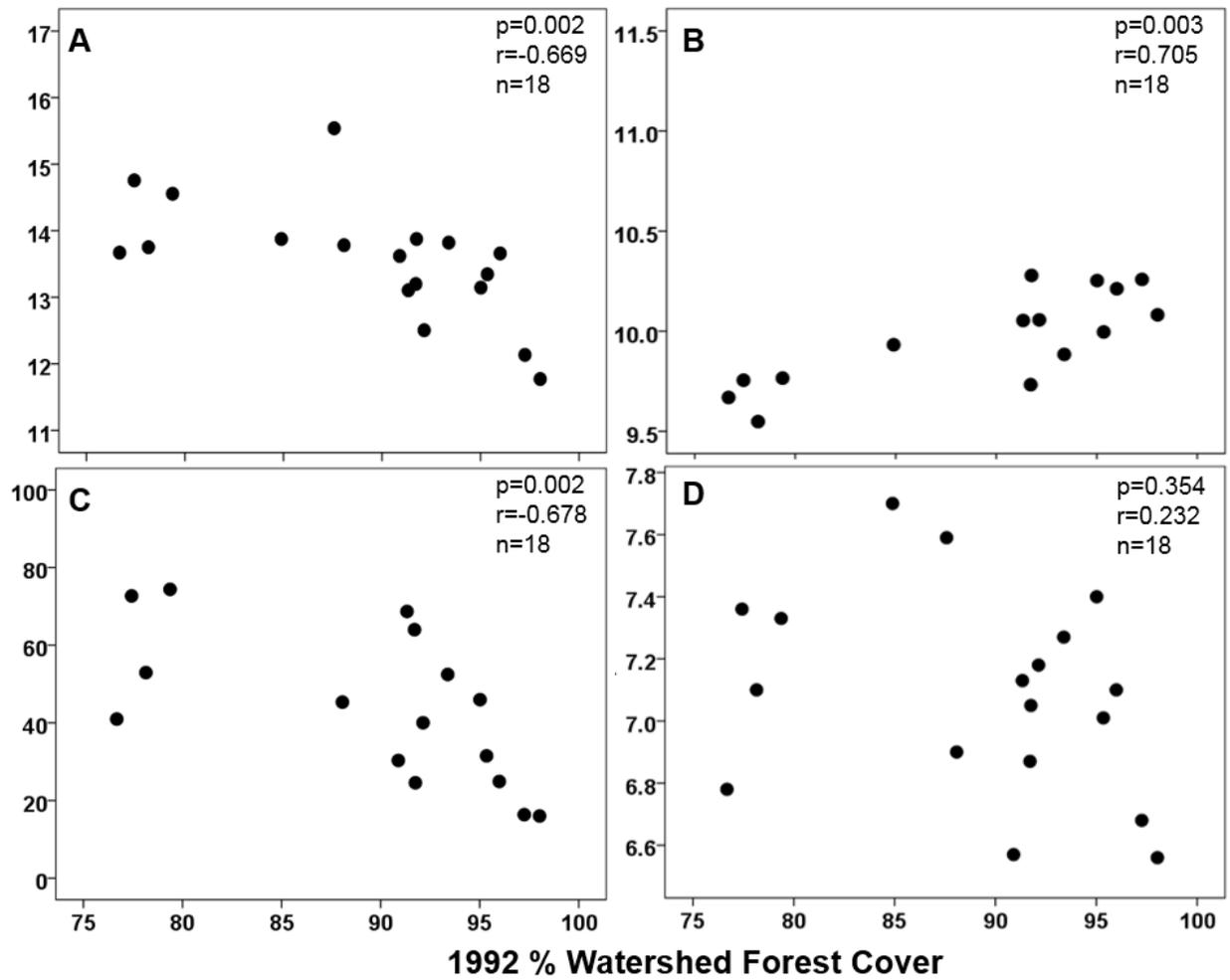


Figure 3. Scatter plot showing Spearman correlation relationships between 1992 % watershed forest cover and A) water temperature (°C), B) dissolved oxygen (mg/L), C) specific conductivity (μS/cm), and D) pH. All trends are significant at the $p \leq 0.05$ level except pH.

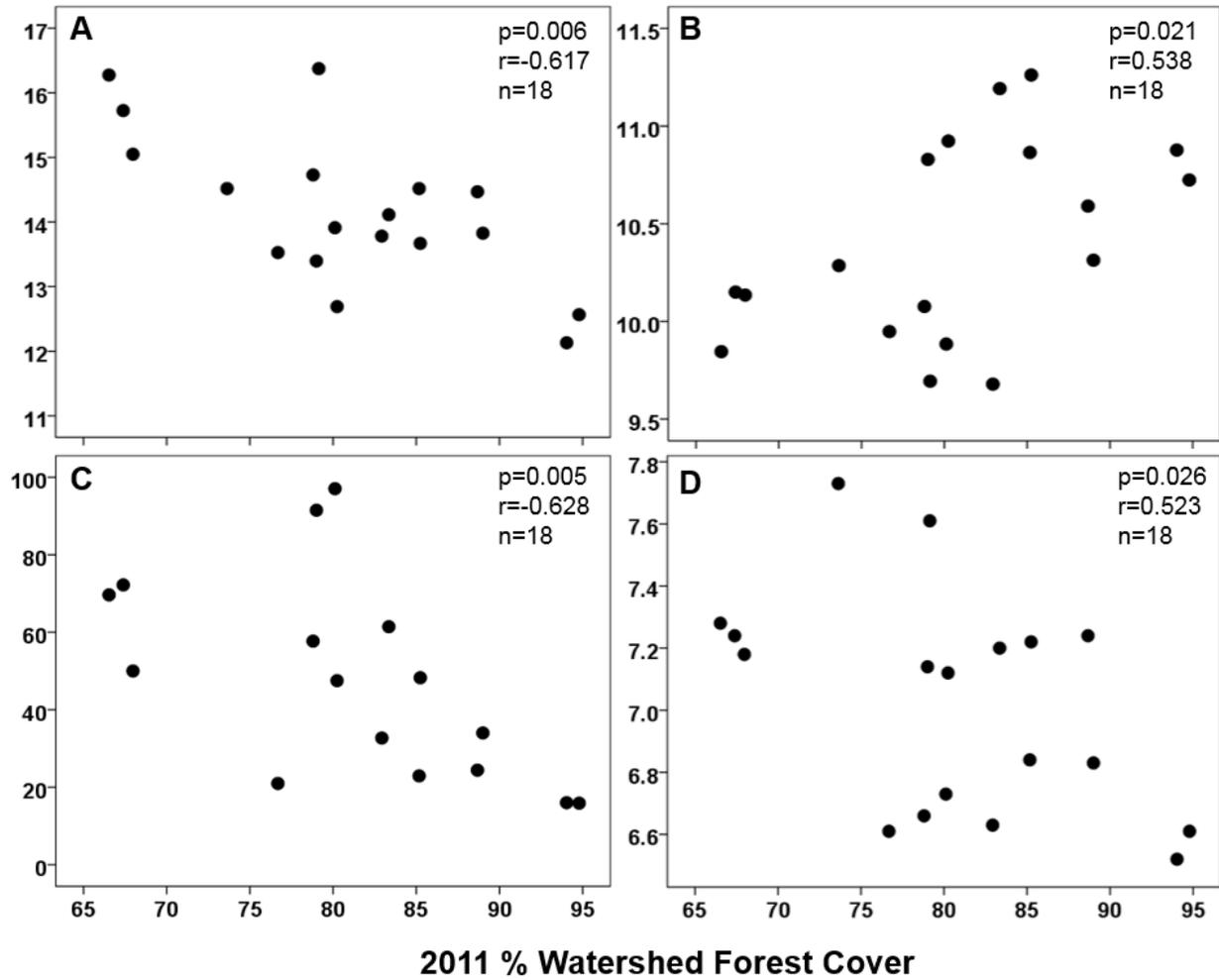


Figure 4. Scatter plot showing Spearman correlation relationships between 2011 % watershed forest cover and A) water temperature (°C), B) dissolved oxygen (mg/L), C) specific conductivity (μS/cm), and D) pH. All trends are significant at the $p \leq 0.05$ level.

CHAPTER 2

Effects of land use and in-stream habitat on Appalachian elktoe (*Alasmidonta raveneliana*) in the Nolichucky River Drainage, North Carolina

ABSTRACT

In-stream habitat variables such as flow velocity, depth, particle size, and water temperature largely impact the location and distribution of mussels and other benthic organisms within the stream continuum. The Appalachian elktoe (*Alasmidonta raveneliana*) is endemic to upper Tennessee River drainage streams draining the Blue Ridge Physiographic Province in North Carolina and Tennessee. Populations of this federally-endangered mussel have declined across parts of its highly-restricted range during the past three decades, but few parsimonious mechanisms have been proposed to explain its near complete extirpation from several former strongholds. Some evidence suggests that Appalachian elktoe are extremely sensitive to subtle environmental changes. Further, Appalachian elktoe appear to be a headwater specialist and occur near what appears to be the upstream limits for bivalves in many Blue Ridge streams. The goal of the current research is to examine how land use, at both the watershed and local scales, influences in-stream habitat conditions and the distribution of Appalachian elktoe populations within the Nolichucky River Drainage. This system supports Appalachian elktoe populations that vary in abundance by several orders of magnitude (0-217 mussels per site). Although Appalachian elktoe were more likely to be detected at sites with more forested watersheds and riparian zones as well as coarser substrates compared to sites with less forest cover and more fine sediments, there were few significant relationships between forest cover and physical habitat parameters. These data suggest that the current distribution of Appalachian elktoe in the Nolichucky Drainage may be driven by recent land use changes. Alternatively, they may suggest

that long-term land use-mediated changes in stream temperature and dissolved ion concentrations may be driving shifts in the distribution of Appalachian elktoe in the Nolichucky Drainage and other watersheds.

INTRODUCTION

Stream ecologists have long noted the important interactions between aquatic systems and their associated terrestrial landscapes. Conditions within terrestrial watersheds strongly influence stream hydrologic, thermal, and geomorphic attributes (Harding et al. 1998, Gergel et al. 2002, Hopkins and Roush 2013). The intensity and frequency of landscape-scale disturbances may have profound effects on water quality as well as the composition of benthic invertebrate and fish communities (Snyder et al. 2003, Allan 2004, Pan et al. 2004, King 2005, Ahern et al. 2005, Weijters et al. 2009). Numerous studies have shown that there is a lag between land use change and subsequent changes to stream habitat and water quality (Harding et al. 1998, Maloney et al. 2008). Sediments derived from historical land use practices may contribute more to overall stream health than current day land use (Scott, 2006). Harding et al. (1998) coined the term “land use legacy” to describe the observation that land use during the 1950s was a better predictor of present day (1990s) fish diversity than were current land use practices. This suggests that the current distribution of organisms may be influenced more strongly by latent effects of past land use than by current land use conditions.

Beginning in the 19th century, the Southern Appalachian region, including much of the Nolichucky River Drainage, experienced large-scale clearing of forests and conversion to agricultural fields (Glenn 1911). However, during the late 20th and early 21st centuries, forests

across much of this region began to regenerate (Harding et al. 1998). Glenn (1911) indicated that ~74% of all lands were still forested around the turn of the century; however, many river valleys and hill slopes were completely denuded of vegetation during intensive logging and conversion to agriculture fields during subsequent decades. Presently, urban and localized ex-urban development have expanded the effects of human populations into formerly rural regions including many upland and steep-sloped habitats in the Appalachian Mountains (Scott 2006). However, the short-and long-term impacts of more recent ex-urban development are not well-understood (Paul and Meyer 2001, Scott 2006, Maloney et al. 2008). In addition to the more obvious impacts of land clearing, road and other infrastructure construction associated with development may also disrupt stream hydrology and geomorphology and lead to stream warming, habitat loss and reduced water quality (Scott 2006).

Changes to land use and land cover (LULC) at multiple scales have the potential to impact freshwater mussel populations (Ellis 1936, Brim-Box and Mossa 1999). Sedimentation from altered land use practices has the potential to fill intestinal spaces in larger particles forming hardpan (Gordon et al. 1994), disrupt filter feeding by clogging and fouling gills (Ellis 1936, Kat 1982, Aldridge et al. 1987) and smothering of juvenile mussels. This change in sedimentation regimes may often produce changes in mussel fauna and the affects can take years to become noticeable (Brim-Box and Mossa 1999). However, it is frequently difficult to link aquatic habitat degradation to land use alteration because landscape disturbances exhibit a high degree of spatial and temporal variability (Brim-Box and Mossa 1999, Allan 2004).

Episodic flooding is an important stressor to benthic organisms, including mussels, and its effects are evident at multiple scales (Di Maio and Corkum 1995, Gangloff and Feminella 2007). Many benthic organisms survive the scouring effects of spates by moving into flow (or

hydraulic) refugia that experience lower-intensity shearing and turbulent forces and exhibit higher levels of substrate stability relative to other parts of the stream (Vannote and Minshall 1982, Lancaster and Hildrew 1993, Strayer et al. 1993, Strayer 1999, Fuller et al. 2010, Pandolfo et al. 2010). However, adult freshwater mussels are relatively sedentary and are generally unable to move quickly enough to avoid high-flow events. Conversely, long-distance displacement by high-flow events is frequently lethal for freshwater mussels. Moreover, individuals may become stranded on sand bars where they are subject to high rates of predation and desiccation when waters recede (Brown and Banks, 2001). Similarly, deep burial by mobile sediments may also result in mass mortalities of mussels as is evidenced by fossil and sub-fossil ‘death assemblages’ (i.e., evidence for mass burial of large aggregations of freshwater bivalves in riverine strata; Vannote and Minshall 1982, Cummins 1994).

Appalachian elktoe

The Appalachian elktoe (*Alasmidonta raveneliana*) is endemic to upper Tennessee River Drainage streams draining the Blue Ridge Physiographical Province in western North Carolina and eastern Tennessee (Clarke 1981). Populations currently occur in 10 streams in the Nolichucky, and Little Tennessee River drainages in North Carolina but only five of these populations are believed to be stable and reproducing (US Fish and Wildlife Service 2009).

The Appalachian elktoe was listed as an Endangered Species under the US Endangered Species Act in November of 1994 (USFWS 1994). During the latest 5-year review conducted by the US Fish and Wildlife Service (USFWS) the Appalachian elktoe was considered to be ‘declining’ (USFWS 2009). Two previously-viable Appalachian elktoe populations largely collapsed during the last 10 years. In the Nolichucky Drainage, one moderately-large population

was greatly reduced in size following the accidental discharge of untreated waste water from a water treatment plant facility into the Cane River. A second, much larger Appalachian elktoe population in the Little Tennessee River collapsed during the past decade for reasons that remain poorly understood. Hypothesized mechanisms for this collapse include land use-mediated changes to water temperature and quality, the effects of invasive species, flooding associated with remnants of hurricanes and long-term climate change (Fraley and Simmons 2006, Levine et al. 2015).

The goals of this research are to understand how variation in stream habitat parameters and local land use conditions influence the distribution, abundance, and demographic structure of Appalachian elktoe populations within a contiguous watershed, the Nolichucky River Drainage. I hypothesize that mussel distributions will be related to in-stream habitat (e.g., substrate, flow) and land-use at the local and watershed scale. This research will provide insights into how variability in physical habitat and land use conditions within a drainage network influence Appalachian elktoe populations that may inform subsequent habitat conservation and management strategies designed to mitigate ongoing declines and recover populations of this critically endangered mussel.

METHODS

Study Site

The Nolichucky Basin drains ~2921 km² (~1600 km² of the drainage is in North Carolina) of primarily upland habitat in western North Carolina and eastern Tennessee. Its headwaters originate on the slope of Mount Mitchell (elevation 2,037 m), the highest point in North America

east of the Mississippi River. The Nolichucky River is formed at the confluence of the Toe and Cane Rivers near Burnsville, North Carolina (elevation 860 m). Along with their primary tributaries the North and South Toe rivers, these streams drain portions of the Blue Ridge Physiographic Province in western North Carolina. The Nolichucky River leaves North Carolina via a narrow canyon and emerges onto the floor of the Tennessee Valley ~9 km northwest of Erwin, Tennessee (elevation 510 m) before joining the French Broad River in Douglas Reservoir near Newport, Tennessee (elevation 321 m). The lower reaches of the drainage flow through portions of the Ridge and Valley Physiographic Province (Glenn 1911, USFWS 1994). Census data for the three North Carolina counties in the headwaters of the Nolichucky Drainage indicate that human population densities are low: Avery County 17,797; Mitchell County 15,579; Yancey County 17,818 (Equinox Environmental 2014, US Census Bureau 2015).

North Carolina reaches of the Nolichucky Drainage currently support populations of two freshwater mussel species. In addition to the Appalachian elktoe, the Nolichucky Drainage supports populations of the Wavy-rayed lamp mussel (*Lampsilis fasciola*). Wavy-rayed lamp mussels are listed as Endangered by the North Carolina Wildlife Resource Commission (NCWRC) but considered a species of least concern by the International Union for the Conservation of Nature (IUCN) RedList and are classified as a G5 or Globally Secure taxon by Nature Serve (Williams et al. 1993, USFWS 1994, Parmalee and Bogan 1998, IUCN 2015, NatureServe 2016). Wavy-rayed lamp mussels occur throughout the Ohio and Great Lakes drainages (Parmalee and Bogan 1998)

Mussel Surveys

I conducted mussel surveys in the Nolichucky Drainage with assistance from personnel with the US Fish and Wildlife Service, US Forest Service (USFS) and Appalachian State University (ASU). Surveys used timed searches to quantify Appalachian elktoe relative abundance at 25 sites located across the Nolichucky River Drainage in Avery, Mitchell and Yancey counties, North Carolina (Table 1, Figure 1). Surveys were conducted within 150 m study reaches. Study reaches were divided into 10 m sections ($n = 16$ sections per site). Experienced personnel searched each 10 m section using visual-tactile survey methods (i.e., searchers examined the streambed and lightly disturbed finer substrates) to detect mussels. I recorded search times for each 10 m section and calculated mussel catch-per-unit-effort (CPUE) for each site by dividing the number of mussels per site by the total search time (hours) conducted at that site. Additionally, I calculated frequency of occupancy (FOO) for Appalachian elktoe as the proportion of sections in which mussels were detected per site. Mussels were identified and measured (total length) before being returned to their point of capture.

In-Stream Habitat

I quantified physical habitat parameters along 16 cross-channel transects within each study reach. Using a modified Wolman Pebble count (Wolman 1954), I quantified size (maximum diameter) or characterized (wood, organic matter, bedrock, boulder, silt and sand) 25 randomly-selected substrate particles along each cross-channel transect ($n = 400$ measurements per reach). This substrate characterization was modified from the original method described by Wolman, 1954 by reducing the number of particles per transect from 100 to 25. A Marsh McBurney Flow Mate (HACH, Loveland, CO) flow meter was used to quantify stream depth and mid-water

column current velocity at 5 equidistant increments across each transect at base flow ($n = 80$ depth and flow measurements per reach).

I estimated bank-full depth by measuring the height of the low bank using a telescoping stadia rod. Three bank-full height measurements were made in each reach and I attempted to get measurements at the upstream and downstream ends of the reach as well as in mid-reach (Gangloff and Feminella 2007). I was not able to measure bank-full depth at 4 sites located in canyon reaches with limited floodplain. I measured wetted width using a fiberglass tape measure and bank-full width using a Nikon AculonTM range finder with an accuracy of ± 0.5 m. Bank-full depth was calculated by adding base flow depth (cm) and the estimated bank-full depth (m) together.

Land use and Land Cover Analysis

I determined LULC cover for 1992 and 2011. I also calculated the change in forest cover from 1992-2011 for all 25 study sites. ArcGIS (v10.3) and the ArcHydro toolset were used to delineate the drainage area for each sampling locality following slightly amended protocol from Merwade (2012). In contrast to Merwade (2012), I used a much smaller (1000) cell convergence requirement to initiate streams. Stream initiation is the point at which a pre-defined number cells all flowing in the same direction converge and form a stream. The default setting for the ArcHydro Toolset was to use the number of pixels contained in 1% of the overall map area. The cell initiation threshold was changed because resulting maps were too coarse and underrepresented small tributary streams.

Drainages were delineated from a Digital Elevation Model (DEM) downloaded from the National Elevation Dataset (30 m cell resolution) operated by the US Geological Survey

(USGS). Land use and land cover layers (30 m spatial resolution, each 30 x 30 m cell equals one LULC type) were downloaded from the USGS Earth Explorer portal. Analysis of LULC occurred at two different spatial scales; 1) Watershed or the entire catchment upstream from each point and 2) Riparian or within 100 m of the entire upstream stream network (Hopkins and Roush 2013).

USGS LULC categories were simplified prior to statistical analysis. Specifically, deciduous, evergreen, and mixed forest classifications were combined into a single category called forest cover for each respective sampling year. I also combined the USGS categories for low, moderate, and highly disturbed areas into one category, disturbed land cover. The agricultural land category used in my analysis was calculated by combining USGS LULC coverage data for row crop and pasture/hay LULC classes.

Stream Gradient

Light Detection and Ranging (LiDAR) data were obtained by the USFWS to delineate a Digital Elevation Model (DEM) of the state of North Carolina. This DEM was created and disseminated by Dave Newcomb of the USFWS Raleigh, NC office which consisted of 6.1 x 6.1 m pixel sizes with elevation represented at the 1.27 cm scale. The DEM was manipulated in ArcMap v10.3.1 (Esri, Redlands CA 2015) and I determined the channel slope for all study sites using the Slope, Hillshade, Buffer and Interpolate Line Spatial Analyst tools.

In order to ensure correspondence between mussel survey sites and the sites used for LiDAR and land use analyses, sites were visually inspected and GPS coordinates were augmented using satellite photos to accurately reflect survey site locations. The Slope tool was used to calculate a layer in which each pixel is assigned a slope value from the original DEM,

then, the Hillshade tool was used to build a layer representing the topography of the area. The Hillshade layer was only used to visualize the study area and not in any analysis. I used the ArcGIS 3D Analyst > Buffer tool to encapsulate all of my sampling sites within a circle with a radius of 150 m. Therefore, the distance from the GPS location on the map to the downstream edge of the buffer circle (the circles radius) was 150 m, which corresponds to my site survey length. Finally, the ArcMap 3D Analysis > Interpolate line tool was used to draw a line traveling down the center of the stream channel over the 150 m survey reach. This tool results in a graphical representation of the topography across which the line is drawn. I then calculated stream power by multiplying the average stream bank-full height (estimated stream bank-full + stream base flow depth) for each site by the stream gradient obtained via ArcGIS (Gordon et al. 1994, Gangloff and Feminella 2007).

Shell Sectioning

Appalachian elktoe shells were collected opportunistically from 2014-2015. These animals most likely died during the previous 2 years as average alkalinity levels are low in this system (4.03 mg/L) and shells quickly dissolve in the South Toe River's mildly acidic waters. Because the Appalachian elktoe is a federally endangered species, I was unable to sacrifice or voucher live individuals. All shells represented in this study were collected from the South Toe River ($n=89$). Further, these shells (65%) were collected primarily from one site in the South Toe River near the highway 19E crossing ($n=58$). While the remaining shells were collected from three South Toe River sites downstream of the 19E crossing 725 m ($n=4$ shells), 3 km ($n=18$ shells) and 4.1 km ($n=13$ shells, Figure 1). Because shell sample sizes from three sites were low, I chose to

include all shells from the South Toe River in a single von Bertalanffy model to determine growth rate and age of Appalachian elktoe in the South Toe River.

I selected Appalachian elktoe shells in good condition (i.e., intact periostracum, no holes in umbo) for thin sectioning. If shells were unstable (e.g. heavily eroded, cracked or broken), valves were placed on clear plastic (Sarran™ wrap) covering loose sand in a Tupperware™ container. The sand filled container allowed precise positioning of the shell while the interior portion was filled with Buehler EpoThin™ epoxy resin which stabilized the shell for cutting. The sectioning process was started by making a cut from the shell umbo, to the shell margin while crossing the longest portion of the valve. Some valves were cut in the reverse direction (margin-to-umbo) as some highly cracked or eroded shells appeared to better withstand cutting in this direction. Shells were cut using a low-speed, Hillquist™ wet table-top saw with a diamond carbide-impregnated blade.

Shell edges were sanded using 400-600 grit silicon carbide sandpaper until smooth. This cut portion was then dipped in Buehler EpoThin™ epoxy resin, affixed to a petrographic slide and air-dried for ~24 h. Once cured, prepared slides were affixed to a saw chuck and re-sectioned on a Hillquist™ saw/grinder to a thickness of ~1.5 mm. Mounted sections were honed on the Hillquist™ saw/grinder to a thickness of ~0.7 mm as measured with a Mitutoyo Digimatic™ micrometer. Finally, prepared slides were wet sanded on 400 and 600 grit silicon carbide paper until smooth. This resulted in shell sections with a final thickness of ~0.5 mm. Growth lines (annuli) were defined as continuous bands extending from the shell margin (periostracum) to the umbo region (Neves and Moyer 1988, Hagg and Commens-Carson 2008, Singer and Gangloff 2013). Shells were aged by two independent reviewers using an Olympus SZ61 0.67x-4.5x

stereo-microscope. Shells that differed in age between readers were averaged before final analysis.

Statistical Analyses

Statistical analyses were conducted in SPSS software (IBM Corp. Version 22, Armonk NY) and R Studio (R Development Team, 2008). Quantitative habitat data was summarized as mean wetted width, mean flow velocity, mean depth, median particle size, and stream power. I calculated the proportion of categorical substrate types (e.g., sand, silt, bedrock, boulder, organic and wood) present within study each reach. To control for uncertainties associated with differentiating small diameter particles, I summed all of the sand, silt and clay particles detected to obtain an estimate of the proportion of fine sediments (<2 mm) present within each site.

I used a principal component analysis (PCA) to identify co-linear habitat variables and reduce the number of parameters in multivariate models. I included site-scale means for all measured physical habitat parameters (stream power, mean depth, mean velocity, median substrate, mean width, % wood, % bedrock, % organic, % boulder and % fines = % silt + % sand, Table 2). I examined the ability of stream habitat variables that loaded strongly on PC axes to independently predict Appalachian elktoe presence. All possible interactions between PCs and Appalachian elktoe population attributes were investigated using Generalized Linear Models.

Spearman correlations were used to investigate trends among percent riparian forest cover in 2011 and frequency of occupied sections (FOO). Spearman correlations were also used to investigate trends among percent 2011 watershed forest cover and percent fine substrate, and Appalachian elktoe presence against percent fine substrates. A histogram was developed to show frequency in total lengths of live Appalachian elktoe encountered.

The von Bertalanffy growth equation is commonly used to create growth curves for freshwater fishes and mussels (von Bertalanffy 1938, Anthony et al. 2001). The von Bertalanffy growth equation is $L_t = L_\infty (1 - e^{-K(t-t_0)})$ where L_t is the shell at a given age (t), L_∞ is the theoretical shell length-at-age infinity, K is a fitted constant showing the rate of L_t approaching L_∞ over time, and t_0 is the theoretical age when the shell length is equal to 0. Thin-section analysis was performed by applying the von Bertalanffy growth equation via R Studio (R Core Development Team, 2008) using the FSA: Fisheries Stock Analysis R package version 0.8.6 to apply the growth equation (Ogle D 2016).

RESULTS

Appalachian elktoe Distribution and Abundance

I found a total of 327 Appalachian elktoe and 29 Wavy-rayed lamp mussels alive during 2015 surveys in the Nolichucky Drainage. Roughly half (12) of the 25 sites sampled had live mussels present. All 12 sites that contained live mussels had Appalachian elktoe present (Figure 1, Table 1). Wavy-rayed lamp mussels were found at only 7 of 25 sites in the Nolichucky Drainage (Table 1). Although Wavy-rayed lamp mussels were not found at all 12 sites that contained Appalachian elktoe, only one of seven sites that had Wavy-rayed lamp mussels did not have Appalachian elktoe.

I found Appalachian elktoe populations at 6 sites in the South Toe River and 3 sites in both the Cane and mainstem Toe Rivers. Contrastingly, Wavy-rayed lamp mussels were found at 3 sites in the South Toe and 4 sites in the mainstem Toe River. Wavy-rayed lamp mussels were found at the most downstream site sampled in 2015 (Toe River mainstem). The vast majority

(>94%) of Appalachian elktoe encountered during 2015 were detected in the South Toe River and the largest number of Appalachian elktoe encountered at any one site during this survey (217 mussels or 66% of the total number detected in this study) were found in the South Toe River. The length of Appalachian elktoe encountered during 2015 surveys in the Nolichucky Drainage ranged from 21 to 87 mm with a mean of 57.5 mm and median of 56 mm (Figure 2).

Land use, Habitat and Mussels

Although the proportion of forest cover was relatively high (>70%) at all sites in this sparsely-populated drainage, there appears to be a strong link between forest cover and Appalachian elktoe occurrence. The frequency of sections occupied by Appalachian elktoe (site-scale FOO) was significantly positively correlated with 2011 % riparian forest cover ($p<0.0001$, $r=0.754$, $n=25$, Figure 3). Habitat PC₂ (substrate size, proportion bedrock, proportion wood) was significantly positively related to the presence of Appalachian elktoe ($p=0.022$, $r=0.455$, $n=25$, Figure 4). Lastly, % fines (proportion of silt + proportion of sand) was negatively related to 2011 watershed % forest cover suggesting that forest cover at multiple scales is associated with benthic substrate conditions in the Nolichucky Drainage ($p=0.006$, $r=-0.534$, $n=25$, Figure 5).

Habitat Parameters

Principal component analyses of habitat data resulted in four PCs explaining 77% of the total variation in physical habitat conditions (Table 2). Habitat PC₁ explained 33.9% of overall habitat variation and stream depth, width, and % boulder loaded positively on PC₁ whereas organic matter loaded negatively. PC₂ explained 20.1% of physical habitat variation. Mean velocity and % wood loaded negatively on PC₂ whereas median substrate and % bedrock and width loaded

positively on PC₂. Habitat PC₃ explained 12.7% of variation in the dataset with stream power and the proportion of boulders loading positively whereas mean velocity and bedrock had negative loadings on PC₃. Finally, PC₄ explained only 10.3% of habitat variability and stream power, mean velocity, and % bedrock loaded positively and substrate size loaded negatively on this component (Table 2).

Regression Analysis

Generalized liner regression model found that Habitat PC₁ and PC₂ produced the most significant model in predicting Appalachian elktoe presence ($AIC_c = 32.37$, $W_i = 0.40$, $X^2 = 9.39$, $p = 0.01$, Table 3). Although Habitat PC₁ explains variation mainly in stream size, Habitat PC₂ loaded strongly on variables appearing to be more related to Appalachian elktoe presence. The second most significant model found was Habitat PC₂ alone predicting Appalachian elktoe presence ($AIC_c = 32.36$, $W_i = 0.15$, $X^2 = 4.81$, $p = 0.03$, Table 3).

Shell Thin-Sections

Analysis of length-at-age data indicate that Appalachian elktoe are short-lived and quick growing mussels. The mean age of sectioned mussels was 5.6 years and the median was 5.5 years. Shell lengths begin to asymptote at around age 8 and growth of all animals ages 8+ appeared relatively slow. The youngest shells sectioned were age 3 years and the mean length of age 3 shells was 49.4 mm with the smallest shell being 34.5 mm in length. I did not find any animals with ages >11 y and the largest individual available for thin-sectioning was 91 mm (Figure 7). Shell erosion on older, larger individual's precluded age and growth analysis.

DISCUSSION

My data demonstrate that the distribution and abundance of Appalachian elktoe populations in the Nolichucky Drainage is extremely patchy but that it is also potentially dynamic. Although I found Appalachian elktoe in most of the streams examined, a number of populations appear to be extirpated from at least 7 sites where they formerly occurred (Fraley and Simmons 2006).

However, several sites including the upper North Toe River had not been previously surveyed for Appalachian elktoe. They were targeted during this study to define the upstream range of Appalachian elktoe in that stream. The upper reach of the North Toe (above Spruce Pine, NC) have not contained Appalachian elktoe populations due to a former impoundment near the town of Spruce Pine and issues with water quality from waste water treatment effluent. The habitat in some areas upstream of Spruce Pine appear suitable for Appalachian elktoe if previously mentioned stressors were relieved.

Appalachian elktoe populations at most of my study sites appear to be small and the vast majority (95%) of individuals were detected in a single stream, the South Toe River. However, surveys in the early 1990s failed to detect Appalachian elktoe populations in the South Toe River. Further, these South Toe populations appear to have been increasing since their discovery in 1998 (USFWS 2009). Given that these populations are large and the surveys were conducted by experienced personnel, it seems likely that Appalachian elktoe have only recently colonized the lower South Toe River as populations were discovered in the South Toe during 1998. Protection of the South Toe watershed will be key to ensuring the survival of Appalachian elktoe in this watershed as there are few viable populations left in other tributaries. The South Toe appears to be the least-impacted sub-watershed in the Nolichucky Drainage and is a key

stronghold for populations of Appalachian elktoe and numerous other sensitive species such as Hellbenders and the Wavy-rayed lamp mussel (NC Wildlife Action Plan 2005).

Size demographics for most Appalachian elktoe populations in the Nolichucky Drainage during 2015 surveys are skewed toward large, older adult individuals. While previous surveys have found large numbers of small, juvenile individuals, my research was unable to detect them. However, analysis of shell thin sections indicate that Appalachian elktoe are relatively short-lived and surprisingly fast-growing mussels. Results from thin section shells also indicate that all year classes (3-11 y) are present in the South Toe River and are indicative of naturally reproducing, stable populations (Figure 7). Demographic anomalies including the relatively low number of young mussels (< 40 mm) detected may reflect the relatively rapid growth of individuals and the relatively low survivorship of early age classes in most mussel populations (Figure 2). These patterns may reflect important differences in Appalachian elktoe life history traits (e.g., growth, life span, reproductive output) relative to other freshwater mussels. These attributes appear to be fairly common among other headwater-adapted mussel taxa (e.g., *Alasmidonta*, *Anodontoides*, *Strophitus*, *Villosa* spp.) (Haag and Warren, 1998).

Physical habitat and land use

Streams are intimately linked to their associated terrestrial ecosystem, including allochthonous organic inputs, stream nutrients, and geomorphic structures (Vannote et al. 1980, Kreutzweiser and Capell 2001). Human modifications of watershed and riparian terrestrial land use have the potential to adversely affect streams on a multitude of characteristics. Altered land use, including deforestation may increase sediment runoff and siltation rates, degrade water quality, and increase the intensity and frequency of high-flow events (Naiman and Decamps 1997, Harding et

al. 1999, Gulis and Suberkropp 2003, Strayer et al. 2004, Arthington et al. 2009). This increase in siltation can result in filling of interstitial shelter space which subsequently decreases the survivorship of benthic organisms. Further, increased turbidity and siltation can restrict the feeding ability of filter feeding organisms including freshwater mussels (Henley et al. 2000). Siltation of streams has long been linked with mussel declines in the southeastern United States since the late 1880s (Kunz 1898).

The factors responsible for the dynamic nature of Appalachian elktoe populations remain unclear but data suggest that riparian disturbances, including forest clearing, may be associated with elevated levels of fine sediments in some reaches. PCA and multiple regression analysis indicated that Habitat PC₂ (substrate size and composition) were the best indicators of suitable Appalachian elktoe habitat.

In addition to long-term changes in land use and habitat conditions, short-term changes to land use may have profound implications for benthic habitats and the persistence of Appalachian elktoe populations. In the South Toe River, where the majority of Appalachian elktoe were found in this study, substantial changes in abundance were observed between 2014 and 2015 at one site impacted by sediments from ongoing highway construction. I observed a 50% decline in Appalachian elktoe abundance and a 63% decline in CPUE from 2014 (18 mussels, 6 mussels per hour) to 2015 (9 mussels, 2.3 mussels per hour). This observation supports the hypothesis that fine sediments negatively influence Appalachian elktoe abundance and suggests that changes may be rapid, underscoring the need to strengthen protection and restoration of riparian buffer zones in streams with Appalachian elktoe populations.

Management Implications

In the Nolichucky Drainage, Appalachian elktoe populations appear to be stable and reproducing only in the South Toe River. Because populations in many other streams appear to be declining, the South Toe watershed is now the only major population stronghold in this drainage and represents one of only 3 or 4 viable populations remaining. Continued monitoring of this population (including quantitative surveys to detect recruits and obtain more complete demographic data) are needed to track population trends and ensure that current management strategies do not need to be augmented. Additionally, float surveys of the mainstem Toe and North Toe Rivers are needed to help determine whether suitable Appalachian elktoe habitats may exist in more remote sections of the drainage. Headwater site catchments and riparian zones were typically more forested than at sites further downstream in the Nolichucky Drainage. This trend is attributable to increased human population sizes and disturbance levels near sites lower in the drainage where the topography is less mountainous and more suitable for residences and agricultural activities.

Results of this and other studies suggest that although mechanistic links between the changes to LULC at the watershed or and riparian scales may not be directly apparent, indirect changes including stream warming and increased levels of fine sediment inputs may have profound consequences for populations of sensitive headwater mussels. Ongoing analyses will examine how age and growth data vary among Appalachian elktoe populations and may shed light on interactions with environmental conditions. Juvenile propagation and population augmentation are on-going for this species (R. Hoch and S. Fraley, NCWRC Pers. Com.). Well-designed studies using sentinel juvenile mussels may hold the key to understanding how habitat factors influence survival and growth rates in this and other imperiled headwater mussel taxa.

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Tables and Figures

Table 1. Number, search time, catch-per-unit-effort (CPUE), and mean mussel length and standard deviation of *Alasmidonta raveneliana* encountered during Nolichucky drainage surveys and number of *Lampsilis fasciola* encountered in surveys between June 1 and August 14, 2015.

CR = Cane River, ST = South Toe, NT = North Toe and Toe = Toe River. Site number corresponds to placement in respective stream, ascending in downstream direction.

Site	Locality	Search Effort (hours)	<i>Alasmidonta raveneliana</i>		<i>Lampsilis fasciola</i>	
			No. Live Mussels CPUE	Mean Length (mm) SD Length (mm)	No. Live Mussels CPUE	Mean Length (mm) SD Length (mm)
CR1	35.901928, -82.311812	2.5	1, 0.41	67 (0)	-	-
CR2	35.902318, -82.328052	4.4	3, 0.68	71.2 (3.5)	-	-
CR3	35.91619, -82.35736	1.5	-	-	-	-
CR4	35.913875, -82.384771	1.3	-	-	-	-
CR5	35.942306, -82.390349	2	-	-	-	-
CR6	36.00019, -82.35673	2.3	1, 0.44	55 (0)	-	-
NT1	35.99587, -82.01877	3.5	-	-	-	-
NT2	35.97805, -82.01685	2	-	-	-	-
NT3	35.944044, -82.00402	3.2	-	-	-	-
NT4	35.929205, -82.114801	2	-	-	-	-
NT5	35.914949, -82.069168	2.2	-	-	-	-
NT6	35.90479, -82.04472	2.3	-	-	-	-
ST1	35.87119, -82.196812	6	30, 5.03	57.7 (9.6)	1, 0.16	59 (0)
ST2	35.9051, -82.191674	6.4	217, 34.17	59.5 (9.8)	-	-
ST3	35.908732, -82.191452	4.8	17, 3.54	55.6 (8.6)	-	-
ST4	35.915051, -82.213237	4	9, 2.27	39.9 (9.8)	1, 0.25	69 (0)
ST5	35.92133, -82.20711	5.3	31, 5.89	46.3 (11.3)	4, 0.75	60.3 (8.3)
ST6	35.926794, -82.202692	4.5	6, 1.33	56 (5.9)	-	-
Toe1	36.005635, -82.19315	10.3	7, 0.68	54.2 (10.9)	9, 0.87	69 (10.5)
Toe2	36.008022, -82.237381	5.4	-	-	-	-
Toe3	35.993532, -82.249744	7.9	4, 0.51	62 (12)	6, 0.75	56.4 (11.2)
Toe4	35.993993, -82.258602	8	1, 0.12	42 (0)	4, 0.50	70.2 (7.9)
Toe5	36.00399, -82.24755	3.2	-	-	-	-
Toe6	36.045831, -82.299447	3.9	-	-	-	-
Toe7	36.02653, -82.321303	8.6	-	-	4, 0.47	59.1 (7.1)
Total		107.1	327, 3.05	57.3 (10.9)	29, 0.27	64.6 (10.7)

Table 2. Loading factors and percent variation explained for principal component analysis for Stream Habitat. Underlined values indicated loading factors with absolute values > 0.4 and bolded percentages indicate variance explained values.

Habitat Variable	PC₁	PC₂	PC₃	PC₄
Stream Power	0.07	0.24	<u>0.73</u>	<u>0.48</u>
Mean Depth	<u>0.84</u>	-0.28	0.27	-0.05
Mean Velocity	0.50	<u>-0.40</u>	<u>-0.40</u>	<u>0.47</u>
Median Substrate	0.37	<u>0.62</u>	0.03	<u>-0.54</u>
Mean Width	<u>0.75</u>	0.38	-0.33	0.09
% Wood	0.22	<u>-0.82</u>	-0.10	-0.01
% Bedrock	<u>0.40</u>	<u>0.62</u>	-0.39	0.38
% Organic	<u>-0.67</u>	0.08	0.16	0.28
% Boulder	<u>0.67</u>	0.18	0.38	0.15
% Fines	<u>0.79</u>	-0.30	0.24	-0.20
% Variation Explained	33.9	20.1	12.7	10.3

Table 3. Akaike Information Criterion with correction for finite sample size (AIC_C), change in Akaike Information Criterion (ΔAIC_C), Akaike weight (W_i), Model likelihood (X^2), p values for Principal Components (PCs). $n = 25$ for all analyses.

Variable	AIC_C	ΔAIC_C	W_i	Model X^2	Model p
PC ₁ , PC ₂	32.37	0.00	0.40	9.39	0.01
PC ₂	34.36	1.98	0.15	4.81	0.03
PC ₁ , PC ₂ , PC ₃	34.38	2.00	0.15	10.24	0.02
PC ₁	36.14	3.76	0.06	3.03	0.08
PC ₂ , PC ₄	36.41	4.04	0.05	5.35	0.07
PC ₂ , PC ₃	36.50	4.13	0.05	5.26	0.72
PC ₁ , PC ₂ , PC ₃ , PC ₄	37.03	4.66	0.04	10.75	0.03
PC ₁ , PC ₄	38.17	5.80	0.02	3.59	0.17
PC ₁ , PC ₃	38.27	5.89	0.02	3.50	0.17
PC ₄	38.66	6.29	0.02	0.50	0.48
PC ₃	38.76	6.39	0.02	0.41	0.53
PC ₂ , PC ₃ , PC ₄	38.80	6.43	0.02	5.82	0.12
PC ₃ , PC ₄	40.84	8.47	0.01	0.92	0.63

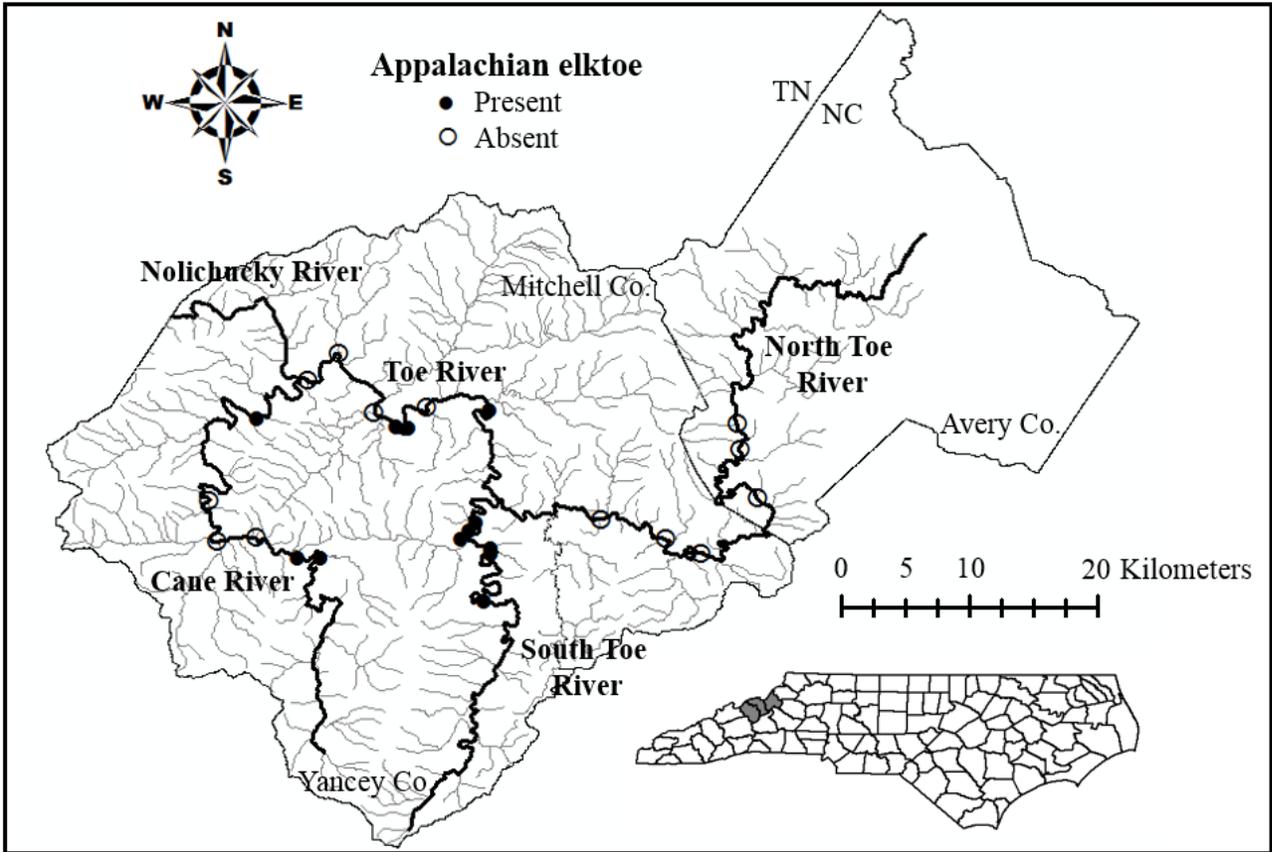


Figure 1. Map of study localities in the Nolichucky drainage in North Carolina. Solid circles represent sites with extant Appalachian elktoe populations whereas open circles indicate non-occupied sites during 2015 surveys.

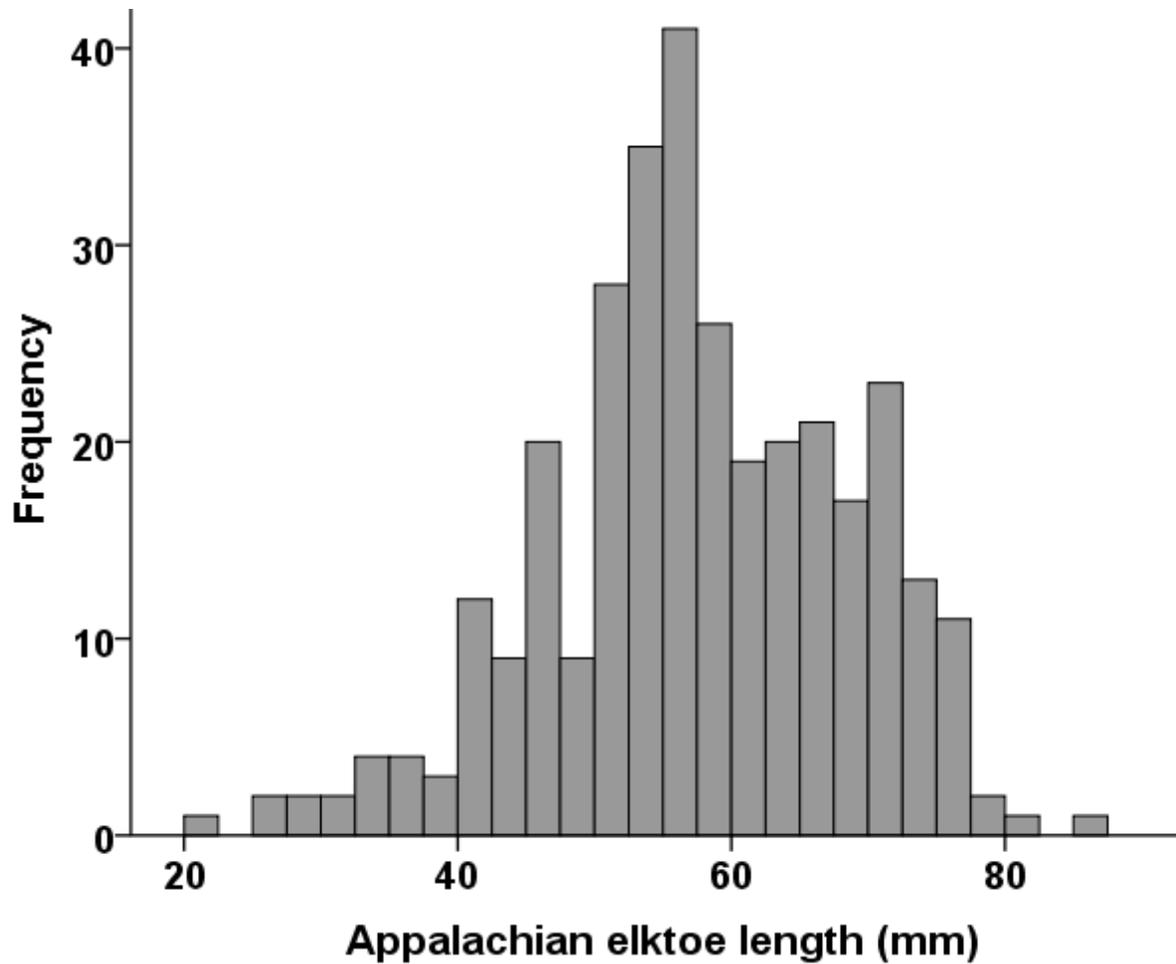


Figure 2. Distribution of Appalachian elktoe size classes for 327 mussels encountered alive at 12 of 25 sites in the Nolichucky River Drainage in 2015. The mean total length of mussels was 57.5 mm, the maximum length was 87 mm and the minimum length was 21 mm.

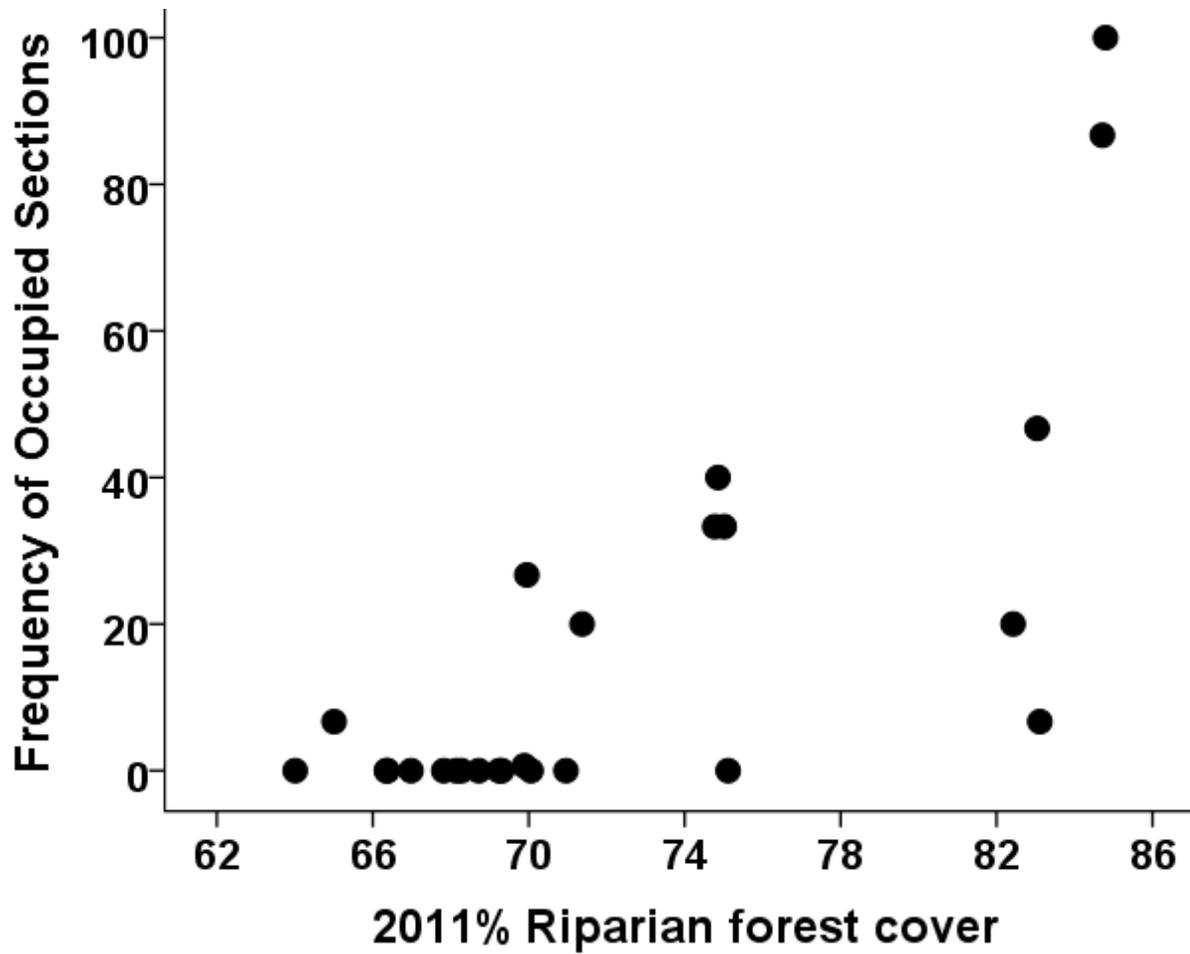


Figure 3. Scatter plot of the relationship between percent 2011 % riparian forest cover and the frequency of occupied (FOO) sections ($n = 16$ per site) within each 150-m focal reach in which Appalachian elktoe were detected (Spearman correlation, $p < 0.0001$, $r = 0.711$, $n = 25$).

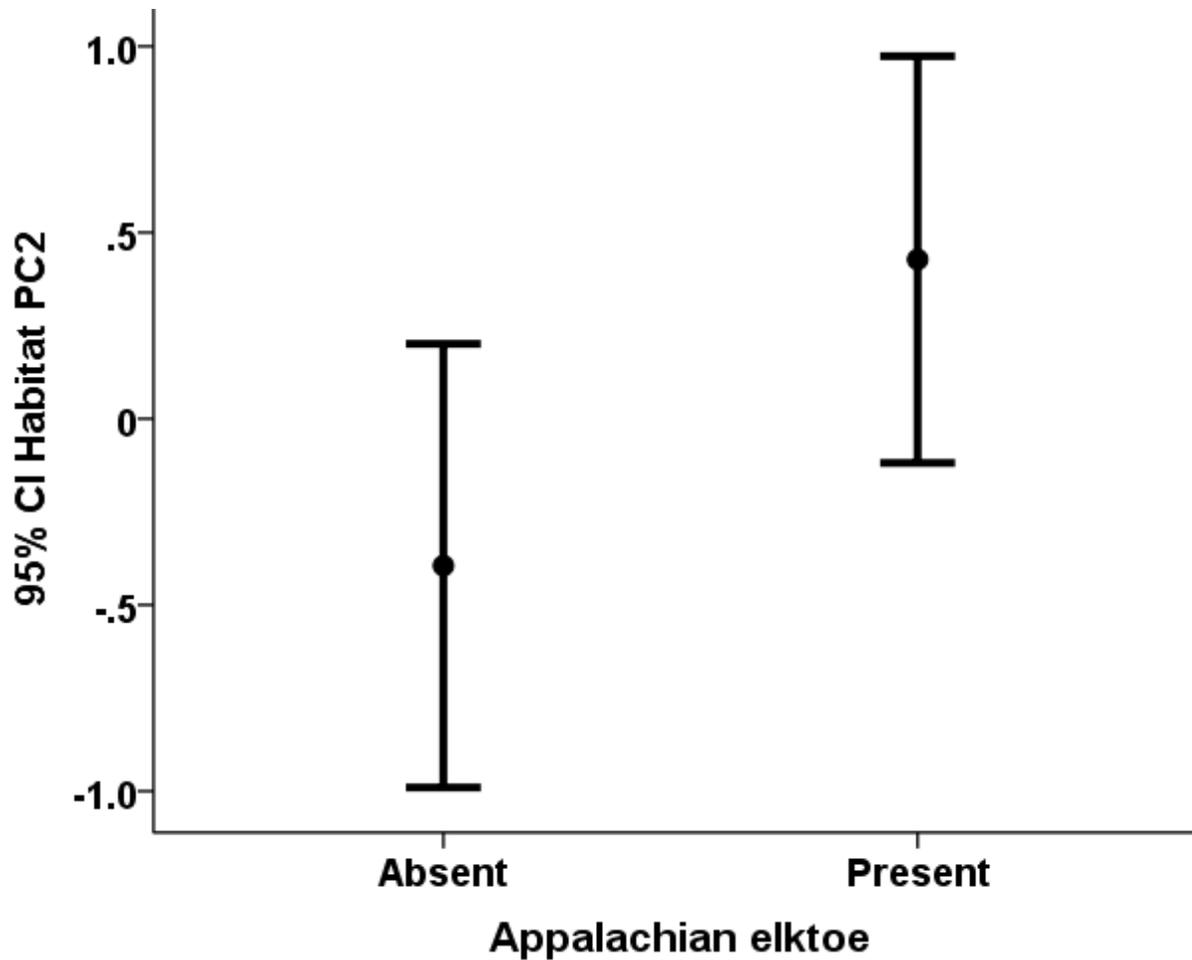


Figure 4. Mean (\pm 95% confidence interval) PC₂ scores for sites where Appalachian elktoe were absent and present in the Nolichucky River Drainage during 2015 surveys. Streams with wider channels, higher flow velocities, larger median substrate diameters more bedrock and fewer fines or organic matter were more likely to support Appalachian elktoe compared to streams with lower flows, smaller particles and more organic matter.

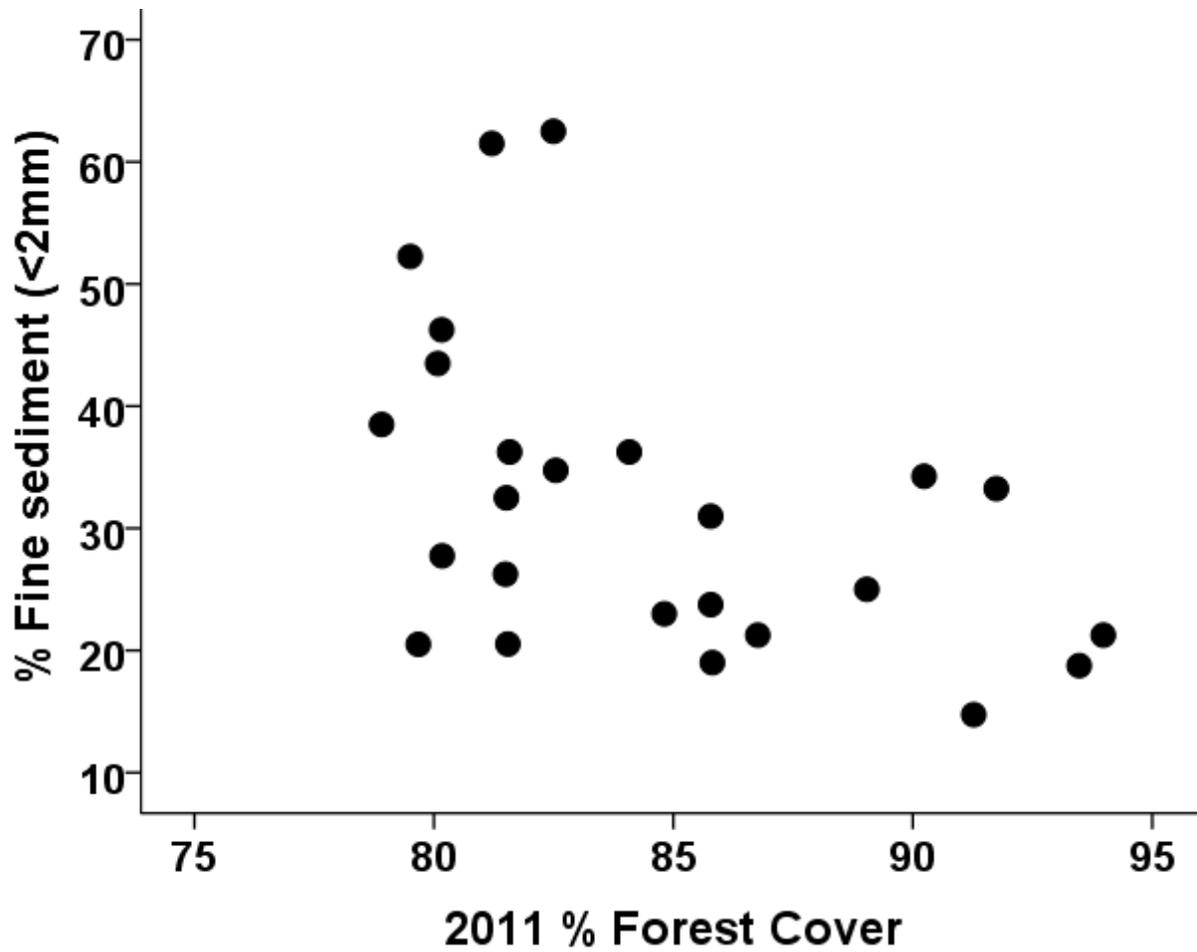


Figure 5. Relationship between the percent of 2011 forest cover at the watershed scale and the percentage of fine substrates (< 2 mm diameter) observed during Wolman pebble counts at sites in the Nolichucky Drainage in 2015 (Spearman correlation, $p < 0.006$, $r = -0.534$, $n = 25$).

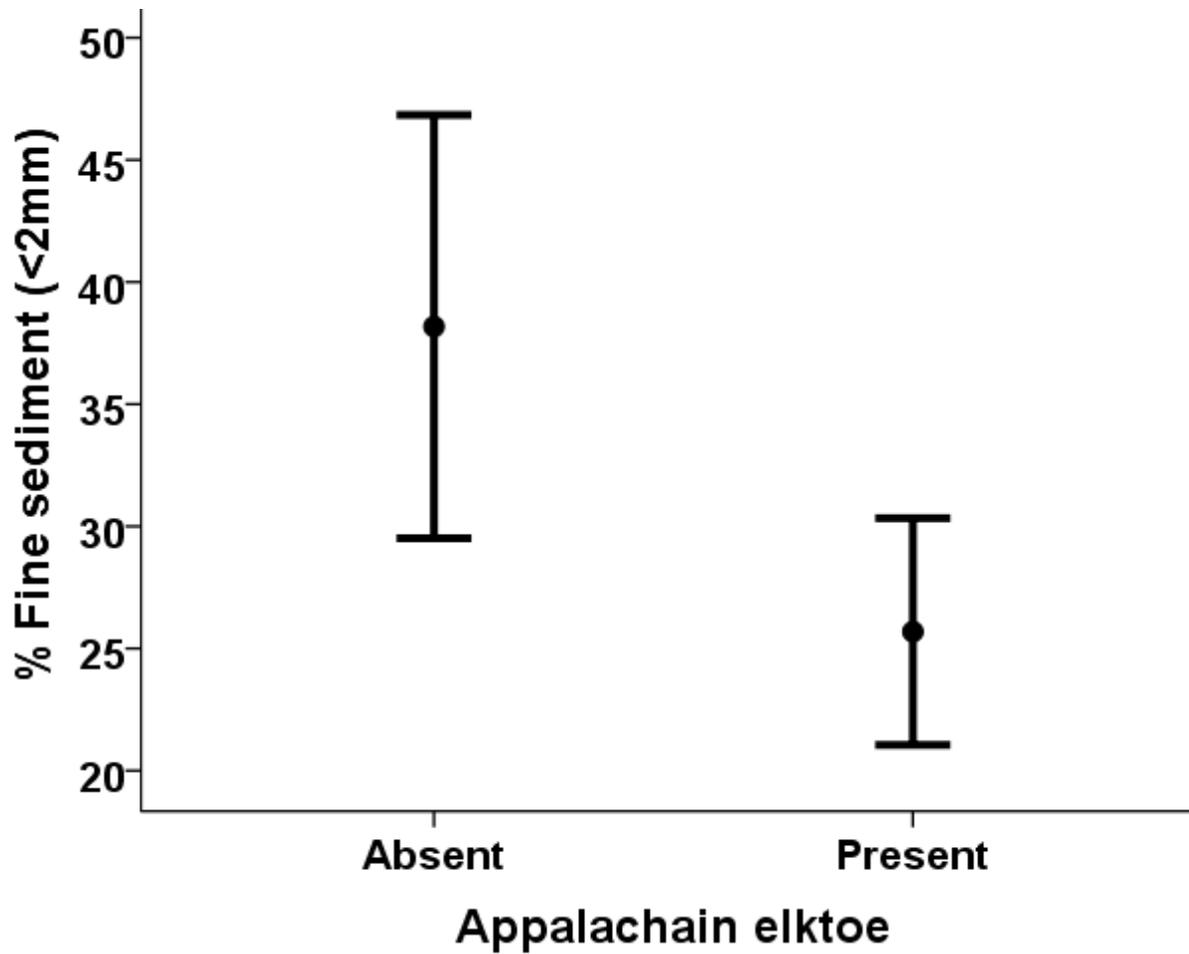


Figure 6. Relationship between Appalachian elktoe presence and the percent of fine substrates (<2 mm diameter) at sites in the Nolichucky Drainage in 2015. Sites with lower amounts of fine sediment (silt + sand) are significantly more likely to have Appalachian elktoe present ($p < 0.011$, $n = 25$). Error bars represent 95% confidence intervals.

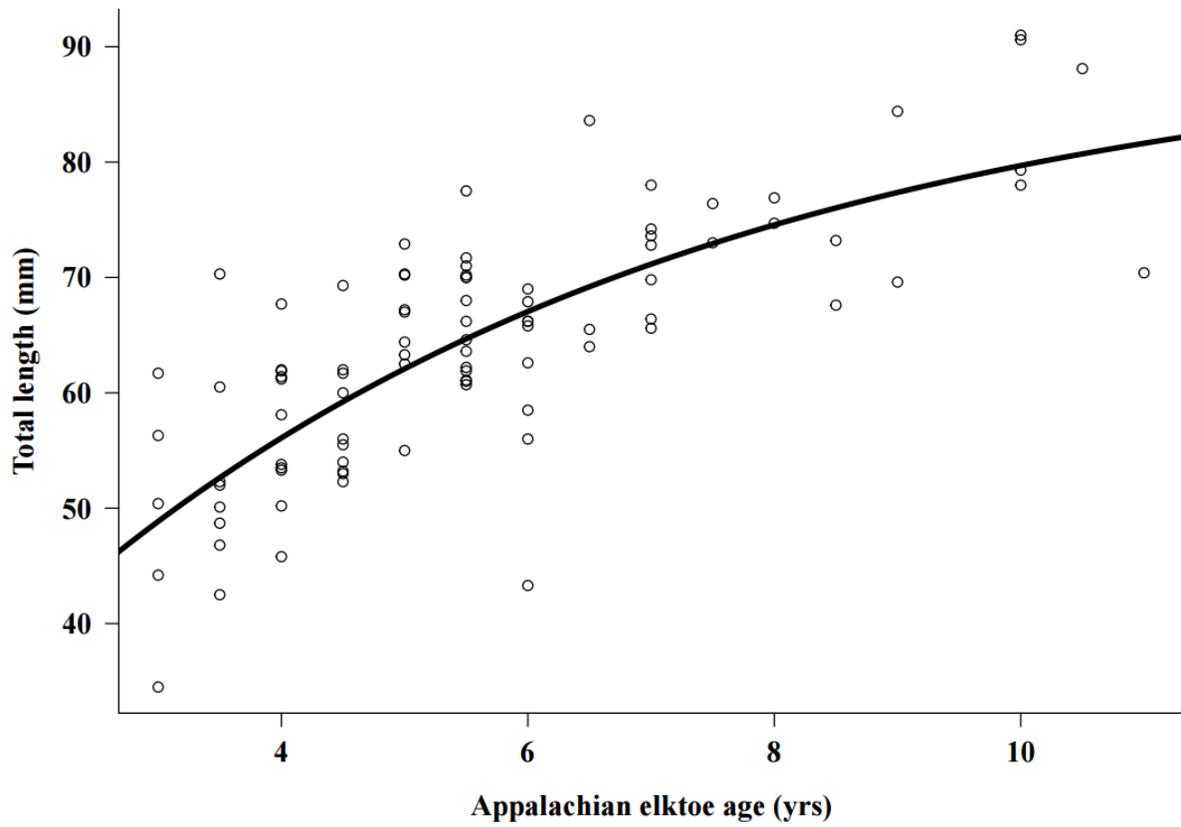


Figure 7. von Bertalanffy length-at-age data representing Appalachian elktoe shells thin-sections collected during 2014-2015 from 4 sites in the South Toe River ($n=89$ shells).

Vita

Gary Pandolfi is a native Appalachian and avid outdoorsman who grew up in the mountains near Burnsville, NC. He spent his formative years hunting bear, deer and turkey and familiarizing himself with all things mechanical. After enrolling at Appalachian State University in the fall of 2009 and briefly flirting with degrees in engineering and physics, Gary wisely decided on a biology major. He joined the lab of Dr. Michael Gangloff in the Fall 2011 and quickly became enamored with freshwater field ecology while assisting Dr. Gangloff's graduate students with their research in mountain streams including the New and Watauga rivers. Once the proverbial 'hook' was set, a career in aquatic biology became inevitable. During the past seven years he has ascended the ranks from undergraduate volunteer to technician, laboratory manager and eventually graduate research assistant while assisting Dr. Gangloff with research in five states and more than a dozen river basins.