Distribution and Life History of *Chrosomus* sp. cf. *saylori* in the Upper Clinch River Watershed, Virginia

Shannon Lynn White

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Donald Orth, Committee Chair

Emmanuel Frimpong

Andrew Dolloff

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Abstract

In 1999, a new species of minnow, *Chrosomus* sp. cf. *saylori* (Clinch dace), was discovered in the Tennessee drainage of Virginia. *Chrosomus* sp. cf. *saylori* are listed as a Federal Species of Concern and on Virginia's Wildlife Action Plan as Tier II- Very High Conservation Need because of potential threats from habitat degradation, high population fragmentation, and a largely unknown distribution. Consequently, a management plan for *C*. sp. cf. *saylori* is of utmost importance, but more information regarding its distribution and life history is required before such a plan can be implemented. In 2011 and 2012 I sampled 60 headwater streams in the upper Clinch River watershed, Virginia. From this and historical data, I conclude that *C*. sp. cf. *saylori* are restricted to eight small tributaries to the Clinch River. Multivariate analysis of habitat correlates indicated that *C*. sp. cf. *saylori* populations are found in small, high elevation streams with gravel substrate and forested watersheds. Three species distribution models were unable to predict *C*. sp. cf. *saylori* distribution. Morphological traits were significantly different between *C*. sp. cf. *saylori* and other *Chrosomus*, thereby providing an initial indication of speciation and differing niche roles. I observed a nest association with *Campostoma anomalum*. Gonad weight was lower for *C*. sp. cf. *saylori* than closely-related congeners. Together, this information indicates that *C*. sp. cf. *saylori* are narrowly distributed and populations are small, fragmented, and of questionable viability. In the future, long-term monitoring efforts and genetics analyses should be completed and additional protection measures pursued.

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General Introduction

With over 2,000 species in 210 genera, the family Cyprinidae is the most diverse family of freshwater fishes (He et al. 2008) and comprises 30% of the North American fish fauna (Johnston 1999). Anthropogenic alternation and exploitation of the natural environment have negatively impacted many species of Cyprinidae, and over 20% of cyprinids in the United States are listed as imperiled (Williams et al. 1989, Jelks et al. 2008). The region of greatest fish diversity and endemism in North America is the southeastern United States, which also supports the greatest number of threatened cyprinids (Warren et al. 2000).

Many cyprinids of the southeastern United States are of the subfamily Leuciscinae, one of five cyprinid subfamilies consisting of over 90 genera distributed widely across North America and Eurasia. The genus *Chrosomus* is a member of the Leuciscinae subfamily, and is one of just two cyprinid genera with a Holarctic distribution (Cunha et al. 2002, Strange and Mayden 2009).

The taxonomic classification of *Chrosomus* has been the topic of much debate and change. Historically, *Chrosomus, Phoxinus,* and *Pfrille* were separate genera (Jordan 1924). However, similar morphological traits among fishes in those three genera and initial genetic analyses prompted taxonomists to reorganize the classification into one monophyletic genus, *Phoxinus* (Banarescu 1964, Howes 1985). While species in *Phoxinus* were morphologically similar, their distribution across North America and Eurasia led many to question the phylogeny. Furthermore, competing studies could never support a monophyletic *Phoxinus* genus (Briolay et al. 1988, Simons and Mayden 1998).

It wasn’t until the development of more advanced molecular DNA sequencing that Mayden et al. (2006) determined that a monophyletic *Phoxinus* was an non-natural grouping; a conclusion that substantiated reorganization of *Phoxinus*. Today, the understood taxonomy is that described by Strange and Mayden (2009) which includes splitting *Phoxinus* into two genera: *Phoxinus* and *Chrosomus.* *Phoxinus* comprises three species that are distributed in Eurasia. Fishes in the genus *Chrosomus* are distributed in North America and include six species in the subgenus *Chrosomus* and one species in subgenus *Pfrille* (the finescale dace, *Chrosomus neogaeus*).

Of interest here are fishes in the subgenus *Chrosomus*. Given their small scales, *Chrosomus* minnows are commonly referred to as the "fine-scaled dace." These fishes are renowned for their sexually-dimorphic nuptial coloration, with males turning bright red and yellow during spawning season. *Chrosomus* fishes typically inhabit cool waters of headwater streams, and commonly prefer slow, shallow pools over faster riffles (Bestgen 1989). They are also well-documented nest associates, usually spawning over mounds or pits created by *Nocomis* spp. or *Campostoma* spp. (Johnston and Page 1992).

Species in the genus include *C. tennesseensis* (Tennessee dace), *C. oreas* (mountain redbelly dace), *C. saylori* (laurel dace), *C. cumberlandensis* (blackside dace), *C. eos* (northern redbelly dace), and *C. erythrogaster* (southern redbelly dace). These six species are distributed in adjacent, yet largely non-overlapping regions of the United States (however, recent bait bucket introductions and translocations have started to change this pattern). This distribution pattern supports the hypothesis that a common ancestor (often thought to be *C. neogaeus*, of the subgenus *Pfrille* (Strange and Mayden 2009) disseminated into North America, and periods of glaciation, stream capture, and other geological events resulted in isolation and subsequent speciation (Starnes and Jenkins 1988).

The resulting taxonomy of the *Chrosomus* subgenus, as described by Strange and Mayden (2009), includes two monophyletic groups. The "mountain clade" consists of *C. oreas* and *C. tennesseensis*. The *C. erythrogaster* clade includes *C. saylori*, *C. cumberlandensis, C. eos*, and *C. erythrogaster*. This taxonomic configuration, which was based on DNA analyses, also reflects differences in morphology as the mountain clade has an uninterrupted upper lateral band and a broken lower lateral band, and the *C. erythrogaster* clade largely consists of fishes with two uninterrupted lateral bands (the exception is *C.* cumberlandensis, which has one thick, uninterrupted lateral band) .

The conservation status of *Chrosomus* minnows is one of two extremes. *Chrosomus erythrogaster, C. eos*, and *C. oreas* are distributed across a fairly broad landscape, and usually exist in high abundance. Several subpopulations of these three species are listed as endangered or critically imperiled at the state level, but the species is of no immediate danger of extirpation or extinction (Slack et al. 1997). The remaining three species, *C. saylori*, *C. cumberlandensis*, and *C. tennesseensis*, have all been identified as vulnerable and in need of conservation and restoration. The Endangered Species Act listed *C. cumberlandensis* as threatened in 1987 (U.S. Fish and Wildlife Service [USFWS] 1987), and recognized *C. saylori* as endangered in 2011 (USFWS 2011) In Tennessee, *C. cumberlandensis* has been listed as threatened, *C. saylori* as endangered, and *C. tennesseensis* as in need of a management plan. Kentucky has also listed *C. cumberlandensis* as threatened.

Populations of *Chrosomus* are most threatened by habitat alteration, in particular road construction, landuse change, and mining. The construction of roads across headwater streams is common and attention to proper fish passage at road crossings, both during road construction and the subsequent maintenance of fish passage devices, is often nonexistent. While the effects of road crossings have not been studied for *Chrosomus* specifically, it has long been determined that culverts are a major impediment to fish movement (Trombulak and Frissell 2000). Many headwater species migrate to access feeding, spawning, and rearing habitats, and the inability to do so can reduce the reproductive potential of individuals (Schlosser and Angermeier 1995). Furthermore, maintaining open migration channels maximizes the combinatory potential of sexual reproduction. When populations do become isolated, inbreeding depression and loss of genetic diversity quickly decrease resiliency to environmental disturbance (Hedrick and Kalinowski 2000). And, with temperature, flow, and water quality of headwater streams being so variable, the inability to withstand disturbance can decrease population size rapidly and lead to complete extirpation (Finn et al. 2011).

Landuse changes are the biggest threat to *Chrosomus* habitat. Development and cultivation of riparian lands increases soil and stream bank erosion, thereby increasing the amount of sediment in streams (Sutherland et al. 2002). Because *Chrosomus* require gravel substrate for spawning, increased siltation is a physical barrier to reproduction. After just a few generations of marginal reproductive success, populations can become extirpated. For example, Slack et al. (1997) determined that populations of *C. erythrogaster* in Mississippi streams are either completely extirpated or decrease in size when watershed development increased.

Chrosomus populations occurring in the Appalachian Mountains are also threatened by coal mining. By increasing turbidity and decreasing pH, coal removal is a major stressor to ichthyofauna, sometimes resulting in complete extirpation (Powell 1988). Coal mining decreases water quality by decreasing pH and increasing turbidity and sedimentation. Coal strata naturally contain high levels of sulphur that, over time, oxidize into sulphuric acid. However, mining operations expose a larger area of strata to weatherization and thus accelerates the oxidation process (Powell 1988). It is estimated that, in the Appalachians, 6,000 tons of sulfuric acid is produced daily by the oxidation of pyrite (Ahmad 1974). In addition to sulphur, there are up to 20 other possible contaminants that may enter a stream such as aluminium, calcium, and lead. The effects of mining are long term, and streams located in a mining watershed may continue to have poor water quality and decreased functionality long after the termination of the mining processes (Fritz et al. 2010).

Given the threatened and endangered status of many *Chrosomus* and the complexity of conservation threats, the discovery of a potential new *Chrosomus* species in 1999 gathered immediate attention from biologists. The fish was discovered by Lingenfelser et al. (2004) in Mudlick Creek in Tazewell County, Virginia as part of a biological survey assessing stream health of the Indian Creek watershed, a tributary to the Clinch River. At the time, *Chrosomus* were not known to inhabit the Clinch River system (Jenkins and Burkhead 1994). But, Mudlick Creek is in close proximity to the distribution of *C. saylori* (Skelton 2001), and similar morphological characteristics between the unknown fish and *C. saylori* led biologists to initially assume that Mudlick Creek supported a disjoint population of *C. saylori*.

Discovery of this new population initiated widespread sampling by Skelton (2007) in order to assess the occupancy status of *Chrosomus* in the Clinch River drainage. In 2002, Skelton (2007) observed the newly-discovered fish in breeding colors, and at that time it was determined that the fish was not *C. saylori*, but a new, undescribed species hereinafter referred to as *C.* sp. cf. *saylori* (Clinch dace).

Sampling efforts by Skelton (2007) lasted from 1999-2007 and revealed that *C.* sp. cf. *saylori* are distributed in 16 streams across eight tributaries to the upper Clinch River watershed. These streams are located in coal mining drainages and, though the effects of mining on

Chrosomus have never been studied, mining has empirically been determined to drastically decrease stream health and function (Fritz et al. 2010). Sampling from Skelton (2007) also indicated that populations of *C*. sp. cf. *saylori* are small, fragmented, and of questionable viability. Because of these threats, *C*. sp. cf. *saylori* were listed as a Federal Species of Concern and placed on Virginia's Wildlife Action Plan as Tier II- Very High Conservation Need.

A conservation plan for *C*. sp. cf. *saylori* is of high priority. However, the sampling done by Skelton (2007) focused on maximizing the number of sites sampled, rather than the length of stream sampled at each site. While this sampling method is preferred for assessing the distribution of rare and endangered species (Joseph et al. 2006), it was not extensive enough to confidently determine the distribution of *C*. sp. cf. *saylori*, nor infer information about population size and stability. Furthermore, habitat correlations, life history, and reproductive mode have never been studied in *C*. sp. cf. *saylori*. Thus, much information about the species is still unknown, and a successful conservation plan cannot be created without a more extensive study of *C*. sp. cf. *saylori*.

The objective of this research was threefold: 1) describe the distribution and habitat correlates of *C*. sp. cf. *saylori* so as to create a predictive model of distribution, 2) determine major life history characteristics of *C*. sp. cf. *saylori* in relation to other *Chrosomus* species, and 3) describe the reproductive behavior and morphology of *C*. sp. cf. *saylori*. This study offers additional insight into the current status of *C*. sp. cf. *saylori*, helps inform future research objectives, and provides information necessary for developing a conservation management plan for the species. In total, this study suggests that additional protection at the state and federal level is warranted for *C*. sp. cf. *saylori*, and data provided here would be supportive for successful petitioning at both levels.

Chapter One: Distribution and habitat correlates of *Chrosomus* sp. cf. *saylori* in the upper Clinch River watershed and surrounding tributaries

Abstract

Identifying the distribution of a species is essential before developing and implementing a recovery plan. To date, distribution studies of the six described species of *Chrosomus* have been minimal, and all lacked detailed descriptions of habitat correlates necessary for developing species distribution models or predicting threats to habitat. Here, I provide an analysis of the distribution and habitat correlates of *C*. sp. cf. *saylori*. *Chrosomus* sp. cf. *saylori* was first discovered in 2009 in Tazewell County, Virginia. The species is currently undescribed, but due to potential threats from habitat degradation, high population fragmentation, and a largely unknown distribution, it is listed as a Federal Species of Concern and on Virginia's Wildlife Action Plan as Tier II- Very High Conservation Need. As such, determining the distribution of *C*. sp. cf. *saylori* is of utmost importance for developing and implementing a management protocol for the species. I sampled 60 locations, mostly in the upper Clinch River watershed, Virginia, to determine *C*. sp. cf. *saylori* occupancy. Data on ten stream habitat variables were collected for each stream. Using logistic regression, classification and regression trees, and random forests, I developed three species distribution models to predict *C*. sp. cf. *saylori* presence in Russell and Tazewell counties, Virginia. I found *C*. sp. cf. *saylori* at 14 sites spanning a total of 1.25 km distributed across eight drainages to the upper Clinch River watershed. The number of individuals at each location ranged from 1 to 13. Habitat correlates were consistent with small, headwater streams and included small substrate size, narrow stream width, shallow depth, and undeveloped watershed landuse. None of the species distribution models were effective for predicting *C*. sp. cf. *saylori*. Together, these data and analyses show

that the distribution of *C*. sp. cf. *saylori* is narrow, fragmented, and possibly driven more by geologic history rather than habitat preference. Because of this, and the small number of individuals captured at each site, increased protection measures for *C*. sp. cf. *saylori* should focus on critical habitat restoration and conservation by decreasing siltation and limiting the amount of watershed development.

Introduction

Monitoring fish populations that are small and potentially a candidate for federal listing represents a challenge. Abundance estimates are a reliable indicator of population size and stability; however, such extensive sampling (often requiring multi-pass removal techniques) is often unfeasible due to time and budget constraints (Rhodes et al. 2006). Because occupancy analysis requires presence/absence data rather than a count of all individuals, it is often more preferable than abundance because it is quicker, thus allowing more streams to be sampled (MacKenzie et al. 2003). It is also often the preferable method for monitoring threatened and endangered species as it gives a relatively quick assessment of population distribution and location of optimal habitat (Joseph et al. 2006).

Occupancy modeling is a common method for assessing species presence at a given location. Studies are designed such that N sites are sampled T times to produce an encounter history with presence/absence defined by a binary random variable (MacKenzie et al. 2002). For example, an encounter history of (0, 1, 0, 0) indicates that a site was surveyed four times and the species was only detected on the second survey. It is important to note that it is necessary to partition zero histories into instances where individuals were truly absent and instances where individuals were present, but not detected (Royle and Nichols 2003).

Successful occupancy modeling hinges on accurate detection probabilities, which are highly variable and dependent on sampling gear, species abundance, and the homogeneity of habitat units sampled (Angermeier and Smogor 1995, Bayley and Peterson 2001). As such, trying to estimate occupancy of stream fishes is extremely difficult. The literature is rich in studies that have modeled capture efficiency as a product of gear choice, length of stream sampled, heterogeneity of habitat units sampled, and abundance of the target species. The consensus is that, the more effort (e.g. increasing the number of electrofishing passes; Meador et al. 2003), and the longer the reach sampled (Paller 1995), the higher probability of encountering all species present in that system. And, if the target species is rare, more sampling and effort will be necessary in order to detect that species (MacKenzie et al. 2003).

Theoretically, if one were to sufficiently sample a large enough reach, occupancy modeling would yield an accurate prediction of species occurrence. However, this is not always possible in small, headwater streams where landowner access and heavy vegetation prevents the sampling of contiguous habitat units and large stretches of stream (Herlihy et al. 2000). In addition, the lack of roads around headwater streams often makes it unfeasible to sample all available habitat units, and biologists are restricted to measuring the habitat directly surrounding a road crossing.

Since investigators cannot sample all possible locations for a species and detection probability is so variable for fishes inhabiting small streams, species distribution models offer a more flexible alternative for creating a spatial representation of a species' range. Distribution models are often more accurate for species that occupy habitat over a small spatial and environmental gradient, thus making it ideal for modeling the distribution of rare fishes (Hernandez et al. 2006). And, though the accuracy of models increases asymptotically with the number of sites sampled, the sample size required for an accurate distribution model is often reasonable enough for most studies of rare species (Hernandez et al. 2006, Wisz et al. 2008). The only qualification for a species distribution model is a set of known presences (though presence/absence or abundance is preferred) and information on the environmental gradient over which the sites occur (Hernandez et al. 2006).

Despite the clear applicability of species distribution models to *Chrosomus,* to date no author has attempted to model the distribution of any of the six North American species. This is partially due to the historic nature of some studies, but otherwise largely owing to insufficient data collected during studies of distribution. Hamed and Alsop (2005), who have done the most well-documented sampling for *C. tennesseensis,* sampled 600 m of 52 streams using electrofishing and seining. However, the amount of habitat data collected by Hamed and Alsop (2005) was limited to substrate size and pool characteristics. The distribution accounts of *C. eos* (Bestgen 1989), *C. cumberlandensis* (Starnes and Starnes 1978, Starnes and Starnes 1981), *C. saylor*i (Skelton 2001) have all been reported without mention of methods, and very little information on habitat preference was collected. Absent from refereed literature are distribution accounts for *C. oreas* and *C. erythrogaster,* and these distributions only appear in state fish identification books which have variable methods and little information on habitat.

Given the aforementioned studies of *Chrosomus* distribution, biologists believe the ranges of *Chrosomus* species are adjacent, but largely non-overlapping (Figure 1.1). This depiction of *Chrosomus* distribution suffices for the more broadly distributed species; however, our understanding of the distribution of rare *Chrosomus* is at a scale much too large for implementing conservation practices (Vaughn and Ormerod 2003). For these species, models of distribution and vulnerability are desirable, but would require a more detailed survey of streams and collection of habitat variables (Hernandez et al. 2006).

The sampling protocol used by Skelton (2007) when assessing for *C.* sp. cf. *saylori* presence had many of the shortcomings of other *Chrosomus* studies. While Skelton (2007) sampled 155 sites (Figure 1.2), sampling was largely localized to one pool at a stream and no habitat data were collected. From Skelton (2007), biologists could make broad inferences of *C.*

sp. cf. *saylori* distribution; however, they could not begin to create a conservation plan until stream occupancy could be more definitively determined and information regarding habitat correlates was collected. This information was particularly necessary given that the distribution proposed by Skelton (2007) showed *C*. sp. cf. *saylori* distributed in streams adjacent to active coal mines, and populations that were highly fragmented and of questionable viability.

The objective of this study was to more clearly define the distribution of *C*. sp. cf. *saylori* so as to create a species distribution model and define critical habitat. This was accomplished by sampling at 60 locations, mostly in the upper Clinch River watershed, in 2011 and 2012. I also collected macro- and micro-habitat variables for each stream, which were used to determine *C*. sp. cf. *saylori* habitat correlates and incorporated into three species distribution models.

Methods

Site selection

I characterized macrohabitat variables for streams sampled by Skelton (2007) in a Geographic Information System (GIS). Variables included in the analysis were proportion of watershed (as defined by National Hydrography Dataset (NHD) Plus catchments) in three landuse classes (urban, agriculture, and forested), watershed area, elevation, stream slope, and number of road crossing. Data were collected from the NHD, National Land Cover Dataset (NLCD), and National Elevation Dataset (NED). For each variable, I determined the “ideal” value for *C*. sp. cf. *saylori* by averaging together the values for each stream that supported *C*. sp. cf. *saylori*. To determine the “suboptimal” value for *C*. sp. cf. *saylori*, I averaged together the values for streams that did not support *C*. sp. cf. *saylori*.

In order to choose samples sites, I ranked all watersheds in Russell and Tazewell counties, Virginia based on their values for each of the macrohabitat variables. Watersheds with a habitat variable similar to the ideal value were ranked as a three, and the ranks declined as values progressed further from the ideal and towards the suboptimal. After ranking each watershed on each individual variable, I summed all ranks for a watershed to get one composite rank for each watershed.

In summer 2011, I sampled 30 sites that Skelton (2007) did not sample. Sites were randomly-selected, but with the stipulation that the number of sites sampled with each composite rank was proportional to the total number of watersheds in Russell and Tazewell counties with that composite rank (i.e. if 10% of all watersheds had a composite rank of 5, then I sampled 3 sites with a composite rank of 5). Using a similar process, I also randomly selected 10 sample sites that Skelton (2007) sampled, but did not find *C*. sp. cf. *saylori*. Without regard to GIS habitat variables, I sampled 10 random sites that Skelton (2007) sampled and did find *C*. sp. cf. *saylori*. Thus, in total, I sampled 50 sites in summer 2011.

Following summer 2011, I selectively-sampled streams that were of particular interest due to close proximity to known populations of *C*. sp. cf. *saylori*. In fall 2011, I sampled five locations in Buchanan, Virginia and McDowell, West Virginia. These streams are not part of the Clinch River drainage, but close proximity to streams inhabited by *C*. sp. cf. *saylori* and no record of previous sampling in those counties made them of questionable occupancy. In summer 2012, I re-sampled larger, more upstream reaches of streams known to support *C*. sp. cf. *saylori* populations. During this sampling, I started collecting data from the last known point of sampling until I reached the stream source (i.e. spring or first area of overland flow). The goal in this sampling was to better understand the population structure of a subset of streams and to gather data on smaller headwaters.

Fish Collection

I sampled at least six 50-m subsections spanning a total of 300 m at most sites (land owner permission prevented a full survey of some locations, but enough data was collected at those sites to substantiate inclusion in this study). Thus, between each sampled subsection, there was a 50-m section of stream that was not sampled. This sampling protocol allowed a larger area of stream to be surveyed, which include a more heterogeneous mix of habitats and fish fauna.

Fish assemblage was determined using one-pass electrofishing. A total count of all species was kept for each 50-m subsection. In addition, length and weights of every *C*. sp. cf. *saylori* were recorded. I retained 82 *C*. sp. cf. *saylori* in formalin for future laboratory study and museum records.

Microhabitat Data Collection and Analysis

In each sampled subsection, I measured habitat at transects spaced 10 m apart. At each transect, I collected data on depth, substrate size (modified Wentworth scale), and cover at nine equidistant points. Stream width was measured at each transect. The volume of every pool in each sampled subsection was also recorded.

I used nonmetric multidimensional scaling (NMDS) with 50 random starts and a Jaccard distance metric on presence/absence data to determine whether community composition was different between sites with and sites without *C*. sp. cf. *saylori*. Appropriate dimensionality was selected *a priori* by creating a scree plot of dimensionality (1 to 6 dimensions) and stress. A Monte Carlo simulation with 50 iterations was calculated from the data to provide a random stress level, and the stress level of the chosen dimensionality and the randomly-produced stress level were compared with a two sample t-test with significance held at α=0.05. Significance of the resulting ordination was tested using a multi-response permutation procedure (MRPP).

Correlations between sites and environmental variables were made using Pearson's r to determine which habitat variables had the strongest correlation to *C*. sp. cf. *saylori* occupancy.

Species Distribution Models

I modeled the distribution of *C*. sp. cf. *saylori* using logistic regression, classification and regression trees (CART), and random forests (RF). Models were completed only for data collected in summer 2011, and abundances were reduced to presence/absence. Spatial autocorrelation was accounted for using principal coordinates of neighborhood matrices (PCNM) with distance between sites being defined using Euclidean distance. A PCNM analysis works by deriving a set of eigenvectors from a principal coordinates analysis (PCoA) on a distance matrix that has been truncated by a user-defined value of maximum neighbor distance (i.e., locations that are separated by a distance lower than the maximum neighbor distance retain the original Euclidean distance whereas locations separated by a distance larger than the maximum neighbor distance receive a distance of 4x the maximum neighbor distance). This results in eigenvectors which can then be used as predictor variables in species distribution models (Borcard and Legendre 2002). However, because the PCoA produces negative eigenvectors and not all eigenvectors are significant for explaining spatial autocorrelation, I ran a significance test on the Moran's I value, a measure of spatial autocorrelation, on each positive spatial eigenvector (negative eigenvectors were removed). All eigenvectors that had a significant Moran's I value were included in all three models.

Because I did not have an *a priori* definition of the maximum neighbor distance, I visualized five definitions of neighborhood based on 1000 m, 2500 m, 5000 m, 7500 m, and 10000 m between site distances. I selected the maximum neighbor distance that resulted in the inclusion of all sites in a neighborhood for PCNM analysis.

Due to a low proportion of presences in the dataset, I did not withhold any data for cross-validation. I evaluated all models using the area under the receiver-operating characteristic curve (AUC), omission rate, sensitivity and specificity, and percentage of correctly classified observations (Fielding and Bell 1997). All analyses were performed using the R statistical program version 2.14.

For logistic regression, I ran 14 models which were based on *a priori* hypotheses of variables that influence *C*. sp. cf. *saylori* distribution. Models were ranked using Akaike's Information Criteria (AIC) and, for each model, I calculated the difference in AIC from the model with the lowest AIC (Δ_i) and Akaike's weights (w_i). All models with $\Delta_i \leq 2$ and high w_i were considered good candidate models for describing *C*. sp. cf. *saylori* distribution (Burnham and Anderson 2002).

Because CART and RF do not require development of *a priori* hypotheses, I included all environmental variables and significant PCNM eigenvectors in these models. The RF model used the same candidate predictors to construct 1000 trees, and both CART and RF models were predicted using classification rather than regression trees. I constructed variable importance plots describing mean change in accuracy and Gini coefficient after removal of a given predictor variable. Negative mean decrease in accuracy is indicative of improved model performance after that variable is removed. The Gini coefficient is also a measure of variable performance wherein the larger the Gini coefficient, the more a variable is able to accurately group presences and absences when it is chosen as a node.

Results

Fish Assemblage

A total of 19,102 individuals were captured during this study, of which 78 (0.4%) were *C*. sp. cf. *saylori*. *Chrosomus* sp. cf. *saylori* were located at 14 of 60 sampling locations across eight drainages to the Clinch River (Figure 1.3), and the average number of individuals at each site was less than 6 (min=1, max=13). Streams in this study were dominated by *Rhinichthys atratulus* (blacknose dace), which accounted for 58% of all fish collected (Figure 1.4, Appendix A). Other common species included *Campostoma anomalum* (central stoneroller; 15%) and *Semotilus atromaculatus* (creek chub; 11%). A total of 31 species were collected, and, on average, there were 5.6 (min=1, max= 12) species at each stream and 6.1 species at streams that contained *C*. sp. cf. *saylori* (min=4, max=10).

Of the 10 sites sampled that Skelton (2007) had sampled and not found *C*. sp. cf. *saylori*, I also did not find *C*. sp. cf. *saylori*. Of the 10 sites sampled that Skelton (2007) sampled and found *C*. sp. cf. *saylori*, I found *C*. sp. cf. *saylori* at eight of those sites. The two sites that I did not find *C*. sp. cf. *saylori* were Big Lick Creek and West Fork Big Creek. Big Lick Creek was reported to support large populations of *C*. sp. cf. *saylori* by Skelton (2007); however, sampling the same reach in 2011 did not produce any *C*. sp. cf. *saylori*. In 2012, I sampled Big Lick Creek 500 m from the original sample site and found *C*. sp. cf. *saylori*, and continued to find *C*. sp. cf. *saylori* in a patchy distribution at four locations in the stream (Figure 1.5). Thus, it appears that *C*. sp. cf. *saylori* had moved further upstream or the downstream populations had been extirpated. West Fork Big Creek was not revisited, and so it is unknown whether that populations has been extirpated, moved further upstream, or was simply missed during 2011 sampling.

Habitat Correlates of *Chrosomus* sp. cf. *saylori*

On average, *C*. sp. cf. *saylori* were located in streams with an average depth of 11.58 cm (SE= 1.58), width of 2.16 m (SE=0.21), and pool volume of 2.45 m^3 (SE=0.49, Figure 1.6). None of these microhabitat variables were statistically significant from streams that did not have *C*. sp. cf. *saylori*. For the macrohabitat variables, average stream slope for sites with *C*. sp. cf. *saylori* was 1.44% (SE= 0.03), elevation was 652.87 (SE=14.07), watershed area was 5.63 km^2 (SE=1.01), and stream order was 2.50 (SE=0.17). Watershed area was significantly smaller (p=0.001) and elevation significantly larger (p=0.002) than streams that did not inhabit *C*. sp. cf. *saylori* (Appendices B-D).

For the NMDS, two dimensions resulted in a stress of 19, which was significantly lower than stress from Monte Carlo simulation (p=0.02). Thus two dimensions were chosen for this analysis.

There was a significant difference in the community assemblage between sites with, and sites without *C*. sp. cf. *saylori* (Figure 1.7, by MRPP, p<0.001). Sites with *C*. sp. cf. *saylori* also supported *S. atromaculatus* and *C. anomalum.* When NMDS axes were correlated to environmental variables, the first axis was positively correlated to stream depth and negatively correlated to substrate size. The second NMDS axes was positively correlated to proportion of forested landuse in the watershed and negatively correlated to stream width (Table 1.1). These correlations support the conclusion that *C*. sp. cf. *saylori* most often inhabited small streams (i.e. low pool volume, stream width) that had gravel substrates and located in watersheds with less development.

Chrosomus sp. cf. *saylori* Distribution Models

In establishing the neighborhoods, I had to use a distance of 10,000 m to include all sites in a neighborhood. Of the 49 eigenvectors produced, 11 had a significant, positive Moran's I and were used to model *C*. sp. cf. *saylori* distribution (Figure 1.8).

Of the 14 logistic models, only the spatial predictor model (w = 0.55) and global model (w = 0.31) had high support (Table 1.2). The spatial predictor model had an AUC of 0.75, an omission rate of 0.42, a sensitivity of 0.58, a specificity of 0.92, and correctly predicted 84% observations. However, because the spatial eigenvalues are only useful in correcting for spatially autocorrelation and not for creating a distribution report, I do not report results of model averaging.

The CART modeled resulted in a tree with three predictors: elevation, and two eigenvectors of spatial autocorrelation (Figure 1.9). This model had an AUC of 0.92, no omissions, a sensitivity of 1, a specificity of 0.84, and correctly predicted 88% of the observations.

Spatial eigenvectors were the most important predictors in the RF classification, and seven of the top 10 predictors were a spatial eigenvectors (Figure 1.10). The RF classification had an AUC of 0.64, an omission rate of 0.17, a sensitivity of 0.83, a specificity of 0.45, and correctly predicted 54% of the observations and included spatial predictors as the most important predictors.

Discussion

Chrosomus sp. cf. *saylori* were located at 25% of sites sampled. As described by the NMDS analysis, streams supporting populations of *C*. sp. cf. *saylori* tended to be smaller with low pool volume and stream width and located in forested watersheds. When trying to model distribution, the CART model performed the best and had the highest AUC, sensitivity, specificity, and lowest omission rate. However, none of the models were particularly effective for predicting *C*. sp. cf. *saylori* distribution, and all placed spatial eigenvectors among the variables most important to *C*. sp. cf. *saylori* distribution.

The failure of the three species distribution models lies in the reliance of the spatial eigenvectors to predict *C*. sp. cf. *saylori* distribution. These eigenvectors are only intended to remove the effects of spatial autocorrelation, thereby increasing the predictive power of environmental variables. By themselves, spatial eigenvectors are incapable of modeling distribution at locations that don't already have a known occupancy. Though the performance of the CART model initially seems promising, the only environmental variable it uses is elevation and it shows that *C*. sp. cf. *saylori* occupy stream at elevations >673 and <673 at approximately equal proportion. Thus, the CART model provides little information about the environmental drivers of *C*. sp. cf. *saylori* distribution.

Although the spatial eigenvectors were among the most important variable in all three distribution models, it must be noted that they were produced using PCNM with Euclidean distance between sample sites as the measure of neighbor proximity. Given that streams are connected linearly, the use of Euclidean distance likely artificially classified many sample sites as neighbors even though the fluvial distance between them is very large. In fact, the results of my PCNM analysis placed sites in the Clinch River drainage and sites in the Big Sandy drainage as neighbors, but it is unlikely that the biological and ecological processes are similar between streams in those two watersheds. It has been shown that linear distance explains more variation in fish distribution than Euclidean distance (Landeiro et al. 2011), and a modified PCNM with linear distance could change the results of my models.

Given this, one could argue that rerunning the distribution analysis using a linearly-defined PCNM and perhaps a different modeling technique (e.g. maximum entropy, which has been proven to be robust to studies with limited sample size (Hernandez et al. 2006)) would be a worthwhile endeavor. However, the measured environmental variables were largely insignificant in all three models, indicating the distribution of *C*. sp. cf. *saylori* is not influenced by any of the measured variables (Elith and Graham 2009). Thus, any further attempts to model *C*. sp. cf. *saylori* with the current dataset would likely be unsuccessful. Furthermore, linear distance in generally believed to be more influential for accounting for spatial autocorrelation that results from fish movement and dispersal and Euclidean distance better for large-scale drivers of autocorrelation (Landeiro et al. 2011). Given that *Chrosomus* are relatively sedentary (Albanese et al. 2004), movement is likely not affecting distribution patterns, and so linear distance may not explain spatial patterns in *C*. sp. cf. *saylori* distribution better than Euclidean distance.

A critical component to any species distribution model is habitat variables collected at an appropriate scale to the organism of interest and across an environmental gradient (Hernandez et al. 2006). The variables used in the distribution analysis were collected at both a macro and micro level, and so it is unlikely that the scale of data collected was inappropriate. Data points were also collected at streams ranging from 0.3 to 13 m in width, and included sites that likely had and likely did not have *C*. sp. cf. *saylori* as determined by an analysis of macrohabitat variables. Thus, a wide range of habitats was sampled. Therefore, it is unlikely that the distribution of *C*. sp. cf. *saylori* could be explained by sampling at different scales. Instead, this result lends to the conclusion that *C*. sp. cf. *saylori* is more likely explainable by a history of vicariance and dispersal, processes that are not currently explicable or applicable to species distribution modeling. However, to substantiate that claim, more variables should be included in the analysis (e.g. base flow, water quality parameters, riparian landuse, and connectivity).

Despite the inability to model *C*. sp. cf. *saylori* distribution, enough sites were sampled to confidently conclude that the distribution of *C*. sp. cf. *saylori* is likely that shown in Figure 1.3. This is alarming because several streams with *C*. sp. cf. *saylori* are adjacent to streams that are seemingly depauperate of *C*. sp. cf. *saylori*, and some populations are separated by at least 30 km of stream and river. Though the movement ability of *Chrosomus* has never been intensively studied, it is unlikely that they are capable of readily moving such great distances without high mortality (Albanese et al. 2004).

Perhaps a better indication of *C*. sp. cf. *saylori* distribution is a consideration of the potential length of stream inhabited. I sampled a total of 17.7 km of headwater stream and found *C*. sp. cf. *saylori* to inhabit only 1.4 km (i.e. *C*. sp. cf. *saylori* were present in 8% of the sampled habitat). Looking at the length of second and third order streams (which is the predominant order of streams inhabited by *C*. sp. cf. *saylori*) located north of the Clinch River in Russell and Tazewell counties, Virginia (which is the only location *C*. sp. cf. *saylori* have ever been found), there are 505 km of habitat available, of which 125 km has not been sampled extensively or is already known to contain *C*. sp. cf. *saylori*. Assuming *C*. sp. cf. *saylori* occupy 8% of available habitat, *C*. sp. cf. *saylori* could conceivably occupy an additional 10 km (i.e. 8% of 125 km).

This estimate assumes *C*. sp. cf. *saylori* only occupy 8% of available habitat, which may be low considering it was developed using all 60 streams sampled in 2011 and 2012, including those streams that did not have ideal *C*. sp. cf. *saylori* habitat. However, the 125 km of unsampled, potential *C*. sp. cf. *saylori* is likely high because it was calculated without regard to habitat preference, and likely includes several kilometers of stream that would not support *C*. sp.

cf. *saylori*. Because I took the product of those two variables, the individual errors are largely negated (i.e. the possibility that 8% is too low is corrected for by 125 km being too high). Thus, the estimate of 10 km of additional habitat occupied by *C*. sp. cf. *saylori* is reasonable.

With such population fragmentation, the threat of extinction for *C*. sp. cf. *saylori* must be considered. Extinction risk is a direct product of the size, number, and arrangement of population in the landscape (Fagan et al. 2002). Population theory predicts that the more fragmented a population becomes, the higher the risk of extinction due to loss of genetic diversity and susceptibility to environmental stochasticity (Cushman et al. 2012). And, the threats of extinction are even greater for dendritic landscapes (i.e. streams) where organisms must move through linearly-connected habitat patches, many of unsuitable quality, in order to access other metapopulations (Fagan 2002, Grant 2011).

Of perhaps even more concern is the population status within a single stream. Between 1 and 13 individuals were captured at a single site across all 300 m sampled. Though single-pass electrofishing cannot be used to estimate population sizes, low number of individuals caught on that one pass would be indicative of a small population (Bateman et al. 2005, Reid et al. 2008).

The presence of multiple culverts, often with improper fish passage, and beaver dams in streams inhabited by *C*. sp. cf. *saylori* leaves one to question whether populations within a stream are interbreeding, or whether they represent smaller subpopulations. The latter hypothesis has been documented in other systems (Benton et al. 2008), and could be possibly confirmed by the sporadic encounter of *C*. sp. cf. *saylori* across the 300 m sampled. It was common for me to find *C*. sp. cf. *saylori* in one section, but then not find *C*. sp. cf. *saylori* for 400 m or more and after passing several road crossings (Figure 1.5).

Although culverts could be a major source of habitat degradation and an impediment to *C*. sp. cf. *saylori* movement, and thus survival, downstream scour pools are also seemingly a source of refuge habitat during the summer months when stream fish mortality has been shown to be highest in small streams (Falke et al. 2012). Many streams inhabited by *C*. sp. cf. *saylori* dry in the summer months, and fish survival is dependent on the continued existence of pools (Capone and Kushlan 1991, Labbe and Fausch 2000). For these streams in the Appalachian Mountains, the presence of bedrock prevents extensive erosion and pool formation, and large, stable pools are most often formed downstream of culverts. These pools, which rarely dry during the summer, could increase yearly survival of *C*. sp. cf. *saylori* by providing critical habitat, particularly since piscivorous fish (e.g. *Semotilus atromaculatus*) larger than 150 mm are not very abundant in the upper headwaters. However, because *C*. sp. cf. *saylori* would ordinarily have to move to find suitable summer habitat, and these movements would occur during spawning season, scour pools may also decrease gene flow and colonization which would occur during periods of movement. Culverts have only been studied in the context of blocking fish migrations, but understanding how culverts affect short- and long-term survival as described above would help further our understanding of the population dynamics in headwater streams.

In the future, the effects of *Castor canadensis* (North American beaver) on *C*. sp. cf. *saylori* should also be assessed. *Castor canadensis* are known ecosystem engineers, and can change the composition of the aquatic community (Smith et al. 1991, Hägglund and Sjöberg 1999). In particular, ponding above dams increases water temperature, reduces stream flow, and decreases average sediment size. Though *Chrosomus* are believed to be able to survive in beaver ponds (Hägglund and Sjöberg 1999), these habitat characteristics are not ideal for *C*. sp. cf. *saylori*, and at the very least are impediments to fish movement. Three streams in this study had heavy beaver activity that took place between sampling by Skelton (2007) and the completion of this study. In two instances, there was a marked decline in *C*. sp. cf. *saylori* populations. Deep pools with muddy substrate prevented sampling of the third stream to assess the *C*. sp. cf. *saylori* population. Population size of *C*. *canadensis* is increasing in the Appalachian Mountains (Fuller and Peckarsky 2011) and, if *C*. sp. cf. *saylori* are unable to use or move through the ponds, then *C*. *canadensis* activity will be a major source of habitat loss and fragmentation as populations become trapped above dams.

Given the dendritic landscape, barriers to fish passage, limited mobility, and already small population sizes, it is highly unlikely that, should one population of *C*. sp. cf. *saylori* go extinct, the habitat would be rapidly recolonized (Fagan 2002). And, because so few populations of *C*. sp. cf. *saylori* are known, loss of even a few individuals could be detrimental to the viability of the species (Harrison 1991). This magnifies the significance of local disturbances, and turns factors that affect fish over small spatial and temporal scales into drivers extinction (Lafferty et al. 1999). Understanding local stressors could also help hypothesize the distribution of extinct populations of *C*. sp. cf. *saylori* and explain why extant populations are so fragmented.

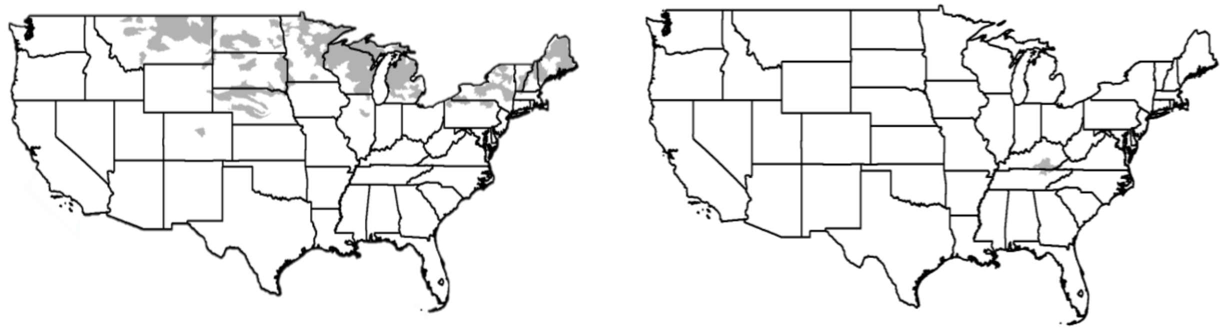
Together, this study shows that populations of *C*. sp. cf. *saylori* are small, narrowly-distributed, and exhibit fragmentation both within and across streams. Habitat preference is for streams that are small with gravel substrate and narrow stream width. However, threats from watershed landuse, improper fish passage at culverts, and ponding from *C*. *canadensis* represent threats to continued *C*. sp. cf. *saylori* survival. Though it has been shown that rare fish populations can exist long-term, the ability to do so depends on an unfragmented spatial distribution (Fagan et al. 2002), which *C*. sp. cf. *saylori* does not have. Thus, future studies should focus on monitoring *C*. sp. cf. *saylori* to determine temporal trends in population stability and identification of stressors. Any identified population of *C*. sp. cf. *saylori* should conserved, managed, and monitored so as to prevent extirpation.

Table 1.1: Pearson's r correlations between environmental variables and NMDS axes. Significant correlations ($P<0.05$) are shown in bold.

Variable	NMDS 1	NMDS 2
Average Stream Width	**0.26**	**-0.34**
Average Stream Depth	**0.39**	**-0.25**
Average Substrate Size	**-0.32**	**-0.03**
Proportion of Stream Covered	0.05	-0.10
Average Pool Width	**0.11**	**-0.29**
Average Pool Depth	**0.27**	**-0.33**
Total Pool Volume	**0.37**	**-0.18**
Number of Pools	0.10	-0.04
Average Pool Volume	**0.39**	**-0.25**
Proportion of Watershed Developed	0.12	-0.17
Proportion of Watershed Farmed	-0.10	-0.21
Proportion of Watershed Forested	**0.18**	**0.31**
Proportion of Watershed Barren Rock	0.13	0.07
Proportion of Watershed Water	-0.15	0.11
Proportion of Watershed Wetland	-0.07	0.01
Stream Slope	0.16	0.16
Stream Elevation	0.13	0.08
Stream Order	**0.21**	**-0.35**

Table 1.2: List of 14 logistic regression models with AIC, ΔAIC, and w_i values used for predicting *C.* sp. cf. *saylori* distribution from summer 2011 data. Models included GIS-defined macrohabitat variables and 11 spatial eigenvectors (E1-E11).

Model	AIC	ΔAIC	w_i
Spatial Eigenvectors (E1 + E2 + E3 + E4 + E5 + E6 + E7 + E8 + E9 + E10 + E11)	54.89	0.00	0.55
Width + Depth + Substrate + Cover + Volume + Number of Pools + Area + Proportion of Watershed Developed + Proportion of Watershed Farmed + Proportion of Watershed Forested + Proportion of Watershed Bare Rock + Proportion of Watershed Water + Proportion of Watershed Wetland + Stream Slope + Elevation + Order + E1 + E2 + E3 + E4 + E5 + E6 + E7 + E8 + E9 + E10 + E11	56.00	1.11	0.31
Depth * Width + Volume	61.00	6.11	0.03
Elevation + Stream Slope + Area	61.26	6.37	0.02
Area + Depth + Elevation + Width	61.43	6.53	0.02
Width + Depth + Volume	61.48	6.58	0.02
Depth + Number of Pools + Volume + Stream Slope	62.23	7.34	0.01
Depth * Area + Stream Slope	62.70	7.81	0.01
Depth * Volume + Width	63.42	8.53	0.01
Proportion of Watershed Farmed + Proportion of Watershed Developed + Substrate + Stream Slope	63.52	8.63	0.01
Area + Stream Slope + Depth + Width	63.86	8.96	0.01
Width + Depth + Substrate + Cover + Volume + Number of Pools	64.89	9.99	0.00
Area + Proportion of Watershed Developed + Proportion of Watershed Farmed + Proportion of Watershed Forested + Proportion of Watershed Bare Rock + Proportion of Watershed Water + Proportion of Watershed Wetland + Stream Slope + Elevation	67.39	12.50	0.00
Width + Depth + Substrate + Cover + Volume + Number of Pools + Area + Proportion of Watershed Developed + Elevation + Proportion of Watershed Farmed + Proportion of Watershed Forested + Proportion of Watershed Bare Rock + Proportion of Watershed Water + Proportion of Watershed Wetland	73.14	18.25	0.00

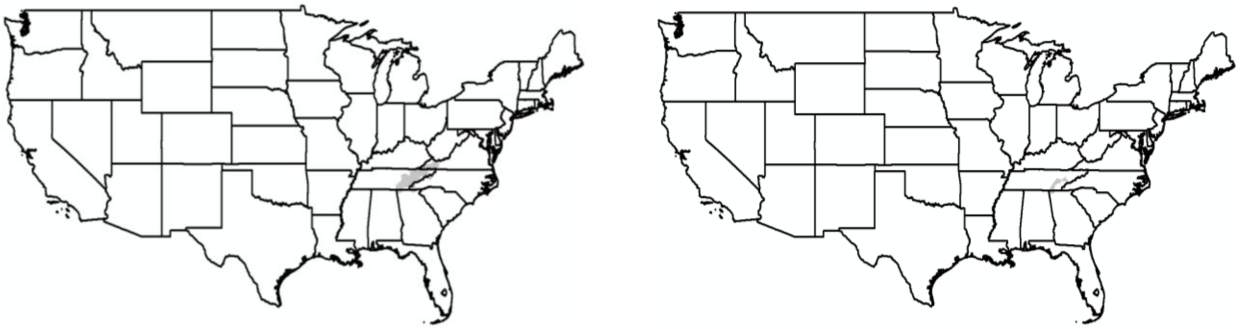


C. eos

C. cumberlandensis

C. erythrogaster

C. oreas



C. tennesseensis

C. saylori

Figure 1.1: Distributions of *C. eos*, *C. erythrogaster*, *C. tennesseensis*, *C. cumberlandensis*, *C. oreas*, *C. saylori* in the United States (data obtained from NatureServe).

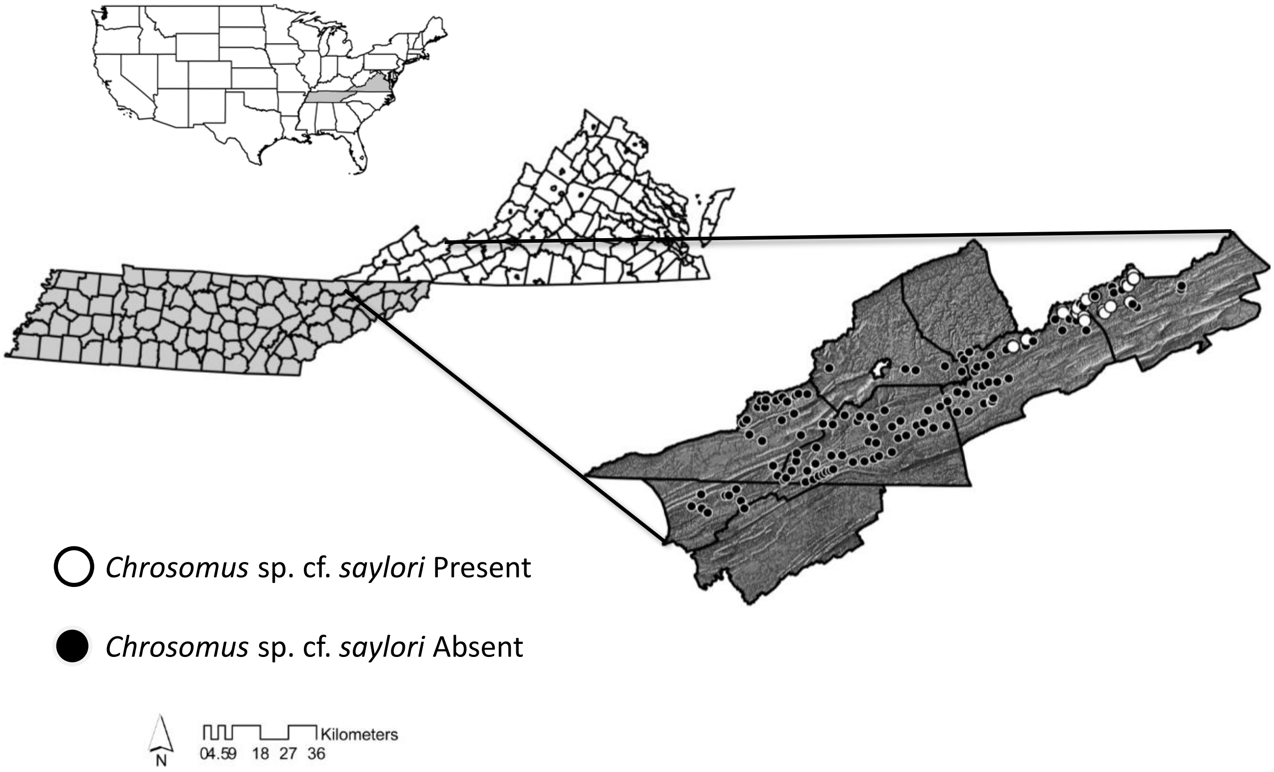


Figure 1.2: Locations sampled by Skelton (2007) showing presence (white) and absence (black) of *Chrosomus* sp. cf. *saylori*.

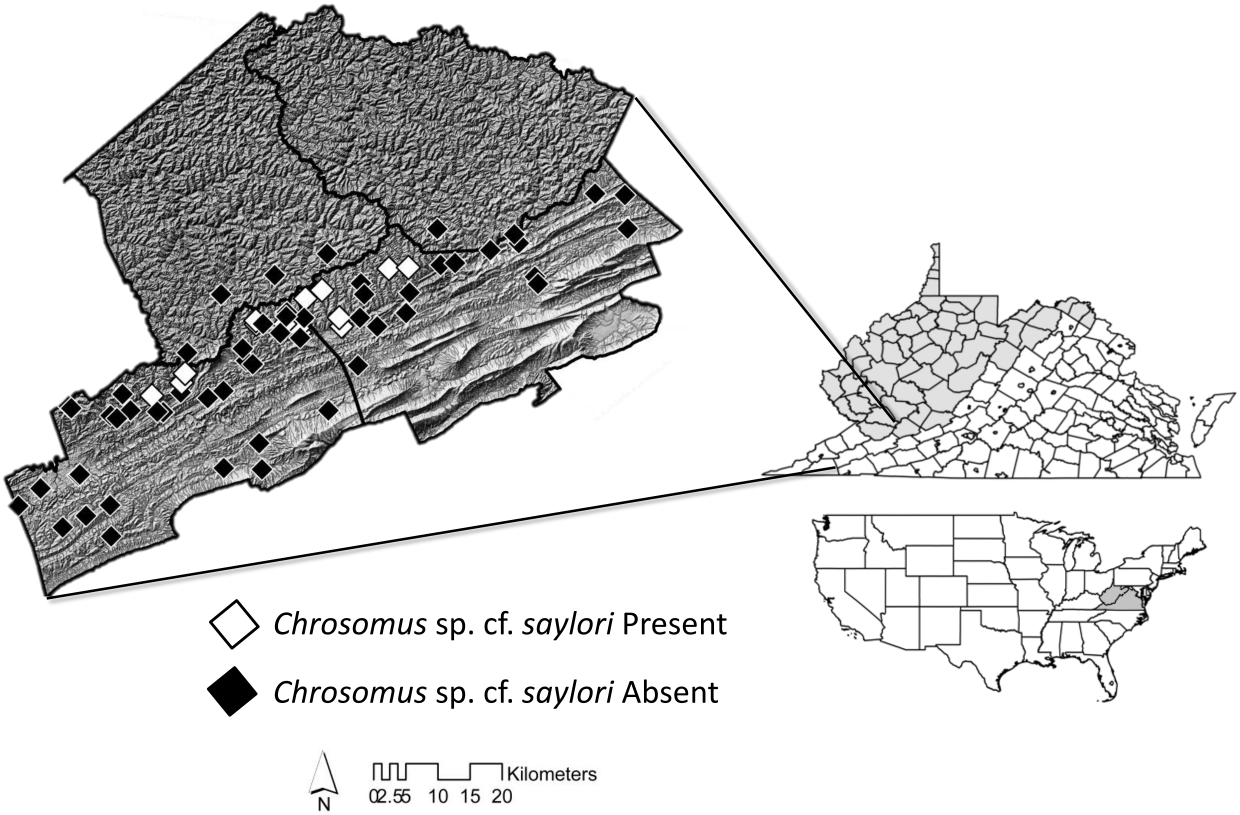


Figure 1.3: Presence (white) and absence (black) of *Chrosomus* sp. cf. *saylori* at sites sampled in Virginia and West Virginia in 2011 and 2012.

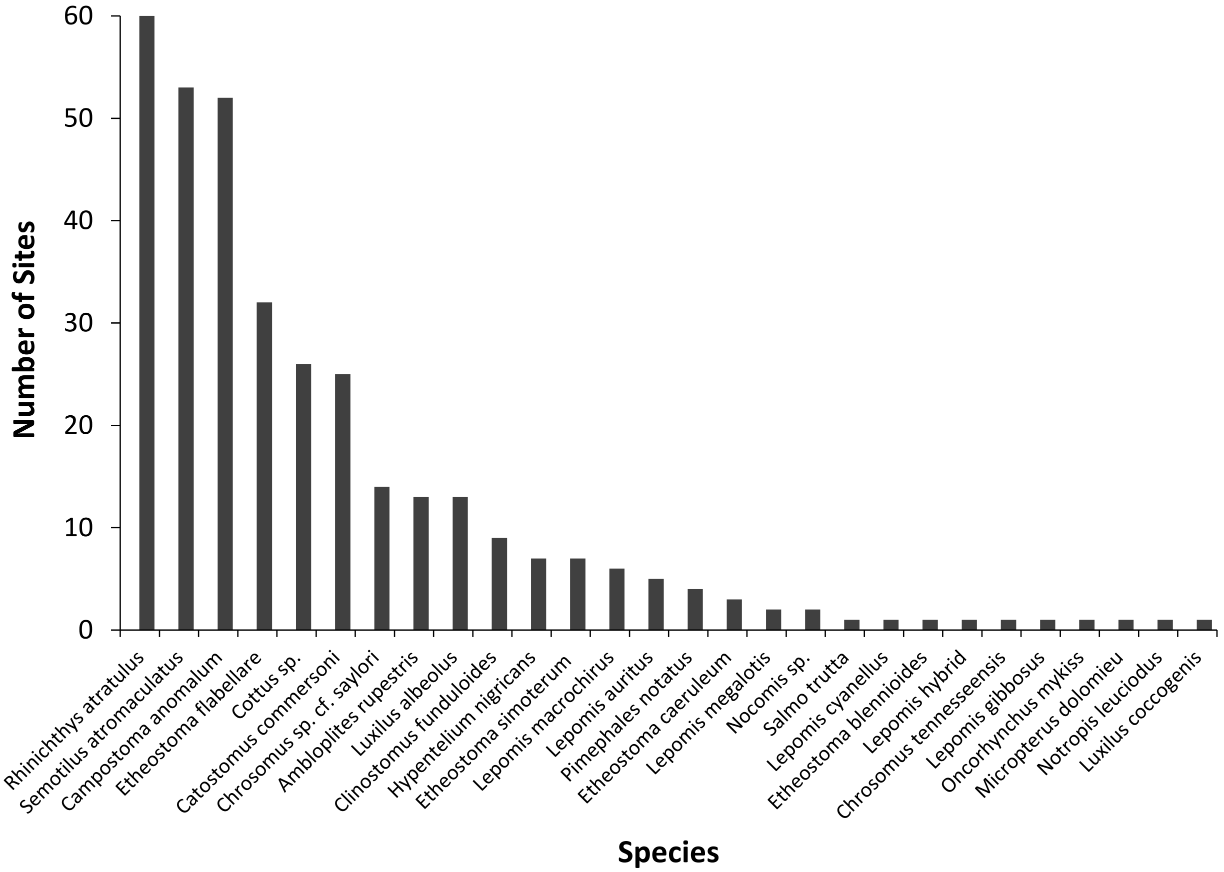


Figure 1.4: Number of sites occupied by each of the 28 species encountered during sampling in 2011-2012.

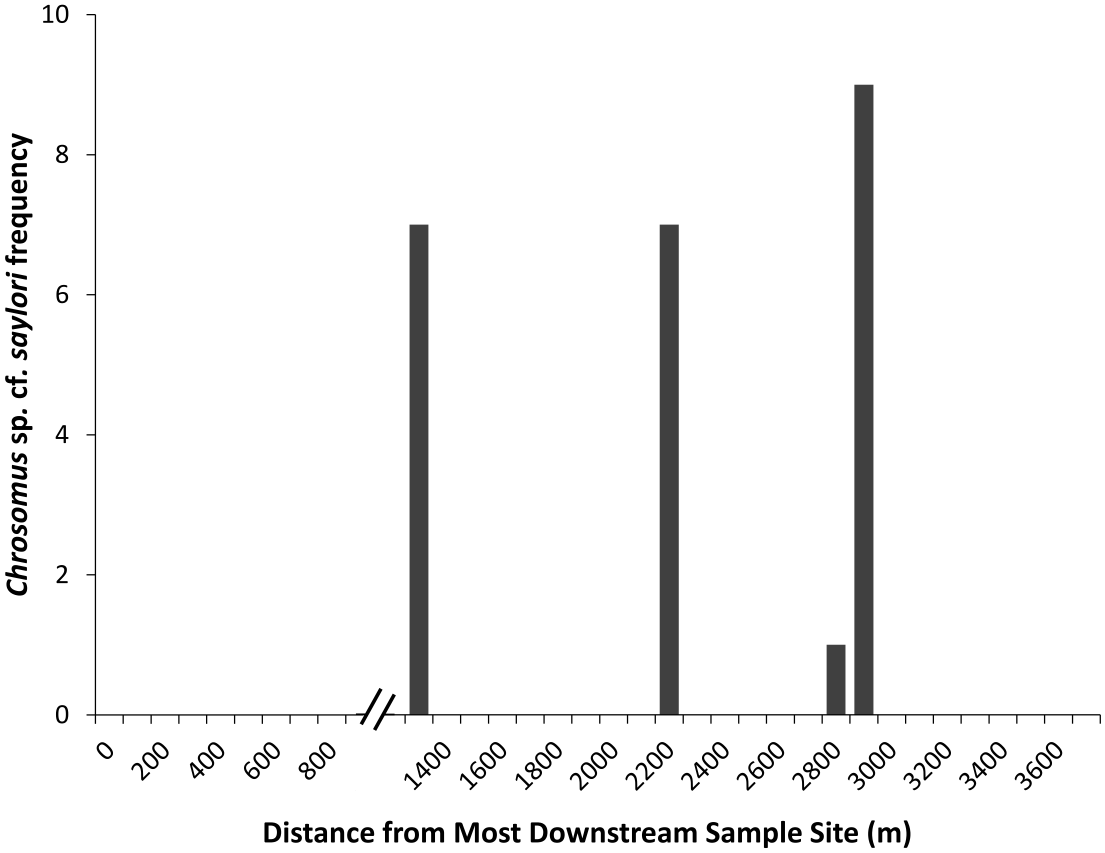


Figure 1.5: Frequency of *C*. sp. cf. *saylori* capture in Big Lick Creek starting at the most downstream sample site at Swords Creek Road and continuing for 3600 m upstream. The break on the x-axis reflects a section of stream not sampled between 1000-1300 m. Sporadic occurrence of *C*. sp. cf. *saylori* at just four locations is possible evidence of multiple subpopulations.

Figure 1.6: Photograph of Pine Creek, a representative stream that supports *C*. sp. cf. *saylori* populations. Photograph was taken on 6 June 2011 and is facing downstream.

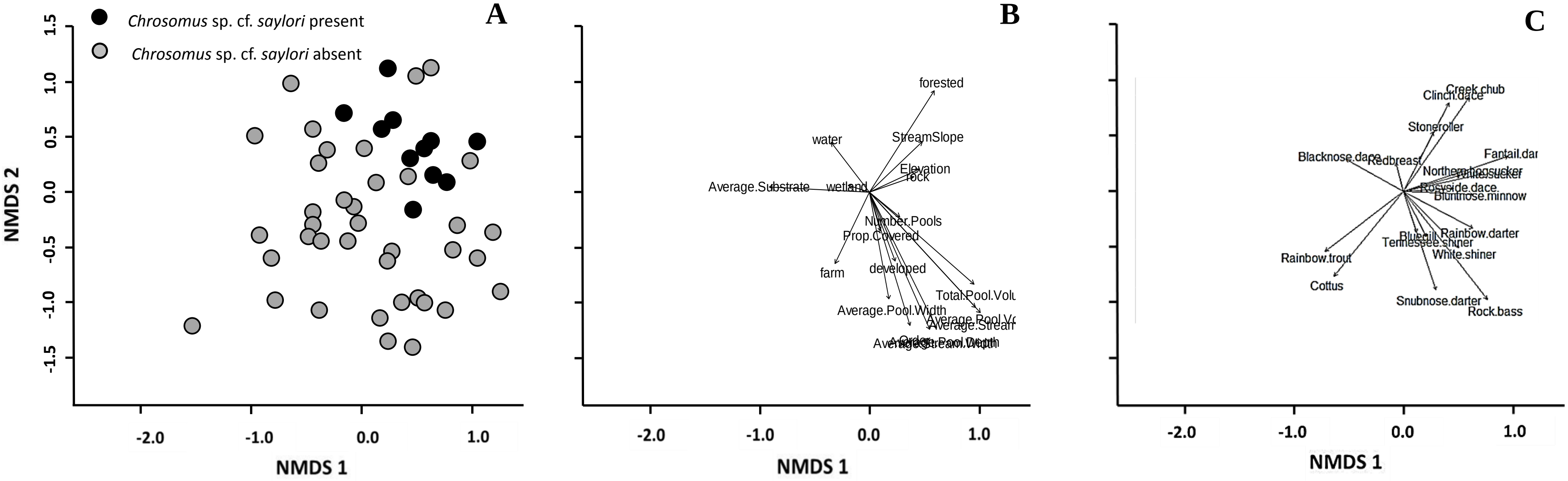


Figure 1.7: NMDS ordination (A) of sites without *Chrosomus* sp. cf. *saylori* (grey) and with *C*. sp. cf. *saylori* (black). Correlation of NMDS axes with environmental variables (B) and species abundance (C) shows that streams with *C*. sp. cf. *saylori* are narrow, shallow, and located in forested watersheds and support populations of *Semotilus atromaculatus* and *Campostoma anomalum*.

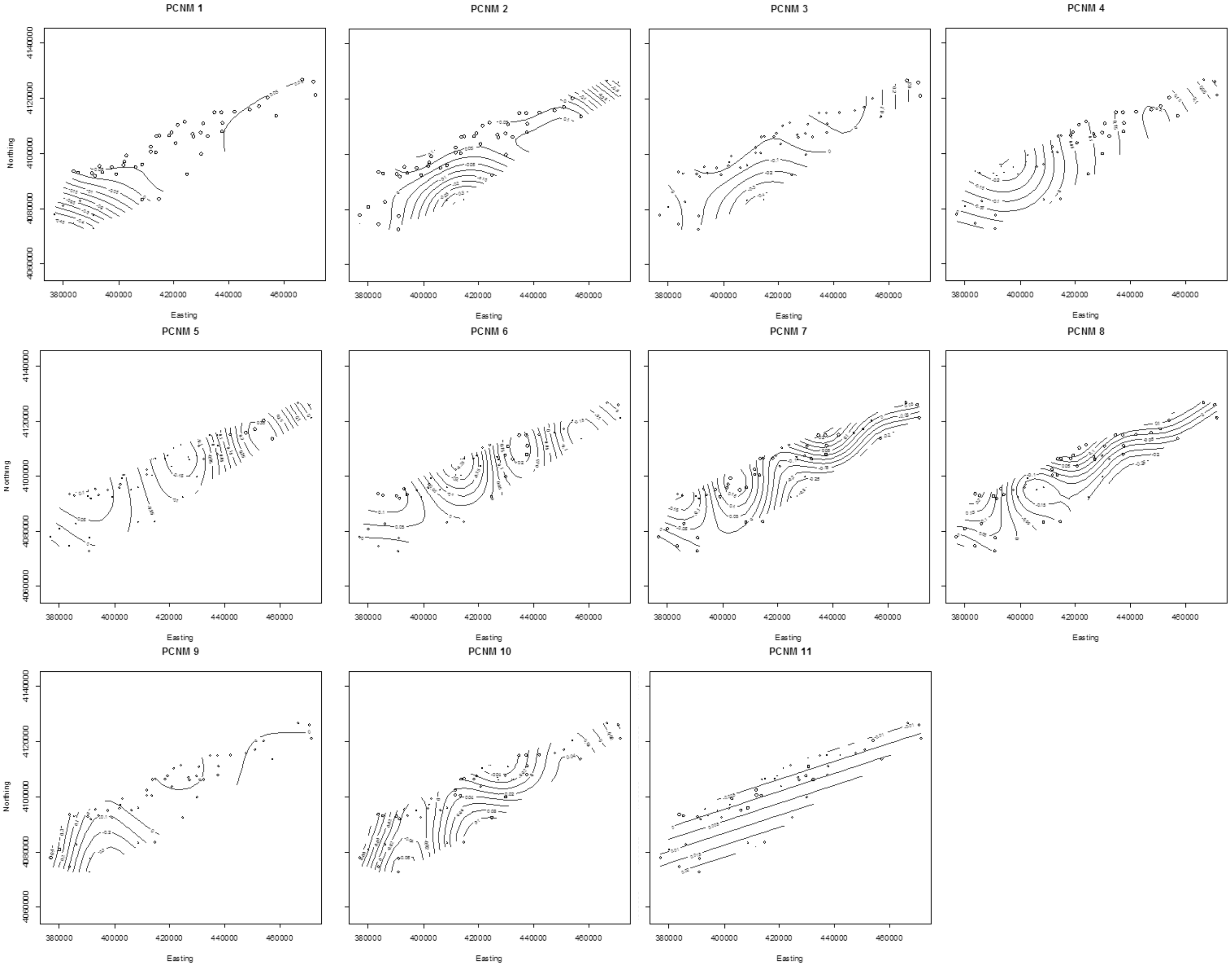


Figure 1.8: Plots of the 11 significant eigenvectors included in the species distribution models. Each plot signifies areas of spatial autocorrelation wherein lines that are closer together are indicative of strong autocorrelation in that region.

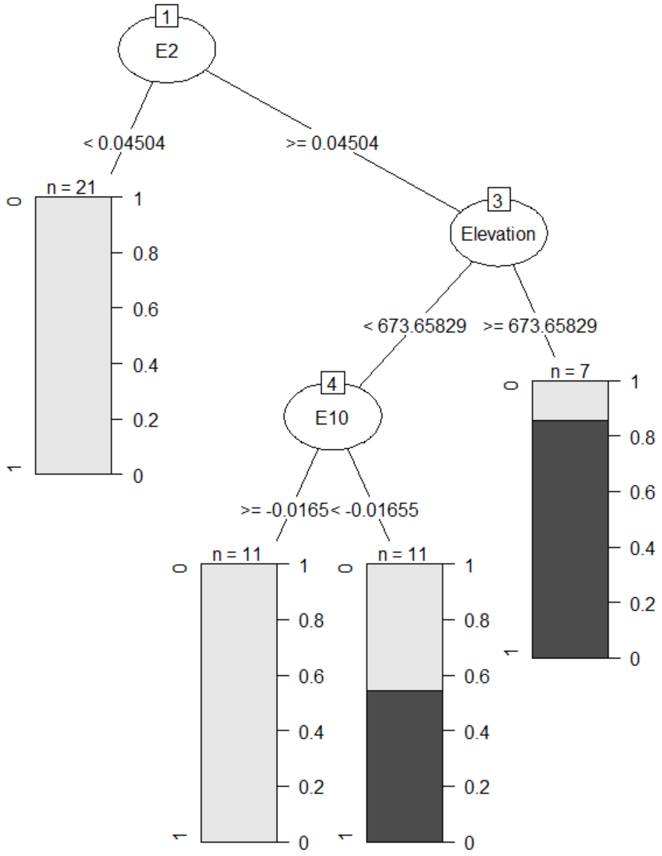


Figure 1.9: CART model predicting the presence of *C*. sp. cf. *saylori* in Russell and Tazewell counties, Virginia, USA. The first node shows a split at the second eigenvector (E2), the second node a split for elevation, and the third node a split for the tenth eigenvector (E10). The threshold values for each of the aforementioned variables are given in the branch. Proportion of presences (black) and absences (grey) are shown in the leaves.

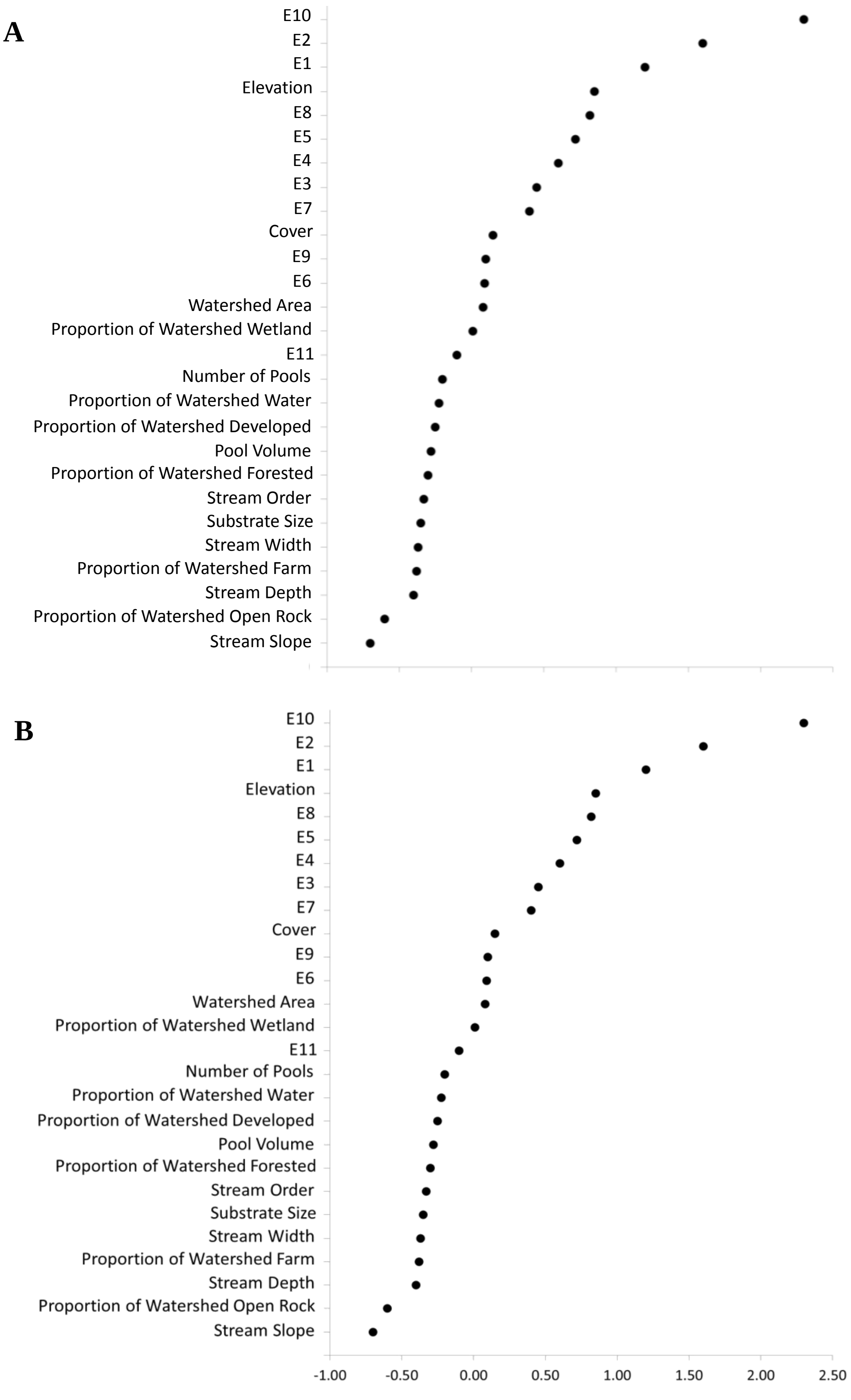


Figure 1.10: Variable importance plots for RF model predicting *Chrosomus* sp. cf. *saylori* presence in Russell and Tazewell counties, Virginia showing mean decrease in accuracy (A) and mean decrease in Gini coefficient (B).

Chapter Two: Ontogenetic and comparative morphology of *Chrosomus* sp. cf. *saylori*

Abstract

A description of *Chrosomus* sp. cf. *saylori* morphology and life history characteristics is necessary to describe the species, which will be beneficial should further conservation measures be pursued. Here, I compare morphometric and meristic features between *C*. sp. cf. *saylori* and closely-related congers. In addition, I test for possible sexual dimorphism in morphology, use otolith age data to make inferences on ontogenetic shifts in morphology, and analyze diet preference through gut content analysis. I found clear external morphological differences between *C*. sp. cf. *saylori* and *C*. *saylori*, and differences in internal morphology between *C*. sp. cf. *saylori* and other *Chrosomus* species. There were also morphological differences owning to ontogenetic development and sexual dimorphism. Size-at-age data were similar to previous *Chrosomus* studies, and the maximum age in my sample was two years. Gut contents were mostly invertebrates and intestine length was much reduced compared to other *Chrosomus*. *Chrosomus* sp. cf. *saylori* is likely a distinct species and isolation of *C*. sp. cf. *saylori* from its sister species, *C*. *saylori*, for many generations has led to distinct, measurable differences in morphology. The maximum age of two was comparable to congeners, and the gut contents and functional morphology of *C*. sp. cf. *saylori* indicates that *C*. sp. cf. *saylori* occupies a different trophic level than other *Chrosomus* species. In the future, genetic analysis should be done to confirm the speciation of *C*. sp. cf. *saylori*.

Introduction

Evolution is the foundation of speciation, and there are numerous evolutionary mechanisms that can alter allele frequencies (Levin 2000). Strong environmental gradients can influence evolution through natural selection, thus resulting in species with unique genotypes and phenotypes. However, in the absence of selective pressures, speciation can still occur through mutation and genetic drift (Slatkin 1987, Charlesworth 2009). Species that form as a result of these random processes often have different genotypes, but phenotypes that are largely the same (Stern and Orgogozo 2009).

The speciation account of *Chrosomus* is complex and includes a hypothesized history of several extinction events and geologic events that have led to either isolation or movement of fishes to neighboring drainages. As such, the six described species of *Chrosomus* in the subgenus *Chrosomus* are hypothesized to be divergent sister species, and the species are widely distributed across North America and are largely non-overlapping (Strange and Mayden 2009).

The exception is in eastern Tennessee where the distributions of four *Chrosomus* (*C. cumberlandensis, C. tennesseensis C. saylori,* and *C. erythrogaster*) are in close proximity (Strange and Mayden 2009). In this region, species identification can be difficult because there are few distinct morphological traits that are present in *Chrosomus,* particularly when fishes are not in breeding season and lateral banding is not distinct (Starnes and Jenkins 1988, Skelton 2001).

A lack of substantial morphological differences between the parapatric *Chrosomus* likely owns to similar environmental pressures over which natural selection act. The habitat correlates of *Chrosomus,* though poorly understand, are similar across species as all *Chrosomus* have been found to inhabit headwater streams with low velocity, moderate gradient, and cover. Thus, the environmental gradient over which *Chrosomus* are distributed is narrow, and the majority of phenotypic and genotypic dissimilarities in *Chrosomus* are likely caused by random gene flow, a process that happens slowly and usually results in less distinct adaptations than evolution through natural selection (Wiley and Mayden 1985).

Given homogeneous morphology of congeners near the Tennessee River basin and the proximity to the parapatric *Chrosomus*, the discovery of *Chrosomus* in the upper Clinch River watershed led to questioning about whether the fish was a new species or a subspecies of an already-described *Chrosomus*. The lack of an interrupted lower lateral band dismissed a possible close association with *C. oreas*, which is the *Chrosomus* most closely distributed to the upper Clinch River basin. The next closest congener to the upper Clinch River is *C. saylori* and, given the near identical morphology, it was assumed *C. saylori* was the closest ancestor to the new *Chrosomus* (Skelton 2007).

The only discernible external differences between *C. saylori* and the new *Chrosomus* is an upper lateral band that diminishes near the caudal fin in *C. saylori* and two yellow spots at the base of the caudal fin in *C.* sp. cf. *saylori* (Figure 2.1). This trait is only present when fish are in breeding colors, but was enough for Skelton (2007) to declare that the new *Chrosomus* was a new undescribed species, *C.* sp. cf. *saylori*.

A detailed comparison of *C. saylori* and *C.* sp. cf. *saylori* has not yet been completed. The two species are geographically isolated, and so one would predict that multiple generations of divergent evolution would have resulted in a speciation event. However, the minimal differences in external morphology that have thus far been identified would lead many taxonomists to question whether these are truly two individual species (Boster and Johnson 2009).

Also absent for *C*. sp. cf. *saylori* is a description on internal morphology and diet. Across all species, *Chrosomus* diet has been shown to consist of mostly invertebrates and algae (Settles and Hoyt 1976, Starnes and Starnes 1981, Starnes and Jenkins 1988). However, the digestive morphology, specifically length and the degree of coiling of the intestines, pharyngeal tooth formula and number of gill rakers are variable across species (Starnes and Starnes 1978, Skelton 2001). A description of these traits could help in taxonomic resolution of *C*. sp. cf. *saylori* as internal anatomy of cyprinids is often more variable than external traits, and is a key component to species identification (Jenkins and Burkhead 1994).

Given that *C*. *saylori* and *C*. sp. cf. *saylori* are state and federally listed, determining the degree of relatedness between the two species has the potential to influence future management plans (Kell et al. 2009). Should the two species be very closely related, one conservation effort that targets both species may be appropriate. However, should *C*. sp. cf. *saylori* be a distant congener to *C*. sp. cf. *saylori*, one all-encompassing management plan could prove to be a detriment to both species (Boersma et al. 2001) as it would fail to account for the variable environments and threats associated specifically to each species. In the long-term this could lead to poor adaptive management and failure of recovery plan implementation, and thus an overall decline in population size of both species (Clark and Harvey 2002, Lundquist et al. 2002).

The objective of this study was to formally describe the internal and external morphology of *C*. sp. cf. *saylori* so as to provide an anatomical description and basis of comparison to other *Chrosomus*. Specific attention was given to comparing the morphologies of *C*. *saylori* and *C*. sp. cf. *saylori* so as to infer the likelihood of speciation. I also investigated whether morphological traits exhibit sexual dimorphism or are influenced by ontogenetic development.

Methods

Fish Collection

Specimens used in this study were collected in 2009, 2011, and 2012. Fish were preserved in formalin for two weeks before being permanently stored in 70% ethyl alcohol. For all external measurements, I used 82 individuals. However, because examining internal features and performing an otolith age analysis required dissection and preservation of some C. *sp. cf. saylori* is desirable for museum collection, I only analyzed internal anatomy for 63 fish (hereafter referred to as the "subset fish"). I selected which fish to dissect using a systematic random sample, thus ensuring all size classes were represented in the subset.

Size-at-Age

I measured standard length and weight for each fish and used a length-frequency histogram to approximate age. Using methods described by Mills (1987), I also aged the subset fish using sagittal otoliths. The two methods were almost always congruent. When there was a discrepancy, I chose to use ototlith age as it has been shown to be more accurate (Campana 2001). I produced a length-weight relationship (LWR) using log transformed lengths and weights in the form log(weight)= b*log(length)+a. A b=3 is indicative of isometric growth and b≠3 of allometric growth (Verreycken et al. 2011). Therefore, to determine if *C.* sp. cf. *saylori* exhibit allometric or isometric growth, I examined the limits of the confidence interval for b from the LWR regression to determine if the slope was significantly different from 3.

External Morphology

Morphometric measurements were collected as described by Hubbs and Lager (1964) using digital calipers rounded to the nearest 0.1mm. In addition, as described by Skelton (2007), several additional morphometric measurements were taken in order to build a truss, which was necessary for analyzing external morphology using multivariate methods. Differences in morphometric characters between *C*. sp. cf. *saylori* and *C. saylori* were assessed using a sheared principal components analysis (SPCA; Humphries et al. 1981) in SAS with a program written by D. Swofford (Statistical Analysis Systems Institute, Inc., Cary, NC). Ideally, this analysis would have been completed for only individuals of the same sex and age; however, I did not have that information for *C. saylori*. Therefore, to minimize variance attributed to differences in age, I only used individuals that were greater than 45 mm standard length, which was a proxy for adults. This resulted in *n*=42 for *C*. sp. cf. *saylori* and *n*=29 for *C. saylori*.

I was also interested in whether there were differences in morphology due to sex or ontogenetic development. Therefore, I ran a SPCA to tests for morphological differences in males and females of each age class. Young-of-year males were excluded from this analysis due to low sample size.

For all SPCAs, I used a MANOVA to test for a significant group effect. For those tests with a significant group effect, I used a one-way ANOVA to determine which morphometric traits were significantly different between which groups. For all tests, significance was held at α=0.0001, which is equivalent to a Bonferonni correction. The corrected level for α was determined by dividing the maximum number of multiple comparisons (363) by the maximum desired Type-I error rate of 0.05.

I counted the number of rays of the anal, caudal, dorsal, pectoral, and pelvic fin rays as described by Jenkins and Burkhead (1994). Horizontal scale rows, predorsal scales, scales above and below the lateral line, midlateral scales, and lateral line scales were also counted. For the scale and ray count formulae, the value found in 80% or more individuals is bracketed by the minimum and maximum values in parentheses. For example, a formula of (9)10-12 would indicate that at least 80% of fish have a count of 10-12, but the absolute minimum encountered was 9.

Diet and Internal Morphology

I determined the diet of *C*. sp. cf. *saylori* by observing *in-situ* feeding behavior and dissecting stomachs from the subset fish. Field observations were conducted from 19 May-2 June 2012 at Big Lick Creek in Tazewell County, Virginia. During each day, I would observe for at least two hours, the behavior of *C*. sp. cf. *saylori* to determine prey preference and feeding mode.

For each fish I dissected, I removed the entire digestive tract from anus to stomach and stretched it to remove coils. Using digital calipers, I measured the length of the intestine to the neared 0.01 mm. Stomach contents were often partially or completely digested, and so contents were identified as sand, plant, or macroinvertebrate. When possible, the order of each macroinvertebrate was identified. For each fish, I counted left and right pharyngeal teeth and the number of gill rakers and filaments on the first gill arch. Pharyngeal tooth formula was written to show the number of teeth on the left arch first, a hyphen, and then the number of teeth on the right arch. On a single side, minor row teeth were separated by major row teeth by a comma. For example, a pharyngeal tooth formula of 2,5-5,2 would indicate 2 minor and 5 major teeth on both arches (Jenkins and Burkhead 1994)

Results

Size at Age

There were four young-of-year (YOY), 33 year-1, and 26 year-2 fish in my sample. Maximum size of YOY fish was 37.97 mm, year-1 fish 50.95 mm, and year-2 fish 61.4 mm (Figure 2.2). Fish had a steady growth rate of approximately 1 mm per month (y=1.01x+29.63, Figure 2.3). The LWR was log(W)=3.4106*log(L)-5.4864, and the slope was found to be significantly greater than 3 (lower 95% confidence interval= 3.26), thereby indicating positive allometric growth.

Internal and External Anatomy

Meristic features were similar to those of other *Chrosomus* with (7)8-9 anal fin rays, (15)17(19) branching caudal fin rays, (7)8-9 dorsal fin rays, (12)14(15) pectoral fin rays, and (7)8(9) pelvic fin rays (Appendix E). The lateral line was incomplete with 0-69 scales. There were 52-86 midlateral scales, 8-23 scales above the lateral line, 7-21 scales below the lateral line, 20-36 horizontal scale rows, 24-64 circumferential scales, 22-34 caudal peduncle scales, and 25-49 predorsal scales (Appendix F).

There was a significant separation between *C.* sp. cf. *saylori* and *C. saylori* in the SPCA (by MANOVA, $p<0.001$, Figure 2.3). This separation owns particularly to axis 2, which was negatively correlated to length of the anal fin base and positively correlated to head depth (Table 2.1). There were several morphometric features that were significantly different between *C.* sp. cf. *saylori* and *C. saylori* (Table 2.1).

A separate SPCA showed that *C.* sp. cf. *saylori* morphology was different between males and females, and there were ontogenetic shifts in morphology (by MANOVA, $p<0.006$, Figure 2.4). The second SPCA axis was most positively correlated to length of the anal fin base and negatively correlated to body width at anal origin. The third SPCA axis was most positively correlated to interorbital distance and most negatively correlated to the distance between the origin of the pelvic fin to the origin of the pectoral fin. Multiple comparisons revealed numerous significant group differences, all attributable to fluctuating fin position (Table 2.2).

Chrosomus sp. cf. *saylori* had 8-16 gill rakers and 27-47 gill filaments on the first gill arch. The most common pharyngeal tooth formula was 0,5-4,0, which was present in 52% of fish. The next most common tooth formula was present in 32% of fish, and was 0,4-4,0 (Appendix G). The basioccipital plate was largely rounded.

Diet Analysis

Average intestine length was 0.63 times the standard length (SE=0.019). The intestine was S-shaped, and lacked extensive coiling. Macroinvertebrates were present in 56% of dissected stomachs were the predominate material in each stomach. Though the macroinvertebrates were largely decomposed, I was able to identify members of the orders Megaloptera, Coleoptera, Diptera, Ixodida, and Hymenoptera. Other items present in the digestive track included plant material and sand. These gut contents corroborate field observations as *C.* sp. cf. *saylori* were seen mostly drift feeding, but would occasionally feed on submerged algae attached to branches beneath undercut banks.

Conclusions

Here, I show that *C*. sp. cf. *saylori* are morphologically distinct from *C. saylori*, and that *C*. sp. cf. *saylori* morphology is variable across ages and sexes. The maximum age in my sample was two years, and the length-weight relationship showed positive allometric growth. Macroinverebrates were the most prevalent food item in dissected stomachs, and the morphology of the digestive system was consistent with a diet low in plant material.

Growth patterns across species and/or populations can be compared using the slope of multiple LWRs. Given that length-weight parameters are affected by food availability, spawning activity, local habitat availability, and stress, comparing length-weight relationships can also be indicative of the relative condition of independent populations. Length-weight relationships are poorly studied in *Chrosomus*, and the only relationship that has thus far been established is for *C. erythrogaster* by Settles and Hoyt (1976). The slope of the length-weight relationship for 864 *C.*

erythrogaster in Kentucky was found to be 3.033. The slope for 82 *C*. sp. cf. *saylori* was 3.4106, which is significantly higher than the slope for *C. erythrogaster*. Thus, initially, it appears as though *C*. sp. cf. *saylori* have an allometric growth pattern whereas *C. erythrogaster* is more isometric. However, this conclusion could be a product of unequal sample sizes and should be used with caution.

Chrosomus size at age has been well documented for *C. tennesseensis* (Hamed et al. 2008), *C. erythrogaster* (Settles and Hoyt 1976, Settles and Hoyt 1978), and *C. cumberlandensis* (Starnes and Starnes 1981). All of these studies, which aged fish using length-frequency histograms and scales, found that young-of-year grow to approximately 30 mm before winter, reach 50 mm at the end of the first year, and reach a maximum length of approximately 60 mm. The only discrepancy among those studies is that (Hamed et al. 2008) found three years to be the maximum age, whereas the maximum age was two years for the studies by Settles and Hoyt (1976, 1978) and Starnes and Starnes (1981).

This is the first study to age *Chrosomus* using otoliths. Otolith age determination is an uncommon method for small North American cyprinids (Simmons and Beckman 2012), but is rapidly becoming the preferred aging method, particularly for studies with limited sample size (Campana 2001) where length-frequency analysis often fail. Using otoliths, I found a size-at-age pattern consistent to those reported for other *Chrosomus*, and with a maximum age of two years.

It is impossible to determine if the absolute maximum age of *C*. sp. cf. *saylori* is two years. However, given that there were relatively few two-year-old fish in fall samples of *C*. sp. cf. *saylori* and *C. erythrogaster* (Settles and Hoyt 1976), and fewer than 1% of fish studied by Hamed et al. (2008) reached three years old, it is likely that there is near 100% mortality of two-year-olds following summer spawning. This conclusion is congruent with all reported

Chrosomus life history patterns, including that for *C. cumberlandensis*, as Hamed et al. (2008) noted high mortality between September and December.

While the cause of mortality in *Chrosomus* is unknown, there are several plausible mechanisms. The first is size-selective predation for larger, more colorful fish. Hamed et al. (2008) hypothesized that the absence of piscivores in streams in their study likely resulted in older fish, and that streams with heavier predation pressures would likely see a decrease in the number of older fish. The second possibility is leaf decomposition in the fall. During this time period, adult fish are already in poor condition due to recent spawning (Settles and Hoyt 1976), and the decrease in dissolved oxygen due to decomposition of allochthonous materials could be a source of stress-induced mortality (Settles and Hoyt 1978, Johnson et al. 2009). Another possibility is that a single spawning event requires a high energetic demand, and that there is rapid senescence following the release of gametes.

Ontogenetic shifts in morphology have been documented for several cyprinids. For *C*. sp. cf. *saylori*, morphological distinctions were not due to fin size or head shape, but only to the location of fins relative to one another. This conclusion likely owns to increased body depth as fishes experience positive allometric growth and fins located ventrally move further away from fins located on the dorsal and lateral axes of the body. A similar conclusion was documented by Simonovíc et al. (1999) for the closely related *Phoxinus phoxinus* (European minnow). Simonovíc et al. (1999) attributed their findings to ontogenetic shifts in habitat and diet, which subsequently altered fish morphology.

Like other investigators (Settles and Hoyt 1978, Starnes and Jenkins 1988, Skelton 2001), I also found sexually dimorphic morphology, particularly for one- and two-year-old fish. Interestingly, the dimorphism wasn't attributable to difference in fin length, a feature that has been shown to be a reliable indicator of sex in *Chrosomus*, but rather fin position. The dimorphism observed in *C*. sp. cf. *saylori* can be attributed to differences in allometric growth rates, which generally fluctuate as fishes reach sexual maturity (Settles and Hoyt 1976), thus making body proportions more variable between sexes. Furthermore, the majority of fish in this study were collected during spawning season, during which time the abdomens of females grow larger to support gonad development (Hood and Heins 2000). Thought the SPCA controls for standard length, it does not control for body weight and depth, which was likely the cause of different fin position in females.

The absence of a complete lateral line and the external meristic count data documented here are traits that are relatively homogenous across *Chrosomus* species. Though many of the scale counts provided in this analysis have a range much larger than that reported for other *Chrosomus*, other studies did not include YOY fish in their analyses. Fish develop more lateral scales and horizontal scales rows through development, and the inclusion of YOY in my analysis increased the variance in my meristic estimates.

Chrosomus diet has been shown to consist of invertebrates, plants, and sand by every investigator. The difference among previous *Chrosomus* diet descriptions and the diet study of *C*. sp. cf. *saylori* presented here is in functional anatomy. Other *Chrosomus* have been reported to have winged basioccipital plates and more pharyngeal teeth than *C*. sp. cf. *saylori* (Starnes and Starnes 1978). The intestine length was also significantly smaller in *C*. sp. cf. *saylori* (0.63 x standard length) than *C. cumberlandensis* (3.6 x standard length; Starnes and Starnes 1978).

Because plant material is harder to digest than insects, herbivorous fish typically have larger intestines with more pharyngeal teeth. The absence of these traits in *C*. sp. cf. *saylori* may reflect the trophic niche of the species, and is an evolutionary adaptation that makes *C*. sp. cf.

saylori more suited for a diet of primarily macroinvertebrates. Whereas *C. eos*, *C. tennesseensis*, *C. oreas*, and *C. cumberlandensis* have been shown to have a diet high in plant matter and algae (Cochran et al. 1988, Starnes and Jenkins 1988), the predominant food item in the intestines of *C.* sp. cf. *saylori* was macroinvertebrates. And, because there is a seasonal shift in diet (Starnes and Starnes 1981), the percent of macroinvertebrates reported here could be less than that for the rest of the year. It is also likely that *C.* sp. cf. *saylori* were ingesting microscopic diatoms (Settles and Hoyt 1979, Starnes and Starnes 1981), particularly for smaller fish, which went undetected in my analysis.

The most notable SPCA result was that showing a distinct morphological separation between *C.* sp. cf. *saylori* and *C. saylori*. Previous studies have found success in using SPCA for discriminating between closely-related congeners in a variety of fish species (Stauffer and Van Snik 1997, Hopkins et al. 2009, Schliewen et al. 2001). Given that the speciation of *C.* sp. cf. *saylori* is in doubt and has never been formally tested, this study provides initial evidence that *C.* sp. cf. *saylori* is not just a subspecies of *C. saylori*, but it a separate species. However, despite differences in the external morphology of *C.* sp. cf. *saylori* and *C. saylori,* the two congeners are still more similar to one another than any of the *Chrosomus*, especially in regard to diet, pigmentation, and internal anatomy. Thus, it is likely that the two are sister species that share an extinct ancestor and geographic isolation has led to a speciation event which has resulted in two species with similar, yet distinct morphologies.

Though this study showed a clear morphological separation between *C.* sp. cf. *saylori* and *C. saylori*, in the future genetic differentiation of the two congeners will need to be completed to definitively determine speciation. Confirming speciation will influence the development of separate, independent management plans for both *C.* sp. cf. *saylori* and *C. saylori,* which is the preferable implementation strategy give that these two species have completely disjoint distributions and are affected by different environmental variables (Gibbs and Currie 2012). Until a genetic analysis is completed, it should be assumed that *C.* sp. cf. *saylori* are independent of *C. saylori*, and protection of streams identified as occupied in this study and by Skelton (2007) should be pursued.

Table 2.1: Means and ranges of morphometric measurements (reported as thousandths of standard length) for *Chrosomus* sp. cf. *saylori* and for *C. saylori*. Measurements that were significantly different (p<0.001) are shown in bold. Measurements for *C. saylori* were provided by C. Skelton (personal communication).

	Chrosomus sp. cf. *saylori* (*n* =82)		*Chrosomus saylori* (*n* =41)	
Measurement	Mean	Range	Mean	Range
Standard length	45.4	29-61	45.7	40-55
Pectoral fin length	**177.1**	**132-230**	**206.0**	**185-225**
Pelvic fin length	**216.0**	**124-259**	**168.0**	**153-180**
Dorsal fin length	**213.6**	**171-241**	**230.0**	**216-259**
Anal fin length	196.5	154-229	203.0	186-217
Interorbital distance	**94.7**	**63-120**	**90.0**	**82-100**
Head width	137.2	117-161	151.0	132-171
Body width at dorsal fin origin	130.0	72-205	138.0	120-165
Body width at anal fin origin	96.3	49-143	107.0	89-133
Snout length	**70.5**	**51-92**	**78.0**	**69-88**
Head length	259.3	229-304	258.0	245-289
Snout–occiput	211.0	187-236	211.0	192-271
Occiput–dorsal origin	336.5	257-373	350.0	314-377
Dorsal fin base	103.0	72-138	106.0	93-120
Dorsal end–caudal base	380.3	342-409	375.0	358-395
Caudal peduncle depth	**102.7**	**81-122**	**126.0**	**118-138**
Anal end–caudal base	**235.6**	**164-309**	**249.0**	**233-266**
Anal fin base	**115.6**	**88-168**	**107.0**	**97-118**
Anal origin–pelvic origin	178.4	116-214	174.0	155-192
Pectoral origin–pelvic origin	145.8	117-194	218.0	197-239
Snout–isthmus	168.7	90-206	172.0	154-183
Gape width	59.9	43-78	67.0	54-74
Upper jaw length	**69.1**	**52-90**	**82.0**	**70-93**
Head depth	**146.2**	**79-172**	**174.0**	**163-189**
Occiput–pectoral origin	163.7	129-206	175.0	155-189
Dorsal origin–pectoral origin	322.3	231-395	341.0	324-367
Dorsal origin–pelvic origin	213.2	170-251	230.0	208-256
Dorsal origin–anal origin	**213.6**	**163-258**	**233.0**	**216-248**
Dorsal end–pelvic origin	235.2	184-292	248.0	220-269
Dorsal end–anal origin	**154.6**	**119-183**	**173.0**	**152-190**
Dorsal end–anal end	187.3	147-226	189.0	169-205
Orbit diameter	73.8	58-114	71.0	62-87
Pectoral origin–isthmus	**123.8**	**93-180**	**132.0**	**116-146**
Pectoral origin–pectoral origin	**136.5**	**99-176**	**167.0**	**149-186**

Table 2.2: Differences (mm) in morphological traits used in SPCA analysis for young-of-year (YOY), Age 1, and Age 2 male and female *Chrosomus* sp. cf. *saylori*. The first two columns compare males, middle six columns compares females to males, and the last three columns compare females. In all cases, the second listed age class was subtracted from the first listed age class to get the difference. YOY males were excluded from this analysis due to slow sample size. Significant differences, as determined during multiple comparisons, are shown in bold.

	Males		Females-Males						Females		
Feature	Age 2-YOY	Age 2-Age 1	YOY-Age 1	Age 1-Age 1	Age 2-Age 1	YOY-Age 2	Age 1-Age 2	Age 2-Age 2	Age 1-YOY	Age 2-YOY	Age 2-Age 1
Pectoral Fin Length	4.8	1.6	-2.9	-0.9	1.3	-4.5	-2.5	-0.3	2.0	4.2	2.2
Pelvic Fin Length	3.5	1.8	-1.7	-0.5	1.2	-3.5	-2.3	-0.7	1.2	2.8	1.6
Dorsal Fin Length	5.3	2.4	-2.4	-0.3	2.0	-4.8	-2.8	-0.4	2.1	4.4	2.3
Anal Fin Length	4.5	2.2	-1.7	-0.4	2.4	-3.9	-2.6	0.2	1.3	4.1	2.8
Interorbital Distance	2.2	1.2	-0.6	0.1	1.5	-1.7	-1.1	0.3	0.6	2.0	1.4
Head Width	3.6	1.6	-1.6	-0.3	1.9	-3.2	-1.9	0.4	1.3	3.5	2.2
Body Width at Dorsal Origin	**5.4**	2.8	-2.0	-0.3	2.6	**-4.8**	**-3.1**	-0.2	1.7	**4.7**	**3.0**
Body Width at Anal Origin	3.5	1.9	-1.2	0.0	**2.0**	-3.2	-2.0	0.1	1.2	3.2	**2.1**
Head Length	7.1	3.2	-3.0	-0.3	3.2	-6.2	-3.5	-0.1	2.7	6.2	3.4
Snout-Occiput	4.3	2.0	-2.3	-0.2	2.3	-4.3	-2.2	0.3	2.1	4.6	2.5
Anal Base End-Hypural	6.1	2.3	-2.7	-0.7	2.1	-5.0	-3.0	-0.2	2.0	4.8	2.8
Anal Fin Base	2.9	1.4	-1.6	-0.3	1.4	-2.9	-1.6	0.1	1.3	3.0	1.7
Anal origin-Pelvic Origin	5.0	**1.8**	-3.3	-0.4	2.0	**-5.1**	-2.2	0.2	**3.0**	**5.3**	2.4
Caudal Peduncle Depth	3.3	1.8	**-1.5**	-0.1	1.5	**-3.3**	**-2.0**	-0.3	1.4	**3.1**	1.7
Dorsal Base End-Anal Base End	4.7	2.5	-2.6	-0.7	2.6	-5.1	-3.2	0.1	1.9	5.2	**3.3**
Dorsal Base End-Anal Origin	4.5	2.6	-2.6	-0.4	2.4	**-5.2**	**-3.0**	-0.2	**2.3**	**5.0**	**2.7**
Dorsal Base End-Hypural	9.5	5.0	**-3.5**	-0.4	4.9	-8.5	-5.4	-0.1	3.1	8.4	5.3
Dorsal Base End-Pelvic Origin	6.9	3.4	-3.7	-0.3	3.4	**-7.0**	-3.7	0.0	**3.4**	**7.0**	3.7
Dorsal Fin Base	2.9	1.1	**-1.8**	-0.4	1.2	-2.9	-1.6	0.1	1.3	3.0	1.7
Dorsal Origin-Anal Origin	6.0	**3.8**	-3.0	-0.3	3.4	**-6.8**	**-4.1**	-0.4	2.7	**6.4**	**3.7**
Dorsal Origin-Pectoral Origin	8.3	4.6	-3.6	-0.5	5.0	-8.3	-5.1	0.4	3.2	8.6	5.5
Dorsal Origin-Pelvic Origin	5.4	3.1	-2.7	-0.1	3.2	-5.7	-3.2	0.1	2.5	5.9	3.3
Gape Width	1.4	0.7	-1.0	-0.1	0.7	-1.8	-0.9	0.0	0.9	1.7	0.8
Head Depth	3.2	1.6	-1.4	0.3	2.2	-3.0	-1.3	0.5	1.7	3.5	1.9
Occiput-Dorsal Origin	8.4	4.3	-3.6	0.0	**5.6**	-7.9	-4.3	1.3	3.6	9.2	5.6
Occiput-Pectoral Origin	5.6	2.8	-1.6	-0.5	2.3	-4.4	**-3.3**	-0.5	1.1	3.8	2.7
Orbit Diameter	1.2	0.5	-0.6	0.0	0.7	-1.1	**-0.4**	0.3	0.7	1.4	**0.7**
Pectoral Origin-Isthmus	3.7	1.6	-1.5	-0.5	1.4	-3.1	-2.1	-0.2	1.0	2.9	1.9
Pectoral Origin-Pectoral Origin	**5.7**	**3.3**	-1.9	-0.5	2.4	**-5.2**	**-3.8**	-0.8	1.4	**4.4**	**3.0**
Pelvic Origin-Pectoral Origin	5.6	2.7	-2.7	-0.3	3.6	-5.4	-2.9	0.9	2.5	6.3	3.8
Snout Length	2.2	1.0	-1.2	-0.4	0.9	-2.1	-1.3	-0.1	0.8	2.1	1.3
Snout-Isthmus	4.0	1.9	-1.8	-0.2	1.5	-3.7	-2.1	-0.3	1.6	3.3	1.8
Upper Jaw Length	1.5	0.9	-0.7	0.0	1.1	-1.6	-0.9	0.2	0.6	1.8	1.1

Figure 2.1: Photograhps of *Chrosomus saylori* (A) and *C*. sp. cf. *saylori* (B). The only discernible difference in external morphology between the two congeners is in the upper lateral band, which ends before the caudal fin in *C*. *saylori* and is complete in *C*. sp. cf. *saylori*, and two yellow spots at the base of the caudal fin in *C*. sp. cf. *saylori*. Photo of *C*. *saylori* by D. Neely and *C*. sp. cf. *saylori* by C. Skelton.

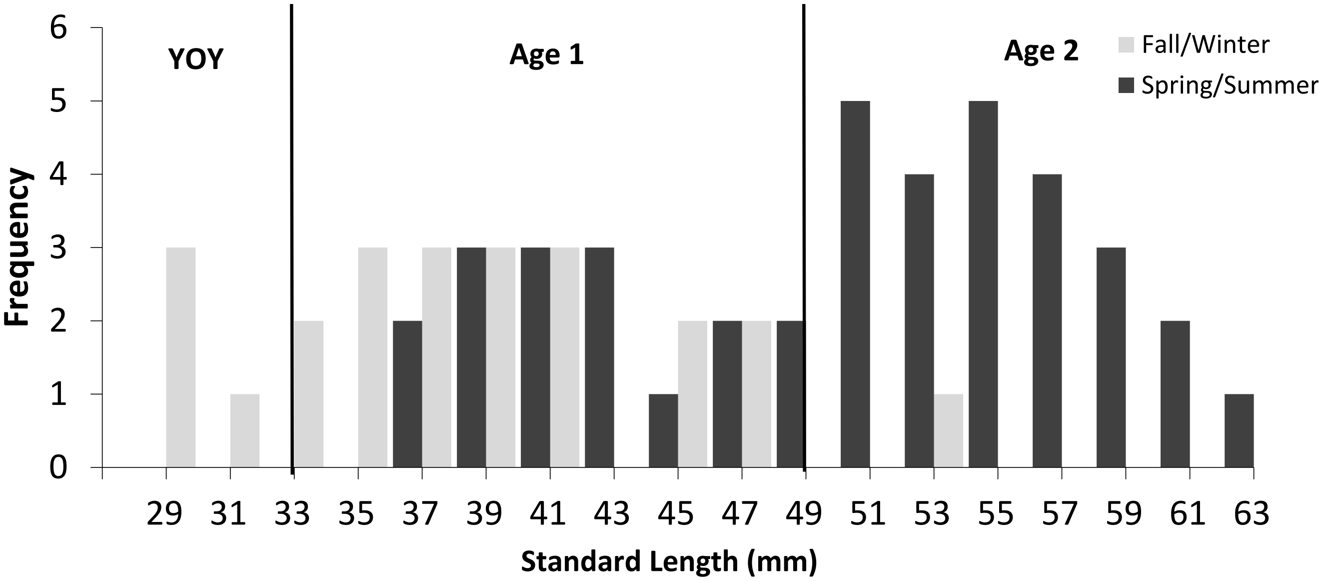


Figure 2.2: Length frequency histogram for *Chrosomus* sp. cf. *saylori* captured in fall/winter (grey, n=23) and spring/summer (black, n=40). Ages of fish, as determined by otolith analysis are also indicated.

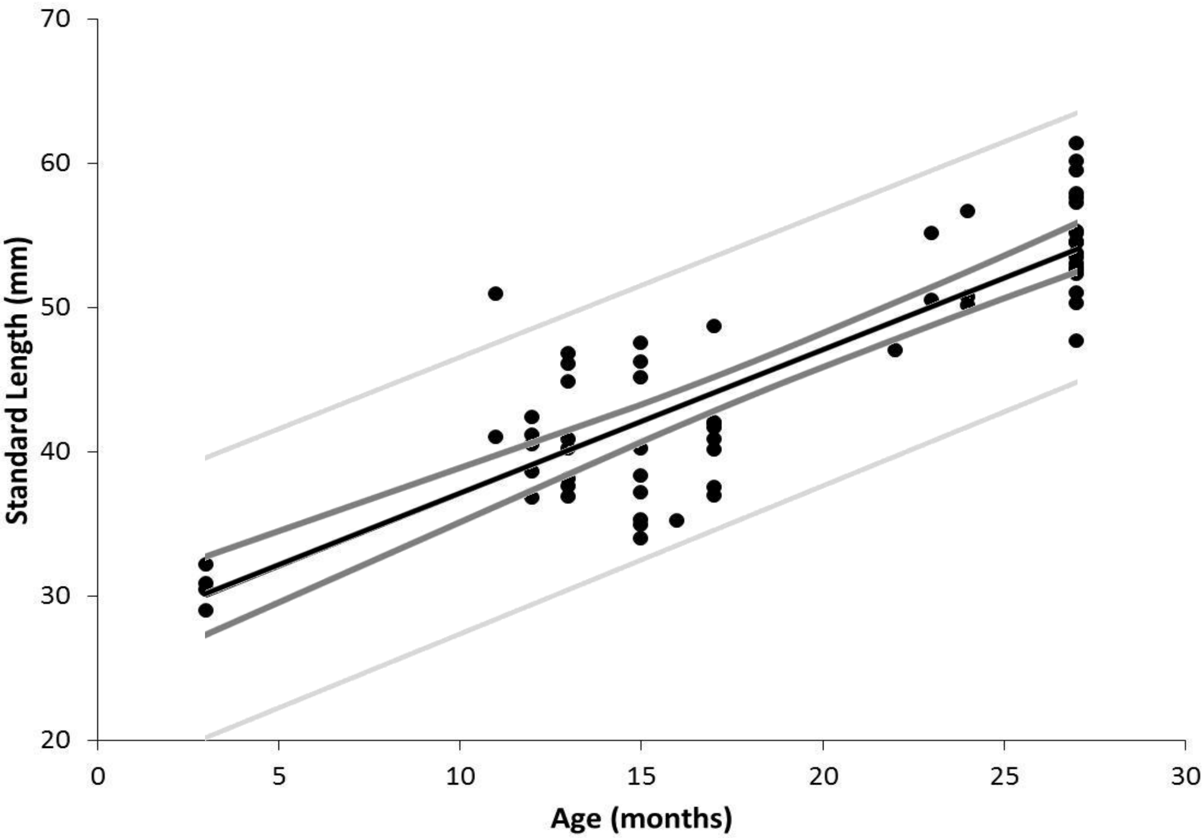


Figure 2.3: Regression of standard length (mm) on age (months) for 63 *C*. sp. cf. *saylori*. Inner black line is the fitted line with equation y=1.01x+29.63 and r=0.75. Middle dark grey bars are 95% confidence intervals and outer light grey bars 95% predictive intervals.

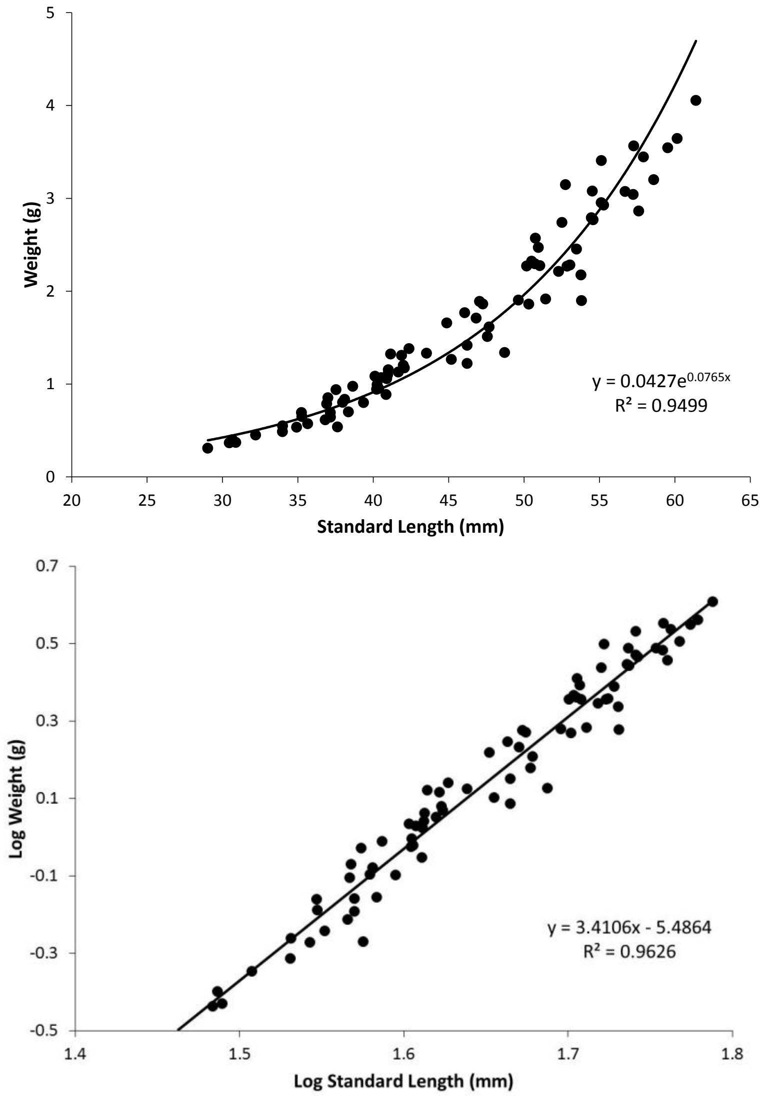


Figure 2.4: Standard length and weight (A) and log length and weight (B) for *Chrosomus* sp. cf. *saylori*. The resulting fitted lines from regressions are shown. The slope of the regression of the log transformed data is significantly larger than 3 ($p<0.001$).

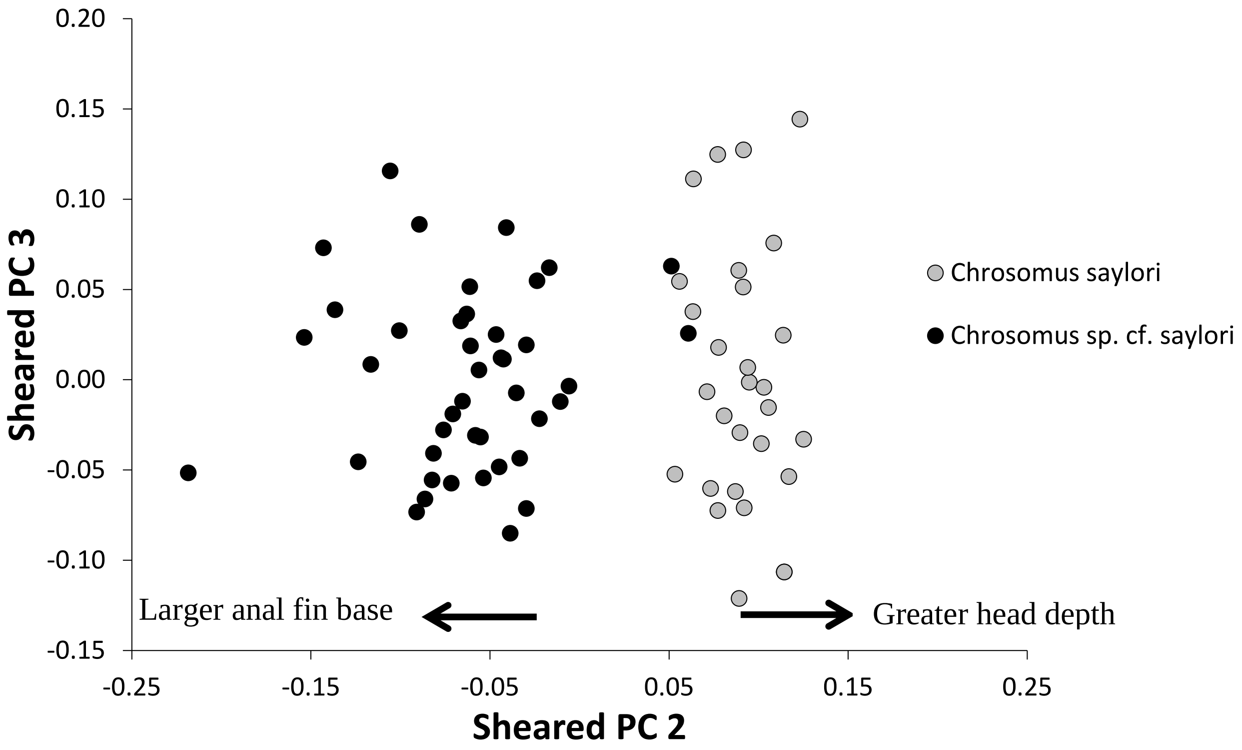


Figure 2.5: Sheared PCA ordination of *Chrosomus* sp. cf. *saylori* (black, n=42) and *C. saylori* (grey, n=29). The significant (by MANOVA, $p<0.0001$) separation owns to SPCA axes 2, which was positively correlated to head depth and negative correlated to length of the anal fin base.

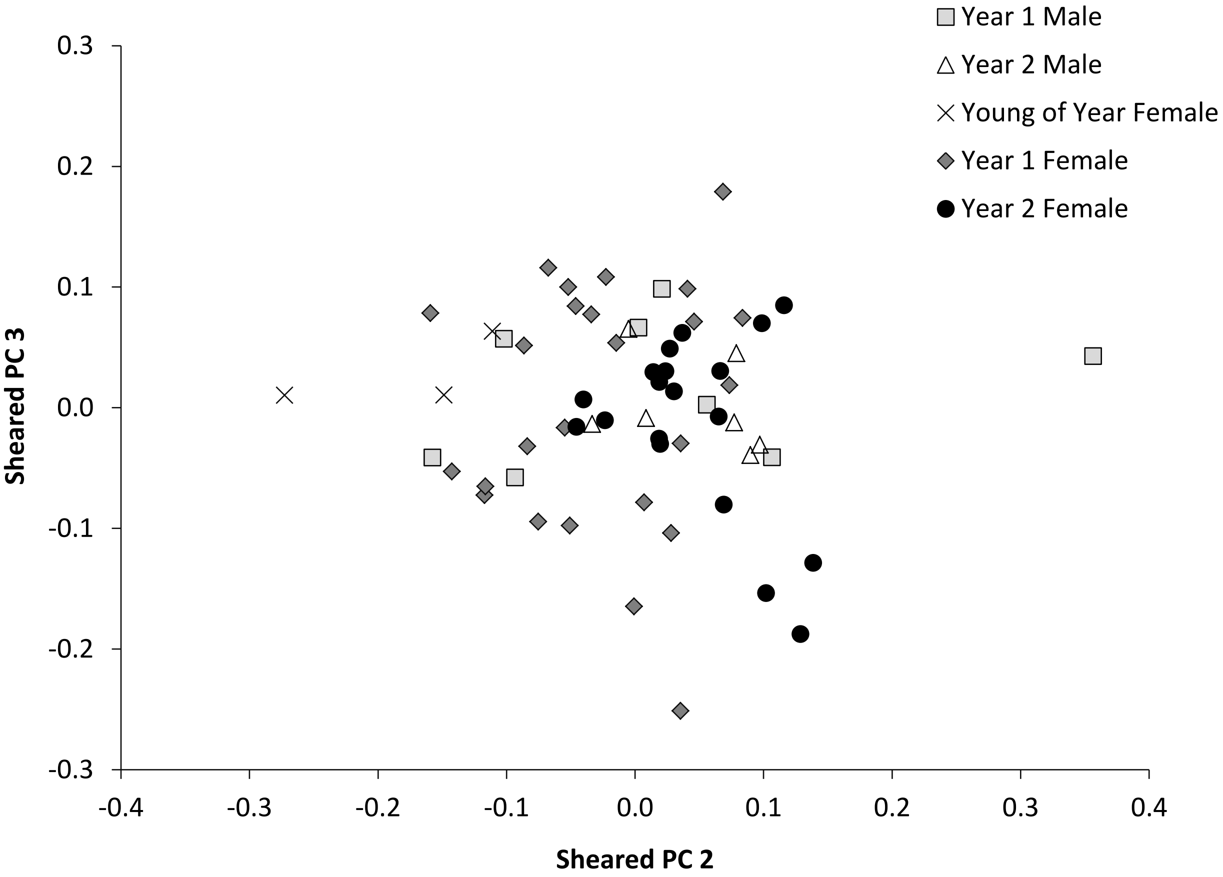


Figure 2.6: Sheared PCA of year-1 males (grey squares), year-2 males (white triangles), young-of-year females (black crosses), year-2 females (grey diamonds), and year-2 females (black circles).

Chapter Three: Reproductive biology of *Chrosomus* sp. cf. *saylori*

Abstract

Chrosomus minnows are typically described as nest associates that display strong sexual dimorphism and spawn from April-July. The reproductive biology of *C*. sp. cf. *saylori* is presently unknown. It is likely that *C*. sp. cf. *saylori* reproduction is similar to its closely-related congeners; however, identification of specific dates of spawning and sexual morphology is critical for determining periods of high vulnerability and population fitness. Here, I use *in-situ* observations of reproduction to describe spawning behavior and laboratory analysis to determine age of reproductive maturity, number of mature ova per female, and gonadosomatic index. I determined that *C*. sp. cf. *saylori* likely spawn from May-July and have a nest association with *Campostoma anomalum* and *Semotilus atromaculatus*. Females did not reach reproductive maturity until two years, and had a lower number of mature eggs per females and gonadosomatic index in comparison to other *Chrosomus*. There was a 3:1 female-biased sex ratio. There was no sexual dimorphism in pigmentation, but males did have more rounded pectoral fins and pearl organs during spawning. Though the fecundity of individuals is lower than typically reported for *Chrosomus*, the skewed sex ratio could be increasing population fecundity, thereby maintaining population persistence. However, conservation efforts for *C*. sp. cf. *saylori* should still focus on increasing reproductive success. Efforts should be made to decrease overland erosion and watershed disturbance year-round, but particularly during spawning season so as to decrease the amount of silt in streams. Due to the nest association, any recovery plans for *C*. sp. cf. *saylori* will also need to consider protection of *Campostoma anomalum*.

Introduction

Fish in the family Cyprinidae have highly versatile, and sometimes plastic, spawning modes that include variable degrees of parental care (Mayden and Simons 2002). The most primitive spawning mode, and that used by over 60% of cyprinids (Johnston 1999), is that of broadcast spawning where eggs and sperm are released onto open substrate, and no parental care is provided (McLennan 1994). More derived spawning modes including pit- and mound-building, which involve fish reorganizing the stream bottom into a desired morphology, and subsequently releasing gametes into the structure (Sabaj et al. 2000). Closely-related are crevice-spawners, which only spawn in the large gaps between substrate. Some pit- and mound-building fishes also provide parental care by covering fertilized eggs with rock, but even more parental care is provided by egg-clumping and egg-clustering fishes, which release eggs into rock cavities and then continue to guard eggs until hatching (Johnston 1999). This list is not exhaustive, as there are other, more specialized spawning modes that are species-specific.

Though Cyprinidae is the largest family of fishes in North America, studies of their spawning behavior are infrequent, and less than half of all cyprinids have a known spawning mode (Johnston 1999). This is problematic because a description of a species' reproductive biology is required for complete understanding of life history traits. Without knowing a species' full life history, it is difficult to hypothesize taxonomic relationships (McLennan 1994), determine speciation (McElroy and Kornfield 1990), and develop accurate sampling protocols (Pope and Willis 1996).

Understanding reproductive mode is also important for identifying sources of imperilment because, as Johnston (1999) concludes, the probability of imperilment is higher for certain spawning modes. At the time of publication, Johnston (1999) noted that 46 North

American cyprinids were listed as endangered, threatened, or of special concern. For many of those fish, the spawning mode is currently unknown. But, none of the imperiled fish have a known mound-building or egg-clustering strategy. All of the remaining reproductive modes (pit-building, crevice-spawning, broadcast, egg-clumping, and pit-ridge-building) are among the strategies used by imperiled cyprinid fishes. And, fishes with a mound- or pit-building or egg-clustering strategy are among the most prolific and populous fishes in the world.

The decline of a species is not likely due to the reproductive mode itself, but rather the decline in critical habitat requirements associated with a particular reproductive mode (Johnston 1999). Heavy siltation can smother clean gravel necessary for broadcast spawners and can fill crevices commonly used by crevice-spawners (Kemp et al. 2011). Multiple road crossings can decrease fish movement, thus preventing broadcast spawners from congregating in larger numbers (Jager 2000). Dams and improper stormwater management can alter hydrology and streamflow, which can inhibit egg fertilization in broadcast spawners (Johnston 1999).

Conversely, those fishes that build mounds are less affected by the aforementioned habitat changes because they can usually construct suitable spawning habitat, even in the midst of high degrees of disturbance (Sutherland et al. 2002). Furthermore, fishes with parental care (i.e. egg- clustering) can ward off predators. This is particularly important in disturbed ecosystems, as habitat degradation and anthropogenic translocations can increase establishment and subsequent predation of nonnative species (Marvier et al. 2004).

Even in degraded habitats, many fishes without complex reproductive modes can still spawn through nest association. With a nest association, a pit or mound is constructed by one species (the host), and then used by one or more additional species for spawning (the associates). Historically, this was considered an act of mutualism as the associates receive critical spawning habitat and the hosts receive reduced egg predation via the dilution effect (Johnston 1994). However, heavy predation of eggs from associates and builders has led some to question whether this behavior is actually commensalistic or parasitic (Phillips et al. 2011). And, because many species exhibit plastic nest association, it is hypothesized that nest associations are sometimes an evolutionary adaptation that develops in response to a decline in suitable habitat (Kerns and Bonneau 2002).

Identifying spawning mode and the presence of nest association is critical when developing restoration plans. Spawning mode can offer insights into habitat features that need to be preserved and/or added to the environment (Albanese 2000). Fish that are known nest associates will require restoration plans that are ecosystem-focused as their recovery could be dependent on the continued existence of the pit or mound builder (Johnston 1999, Pendleton et al. 2012). Furthermore, identification of time and duration of spawning provides time windows where watershed disturbances are most likely to impact fishes (Niemi et al.1990).

Due to their vibrant colors that appear during spawning season, the reproductive behavior of *Chrosomus* is well-documented. *Chrosomus* are broadcast spawners that reproduce from May-July. Most species are nest associates with *Campostoma* spp., *Nocomis* spp., or *Semotilus atromaculatus* (Hamed et al. 2008), but this behavior appears to be plastic and not used in the absence of moderate to severe siltation (Smith 1908, Starnes and Starnes 1981). There is a distinct sexual dimorphism with males achieving brighter, more vibrant colors than females, and having longer, more rounded pectoral fins (Starnes and Starnes 1978, Settles and Hoyt 1978, Starnes and Jenkins 1988). Males also develop pearl organs, a type of breeding tubercle that appears along the entire dorsal and lateral axis of the body (Smith 1908, Skelton 2001).

Given that *Chrosomus* are broadcast spawners and are distributed in areas of heavy watershed disturbance, the placement of over half of *Chrosomus* species on lists of threatened, endangered, and imperiled fishes is unsurprising (Johnson 1999). This includes the undescribed species, *C*. sp. cf. *saylori*, which presently does not have a well-documented spawning mode. While it is likely that *C*. sp. cf. *saylori* have a reproductive behavior similar to other *Chrosomus*, confirming nest association and identifying the exact months (and spawning temperatures) of breeding are critical for the development of a conservation plan. Furthermore, describing the reproductive morphology of *C*. sp. cf. *saylori* could give insights into individual and population fecundity, which will help in determining the viability of populations.

The objective of this study was to describe the reproductive biology of *C*. sp. cf. *saylori*. This included determining the timing and mode of reproduction through field observations, and quantifying the reproductive morphology. Comparisons were made between *C*. sp. cf. *saylori* and other *Chrosomus* so as to make inferences about species-specific traits and fecundity of *C*. sp. cf. *saylori* relative to congeners.

Methods

Field Observations

On 19 May 2012, I observed approximately 20 *C*. sp. cf. *saylori* in full breeding colors in Big Lick Creek in Tazewell County, Virginia. Fish were observed in a shallow run, approximately 4 m upstream of a pool. For the follow three weeks, I visited this location daily and used binoculars and underwater cameras to observe fish behavior in the run, and in the 25 m upstream and downstream of this location.

I attempted to monitor *C*. sp. cf. *saylori* at Mudlick Creek in Tazewell, Virginia in a similar fashion; however, lack of land access permission made detailed observation of that stream impossible. I did observe this location once a week, from which I can infer an approximate spawning time. Stream temperatures were measured with continuous data loggers that recorded temperature every hour. Precipitation, moon phase, and air temperature were obtained from a local meteorology station.

Sexual Morphology

I used 63 fish ranging in size from 29-65 mm in this study. Fish were captured from 2009-2012, and in April-November using backpack electrofishing and seining and were preserved in formalin for two weeks before being transferred to 70% ethyl alcohol. Before preservation, detailed descriptions of external morphology including color, fin position and size, and presence of tubercles were noted in the field. Because pectoral fin length has consistently been shown to be dimorphic between sexes, I compared this trait between males and females using an analysis of covariance (ANCOVA).

I measured gonad weight to the nearest 0.001 g and, for all females, counted the number of mature eggs. Mature eggs were taken to be those eggs that were opaque and yellow and eggs were considered immature if they were translucent and clear. I calculated a gonadosomatic index (GSI) for all fish by dividing gonad weight by total weight and multiplying by 100. The GSI was used to determine the duration of spawning and the age of the first spawning event. Fish were aged using otoliths in a manner described by Mills (1987).

Results

Field Observations

Spawning was observed on 23 May 2012 at Big Lick Creek in a shallow run approximately 7 cm in depth. Average water temperature for this day was 15.4 °C (Figure 3.1) and air temperature 16 °C. There was a waxing crescent moon. On 22 May, there was 1.5 mm of precipitation and 0.02 mm of precipitation the day of observed spawning (Figure 3.2). For two days prior to spawning, *Campostoma anomalum* were seen making three shallow pits, and these pits were subsequently used by *Chrosomus* sp. cf. *saylori* for spawning. When spawning, several brightly-colored fish would swarm a single pit and vibrate rapidly over the depression. The entire event lasted less than 30 seconds, and was repeated every 5-10 minutes for at least an hour. Following a spawning event, fish would swim either underneath the undercut bank or downstream to the pool and then return back to the spawning site several minutes later.

After the spawning event on 23 May, *C*. sp. cf. *saylori* retreated to a downstream pool, and subsequently continued to move further away from the spawning site. Colors were noticeably muted by 28 May. Four *Campostoma anomalum* returned to the original spawning site and began to build pits on 2 June, but *Chrosomus* sp. cf. *saylori* were never seen at the spawning site again.

At Mudlick Creek, fish were observed to be in full breeding coloration by 17 May and in close proximity to a gravel mound. As the rest of the stream bottom was largely silt and sand and *Nocomis* spp. are not in Mudlick Creek, the mound was likely the creation of *Semotilus atromaculatus*. I was unable to observed Mudlick Creek again until 30 May, at which point coloration had begun to fade, and fish had moved to a deep pool.

Sexual Morphology

The average number of mature ova in females was 267.3 (SE=24.2, min=153, max=442, n=12). Only age-two females had mature ova. Age-one females had numerous immature ova, and age-zero fish had several ova that had just begun to form and resembled small bubbles.

For two-year-old females, the GSI was 4.98 in June, which increased to 7.15 in early July before declining (Figure 3.3). The GSI for one-year-old females showed a drop from 1.63 to

1.01 in mid-June, and rose again in July to 1.92 where it stayed for the remainder of the year. There was only one collection point for young-of-year (YOY) females, which was 0.25.

Male GSI for two-year-olds declined from 1.78 in April to 1.10 in June, after which it increased to 1.21 in the middle of July. The GSI for one-year-old males was 2.22 in April, fluctuated between 0.19-1.11 from June-July, and showed steady decline to 0.08 in November. Only one sample point for YOY males was taken, and showed a GSI of 0.08.

There was a 3:1 female-male sex ratio in my sample. Vibrancy of coloration was a poor predictor of sex. The most vibrant colors were often displayed by females, which also had larger abdomens during spawning season. Males did have more rounded pectoral fins and, in the height of spawning season, pearl organs. Pectoral fin length was strongly correlated to standard length in males (r^2=0.72, $p<0.001$, N= 16) and females (r^2=0.76, $p<0.001$, N= 66). Males had a significantly larger pectoral fin (by ANCOVA, p=0.006); however, the growth rate of the pectoral fin was consistent across the sexes (by ANCOVA, p=0.82, Figure 3.4).

Discussion and Conclusion

Here, I provide a detailed description of *C*. sp. cf. *saylori* breeding behaviors. Spawning appears to last from at least May-July and, based on the presence of mature eggs and GSI, fish likely do not reproduce until their second year of life. There was no difference in breeding coloration between males and females, and the only evident sexual dimorphism was in pectoral fin length and shape and the presence of pearl organs in breeding males. A nest association was directly observed with *Campostoma anomalum*, and it is likely there was another association with *Semotilus atromaculatus*.

The spawning behavior outlined here for *C*. sp. cf. *saylori* is indicative of other *Chrosomus*. All previous studies, which used both field observations and GSI, have shown

Chrosomus to spawn from April-July (Starnes and Starnes 1981, Das and Nelson 1990), and with a nest association (Hamed and Alsop 2005). While May was the earliest I observed breeding, I cannot discount the possibility of spawning in April, particularly since I do not have GSI for two-year-old females before June. Field records showed that fish were starting to obtain breeding colors on 1 April, and so there could have been spawning later that month. In addition, it is uncertain whether *C*. sp. cf. *saylori* nest association is a plastic trait, or an obligatory response to habitat degradation. Most streams inhabited by *C*. sp. cf. *saylori* have moderate to heavy siltation, and so the availability of long stretches of clean, gravel substrate is low. Thus, the nest association may maintain reproductive output in an otherwise unsuitable system (Pendleton et al. 2012).

While timing and mode of reproduction is similar between *C*. sp. cf. *saylori* and other *Chrosomus*, the sexual development of *C*. sp. cf. *saylori* is quite different. All previous *Chrosomus* studies have reported female sexual maturation at one year (Starnes and Starnes 1981). However, the absence of mature eggs in year-one female *C*. sp. cf. *saylori* is indicative of delayed sexual maturation. Given that year-one individuals are usually the most abundant and have relatively high fecundities, they typically contribute the most towards *Chrosomus* reproduction (Hamed et al. 2008).

In addition to delayed maturation, *C*. sp. cf. *saylori* also have a lower maximum fecundity. The maximum average GSI of two-year-old *C*. sp. cf. *saylori* was 7.5, which is significantly lower than the maximum GIS for two-year-old *C. erythrogaster* (12.7; Settles and Hoyt 1978) and *C. eos* (19; Das and Nelson 1990). A lower GSI is indicative of fewer and/or smaller eggs per female. The average number of mature eggs per female in *C*. sp. cf. *saylori* was

267.3, whereas the average number of mature eggs in *C. erythrogaster* was 568 (Settles and Hoyt 1978).

Given that male gametes cannot be counted and measured, it is more difficult to draw inferences about age of maturation. However, the GSI of year-one males was lower than year-two males, and so it can be concluded that males do not contribute significantly towards reproduction until year-two. And, similar to females, the GSI of males was significantly lower in *C.* sp. cf. *saylori* than *C. erythrogaster* males (Settles and Hoyt 1978)

Chrosomus have been documented to have multiple reproductive bouts throughout the spawning window (Hamed et al. 2008). There are two competing explanations for this behavior. The first, which has been confirmed in cyprinids but not in *Chrosomus*, is that individuals exhibit fractional spawning and reproduce multiple times (Gale and Gale 1977). The second, which has been confirmed in *Chrosomus* (Hamed et al. 2008) is that there are two life history modes including some fish that were hatched in May (and thus spawn in May) and fish that hatch in July (and thus spawn in July). While my data are inconclusive as to which of those two processes are at play, observations of breeding in May and a rise in the female GSI in July is suggestive of multiple spawning sessions. In addition, stream temperatures in July are approximately 22 °C, which is closer to spawning temperatures reported for other *Chrosomus* (Settles and Hoyt 1978, Hamed et al. 2008). Interestingly, a single stream was sometimes inhabited by parapatrically-distributed May and July spawners. It is presently unknown whether this is an incidence of reproductive isolation owning to geographic isolation or a sampling error.

Previous studies have reported male-biased sex ratios in *Chrosomus* (Smith 1908, Starnes and Starnes 1981, Hamed et al. 2008). However, here I report a female biased ratio of 3:1. Strongly biased sex ratios, especially those favoring males, can make populations more susceptible to extinction (Le Galliard et al. 2005). However, 3:1 is not extreme, and female-bias is the theoretically-predicted sex composition in small, sedentary populations (Nunney 1985). As Hamilton (1967) stated, populations with more or less permanent residents increase productivity by decreasing the proportion of males, who's gametes are the most numerous and least energetically costly, and increasing the proportion of females, who's gametes are less numerous and are more energetically costly to produce. The result is an increase probability of fertilization for each egg, and a population that can maintain viability longer than one with a balanced sex ratio (Nunney and Luck 1988).

Unlike other reports of *Chrosomus*, *C*. sp. cf. *saylori* did not exhibit clear sexual dimorphism in coloration. In fact, observation of individuals in the field immediately after capture and subsequent sexing in the lab showed that females were often the most brightly colored. As such, stream bank observations of *C*. sp. cf. *saylori* that use coloration to determine sex ratio and sex-specific spawning behaviors would be ineffective. Furthermore, studies of any *Chrosomus* that uses coloration to determine sex should be reviewed circumspectly. The only definitive external characteristic that has been shown to be sexually dimorphic across all *Chrosomus* is rounded pectoral fins (Settles and Hoyt 1978, Skelton 2001). This trait is not present until sexual maturation in *C*. sp. cf. *saylori*, and the only way to identify sexes in sexually immature fishes is through dissection.

The absence of strict sexual dimorphism in *C*. sp. cf. *saylori* when *Chrosomus* have previously been reported to have strong sexual dimorphism could be an example of Rensch's rule acting on *C*. sp. cf. *saylori*. Rensch's rule states that sexual dimorphism increases with size when males are the larger sex, but decreases with size when males and females are approximately the same size (Abouheif and Fairbairn 1997). I do not have enough samples to formally test Rensch's rule for *C*. sp. cf. *saylori*. However, it is generally believed that Rensch's rule is seen in populations where severe sexual dimorphism is selected against (i.e. predation on larger, more colorful individuals) and in populations with female-biased sex ratios with less sexual selection for strong secondary sex characteristics (Dale et al. 2007).

Taken together, this study shows that *C*. sp. cf. *saylori* individual performance is much reduced compared to congeners. However, due to the skewed sex ratio, which may have evolved in response to population isolation, *C*. sp. cf. *saylori* fitness may be similar to that of other *Chrosomus*. In fact, long-term population fitness of *C*. sp. cf. *saylori* may actually be higher because it distributes reproduction across more females, thus reducing the effect of individual mortality on offspring production. This conclusion should be heeded with caution as it assumes that the sex ratio is both a beneficial adaption and is stable. If the sex ratio became skewed due to severe inbreeding depression (Sheffer et al. 1999) or differential mortality of males (Brazo et al. 1978), then the ratio is likely to continue to favor increasingly-large proportions of females, which would eventually lead to population collapse.

Chrosomus sp. cf. *saylori* are a species of federal special concern, and identification of the timing and mode of reproduction should influence management decision. In the future, watershed activities and bridge/road construction adjacent to streams with *C*. sp. cf. *saylori* should be minimized from April-July. In addition, year-round efforts should be made to minimize overland erosion so as to decrease the amount of silt in streams. These activities will provide more spawning habitat, which will increase reproductive success and the viability of offspring.

Several species of *Chrosomus* are already listed on state and federal endangered species acts (Starnes and Starnes 1981, Skelton 2001). Though the reproductive potential of *C*. sp. cf.

saylori may be equal to that of congeners, it is still precariously low. In addition, the distribution and population size of *C*. sp. cf. *saylori* is much smaller than congeners. These findings should be viewed as evidence for the need to pursue further listing for *C*. sp. cf. *saylori*.

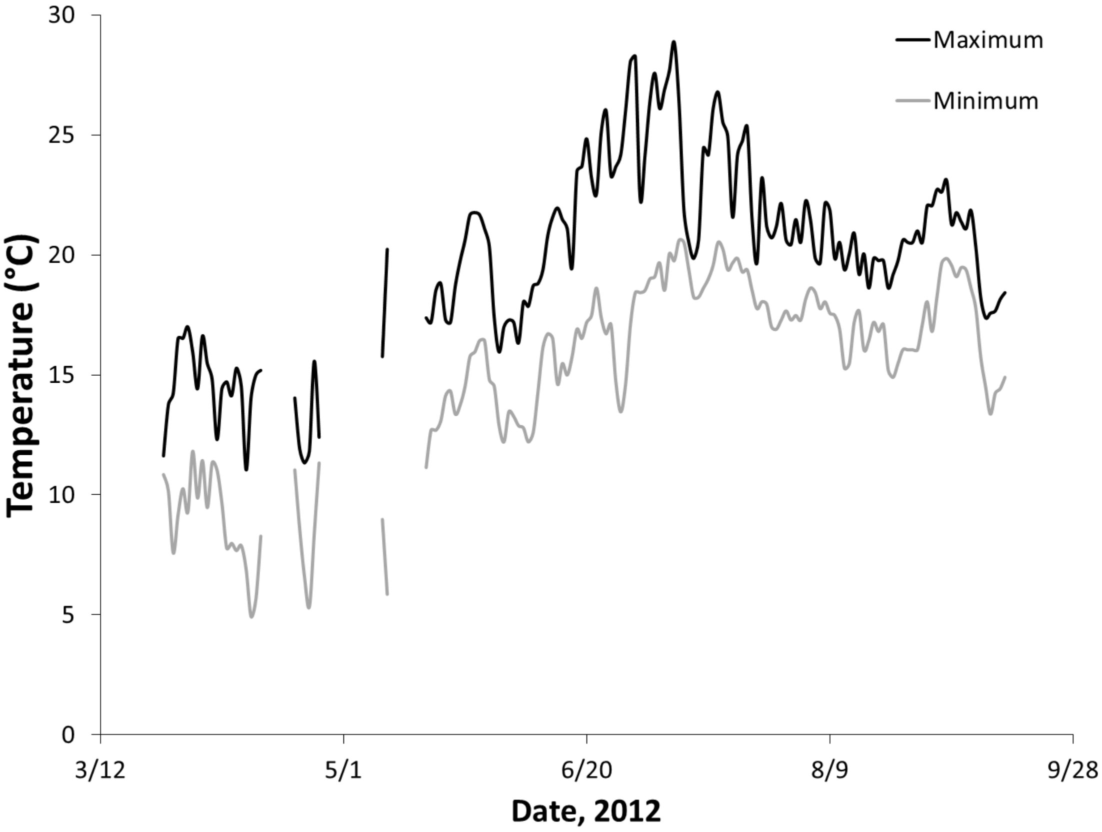


Figure 3.1: Temperature (°C) profile for Big Lick Creek from 26 March to 14 September, 2012 showing maximum (black) and minimum (grey) stream temperatures for each day. Absence of data points indicates malfunctioning equipment.

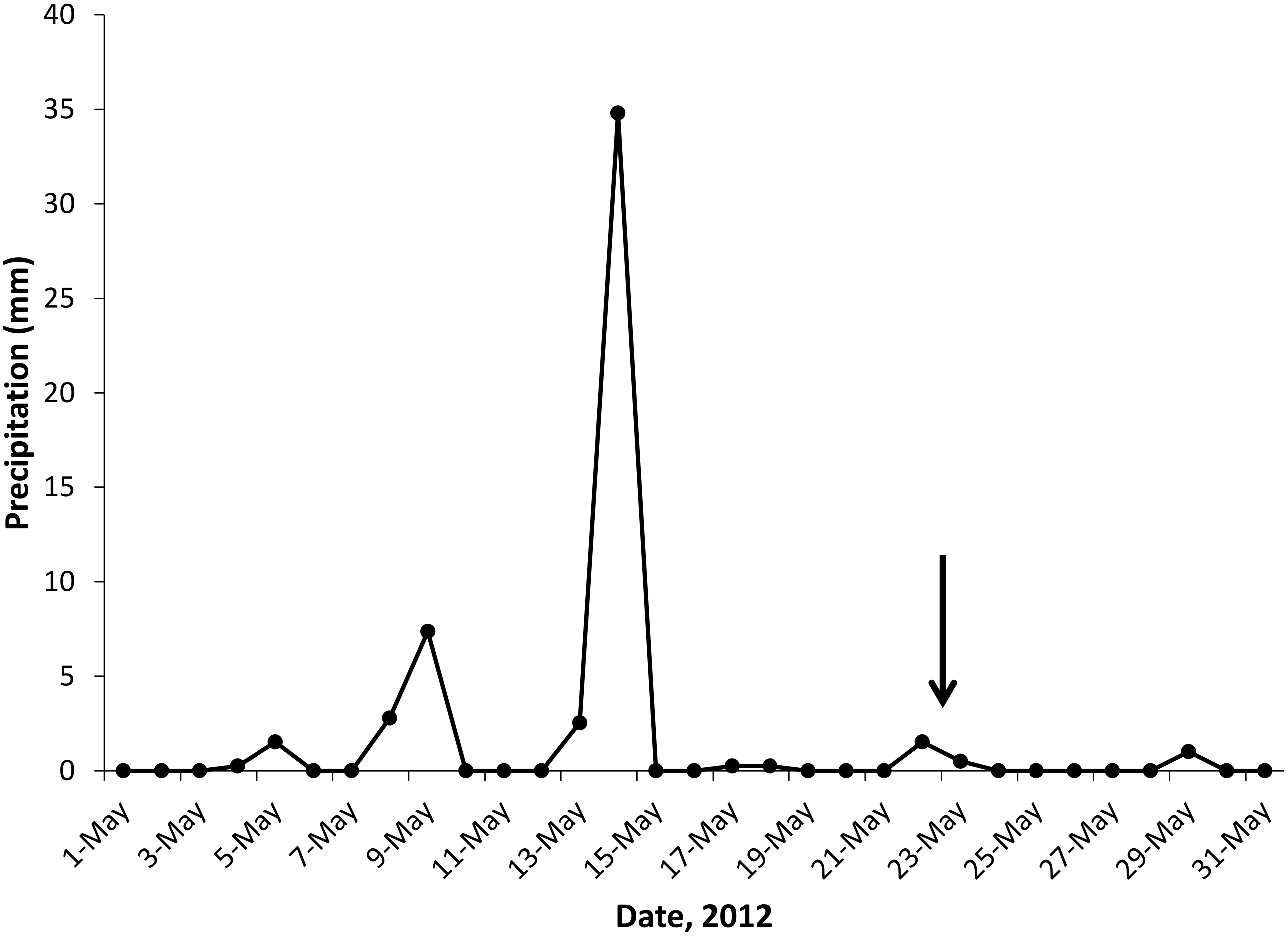


Figure 3.2: Daily precipitation (mm) for May 2012. Arrow points to 23 May, the date the date *Chrosomus* sp. cf. *saylori* were seen spawning in Big Lick Creek.

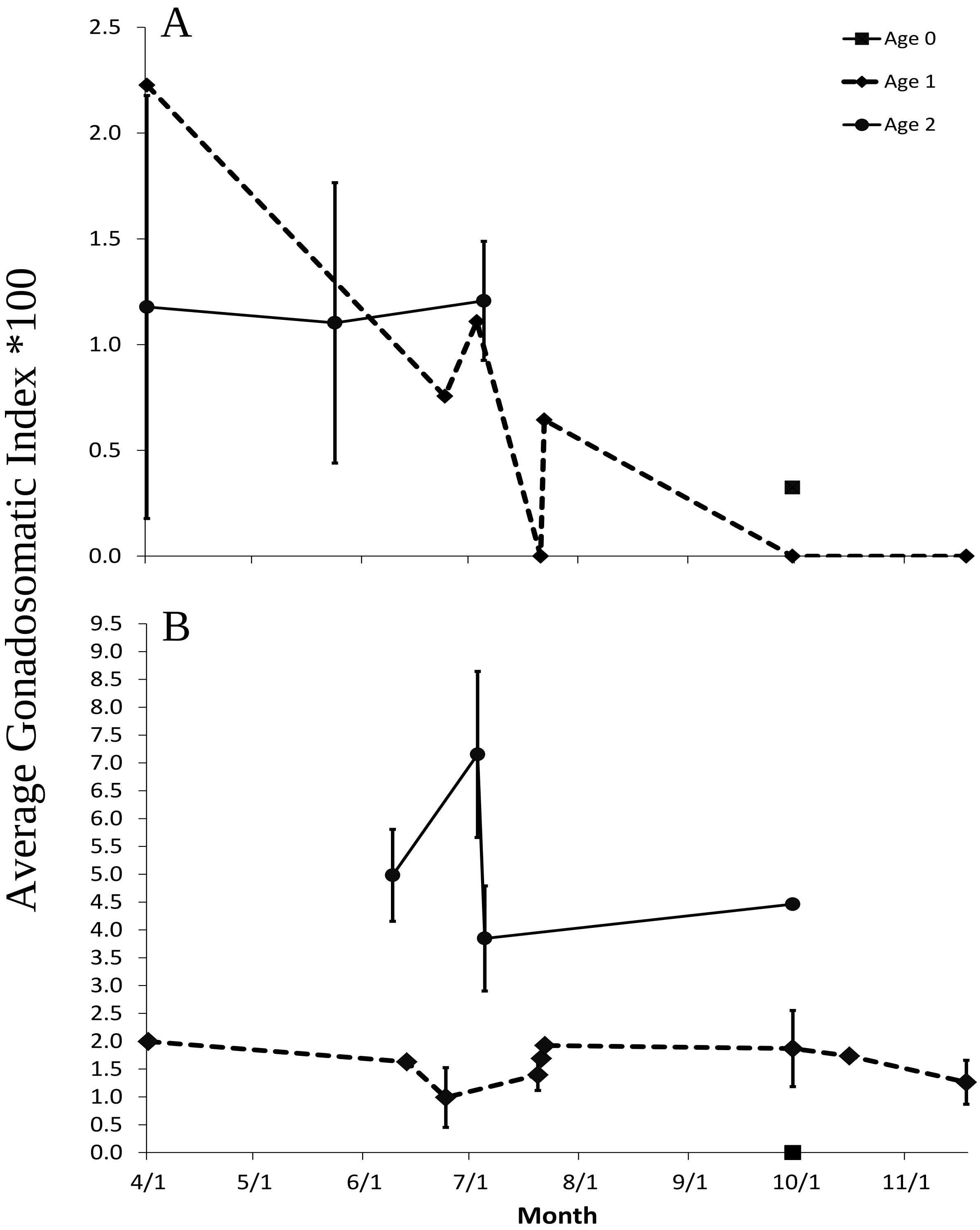


Figure 3.3: Average gonadosomatic index (GSI) for male (A) and female (B) *Chrosomus* sp. cf. *saylori* from April 1-November 18. Fishes used in this analysis were collected in 2009, 2011, and 2012, and dates show the average GSI for all fish across all streams and collection years.

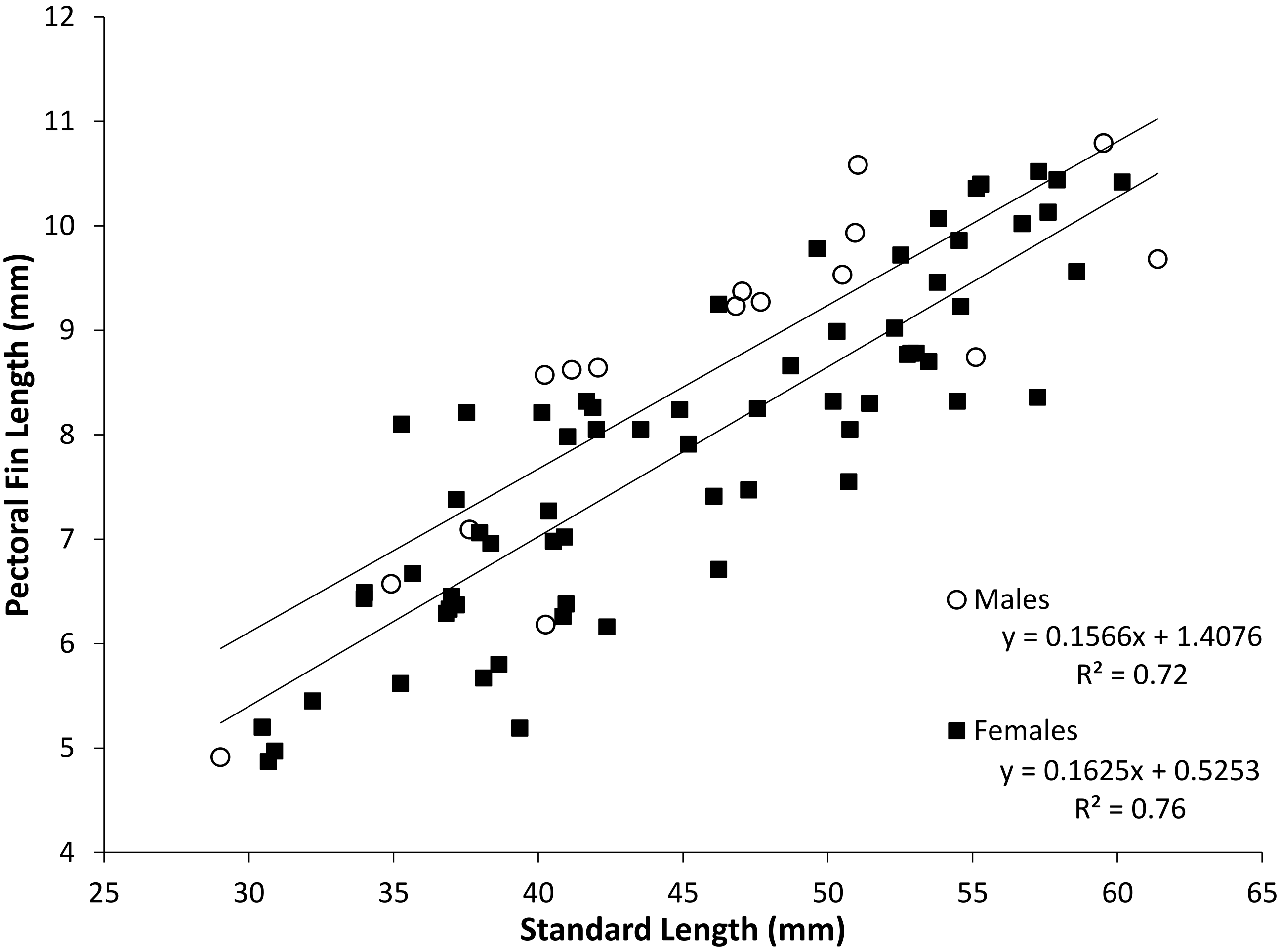


Figure 3.4: Regression of pectoral fin length on standard length for males (open circles; y=0.1566x+1.4074, r^2=0.72, p=<0.001) and females (black squares y=0.1625x+0.5253, r^2=0.75, p=<0.001). Length of pectoral fin was significantly different between males and females (p=0.006).

General Conclusions

Chrosomus sp. cf. *saylori* are distributed in eight small tributaries to the upper Clinch River (Chapter 1). Populations are small, and some of questionable viability. Internal and external morphological characteristics are significantly different between *C*. sp. cf. *saylori* and other congeners, most notably *C. saylori*, which provides an initial conclusion that many generations of isolation has resulted in the speciation of *C*. sp. cf. *saylori* (Chapter 2). Individual fitness of *C*. sp. cf. *saylori* is much lower than that reported for other *Chrosomus* species; however, population fitness is seemingly being maintained through a female-biased sex ratio which is enhancing the probability of fertilization for each egg (Chapter 3).

My efforts provide only an initial baseline, and there remain several uncertainties about *C*. sp. cf. *saylori* that should be addressed. Most notably, genetic analysis of *C*. sp. cf. *saylori* must be completed in order to update the *Chrosomus* phylogeny and definitively prove speciation. While completing a genetic sequence for *C*. sp. cf. *saylori*, an analysis of metapopulation structure should also be considered. Specifically, the sporadic distribution of *C*. sp. cf. *saylori* in a stream and the observation of two life history patterns several hundred meters from one another leads me to question the population structure of *C*. sp. cf. *saylori*. Given the presence of multiple culverts in streams inhabited by *C*. sp. cf. *saylori*, the sedentary behavior of the genus, and patchy mosaic of suitable habitat, it is entirely plausible that *C*. sp. cf. *saylori* have established several isolated subpopulations within some streams. If this is the case, population extinction is almost certain as each subpopulation will be exposed to high incidences of inbreeding and the deleterious effects of genetic drift. Should it be determined that there are subpopulations, conservation should focus on reestablishing within-stream connectivity.

The presence of multiple subpopulations within a single stream is uncommon. However, it is well-established that dendritic organization of stream networks often influences the establishment of sedentary, isolated populations of fish that rarely move away from natal streams (Fagan 2002), and I believe this to be the scenario for *C*. sp. cf. *saylori*. Isolation, and the subsequent genetic collapse associated with isolation, is a major cause of population extinction, particularly when effective population sizes do not adhere to "50-500 rule" posited by Franklin (1980), and substantiated by others (Jamieson and Allendorf 2012), which states that an average effective population size (N_e) of 50 is needed for short-term population persistence and an average N_e of at least 500 is required for long-term population viability.

The current effective population size of *C*. sp. cf. *saylori* is largely unknown. However, it is likely that the average N_e of many *C*. sp. cf. *saylori* populations is less than 50 individuals, particularly if there are multiple subpopulations within a single stream. Furthermore, it is almost certain that average N_e for most, if not all, populations of *C*. sp. cf. *saylori* is less than 500 individuals. I lack the long-term genetic and observational data necessary to make definitive conclusions about the rate of genetic drift, and thus N_e, for *C*. sp. cf. *saylori*. However, if one considers the equation $N_e=4N_mN_f/(N_m+N_f)$, which has been shown to be a reliable predictor of N_e for populations with skewed sex ratios, and my documented 3:1 females biased sex ratio (Chapter 3) , then each population of *C*. sp. cf. *saylori* would have to consist of 167 males and 501 females in order to have an Ne>500. The maximum number of reproducing individuals (i.e. Age 2; Chapter 3) at a stream was 13. Though my sampling protocol likely underestimates population size, it is safe to conclude that populations are not approaching that needed to secure long-term survival.

Not surprisingly, the most obvious threat to *C*. sp. cf. *saylori* is habitat degradation. There is a significant correlation between streams inhabited by C. sp. cf. *saylori* and forested watersheds (Chapter 1), and studies of *Chrosomus* have documented declines in populations following watershed development (Slack et al. 1997). The evolution of nest association has allowed *C*. sp. cf. *saylori* to continue occupying streams with less than favorable habitat. However, heavy siltation is still a major cause for concern for *C*. sp. cf. *saylori*. The presence of multiple culverts, beaver dams, and watershed activities, including agriculture, mining, development, and road construction are all likely sources of increase siltation into stream occupied by *C*. sp. cf. *saylori*. Though cessation of these activities during spawning season would help reproduction, year-round efforts to minimize sediment runoff should be pursued to decrease stress.

The impacts of mining on *C*. sp. cf. *saylori* were not directly addressed in this study, but should be a focus of future investigations. Theoretically, one would predict that *C*. sp. cf. *saylori* would be negatively impacted by mining activities. However, *C*. sp. cf. *saylori* seem to thrive in high elevation, forested watersheds with high densities of deep coal mines. This could be because those populations have decreased substantially from historic highs and are in the process of declining. Conversely, *C*. sp. cf. *saylori* might be adapted to living in streams with high conductivity and low pH, thereby giving them a competitive advantage in systems degraded by mining. And, because deep coal mines are subsurface, they could conserve large tracts of forested land, a critical habitat component to *C*. sp. cf. *saylori* (Chapter 1).

My study, while extensive, was conducted over a small temporal scale and so I cannot make strong predictions about trends in *C*. sp. cf. *saylori* occupancy and abundance. Therefore, a long-term monitoring plan should be established for *C*. sp. cf. *saylori*. This plan should include repeated sampling of fish and habitat to determine population persistence, changes in habitat, movement and colonization of fish within a stream, and how *C*. sp. cf. *saylori* respond to temporal variation in habitat. Streams that are considered to have suitable habitat but were absent of *C*. sp. cf. *saylori* should also be resampled to confirm absence.

Given that the population structure of *C*. sp. cf. *saylori* is in violation of the 50-500 rule and dispersal is limited by the biology of the species and habitat barriers, *C*. sp. cf. *saylori* may benefit from genetic recovery through within- and between-stream translocations and captive breeding programs. Captive breeding of *Chrosomus* has had mixed results. Rakes et al. (1999) were able to captivity breed *C*. *cumberlandensis* when exposed to pheromones from *Nocomis micropogon* (river chub) and *Campostoma anomalum* (central stoneroller). However, in some instances, only 20% of eggs were successfully fertilized and produced viable offspring (Rakes et al. 1999). Thus, establishing captive breeding for *C*. sp. cf. *saylori* may not be a viable option with current population sizes as it could result in unnecessary mortality and potentially only limited success.

Translocations may also prove to be an effective tool for population recovery. However, to reduce the probability of ineffective or detrimental translations, a genetic analysis should be completed in order to determine the influence of geographic location on the genetic structure of populations (George et al. 2009). Populations that are genetically dissimilar likely have local adaptations, thus reducing the viability of translocated fish and increasing the probability of outbreeding depression (Moritz 1999). Conversely, populations that are extremely similar will contribute very little new genetic material, which would do little to relieve problems of inbreeding depression and genetic drift. Therefore, translocations should focus on populations that share an intermediate amount of genetic similarity (Vrijenhoek 1998). Furthermore, determining the presence and relatedness of within-stream subpopulations would determine whether translocations within a single stream are an appropriate action. Should translocations be deemed a viable recovery option, long-term partnerships would have to first be pursued between biologists, geneticists, and land owners to ensure that, not only do fish get moved, but that there are long-term monitoring efforts that enable adaptive management opportunities (George et al. 2009).

Because distribution seems to be affected more by geologic history and possible local extinction events (Chapter 1), introductions of *C*. sp. cf. *saylori* into streams with suitable habitat may also be a worthwhile effort. However, until present populations have increased or it has been determined that N_e is larger than currently predicted, introductions should not be attempted in order to minimize unnecessary mortality.

A public outreach effort to riparian landowners would also be a worthwhile endeavor. Many homeowners were unaware of the presence of *C*. sp. cf. *saylori* in their neighboring streams but, upon further communication, they were extremely receptive to my sampling and wanted to remain apprised of my findings. Educating these homeowners and discouraging common landowner activities (i.e. stream dumping) could protect critical *C*. sp. cf. *saylori* habitat. Furthermore, minnow traps were common in *C*. sp. cf. *saylori* streams, and I frequently found *C*. sp. cf. *saylori* in traps. Trap owners often commented that *C*. sp. cf. *saylori* were their preferred bait, and this could be a significant source of mortality. Though it is unlikely that minnow traps could be policed in these small headwater streams, educating trap users to release *C*. sp. cf. *saylori* could only help *C*. sp. cf. *saylori* populations. This effort could be implemented during follow-up surveying, thus decreasing the amount of effort required to initiate a landowner education program.

Attempts to form working relationships with coal companies that own land with streams suspected to inhabit *C*. sp. cf. *saylori* should also be made. Coal companies are fearful of new laws and regulations which may stem from federal listing of *C*. sp. cf. *saylori*, and so they are reluctant to grant stream access. However, the majority of streams, especially segments of headwater streams that have thus far not been sampled, are on coal company land. Until an assessment of these locations is complete, estimates of the length of stream occupied and population sizes are complete conjecture.

This study elucidates the current status of *C*. sp. cf. *saylori*, and indicates that more stringent protection is necessary. Given the narrow distribution, small local abundance, and habitat specificity (Pritt and Frimpong 2010), *C*. sp. cf. *saylori* are among the rarest of fish species and should receive further protection. In addition, other, more abundant, *Chrosomus* have already been included on the Endangered Species Act (ESA), and so it is reasonable to pursue listing of *C*. sp. cf. *saylori* under the ESA. With this study, I have identified all the major items of information that are typically required for a successful petition for listing. This includes identification of critical habitat, threats to population persistence, distribution, likely speciation, and ineffectiveness of current protection measures. Thus, a petition for listing would likely be successful.

Even if protection under the federal ESA is not pursued, *C*. sp. cf. *saylori* would benefit from increased protection at the state level. Identification of habitat and threats would justify upgrading the species from Tier II to Tier I on the Virginia Wildlife Action Plan and *C*. sp. cf. *saylori* are presently not included in the Virginia Endangered Species Act. Pursuing listing under both of these entities would likely increase protection measures for the species, thereby increasing the probability of implementing a successful species recovery plan.

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Appendix A: Count of individuals, by species, encountered during 2011-2012 sampling of 60 stream. Fish were captured using single-pass electrofishing.

Stream	Species	Count
Alvy Creek	*Rhinichthys atratulus*	345
	Cottus spp.	48
	Semotilus atromaculatus	12
	Etheostoma flabellare	2
	Campostoma anomalum	2
Beech Fork	*Rhinichthys atratulus*	406
	Cottus spp.	103
	Semotilus atromaculatus	4
	Campostoma anomalum	3
	Catostomus commersoni	18
Benny Creek	*Rhinichthys atratulus*	248
	Cottus spp.	99
	Semotilus atromaculatus	7
	Clinostomus funduloides	19
	Campostoma anomalum	49
Big Branch	*Rhinichthys atratulus*	93
	Semotilus atromaculatus	76
	Etheostoma flabellare	44
	Clinostomus funduloides	43
	Luxilus sp.	1
Big Creek	*Rhinichthys atratulus*	133
	Semotilus atromaculatus	28
	Campostoma anomalum	53
	Catostomus commersoni	9
Big Lick	*Rhinichthys atratulus*	95
	Chrosomus sp. cf. *saylori*	7
	Semotilus atromaculatus	95
	Etheostoma flabellare	16
	Clinostomus funduloides	1
	Campostoma anomalum	29
Chaney Creek	*Rhinichthys atratulus*	469
	Campostoma anomalum	242
Claypool Branch	*Rhinichthys atratulus*	243
	Lepomis macrochirus	1
	Cottus spp.	5
	Semotilus atromaculatus	9
	Etheostoma flabellare	26
	Hypentelium nigricans	3
	Ambloplites rupestris	3

Appendix A: continued.

	Campostoma anomalum	24
	Notropis leuciodus	15
	Luxilus albeolus	27
	Catostomus commersoni	2
Copper Creek	*Rhinichthys atratulus*	47
	Lepomis macrochirus	1
	Cottus spp.	77
	Semotilus atromaculatus	3
	Ambloplites rupestris	8
	Campostoma anomalum	28
	Luxilus coccogenis	2
	Luxilus albeolus	2
	Catostomus commersoni	6
Dix Creek	*Rhinichthys atratulus*	77
	Cottus spp.	29
	Semotilus atromaculatus	1
	Campostoma anomalum	7
Downstream Big Lick Creek	*Rhinichthys atratulus*	127
	Semotilus atromaculatus	34
	Etheostoma flabellare	31
	Campostoma anomalum	47
Downstream Hart Creek	*Rhinichthys atratulus*	92
	Chrosomus sp. cf. *saylori*	1
	Semotilus atromaculatus	65
	Etheostoma flabellare	17
	Campostoma anomalum	51
Downstream Hurricane Fork	*Rhinichthys atratulus*	204
	Semotilus atromaculatus	14
	Etheostoma flabellare	19
	Etheostoma blennioides	6
	Lepomis megalotis	1
	Hypentelium nigricans	1
	Campostoma anomalum	170
	Catostomus commersoni	1
Dumps Creek	*Rhinichthys atratulus*	112
	Ambloplites rupestris	2
	Campostoma anomalum	44
	Catostomus commersoni	2
Goose Creek	*Rhinichthys atratulus*	33
	Lepomis macrochirus	6
	Cottus spp.	5

Appendix A: continued.

	Semotilus atromaculatus	12
Greasy Creek	*Rhinichthys atratulus*	85
	Chrosomus sp. cf. *saylori*	4
	Semotilus atromaculatus	195
	Etheostoma flabellare	13
	Clinostomus funduloides	37
	Campostoma anomalum	21
	Catostomus commersoni	53
Hess Creek	*Rhinichthys atratulus*	271
	Chrosomus sp. cf. *saylori*	2
	Semotilus atromaculatus	63
	Etheostoma flabellare	16
	Chrosomus tennesseensis	1
	Campostoma anomalum	73
	Catostomus commersoni	5
Hogwallow Branch	*Rhinichthys atratulus*	141
	Cottus spp.	44
	Oncorhynchus mykiss	18
Hurricane Fork	*Rhinichthys atratulus*	100
	Pimephales notatus	20
	Chrosomus sp. cf. *saylori*	6
	Semotilus atromaculatus	104
	Etheostoma flabellare	25
	Lepomis megalotis	1
	Lepomis auritus	2
	Campostoma anomalum	55
	Luxilus albeolus	5
	Catostomus commersoni	48
Indian Creek	*Rhinichthys atratulus*	193
	Semotilus atromaculatus	44
	Lepomis auritus	1
	Campostoma anomalum	12
Jackson Fork	*Rhinichthys atratulus*	65
	Chrosomus sp. cf. *saylori*	1
	Semotilus atromaculatus	33
	Etheostoma flabellare	4
	Ambloplites rupestris	1
	Clinostomus funduloides	10
	Campostoma anomalum	1
	Catostomus commersoni	12
Jacobs Fork	*Rhinichthys atratulus*	50

Appendix A: continued.

	Cottus spp.	283
	Campostoma anomalum	3
Katie Branch	*Rhinichthys atratulus*	134
	Lepomis macrochirus	49
	Cottus spp.	1
	Semotilus atromaculatus	17
	Etheostoma flabellare	11
	Campostoma anomalum	105
	Luxilus albeolus	5
	Catostomus commersoni	3
Laurel Branch	*Rhinichthys atratulus*	224
	Salmo trutta	1
	Cottus spp.	47
	Semotilus atromaculatus	11
	Etheostoma flabellare	6
	Nocomis micropogon	1
	Etheostoma simoterum	7
	Campostoma anomalum	19
	Luxilus albeolus	9
	Catostomus commersoni	12
Laurel Fork	*Rhinichthys atratulus*	26
	Lepomis macrochirus	9
	Pimephales notatus	5
	Semotilus atromaculatus	17
	Etheostoma caeruleum	46
	Lepomis auritus	3
	Ambloplites rupestris	14
	Clinostomus funduloides	24
	Campostoma anomalum	10
	Luxilus albeolus	5
	Catostomus commersoni	2
Left Fork Coal Creek	*Rhinichthys atratulus*	57
	Chrosomus sp. cf. *saylori*	6
	Semotilus atromaculatus	21
	Campostoma anomalum	22
Left Fork Lick Creek	*Rhinichthys atratulus*	316
	Semotilus atromaculatus	10
	Etheostoma flabellare	43
	Hypentelium nigricans	8
	Ambloplites rupestris	1
	Etheostoma simoterum	4

Appendix A: continued.

	Campostoma anomalum	165
	Catostomus commersoni	3
Levisa Fork	*Rhinichthys atratulus*	360
	Semotilus atromaculatus	118
	Etheostoma flabellare	2
	Hypentelium nigricans	44
	Etheostoma caeruleum	6
	Micropterus dolomieu	2
	Campostoma anomalum	223
Lewis Creek	*Rhinichthys atratulus*	42
	Cottus spp.	2
	Semotilus atromaculatus	4
	Etheostoma flabellare	7
	Campostoma anomalum	13
	Luxilus albeolus	1
Little Town Hill Creek	*Rhinichthys atratulus*	40
	Chrosomus sp. cf. *saylori*	1
	Cottus spp.	2
	Semotilus atromaculatus	7
	Etheostoma flabellare	3
	Catostomus commersoni	3
Loop Creek	*Rhinichthys atratulus*	265
	Cottus spp.	58
	Etheostoma flabellare	20
	Ambloplites rupestris	8
	Etheostoma simoterum	17
	Campostoma anomalum	19
	Luxilus albeolus	4
Lowe Branch	*Rhinichthys atratulus*	105
	Cottus spp.	27
	Semotilus atromaculatus	20
	Etheostoma flabellare	10
	Clinostomus funduloides	1
	Campostoma anomalum	3
Middle Creek	*Rhinichthys atratulus*	70
	Pimephales notatus	3
	Cottus spp.	2
	Semotilus atromaculatus	15
	Etheostoma flabellare	10
	Lepomis hybrid	2
	Hypentelium nigricans	9

Appendix A: continued.

	Nocomis micropogon	2
	Ambloplites rupestris	4
	Etheostoma simoterum	2
	Campostoma anomalum	126
Mill Creek	*Rhinichthys atratulus*	332
	Semotilus atromaculatus	77
	Campostoma anomalum	134
Moll Creek	*Rhinichthys atratulus*	254
	Cottus spp.	63
	Semotilus atromaculatus	12
	Hypentelium nigricans	1
	Campostoma anomalum	28
Moses Branch	*Rhinichthys atratulus*	275
	Semotilus atromaculatus	58
	Lepomis auritus	16
	Campostoma anomalum	221
Mountain Branch	*Rhinichthys atratulus*	699
	Cottus spp.	1
	Campostoma anomalum	5
Mudlick Creek	*Rhinichthys atratulus*	33
	Chrosomus sp. cf. *saylori*	12
	Semotilus atromaculatus	223
	Etheostoma flabellare	71
	Ambloplites rupestris	9
	Campostoma anomalum	73
	Catostomus commersoni	73
Panther Branch	*Rhinichthys atratulus*	124
	Semotilus atromaculatus	24
Pine Creek	*Rhinichthys atratulus*	267
	Chrosomus sp. cf. *saylori*	5
	Semotilus atromaculatus	122
	Etheostoma flabellare	37
	Campostoma anomalum	43
	Catostomus commersoni	14
Right Fork Garden Creek	*Rhinichthys atratulus*	286
	Semotilus atromaculatus	33
	Hypentelium nigricans	42
	Etheostoma caeruleum	8
	Ambloplites rupestris	3
	Luxilus sp.	3
	Campostoma anomalum	168

Appendix A: continued.

	Catostomus commersoni	2
Right Fork Lick Creek	*Rhinichthys atratulus*	1002
	Semotilus atromaculatus	3
	Campostoma anomalum	197
Rocky Fork	*Rhinichthys atratulus*	90
	Cottus spp.	16
	Semotilus atromaculatus	48
	Etheostoma flabellare	1
	Luxilus sp.	1
	Etheostoma simoterum	1
	Campostoma anomalum	14
	Catostomus commersoni	1
Seven Spring Creek	*Rhinichthys atratulus*	175
	Cottus spp.	118
	Semotilus atromaculatus	2
	Campostoma anomalum	17
	Luxilus albeolus	1
Sinking Creek	*Rhinichthys atratulus*	94
	Semotilus atromaculatus	30
	Ambloplites rupestris	13
	Campostoma anomalum	26
	Luxilus sp.	3
	Luxilus albeolus	67
	Catostomus commersoni	5
Stone Branch	*Rhinichthys atratulus*	59
	Cottus spp.	2
	Semotilus atromaculatus	5
Strow Creek	*Rhinichthys atratulus*	118
	Semotilus atromaculatus	34
	Etheostoma flabellare	24
	Lepomis auritus	1
	Campostoma anomalum	62
	Catostomus commersoni	2
Sulphur Spring Creek	*Rhinichthys atratulus*	248
	Semotilus atromaculatus	63
	Etheostoma flabellare	4
	Campostoma anomalum	62
Town Hill Creek	*Rhinichthys atratulus*	61
	Chrosomus sp. cf. *saylori*	3
	Semotilus atromaculatus	12
	Etheostoma flabellare	2

Tributary to Big Moccasin Creek	*Rhinichthys atratulus*	153
	Semotilus atromaculatus	26
	Campostoma anomalum	21
	Luxilus albeolus	1
Tributary to North Fork Clinch	*Rhinichthys atratulus*	50
	Lepomis macrochirus	1
	Cottus spp.	56
	Semotilus atromaculatus	4
	Etheostoma flabellare	2
	Pumpkinseed	2
	Ambloplites rupestris	10
	Etheostoma simoterum	1
	Campostoma anomalum	7
	Catostomus commersoni	4
Tributary to Thompsons Branch	*Rhinichthys atratulus*	421
	Cottus spp.	1
	Semotilus atromaculatus	73
	Campostoma anomalum	11
Upstream Big Lick	*Rhinichthys atratulus*	99
	Chrosomus sp. cf. *saylori*	10
	Semotilus atromaculatus	53
	Campostoma anomalum	8
Upstream Chaney Creek	*Rhinichthys atratulus*	265
	Campostoma anomalum	64
Upstream Hart Creek	*Rhinichthys atratulus*	108
	Chrosomus sp. cf. *saylori*	7
	Semotilus atromaculatus	14
	Etheostoma flabellare	20
	Campostoma anomalum	33
Upstream Hess Creek	*Rhinichthys atratulus*	258
	Chrosomus sp. cf. *saylori*	13
	Semotilus atromaculatus	48
	Etheostoma flabellare	4
	Campostoma anomalum	79
	Catostomus commersoni	2
Upstream Pine Creek	*Rhinichthys atratulus*	84
	Semotilus atromaculatus	22
	Etheostoma flabellare	5
	Campostoma anomalum	2
Vall Creek	*Rhinichthys atratulus*	134

Appendix A: continued.

	Cottus spp.	44
	Semotilus atromaculatus	86
	Clinostomus funduloides	26
	Catostomus commersoni	2
West Fork Big Creek	*Rhinichthys atratulus*	175
	Cottus spp.	2
	Semotilus atromaculatus	5
	Etheostoma flabellare	2
	Campostoma anomalum	29
Wrights Valley Creek	*Rhinichthys atratulus*	35
	Pimephales notatus	4
	Cottus spp.	5
	Semotilus atromaculatus	32
	Etheostoma flabellare	24
	Lepomis cyanellus	4
	Ambloplites rupestris	30
	Clinostomus funduloides	18
	Etheostoma simoterum	8
	Campostoma anomalum	17
	Luxilus albeolus	7
	Catostomus commersoni	9

Appendix B: Location of streams found to support *Chrosomus* sp. cf. *saylori* during sampling in 2011-2012. Average values for four microhabitat and ten macrohabitat variables collected for each stream are listed

									Proportion of Watershed							
Stream Name	Latitude	Longitude	Average Stream Width (m)	Average Stream Depth (cm)	Total Number of Pools	Average Pool Volume (m^3)	Stream Slope (%)	Elevation (m)	Developed	Farmland	Forested	Bare Rock	Open Water	Wetland	Watershed Area (km^2)	Stream Order
Big Lick	37.08775	-81.89417	2.10	11.53	0	0.00	1.48	665.39	0.00	0.00	1.00	0.00	0.00	0.00	4.15	2
Downstream Hart Creek	37.01142	-82.09882	3.10	12.53	9	3.52	1.60	565.24	0.00	0.66	0.95	0.00	0.00	0.00	7.99	3
Greasy Creek	37.17712	-81.73330	3.17	26.65	21	5.82	0.53	706.52	0.00	0.00	0.99	0.00	0.00	0.00	4.99	2
Hess Creek	37.09872	-81.97063	1.52	9.20	6	1.00	1.33	677.90	0.00	0.01	0.92	0.06	0.01	0.00	3.77	2
Hurricane Fork	36.99665	-82.15272	2.29	14.51	3	5.31	0.36	577.86	0.00	0.00	0.99	0.01	0.00	0.00	16.70	4
Jackson Fork	37.18028	-81.70553	3.81	13.61	7	2.50	2.15	692.63	0.00	0.01	0.97	0.02	0.00	0.00	7.12	3
Left Fork Coal Creek	37.13520	-81.88470	2.05	10.49	2	3.20	0.83	706.42	0.00	0.18	0.82	0.00	0.00	0.00	2.21	2
Little Town Hill Creek	37.09603	-81.82280	1.51	7.10	9	0.73	1.93	586.12	0.09	0.02	0.89	0.00	0.00	0.00	3.79	3
Mudlick Creek	37.14423	-81.85542	2.75	20.64	11	4.83	0.93	702.75	0.00	0.01	0.96	0.03	0.00	0.00	4.62	3
Pine Creek	37.09932	-81.92083	1.88	8.38	5	1.62	1.46	678.67	0.00	0.09	0.90	0.00	0.00	0.00	4.98	2
Town Hill Creek	37.10710	-81.82437	2.21	8.50	5	1.40	2.55	632.12	0.01	0.01	0.97	0.01	0.00	0.00	9.35	2
Upstream Big Lick	37.09372	-81.89733	1.28	5.35	3	2.02	2.20	688.80	0.00	0.00	1.00	0.00	0.00	0.00	2.81	2
Upstream Hart Creek	37.02813	-82.09460	1.51	6.79	5	0.68	0.94	583.96	0.00	0.15	0.85	0.00	0.00	0.00	3.23	3
Upstream Hess Creek	37.10373	-81.97143	1.07	6.79	2	1.74	1.90	675.85	0.00	0.00	1.00	0.00	0.00	0.00	3.04	2

Appendix C: Location of streams found to not support *Chrosomus* sp. cf. *saylori* during sampling in 2011-2012. Average values for four microhabitat and ten macrohabitat variables collected for each stream are listed.

									Proportion of Watershed							
Stream Name	Latitude	Longitude	Average Stream Width (m)	Average Stream Depth (cm)	Total Number of Pools	Average Pool Volume (m^3)	Stream Slope (%)	Elevation (m)	Developed	Farmland	Forested	Bare Rock	Open Water	Wetland	Watershed Area (km^2)	Stream Order
Alvy Creek	37.00102	-82.10188	2.29	7.73	12	1.78	2.50	511.39	0.00	0.00	1.00	0.00	0.00	0.00	11.33	3
Beech Fork	37.18462	-81.64808	2.32	7.84	11	2.99	1.55	540.01	0.00	0.00	1.00	0.00	0.00	0.00	19.88	4
Benny Creek	37.19907	-81.84740	3.12	11.81	7	3.30	0.30	569.00	0.00	0.00	1.00	0.00	0.00	0.00	11.00	2
Big Branch	37.28488	-81.32482	1.96	16.84	5	1.49	1.95	727.69	0.00	0.47	0.99	0.00	0.00	0.00	2.69	2
Big Creek	37.15783	-81.78772	2.53	12.58	11	1.93	0.59	681.80	0.30	0.25	0.99	0.39	0.00	0.00	12.51	3
Chaney Creek	36.96333	-82.21292	2.59	6.83	3	3.17	3.28	527.71	0.00	0.00	0.99	0.01	0.00	0.00	14.24	2
Claypool Branch	37.00407	-82.02708	2.41	8.16	9	1.78	2.06	518.47	0.00	0.73	0.92	0.00	0.00	0.00	11.34	3
Copper Creek	36.82517	-82.26485	3.53	16.21	8	6.06	1.11	587.33	0.44	0.70	0.92	0.00	0.00	0.18	21.87	4
Dix Creek	37.18747	-81.62267	3.14	11.93	4	2.25	0.75	523.14	0.00	0.00	1.00	0.00	0.00	0.00	13.41	3
Downstream Big Lick Creek	37.08012	-81.89342	1.46	5.78	2	0.91	1.45	644.35	0.00	0.07	0.92	0.00	0.00	0.00	4.59	2
Downstream Hurricane Fork	36.97482	-82.18950	3.60	11.64	10	1.82	1.49	506.86	0.00	0.23	0.99	0.37	0.18	0.18	29.07	4
Dumps Creek	36.99773	-82.20365	3.88	16.96	16	3.96	0.41	567.02	0.25	0.18	0.99	0.41	0.00	0.00	7.79	3
Goose Creek	37.21613	-81.51087	1.89	8.06	3	3.29	1.95	618.14	0.00	0.02	0.96	0.01	0.00	0.00	3.57	2
Hogwallow Branch	36.97783	-81.84287	2.50	12.43	6	1.38	2.04	691.13	0.00	0.37	0.63	0.00	0.00	0.00	7.57	3
Indian Creek	37.05752	-82.09132	2.68	14.63	8	1.93	0.50	567.61	0.00	0.00	1.00	0.00	0.00	0.00	6.40	2
Jacobs Fork	37.20595	-81.56088	3.66	18.05	1	1.01	0.23	543.81	0.00	0.05	0.92	0.03	0.00	0.00	11.66	3
Katie Branch	37.04170	-81.79182	1.26	11.66	4	4.23	0.69	630.63	0.12	0.45	0.43	0.01	0.00	0.00	11.26	3
Laurel Branch	37.04705	-81.98772	3.08	9.59	9	3.01	1.26	591.29	0.00	0.11	0.86	0.03	0.00	0.00	33.25	3
Laurel Fork	37.28670	-81.37720	2.74	18.07	6	4.01	0.39	741.22	0.01	0.10	0.88	0.00	0.00	0.01	23.48	3
Left Fork Lick Creek	36.97982	-82.29788	3.34	12.69	1	7.10	1.53	541.15	0.03	0.01	0.96	0.00	0.00	0.00	1.16	2
Levisa Fork	37.16837	-81.93918	3.18	4.97	3	1.35	12.90	517.82	0.02	0.00	0.98	0.00	0.00	0.00	14.38	3
Lewis Creek	37.06420	-81.99405	2.99	10.35	6	2.29	3.08	655.19	0.00	0.01	0.92	0.07	0.00	0.00	15.36	3
Loop Creek	36.93070	-81.96345	3.64	18.05	7	7.44	0.14	663.45	0.00	0.08	0.92	0.00	0.00	0.00	25.46	4
Lowe Branch	37.11638	-81.70800	2.64	10.06	7	1.24	2.31	636.99	0.00	0.75	0.25	0.00	0.00	0.00	10.55	3
Middle Creek	37.09705	-81.75852	3.74	17.03	7	3.29	0.56	611.44	0.01	0.03	0.95	0.01	0.00	0.00	27.84	3
Mill Creek	37.10838	-81.88692	1.85	7.10	7	1.53	1.16	665.25	0.01	0.06	0.93	0.00	0.00	0.00	7.03	3
Moll Creek	36.80838	-82.30540	2.92	11.77	9	3.52	1.10	605.34	0.00	0.34	0.66	0.00	0.00	0.00	11.11	3
Moses Branch	36.86310	-82.34510	1.76	10.59	6	1.83	1.60	507.32	0.00	0.31	0.69	0.00	0.00	0.00	3.18	2
Mountain Branch	36.89500	-82.02500	2.17	9.64	6	1.09	2.29	617.10	0.00	0.45	0.55	0.00	0.00	0.00	5.73	3
Panther Branch	37.14535	-81.70230	1.24	7.02	7	0.56	4.99	657.00	0.00	0.00	0.98	0.01	0.00	0.00	2.15	2
Right Fork Garden Creek	37.14030	-82.03300	3.64	16.59	4	5.58	0.41	519.06	0.00	0.00	1.00	0.00	0.00	0.00	17.02	3
Right Fork Lick Creek	36.97690	-82.29325	1.95	8.46	15	1.34	3.56	558.09	0.01	0.00	0.98	0.00	0.00	0.00	5.52	3

Appendix C: continued.

Rocky Fork	36.97290	-82.13223	1.70	7.32	7	1.68	0.74	472.20	0.00	0.08	0.92	0.00	0.00	0.00	6.91	2
Seven Spring Creek	36.88210	-82.27940	3.22	16.89	5	3.36	1.80	486.18	0.05	0.42	0.52	0.00	0.00	0.00	6.40	2
Sinking Creek	36.83795	-82.38347	2.79	15.31	19	4.40	1.77	472.28	0.11	0.04	0.85	0.01	0.00	0.00	4.54	3
Stone Branch	37.04207	-81.97615	1.82	6.50	4	0.82	2.45	601.60	0.00	0.06	0.93	0.01	0.00	0.00	4.42	3
Strow Creek	37.09923	-81.95885	1.64	9.35	5	2.30	0.49	669.42	0.00	0.00	0.98	0.02	0.00	0.00	4.80	3
Sulphur Spring Creek	37.08675	-81.92847	1.94	10.97	3	3.35	1.30	648.05	0.00	0.00	1.00	0.00	0.00	0.00	7.63	2
Tributary to Big Moccasin Creek	36.79667	-82.22045	1.39	7.38	6	0.45	4.01	631.28	0.00	0.37	0.63	0.00	0.00	0.00	1.70	2
Tributary to North Fork Clinch River	37.16877	-81.48237	2.48	15.92	2	2.74	1.02	751.89	0.00	0.49	0.50	0.00	0.00	0.00	5.97	4
Tributary to Thompsons Branch	36.99368	-82.05432	1.41	5.90	20	0.45	2.99	498.71	0.00	0.33	0.67	0.00	0.00	0.00	2.12	2
Upstream Chaney Creek	36.96738	-82.22428	3.38	8.85	10	0.75	2.08	508.50	0.21	0.46	0.98	0.31	0.42	0.29	8.15	3
Upstream Pine	37.11007	-81.91823	1.08	3.76	0	0.00	0.82	691.54	0.00	0.03	0.98	0.00	0.00	0.00	3.31	2
Vall Creek	37.23553	-81.65428	2.33	11.17	6	1.56	0.84	589.25	0.00	0.01	0.99	0.00	0.00	0.00	11.56	2
West Fork Big Creek	37.10922	-81.78900	3.96	11.41	4	3.13	2.13	618.58	0.00	0.01	0.98	0.01	0.00	0.00	15.17	3
Wrights Valley Creek	37.23733	-81.31975	3.63	17.29	8	3.58	0.47	747.23	0.04	0.13	0.82	0.01	0.00	0.00	15.52	2

Appendix D: Correlation matrix of 60 sample sites developed from environmental variables.

	Alvy Creek	Beech Fork	Benny Creek	Big Branch	Big Creek	Chaney Creek	Claypool Branch	Copper Creek	Dix Creek	DS Hart Creek	DS Hurricane Fork	Dumps Creek	US Hess Creek	Goose Creek	Greasy Creek	Hess Creek	Hogwallow Branch	Hurricane Fork	Indian Creek	Jackson Fork	Jacobs Fork	Katie Branch	Laurel Branch	Laurel Fork	Left Fork Coal Creek	Left Fork Lick Creek	Levisa Fork	Lewis Creek	Little Town Hill Creek	Loop Creek	Lowe Branch	DS Big Lick Creek	Big Lick	Middle Creek	Mill Creek	Moll Creek	Moses Branch	Mountain Branch	Mudlick Creek	Panther Branch	Pine Creek	Right Fork Garden Creek	Right Fork Lick Creek	Rocky Fork	Seven Spring Creek	Sinking Creek	Stone Branch	Strow Creek	Sulphur Spring Creek	Town Hill Creek	Trib to Big Moccasin Creek	Trib to North Fork Clinch	Trib to Thompsons Branch	US Big Lick	US Hart Creek	US Pine	US Chaney Creek	Vall Creek	West Fork Big Creek	Wrights Valley Creek
Alvy Creek	1.00																																																											
Beech Fork	0.90	1.00																																																									
Benny Creek	0.87	0.76	1.00																																																							
Big Branch	0.91	0.97	0.77	1.00																																																					
Big Creek	0.92	0.98	0.84	0.97	1.00																																																			
Chaney Creek	0.89	0.97	0.79	0.96	0.98	1.00																																																	
Claypool Branch	0.91	0.99	0.75	0.98	0.96	0.97	1.00																																															
Copper Creek	0.88	0.97	0.79	0.95	0.97	0.96	0.96	1.00																																													
Dix Creek	0.88	0.99	0.75	0.98	0.97	0.96	0.99	0.96	1.00																																											
DS Hart Creek	0.92	1.00	0.77	0.97	0.97	0.96	1.00	0.97	0.99	1.00																																									
DS Hurricane Fork	0.91	0.97	0.77	0.95	0.97	0.98	0.96	0.97	0.96	0.97	1.00																																								
Dumps Creek	0.82	0.86	0.81	0.87	0.93	0.88	0.82	0.91	0.85	0.84	0.88	1.00																																							
US Hess Creek	0.57	0.44	0.50	0.48	0.54	0.53	0.42	0.54	0.42	0.43	0.59	0.61	1.00																																						
Goose Creek	0.77	0.77	0.86	0.77	0.84	0.84	0.76	0.80	0.76	0.78	0.82	0.80	0.52	1.00																																					
Greasy Creek	0.88	0.83	0.78	0.82	0.88	0.84	0.81	0.83	0.80	0.83	0.87	0.86	0.75	0.80	1.00																																				
Hess Creek	0.86	0.83	0.73	0.85	0.87	0.86	0.82	0.82	0.82	0.83	0.91	0.84	0.60	0.87	0.87	1.00																																			
Hogwallow Branch	0.82	0.87	0.74	0.91	0.93	0.89	0.85	0.89	0.87	0.84	0.89	0.96	0.58	0.74	0.79	0.85	1.00																																		
Hurricane Fork	0.87	0.79	0.79	0.81	0.87	0.81	0.77	0.82	0.79	0.79	0.84	0.88	0.78	0.79	0.97	0.87	0.84	1.00																																	
Indian Creek	0.88	0.74	0.83	0.79	0.83	0.76	0.72	0.78	0.74	0.73	0.79	0.90	0.68	0.70	0.84	0.83	0.89	0.90	1.00																																
Jackson Fork	0.74	0.68	0.66	0.72	0.76	0.74	0.66	0.71	0.67	0.67	0.80	0.82	0.67	0.83	0.81	0.95	0.81	0.84	0.83	1.00																															
Jacobs Fork	0.66	0.67	0.82	0.73	0.81	0.77	0.65	0.74	0.70	0.66	0.70	0.87	0.58	0.82	0.73	0.70	0.84	0.80	0.78	0.72	1.00																														
Katie Branch	0.71	0.81	0.65	0.79	0.83	0.82	0.78	0.82	0.80	0.80	0.85	0.80	0.52	0.90	0.81	0.90	0.77	0.80	0.64	0.87	0.72	1.00																													
Laurel Branch	0.90	0.93	0.77	0.92	0.96	0.93	0.91	0.92	0.91	0.92	0.94	0.91	0.68	0.83	0.97	0.89	0.88	0.95	0.83	0.81	0.77	0.86	1.00																												
Laurel Fork	0.76	0.83	0.78	0.80	0.83	0.87	0.80	0.89	0.81	0.82	0.89	0.79	0.48	0.87	0.69	0.82	0.75	0.68	0.67	0.75	0.68	0.83	0.76	1.00																											
Left Fork Coal Creek	0.82	0.84	0.75	0.90	0.91	0.85	0.82	0.88	0.86	0.82	0.86	0.95	0.59	0.73	0.79	0.83	0.98	0.87	0.91	0.81	0.85	0.77	0.87	0.74	1.00																										
Left Fork Lick Creek	0.91	0.87	0.95	0.89	0.91	0.88	0.87	0.89	0.88	0.88	0.87	0.85	0.52	0.87	0.82	0.80	0.83	0.85	0.84	0.71	0.83	0.76	0.86	0.82	0.85	1.00																									
Levisa Fork	0.93	0.77	0.94	0.80	0.82	0.81	0.79	0.77	0.76	0.79	0.79	0.73	0.54	0.81	0.78	0.77	0.73	0.79	0.82	0.67	0.73	0.62	0.78	0.72	0.73	0.94	1.00																								
Lewis Creek	0.96	0.87	0.85	0.89	0.92	0.89	0.87	0.87	0.86	0.88	0.91	0.86	0.71	0.82	0.95	0.90	0.86	0.96	0.90	0.83	0.76	0.78	0.95	0.73	0.86	0.90	0.90	1.00																							
Little Town Hill Creek	0.96	0.92	0.92	0.93	0.95	0.94	0.92	0.91	0.91	0.92	0.92	0.88	0.53	0.84	0.85	0.85	0.88	0.85	0.87	0.73	0.79	0.73	0.90	0.81	0.86	0.95	0.94	0.93	1.00																						
Loop Creek	0.92	0.94	0.83	0.93	0.97	0.91	0.92	0.96	0.94	0.94	0.94	0.93	0.54	0.78	0.88	0.86	0.91	0.89	0.88	0.76	0.76	0.80	0.93	0.82	0.92	0.91	0.79	0.91	0.92	1.00																					
Lowe Branch	0.79	0.86	0.69	0.90	0.91	0.88	0.84	0.88	0.87	0.84	0.87	0.94	0.53	0.71	0.76	0.81	0.99	0.80	0.84	0.78	0.81	0.76	0.86	0.73	0.96	0.79	0.68	0.82	0.85	0.88	1.00																				
DS Big Lick Creek	0.73	0.77	0.79	0.78	0.85	0.84	0.76	0.78	0.77	0.76	0.78	0.80	0.62	0.88	0.85	0.75	0.76	0.84	0.67	0.69	0.89	0.80	0.88	0.69	0.74	0.83	0.77	0.83	0.82	0.75	0.74	1.00																			
Big Lick	0.92	0.74	0.82	0.81	0.79	0.77	0.78	0.73	0.77	0.78	0.79	0.67	0.59	0.71	0.78	0.80	0.73	0.82	0.83	0.69	0.65	0.61	0.78	0.65	0.75	0.88	0.93	0.91	0.88	0.79	0.68	0.69	1.00																		
Middle Creek	0.85	0.85	0.87	0.85	0.93	0.90	0.83	0.88	0.84	0.85	0.87	0.91	0.67	0.91	0.93	0.83	0.84	0.92	0.81	0.79	0.90	0.85	0.95	0.78	0.83	0.90	0.83	0.92	0.89	0.88	0.82	0.96	0.75	1.00																	
Mill Creek	0.79	0.82	0.81	0.81	0.88	0.87	0.81	0.82	0.80	0.82	0.83	0.83	0.64	0.89	0.91	0.79	0.77	0.87	0.70	0.72	0.85	0.83	0.93	0.72	0.75	0.84	0.78	0.87	0.85	0.80	0.75	0.99	0.70	0.98	1.00																
Moll Creek	0.93	0.99	0.80	0.98	0.99	0.97	0.99	0.98	0.98	0.99	0.97	0.89	0.49	0.79	0.86	0.86	0.90	0.84	0.81	0.73	0.71	0.81	0.94	0.82	0.88	0.90	0.81	0.91	0.94	0.96	0.89	0.78	0.80	0.87	0.82	1.00															
Moses Branch	0.92	0.96	0.79	0.99	0.98	0.96	0.96	0.96	0.96	0.96	0.96	0.92	0.53	0.78	0.84	0.87	0.95	0.85	0.85	0.77	0.77	0.80	0.93	0.80	0.94	0.89	0.80	0.91	0.94	0.95	0.94	0.78	0.81	0.87	0.81	0.98	1.00														
Mountain Branch	0.92	0.98	0.76	0.98	0.96	0.97	0.99	0.94	0.98	0.99	0.95	0.81	0.43	0.75	0.80	0.83	0.86	0.78	0.74	0.66	0.67	0.77	0.90	0.79	0.83	0.88	0.81	0.88	0.93	0.91	0.86	0.77	0.82	0.83	0.80	0.98	0.96	1.00													
Mudlick Creek	0.78	0.76	0.84	0.75	0.83	0.82	0.73	0.79	0.73	0.76	0.83	0.84	0.55	0.98	0.82	0.91	0.76	0.80	0.76	0.90	0.79	0.90	0.82	0.89	0.75	0.84	0.79	0.82	0.82	0.80	0.73	0.81	0.69	0.88	0.84	0.78	0.78	0.72	1.00												
Panther Branch	0.90	0.80	0.77	0.84	0.87	0.84	0.81	0.79	0.79	0.80	0.85	0.82	0.76	0.78	0.94	0.88	0.84	0.94	0.87	0.82	0.76	0.74	0.93	0.64	0.82	0.82	0.85	0.98	0.88	0.83	0.80	0.85	0.88	0.90	0.87	0.84	0.86	0.83	0.77	1.00											
Pine Creek	0.69	0.75	0.76	0.74	0.81	0.83	0.73	0.77	0.73	0.74	0.80	0.79	0.50	0.98	0.77	0.86	0.73	0.74	0.64	0.85	0.81	0.93	0.81	0.85	0.71	0.78	0.71	0.76	0.77	0.73	0.71	0.88	0.61	0.88	0.88	0.75	0.75	0.72	0.96	0.74	1.00										
Right Fork Garden Creek	0.62	0.46	0.89	0.50	0.59	0.55	0.45	0.54	0.43	0.46	0.50	0.66	0.48	0.77	0.57	0.53	0.58	0.61	0.69	0.57	0.79	0.49	0.53	0.62	0.58	0.78	0.81	0.64	0.71	0.55	0.53	0.69	0.62	0.73	0.67	0.51	0.54	0.46	0.76	0.60	0.68	1.00																		
Right Fork Lick Creek	0.87	0.80	0.88	0.81	0.88	0.86	0.80	0.81	0.77	0.81	0.83	0.83	0.69	0.90	0.92	0.82	0.77	0.90	0.79	0.77	0.83	0.78	0.92	0.72	0.75	0.88	0.88	0.93	0.89	0.81	0.74	0.95	0.79	0.97	0.97	0.83	0.82	0.80	0.86	0.93	0.86	0.76	1.00																	
Rocky Fork	0.95	0.96	0.83	0.98	0.98	0.95	0.96	0.95	0.96	0.96	0.95	0.91	0.54	0.77	0.87	0.87	0.93	0.88	0.88	0.76	0.75	0.77	0.94	0.79	0.92	0.91	0.84	0.94	0.96	0.97	0.91	0.77	0.85	0.87	0.81	0.98	0.99	0.96	0.78	0.88	0.73	0.56	0.84	1.00																
Seven Spring Creek	0.70	0.78	0.71	0.79	0.86	0.83	0.75	0.81	0.77	0.77	0.80	0.87	0.69	0.85	0.89	0.78	0.82	0.87	0.71	0.78	0.87	0.86	0.92	0.69	0.80	0.78	0.66	0.83	0.77	0.79	0.81	0.95	0.61	0.96	0.96	0.80	0.81	0.75	0.81	0.84	0.87	0.61	0.91	0.78	1.00															
Sinking Creek	0.85	0.86	0.81	0.84	0.89	0.86	0.85	0.87	0.85	0.87	0.87	0.82	0.63	0.86	0.94	0.81	0.75	0.91	0.73	0.73	0.75	0.85	0.94	0.76	0.77	0.89	0.80	0.91	0.85	0.88	0.72	0.89	0.76	0.94	0.93	0.87	0.83	0.83	0.83	0.86	0.81	0.61	0.92	0.85	0.89	1.00														
Stone Branch	0.88	0.88	0.74	0.90	0.92	0.91	0.88	0.86	0.88	0.88	0.90	0.84	0.69	0.80	0.93	0.88	0.86	0.93	0.80	0.80	0.77	0.83	0.97	0.69	0.85	0.84	0.80	0.96	0.88	0.87	0.84	0.90	0.82	0.92	0.92	0.90	0.91	0.90	0.77	0.96	0.79	0.52	0.92	0.91	0.90	0.91	1.00													
Strow Creek	0.93	0.85	0.82	0.86	0.90	0.85	0.84	0.85	0.84	0.85	0.88	0.86	0.73	0.80	0.97	0.88	0.83	0.98	0.90	0.81	0.76	0.79	0.96	0.70	0.85	0.88	0.85	0.98	0.90	0.92	0.79	0.83	0.87	0.92	0.88	0.88	0.88	0.84	0.80	0.96	0.74	0.60	0.91	0.91	0.84	0.92	0.95	1.00												
Sulphur Spring Creek	0.66	0.48	0.61	0.54	0.61	0.55	0.47	0.51	0.47	0.48	0.58	0.68	0.86	0.63	0.86	0.72	0.64	0.89	0.78	0.77	0.68	0.60	0.76	0.39	0.65	0.60	0.64	0.82	0.62	0.61	0.58	0.74	0.68	0.77	0.75	0.55	0.59	0.49	0.65	0.88	0.61	0.56	0.81	0.62	0.77	0.73	0.79	0.84	1.00											
Town Hill Creek	0.87	0.83	0.89	0.84	0.90	0.88	0.83	0.84	0.82	0.84	0.85	0.84	0.67	0.91	0.92	0.82	0.79	0.91	0.79	0.76	0.85	0.81	0.93	0.75	0.78	0.91	0.89	0.93	0.91	0.84	0.76	0.96	0.82	0.98	0.97	0.85	0.85	0.83	0.86	0.92	0.87	0.75	0.99	0.86	0.91	0.94	0.93	0.92	0.78	1.00										
Trib to Big Moccasin Creek	0.91	0.95	0.73	0.97	0.93	0.95	0.98	0.90	0.96	0.96	0.93	0.77	0.41	0.72	0.77	0.81	0.85	0.75	0.72	0.64	0.65	0.72	0.88	0.75	0.81	0.85	0.81	0.87	0.92	0.87	0.84	0.75	0.82	0.79	0.78	0.95	0.95	0.99	0.69	0.83	0.69	0.44	0.78	0.94	0.71	0.79	0.89	0.82	0.48	0.81	1.00									
Trib to North Fork Clinch	0.60	0.70	0.67	0.70	0.77	0.80	0.67	0.81	0.69	0.68	0.76	0.78	0.74	0.75	0.71	0.63	0.74	0.72	0.61	0.63	0.82	0.72	0.77	0.78	0.72	0.72	0.60	0.69	0.70	0.70	0.74	0.84	0.53	0.84	0.83	0.71	0.72	0.66	0.71	0.68	0.76	0.62	0.78	0.68	0.87	0.76	0.75	0.68	0.58	0.79	0.63	1.00								
Trib to Thompsons Branch	0.90	0.96	0.76	0.98	0.95	0.94	0.96	0.94	0.96	0.96	0.93	0.85	0.47	0.75	0.80	0.83	0.91	0.81	0.79	0.71	0.72	0.78	0.90	0.76	0.90	0.89	0.81	0.89	0.92	0.92	0.90	0.76	0.82	0.83	0.78	0.97	0.98	0.97	0.73	0.84	0.71	0.50	0.79	0.97	0.77	0.84	0.90	0.85	0.54	0.82	0.96	0.68	1.00							
US Big Lick	0.82	0.57	0.78	0.64	0.67	0.60	0.57	0.61	0.56	0.57	0.64	0.74	0.68	0.61	0.75	0.74	0.74	0.83	0.95	0.75	0.66	0.50	0.69	0.53	0.78	0.77	0.83	0.85	0.78	0.73	0.67	0.57	0.86	0.69	0.59	0.65	0.70	0.59	0.67	0.83	0.51	0.71	0.72	0.76	0.56	0.64	0.70	0.84	0.79	0.71	0.59	0.46	0.66	1.00						
US Hart Creek	0.93	0.96	0.79	0.98	0.97	0.96	0.97	0.93	0.97	0.96	0.95	0.86	0.49	0.75	0.82	0.86	0.91	0.83	0.83	0.72	0.74	0.75	0.91	0.77	0.90	0.89	0.83	0.91	0.95	0.94	0.90	0.77	0.85	0.84	0.80	0.97	0.98	0.98	0.73	0.87	0.71	0.50	0.81	0.99	0.75	0.81	0.91	0.88	0.57	0.84	0.98	0.67	0.97	0.70	1.00					
US Pine	0.79	0.72	0.74	0.81	0.81	0.75	0.71	0.75	0.76	0.71	0.76	0.84	0.61	0.66	0.73	0.79	0.90	0.84	0.92	0.76	0.81	0.65	0.78	0.64	0.94	0.81	0.74	0.83	0.83	0.84	0.86	0.69	0.83	0.76	0.67	0.78	0.85	0.75	0.68	0.82	0.62	0.59	0.70	0.86	0.69	0.67	0.79	0.83	0.69	0.74	0.74	0.62	0.82	0.86	0.85	1.00				
US Chaney Creek	0.70	0.62	0.62	0.66	0.71	0.68	0.60	0.66	0.61	0.62	0.75	0.78	0.71	0.82	0.81	0.93	0.76	0.85	0.80	0.99	0.70	0.86	0.79	0.69	0.77	0.67	0.65	0.82	0.68	0.71	0.72	0.70	0.68	0.77	0.72	0.67	0.71	0.61	0.87	0.83	0.83	0.56	0.76	0.71	0.78	0.73	0.79	0.81	0.82	0.76	0.58	0.61	0.66	0.75	0.66	0.74	1.00			
Vall Creek	0.87	0.79	0.81	0.86	0.88	0.82	0.78	0.83	0.80	0.78	0.84	0.93	0.65	0.72	0.83	0.85	0.95	0.90	0.98	0.84	0.82	0.69	0.86	0.70	0.96	0.85	0.81	0.91	0.89	0.90	0.91	0.71	0.82	0.83	0.73	0.85	0.91	0.80	0.77	0.88	0.68	0.66	0.79	0.92	0.76	0.74	0.84	0.89	0.75	0.80	0.78	0.66	0.86	0.90	0.88	0.94	0.80	1.00		
West Fork Big Creek	0.65	0.61	0.75	0.64	0.73	0.72	0.59	0.67	0.60	0.60	0.72	0.80	0.60	0.93	0.74	0.85	0.75	0.76	0.74	0.92	0.84	0.85	0.74	0.77	0.73	0.73	0.69	0.75	0.72	0.67	0.72	0.80	0.61	0.84	0.79	0.66	0.69	0.59	0.95	0.75	0.94	0.76	0.82	0.68	0.82	0.72	0.73	0.72	0.71	0.81	0.57	0.72	0.64	0.66	0.63	0.69	0.91	0.75	1.00	
Wrights Valley Creek	0.71	0.76	0.76	0.77	0.86	0.81	0.72	0.81	0.75	0.74	0.79	0.93	0.70	0.84	0.89	0.79	0.87	0.90	0.80	0.81	0.91	0.83	0.90	0.70	0.86	0.79	0.67	0.83	0.79	0.82	0.85	0.91	0.61	0.95	0.92	0.79	0.82	0.71	0.84	0.84	0.84	0.67	0.89	0.80	0.97	0.86	0.86	0.85	0.80	0.89	0.67	0.85	0.75	0.66	0.75	0.76	0.80	0.83	0.85	1.00

Appendix E: Fin ray counts taken from 82 *Chrosomus* sp. cf. *saylori*. Age ranged from young-of-year (YOY) to two, and 19 fish were no aged. Missing data points denotes absence or damage to the fin.

Stream	Collection Date	Age	Standard Length	Anal Fin	Caudal Fin	Dorsal Fin	Pectoral Fin	Pelvic Fin
Grassy Branch	9/30/2009	YOY	32.20	8	17	8	14	8
Grassy Branch	9/30/2009	YOY	30.46	8	17	8	14	8
Grassy Branch	9/30/2009	YOY	29.03	8	17	8	14	8
Grassy Branch	9/30/2009	YOY	30.89	8	17	8	14	8
Big Lick Creek	4/1/2012	1	50.95	8	17	8	14	7
Big Lick Creek	4/1/2012	1	41.01	8	17	8	14	9
DS Hart Creek	6/13/2011	1	36.82	8	17	8	14	9
Grassy Branch	9/30/2009	1	45.18	9	17	8	14	8
Grassy Branch	9/30/2009	1	38.36	9	17	8	14	8
Grassy Branch	9/30/2009	1	40.23	8	17	8	14	8
Grassy Branch	9/30/2009	1	34.92	8	17	8	14	8
Grassy Branch	9/30/2009	1	47.57	8	18	8	14	8
Grassy Branch	9/30/2009	1	46.23	8	16	8	14	8
Grassy Branch	9/30/2009	1	33.98	8	17	8	14	8
Grassy Branch	9/30/2009	1	37.17	8	17	8	14	8
Grassy Branch	9/30/2009	1	35.27	8	17	8	14	8
Hess Creek	7/22/2011	1	46.83	8	17	8	14	8
Hess Creek	7/22/2011	1	46.07	8	17	8	14	9
Hurricane Fork	7/20/2011	1	38.11	8	15	8	14	8
Hurricane Fork	7/20/2011	1	36.92	9	17	9	14	8
Hurricane Fork	7/20/2011	1	40.85	8	17	8	14	8
Left Fork Coal Creek	11/18/2009	1	48.72	8	16	8	14	8
Little Town Hill	11/18/2009	1	41.67	8	17	8	14	8
Little Town Hill	11/18/2009	1	42.00	8	17	8	14	8
Little Town Hill	11/18/2009	1	37.00	8	17	8	14	8
Little Town Hill	11/18/2009	1	40.90	8	17	8	14	8
Little Town Hill	11/18/2009	1	37.53	8	17	8	14	8
Little Town Hill	11/18/2009	1	42.07	8	17	8	14	8
Little Town Hill	11/18/2009	1	40.12	8	17	8	14	8
North Branch Indian Creek	10/16/2009	1	35.24	8	17	8	14	8
Pine Creek	6/24/2011	1	41.16	8	17	8	14	7
Pine Creek	6/24/2011	1	42.37	8	17	8	14	7
Pine Creek	6/24/2011	1	40.52	8	17	8	14	8
Pine Creek	6/24/2011	1	38.64	8	17	8	13	8
Town Hill Creek	7/21/2011	1	44.88	8	17	8	14	8

Town Hill Creek	7/21/2011	1	37.63	9	15	8	14	8
Upstream Big Lick	7/3/2012	1	40.26	8	17	8	14	8
Big Lick Creek	4/1/2012	2	47.05	8	17	8	14	9
Grassy Branch	9/30/2009	2	53.78	8	17	8	14	8
Left Fork Coal Creek	5/24/2011	2	55.12	8	16	8	14	8
Left Fork Coal Creek	5/24/2011	2	50.51	8	17	8	14	8
Upstream Big Lick	7/3/2012	2	55.13	8	17	8	14	8
Upstream Big Lick	7/3/2012	2	54.53	8	16	8	14	8
Upstream Big Lick	7/3/2012	2	54.59	8	16	8	14	8
Upstream Big Lick	7/3/2012	2	57.92	8	17	8	14	8
Upstream Big Lick	7/3/2012	2	52.75	8	17	9	14	8
Upstream Big Lick	7/3/2012	2	57.28	8	17	8	14	8
Upstream Hess	7/5/2012	2	59.53	8	17	9	13	8
Upstream Hess	7/5/2012	2	51.05	8	17	9	14	8
Upstream Hess	7/5/2012	2	52.30	8	17	9	14	8
Upstream Hess	7/5/2012	2	55.28	8	17	8	14	8
Upstream Hess	7/5/2012	2	53.05	8	16	8	15	8
Upstream Hess	7/5/2012	2	47.69	8	17	8	14	8
Upstream Hess	7/5/2012	2	57.61	8	17	8	12	8
Upstream Hess	7/5/2012	2	61.40	8	17	8	14	8
Upstream Hess	7/5/2012	2	52.52	9	17	9	14	8
Upstream Hess	7/5/2012	2	50.32	9	17	9	14	8
Upstream Hess	7/5/2012	2	60.16	8	17	8	14	8
Upstream Hess	7/5/2012	2	54.47	8	17	9	14	8
Upstream Hess	7/5/2012	2	53.49	8	17	8	14	8
US Hart Creek	6/9/2011	2	56.71	8	19	8	14	8
US Hart Creek	6/9/2011	2	50.76	8	17	8	14	9
US Hart Creek	6/9/2011	2	50.18	8	19	8	14	8
Grassy Branch	9/30/2009	Unaged	37.16	8	17	8	14	8
Grassy Branch	9/30/2009	Unaged	30.67	8	16	8	14	8
Grassy Branch	9/30/2009	Unaged	37.97	8	17	8	14	8
Grassy Branch	9/30/2009	Unaged	35.66	8	17	8	14	8
Grassy Branch	9/30/2009	Unaged	33.99	8	17	8	14	8
Hurricane Fork	7/20/2011	Unaged	58.60	9	17	9	14	9
Hurricane Fork	7/20/2011	Unaged	40.96	9	17	8	14	8
Hurricane Fork	7/20/2011	Unaged	39.36	8	17	8	14	8
Left Fork Coal Creek	5/24/2011	Unaged	49.63	8	17	9	13	8
Little Town Hill	11/18/2009	Unaged	43.54	8	17	8	14	8
Little Town Hill	11/18/2009	Unaged	41.88	8	17	8	14	8

Appendix E: continued.

Town Hill Creek	11/18/2009	Unaged	40.36	8		8	14	8
Town Hill Creek	7/21/2011	Unaged	46.23	7	17	8	14	7
Tributary to Jackson Fork	5/30/2011	Unaged	53.82	9	17	9	14	7
Upstream Big Lick	7/3/2012	Unaged	47.27	8	16	8	14	8
Upstream Big Lick	7/3/2012	Unaged	52.86	8	17	8	14	8
Upstream Hess	7/5/2012	Unaged	51.44	8	17	9	14	8
US Hart Creek	6/9/2011	Unaged	50.72	8	17	8	14	7
US Hart Creek	6/9/2011	Unaged	57.24	9	17	9	15	9

Appendix F: Scale counts taken from 82 *Chrosomus* sp. cf. *saylori*. Ages ranged from young-of-year (YOY) to two, and 19 fish were not aged. Missing data points denotes absence of scales.

Stream	Collection Date	Age	Midlateral	Lateral Line	Above Lateral Line	Below Lateral Line	Caudal Peduncle	Circumferential	Horizontal Rows	Predorsal
Grassy Branch	9/30/2009	YOY	62	19	8	13	22	32	20	30
Grassy Branch	9/30/2009	YOY	52				18	40	22	37
Grassy Branch	9/30/2009	YOY	63				16	30	22	25
Grassy Branch	9/30/2009	YOY	55				20	32	24	26
Big Lick Creek	4/1/2012	1	68	30	21	11	32	48	34	42
Big Lick Creek	4/1/2012	1	68	17	16	11	24	50	24	30
DS Hart Creek	6/13/2011	1	69	0	14	21	20	42	26	32
Grassy Branch	9/30/2009	1	75	14	15	11	26	52	24	36
Grassy Branch	9/30/2009	1	74	8	16	10	22	46	24	38
Grassy Branch	9/30/2009	1	64	8	13	10	26	48	22	32
Grassy Branch	9/30/2009	1	62	17	20	11	22	32	20	32
Grassy Branch	9/30/2009	1	64	50	12	16	24	48	24	38
Grassy Branch	9/30/2009	1	74	20	16	13	22	54	25	38
Grassy Branch	9/30/2009	1	57				20	44	23	32
Grassy Branch	9/30/2009	1	70				18	24	25	27
Grassy Branch	9/30/2009	1	67	10	16	11	24	48	23	30
Hess Creek	7/22/2011	1	71	21	17	9	28	52	26	32
Hess Creek	7/22/2011	1	65	20	17	10	24	50	22	33
Hurricane Fork	7/20/2011	1	70	27	18	10	22	44	25	30
Hurricane Fork	7/20/2011	1	78	22	21	11	24	48	34	39
Hurricane Fork	7/20/2011	1	72	19	19	10	22	48	32	35
Left Fork Coal Creek	11/18/2009	1	67	22	14	11	26	50	24	37
Little Town Hill	11/18/2009	1	68	22	15	10	22	50	26	30
Little Town Hill	11/18/2009	1	64	18	10	15	24	52	22	30

Appendix F: continued.

Little Town Hill	11/18/2009	1	58	8	12	9	22	50	22	25
Little Town Hill	11/18/2009	1	64	11	14	9	22	46	23	26
Little Town Hill	11/18/2009	1	65	14	16	11	24	48	26	32
Little Town Hill	11/18/2009	1	67	10	16	11	22	48	23	30
Little Town Hill	11/18/2009	1	65				24	50	24	34
North Branch Indian Creek	10/16/2009	1	62	13	15	11	11	50	24	35
Pine Creek	6/24/2011	1	64	19	12	9	24	52	23	32
Pine Creek	6/24/2011	1	62	26	15	9	18	44	21	34
Pine Creek	6/24/2011	1	64	20	17	13	22	48	25	31
Pine Creek	6/24/2011	1	74	14	18	11	30	48	34	49
Town Hill Creek	7/21/2011	1	67	14	17	12	28	54	25	34
Town Hill Creek	7/21/2011	1	67	27	16	12	22	52	23	26
Upstream Big Lick	7/3/2012	1	84	11	17	11	24	44	26	32
Big Lick Creek	4/1/2012	2	70	22	23	11	34	46	32	42
Grassy Branch	9/30/2009	2	82	22	17	11	28	58	28	38
Left Fork Coal Creek	5/24/2011	2	74	35	19	11	32	64	32	40
Left Fork Coal Creek	5/24/2011	2	78	24	20	11	28	48	28	34
Upstream Big Lick	7/3/2012	2	72	40	16	11	24	48	32	33
Upstream Big Lick	7/3/2012	2	73	62	19	14	32	56	34	38
Upstream Big Lick	7/3/2012	2	72	40	16	11	28	50	26	36
Upstream Big Lick	7/3/2012	2	75	26	16	11	24	48	25	35
Upstream Big Lick	7/3/2012	2	73	27	14	10	24	48	29	32
Upstream Big Lick	7/3/2012	2	73	69	16	10	26	48	27	35
Upstream Hess	7/5/2012	2	79	28	20	12	30	56	32	35
Upstream Hess	7/5/2012	2	70	25	14	10	28	46	26	34
Upstream Hess	7/5/2012	2	78	21	16	12	26	54	34	38
Upstream Hess	7/5/2012	2	69	41	20	13	30	56	32	32

Appendix F: continued.

Upstream Hess	7/5/2012	2	66	31	18	12	24	48	28	34
Upstream Hess	7/5/2012	2	67	7	21	11	24	48	27	37
Upstream Hess	7/5/2012	2	76	22	16	12	26	50	36	36
Upstream Hess	7/5/2012	2	84	28	20	10	28	48	32	41
Upstream Hess	7/5/2012	2	74	27	20	11	24	50	26	39
Upstream Hess	7/5/2012	2	69	33	16	11	22	60	30	42
Upstream Hess	7/5/2012	2	86	27	18	11	24	62	33	45
Upstream Hess	7/5/2012	2	77	22	20	10	28	52	32	38
Upstream Hess	7/5/2012	2	84	20	21	9	23	54	32	36
US Hart Creek	6/9/2011	2	78	21	19	12	30	56	25	47
US Hart Creek	6/9/2011	2	74	24	16	11	26	48	32	34
US Hart Creek	6/9/2011	2	69	12	17	12	24	50	33	41
Grassy Branch	9/30/2009	Unaged	70	14	15	10	24	52	26	35
Grassy Branch	9/30/2009	Unaged	56				22	44	24	31
Grassy Branch	9/30/2009	Unaged	68	13	13	10	24	46	22	32
Grassy Branch	9/30/2009	Unaged	69	15	16	11	18	38	22	33
Grassy Branch	9/30/2009	Unaged	64	7	15	11	24	50	24	32
Hurricane Fork	7/20/2011	Unaged	74	21	18	9	28	60	25	39
Hurricane Fork	7/20/2011	Unaged	70	24	17	11	28	48	30	32
Hurricane Fork	7/20/2011	Unaged	73				18	30	50	33
Left Fork Coal Creek	5/24/2011	Unaged	78	54	20	11	34	54	29	39
Little Town Hill	11/18/2009	Unaged	74	22	19	11	26	52	25	34
Little Town Hill	11/18/2009	Unaged	68	12	14	11	26	52	27	33
Town Hill Creek	11/18/2009	Unaged	67	22	16	11	26	50	26	32
Town Hill Creek	7/21/2011	Unaged	69	20	17	12	22	54	26	33
Tributary to Jackson Fork	5/30/2011	Unaged	74	50	14	9	24	56	24	40
Upstream Big Lick	7/3/2012	Unaged	68	11	19	11	28	52	29	36
Upstream Big Lick	7/3/2012	Unaged	67	17	17	12	30	54	25	32

Appendix F: continued.

Upstream Hess	7/5/2012	Unaged	73	27	18	12	28	56	34	42
US Hart Creek	6/9/2011	Unaged	72	19	17	13	24	52	34	32
US Hart Creek	6/9/2011	Unaged	79	19	18	12	32	54	29	39

Appendix G: Counts of three internal morphological features for 63 *Chrosomus* sp. cf. *saylori*. Ages ranged from young-of-year (YOY) to two.

Stream	Collection Date	Age	Pharyngeal Tooth Formula	Gill Filaments	Gill Rakers
Grassy Branch	9/30/2009	YOY	0,4-4,0	30	14
Grassy Branch	9/30/2009	YOY	0,4-4,0	28	14
Grassy Branch	9/30/2009	YOY	0,4-4,0	27	14
Grassy Branch	9/30/2009	YOY	0,4-4,0	28	14
Big Lick Creek	4/1/2012	1	0,5-5,0	46	15
Big Lick Creek	4/1/2012	1	0,5-3,0	38	14
DS Hart Creek	6/13/2011	1	0,4-3,0	40	13
Grassy Branch	9/30/2009	1	0,5-4,0	36	12
Grassy Branch	9/30/2009	1	0,5-4,0	34	14
Grassy Branch	9/30/2009	1	0,5-4,0	34	12
Grassy Branch	9/30/2009	1	0,5-4,0	32	14
Grassy Branch	9/30/2009	1	0,5-4,0	32	14
Grassy Branch	9/30/2009	1	0,5-4,0	38	14
Grassy Branch	9/30/2009	1	0,4-4,0	32	12
Grassy Branch	9/30/2009	1	0,4-4,0	32	10
Grassy Branch	9/30/2009	1	0,4-4,0	33	12
Hess Creek	7/22/2011	1	0,5-4,0	36	14
Hess Creek	7/22/2011	1	0,4-4,0	33	12
Hurricane Fork	7/20/2011	1	0,5-4,0	33	14
Hurricane Fork	7/20/2011	1	0,5-4,0	33	8
Hurricane Fork	7/20/2011	1	0,4-4,0	36	14
Left Fork Coal Creek	11/18/2009	1	0,5-4,0	37	14
Little Town Hill	11/18/2009	1	0,4-4,0	40	12
Little Town Hill	11/18/2009	1	0,4-4,0	38	14
Little Town Hill	11/18/2009	1	0,5-4,0	30	14
Little Town Hill	11/18/2009	1	0,4-4,0	36	14
Little Town Hill	11/18/2009	1	0,5-4,0	32	12
Little Town Hill	11/18/2009	1	0,4-4,0	32	12
Little Town Hill	11/18/2009	1	0,5-4,0	35	14
North Branch Indian Creek	10/16/2009	1	0,4-4,0	29	8
Pine Creek	6/24/2011	1	0,5-4,0	33	13
Pine Creek	6/24/2011	1	0,5-4,0	43	14
Pine Creek	6/24/2011	1	0,5-4,0	38	14
Pine Creek	6/24/2011	1	0,5-4,0	33	16
Town Hill Creek	7/21/2011	1	0,5-4,0	38	12
Town Hill Creek	7/21/2011	1	0,3-4,0	32	13
Upstream Big Lick	7/3/2012	1	0,3-4,0	31	8

Appendix G: continued.

Big Lick Creek	4/1/2012	2	0,5-4,0	40	14
Grassy Branch	9/30/2009	2	0,5-4,0	36	14
Left Fork Coal Creek	5/24/2011	2	0,5-3,0	34	13
Left Fork Coal Creek	5/24/2011	2	0,4-4,0	35	13
Upstream Big Lick	7/3/2012	2	0,5-5,0	34	8
Upstream Big Lick	7/3/2012	2	0,5-4,0	44	14
Upstream Big Lick	7/3/2012	2	0,4-4,0	36	12
Upstream Big Lick	7/3/2012	2	0,4-4,0	34	8
Upstream Big Lick	7/3/2012	2	0,4-3,0	35	9
Upstream Big Lick	7/3/2012	2	0,5-4,0	32	11
Upstream Hess	7/5/2012	2	0,4-4,0	40	8
Upstream Hess	7/5/2012	2	0,5-4,0	43	13
Upstream Hess	7/5/2012	2	0,4-4,0	37	11
Upstream Hess	7/5/2012	2	0,5-4,0	38	12
Upstream Hess	7/5/2012	2	0,5-4,0	38	9
Upstream Hess	7/5/2012	2	0,5-4,0	34	15
Upstream Hess	7/5/2012	2	0,5-4,0	36	8
Upstream Hess	7/5/2012	2	0,5-4,0	42	13
Upstream Hess	7/5/2012	2	0,5-4,0	41	13
Upstream Hess	7/5/2012	2	0,5-4,0	35	9
Upstream Hess	7/5/2012	2	0,5-4,0	40	14
Upstream Hess	7/5/2012	2	0,5-5,0	35	8
Upstream Hess	7/5/2012	2	0,5-4,0	39	12
US Hart Creek	6/9/2011	2	0,4-3,0	40	12
US Hart Creek	6/9/2011	2	0,5-4,0	40	14
US Hart Creek	6/9/2011	2	0,4-4,0	40	15