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

Final Report

Contract Number: 2010-12295

Submitted to:

Virginia Department of Game and Inland Fisheries

by:

Shannon White, Graduate Research Assistant
Donald Orth, Principle Investigator

Department of Fish and Wildlife Conservation
Virginia Polytechnic Institute and State University
Blacksburg, VA 24061-0321

March 2013

Table of Contents

Acknowledgment

This report was completed with funds provided by the Virginia Department of Game and Inland Fisheries through a State Wildlife Grant from the U.S. Fish and Wildlife Service. We thank Michael Pinder and Brian Evans for assistance and encouragement in development of the project scope and methods. Christopher Skelton provided raw data and SAS codes and photo of *Chrosomus* sp. cf. *saylori*. Dave Neely provided the photo of *Chrosomus saylori*. We further thank Nate Adkins, Laci Coleman, Toby Coyner, Andy Dolloff, Chas Gowan, Emmanuel Frimpong, Bonnie Myers, Brandong Peoples, Alex Silvis, Patrick Snellings, Matt Vincent, and Caitlin Worsham, and other volunteers for their special contributions that contributed to successful completion of the project.

Executive Summary

In 1999 Clinch dace, *Chrosomus* sp. cf. *saylori*, was discovered in the Tennessee drainage of Virginia. Subsequent sampling of southwest Virginia and portions of Tennessee indicated that Clinch dace populations are small, fragmented, and of question viability. Further, riparian landuse and mining posed significant threats to critical habitat. As such, Clinch dace were listed as a Federal Species of Concern and on Virginia's Wildlife Action Plan as Tier II- Very High Conservation Need. A management plan and species description for Clinch dace is of utmost importance, but data on distribution and life history is needed before these objectives can be realized.

The objective of this study was threefold: 1) more narrowly describe the distribution of Clinch dace in the upper Clinch River watershed, particularly in Russell and Tazewell counties, Virginia, 2) describe internal and external morphology to determine diet and life history characteristics, and 3) make *in-situ* breeding observations to determine timing and mode of reproduction. To complete these objectives, we sampled 60 streams and measured 12 habitat variables in 2011 and 2012. During sampling, we preserved 82 specimens which were used to

measure internal and external morphological characteristics. We also used streambank observations and underwater videos to document spawning behavior.

We conclude that Clinch dace are restricted to eight small tributaries to the Clinch River. Multivariate analysis of habitat correlates indicated that Clinch dace most commonly inhabit small, high elevation streams with gravel substrate and forested watersheds. Morphologic, meristic, and size-at-age characteristics were similar between Clinch dace and other *Chrosomus*; however, significant differences in digestive anatomy suggest Clinch dace occupy a different trophic niche than congeners. Like many *Chrosomus,* Clinch dace had an observed nest association with central stonerollers (*Campostoma anomalum*); however, gonad weight and mature egg counts were significantly lower for Clinch dace compared to congeners.

Small population sizes coupled with several habitat threats suggest that Clinch dace viability is low in the present condition. As such, a proactive management protocol must be implemented in an attempt to conserve and recover remaining populations. We suggest that management be approached from three outlets including continued research, implementing conservation initiatives, and listing at the state and federal level. Future studies should focus on assessing perceived threats, in particular watershed development and barriers to connectivity, while maintaining a routine sampling protocol to determine spatial and temporal trends in populations. Conservation of Clinch dace habitat through preservation of riparian land and removing barriers to migration will increase juvenile viability and recruitment. Further, additional listing at the state and federal level could offer more legal protection for Clinch dace and allow for mandated riparian conservation.

Introduction

Given their small scales, minnows in the genus *Chrosomus* (family: Cyprinidae) 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).

Of interest here are the *Chrosomus* minnows in the subgenus *Chrosomus* including Tennessee dace (*C. tennesseensis*), mountain redbelly dace (*C. oreas*), laurel dace (*C. saylori*), blackside dace (*C. cumberlandensis*), northern redbelly dace (*C. eos*), and southern redbelly (*C. erythrogaster*). 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).

The conservation status of *Chrosomus* minnows is one of two extremes. Southern redbelly dace, northern redbelly dace, and mountain redbelly dace are distributed across a fairly broad landscape and usually exist in high abundance. The remaining three species, laurel dace, blackside dace, and Tennessee dace, have all been identified as vulnerable and in need of conservation and restoration due to small population sizes and habitat threats. The Endangered Species Act listed blackside dace as threatened in 1987 (U.S. Fish and Wildlife Service [USFWS] 1987), and recognized laurel dace as endangered in 2011 (USFWS 2011). In Tennessee, blackside dace has been listed as threatened, laurel dace endangered, and Tennessee dace as in need of a management plan. Kentucky has also listed blackside dace as threatened.

In 1999, a new species of *Chrosomus* was discovered in the upper Clinch River watershed in Mudlick Creek, in Tazewell County, Virginia. 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 laurel dace (Skelton 2002), and similar morphological characteristics between the unknown fish and laurel dace initially led biologists to assume that Mudlick Creek supported a disjoint population of laurel dace.

In 2002, Skelton (2007) observed the newly-discovered fish in breeding colors and noticed that the fish had two white spots at the base of the caudal fin and a more complete upper lateral band (Figure 1). As laurel dace do not have these features, Skelton (2007) determined that the fish was likely a new, undescribed species hereinafter referred to as Clinch dace (*C*. sp. cf. *saylori*). At present, a genetic description of Clinch dace is underway, and initial evidence supports the conclusions that Clinch dace and laurel dace are separate species (Dave Neely, Tennessee Aquarium Conservation Institute [TACI], personal communication).

Figure 1. *Photographs of laurel dace (top, D. Neely) and Clinch dace (bottom, C. Skelton).*

Discovery of this new population initiated widespread sampling by Skelton (2007) to assess the distribution of *Chrosomus* in the Clinch River drainage and surrounding watersheds. His efforts lasted from 1999-2007 and concluded that Clinch dace are distributed in 16 streams across eight tributaries to the upper Clinch River watershed (Figure 2). 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 decrease stream health and function (Fritz et al. 2010). Sampling from Skelton (2007) also indicated that populations of Clinch dace are small, fragmented, and of questionable viability. Because of these threats, Clinch dace 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 Clinch dace is of high priority. However, sampling completed 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 Clinch dace, nor infer information about population size and stability. Furthermore, habitat correlates, life history, and reproductive mode have never been studied for Clinch dace. Thus, critical information about the species is still unknown, and a successful conservation plan and species description cannot be completed without a more extensive study of distribution, life history, and morphology.

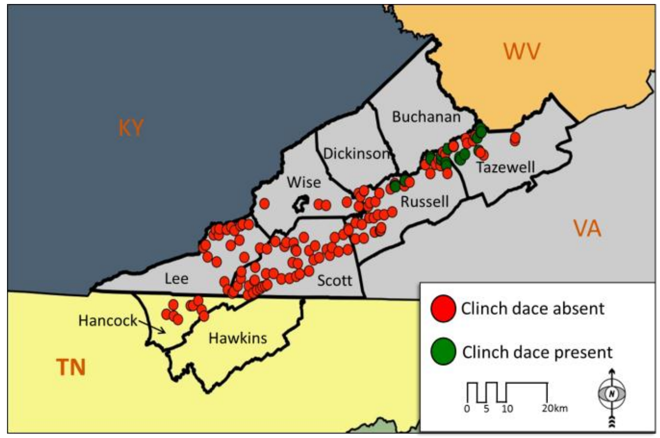

Figure 2. *Distribution of Clinch dace as determined by Skelton (2007).*

The objective of this research was threefold: 1) describe the distribution and habitat correlates of Clinch dace and comment on the availability of critical habitat, 2) determine major life history characteristics of Clinch dace and compare to other *Chrosomus*, and 3) describe the reproductive behavior and morphology of Clinch dace. This document reports the major conclusions of the study and provides comments on future conservation initiatives.

Methods

Stream occupancy and habitat

To describe the distribution of Clinch dace in the upper Clinch River watershed, we sampled (one-pass backpack electrofishing) 60 streams. Streams were primarily in Russell and Tazewell counties, Virginia, with a few in Buchanan, Virginia and McDowell, West Virginia. For most streams, we sampled at least six 50-m subsections spanning across 600 m (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 composition of habitats and fish fauna.

In each sampled subsection, habitat was measured at transects spaced 10 m apart. At each transect, we 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. In addition to these microhabitat variables, we quantified macrohabitat using a Geographic Information Systems (GIS). Variables included 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). Clinch dace habitat correlates were determined using nonmetric multi-dimensional scaling (NMDS).

Life history and morphology

We measured standard length, 33 external morphological features, and 11 meristic variables for 82 Clinch dace. In addition, we dissected 63 fish to remove otoliths for age analysis, determine stomach contents and shape, number of pharyngeal teeth, and number of gill rakers. From these data, we calculated size-at-age, and compared Clinch dace morphology and trophic placement to other *Chrosomus*.

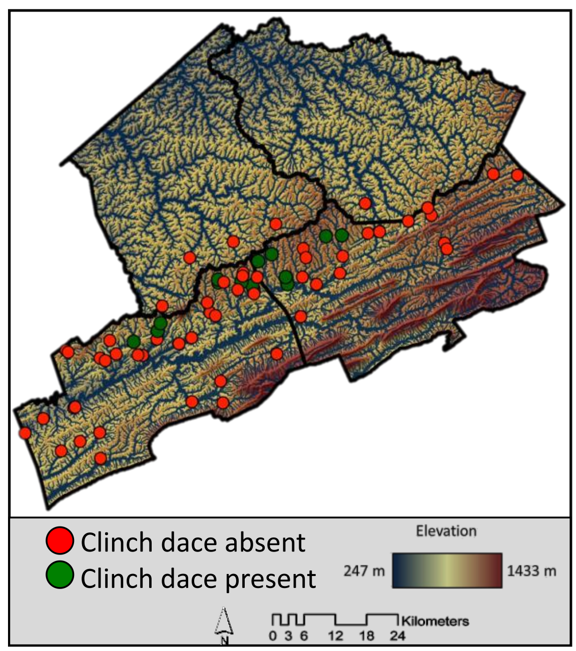

***Figure 3**. Sites with (green) and without (red) Clinch dace in 2011-2012 sampling.*

Reproductive biology

We monitored breeding behavior at Big Lick Creek in Tazewell County, Virginia from 19 May -9 June 2012 using stream bank observations and underwater cameras. We calculated a gonadosomatic index (GSI) for all dissected fish by dividing the gonad weight by the body weight and multiplying by 100. The number of mature eggs in each female was also counted. The GSI was used to infer the timing and duration of spawning.

Results

Stream occupancy and habitat

We captured a total of 78 Clinch dace. Only 14 of the 60 sampling locations were occupied (Figure 3, Appendix A). These locations were distributed in eight drainages to the Clinch River, and the average number of individuals at each site was less than six. On average, Clinch dace 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). None of these microhabitat variables were statistically significant from streams that did not have Clinch dace. For macrohabitat variables, average stream slope for sites with Clinch dace 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 were not inhabited by Clinch dace (Appendices B, C). The results of the NMDS support the conclusion that Clinch dace are most often located in small streams (i.e. low pool volume and stream width and depth) that have gravel substrates and located in watersheds with little development (Figure 4).

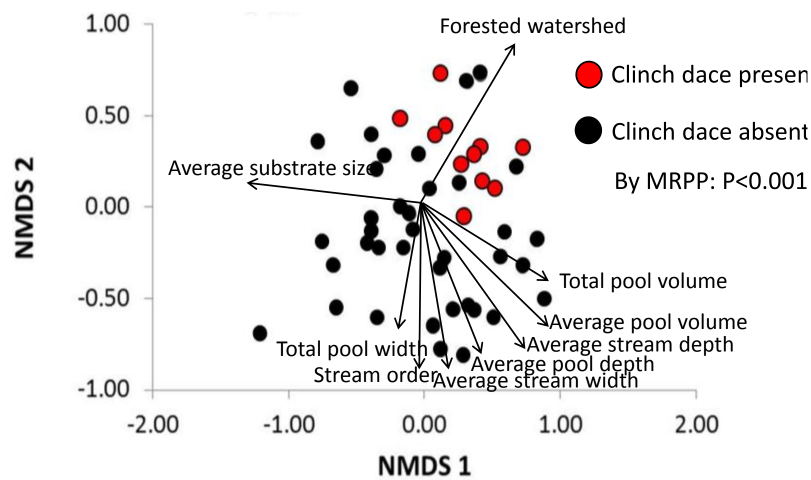

Figure 4. *NMDS ordination with significantly correlated environmental variables.*

Life history and morphology

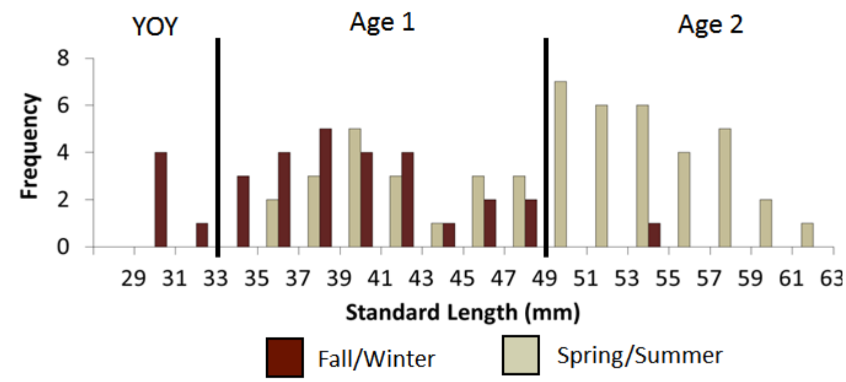

Figure 5. *Length frequency histogram for Clinch dace captured in fall/winter (red, n=23) and spring/summer (brown n=40). Ages of fish, as determined by otolith analysis are also indicated.*

There were four young-of-year (YOY), 33 year-one, and 26 year-two fish in our sample. Maximum size of YOY fish was 37.97 mm, year-one fish 50.95 mm, and year-two fish 61.4 mm (Figure 5). Fish had a steady growth rate of approximately one mm per month (y=1.01x+29.63). 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.

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. 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.

Clinch dace 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. The basioccipital plate was largely rounded. The average intestine length was 0.63 times the standard length (SE=0.019), and had an S-shape without extensive coiling. Macroinvertebrates were the predominant stomach content.

Reproductive biology

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 and air temperature 16 °C. For two days prior to spawning, central stonerollers (*Campostoma anomalum*) were seen making three shallow pits, and these pits were subsequently used by Clinch dace 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 an 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, Clinch dace retreated to a downstream pool, and subsequently continued to move further away from the spawning site. Colors were noticeably muted by 28 May. Four central stonerollers returned to the original spawning site and began to build pits on 2 June, but Clinch dace were never seen at the spawning site again.

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.

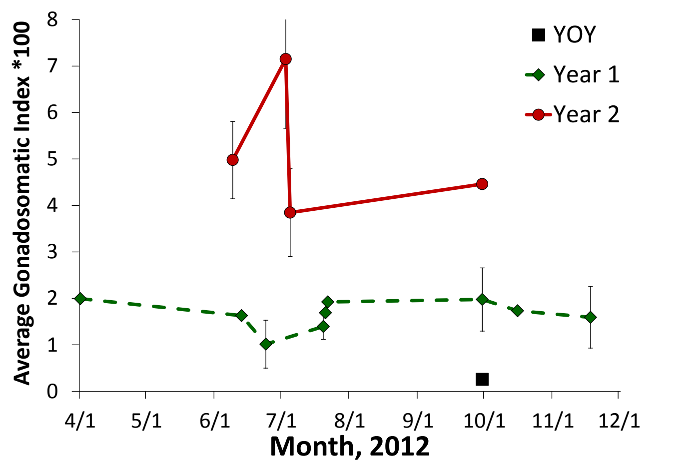

Figure 6. *Average GSI for three age classes of female Clinch dace from 1 April-18 November, 2012.*

For two-year-old females, the GSI was 4.98 in June, which increased to 7.15 in early July before declining (Figure 6). The GSI for one-year-old and young-of-year females was significantly lower than two-year-olds, indicating reproductive maturity is not reached until year-two.

There was a 3:1 female-male sex ratio in our 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.

Conclusions

Combined with historical surveys (Skelton 2007, Appendix D), we conclude that Clinch dace have a narrow distribution and occur in just eight tributaries to the upper Clinch River. Populations are small and fragmented, many with questionable within-and between-stream connectivity. As such, many streams are likely inhabited by populations that do not have long-term viability. Clinch dace morphology and life history were similar compared to closely-related congeners. However, a diet composed primarily of macroinvertebrates and a shorter intestine indicates Clinch dace may occupy a different trophic niche compared to other *Chrosomus*. While breeding behavior of Clinch dace was similar to that of all other documented *Chrosomus* spawning, lower egg counts and delayed age of maturation indicate Clinch dace fecundity may be lower.

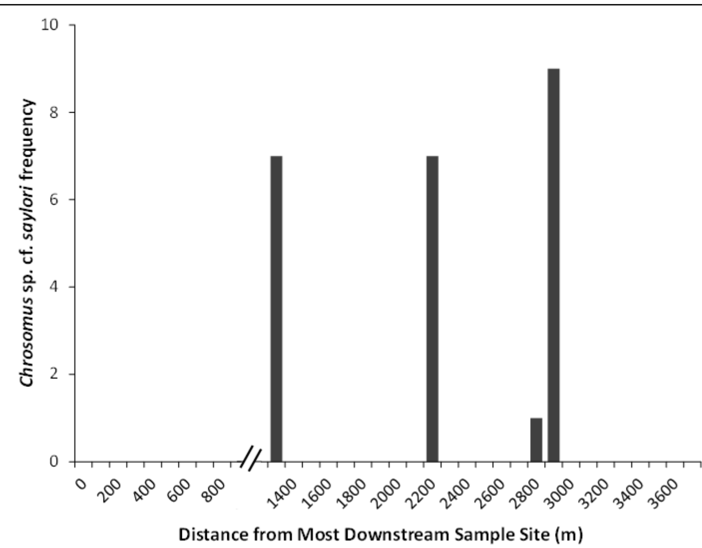

Figure 7. *Frequency of Clinch dace capture in Big Lick Creek starting at the most downstream sample site and continuing for 3600 m upstream. The break on the x-axis reflects a section of stream not sampled.*

Between 1 and 13 individuals were captured at a single site. Though single-pass electrofishing cannot be used to estimate population sizes, a low number of individuals caught on that one pass would be indicative of a small population (Bateman et al. 2005, Reid et al. 2008). Of more concern is the sporadic occurrence of Clinch dace within a stream as it was common to find Clinch dace occurrences separated by 400 m or more (Figure 7). This patchy distribution combined with multiple beaver dams and road crossings at most streams questions whether there are multiple subpopulations within a single stream.

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). This combined with the possibility of within-stream subpopulations indicates

Clinch dace likely do not adhere to "50-500 rule" posited by Franklin (1980), and substantiated by others (Jamieson and Allendorf 2012). This theory 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 Clinch dace is unknown. However, it is likely that the average N_e of many Clinch dace 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 Clinch dace is less than 500 individuals. We lack the long-term genetic and observational data necessary to make definitive conclusions about the rate of genetic drift, and thus N_e, for Clinch dace. However, consider the equation $N_e=4N_mN_f/(N_m+N_f)$, where N_f is the number of reproducing females and N_m is the number of reproducing males, an equation that has been shown to be a reliable predictor of N_e for populations with skewed sex ratios. Applying this to the documented 3:1 females biased sex ratio for Clinch dace, each population of Clinch dace would have to consist of 167 males and 501 females for $N_e>500$. The maximum number of reproducing individuals (i.e. age-two) at a single stream was 13. Though our sampling protocol likely underestimates population size, it is reasonable to conclude that populations are not approaching that needed to secure long-term survival.

The biggest threat to Clinch dace is habitat degradation. We found a significant correlation between streams occupied by Clinch dace and forested watersheds, and studies of *Chrosomus* have documented declines in populations following watershed development (Slack et al. 1997). The evolution of nest association has allowed Clinch dace to continue occupying streams with suboptimal habitat. However, heavy siltation is still a major cause for concern for Clinch dace. The presence of multiple culverts, beaver dams, and watershed activities, including agriculture, mining, development, and road construction are all likely sources of increased siltation into streams occupied by Clinch dace.

The size-at-age estimates in this study are consistent with those from Tennessee dace (Hamed et al. 2008), southern redbelly dace (Settles and Hoyt 1976, Settles and Hoyt 1978), and blackside dace (Starnes and Starnes 1981; Appendix E). It is impossible to determine if the absolute maximum age of Clinch dace is two years. However, given that there were relatively few two-year-old fish in fall samples of Clinch dace and southern redbelly dace (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.

While *Chrosomus* have been reported to reach sexual maturation at one-year (Starnes and Starnes 1981), only two-year-old female Clinch dace had mature ova. Maximum GSI for Clinch was 7.5, which is significantly lower than that for southern redbelly dace (12.6; Settles and Hoyt 1978) and northern redbelly dace (10; Das and Nelson 1990). Furthermore, female Clinch dace only had 267.3 mature eggs per female, whereas southern redbelly dace had 568 (Settles and Hoyt 1978). Loss of a year of spawning coupled with fewer eggs per female suggests that Clinch dace fecundity and overall fitness is likely lower than that of closely related congeners.

Management and Conservation Recommendations

Given the narrow distribution and relative low reproductive output, proactive management of Clinch dace is a necessity and should be accomplish through a three-pronged approach. This includes ongoing research to monitor changes in population size and distribution, pursuit of more stringent listing at the state and federal level, and immediate conservation measures to protect the current status of Clinch dace. This multifaceted approach will be necessary to address remaining uncertainties about the species while preventing further population collapse.

Continuing studies

The present study elucidated the basic distribution and ecology of Clinch dace. There are several withstanding questions; most notably the degree to which various perceived stressors negatively impact Clinch dace populations. The known distribution of Clinch dace completely overlaps the distribution of bituminous coal in Virginia and, while mining has been empirically shown to decrease stream health, the effects on Clinch dace are presently uncertain. Because the majority of mining operations in Russell and Tazewell counties are subsurface, mining may preserve forest cover, thereby improving overall Clinch dace habitat. Likewise, *Chrosomus* are believed to be able to survive in beaver ponds (Hägglund and Sjöberg 1999), but extensive ponding and siltation are suboptimal habitat types for Clinch dace. Three streams in this study had heavy beaver activity that took place between sampling by Skelton (2007) and the completion of this study, and in two instances there was a decline in Clinch dace populations (the third stream could not be sampled due to deep ponding). A formal study to document response of Clinch dace to mining and beaver activity should be completed, particularly since beaver populations in the Appalachian Mountains are increasing (Fuller and Peckarsky 2011). This should include extensive sampling upstream of beaver dams to determine if Clinch dace occupy ponds and monitoring of populations adjacent to ponds to determine how populations respond to dams.

A genetic description of Clinch dace by TACI is presently underway with the goal of providing a species description. However, of greatest conservation need is a description of the genetic structure of the species across the landscape. Knowing the degree of genetic relatedness within and between streams will provide insights into the movement potential of the species, effective population sizes, and whether there are subpopulations forming within single streams. Furthermore, continued genetic analyses could prove beneficial for future studies using environmental DNA, a sampling protocol that may be ideal given that stream access is limited by private ownership, stream temperatures are cool (thus preserving genetic material longer), habitat volumes are low, and Clinch dace are distributed allopatrically to other *Chrosomus* in the upper Clinch River watershed.

In 2012, a population of Clinch dace was discovered in Tennessee in 2012 in the Rugby State National Area (Dave Neely, TACI, personal communication). Initial genetic analysis suggested that these fish were not simply translocated, but are a historic population that was fragmented from Virginia populations many generations ago. Subsequent sampling of neighboring tributaries did not reveal additional populations of Clinch dace. In the future, the degree of relatedness between Virginia and Tennessee populations of Clinch dace should be determined and additional sampling of Tennessee populations should be completed to determine population size.

Of greatest need is a long-term monitoring program to determine the degree of spatial and temporal variability of Clinch dace populations across the landscape. We noted a decline or loss of populations reported by Skelton (2007), but it is unknown if this represents a normal fluctuation or a trend towards population collapse. As such, we recommend a routine monitoring program for all accessible streams that Clinch dace occupy as well as surrounding tributaries in accordance with protocols established by Vesely et al. (2006). This would ideally include assessments at least every four years (i.e. every other generation) using either seining or streambank observations to reduce sampling-induced mortality. The later technique would be ideal given that breeding dress distinguishes Clinch dace from all other species from April-August, even without binoculars. Alternatively, sampling could be through minnow traps (Bryant 2002), as Clinch dace are commonly found in traps and a passive sampling design may be more time efficient. Regardless of technique, we suggest that observations be completed during summer to aid in identification and be completed for only pools as this is the habitat unit that Clinch dace most often occupy during summer months.

State and Federal Listing

Given the narrow distribution, small local abundance, and habitat specificity (Pritt and Frimpong 2010), Clinch dace are characterized as one of the rarest of fish species in the country. As such, more stringent protection is warranted. The precedence is already in place as three other species of *Chrosomus* are listed at state and federal levels and, on average, these listed species have larger population sizes and distributions.

Among other criteria, successful petitions for listing of a species (even if the species is presently undescribed) under the U.S. Endangered Species Act (ESA) must show that there is a significant threat to critical habitat, existing regulations are inadequate, and there are other natural or anthropogenic factors that may threaten continued existence. This study identified clear examples under which the three aforementioned criteria apply to Clinch dace populations. As such, an ESA petition would likely be successful. Following federal listing, landowner agreements such as Safe Harbor Agreements (Bean et al. 2001) and Habitat Conservation Agreements (HCAs) could then be formed in order to protect Clinch dace populations. These agreements offer binding, legal contracts which specify specific actions required of each landowner to reduce threats to listed populations. For Clinch dace, these agreements may be particularly useful for owners of large tracts of land, such as timber and coal companies, and any owners of point source problems that are unwilling to collaborate with conservation practices.

At the state level, identification of critical habitat and clear threats to that habitat would justify upgrading the species from Tier II to Tier I on the Virginia Wildlife Action Plan. Furthermore, Clinch dace are presently not included in the Virginia Endangered Species Act, and listing at that level would provide more protection while allowing additional funds for research and conservation.

Conservation initiatives

While listing would legally force landowners to institute conservation practices, petitioning for recognition as threatened or endangered is a long process. Further, listing may not be the most appropriate option given the desire to continue handling and studying the species and the potential to lose public support for further study. However, delaying the implementation of conservation initiatives until a decision about listing has been reached should not be viewed as a viable strategy for Clinch dace management. There are options for contractual, federal conservation agreements for nonlisted species. For example, Candidate Conservation Agreements with Assurances (CCAAs) operate much like HCAs and SHAs, but are specific to candidate or potential candidate species. As Clinch dace is currently recognized as a Federal Species of Concern, CCAAs would be appropriate even without further listing. VDGIF's Landowner Incentive Program (LIP), which is currently available for the majority of streams occupied by Clinch dace, could be used by private landowners to mitigate costs associated with stream restoration, culvert replacement, and riparian buffering to aid in habitat restoration. Because both CCAAs and LIPs can be used for nonlisted species, they may be particularly appealing to landowners, VDOT, and mining agencies in hopes of preventing or delaying further listing of Clinch dace (and thus more stringent, mandated federal policies).

Even if CCAAS and LIPs are not pursued, habitat conservation is of upmost importance for Clinch dace survival. Because the species is a broadcast spawner, fish depend on clean gravel substrate. However, many streams are dominated by silt from runoff from riparian landuse. While year-around efforts to decrease the amount of silt in streams would benefit Clinch dace, the establishment of spawning windows would offer the greatest benefit. During this time road construction, riparian land disturbance, and stream modifications should be minimized. These windows would ideally be from 15 April -1 August to protect spawning activity of Clinch dace and nest associates while also improving egg viability and juvenile survival.

The establishment of habitat protection agreements and long-term monitoring programs will only be successful if professional relationships are developed with stakeholders. This includes landowners, but also other vested companies and organizations. Virginia Department of Mines, Minerals, and Energy (VDMME) and Virginia Department of Transportation (VDOT) should be made aware of the current status of Clinch dace as many permits and regulations that affect Clinch dace are advised by these organizations. Of particular interest is a partnership with VDOT as future conservation efforts will surely focus on replacement of road crossing. As such, completing a Crossing Assessment Decision Support System, a method developed by the U.S. Department of Agriculture to determine the impact of fish migration barriers, and working with VDOT to implement necessary changes will be important.

If studies of genetic structuring show that Clinch dace populations have intermediate relatedness (Vrijenhoek 1998, George et al. 2009), captive breeding and translocations may be a viable mechanism for increasing population sizes. Captive breeding of *Chrosomus* has had mixed results. Rakes et al. (1999) were able to breed blackside dace in captivity when exposed to pheromones from river chubs (*Nocomis micropogon*) and central stoneroller. However, in some instances, only 20% of eggs were successfully fertilized and produced viable offspring. Thus, establishing captive breeding for Clinch dace may not be a feasible option with current population sizes as it could result in unnecessary mortality with only limited success. Given this, translocations may offer the best initial option. If translocations are used, priority should focus on first re-establishing a connected population within a single stream, and then transferring individuals across streams to aid in population recovery. Only after fragmented populations have been reconnected should reintroductions into unoccupied streams be attempted. Regardless of the methods, long-term monitoring efforts that enable adaptive management opportunities should be pursued to ensure transplanted fish are actually improving population sizes. However, it should be noted that if Clinch dace are listed on the U.S. ESA, more research would be needed to assign populations as a Nonessential Experimental Population in order for translocations to be attempted.

At present, Clinch dace are not included on VDGIF's list of no take species, and a significant source of mortality is from minnow traps. Nearly 30% of all streams occupied by Clinch dace had at least one active minnow trap during sampling. Furthermore, while blackside dace and Tennessee dace are on restriction, *Chrosomus* identification is extremely difficulty making this restriction difficult to follow and enforce. As such, *Chrosomus* minnows remain a popular bait fish, and unintentional translocations pose a significant threat to current populations of threated *Chrosomus*. At present, translocated mountain redbelly dace introduced into the North Fork Holston drainage are threatening populations of Tennessee dace. Furthermore, constant changes in distribution of blackside dace are causing significant delays in permitting for agencies such as VDOT and DMME which must adhere to ESA restrictions when setting and upholding regulations.

In order to reduce translocation, a measure that would aid *Chrosomus* conservation and reduce permitting burdens for state agencies, we recommend a no take restriction be placed on all Virginia *Chrosomus*. To make this restriction easy to interpret and enforce, we suggest marketing it as not simply a restriction of *Chrosomus*, but using a slogan such as "red and black, put it back." This slogan posted with pictures on VDGIF websites, at bait and tackle shops, and potentially publicized on local news (both television and paper) and radio would offer a clear guideline for handling *Chrosomus*. This strategy will reduce adult fishing mortality, which is the age class most vulnerable to minnow traps.

Literature Cited

Bean, M. J., J. P. Jenny, and B. van Eerden. 2011. Safe Harbor Agreements: carving out a new role for NGOs. *Conservation in Practice* 2: 8-15.

Bestgen, K. R. 1989. Distribution and notes on the biology of *Phoxinus eos* (Cyprinidae) in Colorado. *The Southwestern Naturalist* 34: 225-231.

Das, M. K., and J. S. Nelson. 1990. Spawning time and fecundity of northern redbelly dace, *Phoxinus eos*, finescale dace, *Phoxinus neogaeus*, and their hybrids in Upper Pierre Grey Lake, Alberta. *The Canadian Field-Naturalist* 104: 409-413.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135-150 *in* M. E. Soule and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts.

Fritz, K. M., S. F. Fulton, B. R. Johnson, C. D. Barton, J. D. Jack, D. A. Word, and R. A. Burke. 2010. Structure and functional characteristics of natural and constructed channels draining a reclaimed mountaintop removal and valley fill coal mine. *Journal of the North American Benthological Society* 29: 673-689.

Fuller, M. R., and B. L. Peckarsky. 2011. Ecosystem engineering by beavers affects mayfly life histories. *Freshwater Biology* 56: 969-979.

George, A. L., B. R. Kuhajda, J. D. James, M. A. Cantrell, P. L. Rakes, and J. R. Shute. 2009. Guidelines for propagation and translocation for freshwater fish conservation. *Fisheries* 34: 529-545.

Hägglund, Å., and S. Göran. 1999. Effects of beaver dams on the fish fauna of forest streams. *Forest Ecology and Management* 115: 259-266.

Hamed, M. K., F. J. Alsop, and T. F. Laughlin. 2008. Life history traits of the Tennessee dace (*Phoxinus tennesseensis*) in northeast Tennessee. *American Midland Naturalist* 160: 289-299.

Hubbs, C. L., and K. F. Lagler. 1964. Fishes of the Great Lakes region. University of Michigan Press, Ann Arbor.

Jamieson, I. G., and F. W. Allendorf. 2012. How does the 50/500 rule apply to MVPs? *Trends in Ecology and Evolution* 27: 578-584.

Jenkins, R. E., and N. M. Burkhead. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Maryland, USA.

Johnston, C. E., and L. M. Page. 1992. The evolution of complex reproductive strategies in North American minnows (Cyprinidae). Pages 600-621 i*n* R. L. Mayden, editor.

Systematics, historical ecology, and North American freshwater fishes. Stanford University Press, Stanford.

Joseph, L. N., S. A. Field, C. Wilcox, and H. P. Possingham. 2006. Presence-absence versus abundance data for monitoring threatened species. *Conservation Biology* 20: 1670-1687.

Pritt, J. J., and E. A. Frimpong. 2010. Quantitative determination of rarity of freshwater fishes and implications for imperiled-species designations. *Conservation Biology* 24: 1249-1258.

Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. *Environmental Biology of Fishes* 55: 31-42.

Settles, W. H., and R. D. Hoyt. 1976. Age structure, growth patterns, and food habits of the southern redbelly dace *Chrosomus erythrogaster* in Kentucky. *Transactions of the Kentucky Academy of Science* 37: 1-10.

Settles, W. H., and R. D. Hoyt. 1978. The reproductive biology of the southern redbelly dace, *Chrosomus erythrogaster* Rafinesque, in a spring-fed stream in Kentucky. *American Midland Naturalist* 99: 290-298.

Skelton, C. E. 2002. New dace of the genus *Phoxinus* (Cyprinidae: Cypriniformes) from the Tennessee River drainage, Tennessee. *Copeia* 2001: 118-123.

Skelton, C. E. 2007. Distribution and status of blackside dace (*Phoxinus cumberlandensis*) and C. sp. cf. saylori (*Phoxinus* sp. cf. *saylori*) in upper Clinch River system, Virginia. Final Report, Virginia Department of Game and Inland Fisheries, Richmond, VA.

Slack, W. T., M. T. O'Connell, T. L. Peterson, J. A. Ewing III, and S. T. Ross. 1997. Ichthyofaunal and habitat associations of disjunct populations of southern redbelly dace. *Phoxinus erythrogaster* (Teleostei: Cyprinidae) in Mississippi. *American Midland Naturalist* 137: 251-265.

Starnes, W. C., and L. B. Starnes. 1978. A new cyprinid of the genus *Phoxinus* endemic to the upper Cumberland River drainage. *Copeia* 1978: 508-516.

U.S. Fish and Wildlife Service. 1987. Determination of endangered status for the blackside dace. *Federal Register* 52: 22580-22585.

U.S. Fish and Wildlife Service. 2011. Endangered status for the Cumberland darter, rush darter, yellowcheek darter, chucky madtom, and laurel dace. *Federal Register* 76: 48722-48741.

Vesely, D., B.C. McComb, C. D. Vojta, L. H. Suring, J. Halaj, R. S. Holthausen, B. Zuckerberg, and P. M. Manley. 2006. Development of protocols to inventory or monitor wildlife, fish, or rare plants. Gen. Tech. Rep. WO-72. Washington, DC: U.S. Department of Agriculture, Forest Service. 100 p.

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
	Campostoma anomalum	24
	Notropis leuciodus	15
	Luxilus albeolus	27
	Catostomus commersoni	2
Copper Creek	*Rhinichthys atratulus*	47

Appendix A: continued		
	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
	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

Appendix A: continued		
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
	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

Appendix A: continued		
	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
	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

Appendix A: continued		
	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
	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

Appendix A: continued		
	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
	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

Appendix A: continued		
	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

Appendix A: continued		
	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
	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 Clinch dace 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 Clinch dace 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. Presence (1) and absence (0) of ten species at ten sites sampled in 2009 by Toby Coyner (Virginia Tech).

Stream Name	Clinch dace	Blacknose dace	Whitetail shiner	Sculpin spp.	Green sunfish	Creek chub	Snubnose darter	Rosyside dace	Fantail darter	Central stoneroller
Grassy Branch	1	1	0	0	0	0	0	0	0	0
Unnamed tributary to Swords Creek	0	1	1	1	1	1	1	0	0	0
Hart Creek	1	1	0	0	0	1	0	1	1	0
Greasy Creek	0	0	0	0	0	1	0	0	0	0
Greasy Creek	0	1	0	0	0	1	0	0	0	0
North Branch of Indian Creek	0	1	0	0	0	1	0	1	0	0
Left Fork Coal Creek	1	1	0	0	0	1	0	0	0	1
Mudlick Creek	0	1	0	1	1	0	0	0	1	1
Town Hill Creek	1	1	0	0	0	1	0	0	0	1
Little Town Hill Creek	1	1	0	1	0	1	0	0	0	1

Appendix E. Life history and meristic data gathered from historic literature for six described *Chrosomus* species and Clinch dace. Missing information denotes absence of data in literature. N/A indicates that the field is not applicable for that species.

	Size (mm) at Age													
Species	0	1	2	3	Age of Sexual Maturity	Duration of Spawning Season	Average Number of Mature Ova per Mature Female	Maximum Female GSI	Slope of Length-Weight Regression	Ray Counts	Pharyngeal Tooth Formula	Number of Gill Rakers	Predominant Food Source	Intestine Shape and Length
Blackside dace	20-34	39-57	62-64	N/A	1	April-June				8-9 anal 14-16 pectoral	5-5	11-13	sand	highly coiled, 3.6x SL
Mountain redbelly					1					8 dorsal 8 anal 8 pelvic 13-16 pectoral	5-5			
Northern redbelly					1	May- August		19		8 dorsal 8 anal				
Southern rebelly	29-45	37-60	56-65	N/A	1	mid April-June	568	12.7	3.033	10 dorsal 11 anal 16 pectoral				
Tennessee dace	15-30	31-45	40-56	57+	1					8 dorsal 7 anal 8 pelvic 15 pectoral 19 caudal	5-5	11-13	few invertebrates	coiled
Laurel dace										8 dorsal 8 anal 8 pelvic 16 pectoral	5-4	8-9	mostly invertebrate	S-shape lacking extensive coils
Clinch dace	29-32	34-50	47-61	N/A	2	mid May-mid July	267.3	7.5	3.4106	8-9 dorsal 8-9 anal 8 pelvic 14 pectoral 17 caudal	5-4	8-16	mostly invertebrate	S-shape lacking extensive coils, 0.63 x SL