Distribution and Population Characterization of Clinch Dace (*Chrosomus* sp. cf. *saylori*) in the Upper Clinch River System, Virginia

By

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Abstract

The Clinch Dace *Chrosomus* sp. cf. *saylori* is a species of minnow known from only two counties in Virginia. Prior surveys established the species' presence in just eight tributaries to the upper Clinch River. A management plan, which should include both population monitoring and habitat restoration, is still lacking for the species. Population monitoring must balance high detection probability with low risk of injury to captured individuals. I sampled 98 sites in 2014 and 2015 within the putative range of Clinch Dace to refine estimates of its distribution. I sampled 70 of the 98 sites with multiple gears and replication in an occupancy modeling framework. Clinch Dace occupied low-gradient headwater streams with relatively low conductivity in forested watersheds. My surveys uncovered two new tributaries occupied by Clinch Dace, and I was unable to find Clinch Dace in two historically occupied streams. Species detection probability was higher with backpack electrofishing than minnow trapping. *N*-mixture models suggest that Clinch Dace are more abundant in watersheds with high forest cover although forest cover is highly correlated spatially in the nested stream network. Density estimates from mark-recapture sampling suggest that Clinch Dace occur at low densities in approximately 31.5 km of headwater streams. The mean estimate of global population size was 6,706 individuals. Some populations could be affected by low genetic diversity. I conclude by developing a prioritization framework for restoration and protection of 15 candidate conservation areas. Managers should work with private landowners to implement best management practices in high priority watersheds.

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Preface

This thesis is organized as three chapters. I intend to submit each chapter for consideration for publication in the peer-reviewed literature. With the advisement of my committee I have identified journals that would be appropriate destinations for each chapter.

Chapter One: Using presence-absence data to inform monitoring protocols and habitat management for *Chrosomus* sp. cf. *saylori* (Clinch Dace)- **Transactions of the American Fisheries Society**

Chapter Two: Estimating Range and Density of *Chrosomus sp. cf. saylori* in the Upper Clinch River Basin in Virginia- **Copeia**

Chapter Three: Systematic Conservation Planning for *Chrosomus sp. cf saylori*- **Aquatic Conservation**

General Introduction

In local vernacular, the "hollers" ruggedly etched out of the Central Appalachian hills and mountains by a tined network of headwater streams have long concealed undiscovered biological diversity from the nets and eyes of ecologists. This region is known as a globally important biological diversity hotspot for the high number of endemic aquatic species, including fishes, mussels, and amphibians. The Clinch River in Virginia contains more aquatic diversity than any other river basin in the state. Unfortunately, many of these species are rare or imperiled due to a landscape that has been highly altered by anthropogenic development (Bernhardt and Palmer 2011). In response to these threats, The Nature Conservancy named the Clinch Basin its number one hotspot for imperiled species. The organization reports that 29 species of imperiled or vulnerable freshwater mussels and 19 species of fish call the Clinch River home (Master et al. 1998). Some of the biological diversity still lacks taxonomic recognition within the scientific community. It is likely that the list of endemic and imperiled fauna will grow if (when) a new species of *Chrosomus* dace is officially described.

The Cyprinid genus *Chrosomus*, formerly known as *Phoxinus*, includes seven described species in North America: Finescale Dace *Chrosomus neogaeus*, Northern Redbelly Dace *C. eos*, Southern Redbelly Dace *C. erythrogaster*, Mountain Redbelly Dace *C. oreas*, Blackside Dace *C. cumberlandensis*, Laurel Dace *C. saylori*, and Tennessee Dace *C. tennesseensis*. The most recent taxonomic investigation of the genus (Strange and Mayden 2009) assigned *C. neogaeus* to the subgenus *Pfrille* and the other six described species to the subgenus *Chrosomus*. This thesis is focused on an undescribed species of *Chrosomus*.

In 1999, populations of a *Chrosomus* dace were discovered in Mudlick Creek (Skelton 2007), and subsequently in the Indian Creek watershed in Tazewell County, Virginia (Lingenfelser et al. 2004). Up until this point, *Chrosomus* daces were not known from the upper Clinch watershed (Jenkins and Burkhead 1994). Christopher Skelton realized that this population likely represented an undescribed species separate from the closely related Laurel Dace (Skelton 2001). The Laurel Dace is restricted to six

streams on Walden Ridge in the upper Tennessee River drainage (Skelton 2007). Skelton based his

distinction on variation in nuptial coloration. The new *Chrosomus* taxon had two gold spots on the caudal

peduncle that were absent in the Laurel Dace. Since his discovery, researchers have referred to this form

as the Clinch Dace *Chrosomus* sp. cf. *saylori*, which remains undescribed, although life history,

morphometric and meristic data support its classification as a distinct species (White and Orth 2013b,

White and Orth 2014a). Biologists believed the Clinch Dace was endemic to headwaters of Clinch River

tributaries north of the mainstem river in Russell and Tazewell counties (White and Orth 2013a),

Virginia; however, in 2012, distant and presumably native populations of what appear to be Clinch Dace

were discovered in Rugby State Natural Area, Tennessee in the upper Cumberland basin and in two

streams in the Emory basin (a tributary to the Clinch River) in Tennessee. These discoveries further

complicate the phylogenetic understanding of the genus (Dave Neely, Tennessee Aquarium Conservation

Institute, personal communication 2014).

In addition to nuptial coloration, the Clinch Dace is distinguished from the Laurel Dace by anal

fin base length, head depth, and pectoral and caudal ray counts (White and Orth 2013b). The sexes are

difficult to distinguish without dissection, although males can develop pearl organs when reproductively

active and relative body position of fins changes as females become gravid (White and Orth 2013b).

Comparisons of internal morphology show that Clinch Dace have shorter intestine lengths and fewer

pharyngeal teeth than other *Chrosomus* species, adaptations for a diet higher in animal matter (White and

Orth 2013b). Despite the morphological differences, discrimination of closely related species may be

difficult in juveniles and nonbreeding adults.

Most species of *Chrosomus* dace, excluding *C. eos* and *C. neogaeus*, are headwater stream

specialists. For instance, Clinch Dace populations are rarely found in streams larger than third order and 4

m in average wetted width. This headwater ecological niche is uncommon among Virginia stream fishes.

Jenkins and Burkhead (1994) name only one other species that is restricted to these types of habitats, the

Rosyside Dace (*Clinostomus funduloides*), which co-occurs and even occasionally may hybridize with

Clinch Dace in the Middle and Indian Creek systems in Tazewell Co, VA (Michael Moore, personal

observation 2014 and 2015). This headwater restriction suggests that *Chrosomus* populations are sensitive to piscine predation or competition from cyprinids with similar ecological niches. Clinch Dace are rarely found alongside centrarchid or other piscivorous fishes other than Creek Chub (*Semotilus atromaculatus*).

The Clinch Dace is considered a Tier I species (very high conservation need) in the Virginia Wildlife Action Plan, endangered by Jelks et al. (2008), and "critically imperiled" by NatureServe (2015), but does not appear on the Virginia or Federal endangered species lists. During sampling conducted from 1999-2007, Skelton located only 16 streams of eight larger tributaries in the upper Clinch basin occupied by Clinch Dace (Skelton 2007). These tributary systems were Big Creek, Coal Creek, Indian Creek, Middle Fork Clinch River/Dumps Creek, Mudlick Creek, Swords Creek, Town Hill Creek, and Weaver Creek (Figure 1).
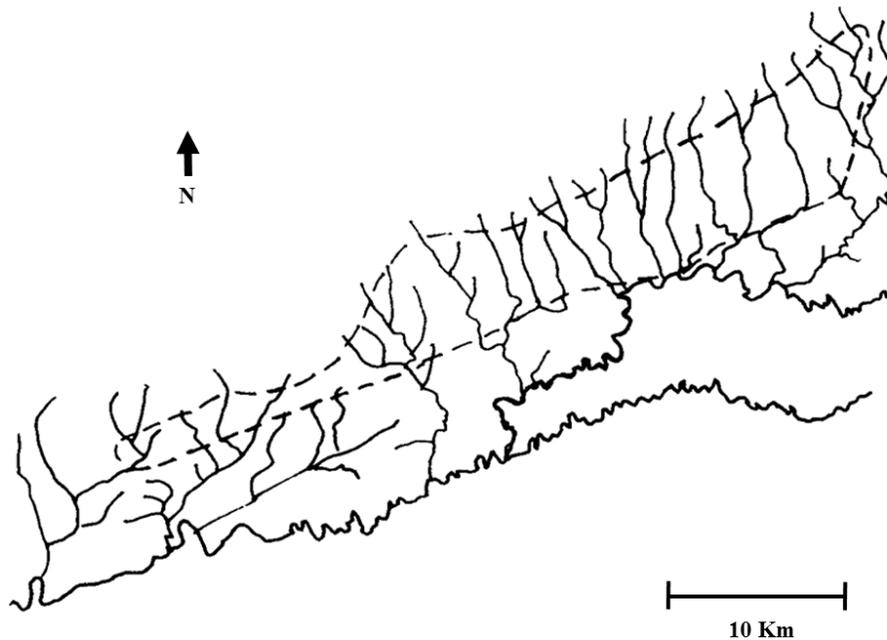
Figure 1: Map depicting the putative range of Clinch Dace following Skelton's (2007) surveys in the Upper Clinch River watershed

Additional surveys conducted in 2011 and 2012 established Clinch Dace presence at 14 of 60 sites sampled (23% naïve occupancy, also known as sampling prevalence in the distribution modeling literature) (White and Orth 2014a). These surveys discovered Clinch Dace in only one new stream,

namely Jackson Fork, a tributary to Indian Creek in Tazewell County. Nevertheless, ~125 km of unsampled 2nd-and 3rd-order streams remained within the species' presumed range (White 2012).

The few populations of Clinch Dace that exist are likely small and fragmented (White and Orth 2012; White and Orth 2014a). Clinch Dace may occur at lower local densities than any of the other *Chrosomus* daces due to the Clinch Dace's more specialized diet, higher trophic position, shorter lifespan, and lower fecundity. Clinch Dace sex ratios were female-biased (3:1 female to male), while other congeners have male-biased sex ratios (Hamed et al. 2008; White 2012; White and Orth 2014b). Other *Chrosomus* mature at age 1 and have higher average fecundities than Clinch Dace (Settles and Hoyt 1978; Das and Nelson 1990; Hamed et al. 2008; White and Orth 2014b). Clinch Dace usually reach maturity at age 2 and die following spawning, which reduces the intrinsic population growth rate (White and Orth 2014a).

Observed reproductive timing is similar to that of other *Chrosomus* species. Reproduction may occur from April-July, but the peak period seems to occur in late May and early June. Clinch Dace were observed on Stoneroller (*Campostoma* spp.) or Creek Chub nests on the 23 May and 6 June in two streams in 2011 (White and Orth 2014b), on 4-6 June in 2014 (Hatcher et al., Virginia Tech University, unpublished data), and 2-5 June, 2015.

A taxonomic species description will motivate interest in Clinch Dace conservation. Clinch Dace may then be a candidate for listing under the Federal Endangered Species Act. The rarity of Clinch Dace is comparable to those of other federally protected *Chrosomus* species. The most closely related taxon, the Laurel Dace, is listed as endangered and the Blackside Dace is listed as threatened. Laurel Dace occupy six streams in three stream clusters (George et al. 2015), while Blackside Dace occupy roughly 125 streams in 52 stream clusters or populations (Biggins 1988; Bivens et al. 2013; O' Bara 1990; Floyd 2015; Skelton 2013). Prior to this study, Clinch Dace were known from only 15 streams in eight stream clusters (Skelton 2007; White and Orth 2014a).

Past surveys for Clinch Dace characterized broad-scale patterns of presence which remains an imperative research goal. Temporal sampling replication is needed to quantify detection probability for

Clinch Dace (White and Orth 2014a). Conducting surveys with multiple collection gears can inform the design of future monitoring protocols. Additionally, little attention has focused upon individual population units. Population-level research goals should include delineating upstream and downstream population boundaries within streams and estimating population density at particular sites.

The three main research objectives were defined for this thesis: 1) Identify appropriate sampling gears and protocols to monitor Clinch Dace populations and characterize the habitat factors that may influence Clinch Dace occupancy in south-flowing tributaries to the Clinch River in Russell and Tazewell counties, Virginia. 2) Refine estimates of the Clinch Dace's range and population density within Virginia and assess the habitat factors that are correlated with density. 3) Recommend specific streams for conservation emphasis using a novel multi-metric systematic conservation plan. All three objectives are intended to provide guidance to state and federal agencies to restore populations of this critically imperiled fish.

Chapter One: Using presence-absence data to inform monitoring protocols and habitat management for *Chrosomus* sp. cf. *saylori* (Clinch Dace)

Abstract

In the summers of 2014 and 2015, I conducted stream surveys at 70 sites throughout the presumed range of the Clinch Dace. I selected sites using a random sampling approach stratified by stream order. I gave priority to 2nd-and 3rd-order stream segments, which are most commonly occupied by Clinch Dace. Sites consisted of four-50-m sub-reaches, which I sampled on consecutive days. I randomly allocated two sampling gears, minnow trapping and backpack electrofishing to each sub-reach in order to compare detection probability between these two gears. I detected Clinch Dace at 13 out of 70 sites ($\psi=0.19$) and discovered new occurrences of Clinch Dace in four streams. I used multi-scale occupancy models based on *a priori* hypotheses to determine which factors influenced Clinch Dace occupancy. I ran all analyses first using the "raw" (scaled) and "spatially transformed" habitat data to account for spatial autocorrelation among environmental conditions at clustered sites in my stream network. Habitat data suggested that Clinch Dace occur in streams with higher substrate embeddedness, percent forest in the watershed, and elevation, and lower channel gradient and conductivity. Analysis of the "spatially transformed" habitat data suggested that some of these relationships may be influenced by spatial autocorrelation, although models incorporating the "raw" habitat data outperformed those with the "spatially transformed" data. Clinch Dace detection probability for 50-m reaches was higher with backpack electrofishing: 0.65 (95% CI=0.49-0.77) than minnow trapping: 0.42 (95% CI=0.28-0.56). Clinch Dace are rare and only occur in 19 streams in 10 tributaries to the Upper Clinch River in two counties in Virginia. Monitoring protocols should be developed using periodic surveys with either backpack electrofishing or minnow trapping. Management actions should focus on preserving forested cover in occupied watersheds and minimizing the future impact of mining activities that could alter water chemistry in close proximity to Clinch Dace populations. Translocations might be implemented among low-gradient headwater streams with sufficient pool habitat for populations to persist.

Introduction

Knowledge of habitat and ecology is necessary in managing for imperiled aquatic species like the Clinch Dace. Previous analyses using multivariate ordination to analyze habitat associations of Clinch Dace (White and Orth 2014a) suggested that they occupy small, high elevation, headwater streams, with gravel substrate, located in mostly forested watersheds. Threats to Clinch Dace may include: fine sediment deposition and increased total dissolved solids from mining activities; introduction of invasive species; habitat alteration favoring native piscivorous predators; forest clearing for timber harvest, livestock grazing, crop agriculture; bait harvest; population fragmentation through the construction of impassible road crossings; discharge of chemicals and other household or sewage wastes; climate change; and surface or subsurface water withdrawals.

Northern Russell and Tazewell counties, Virginia skirt the southern edge of the Appalachian coalfields that begin just north of the mainstem Clinch River. Near the coal field boundary exists a major geological and ecological boundary as well. The northern half of these counties belongs to the Cumberland Mountain ecoregion, a part of the broader Central Appalachian Plateau ecoregion. To the south, the Ridge and Valley ecoregion dominates. All known Clinch Dace populations occur in or near the boundary of the Central Appalachian Plateau ecoregion, which may suggest that Clinch Dace are adapted to the unique geological and thermal characteristics as well as the forested land cover found in these watersheds. "The boundary between the Central Appalachian Plateau and the Ridge and Valley "approximates a major structural topographical, lithological, elevational, and land-use break" (Woods et al. 2009). Narrower stream valleys, with an underlying geology of sandstone, shale, conglomerate and coal and a largely forested vegetation cover typify the Central Appalachian Ecoregion. Ridgetops in this ecoregion range between 1200 and 3600 feet and are approximately 350 to 550 feet above the streambeds at the valley floors (Woods et al. 2009, National Hydrography Dataset V.1). In contrast, the Ridge and Valley ecoregion in the southern areas of these counties contains a more karst-based geology of limestone and dolomite where springs and underground seeps are common. Ridge and Valley watersheds contain

east-west running streams with wide valleys that drain primarily agricultural lands with a clay-based surface geology (National Hydrography Dataset V.1). The calcareous geology of the Ridge and Valley contributes to elevated hardness and reduced stream density across the landscape (Lingenfelser et al. 2004; Woods et al. 1999).

Presumably, mining operations and Clinch Dace populations have overlapped in northern Russell and Tazewell counties for over 100 years even though the species was first discovered only in 1999. Coal has historically been the major source of electricity generation in the United States. In 2009 coal still generated approximately 46% of the electricity in the United States (USDOE 2009). However, coal mining trends are changing. Coal production in Central Appalachian region is gradually declining, being replaced by mining in the Western United States (U.S. Energy Information Administration 2010). Since 1975, mining is increasingly from the surface instead of from underground mines (Bernhardt et al. 2012). The mechanization of large-scale surface mines has resulted in a disproportionate decline in the workforce relative to coal production. From 2012 to 2013, coal production declined by 3.1% and the average number of employees/mine declined by 10.5% nationally (USDOE 2013) A workforce of 8,600 Virginians was still employed in mining-related careers in 2014 (Virginia Employment Commission 2014). Despite the decline, coal is deeply engrained in the economic and energy fabric of our nation and in the cultural identity of southwest Virginia.

Viewed broadly, mining practices can be divided into surface and sub-surface operations. "Mountaintop removal" mining is essentially a surface mining project that displaces entire mountaintops to expose coal seams; this type of mining is less common in the Clinch Drainage in Virginia. Nevertheless, other surface mining techniques have similar effects on a reduced scale. The alterations to topography and the deposition of overburden material in adjacent valleys can have lasting physical, chemical, and biotic impacts upon a watershed (Griffith et al. 2012). Mined materials contain many impurities including rock, sulfuric compounds, and low-quality coal that must be removed during the preparation process. Coal preparation involves coal washing, hold of wash water in slurry ponds, and wastewater discharges. Operation of heavy machinery leads to soil compaction, decreased water

infiltration, increased surface runoff, increased peak flows, and eroded stream banks (Griffith et al. 2012; Wolman 1967). Moreover, deforestation, earth moving, and mining roads lead to significant mobilization and deposition of fine sediments in stream channels, although some studies have not shown an increase in fine sediments downstream of valley fills (Pond et al. 2008), suggesting that sediments may be flushed from high gradient stream channels (Hartman et al. 2005). A large portion of sediments deposited in channels may be eroded from streambanks when hydrologic regimes change downstream of mines, which causes stream channels to widen (Fox 2009). Physical alterations in the headwaters can extend downstream and affect the biogeochemistry of receiving streams as well (Bernhardt and Palmer 2011). Studies in West Virginia indicated that species and functional ecological diversity are lower at sites with "mountaintop removal" mining and, because of the limited dispersal capability of fishes, little to no recovery of fish communities follows cessation of mining (Hitt and Chambers 2014). Other studies have found positive relationships between proportion of watersheds in surface mines and measures of stream impairment based on benthic macroinvertebrate community data (Bernhardt and Palmer 2011).

Water quality alterations can cause more severe effects on stream biota than physical habitat alterations in mined watersheds (Freund and Petty 2007; Hitt and Chambers 2014; Pond et al. 2008). Acid mine drainage is not common in Virginia coal deposits due to the large amounts of carbonate material that binds together the particles of sandstone (Griffith 2012; Daniels et al. 2009). Alkaline mine drainage, however, leads to increases in conductivity or total dissolved solids as water dissolves minerals from the exposed unweathered rock (Hartman et al. 2005). Mixtures of predominantly HCO_3^- and SO_4^- leach off of valley fills and into streams which are a cause, and not just a correlate, of the decline of macroinvertebrate taxa in Central Appalachian streams (Cormier et al. 2013). Conductivity alone can limit the biotic condition of headwater streams in the Central Appalachian region, even when all other physical and chemical habitat variables resemble reference conditions (Timpano et al. 2015). Elevated specific conductance stresses sensitive macroinvertebrate taxa, such as mayflies, that struggle to maintain an osmotic balance in the presence of elevated ionic concentrations (Bernhardt and Palmer 2011). The

consequences to ecosystem function can be severe, as mayfly taxa can make up 25-50% of the macroinvertebrate abundance in reference streams in the Central Appalachian region (Pond et al. 2008). Some hypothesize that populations of headwater benthic insectivorous fishes like Blackside Dace and the Kentucky Arrow Darter (*Etheostoma spilotum*) exhibit a threshold response to elevated conductivity as the energetic demands for foraging increase as macroinvertebrate populations decline (Hitt et al. 2016). Coal deposits also can contain heavy metals like copper, nickel, and zinc that may be toxic (Daniels et al. 2010). In alkaline waters draining mined sites, iron may precipitate on the stream bed, hampering organic matter decomposition by micro and macrofauna (Schlief and Mutz 2005). Increased concentrations of selenium below valley fills accumulates in fish and invertebrate tissues (Arnold et al. 2014) and can be toxic at high concentrations (Hitt and Chambers 2014). Selenium exposure to developing embryos can lead to skeletal, eye, and fin deformities; accumulation of fluid in internal organs; and hence, reproductive failure or inhibition in fish populations (Lemly 1997).

Some legal frameworks — including the Clean Water Act of 1972, Section 404 and the Surface Mining Control and Reclamation Act of 1979 — prescribe restoration and mitigation of surface mining impacts. However, current practices for reclamation of mined sites and stream restoration are highly criticized in some quarters. Some argue that mining impacts clearly outweigh mitigation actions — such as stream creation, habitat restoration, or sediment pond removal — and thus violate the Clean Water Act's mandates (Palmer and Hondula 2014). The length of impacted reaches is generally longer than the length of stream restored as mitigation (Palmer and Hondula 2014). Sub-par mitigation projects often are not required to meet specific biological or water quality standards, but instead are often assessed against the lax regulatory performance criteria for success outlined in permit plans issued by the Army Corps of Engineers or by state agencies. Such criteria may consist of qualitative habitat goals such as meeting a "suboptimal" or increasing habitat metric score (Palmer and Hondula 2014). Another main criticism is that the restoration, which often uses the "Natural Channel Design" (NCD) approach, is aimed at restoring physical habitat structure and not ecosystem function (Bernhardt and Palmer 2011; Tullos et al.

2009). For example, macroinvertebrate community metrics in "restored" Virginia coalfield streams did not show improvement in restored versus unrestored stream channels in mined watersheds, and there was not a discernable influence of restoration on ecosystem processes (Northington et al. 2011; Palmer and Hondula 2014).

The preference of *Chrosomus* species for headwater streams leads to sampling and monitoring difficulties. The dendritic stream networks in the Upper Clinch Basin provide hundreds of stream kilometers to assess for Clinch Dace presence, including areas outside of Russell and Tazewell counties. Sampling protocols for rare species must address the risk of mortality or sub-lethal stress upon individuals. For example, the discovery of the Clinch Dace's sister species, the Laurel Dace, was first recognized from a rotenone survey collection housed in a museum. Although other populations of Laurel Dace were subsequently found in nearby tributaries, the rotenone survey had wiped out the population at its type locality (George et al. 2015). Past surveys for Clinch Dace have used seining (Skelton 2007) and backpack electrofishing (White and Orth 2014a). Each of these methods has drawbacks. Seining is often difficult in these streams because of rocky substrate and dense, thorny riparian vegetation. Pulsed DC backpack electrofishing has generally been proven safe for small-bodied fish, but it requires proper set up and operation of the sampling device (Whaley et al. 1978; Holliman et al. 2003a; 2003b). Safety precautions include maintaining safe distances with the probes and setting voltage and frequency of the unit to the minimum immobilization threshold of the target species. Inevitably, fish still elude capture by hiding in undercut banks and root wads.

Environmental DNA has recently emerged as a viable sampling method for rare and cryptic species, including fishes (Goldberg et al. 2011; Jerde et al. 2011; Minamoto et al. 2012; Olson et al. 2012; Mahon et al. 2013; Jane et al. 2014). This technology involves filtering stream-collected water samples, extracting trapped DNA, and then amplifying the DNA of only the target species using species- specific primers. I investigated eDNA detection of Clinch Dace and Tennessee Dace (*Chrosomus tennesseensis*) in a companion study (Moore et al. 2015). Originally, I had hoped to incorporate eDNA results into the

occupancy modeling framework to compare its detection probability relative to the other traditional gears. During my first field season, however, I dealt with low detection sensitivity and false-negative results likely caused by low DNA copy number and PCR inhibitors present in the water samples (Moore et al. 2015). In my second field season, methodological changes increased detection probabilities to around 73% with three PCR replicates. However, this level of detection probability is still likely too low to justify the time required to filter and process water samples. More research is needed before eDNA can become a reliable and effective method of detection for *Chrosomus* daces.

This risk of not detecting a rare or cryptic species when it is present at a site has contributed to the popularity of occupancy models, which correct presence estimates to account for imperfect detection (MacKenzie et al. 2002). The formula for naïve occupancy without accounting for species detection probability (*p*) is simply the number of sites where the species was present (*x*) divided by the total number of sites sampled (*s*): $\psi = x/s$ (MacKenzie et al. 2002). Occupancy analyses require spatial or temporal replication of site sampling, and are analogous to mark-recapture population analyses except that sites, not individual organisms, become the focal study unit. For each sampled site, the researcher collects detection histories for the target taxa. If one assumes that occupancy and detection probability are constant across all sites, incorporation into maximum likelihood analyses can generate estimates of two crucial parameters ψ – or the probability that a species is present at a site (occupancy), and *p*- the probability that a species is detected given that it is present at a site (detection probability). The addition of site-specific covariates using logistic modeling allows occupancy probability to vary among sites as a function of environmental or other variables. An increased number of sampling replicates per site will improve the precision of occupancy estimates, at the expense of increased sampling effort and time. Nevertheless, occupancy-style surveys are usually cost- and time-efficient relative to traditional survey methods based on counts of individuals (MacKenzie et al. 2002).

The research objectives for this chapter were not to predict Clinch Dace occupancy probability at unsampled sites, but rather to better understand the effect of gear type on detection probability of Clinch Dace and relationships between habitat and occupancy of Clinch Dace. To accomplish these goals, I

modeled occupancy using sampling gear as a covariate of detection probability, and site- and landscape-scale habitat variables as site-specific covariates of occupancy.

Methods

Study Area and Site Selection

Previous surveys for Clinch Dace have focused on broad areas of the Clinch Basin and adjacent watersheds. These surveys suffered from a low number of sites where Clinch Dace were detected. To ensure that I would detect Clinch Dace at a reasonable number of sites and increase my odds of discovering new populations, I narrowed my focus to include streams within 15 south-flowing tributary systems to the Clinch River in Russell and Tazewell counties, Virginia (Figure 1). The region comprising the northern half of these two counties narrowly bounds the only records of Clinch Dace in Virginia. I selected a total of 70 stream segments to survey using a two-visit, two-gear, standardized sampling design. I set the stream segment boundaries as the nodes of stream intersections in the National Hydrography Dataset (NHD) stream layer (Figure 1.1). I selected stream segments using a stratified random sampling design with higher weight for the selection of second- and third- Strahler order streams. Placement of the 200-m sampling reach within each selected segment was contingent on site access with all sites having to be perennially watered. The breakdown of sites by order was: 46 second-order, 15 third-order, and 9 fourth-order. I sampled 48 sites in 2014 and 22 sites in 2015. I sampled an additional 28 sites in 2014 and 2015 in an opportunistic manner to fill gaps in the geographic scope of the survey.

The standardized sampling protocol that I used at all 70 sites consisted of four 50-m sub-reaches, for a total of 200 m of sampled length. I marked and geo-referenced the boundaries of each sub-reach. Usually a short gap of approximately 10 m of un-sampled habitat was left between each sub-reach.

Fish Collection

I tailored my sampling design to an occupancy modeling approach using gear types as a covariate of detection probability (MacKenzie et al. 2002, 2003; Nichols et al. 2008). During both

primary sampling periods, I placed 12 Promar® minnow traps (Figure 1.2), baited with half of a slice of bread and a handful of dry dog food, in pool or run habitat (six within each sub-reach), Baited traps have higher catch rates than un-baited traps for Blackside Dace (Detar and Mattingly 2013). I oriented traps with the long axis parallel to the direction of flow. At the other two sub-reaches, a two-person crew conducted single-pass backpack electrofishing with pulsed DC current using a Smith-Root LR-24 backpack electrofishing unit. Both crew members netted fish. One member was responsible for toting the bucket and the other for operating the backpack electrofishing unit. I recorded the abundance of each species in the sample and measured total length (mm) for all Clinch Dace that I captured. In the second primary period, I repeated the sampling procedure from the first visit.

Instream and Landscape Habitat

To characterize instream habitat conditions and watershed land use in streams occupied by Clinch Dace, I assembled a database of 14 micro- and macrohabitat variables for all 70 sites sampled. Instream habitat variables recorded included substrate composition, substrate embeddedness, depth, width, pool-to-riffle ratio, canopy cover, water transparency, woody debris cover, water temperature, pH, and conductivity.

I assessed stream substrate using a modified Wolman pebble count method classifying the size of 100 pebbles at a site (Wolman 1954). Rather than using the traditional grid approach, the sampler paced diagonally from bank to bank and at a predetermined number of steps picked up the piece of substrate at the tip of their boot. Operator bias was limited by designating a single crew member to conduct habitat surveys each year and by selecting a substrate particle at a fixed point (tip of boot) (Daniels and McCusker 2010). I measured particle size on a Wentworth scale using a gravelometer using the "larger than" approach by noting the smallest-sized hole in the gravelometer through which the substrate particle would not pass. This approach produced an estimate of the particle diameter along the *b-axis.* Measuring particle sizes less than 2 mm is difficult in the field (Wolman 1954); therefore, I combined all particles <2 mm into a single category representing fine sediments, namely sands and silts.

Additionally, the presence of fine sediments at each site was captured using measures of embeddedness. While conducting pebble counts, I measured the embeddedness of each substrate particle as the percent of the vertical extent of a particle that was embedded in fine sediment (Bunte and Abt 2001) within one of five categories: <5%, 5-25%, 25-50%, 50-75%, >75%. I characterized points where bedrock or silt was the dominant substrate as 100% embedded unless the bedrock was free of all silt in which case embeddedness was considered <5%.

At every 10th substrate observation I measured other habitat variables along a transect. 10 habitat transects were completed at each 200 m sampling site. First, I measured percent canopy cover using a convex spherical densiometer. I held the instrument level in the palm of my hand while standing in the center of the stream channel and counted the number of squares in which canopy cover was reflected. This count, multiplied by 4.17, was an estimate of percent canopy cover (Lemmon 1957). I also measured wetted stream width, maximum depth, percent wetted-width occupied by woody debris, and the type of mesohabitat (riffle, run, or pool) at each transect. I recorded water quality parameters at the lowermost and uppermost points of each 200 m sample site, including water temperature, transparency using a transparency tube, pH using a Hanna Phep 5 pH meter, and specific conductance (conductivity) using a Hanna HI98311 DiST® 5 EC/TDS or YSI EcoSense EC300A meter.

I used a geographic information system to calculate other landscape-level habitat variables. I delineated watershed areas draining to each survey site using the 1 arc-second (30-m) National Elevation Dataset (United States Geological Survey 2002) using the watershed tool in the spatial analyst package of ArcMap version 10.1. I calculated land cover of each watershed by overlaying the watershed polygons with the 2011 National Land Cover Database (Homer et al. 2015). I counted road crossings as intersections of the National Hydrography Dataset stream layer with the 2010 Tiger/Lines roads shapefile (U.S. Census Bureau 2012). I estimated stream gradient using the change in elevation of the digital elevation model (DEM) divided by the length of the stream segment in which the survey site occurred (Table 1.1 summarizes habitat variables and their sources).

Before proposing a candidate model set for occupancy modeling, I examined Pearson correlation coefficients among scaled habitat variables. Highly correlated variables may lead to misleading causal assumptions, and it is best to avoid using highly correlated variables together as site covariates in the same occupancy models.

I used box plots to display the median, 95% confidence interval of the median, interquartile range, and maximum and minimum values at sites where I collected Clinch Dace and at sites where I did not collect Clinch Dace. I conducted one-tailed bootstrapped Kolmogorov-Smirnoff tests α=0.10 with 5000 bootstrap iterations to compare the distributions of 12 habitat variables in order to test for differences in environmental variables between sites where Clinch Dace were present and those where they were absent. The bootstrapped version of this test is used when there are ties (Bickel 1969) in the data resulting from my habitat measurements not having completely continuous distributions. I adjusted the critical *P*-value for multiple comparisons using the Bonferroni correction factor. The Bonferroni corrected threshold of significance was α= 0.10/12=0.008.

Occupancy Models

I used multi-model inference in an information theoretic approach to analyze my records of Clinch Dace presence at the 70 sites with repeat sampling data. I selected multi-state occupancy models in program PRESENCE version 7.5 (United States Geological Survey, Patuxent, Maryland) to test the impact of gear type on detection probability (Nichols et al. 2008). These models contained parameters for large-scale or site-level occupancy (ψ), detection probability (p), and small-scale or sub-reach-level occupancy (θ). In this framework, each 50-m sub-reach was considered a unique detection/non-detection event, such that over two days each site had 8 possible detection events. A sampling covariate was coded for each of the 8 sampling events to indicate which sub-reaches had been sampled using minnow trapping and which had been sampled with electrofishing to allow for heterogeneity in *p* for each. In all models, θ was coded to be equal on both days and independent from large-scale occupancy.

Detection probability can be allowed to vary among sampling replicates through the inclusion of sampling covariates. Although detection probability is usually a nuisance parameter that improves estimates of occupancy, I was interested in the information that it can provide about the effectiveness of different sampling strategies and the amount of sampling effort needed to reliably detect Clinch Dace. A detection history where the species was detected on the first and third events, but not the second and fourth, is coded as 1010. The probability of observing this detection history would be represented by:

$$\psi_i p_{i1} (1 - p) p_{i3} (1 - p_{i4})$$

Where ψ_i is the probability of species occupancy at site i and P 1-4 refer to the probability of species detection on four successive sampling events.

Mackenzie et al. (2006) defined four assumptions that must be met by single-season occupancy models: (1) occupancy status at each site does not change over the survey season, (2) the probability of occupancy is constant across sites, or differences in occupancy probability are modeled using covariates, (3) probability of detection is constant across all sites or is a function of site-survey covariates, and (4) detection of species and detection histories at each location are independent. I assumed that site occupancy did not change overnight because I revisited all sites on consecutive days. I satisfied assumption two through the incorporation of site covariates in my models. I addressed assumption three by using site and sampling covariates. Assumption four has not been adequately addressed directly in some published occupancy studies, and can become a problem when one has clusters of sites that are near one another. To address the potential issues imposed by spatial bias, I used an approach to "spatially transform" my environmental variables (Brind'Amour et al. 2011; Peoples and Frimpong 2015).

To account for spatial autocorrelation, I calculated the pairwise network (fluvial) distances among my 70 sites using network analyst tools in ArcGIS 10.1. Second, I used a principal coordinates of neighborhood matrices (PCNM) analysis with the *PCNM* function within the *PCNM* package in R in order to identify the significant spatial eigenvectors within my dataset. The significance test on eigenvalues associated with each spatial eigenvector uses a permutation approach for the Moran's *I* statistic. I tested what subset of spatial eigenvectors best predicted each habitat variable using a stepwise

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model selection technique. Selection eigenvectors was completed with the *stepAIC* function in the *MASS* package of R based on minimum value of Akaike's information criterion (*AIC*). The spatially significant eigenvectors were used in multiple linear regression models to predict "spatially transformed" habitat data. I again normalized the final matrix of predicted values prior to use in occupancy models.

I developed specific *a priori* hypotheses about the habitat factors that drive Clinch Dace occupancy and detection probability. These hypotheses incorporate both the GIS and field-measured habitat variables collected at each site as model site-specific covariates. Testing of these hypotheses aims at improving understanding of the habitat factors that may be most important for managing Clinch Dace habitat and for selecting sites for translocation. I organized the 17 hypotheses — which contain from one to three site covariates — under broader model categories. All habitat data were scaled by subtracting the mean value and dividing by the standard error to make the data symmetrical around 0. Where I included multiple site covariates in a single model, I chose to use only additive relationships. The model *categories* and nested hypotheses were as follows: *Landscape* (gradient, elevation, watershed area, gradient+elevation, gradient+watershed area, elevation+watershed area, and gradient+elevation+watershed area), *water quality* (conductivity, pH, conductivity+pH), *channel morphology* (width:depth, embeddedness, width:depth+embeddedness), *watershed land cover* (all forest, evergreen forest), *resource extraction* (historical mining in watershed), and *riparian* (canopy cover). Correlation among predictors in models can be problematic. I checked correlation between site covariates and identified models that included pairs of variables that had a Pearson correlation coefficient greater than 0.7 in the single model (Figures 1.3 and 1.4). Pearson correlation coefficients were usually much greater in the "spatially transformed" habitat data (Figure 1.4). I ran the models representing each hypothesis both with and without the sampling gear covariates for detection probability and with both the "raw" and "spatially transformed" habitat data, giving me a total of 71 models.

I ranked the 71 models (hypotheses) using an information theoretic approach based on minimum *AICc*. *AICc* is a version of *AIC* corrected for a small sample size with a penalty for each parameter added to the model. I also calculated Δ*AICc*, which was the difference between a model's *AICc* score and the top

model's *AICc* score, and Akaike weight for each model. Models with Δ*AICc* ≤2 are considered to have relatively similar support, while models with Δ*AICc* ≥9-11 have very little support (Burnham and Anderson 2011). I present upper 95% confidence sets for the standard coefficients of ψ and p estimates (Anderson 2008). I tested for goodness-of-fit with my null model using a Pearson chi-square test with 1000 bootstraps. $\hat{C}$ is a measure of overdispersion should be ≤1. If $\hat{C}$ is much larger than 1, *QAIC* can be calculated. *QAIC* inflates the variance of the model to adjust *AICc* to be more conservative toward selecting models with fewer covariates (MacKenzie and Bailey 2004).

Using the gear-specific estimates of detection probability, I developed a cumulative detection curve to determine how many 50-m spatial replicates would be needed for each gear to reach a desired detection probability. The equation for this was $P = 1 - ((1 - p)^x$, where *P* is cumulative detection probability, *p* is the detection probability per sampling event, and *x* is the number of sampling events.

Size Selectivity

I compared the length-frequency distribution of Clinch Dace captures to the maximum size and inferred longevity of Clinch Dace as described in previous research (White and Orth 2013b). Previous studies have measured standard length (SL) instead of total length (TL), so I developed a simple linear model in the statistical program R to convert total length in my records to standard length based on a collection of 19 preserved Clinch Dace specimens housed at Virginia Tech (Adjusted R^2 =0.99).

Size selectivity bias is another common issue with fisheries sampling gear. I divided all lengths into 10-mm bins and used a chi-square test to test for differences in size distributions of individuals captured with backpack electrofishing and minnow trapping.

Results

Instream and Landscape Habitat

The most common land cover across my study area is deciduous forest. Catchments above sample sites were on average 75% forested. Pasture and crop cultivation are most common in the Thompson Creek and North Fork Clinch watersheds, which fall in the Ridge and Valley ecoregion and where Clinch Dace do not occur. Overall, agriculture averages 13% of the area catchments in my study area. Grassland makes up 3% of catchment area on average. Some of the grassland is most likely at reclaimed mined sites that have not supported forest regeneration. Narrow strips of residential and small-scale agriculture exist in some narrow valleys. Developed land makes up 8% of catchments on average and is mostly low intensity. Sites permitted for coal mining by the Virginia Department of Mines Minerals and Energy currently exist in 11 of 15 watersheds with historical records of Clinch Dace. Active or abandoned mines are sometimes classified as "barren", and barren lands averaged 1% of catchment land cover. Only two of my field sites, Middle Fork Clinch River and Coal Creek (both heavily mined watersheds), had percent barren land cover >5% in their catchments. Some streams that I sampled — including Stonecoal Creek and Laurel Branch — have undergone channel reconstruction following mining (Northington et al. 2011).

The bootstrapped Kolmogorov-Smirnoff tests revealed significant differences in some habitat variables between sites occupied and sites not occupied by Clinch Dace ($P<0.1$). Substrate embeddedness ($P=0.056$), forest in watershed ($P=0.05$), evergreen forest in watershed ($P=0.07$), pH ($P=0.05$), channel gradient ($P=0.02$), conductivity ($P=0.073$), and elevation ($P=0.025$) all had significantly different distributions (Figure 1.5). Watershed area ($P=0.44$), canopy cover ($P=0.56$), historical mining ($P=0.45$), width:depth ratio ($P=0.39$), and proportion bedrock ($P=0.145$) were not significant factors in explaining Clinch Dace Distribution at this threshold (Figure 1.5). However, none of these relationships were considered significant once the Bonferroni correction for multiplicity was applied to the critical P-value as all observed P-values were >0.008.

Detection Probability (p)

Null model fit was sufficient (P=0.88, $\hat{C}$=0.90). A value of $\hat{C}$<1 suggests that overdispersion was not a problem. Backpack electrofishing was more effective at detecting Clinch Dace given that the species is present at a site. Detection probability was 0.65 (95% CI=0.49-0.78) for electrofishing and 0.42 (95% CI=0.28-0.56) for minnow trapping. There was strong support for a gear-specific effect on detection probability. Models with a gear-specific sampling covariate always had a higher *AICc* weight value relative to the corresponding model that lacked the gear specific sampling covariate. Accounting for spatial autocorrelation did not alter estimates of detectability.

Cumulative detection probability curves indicate that an estimated 95% probability of detection given presence was reached within just three 50-m replicates using backpack electrofishing. Minnow trapping took twice as many, six 50-m replicates, to reach the 95% detection probability threshold (Figure 1.7).

Occupancy (ψ)

Correlation between predictors was not a major issue with my data that were not "spatially transformed". The only pairs of variables that had correlation coefficients > 0.5 were conductivity and historical mining (0.51), which makes sense as mining can lead to increases in conductivity (Figure 1.3). However, once my habitat data were "spatially transformed", some variables that were included in the top-ranking models based on *AICc* were more correlated but correlations were still <0.7. This is because certain habitat variables were predicted by some of the same spatial eigenvectors. For example, gradient alone was negatively correlated with elevation (-0.63), watershed area (-0.53), all forest (-0.51), and positively correlated to conductivity (0.68), evergreen forest (-0.56), and historical mining (0.68) (Figure 1.4).

The channel morphology hypothesis, driven by a strong positive relationship with substrate embeddedness, best explained Clinch Dace occupancy (Table 1.3). Contrary to expectations, Clinch Dace occupancy probability was higher at sites with high substrate embeddedness. The standardized regression covariate or beta value for the substrate embeddedness covariate in the top-ranked *a priori* model was

0.85 (95% CI=0.24-0.61), which yields strong support for a positive relationship (Figure 1.6). However, the support for this being the single best model is not overwhelming, as it received an *AICc* weight of only 0.23.

There was also some support for other specific watershed and landscape effects using the "raw" habitat data. Clinch Dace were more likely to be found in highly forested watersheds and at higher elevations. The model that included proportion of all forest types in the watershed as a covariate for ψ had a *ΔAICc* of 2.49, which corresponds to an *AICc* weight of only 0.07. In this model, proportion forest had a positive relationship with Clinch Dace occupancy (β=0.95, 95% CI=0.051-1.86).

The only other model hypothesis that received a model weight > 0.05 was that including "spatially transformed" gradient and "spatially transformed" watershed area under the landscape hypothesis. This model had an *AICc* weight of 0.06. Clinch Dace occupancy was driven by negative relationships with both "spatially transformed" gradient (β=-1.07 95% CI=-2.04- -0.10) and "spatially transformed" watershed area (β=-0.805 95% CI=-1.58- -0.03) (Figure 1.6).

The top model including the influence of substrate embeddedness on Clinch Dace occupancy had little support once spatial autocorrelation was accounted for, with a *ΔAICc* of 7.10 relative to the top-ranked model.

 Size Selectivity

I recorded lengths for 714 Clinch Dace captured over 2014-2015. I may have captured some of the same fish on repeat visits, and thus some individuals may have been counted twice within this total. Clinch Dace in my sample ranged from 26-83 mm total length and the distribution of lengths was bimodal, suggesting two distinct age-classes (Figure 1.8 A.). On July 15, 2014, I captured an 83-mm TL Clinch Dace in Hurricane Fork. I also captured a 23-mm juvenile Clinch Dace on June 26 2015 and a several 26-27 mm juveniles both in lotic and lentic areas (impounded by a beaver dam) of upper Pine

Creek on May 25, 2014. These individuals likely originated from spawning events in early June and early May, respectively. The simple linear model to transform TL back into SL was $SL=-0.61+0.83{\times}TL$ (N=19; adjusted R^2=0.99; P=0.00). Using this equation, the estimates of SL for the largest and smallest Clinch Dace I captured were 67.9 mm SL (83 mm TL) and 18.4 mm SL (23 mm TL). The length-frequency distributions of Clinch Dace captured with each gear were not significantly different (chi-square p= 0.06), which suggests that size-selectivity bias is not a major issue with these gear types (Figure 1.9).

Discussion

Results from analysis of presence-absence data provide three major insights into the management of the imperiled Clinch Dace: gear selection is a crucial component of monitoring plans for rare species, Clinch Dace populations may persist despite siltation of streambeds, and finally Clinch Dace populations are likely sensitive to conversion of forested watersheds from logging or surface mining which can lead to elevated levels of conductivity. All three of these discoveries may be generalized to managing populations of other rare fishes in the region.

Instream and Landscape Habitat

The univariate Kolmogorov-Smirnoff tests to test for differences between the habitat at sites occupied and unoccupied by Clinch Dace initially suggested that there may be differences in a few variables, however, due to the large number of comparisons conducted (12) the Bonferroni correction adjusted the critical P-value low enough to suggest that the observed significant relationships may be due to chance. I would need a larger sample size of occupied and unoccupied sites or reduce the number of habitat comparisons for the Kolmogorov-Smirnoff tests to reveal significant habitat associations.

Detection Probability (p)

Both backpack electrofishing and minnow trapping may be valuable sampling techniques in future monitoring protocols, The higher detection probabilities associated with backpack electrofishing indicate that this sampling method is superior in streams with low densities of Clinch Dace when the sampling protocol requires fewer than three passes at a site. Minnow traps could be used in multi-session demographic assessments to estimate survival, population size, or movement (Detar and Mattingly 2013), where individual Clinch Dace would receive unique marks with elastomer tags. Minnow traps are also better suited for wide, deep, and homogenous habitats where Clinch Dace have a greater ability to elude capture by electrofishing. Biologists may find that minnow traps are the only option to capture Clinch Dace in non-wadeable beaver ponds.

The Clinch Dace's vulnerability to minnow traps underscores the threat local fishermen who harvest bait pose to populations. Virginia outlaws bait harvest in six streams containing Tennessee Dace and special regulations on harvest also may be warranted for Clinch Dace (Virginia Department of Game and Inland Fisheries 2016). Bait regulations often differ on a state-by-state basis, and can be confusing to anglers (Meronek et al. 1995). As such, place-based trapping bans may be ineffective and poorly enforced. Managers should teach local landowners to recognize Clinch Dace and voluntarily avoid harvesting them for bait.

Occupancy (ψ)

The strong positive relationship between substrate embeddedness and Clinch Dace occupancy in the occupancy models with the "raw" habitat data was unexpected, but may be attributable to multiple factors: 1. Stream gradient may be correlated to substrate embeddedness. As the box plots and Kolmogorov-Smirnoff tests revealed, streams containing Clinch Dace often have lower gradients, lower current velocity, and more pools, which allow fine sediments to accumulate. My measurements of stream gradient did not correlate strongly with embeddedness, but I calculated gradient by measuring the elevation change between nodes on the stream network using GIS, and thus my measurements may not reflect local channel slope. Once the spatial position of my sites were accounted for, low stream gradient

also emerged as a predictor of Clinch Dace occupancy. 2. Nest building by Stonerollers and Creek Chubs may provide enough clean substrate for Clinch Dace to successfully spawn even in degraded habitats. The two instances when I observed spawning aggregations of Clinch Dace occurred on gravel nests in pool tails where the surrounding surface substrate was almost entirely fine sand; however, nests still may be smothered with fine sediments if a high flow event occurs during or after spawning occurs. 3. Previous studies have shown that altered riparian corridors, which may lead to increased inputs of fine sediments, are more detrimental to benthic-dwelling or -feeding species than water column drift- or surface- feeding species (Jones et al. 1999). Few benthic species were syntopic with Clinch Dace. Although I commonly collected Fantail Darters (*Etheostoma flabellare*) alongside Clinch Dace (17/20 sites), I rarely found sculpin (*Cottus spp.*) alongside Clinch Dace, (1/20 sites). The occupancy models in this chapter ignored the relative abundance of Clinch Dace, which may be lower at sites with substantial fine sediment deposition. The associations of habitat and Clinch Dace abundance will be discussed in the chapter two.

Finally, the high level of siltation at some Clinch Dace sites may be an artifact of the spatial position of these sampling points. The pattern with substrate embeddedness disappeared after accounting for the location of survey sites. Three of the sites with the highest substrate embeddedness, Laurel Fork and two sites on Greasy Creek, were located within network distances of 11 km of one another. Overall, Clinch Dace may be able to tolerate some siltation; however, it would be presumptuous to say that increased deposition of fine sands and silts do not negatively affect their populations. Similarly, fine sediment depth was not a strong predictor of Blackside Dace presence in logistic distribution models for that species (Black et al. 2013).

My results corroborate the association between Clinch Dace and forested watershed land cover from White and Orth (2014a). Strong support existed for a positive relationship with Clinch Dace occupancy and "raw" forest cover, however, this relationship was not strongly supported by the "spatially transformed" data. A positive relationship with evergreen forest in particular had more support in my "spatially transformed" models. I hypothesized that remnant riparian stands of hemlock may exist in unlogged streams that may correlate with Clinch Dace presence. However, other evergreen stands within

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the study region are likely hardy ridge-top white pines that were replanted to reclaim mined lands. Future research should note the dominant riparian tree communities at sites such as rhododendron, hemlock, or hardwood to assess potential relationships with Clinch Dace occupancy.

Most of the area that is not forested within the study area has been cleared for surface mining or timber harvest. Large forested tracts in catchments reduce sediment inputs, as well as fluctuations in water clarity, temperature, and hydrologic regimes. Forest could also indicate the absence of recent mining or residential development that will directly lead to degradation of water quality. Occupancy models with "spatially transformed" covariates were not as strongly supported as those using the "raw" habitat data which suggests that environmental factors and not spatial factors may be more important drivers of Clinch Dace distribution (Falke and Fausch 2010).

The pH values of the streams in my study area do not vary much, and are all basic (>7 and <9.5). While the distribution of pH was lower at sites where Clinch Dace were present (P=0.06), due to the limited spread of these data, and the lack of support in occupancy models using the "raw" data, and their overlapping ranges, I did not consider this trend to be biologically significant. This portion of the Appalachian coal seam in Southwest Virginia does not produce acid mine drainage like deposits farther north. This is due to large amounts of complex carbonates within the geology of the region capable of neutralizing any acid-producing materials (Daniels 2009). More commonly, mining can lead to alkaline mine drainage where pH rises as basic cations leach from crushed rocks (Griffith et al. 2012). Hence, conductivity is an informative mining-related water quality parameter within this study area.

Conductivity was elevated at 64 of 70 study sites over the general reference conditions for Appalachian streams, which is usually <133 µS/cm where mining or urbanization has not occurred (Pond 2008). At the same time, only 9 sites that I surveyed had conductivity >500 µS/cm, which may explain the lack of stronger relationship with conductivity in the occupancy models. Timber harvest may increase productivity of waterbodies by increasing nitrogen and phosphorus inputs into streams but usually does not result in the ionic increases observed in mined watersheds (Arthur et al. 1998). All streams examined in a study in West Virginia with a conductivity >500 µS/cm were biologically impaired in terms of

macroinvertebrate communities present (Pond et al. 2008). Cormier et al. (2013) found that between 11 and 17% of total macroinvertebrate genera disappeared at conductivities >500 µS/cm. Other studies suggested that the threshold of conductivity where biological impairment occurs may be as low as 300 µS/cm in the Central Appalachian region (EPA 2011). The effect of conductivity on fish may be a direct physiological response or an indirect result of declines in macroinvertebrate prey populations. Certain taxa of macroinvertebrates such as Ephemeroptera are highly sensitive to increases in TDS (Pond et al. 2008), and these taxa may or may not be important components of Clinch Dace diets. A diet study by White (2012) was able to identify macroinvertebrates from only four orders: Megaloptera, Coleoptera, Diptera, Ixodida, and Hymenoptera in 63 Clinch Dace. Interestingly, two of these orders are largely terrestrial, and Ephemeroptera, Plecoptera, and Trichoptera, often considered the macroinvertebrate orders most indicative of water quality, were not identified in Clinch Dace stomachs. Furthermore, conductivity and Clinch Dace occupancy may follow a non-linear relationship. A threshold in conductivity may exist, above which Clinch Dace cannot persist (Hitt et al 2016).

Point-source discharges of pollutants from surface mines can lead to sharp increases in conductivity well above the tolerances of some species. For instance, I observed an isolated population of Clinch Dace upstream of a large surface mine in Left Fork Coal Creek. Where this population persists, conductivity readings are less than 200 µS/cm; however, downstream of the discharge point from the surface mine, conductivity values rapidly increased over 1000 µS/cm (Figure 1.10). As freshwaters in Appalachia become saltier, scientists are recommending location-specific total dissolved solids criteria to protect sensitive species (Cañedo-Argüelles et al. 2016).

Additionally, little is known about how the proportion of ionic constituents — that lead to elevated total dissolved solids or conductivity — influences fish. As a result, regulation of this water quality parameter has lagged behind pH regulation. Cook et al. (2015) found elevated levels of P ions in untreated household waste discharge; Mn, Si, and HCO_3^- in underground mining discharge; and Ni^{2+}, Ca^{2+}, K^+, and SO_4^{2-}, and Se^{2-} in surface mining discharge. Other studies suggest that surface mine spoils are high in Ca^{2+}, K^+, SO_4^{2-}, and HCO_3^- ions (Daniels et al. 2014). To determine the specific biological

impacts of individual ionic constituents would require experimental manipulations of water quality, and most studies in mined watersheds have been of an observational nature (Freund and Petty 2007). Such experimental studies would be inappropriate with an imperiled species like Clinch Dace. However, captive propagation may support toxicity tests with Clinch Dace in the future.

Past research established that *Chrosomus* dace are headwater specialists (Skelton 2007; White and Orth 2014a). A significant negative relationship with watershed area only existed in the "spatially transformed" occupancy models. The presence data were biased by two downstream sites on Mudlick and Pine creeks; only a single Clinch Dace was discovered. These individuals are likely strays from larger populations further upstream, which also may suggest a greater dispersal capability of Clinch Dace than was previously thought. Additionally, because I directed most survey effort at small streams, there is a limited range of watershed areas within the data.

Historical mining was not strongly associated with Clinch Dace occupancy. Historical mining was included to investigate whether the legacy of past mining is reflected in the current distribution of Clinch Dace. Research has shown that the streams draining reclaimed mine land do recover at a slow pace. Total dissolved solids levels in streams follow a negative quadratic pattern following valley fill creation, reaching a peak before declining to <500 µS/cm an average of 19.6 years later (Evans et al. 2014). The largest percentage of mining in a watershed where Clinch Dace are currently present was Town Hill Creek with 21.4%. It may not be a coincidence that thorough surveys in lower Town Hill Creek in 2014 did not capture any Clinch Dace. I also observed that the water remained perpetually turbid even at low flow, suggesting possible inputs of fine sediments from access roads and surface mining operations. Isolated refuge populations may have allowed Clinch Dace to persist during past disturbances and make short-distance recolonizations once water quality recovered. Water quality in some streams may have recovered following past disturbances to the point where it would now be possible to translocate individuals to establish new populations.

Other habitat variables that I collected and chose not to include in the modeling exercise also may be important for Clinch Dace occupancy. It is clear that Clinch Dace prefer pool microhabitat. I did not

include the measured variable "proportion pools" in this analysis because I characterized microhabitat type only at 10 random transects per site. With pools being rare in these small streams, there was little variation in the proportion of pools across my sample sites. I proposed mean-width-to-mean-depth ratio as a surrogate for pool, however, this covariate received little support in the models.

Some variables such as water temperature and transparency depended on the time of collection. Recorded temperatures from 26 May to 1 August at 12 Clinch Dace sites ranged from 12.0-21.6 ˚C although there may be larger daily fluctuations in temperature that I did not capture. Thermal tolerance of Clinch Dace and thermal characteristics of their habitats are not well known. In Central Appalachia, air temperatures have been rising since a cool period in the 1960s and 1970s and mean summer air temperatures are predicted to rise between 0.8 and 3.8 ˚C from 2010-2069 (Butler et al. 2015). Clinch Dace are headwater specialists and poor dispersers, and as such will find it difficult to find cooler refugia by moving upstream. Shade from riparian corridors would contribute to lower and less variable thermal regimes. Studies of some southern Appalachian streams have shown that clearcutting watersheds can increase water temperature by as much as 6.7˚C (Swift and Messer 1971). Finally, groundwater extractions for gas wells and the flooding of underground mines could eliminate the cooling influence of springs and hyporheic flow in and therefore also disrupt thermal regimes in Clinch Dace streams. Specific streams, such as Hart Creek, have streamside springs that in the past residents used to store perishable foods (resident, personal communication). Some of these springs no longer flow. Many residents report observing declining base-flows in many creeks. The combined effect of lower flows that would push Clinch Dace downstream to find pool habitats, and the warming temperatures that would have the reverse effect, could squeeze Clinch Dace populations out of some of these creeks. Future research should deploy temperature loggers to better document temperature regimes in these streams.

There may be other worthwhile analytical approaches to deal with complex habitat associations of Clinch Dace. The occupancy models I used are essentially a form of logistic regression analysis with an added parameter to account for detection probability. The assumptions of logistic regression may

constrain my ability to detect complex marginal, non-linear, and threshold relationships of individual habitat covariates with the presence of the Clinch Dace. As discussed earlier the data suggests that some of these non-linear relationships may occur with variables such as conductivity. Some of which could be elucidated through machine-learning approaches such as boosted regression trees. Due to potential interaction and correlation (including spatial) among the predictors which were difficult to model explicitly, a machine-learning approach might be a robust addition to future analytical efforts.

Size Selectivity

My surveys captured both the smallest and largest Clinch Dace ever recorded. The maximum recorded age for Clinch Dace in a previous study was two years, and age-two individuals varied in length from approximately 45 to 61 mm SL (White and Orth 2013b, Figure 1.8 B.). The length-frequency data from my collection imply the presence of at least two age-classes (Figure 1.8 A). Given estimates of 1 mm growth per month, a 67.9 mm SL fish would seem to be 6-7 months older than the largest age-two Clinch Dace aged in White and Orth (2013b). It remains unclear whether the 67.9-mm SL individual captured in our study reached a third year of life or simply experienced higher growth rates. Given that small proportions of Tennessee Dace reach age-three, it is plausible that a small proportion of the Clinch Dace population may live to see a fourth summer before dying (Hamed et al. 2008). Other *Chrosomus* species including Blackside Dace reportedly only reach two years of age (Starnes and Starnes 1981).

The length-frequency distributions of Clinch Dace captured with each gear were largely similar except for an under-representation of individuals in the 30-40 mm size class with minnow trapping. This may suggest that minnow traps are somewhat less successful catching small Clinch Dace, but more data will need to be collected to test this hypothesis.

Applications to Monitoring and Managing Rare Fishes

This study has broad implications for both monitoring of and habitat management for rare fishes in wadeable streams. Tradeoffs often exist between detection probability and stress to individuals. Although backpack electrofishing produced the highest detection probabilities and rarely produced noticeable injury to fish in my study, it is a more intrusive sampling gear than minnow trapping. Managers should continue to refine technologies such as environmental DNA sampling (Goldberg et al. 2011; Jerde et al. 2011; Minamoto et al. 2012; Olson et al. 2012; Mahon et al. 2013; Jane et al. 2014) which may maximize detection probability and eliminate physical contact with the fish. Vegetation management at the watershed scale may be important for rare fishes in the region. Pollution regulators and fish management agencies must begin to consider conductivity as a potentially important water quality parameter for the persistence of insectivorous fishes as surface mining in Central Appalachians may threaten populations of their macroinvertebrate prey. Currently, the success of most mitigation projects related to coal mining are not assessed using biological and water quality parameters remain impaired (Palmer and Hondula 2014). Specific management actions to address these threats could include promoting best management practices for timber harvest such as maintaining vegetated buffer strips around streams, minimizing road construction, and controlling runoff from logging roads, should be implemented in commercial logging operations that occur in watersheds containing rare fishes. Best management practices (BMPs) have been shown to reduce hydrologic and sediment alterations in stream channels (Arthur et al. 1998). Surface mining valley fills which can elevate conductivity, should not occur upstream of populations of sensitive rare fishes. Managers should monitor permitted surface mining activities near populations of rare fishes and ensure that state and federal water quality standards are met. Where the threat of large-scale land cover alteration exists lists of suitable sites for translocation or founding of experimental populations of Clinch Dace should be compiled

Table 1.1: Habitat variables and descriptions that I collected for all 70 sites.

Habitat Variable	Measurement Method
Flow	Visual estimate (Below Average, Average, Above Average)
Elevation	Calculated from DEM in ArcGIS 10.1 at downstream node of stream reach.
Gradient	Calculated from DEM in ArcGIS 10.1 at downstream node of stream reach. Elevation change/length of stream reach
Watershed Area	Delineated watershed upstream of sample points using DEM in ArcGIS 10.1.
Strahler Order	Visually assessed using full NHD stream layer
Air Temperature	Estimated at site at time of sampling
Water Temperature	Measured downstream and upstream of sample site.
Transparency	Used a 120-cm transparency tube.
Conductivity	Measured specific conductance downstream and upstream of sample site using meter.
pH	Measured downstream and upstream of sample site using meter.
Substrate	100 pebble count. Pebbles decided by randomly picking up stone at tip of boot.
Mesohabitat	Recorded mesohabitat (run, riffle, pool) at 10 evenly spaced transects.
Width	Measured width in meters at 10 evenly spaced transects.
Depth	Measured depth in centimeters at 10 evenly spaced transects.
Canopy Cover	Spherical densioimeter at 10 evenly spaced transects.
Woody Debris	Estimated width of channel occupied by woody debris at 10 evenly spaced transects.
Historical Mining	Average proportion of surface mines in watershed for three historical mining layers.

Watershed Land cover

Proportional coverage of 13 land-cover types in watershed calculated using ArcGIS 10.1.

Table 1.2: Occupancy model hypotheses on the factors that influence the parameters *p* (detectability) and *ψ* (occupancy). Abbreviations of variables in parentheses are used in later table and figures.

A priori Occupancy Model Hypotheses

Parameter (Abbreviation in Tables and Figures 1.3-1.4)	Hypothesized Effect	Justification and Supporting Evidence
p		
Sampling Gear	Electrofishing will have greater detection probability than minnow trapping	Electrofishing is an active surveying method versus minnow trapping which is a passive sampling gear. There are few places for Clinch Dace to avoid the electric field in a small stream channel. Electrofishing has been the preferred gear type in previous studies of Clinch Dace and other *Chrosomus* species (White and Orth 2014a).
ψ		
Canopy Cover (Canopy)	Positive	Higher canopy cover indicates less disturbed riparian corridors. Near-stream vegetation has been shown to correlate with ecological community (Lammert and Allan 1999) through its role in sediment stabilization, thermal regulation (Swift Jr and Messer 1971), pool formation (Bilby and Ward 1991) and pollutant filtration.
Conductivity (condcomp)	Negative	Increased conductivity can lead to declines in fish populations although the mechanisms are still not well understood (Hitt and Chambers 2014).
pH (pH)	Negative	Increased pH can occur below mined sites due to alkaline mine drainage as crushed rock begins to weather which may correlate with high conductivities that are harmful to fish and macroinvertebrate populations.
Elevation (elev)	Positive	Streams at higher elevations will generally have lower water temperatures and smaller channels.
Gradient (Grad)	Negative	Lower gradient streams may contain slower current velocities and well-defined pool habitat preferred by *Chrosomus* dace (Black et al. 2013).
Historical Mining (HistMining)	Negative	Historical surface mining can leave a legacy of increased sediment erosion and altered water quality parameters in

		streams that may induce stress in Clinch Dace and macroinvertebrate populations.
Proportion>75% embeddedness (X75.embed)	Negative	Substrate embeddedness is related to deposition of fine sediment. Fine sediment can smother fish eggs, clog gills, alter foraging behavior, contain metals and other contaminants, and impact populations of macroinvertebrate prey thereby decreasing fish growth, reproductive success, and survival (Kjelland et al. 2015).
Proportion Evergreen Forest (Prop42)	Positive	Most of the evergreen forest that occurs in the National Land cover dataset for this region is concentrated along riparian corridors. The mature evergreen community is composed largely of Hemlock in this region. The unique shading and allochthonous inputs of litter in hemlock stands has been shown to structure macroinvertebrate communities and the effects also may also affect fish (Adkins and Rieske 2015).
Proportion Total Forest (Forest)	Positive	Forest is the natural land cover type for the study region (Woods et al. 1999) and therefore higher proportions of forest would suggest less human alteration.
Watershed Area (wsa)	Negative	Clinch Dace are primarily found in headwater streams 2nd to 3rd order which would have a smaller watershed area (White and Orth 2014a).
Width:Depth (Wid.Dep)	Negative	A low width to depth ratio would suggest a narrower deeper channel that may represent a smaller stream with more abundant low gradient pools. Pools are preferred habitat of *Chrosomus* (White and Orth 2014a)

Table 1.3: Rankings of 71 occupancy models. Table includes *AICc, ΔAICc*, *AICc* weight, Model Likelihood, and number of parameters for each model. Models correspond to the habitat specific occupancy hypotheses and gear specific detection probability hypothesis for *Chrosomus* sp. cf. *saylori*. Models using "raw" habitat data are indicated by "raw" in the data column while models using "spatially transformed" data are indicated with an "sp" in the data column.

Data	Model	No. Par.	*AICc*	*ΔAICc*	*wᵢ*	Model Likelihood
raw	ψ(75%embed),θ(.),p(gear)	5	207.23	0	0.226	1.00
raw	ψ(75%embed+widdep),θ(.),p(gear)	6	208.92	1.69	0.097	0.43
raw	ψ(Forest),θ(.),p(gear)	5	209.72	2.49	0.065	0.29
raw	ψ(75%embed),θ(.),p(.)	4	209.98	2.75	0.057	0.25
sp	ψ(grad+wsa),θ(.),p(gear)	6	210.07	2.84	0.055	0.24
raw	ψ(Forest+Prop42),θ(.),p(gear)	6	210.99	3.76	0.035	0.15
raw	ψ(elev),θ(.),p(gear)	5	211.32	4.09	0.029	0.13
sp	ψ(grad+elev+wsa),θ(.),p(gear)	7	211.52	4.29	0.027	0.12
raw	ψ(Wid.Dep+75%embed),θ(.),p(.)	5	211.6	4.37	0.025	0.11
raw	ψ(Prop42),θ(.),p(gear)	5	211.79	4.56	0.023	0.10
sp	ψ(pH),θ(.),p(gear)	5	211.97	4.74	0.021	0.09
raw	ψ(condcomp),θ(.),p(gear)	5	212.17	4.94	0.019	0.08
sp	ψ(grad),θ(.),p(gear)	5	212.27	5.04	0.018	0.08
raw	ψ(Forest),θ(.),p(.)	4	212.48	5.25	0.016	0.07
sp	ψ(prop42),θ(.),p(gear)	5	212.6	5.37	0.015	0.07
sp	ψ(grad+wsa),θ(.),p(.)	5	212.76	5.53	0.014	0.06
raw	ψ(elev+wsa),θ(.),p(gear)	6	212.91	5.68	0.013	0.06
sp	ψ(Forest),θ(.),p(.)	5	213.28	6.05	0.011	0.05
sp	ψ(Forest),θ(.),p(gear)	5	213.28	6.05	0.011	0.05
raw	ψ(.),θ(.),p(gear)	4	213.31	6.08	0.011	0.05
raw	ψ(grad+elev),θ(.),p(gear)	6	213.66	6.43	0.009	0.04
raw	ψ(Forest+Prop42),θ(.),p(.)	5	213.67	6.44	0.009	0.04
raw	ψ(condcomp+pH),θ(.),p(gear)	6	213.94	6.71	0.008	0.03
raw	ψ(pH),θ(.),p(gear)	5	213.96	6.73	0.008	0.03
raw	ψ(elev),θ(.),p(.)	4	214.08	6.85	0.007	0.03
raw	ψ(wsa),θ(.),p(gear)	5	214.12	6.89	0.007	0.03
sp	ψ(grad+elev+wsa),θ(.),p(.)	6	214.12	6.89	0.007	0.03
sp	ψ(condcomp+pH),θ(.),p(gear)	6	214.17	6.94	0.007	0.03
sp	ψ(75%embed),θ(.),p(gear)	5	214.33	7.1	0.007	0.03
raw	ψ(Prop42),θ(.),p(.)	4	214.55	7.32	0.006	0.03
sp	ψ(grad+elev),θ(.),p(gear)	6	214.55	7.32	0.006	0.03

sp	ψ(canopy),θ(.),p(gear)	5	214.56	7.33	0.006	0.03
sp	ψ(Forest+prop42),θ(.),p(gear)	6	214.59	7.36	0.006	0.03
sp	ψ(wsa),θ(.),p(gear)	5	214.63	7.4	0.006	0.02
sp	ψ(pH),θ(.),p(.)	4	214.72	7.49	0.005	0.02
raw	ψ(condcomp),θ(.),p(.)	4	214.93	7.7	0.005	0.02
sp	ψ(elev),θ(.),p(gear)	5	215	7.77	0.005	0.02
sp	ψ(grad),θ(.),p(.)	4	215.03	7.8	0.005	0.02
raw	ψ(grad+elev+wsa),θ(.),p(gear)	7	215.05	7.82	0.005	0.02
sp	ψ(histminin),θ(.),p(gear)	5	215.3	8.07	0.004	0.02
sp	ψ(condcomp),θ(.),p(gear)	5	215.35	8.12	0.004	0.02
sp	ψ(prop42),θ(.),p(.)	4	215.36	8.13	0.004	0.02
raw	ψ(Wid.Dep),θ(.),p(gear)	5	215.41	8.18	0.004	0.02
raw	ψ(HistMining),θ(.),p(gear)	5	215.46	8.23	0.004	0.02
raw	ψ(grad),θ(.),p(gear)	5	215.49	8.26	0.004	0.02
raw	ψ(Canopy),θ(.),p(gear)	5	215.58	8.35	0.003	0.02
raw	ψ(elev+wsa),θ(.),p(.)	5	215.59	8.36	0.003	0.02
sp	ψ(width:depth),θ(.),p(gear)	5	215.63	8.4	0.003	0.01
raw	ψ(grad+wsa),θ(.),p(gear)	6	215.64	8.41	0.003	0.01
sp	ψ(width:depth+75%embed),θ(.),p(gear)	6	216.01	8.78	0.003	0.01
sp	ψ(elev+wsa),θ(.),p(gear)	6	216.03	8.8	0.003	0.01
raw	ψ(.),θ(.),p(.)	3	216.12	8.89	0.003	0.01
raw	ψ(condcomp+pH),θ(.),p(.)	5	216.63	9.4	0.002	0.01
raw	ψ(pH),θ(.),p(.)	4	216.71	9.48	0.002	0.01
raw	ψ(grad+elev),θ(.),p(.)	5	216.79	9.56	0.002	0.01
raw	ψ(wsa),θ(.),p(.)	4	216.88	9.65	0.002	0.01
sp	ψ(canopy),θ(.),p(.)	4	217.31	10.08	0.001	0.01
sp	ψ(wsa),θ(.),p(.)	4	217.38	10.15	0.001	0.01
raw	ψ(grad+elev+wsa),θ(.),p(.)	6	217.64	10.41	0.001	0.01
sp	ψ(elev),θ(.),p(.)	4	217.76	10.53	0.001	0.01
sp	ψ(HistMining),θ(.),p(.)	4	218.05	10.82	0.001	0.00
sp	ψ(condcomp),θ(.),p(.)	4	218.1	10.87	0.001	0.00
raw	ψ(Wid.Dep),θ(.),p(.)	4	218.16	10.93	0.001	0.00
raw	ψ(HistMining),θ(.),p(.)	4	218.22	10.99	0.001	0.00
raw	ψ(grad),θ(.),p(.)	4	218.24	11.01	0.001	0.00
raw	ψ(grad+wsa),θ(.),p(.)	5	218.32	11.09	0.001	0.00
raw	ψ(Canopy),θ(.),p(.)	4	218.33	11.1	0.001	0.00
sp	ψ(width:depth),θ(.),p(.)	4	218.38	11.15	0.001	0.00
sp	ψ(width:depth+75%embed),θ(.),p(gear)	5	218.69	11.46	0.001	0.00
sp	ψ(elev+wsa),θ(.),p(.)	5	218.71	11.48	0.001	0.00
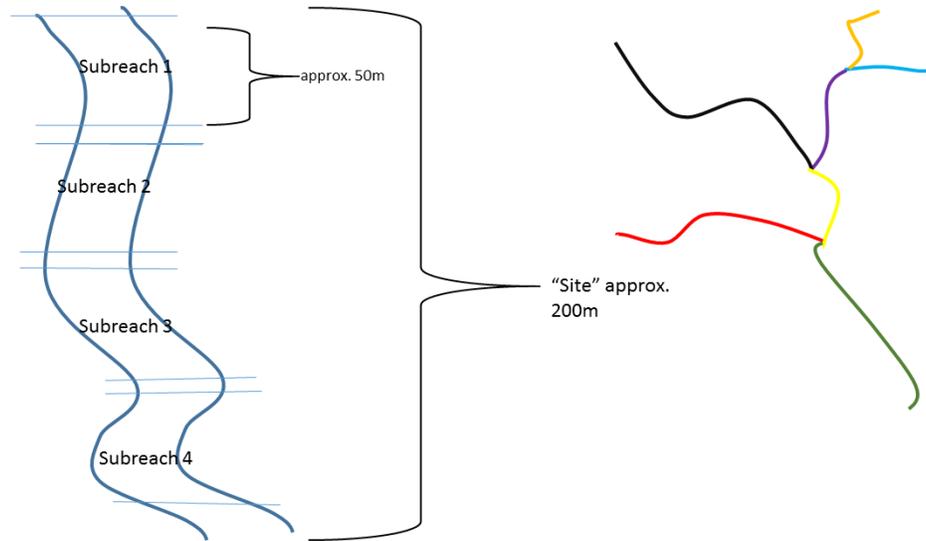
Figure 1.1: Left-A diagram of a 200-m "site" composed of 4 50-m sub-reaches. Right-hypothetical stream network which each segment split by stream nodes and with each labeled a different color
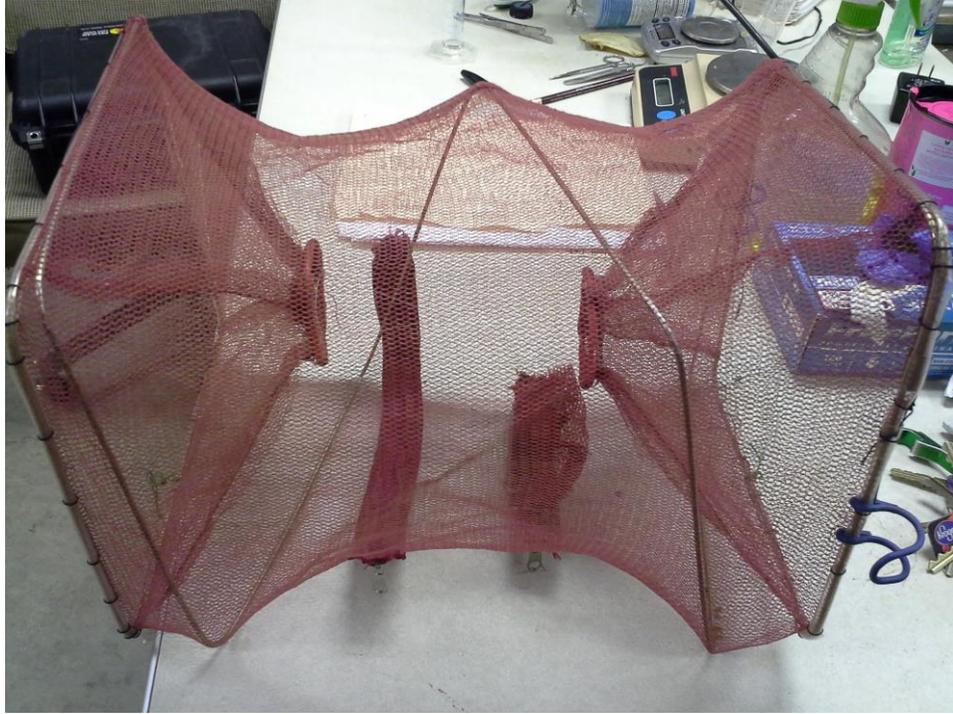
Figure 1.2: Promar® mesh minnow trap used in fish sampling.
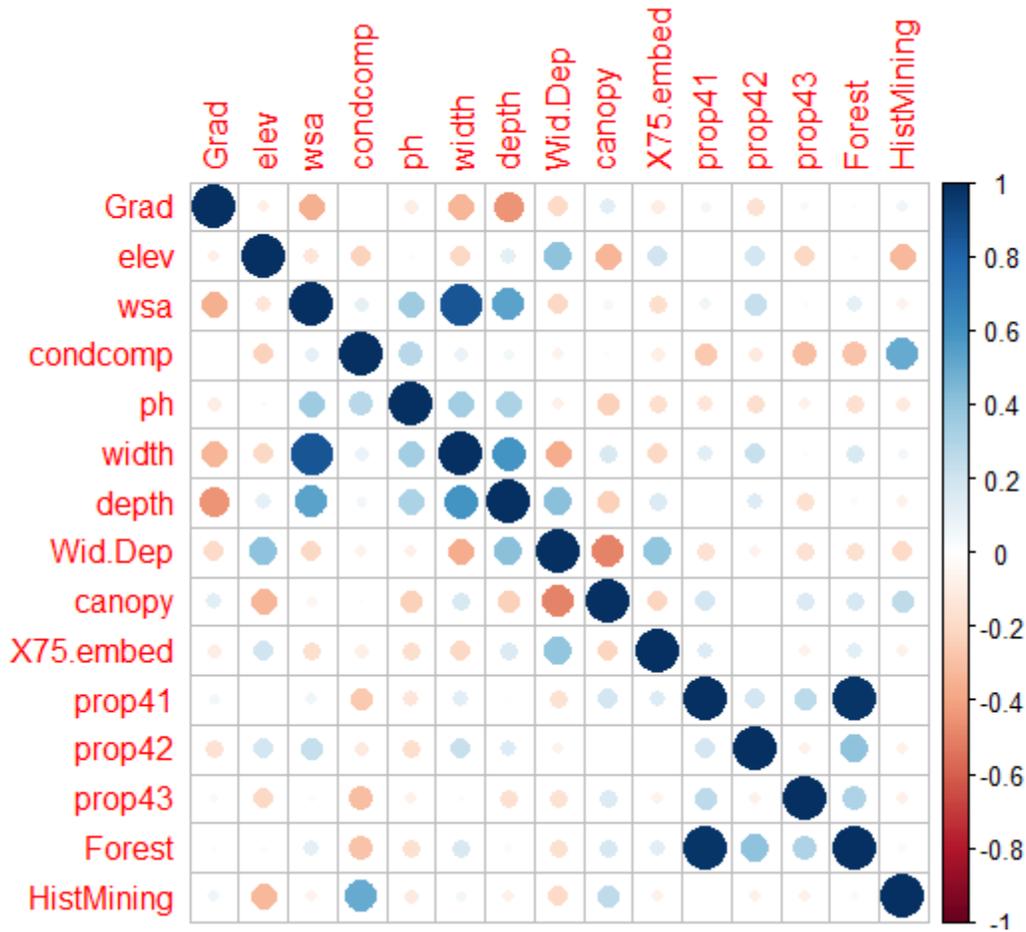
Figure 1.3: Pearson correlation matrix plot for *z*-scored habitat variables. See Table 1.2 for variable abbreviations and descriptions.
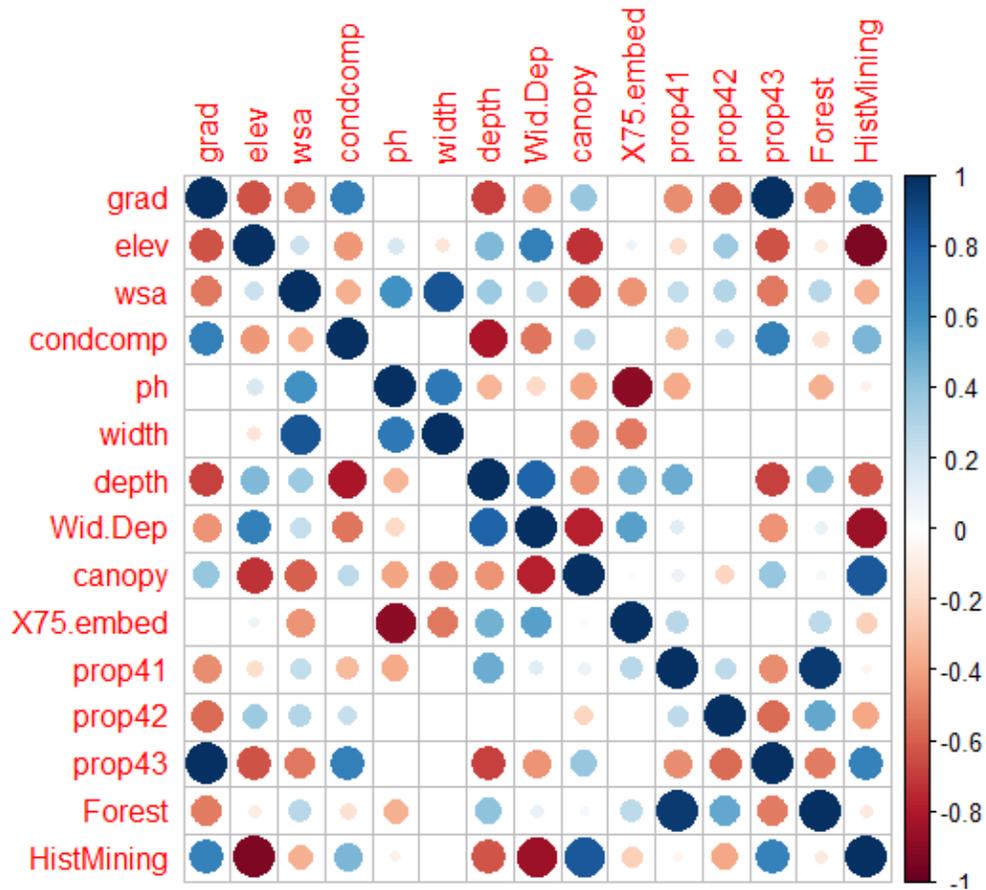
Figure 1.4: Pearson correlation matrix plot for spatially transformed *z*-scored habitat variables.

See Table 1.2 for variable abbreviations and descriptions.

Model Hypothesis Categories:

Landscape ▬ (green)	Land Use ▬ (purple)	Channel Morphology ▬ (yellow)
Water Quality ▬ (blue)	Mining ▬ (orange)	Riparian ▬ (dark red)
Other ▬ (black)		
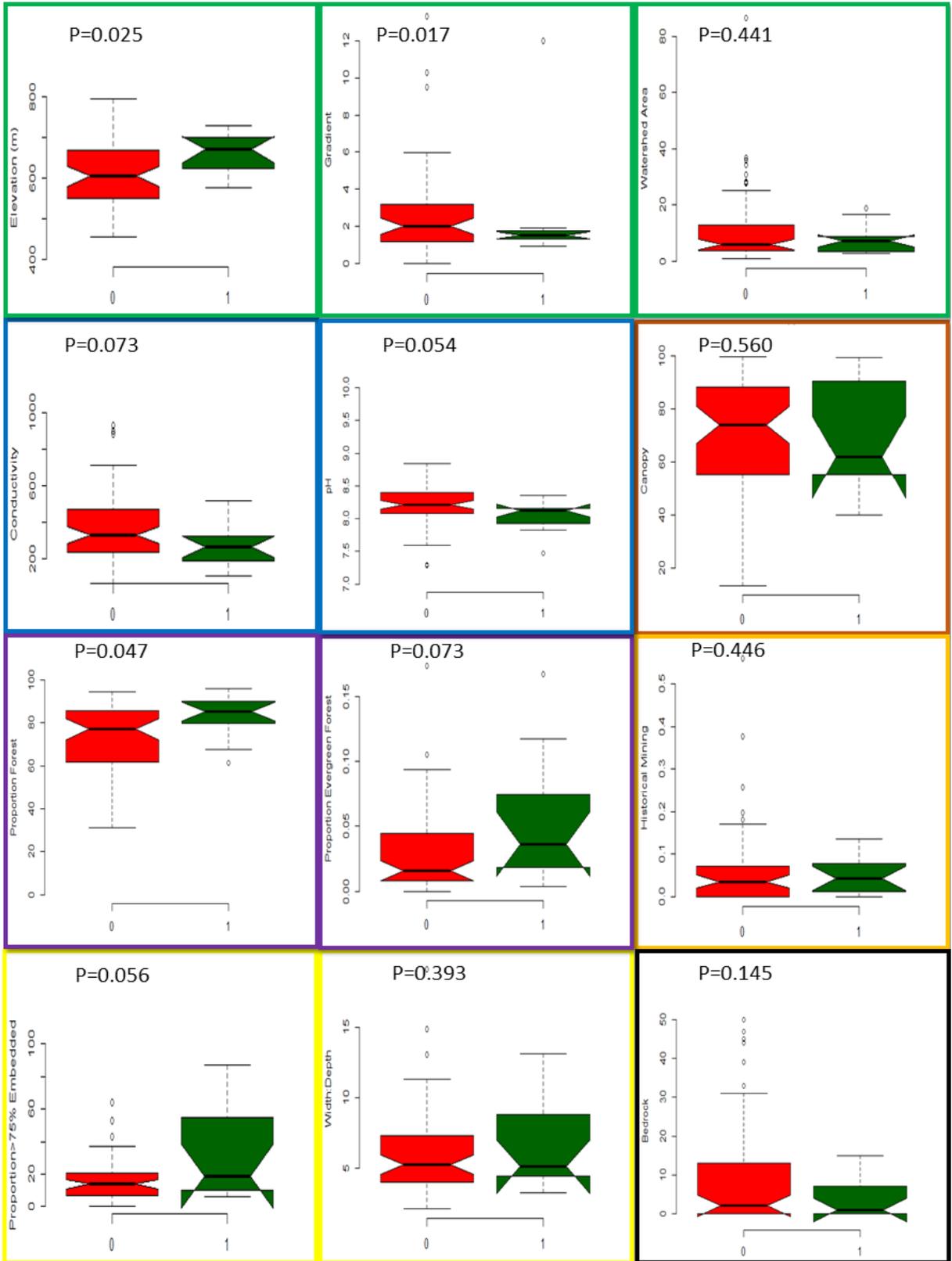
Figure 1.5: Box plots illustrating the distribution of environmental variables incorporated into occupancy modeling. Variable values are the raw values before normalization or transformation to account for spatial autocorrelation. Green box plots (1) are values at sites where Clinch Dace were detected and red boxes (0) were values for sites where Clinch Dace were not detected. The borders of the boxes are color-coded to group the environmental variables in the model hypothesis categories used in the occupancy modeling framework. *P*-values correspond to 1-sided bootstrapped Kolmogorov-Smirnoff tests to test whether the distributions of each environmental differ between sites where Clinch Dace were present and those where they were absent.
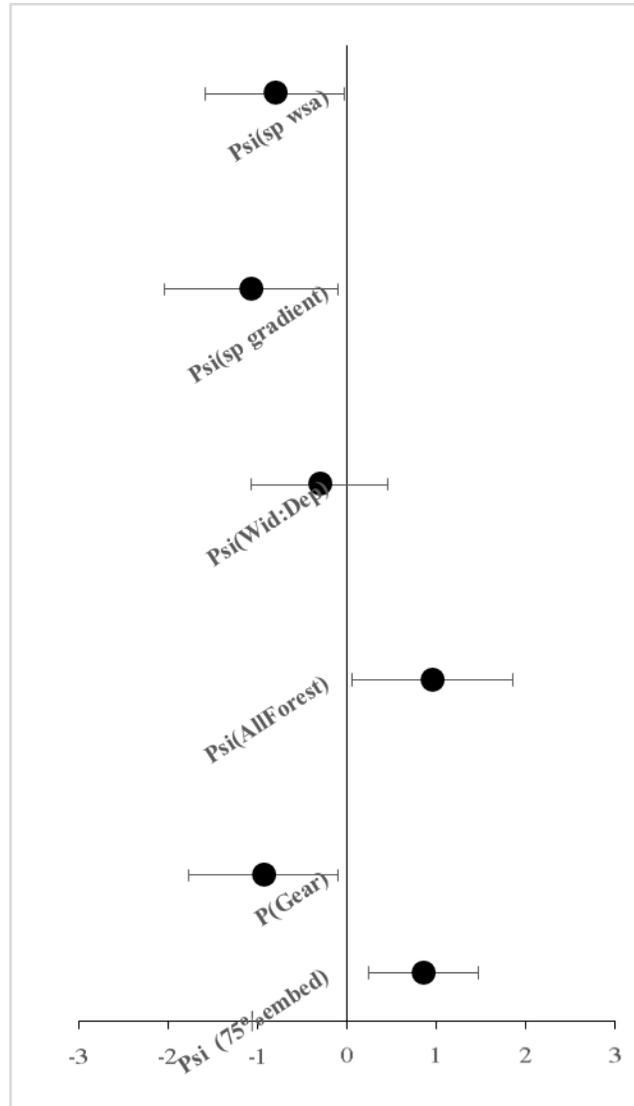
Figure 1.6: Average beta values and associated 95% confidence intervals for covariates of occupancy and detection probability in models with >0.05 model weight. "spatially transformed" covariates are indicated with an "sp". Note* minnow trapping was coded with a 1 in our data entry form and electrofishing with a 0, a negative beta value at -0.94 (95% CI= -0.177- -0.10) suggests that minnow trapping has a lower associated probability of detection for Clinch Dace.
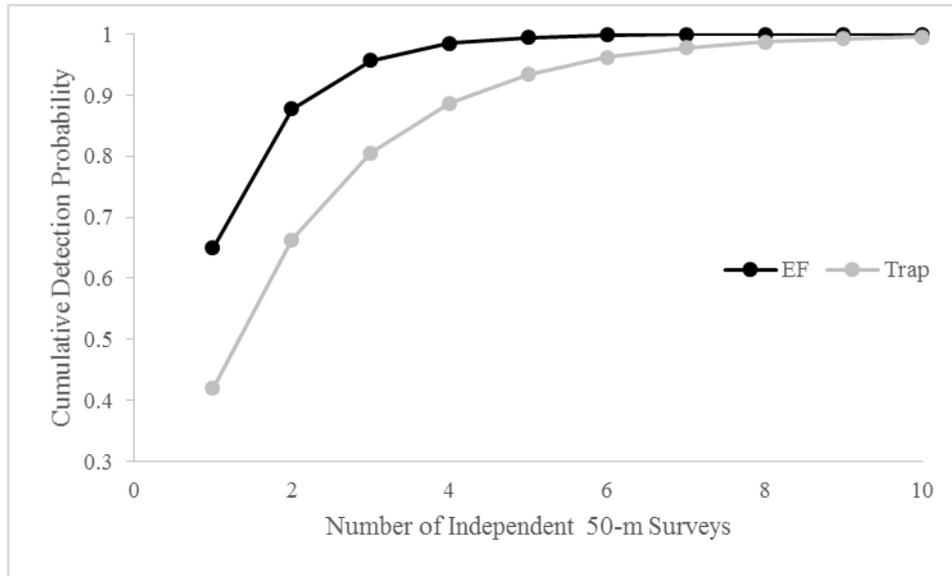
Figure 1.7: Cumulative detection probability assuming independent replicates of a 50-m survey site. Electrofishing refers to single-pass surveys and minnow trapping refers to the deployment of six baited cloth mesh minnow traps per 50 m
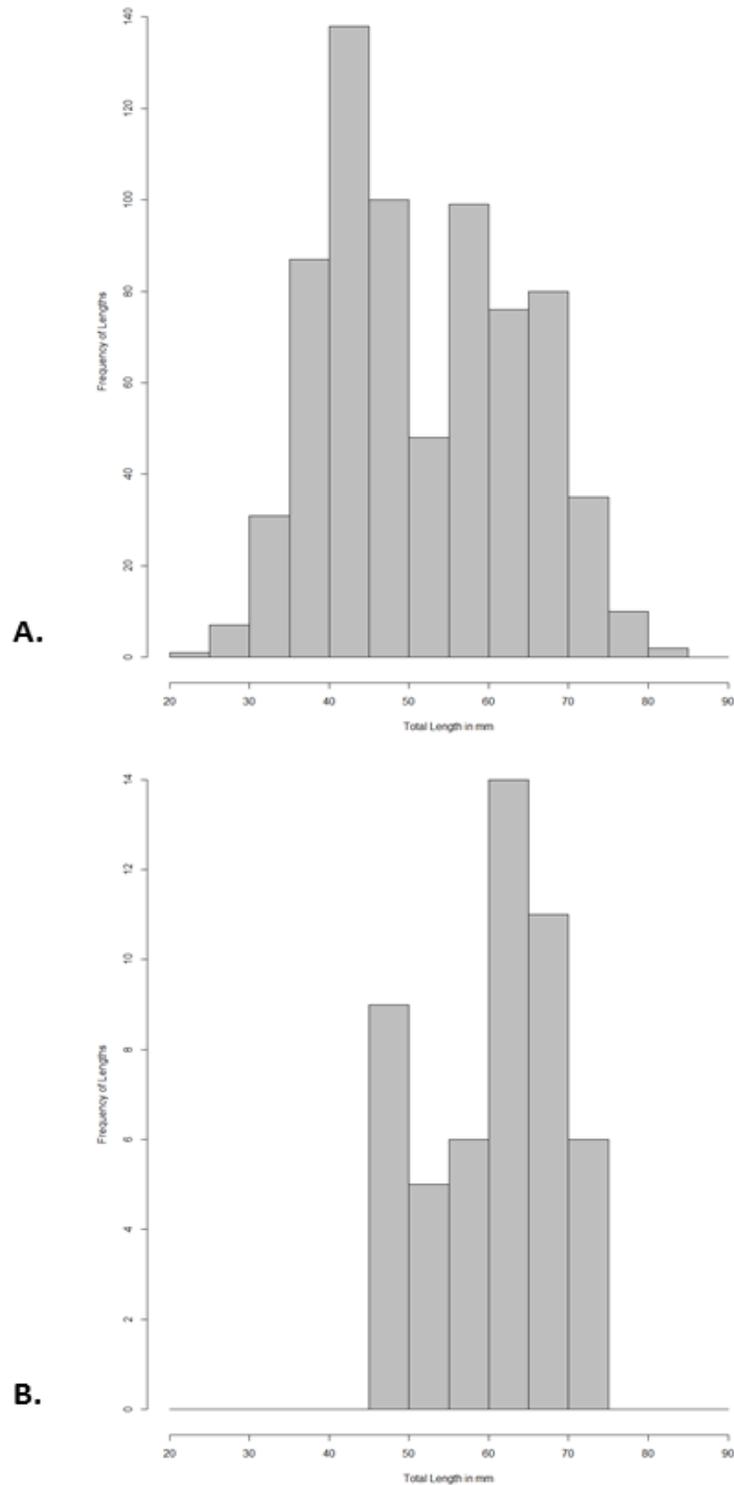
Figure 1.8: A. Length-frequency histogram (total length) with 5-mm bins for all Clinch Dace captures during 2014-2015 N=714 **B.** Length frequency histogram (total length) with 5-mm bins for available Clinch Dace captured by Shannon White 2011-2012 N=51.
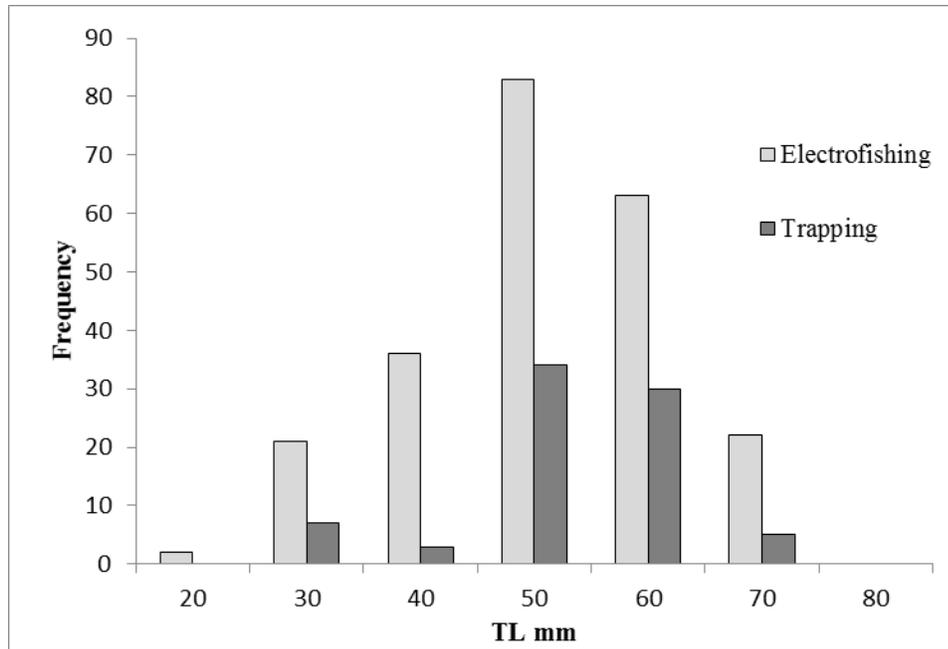
Figure 1.9: Length-frequency histogram with 10-mm bins for all Clinch Dace captured during 2014-2015 at 70 occupancy modeling sites divided by gear type.

Figure 1.10: Aerial photograph of a large surface mine in Fork Ridge, VA approximately 4 miles north of Raven, VA. An isolated population of Clinch Dace exists upstream of the mine in Left Fork Coal Creek, where conductivity readings were measured at <200 μS/cm in 2015. Downstream of the mine at Jamison Creek, a tributary of Left Fork Coal Creek, we measured conductivity values >1000 μS/cm. In Jamison Creek, an orange precipitate likely of oxidized iron coated the stream bottom.

Chapter Two: Estimating Range and Density of *Chrosomus* sp. cf. *saylori* in the Upper Clinch River Basin in Virginia.

Abstract

Estimates of Clinch Dace abundance and density are needed across its range in Virginia. These data will help biologists prepare monitoring and management plans for the species that indicate where distinct populations exist and their long-term viability. I generated estimates of population extent, measured in km of potentially occupied habitat, based on 206 site visits from four independent fish surveys within the Clinch Dace's putative range since 2007. I used *N*-mixture models with repeated count data to test hypotheses on factors that influence Clinch Dace abundance. Next, I used mark-recapture techniques to estimate Clinch Dace densities in five occupied streams. A linear regression model generated from these data allowed me to transform relative abundance estimates from single-pass electrofishing data into absolute abundance estimates with 90% prediction intervals (PI) for Clinch Dace in occupied streams. I developed estimates of total census and genetic size of individual populations and summed to estimate global abundance. I estimated that Clinch Dace occur at low densities in 31.5 km out of the approximately 351 km of 2nd- and 3rd-order streams in Russell and Tazewell counties in Virginia. Individual population census sizes ranged from 11 to 1675 individuals. Estimates of combined global census population size for 10 of 16 populations was just 6,706 (90% PI=2,847-22,594) individuals larger than 45 mm. Clinch Dace populations are small, isolated, and likely lack genetic diversity. These results underscore the need for regular population monitoring and confirm that prompt conservation action is needed to protect most Clinch Dace populations in Virginia.

Introduction

Clinch Dace (*Chrosomus* sp. cf. *saylori*), a newly discovered species of finescale or redbelly dace, is known from only 10 tributaries to the upper Clinch basin in Russell and Tazewell counties Virginia. Surveys conducted in 2007 and 2010-2012 suggest that the species occurs at low densities in scattered pool habitat in these small headwater streams (Skelton 2007; White and Orth 2014a). Due to its small range and low densities, Clinch Dace are listed as a tier I species (very high conservation need) in the Virginia Wildlife Action Plan (Virginia Department of Game and Inland Fisheries 2015), endangered by Jelks et al. (2008), and "critically imperiled" by NatureServe (2015). Yet until now, any estimates of Clinch Dace abundance and total occupied stream length have been rough estimates at best. The upper and lower extents of Clinch Dace populations in occupied streams had been based on scattered sampling records. White (2012) estimated that Clinch Dace occupied 8%, or 1.4 km, of the 2nd-and 3rd-order stream length that she sampled. She noted that 125 km of stream habitat within 2nd and 3rd order tributaries to the Clinch River in Northern Russell and Tazewell counties (my present study area) still needed to be sampled to refine this estimate. This led to an estimation of 11.4 km of potentially occupied stream habitat.

Differences in sampling methodology confound direct comparisons of abundance across space and time in occupied streams. Thompson (2004) highlights four levels of uncertainty that may affect sampling and management for rare or elusive species: environmental variation, partial observability, structural uncertainty, and partial controllability. The first two are of greater importance when developing monitoring protocols while the latter dictate species management. Environmental variation in stream habitat, such as flow or turbidity, influence a biologist's ability to detect rare species. Secondly, partial observability can make large populations seem small if survey methods capture only a small fraction of individuals present. Both factors hinder biologists' attempts to confidently assess whether management actions lead to the desired responses in population abundance.

During the first surveys for the species, the largest number of individual Clinch Dace captured at a site by Lingenfelser et. al. (2004) was 16. Later, Skelton (2007) captured over 40 individuals in just two

seine hauls in Big Lick Creek. White and Orth (2014a) observed much lower maximum densities, capturing at most 13 individuals in 250 meters of backpack electrofishing. Was Skelton able to capture a larger proportion of the local population with seining, or do Clinch Dace populations exhibit inter-annual stochasticity, or did White et al. (2013a) observe a declining trend in these populations? These questions are impossible to answer without standardized survey methods specifically designed to generate estimates of relative or absolute abundance over longer time frames. Data on abundance is similarly lacking for other species in the genus. Only one other published study has aimed to measure population densities of *Chrosomus* daces. In reaches two-thirds as long as those sampled by White and Orth (2012), the maximum single-pass catch for Blackside Dace (*Chrosomus cumberlandensis*) was much higher at 151 individuals (Black et al. 2013).

Management plans present recovery goals at the population level. For example, the most recent draft of the Recovery Plan for the closely related, Federally Endangered Laurel Dace states that in order to delist the species, biologists must ensure "viable populations are present throughout suitable habitat in Bumbee, Moccasin, Youngs, Soddy, and Cupp creeks, and Horn Branch, and one additional viable population exists either through reintroduction into Laurel Branch or discovery of an additional wild population" (George et al. 2015). The Recovery Plan for the Federally Threatened Blackside Dace also mandates recovery goals at the population-level; stating that at least three inhabited stream reaches must be present in eight populations (Biggins 1988). Such recovery goals prompt two questions that are paramount for rare species recovery: 1. How do we define a population? And 2. How do we determine population viability?

The two preeminent standards for defining populations are the ecological and evolutionary paradigms. The ecological paradigm focuses on the location where individuals live while the evolutionary paradigm is concerned with breeding patterns and gene flow. Waples and Gaggiotti (2006) provide the following definitions of each: ecological paradigm-"a group of individuals of the same species that co-occur in space and time and have an opportunity to interact with each other," and evolutionary paradigm-"a group of individuals of the same species living in close enough proximity that any member of the

group can potentially mate with any other member." Conflicts between demographic and genetic

worldviews are widespread in conservation biology theory. Examples are the declining (demographic)

and small (genetic) population paradigm frameworks, which are pitted against one another to describe the

factors influencing imperiled populations. The declining population paradigm centers on recruitment and

mortality rates that influence population size, while the small population paradigm describes the impacts

of low genetic variability leading to local extirpation or species extinction. However, one approach is not

necessarily superior to the other, and researchers should try to incorporate demographic and genetic

principles into management plans or choose the one worldview that best meets their research capabilities

and knowledge goals (Lande 1988; Caughley 1994).

The ecological and evolutionary paradigms develop estimates of migration among populations

differently. A migration rate is calculated for the ecological paradigm, whereas the number of genetically

effective migrants per generation, i.e. gene flow, is calculated for the evolutionary paradigm. Population

boundaries of small-bodied fishes under the ecological paradigm are usually determined through studies

of physical migration and dispersal capabilities through mark-recapture studies (Detar and Mattingly

2013; Walker et al. 2013).

Individual movement under the evolutionary paradigm is determined using records of familial

lineage stored in DNA (Roberts et al. 2016). The field of population genetics includes a variety of genetic

markers and statistical models to test patterns of geneflow over evolutionary and contemporary time

frames (Gharrett and Zhivotovsky 2003). For instance, an assessment of Laurel Dace mitochondrial DNA

variation indicated evolutionarily significant genetic divergence between northern and southern

populations that should not be mixed in stocking or translocation (Strange and Skelton 2005).

Estimates of population size are based on count data corrected for partial observability or the fact

that not all individual organisms are observed. Catch-per-unit-effort is a measure of relative abundance

which one assumes is representative of absolute abundance. While sampling and analytical methods vary,

the general premise is that the number of individuals in a population is $N = \frac{C}{Parea * Pd}$, where C equals the

count during sampling and *Parea* is the area occupied by the species and *Pd* is the proportion of total individuals that are captured (Thompson 2004). Mark-recapture sampling protocols are commonly used to assess population size. Researchers mark captured individuals on the first sampling occasion and then record the proportion of marked individuals captured during a second sampling period. Individual detection probability also impacts the probability of species detection, as highlighted in Chapter 1 Figure 1.7. If there are more individuals in an area and each individual has a high probability of capture, then the odds of detecting at least one individual of that species will also be high. Species detection can be estimated with the equation $p = 1 - (1 - r)^N$, where r is individual detection probability and *N* is population abundance (Royle and Nichols 2003).

Royle and Nichols (2003) introduced a class of models referred to as *N*-mixture models that use temporally repeated count data on unmarked individuals at a set of sites to estimate average abundance over the study area and the habitat covariates correlated to species abundance, all while accounting for imperfect detection of individuals. These hierarchical models integrate likelihood estimates of *p* and *N* over the prior user-selected mixing distribution for the parameter (Poisson, zero-inflated Poisson, or negative binomial) (Royle and Nichols 2003; Royle 2004). The Poisson is the most commonly used distribution in ecological data because it assumes that individuals are distributed randomly over space. Patchiness of populations are accommodated using model covariates or by specifying a distribution such as negative binomial that accounts for extra variance. Within a site, a binomial distribution is used to model the variation in counts. Essentially these models are nested generalized linear models, a Poisson or negative binomial regression for abundance, and a binomial model for detection. Assumptions of the model are similar to the single-season occupancy models used in Chapter 1. Populations must be closed, individuals of the species must not be falsely counted, individuals must be detected independently, and all individuals must have identical detection probability.

The *N*-mixture models can provide biased site-specific estimates of *N* and these estimates are likely to be inferior to the results of mark-recapture methods. However, the utility of these models is that such count estimates allow for heterogeneity in detection probability based on abundance at a site

(Mackenzie 2006). More useful from my perspective, these models can provide additional insight into habitat suitability once species abundance and not just presence or absence are considered.

After estimating absolute abundance, biologists must ascertain whether populations are viable. Viability relates to the census size (*Nc*) and genetic diversity of a population. Estimating population growth or decay rates also can be answered with traditional "fish-in-hand" methods or through estimating the genetically effective population size (Caballero 1994; Crow and Kimura 1970; Wright 1931; Wright 1938). Low genetic diversity in small populations reduces individual fitness, thereby increasing the risk of population extirpation (Leberg 1990; Spielman et al. 2004). Higher genetic variability within a species can produce more stable population sizes, larger distributions, and reduce overall extinction risk (Forsman and Wennersten 2015). The genetic effective population size (*Ne*) is one measure of genetic variability and viability of a population. A commonly used standard for minimum viable *Ne* is 50 to reduce the risk of inbreeding depression or genetic drift in the short term, and an Ne of 500 to preserve the population's potential for evolutionary adaptation over longer periods (Jamieson and Allendorf 2012).

The ratio of genetically effective population to census population sizes varies among taxa depending on life history strategy. Clinch Dace mature early, have short generations, and bear relatively small clutches compared to other fishes, all of which may allow it to cope with highly variable environmental conditions in headwater streams. These are characteristics of an opportunistic life-history strategy, which may cause dramatic population fluctuations in response to environmental stochasticity (Winemiller and Rose 1992). Species with large variation in population size over generations, usually have lower genetic variability and smaller *Ne*:*Nc* ratios (Jamieson and Allendorf 2012). A literature review of studies published prior to 1995 estimated mean *Ne*:*Nc* across broad taxonomic groups to be around 0.10 (Frankham 2007). Therefore, estimates of minimum census populations needed to meet the 50-500 rule in Clinch Dace might equate to census sizes on the order of 500-5000 or larger.

There is little doubt that a thorough comparison of both ecological and evolutionary population paradigms for the Clinch Dace, is warranted. On the strength of four independent surveys for Clinch Dace in Virginia since 2007, I set three goals to begin characterizing the extent and viability of each population

of Clinch Dace. First, I define the geographic extent of populations of Clinch Dace by converting sample points to reasonable estimates of contiguous occupied stream length. Second, I use *N*-mixture models to explore the habitat variables that may affect Clinch Dace abundance using both the raw data and accounting for spatial autocorrelation. Third, I conduct the first estimate of Clinch Dace density using a mark-recapture protocol. Fourth, I combine estimates of distribution and density to estimate census population size and through use of published *Ne*:*Nc* ratios, genetically effective population size.

Methods

Population Extent

      To develop an estimate of occupied habitat length, I compiled all sampling records collected since 2007 (Skelton 2007; White and Orth 2014a). Fish surveys have been conducted at 93 different sites across the putative range of Clinch Dace in Virginia by four researchers over the past eight years (Skelton 2007; T. Coyner 2009, unpublished; White and Orth 2012, Z. Martin 2015, unpublished). In 2014-2015, I surveyed another 114 sites and added them to the record database in an effort to fill in gaps in the 125 km of unsampled 2nd-and 3rd-order habitat in northern Russell and Tazewell counties. The 113 sites were composed of the 70 randomly selected sites for my occupancy analysis, the 15 sites sampled for mark-recapture density estimates, and 28 additional targeted sites that were sampled opportunistically. Different survey protocols were used in each of the three independent surveys. Consequently, detection probability given species presence may differ among surveys and it would be difficult to include this heterogeneity in distribution models (See Chapter 1). Therefore, I used only records of confirmed Clinch Dace presence in my estimation of range extent.

      I set population boundaries under the assumption that Clinch Dace would have limited dispersal capability that would be further reduced in the presence of culverts, predators, and other barriers. I defined populations as sets of historical records of Clinch Dace presence within a stream network separated by at least 1.5 km of uninhabited stream length from all other records of Clinch Dace presence. The maximum dispersal threshold of 1.5 km was based on a study of movements of 226 individual

Blackside Dace; in this study, fewer than 5% moved more than 1000 m across mostly occupied or suitable habitat (Detar and Mattingly 2013). To estimate range extent, I calculated the distance between the furthest upstream and the furthest downstream records of Clinch Dace in each stream and assumed that Clinch Dace occupied the intervening habitat.

N-Mixture Models with Repeated Count Data

I used the same candidate model set from the multi-scale single-season occupancy modeling exercise from Chapter 1, except that site-level covariates for species occupancy (ψ), became site-level covariates for mean abundance (λ or hereafter *N*), and species-level covariates for detection (p) became detection covariates for individuals (r). The researcher must also set a value for *K* which is the estimate of the maximum number of individuals that could occur at a site. The model then integrates the abundance estimate below this value. I adjusted the value of K in my models to 240, high enough that AIC was relatively stable but top model still satisfied the GOF test. 240 individuals/50 m also likely exceeds the biological carrying capacity of Clinch Dace in the wild. The maximum estimated density of Blackside Dace in Detar and Mattingly (2013) was 153 individuals/50 m in Breeden's Creek, KY. The carrying capacity for Clinch Dace is also likely lower for Clinch Dace as evidenced by our mark-recapture population estimates and the unique life-history traits of the species (White and Orth 2014b). This approach allowed me to test whether the environmental factors most strongly related to Clinch Dace abundance differ from those most strongly related to Clinch Dace occupancy. The model categories and hypotheses related to abundance are as follow: *Landscape* (gradient, elevation, watershed area, gradient+elevation, gradient+watershed area, elevation+watershed area, and gradient+elevation+watershed area), *water quality* (conductivity, pH, conductivity+pH), *channel morphology* (width:depth, embeddedness, width:depth+embeddedness), *watershed land cover* (all forest, evergreen forest), *resource extraction* (historical mining in watershed), and *riparian* (canopy cover). I assessed correlation between site covariates and highlighted instances when pairs of variables in the same category had a Pearson correlation coefficient greater than 0.7. I scaled and centered all habitat variable

measurements. I considered all spatial and temporal replicates as equal. This gave me eight 50-m survey replicates for each site. I then collapsed the detection history and combined data for the two 50-m reaches for each gear type on each day. This gave me a dataset consisting of 70 sites with four sampling repeats at each site (two temporal replicates of 100 m of backpack electrofishing and two replicates of 100 m of minnow trapping). I conducted a goodness of fit (GOF) test based on the Pearson's chi-square statistic using 100 simulations on the top-ranked model. I ran GOF tests multiple times to gain a larger sample size.

To account for spatial autocorrelation in the habitat data and remove the spatial signature from my habitat data, I implemented the same procedure to "spatialize" my habitat measurements as in Chapter 1. First, I calculated the pairwise network (fluvial) distances among all of my 70 sites using network analyst tools in ArcGIS 10.1. Second, I used a principal coordinates of neighborhood matrices (PCNM) analysis with the *PCNM* function within the *PCNM* package in R in order to identify the significant spatial eigenvectors within my dataset. The significance test on eigenvalues associated with each spatial eigenvector uses a permutation approach for the Moran's I statistic. I tested what subset of spatial eigenvectors best predicted each habitat variable using a stepwise model selection technique. Selection eigenvectors was completed with the *stepAIC* function in the *MASS* package of R based on minimum value of Akaike's information criterion (*AIC*). The spatially significant eigenvectors were used in multiple linear regression models to predict "spatially transformed" habitat data. I again normalized the final matrix of predicted values prior to use in occupancy models.

I ranked all 71 models including those produced with the "raw" and "spatially transformed" habitat data in a single model-ranking table to assess the effect of spatial autocorrelation in the count data. I judged model support in an information theoretic framework based on minimum *QAIC*. The *QAIC* weight helps assess the level of support for a top model versus other models in the candidate set and is a value that ranges from 0-1 and sums to 1 for all models. I conducted all analyses using the *pcoun*t() function within the "unmarked" package of R.

Mark-Recapture Density Estimation

The second component of population size estimation requires more informed estimates of density in each occupied stream. A low proportion of recaptures of fin-clipped individuals during 2014 suggested that the individual capture efficiency with backpack electrofishing may be poor and that actual populations may be larger than single-pass sampling catch rates.

A probabilistic sampling methodology is required to extend abundance estimates to unsampled sites (Thompson 1992). Therefore, in July and August, 2015, I used a stratified approach to randomly select three 100-m reaches in six streams containing Clinch Dace to conduct a two-pass mark-recapture estimate of abundance. The streams I selected for mark-recapture — Big Lick Creek, Greasy Creek, Hart Creek, Hurricane Fork, Middle Creek, and Pine Creek — previously had been sampled thoroughly to estimate the downstream and upstream extent of occupancy, had strong or moderately strong historical catch rates of Clinch Dace, and had good stream access throughout the occupied area.

Another requirement of sampling schemes to measure population abundance is that all individuals must be available for capture at the time of sampling (Thompson 2004). For example, if conducting visual surveys for a marine mammal, the only individuals that are available to count are those that surface during the survey period. I assumed that all Clinch Dace in a stream were available for capture during backpack shocking since all would be within range of our nets and electric field.

I implemented a mark-recapture population estimation protocol using the Chapman estimator following methods used by Black et al (2013) for Blackside Dace. I enclosed the 100-m reaches using minnow seines tied to t-posts and weighed the bottom of the seines with large rocks to satisfy the assumption of closure. On the first day, I used single-pass electrofishing to capture Clinch Dace within each 100-m reach using a Smith-Root LR24 electrofishing unit adjusted to the minimum immobilization threshold in order to limit injury, mortality, or stress during capture. I recorded electrofishing time from a

subset of surveys to ensure that effort was roughly even across sampling events. I retained captured

Clinch Dace in an aerated bucket until the entire site had been sampled. I measured total length (TL) mm

and marked all individual Clinch Dace larger than 45mm (roughly the transitional age of age 0-age 1

individuals indicated by the length-frequency histogram assembled during my first field season) (Figure

1.1) with a small clip of one caudal fin lobe. I allowed Clinch Dace to recover from the procedure in the

bucket and then released all fish in pool habitat throughout the reach.

The next day, I returned and sampled each reach again using single-pass backpack electrofishing.

I measured and counted each dace and noted whether it had a fin clip. I used a Chapman modification of

the Petersen mark-recapture estimator, which is less biased at small sample sizes. The equation for the

Chapman estimator is $N=\frac{(K+1)(n+1)}{(k+1)} - 1$. Where K is the number of individuals marked on the first visit,

n is the number of individuals captured on the second visit, and k is the number of individuals recaptured

on the second visit. Following sampling, I thoroughly examined the downstream blocknet for any fish that

had been swept downstream during the sampling period. I also estimated 90% confidence intervals for

densities at each of my mark-recapture sites by assuming recaptures fit a binomial distribution. A

binomial distribution allows for extra variation to more closely approximate overdispersed data.

I developed a linear regression model relating the Log_{10} first day single pass catch to the Log_{10}

population estimation for all reaches with >0 first day catch (N=13). I then used this regression to

estimate population size based on all surveys conducted by Shannon White and myself that used single-

pass electrofishing. I calculated electrofishing sampling efficiency by taking single-pass capture /100 m

from the first day of sampling divided by the Chapman-estimated population density/100 m and averaged

across all mark-recapture sampling reaches.

The mean density estimates for each stream were composed of population estimates for the 100-

m reaches sampled in the mark-recapture analysis, transformed single-pass catches I conducted in 2014

and 2015, and all transformed single-pass catches from White (2012) standardized to a length of 100 m. I

calculated mean 90% upper and lower prediction boundaries from all available data for each occupied stream.

Total Population Estimation

I multiplied the mean, 10%, and 90% estimates of density of >45 mm Clinch Dace/100 m by the estimated number of occupied 100-m reaches in each stream to estimate the total number of >45 mm Clinch Dace in each stream. Using data across multiple years and streams helped account for spatial and temporal variation in Clinch Dace abundance. A few populations lacked single-pass electrofishing data, and thus I did not estimate abundance for these populations. I applied an Nc:Ne ratio of 0.1 to all populations to generate a rough estimate of genetic effective population size (Frankham 2007), allowing me to identify populations that may be threatened by low genetic diversity.

Results

Population Extent

Clinch Dace occupied 16 contiguous or nearly contiguous areas of habitat in Virginia, assemblages of individuals that I will henceforth refer to as "populations." I documented Clinch Dace presence at all but five historically occupied streams. I was unable to capture Clinch Dace in Town Hill Creek, Little Town Hill Creek, and Jackson Fork, and was unable to access the North and South Branches of Indian Creek, leaving the current status of these populations unknown. The estimates of potentially occupied stream length for each population ranged from a single point in Jackson Fork to 6,995 meters in Mudlick/Zeke Creek. The mean occupied length was 2,250 m. The total across all 15 populations was 31,502 m or 31.5 km (Table 2.1). In 2016, Zachary Martin, a graduate student at Virginia Tech, discovered Clinch Dace in Grassy Creek, a tributary of Lewis Creek in Russell County. His sampling record was a few hundred meters upstream of previous sampling events conducted by White and Orth (2014b) and by myself in 2015 that had failed to detect Clinch Dace. This new population is not contained in this analysis.

N-Mixture Models with Repeated Count Data-

The mean *P*-value for the GOF test with this top model was 0.06 indicating marginal fit. Gear had a strong effect on the number of Clinch Dace captured. *ΔAIC* increased by >13 when the gear covariate was removed from a given model.

For abundance, only one model outperformed the null model. This model was from the landscape model category and contained the "raw" measurement of "watershed forest cover". However, support for this model being the best among the hypothesis set was weak, with a ΔAIC of 0.49 and a weight of 0.11. The null model for abundance also had good support *ΔQAIC*=0.49, w_i = 0.08. Clinch Dace did not occur at high densities when watershed forest cover was <80% (Figure 2.6).

The only other model with a ΔQAIC <2 was the model containing the "spatially transformed" canopy cover data (*P=0.08*) ΔQAIC=1.42, w_i = 0.05. Sites with "spatially transformed" canopy cover values <60% did not have high densities of Clinch Dace.

Although substrate embeddedness was the most influential site level covariate in occupancy models based on presence-absence data, the model with substrate embeddedness as a covariate of abundance had very little support *ΔQAIC*=2.11, w_i = 0.03. Greasy Creek and Laurel Fork, the sites with the highest embeddedness, had very low estimates of Clinch Dace density.

Mark-Recapture Density Estimation

Mean effort based on electrofishing time for 22 of the 30 sampling events was 2013 seconds (SD=440.1). Sampling time likely had more to do with the width and habitat complexity of each reach than inconsistent survey effort. Wide reaches with complex habitats simply took longer to sample (maximum, 3287 seconds). Electrofishing times measured at sites on both days only deviated by 174 seconds on average (*N*=6).

Single-pass catches for Clinch Dace in the 100-m mark-recapture reaches ranged from 0 individuals >45 mm at Greasy Creek to 23 individuals >45 mm at Middle Creek. I recorded few recaptures during the second sampling event at most sites. On 5 occasions, I had 0 recaptures of individuals that I had captured on the first day. Sites with few recaptures such as those in Hart Creek yielded wide confidence intervals using the binomial distribution. Comparisons of the number of Clinch Dace captured on day one with the Chapman population estimate of the 100-m reach led to an average individual capture probability of 0.28, suggesting that single-pass electrofishing may result in the capture of only one out of every four Clinch Dace within a given stream reach. I did not complete sampling at Big Lick Creek because flash floods following the first sampling event overtopped the block nets and violated the assumption of closure. On the first day of sampling at Big Lick Creek, I captured a moderate number of Clinch Dace (mean=9.3 >45 mm individuals/100 m).

The mean Chapman estimated density of Clinch Dace for 13 reaches with day 1 captures of ≥ 1 was 30.67 Clinch Dace/100 m. Hart Creek (63.3/100 m), Pine Creek (39.7/100 m), and Middle Creek (38/100 m) had high estimated densities of Clinch Dace, while Hurricane Fork (11/100 m) and Greasy Creek (1.3/100 m) had low estimated densities.

After removing the two sites at Greasy Creek where day one catch was 0, I calculated the following linear model to relate Log_{10} single-pass catch rate to mean Log_{10} population estimate: $y=1.071x+0.48$ ($R^2 = 0.8451$) (Figure 2.2). I used this model to estimate absolute densities at all sites sampled where I had obtained 100-m single-pass electrofishing data (Chapter 1) with the equation $y= 3.022x^{1.071}$ with x being the combined day-one catch at the two 50-m electrofishing reaches and y being the estimated abundance. I predicted Clinch Dace density in 35 100-m reaches. The resulting estimates of density at mark-recapture sites ranged from 3 to 100 dace/100 m with a mean density of (31 Clinch Dace >45 mm/100 m). The mean density in predicted reaches was 23.6 Clinch Dace >45 mm/100 m. Estimated abundance from White (2012) were much lower than recent collections; with a mean of density of 5.7 Clinch Dace ≥ 45 mm/100 m.

Total Population Estimation

I estimated Clinch Dace abundance for 10 of the 15 populations (Table 2.2). Five populations lacked reliable estimates of occupied stream length or Clinch Dace density due to limited sampling access or low densities. Hurricane Fork/Grassy Branch had the largest estimated population size of 1,764 individuals (90% PI=640-4768). The smallest estimated population was in Left Fork Coal Creek with an estimate of only 10 (3-27) individuals. Other demographically strong populations included Pine Creek-1,675 (868-4111), Hart Creek-1,097 (443-7,955), Big Lick Creek-662 (254-1,727), and Mudlick/Zeke Creek-571 (218-1,502). Other demographically weak populations included Town Hill Creek-70 (25-200) and Hess Creek-32 (12-89). The combined estimate of population size for all 10 estimated populations was 6,706 (2,847-22,594) individuals >45 mm (Table 2.3).

Applying a *Ne*:*Nc* ratio of 0.1 suggested by a review of the genetics literature to these populations suggests all populations are threatened by a lack of genetic diversity. The estimated of *Ne* of four populations would fall below the recommended short-term conservation threshold of 50, and the remaining five would fall below the long-term threshold of 500.

Of the populations where I was unable to estimate population size, only North and South Branch Indian Creek, Laurel Fork, and West Fork Big Creek have the potential to substantially increase the estimate of global population size. Lewis Creek is perhaps the smallest Clinch Dace population and lacks an abundance estimate because Clinch Dace were captured at this site only with minnow traps in a single pool during occupancy surveys and at a single location further upstream during opportunistic electrofishing sampling. Clinch Dace may no longer exist in Jackson Fork or the population was so small that I did not detect Clinch Dace despite thorough surveys in 2014.

Discussion

This study represents the first effort to characterize the density and spatial extent of discrete aggregations of Clinch Dace. My surveys confirm the isolated nature of Clinch Dace populations and the low densities of individuals within occupied streams. These data are essential elements for creating management plans tailored to protection and recovery of individual populations.

Population Extent

My estimate of 31.5 km of occupied habitat is significantly larger than the estimate of 11.4 km given by White (2012). The difference results from observations of new populations and range extensions discovered in 2014 and 2015 and from my assumption that Clinch Dace occupied all habitat between the most upstream and downstream records of occurrence. White (2012) only counted reaches where she personally observed Clinch Dace. The two branches of uppermost Indian Creek, Laurel Fork, Upper West Fork Big Creek, Left Fork Coal Creek and Upper Town Hill Creek remain the least monitored Clinch Dace streams. Uncertain current status of these historical populations may lead to inaccuracies in the estimate of occupied stream length. However, overly conservative estimates of total length occupied for some streams where a lack of upstream access limited sampling opportunities is likely balanced by more generous downstream estimates of population boundaries where I captured a single Clinch Dace.

Stream access continues to pose operational obstacles to monitoring Clinch Dace populations. I was unable to sample North and South Branch Indian Creek. A large landholding company denied sampling access in 2015 citing injury liability concerns. Clinch Dace were discovered in Lower Town Hill and Little Town Hill Creeks in 2007 and 2011 (Skelton 2007; White 2012). I sampled the lower reaches of Town Hill Creek and Little Town Hill Creek extensively in 2014 and did not find a single Clinch Dace. The landowner denied access to the middle and upper reaches of this stream in 2014. I frequently observed trucks hauling coal on the road that parallels Town Hill Creek. Mining activities and sediment inputs from roads and mines upstream reduced water-transparency in Lower Town Hill Creek

even at base-flow in 2014. In the future, biologists should continue to work with landowning companies to confirm the status of the species further upstream and to encourage erosion control measures.

I also sampled extensively for the historically observed population of Clinch Dace in Jackson Fork. I sampled approximately 1.2 km of stream habitat in 2014 with backpack electrofishing without capturing a single Clinch Dace. The only record of Clinch Dace in Jackson Fork comes from a single individual in 2011 (White and Orth 2012). This individual may have been a migrant from the nearby populations in North or South Branch Indian Creek. A complex of beaver ponds prevented sampling further upstream. This stream had many deep pools suitable for Clinch Dace that were often occupied by Rosyside Dace and predatory Rock Bass (*Ambloplites rupestris*) instead. The pool where White captured the dace had been altered following the construction of a new road crossing. Photos of the old road crossing from the Virginia Department of Mines Minerals and Energy show it as a potential barrier to upstream movement by fishes. Shannon White captured only one Rock Bass in 250 m of backpack electrofishing in 2011. In 2014, I captured several Rock Bass and Redbreast Sunfish (*Lepomis auritus*) both above and below the new, passable road crossing. Future barrier removal decisions should consider the movement dynamics of potential predators in addition to Clinch Dace.

Upper West Fork Big Creek has never been sampled. A gated mining road allows access to the upper portions of the watershed, but requests for access were denied in 2015. Clinch Dace densities are low in the downstream portions of the creek, but there the stream is wide and high gradient, with a predominantly bedrock substrate. Habitat conditions that are more suitable for Clinch Dace may exist further upstream.

Left Fork Coal Creek has been sampled sporadically in the past by Skelton (2007) and White and Orth (2014). I made visual confirmation of several Clinch Dace occupying a large pool below an impassable culvert in the upper part of the creek in June 2015.

The population boundaries for Clinch Dace in Big Lick Creek, Pine Creek, Mudlick Creek are also ill-defined. A single individual Clinch Dace was captured in two days of sampling in lower Pine Creek and lower Mudlick Creek in 2014. The downstream record of Clinch Dace in Big Lick Creek

comes from Skelton (2007). White (2012) thoroughly sampled this entire creek in 2011 and 2012 and found the lower population boundary 1.3 km further upstream. Population extent estimates in adjacent Zeke and Mudlick creeks also may be misleading. Sites in these creeks were positioned lower in the watershed and were much wider than typical Clinch Dace habitats with little pool habitat and only a few Clinch Dace captured. The upper reaches of Zeke Creek have never been sampled. Access is limited, with only gated roads leading to gas wells and contour mining operations. Survey data in Mudlick Creek from White 2012 and Skelton 2007 as well as the largely forested land cover of the watershed suggest that larger populations may exist further upstream in both of these creeks with the possible exchange of migrants.

Future efforts to survey populations with limited access hinge either upon developing relationships with large private landholding companies or improving sensitivity of remote detection methods such as environmental DNA sampling (Moore et al. 2015).

Migration through the mainstem Clinch River or 4th-order or larger tributaries would expose Clinch Dace to suboptimal habitat conditions and the risk of predation. This view suggests that dispersal between the 10 occupied sub-tributary networks is not likely. Within tributary networks, particularly Swords and Indian creeks, the potential exists for reestablishing metapopulations. Both watersheds contain multiple occupied tributaries in relatively close proximity. In the Swords Creek system, Pine Creek and Big Lick Creek are separated by only about 2 km of unoccupied stream habitat. An individual that washes out of Big Lick Creek during a high flow event may be able to immigrate to the population in Pine Creek. Future habitat restoration should focus on these unoccupied stream reaches between neighboring Clinch Dace populations. Creation of habitat corridors that are more hospitable for Clinch Dace may encourage migration through Big Lick and Indian Creeks as has proven effective in promoting movement of other small fish species across inhospitable habitats (Roberts and Angermeier 2007). Geneflow among populations also would boost genetic variability and effective population sizes (Jamieson and Allendorf 2012). Other studies on *Chrosomus* suggest that movement is correlated with high-flow events and day length (Albanese et al. 2004; Detar and Mattingly 2013). I encourage future

mark-recapture movement studies or genetic analysis of the Indian Creek (North and South Branch Indian Creek, Greasy Creek, Laurel Fork, Jackson Fork) and Swords Creek (Pine, Big Lick, and Hess Creeks) populations to determine the magnitude of emigration/immigration processes and the environmental contexts under which they are likely to occur.

Refining our knowledge of the distribution of Clinch Dace should remain an ongoing endeavor. Undiscovered populations of Clinch Dace likely exist elsewhere in Virginia or Tennessee. Much more research attention has been directed towards Blackside Dace, which had its Federal Species Recovery Plan published in the late 1980s and for which biologists are still discovering new populations (Bivens et al. 2013; Skelton 2013). The estimated occupied stream length for Clinch Dace (19.6) miles is slightly higher than the estimated occupied length for Blackside Dace (17 miles) when its recovery plan was written in 1988 (Biggins 1988). However, since 1988, biologists have located around 90 additional streams occupied by Blackside Dace (Biggins 1988; Floyd 2015). More concerning is that since its discovery, Blackside Dace are believed to have been extirpated from 31 streams (Floyd 2015). Declines of similar magnitude could result in the extinction of the Clinch Dace.

The distributional ranges of *Chrosomus* species are not always constrained by logical watershed boundaries. The propensity of the genus to occupy headwater streams makes them some of the most likely fish species to have crossed drainage divides through natural stream-capture processes (Burridge et al. 2006) and through bait-bucket transfer (Skelton 2013). Large tracts of land owned by the U.S. Forest Service exist within the Clinch watershed in northern Scott County, Virginia. This area within the Central Appalachian ecoregion has sparse sampling records and should become a sampling priority for Clinch Dace occupancy. Logistic distribution models incorporating habitat data from the NHDPlus version 2 dataset and all of the survey data for Clinch Dace from multiple researchers suggests that several streams within this area have habitat conditions suitable for supporting undiscovered Clinch Dace populations (J. Huang and M. J. Moore, unpublished).

N-Mixture Models with Repeated Count Data

The strong support for the inclusion of a sampling gear detection covariate in occupancy models again confirms that electrofishing is more effective at capturing a larger proportion of the individuals present than minnow trapping. Managers can use this information to select sampling gears for the species based on a desired level of individual detection probability.

The *N*-mixture models suggest that the habitat factors most strongly correlated with Clinch Dace presence-absence are not necessarily the same as those that are most strongly correlated with abundance. Watershed forest cover received the most support in the N-mixture models as a covariate of *N*. The additional information provided by the count data can help us understand the strange positive relationship with substrate embeddedness that I observed. The two streams occupied by Clinch Dace that had very high levels of fine sediment deposition both had very low densities of Clinch Dace. One of these streams, Laurel Fork, had a sediment load that was likely a product of beaver impoundment that entrained large quantities of fine sediments. The *N*-mixture models do not provide evidence to support the hypothesis that Clinch Dace thrive in areas with silty substrate. Although their nest associate reproductive strategy can help them cope with these silty conditions, reproductive and foraging success may be increased with substrates that support more diverse assemblages of macroinvertebrate prey. Consequently, managing for abundant Clinch Dace populations likely involves protecting forested land cover in watersheds; limiting surface mining near Clinch Dace streams to keep conductivity below a critical threshold that may be harmful to Clinch Dace and their prey resources (Hitt et al. 2016); eliminating barriers that restrict Clinch Dace movement and colonization of new habitat; ensuring vegetated riparian corridors for bank stabilization, reduced sediment transport, and thermal regulation; and increasing natural pool-forming structures.

Other streams with highly forested watersheds that did not have Clinch Dace but could be candidates for future translocations include Rock Lick Branch, Weaver Creek, Honey Branch, and Alvy Creek. Of these, Alvy Creek is the strongest candidate based on its low measured conductivity, undisturbed riparian buffer, and stream channel size.

The contrast between the model rankings with "raw" and "spatially transformed" data suggest that autocorrelation exists among habitat variables, especially those that are measured at larger scales such as watershed land cover and elevation. The adjusted R^2 values for the multiple linear models that used spatial eigenvectors to predict the "spatially transformed" data suggested that spatial gradients were much stronger in large-scale habitat variables, such that removing the spatial signature removed most of the explanatory power of the variable (Table 2.2). For example, 77% of the variation in elevation and 31% of the variation in watershed forest cover was explained by spatial predictors. This explains why the models including the riparian model category, which was measured at the site scale performed better with the "spatially transformed" data.

Landscape ecologists have long wrestled with the influence of scale in riverine management and the issue of whether riparian or watershed land cover is more important to structuring fish communities (Jones et al. 1999; Rabeni and Sowa 2002; Allan 2004; Frimpong et al. 2005). The River Continuum Concept (Vannote et al. 1980) suggests that headwater stream ecology is tightly linked with riparian vegetation responsible for shading and providing allochothonous organic matter. Similarly, in a study in Indiana, Frimpong et al. (2005) found that the strongest correlation between fish community metrics and percent forest cover occurred within buffer strips 30 m on either side of the stream and that correlation decreased rapidly as the buffer size increased in width. However, other studies have found that land cover at the watershed scale has a stronger influence on biotic communities (Roth et al. 1996; Wang 1997). Perhaps, I would have seen an even stronger correlation with riparian land cover had I calculated land use within a buffer width of the stream instead of measuring canopy cover. However, the anthropogenic land alterations in Appalachia are much different than in the agriculture dominated Midwest. Mining and forestry and steeply sloped watersheds may lead to increased importance of large-scale habitat management. Regardless of scale, intact forests appear important for maintaining Clinch Dace populations.

Other habitat variables, such as water quality may emerge with increased statistical power provided by a larger sample size and that better fit the distributional assumptions of the model. There also

can be a significant time lag between changes in land cover patterns and responses in stream ecological communities, such that present-day communities are a product of land-use conditions 50 or more years ago (Harding et al 1998). This dataset is smaller than the size recommended by Royle and Nichols (2003) (T=5 sampling replicates at R=100 sites) for repeated count occupancy models; however, I believe that this available count data from our survey still provides useful insight into Clinch Dace habitat associations.

Mark-Recapture Density and Total Population Estimates

The capture efficiency of 0.28 for Clinch Dace in the current study aligns closely with the estimate of 0.30 for Blackside Dace (Black et al. 2013). Clinch Dace populations are not as small as suggested by single-pass electrofishing catch data. Regardless, Clinch Dace are usually one of the least abundant fish species in the streams that they inhabit. Only in one stream, Grassy Branch, were Clinch Dace among the most abundant fish species, comprising 26% of the total fish community. I do not believe that significant delayed mortality from the fin clipping or learned avoidance behavior influenced recapture rates. Published studies suggest that excision of pectoral fins or rear halves of caudal fins in Brown Darters (*Etheostoma edwini*) did not affect their survival or ability to swim against strong currents (Champagne et al. 2008). Fin-clipped fish showed no obvious swimming impairment in my study and catch rates on the second day were similar to catch rates on the first.

One downside to my sampling approach is that while it accounts for partial observability, it does not consider environmental variation and its impacts on individual catchability. Future efforts to estimate population size may take into account the amount of woody cover, flow level, turbidity, canopy cover, and other factors that may alter the capture efficiency during surveys.

The physical habitat conditions varied across mark-recapture reaches and may be examined in relationship to Clinch Dace density. Hurricane Fork appears to contain quality physical habitat, but Clinch Dace densities were low. Mature forest shades the stream channel, and fine sediment deposition is low. Much of the watershed is owned by a forestry management company; so there is the threat of future

logging. Surprisingly, conductivity in the stream is higher than in any other stream known to contain Clinch Dace (516 µS/cm). Mining once occurred in this system, although the most intense activity was downstream of extant Clinch Dace populations. Much of Hurricane Fork is larger than streams typically favored by Clinch Dace, averaging 3-4 m wide. This population was estimated to be the largest because of higher densities in Grassy Branch, a tributary of Hurricane Fork. Lower Grassy Branch contains a culvert which although not perched, may be impassible to small fishes. Efforts should be made to increase connectivity with Hurricane Fork to support the functionality of the metapopulation that likely exists in these streams.

Hart Creek, Pine Creek, and Big Lick Creek also harbor strong populations of Clinch Dace. In contrast to Hurricane Fork, land use in these watersheds is mostly residential, which has led to riparian habitat degradation and siltation in places. I recommend that harvest of Clinch Dace for translocation or captive breeding projects should be from one of these four populations after genetically assessing populations for existence of evolutionarily significant units that should be managed separately (Strange and Skelton 2005).

In contrast, mark-recapture surveys indicate that the densities of Clinch Dace in Greasy Creek are alarmingly low. Here, anthropogenic habitat alterations are high. The streambed is composed primarily of sand, and Rosyside Dace, a potential competitor for food or spawning habitat, are far more abundant than Clinch Dace. In 2014-2015, I captured a few juveniles and observed spawning behavior in 2015 which suggests that natural reproduction of Clinch Dace is occurring. In 2015 I discovered potential hybrids between the two species in Greasy Creek and Laurel Fork based on outward phenotype (Figure 2.7). An unnamed tributary to Greasy Creek harbored a small population of Clinch Dace in 2014, but in 2015 its connection to Greasy Creek was blocked by a beaver dam constructed at a culvert, leaving much of the small stream impounded into a large marsh. The suitability of impounded habitat for Clinch Dace is unknown.

Clinch Dace may occur in even lower densities in other streams. The population in Lewis Creek appears perilously small. Surveys suggest Clinch Dace are restricted to a few pools within a few hundred

meters of this stream. Anthropogenic activities have degraded instream and riparian habitat. The riparian vegetation is shrubby grassland, horse pasture, or residential lawn. The substrate is almost entirely fine silt or sand. Human sewage is entering the stream from residential areas along the creek.

Clinch Dace were also rare in Laurel Fork. Sampling access to the upper reaches of this stream is limited by lack of road access. Beaver activity is high in this watershed. Some timber harvest is occurring in the upper portions of this watershed.

It is unclear why the estimates of population size using the White (2012) data for 2011 and 2012 were so much lower than the estimates for surveys conducted in 2014 and 2015. Clinch Dace populations likely exhibit high annual variability. Studies in a North Carolina stream showed that while the fish assemblage structure remained relatively stable across years, the absolute abundance of the rarer species exhibited high coefficients of variation (>76%) as a result of strong and weak year-classes (Freeman et al. 1988). Research has not examined whether and to what extent climatological conditions effect Clinch Dace populations. It is possible that Clinch Dace recruitment declined as a result of drought conditions in southwestern Virginia over the period of 2007 to 2008 when Richlands, VA recorded a precipitation deficit of 12.56 inches (National Centers for Environmental Information 2007-2009). Strong-year classes may have emerged during favorable conditions in 2013 and 2014, increasing my capture totals. However, Freeman et al. (1988) and Grossman et al. (2016) suggested that recruitment of Rosyside Dace increased during drought years and declined during flood years, although, these studies occurred in streams that were larger than those containing Clinch Dace.

Average population densities of Clinch Dace are lower than those published for Blackside Dace, such that the global population of Clinch Dace is likely less than for Blackside Dace. The U.S. Fish and Wildlife Service has set precedent in providing federal protection for the Clinch Dace's congeners, the Blackside Dace and Laurel Dace. Regardless of federal conservation status, protection, collaborative restoration, and attention toward captive propagation are urgently needed to maintain viable populations of Clinch Dace. I urge the prompt description of the Clinch Dace as a species in order to draw attention to its critically imperiled status.

Population genetic investigation is needed for Clinch Dace. Extraction of DNA and sequencing of mitochondrial and characterization of microsatellite DNA length variation among fin-clipped individuals collected in 2014 and 2015 will produce more reliable estimates of both historical and contemporary population sizes. The total population estimates indicate that inbreeding depression or genetic drift could already be occurring in half of the extant populations. Genetic rescue through mixing individuals from adjacent populations may help boost effective population sizes, although considerations should be made not to mix individuals among widely genetically divergent populations which could lead to outbreeding depression. Increased genetic variability may help the Clinch Dace to adapt to a changing environment especially under predicted climatic and land use shifts.

This study confirmed that periodic two-pass mark-recapture surveys can provide quick assessments of Clinch Dace abundance in streams. Although 90% confidence intervals are wide when recapture rates are low, the data generated through these methods is sufficiently sensitive to alert biologists of major population declines. To reduce stress on populations, multiple-pass shocking could be conducted in a few high priority populations every two to three years, or roughly the span of one Clinch Dace generation. Future efforts to monitor Clinch Dace populations should attempt to standardize electrofishing technique and effort and should be coordinated by a single biologist over time.

Table 2.1: Candidate model set rankings for *N*-mixture count based abundance occupancy models determined by minimum *QAIC*. Models with spatialized covariates are designated with "sp" while "raw" covariates are indicated "raw" in the "data" column.

Data	Model	*AIC*	*QAIC*	*ΔQAIC*	exp	w_i
raw	N(forest) r(gear)	336.83	171.99	0.00	1.00	0.11
raw	N(.) r(gear)	340.77	172.47	0.49	0.78	0.08
sp	N(.) r(gear)	340.77	172.47	0.49	0.78	0.08
sp	N(canopy) r(gear)	339.77	173.41	1.43	0.49	0.05
raw	N(pH) r(gear)	340.95	173.99	2.00	0.37	0.04
raw	N(wsa) r(gear)	341.09	174.06	2.07	0.36	0.04
raw	N(evergreen) r(gear)	341.18	174.10	2.11	0.35	0.04
sp	N(wsa) r(gear)	341.64	174.32	2.33	0.31	0.03
sp	N(wid:depth) r(gear)	341.76	174.38	2.39	0.30	0.03
raw	N(embeddedness) r(gear)	341.80	174.40	2.41	0.30	0.03
raw	N(elevation) r(gear)	341.96	174.48	2.49	0.29	0.03
raw	N(cond) r(gear)	341.97	174.48	2.50	0.29	0.03
sp	N(histmining) r(gear)	342.05	174.52	2.53	0.28	0.03
raw	N(grad) r(gear)	342.22	174.61	2.62	0.27	0.03
sp	N(elevation) r(gear)	342.35	174.67	2.68	0.26	0.03
sp	N(pH) r(gear)	340.95	174.76	2.77	0.25	0.03
raw	N(wid:depth) r(gear)	342.56	174.77	2.78	0.25	0.03
sp	N(cond) r(gear)	342.61	174.79	2.81	0.25	0.03
raw	N(canopy) r(gear)	342.64	174.81	2.82	0.24	0.03
sp	N(grad) r(gear)	342.69	174.83	2.85	0.24	0.03
sp	N(evergreen) r(gear)	342.75	174.86	2.88	0.24	0.02
raw	N(histmining) r(gear)	342.76	174.87	2.88	0.24	0.02
sp	N(embeddedness) r(gear)	342.76	174.87	2.88	0.24	0.02
sp	N(embeddedness+wid:depth) r(gear)	341.86	175.93	3.94	0.14	0.01
raw	N(grad+elevation) r(gear)	342.13	176.06	4.07	0.13	0.01
raw	N(elevation+wsa) r(gear)	342.82	176.40	4.41	0.11	0.01
raw	N(cond+pH) r(gear)	342.95	176.46	4.48	0.11	0.01
raw	N(grad+wsa) r(gear)	343.00	176.49	4.50	0.11	0.01
sp	N(elevation+wsa) r(gear)	342.82	176.53	4.54	0.10	0.01
raw	N(embeddedness+wid:depth) r(gear)	343.60	176.78	4.79	0.09	0.01
sp	N(grad+wsa) r(gear)	343.63	176.79	4.81	0.09	0.01
sp	N(grad+elevation) r(gear)	342.13	176.91	4.92	0.09	0.01
sp	N(cond+pH) r(gear)	342.95	177.21	5.22	0.07	0.01
sp	N(grad+elevation+wsa) r(gear)	341.99	177.58	5.59	0.06	0.01
raw	N(grad+elevation+wsa) r(gear)	344.09	178.60	6.61	0.04	0.00
raw	N(forest) r(.)	368.20	185.79	13.81	0.00	0.00
raw	N(.) r(.)	372.19	186.38	14.39	0.00	0.00

sp	N(.) r(.)	372.19	186.38	14.39	0.00	0.00
sp	N(canopy) r(.)	371.18	187.24	15.25	0.00	0.00
raw	N(pH) r(.)	372.35	187.81	15.82	0.00	0.00
raw	N(wsa) r(.)	372.51	187.89	15.90	0.00	0.00
raw	N(evergreen) r(.)	372.61	187.93	15.95	0.00	0.00
raw	N(cond) r(.)	373.38	188.14	16.15	0.00	0.00
sp	N(wsa) r(.)	373.05	188.15	16.16	0.00	0.00
sp	N(wid:depth) r(.)	373.18	188.21	16.22	0.00	0.00
raw	N(embeddedness) r(.)	373.22	188.23	16.24	0.00	0.00
raw	N(elevation) r(.)	373.37	188.30	16.31	0.00	0.00
sp	N(histmining) r(.)	373.48	188.35	16.37	0.00	0.00
raw	N(grad) r(.)	373.66	188.44	16.45	0.00	0.00
sp	N(elevation) r(.)	373.78	188.50	16.51	0.00	0.00
sp	N(pH) r(.)	373.95	188.58	16.60	0.00	0.00
raw	N(wid:depth) r(.)	373.98	188.60	16.61	0.00	0.00
sp	N(cond) r(.)	374.03	188.62	16.64	0.00	0.00
raw	N(canopy) r(.)	374.05	188.63	16.64	0.00	0.00
sp	N(grad) r(.)	374.12	188.66	16.68	0.00	0.00
sp	N(evergreen) r(.)	374.17	188.69	16.70	0.00	0.00
sp	N(forest) r(.)	374.18	188.70	16.71	0.00	0.00
raw	N(histmining) r(.)	374.18	188.70	16.71	0.00	0.00
sp	N(embeddedness) r(.)	374.18	188.70	16.71	0.00	0.00
sp	N(embeddedness+wid:depth) r(.)	373.28	189.68	17.69	0.00	0.00
raw	N(grad+elevation) r(.)	373.55	189.81	17.83	0.00	0.00
raw	N(elevation+wsa) r(.)	374.23	190.15	18.16	0.00	0.00
raw	N(cond+pH) r(.)	374.35	190.20	18.22	0.00	0.00
raw	N(grad+wsa) r(.)	374.43	190.24	18.25	0.00	0.00
sp	N(elevation+wsa) r(.)	374.52	190.28	18.30	0.00	0.00
raw	N(embeddedness+wid:depth) r(.)	375.03	190.53	18.55	0.00	0.00
sp	N(grad+wsa) r(.)	375.05	190.54	18.55	0.00	0.00
sp	N(grad+elevation) r(.)	375.30	190.66	18.67	0.00	0.00
sp	N(cond+pH) r(.)	375.91	190.96	18.97	0.00	0.00
sp	N(grad+elevation+wsa) r(.)	373.39	191.24	19.25	0.00	0.00
raw	N(grad+elevation+wsa) r(.)	375.51	192.27	20.28	0.00	0.00

Table 2.2: Spatial eigenvectors e1-e6 used to predict each habitat variable in the process of "spatial transformation" and the model fit. The top model for each variable selected in the step-wise selection procedure only included a subset of the 6 eigenvectors deemed significant according to Moran's *I*.

Variable	Spatial Predictors	*P*-value	R^2_{adj}
Elevation	e1+e3+e4+e6	$2.2*10^{-6}$	0.77
Ev Forest	e1+e2	$1.5*10^{-7}$	0.36
Dec Forest	e1+e3+e4+e5	$1.2*10^{-5}$	0.31
Forest	e1+e2+e3+e4+e5	$1.8*10^{-5}$	0.31
Embeddedness	e5+e6	$5.8*10^{-5}$	0.23
pH	e3+e5+e6	$5*10^{-3}$	0.2
Historical Mining	e1+e3+e4	$7.0*10^{-4}$	0.19
Width:Depth	e1+e2+e3+e5	$6.9*10^{-4}$	0.18
Canopy Cover	e1+e3+e5+e6	$1.8*10^{-3}$	0.18
Watershed Area	e1+e6	0.01	0.1
Depth	e1+e2+e5	0.03	0.08
Conductivity	e1+e2	0.05	0.06
Gradient	e1	0.04	0.05
Width	e6	0.09	0.03

Table 2.3: The estimated stream length occupied, number of sites used to calculate mean density (*N)*, mean density/100 m, census population size, and genetically effective size of Clinch Dace populations based on mark-recapture sampling and transformation of single-pass electrofishing data.

Stream Name	Occupied Length (m)	N	Mean Density/100 m	Estimated Pop.	Genetic Effective Pop.
Hurricane Creek/Grassy Branch	4,269	6	41.33	1,764 (640-4768)	176 (64-477)
Pine Creek	4,416	5	38.98	1,721 (868-4111)	167 (87- 411)
Hart Creek	2,224	4	49.37	1,097 (443-7955)	109 (44-795)
Big Lick Creek	2,404	6	27.54	662 (254-1727)	66 (25-172)
Middle Creek	1582	4	40.27	637 (324-1433)	63 (32-143)
Mudlick/Zeke Creeks	6,995	2	8.175	571 (218-1502)	57 (22-150)
Greasy Creek	2,932	4	4.71	138 (56-783)	13 (5-78)
Town Hill/Little T. Hill Creek	2,336	1	3.02	70 (25-200)	7 (2-20)
Hess Creek	790	2	4.15	32 (12-89)	3 (1-9)
Left Fork Coal Creek	158	1	6.35	10 (3-27)	1 (0-3)
Jackson Fork	N/A	1	0.92	N/A	N/A
Laurel Fork	132	0	N/A	N/A	N/A
Lewis Creek	734	0	N/A	N/A	N/A
West Fork Big Creek	548	0	N/A	N/A	N/A
Indian Creek	1,982	0	N/A	N/A	N/A
Total	**31,502**			**6,706 (2,847-22,594)**	**666 (285- 2,259)**
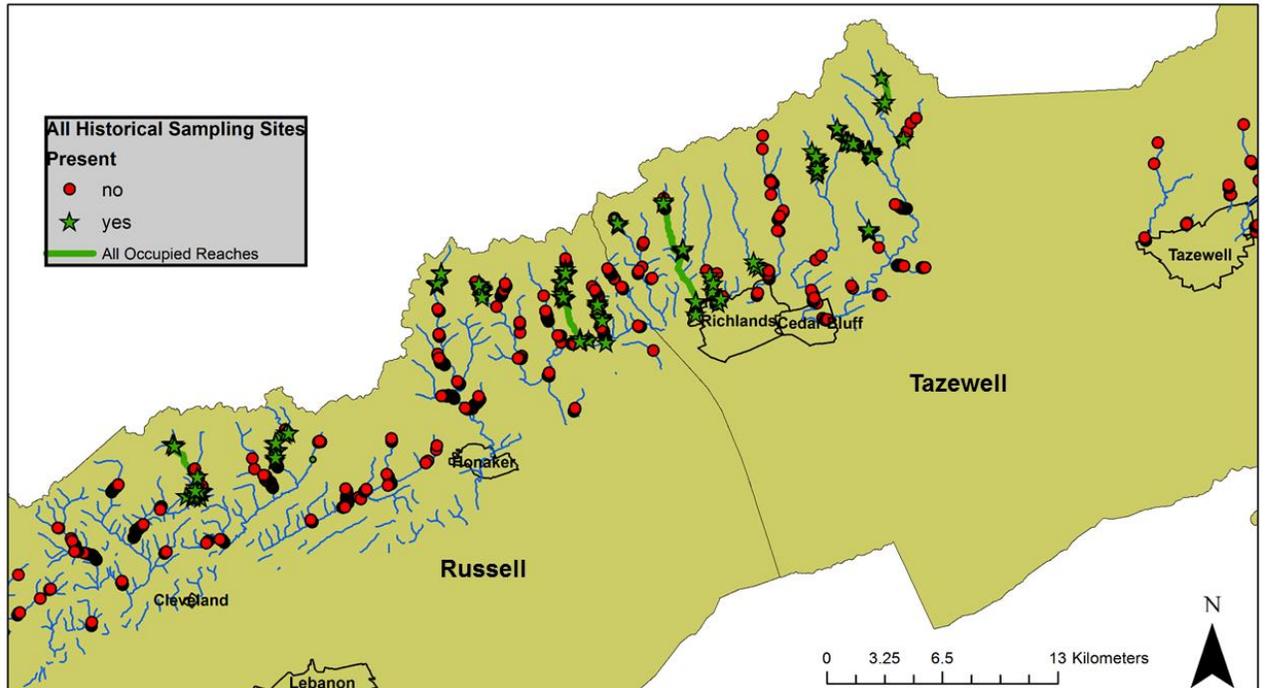
Figure 2.1: Map of Russell and Tazewell counties in Virginia, and the streams included in study area. All sampling records since 2007 included in the population-extent analysis are included. Red circles indicate sites where Clinch Dace were absent, and green stars indicate sites where they were present. The occupied reaches from the upstream-most to downstream-most records of presence are highlighted in green for each population.
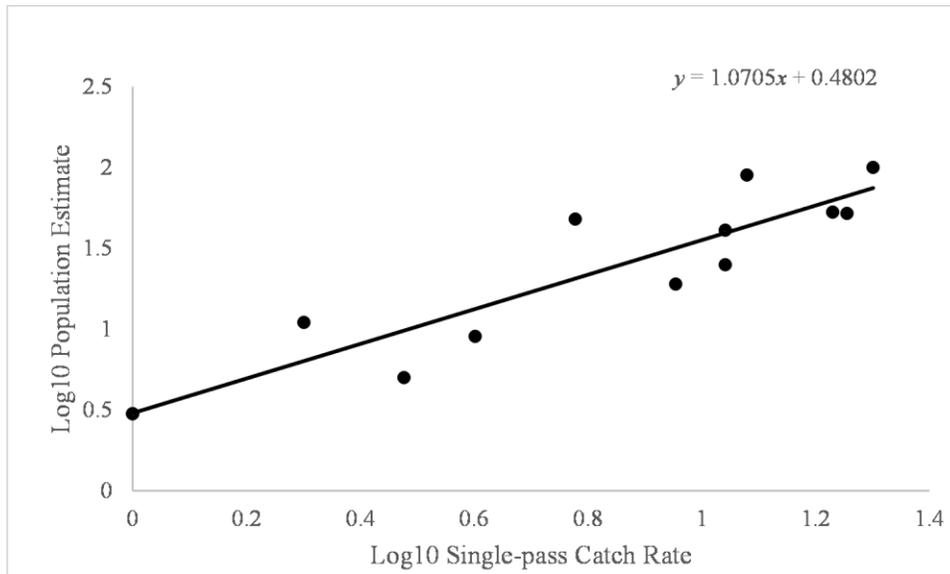
Figure 2.2: Log_{10} population estimates as a function of Log_{10} single-pass catch for the 13 mark-recapture stream reaches. The equation for the regression line is included.
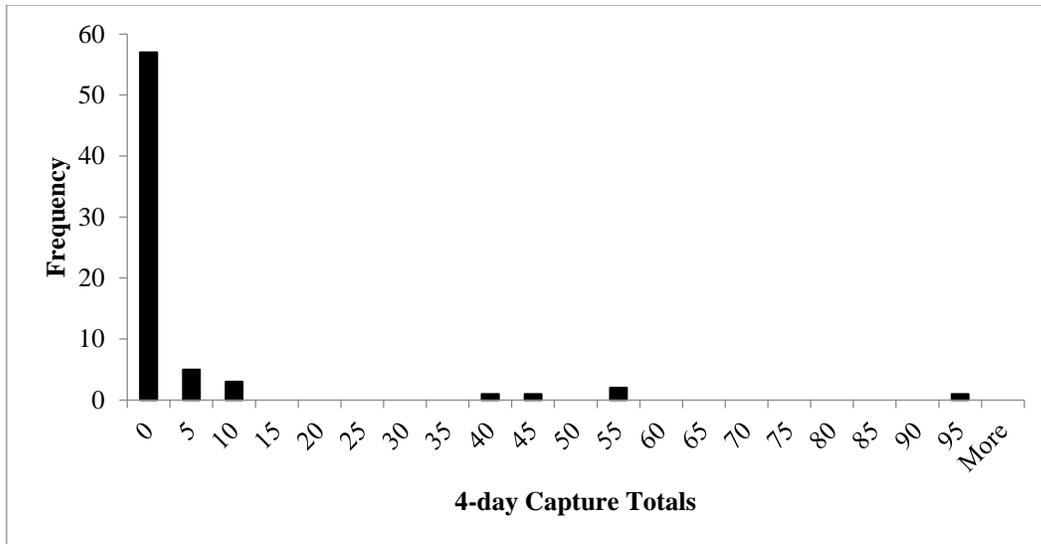
Figure 2.3: Distribution of count-data from occupancy surveys at 70 sites. The distribution does not fit a traditional Poisson distribution.
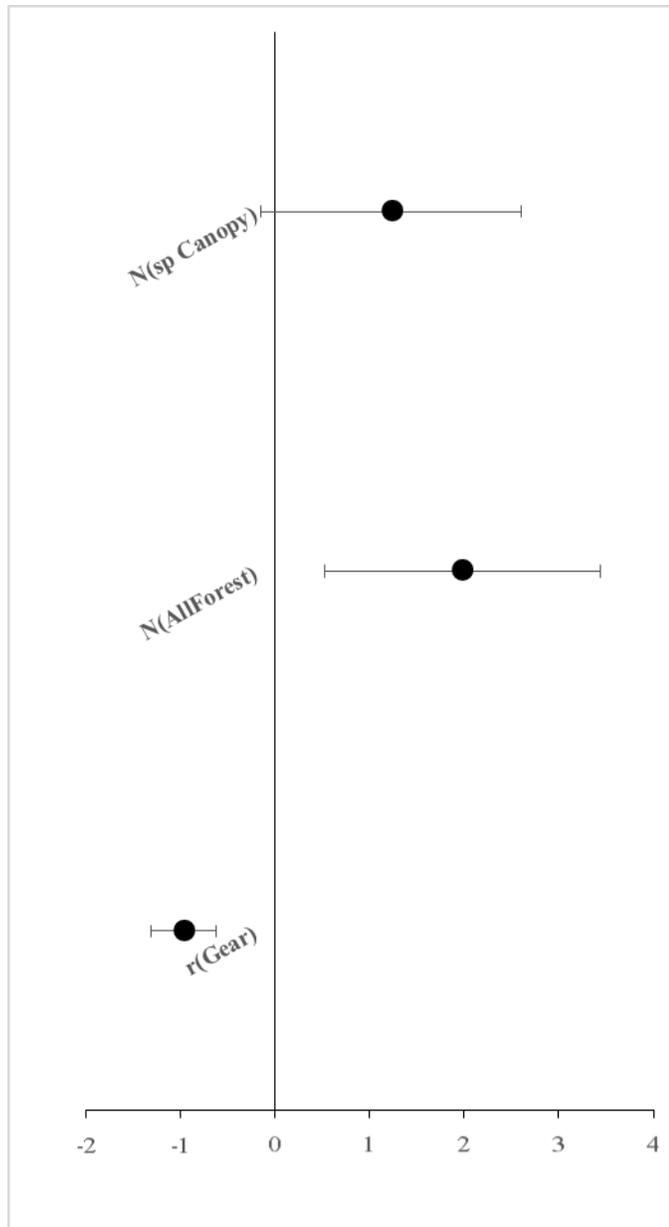
Figure 2.4: Beta values and associated 95% confidence intervals for each covariate of abundance and detection probability for models with weights >0.05.
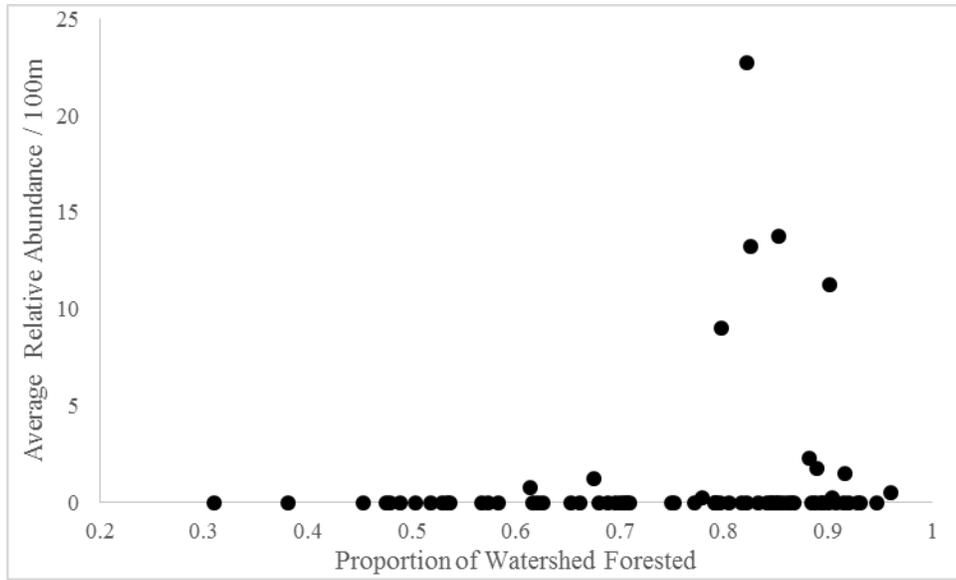
Figure 2.5: Proportion of watershed forested versus average relative abundance of Clinch

Dace/100 m in 70 sites where repeat surveys occurred.
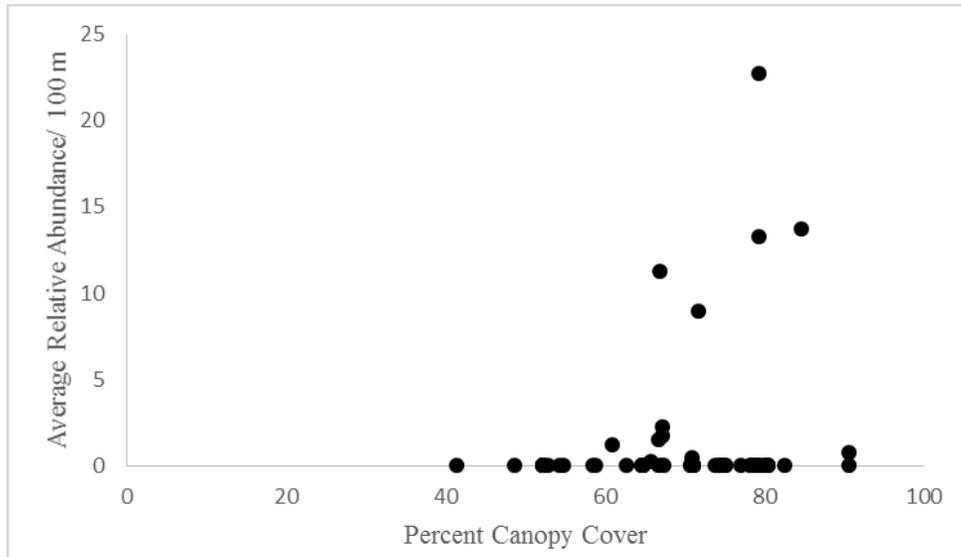
Figure 2.6: Percent "spatially transformed" stream channel canopy cover versus average relative abundance of Clinch Dace/100 m in 70 sites where repeat surveys occurred.

Figure 2.7: Top: Clinch Dace. Middle: potential hybrid Clinch Dace x Rosyside Dace. Bottom: Rosyside Dace. Middle fish is intermediate in coloration, scale size, mouth size, and mouth position between the two species suggesting it could be a hybrid. Microsatellite genetic analysis from such an individual is needed to confirm that it is a true F1 hybrid

Chapter Three: Systematic Conservation Planning for *Chrosomus* sp. cf *saylori*.

Abstract

Conservation planning frameworks allow managers to prioritize actions in order to efficiently meet predefined targets. Traditional planning frameworks often are developed at the ecological community or ecosystem level, which may neglect imperiled taxa occupying species-poor assemblages. I developed a novel multimetric systematic planning framework based on indices of irreplaceability, condition, complementarity, opportunity and feasibility for the 15 geographically distinct candidate conservation areas occupied by the imperiled Clinch Dace. I adopted principles of community-based planning such as metrics of irreplaceability, condition, complementarity, opportunity and feasibility. Irreplaceability is a metric of population demographic strength. Habitat condition is related to instream physical habitat, water quality and watershed land use. Complementarity considered total headwater fish assemblages in the upper Clinch basin. Finally, opportunity and feasibility related to opportunities for active and abandoned mine-land reclamation and landownership patterns. First, I assigned conservation priority rankings to all 15 candidate conservation areas based on the irreplaceability and condition indices. My analysis suggested that conservation action for Clinch Dace should first be focused on Pine Creek, Big Lick Creek, Hurricane Fork, and Mudlick Creek. Next, I examined opportunity and feasibility in highly ranked watersheds. Active coal mine permits exist in 10 candidate conservation areas, and 9 areas contained abandoned mine sites in need of reclamation. Considerable variation exists in the average lot parcel size along occupied stream reaches; therefore, I recommend that managers develop specific outreach and management plans for individual watersheds. Any management will be contingent on developing relationships with stakeholders within the tight-knit rural communities of Southwestern Virginia.

Introduction

   The headwater streams of the central Appalachian Plateau ecoregion have historically been among the most neglected freshwater ecosystems in the state and mostly occur on private lands where sampling access is limited. As a result, Clinch Dace remained undiscovered until 1999 and have yet to benefit from targeted conservation action. Clinch Dace populations are vulnerable to extirpation resulting from habitat alteration at multiple spatial scales. In addition to the persistent threats of watershed modification and riparian forest removal, nutrient enrichment, introduced species, and bait harvest, catastrophic pollution events in the upper Clinch basin periodically lead to large-scale extirpations of native fish and mussel species. Major chemical spills in 1967, 1970, and 1998 occurred in the lower reaches of Clinch River tributaries (Dumps and Indian creeks) downstream of known Clinch Dace populations and decimated populations of aquatic organisms for several kilometers (Crossman 1973; Lingenfelser et al. 2004).

   Traditional approaches to multi-species conservation planning assign each species in a fish community a value based on conservation status or taxonomic or ecological distinctiveness (Dunn 2003; Filipe et al. 2004). The Clinch Dace has high conservation value due to having one of the smallest ranges among North American cyprinid species, being one of only three *Chrosomus* dace species in Virginia, and possessing a distinctive headwater-specialist ecological niche (Jenkins and Burkhead 1993). Yet, due to their small sizes, the streams in which Clinch Dace occur have low fish species richness and serve little human recreational value for fishing or floating. Further, while many conservation areas in the United States exist in places that lack competing anthropogenic uses (Pressey et al. 1994), watersheds in Southwest Virginia have historically been heavily utilized for coal mining and timber harvest, both of which have degraded instream habitat. As a result, the catchments in which Clinch Dace occur would be unlikely candidates for conservation attention under traditional planning schemes that prioritize larger, downstream reaches with higher species richness (Filipe et al. 2004). A strategic conservation planning framework to promote persistence of Clinch Dace populations is needed to address the prevailing threats and to choose among numerous possible conservation options.

Systematic conservation planning (Margules and Pressey 2000) seeks to create defensible and objective decision strategies to best allocate sparse resources for conservation, and effectively bridges the gap between basic research and applied management. The backlog of 60 candidate species that have been assessed and determined to be warranted of listing but are still awaiting protection under the United States Endangered Species Act (ESA gttp://ecos.fws.gov/tess_public/reports/candidate-species-report) signifies a need for alternative conservation approaches for Clinch Dace. So called "ecopragmatists" have envisioned new conservation strategies that side-step ESA regulation for rare species. For example, in 2015, the U.S. Fish and Wildlife service withdrew the Greater Sage Grouse (*Centrocercus urophasianus*) from the ESA candidate species list in favor of state-specific management plans (U.S. Office of the Federal Register. 2015). Others insist on a biocentric "at-any-cost" approach to conservation planning (Cowling et al. 2003; Jenkins et al. 2015). Examples of single-species prioritization frameworks (Sanderson et al. 2002; Wall et al. 2004), may be rare in the literature, either because conservation agencies are reluctant to accept the loss of any population units or because they do not publish their management plans in peer-reviewed scientific journals. The biocentric mindset ultimately may lead managers to spend disproportionate amounts of resources upon restoring marginal populations at the expense of safeguarding stronger ones. It is important to recognize the need for flexibility amidst the reality of a landscape altered by human actions (Kareiva et al. 2011).

In response to growing threats to freshwater ecosystems (Dudgeon et al. 2006), freshwater conservation plans have emerged that draw from well-established terrestrial frameworks. Although most concepts are transferrable between both aquatic and terrestrial conservation plans, adjustments may provide better fit with the linear habitat connectivity that characterizes rivers and streams (Dunn 2003; Nel et al. 2009). Many conservation NGOs, including the Nature Conservancy (Master et al. 1998) and the World Wildlife Fund (Abell 2000), have developed their own systematic plans for preserving aquatic biodiversity. Moreover, the Nature Conservancy ranked the upper Clinch River watershed as its number one biodiversity hotspot due to the presence of 48 species of rare mussels or fishes (Master et al. 1998). The World Wildlife Fund also placed the Clinch River watershed on its list of important areas for

conservation of freshwater biodiversity (Abell 2000). Another related and widely used field of conservation planning is "Gap" analysis which overlays the modeled distributions of aquatic species on existing conservation lands. Such analysis aids in identifying ecosystem features that are underrepresented in managed or protected areas so that they can be prioritized for future protection (Scott et al. 1993). A National "Gap" program funded by the United States Geological Survey currently exists for seven states, and six major river basins. (http://gapanalysis.usgs.gov/aquatic-gap/). Most systematic plans are developed at the catchment or watershed scale due to the influence of watershed management on instream processes (Strayer et al. 2003).

A conservation area network represents the final product of a proper systematic conservation planning process that addresses several key requirements outlined by Nel et al. (2009), such as setting quantitative conservation targets, identifying current ecological conditions and future threats, planning for representation of the entire biotic community, and designing for persistence and long-term success. The values that managers emphasize in setting objectives will differ among projects. Some plans center on aspects of biodiversity, such as biogeography, species richness, rare and threatened species and communities, and representativeness (Cowling et al. 2003; Pressey et al. 1994; Woods et al. 1999). The targets of other plans may be landforms (Beier et al. 2015; Lombard et al. 2003); ecosystem services such as carbon storage, pollination, or flood control (Chan et al. 2006; Egoh et al. 2010); economics (Moore et al. 2004; Brown et al. 2015) ; or culture and tradition (McNeely and Pitt 1985; Angelstam et al. 2003; Dunn 2003). Managing for the presence of umbrella species shelters entire communities of plants and animals, which combines elements of single-species and multi-species centered plans (Fleishman et al. 2000; Hitt and Frissell 2004). Just as systematic conservation frameworks have been developed to guide conservation of aquatic communities, minor adjustments will make the approach valuable for single-species conservation planning.

Quantitative metrics often used in conservation planning include "irreplaceability" (Pressey et al. 1994; Pressey and Taffs 2001; Noss et al. 2002; Cowling et al. 2003; Linke et al. 2007; Linke et al. 2008)

and "condition" (Boon et al. 1998; Linke et al. 2007). Composite rankings of candidate conservation areas (CCAs) along these or related dimensions help managers decide where to allocate effort in order to advance conservation goals (Pressey et al. 1994).

Modifying the concept developed by Pressey et al. (1994), Linke et al. (2008) defined irreplaceability as "the likelihood that an area will be required as part of a conservation system that achieves all conservation targets and the extent to which the options for achieving all targets are reduced if the area is unavailable for conservation." Irreplaceability is influenced by complementarity or spatial efficiency, and managers seek to avoid excessive overlap of the important features or species shared among selected conservation areas. It is the inclusion of "complementarity" and "efficiency" that some have used to demarcate the line between systematic conservation planning and earlier score-based planning methods (Wilson et al. 2005). Heuristic algorithms have been developed to aid in the selection of complementary networks of conservation areas (Linke et al. 2008; Noss et al. 2002; Pressey et al. 1994).

Comprehensive biological data may not be available for all CCAs, and in these cases, researchers rely on species distribution models to help predict species occupancy (Noss et al. 2002; Wall et al. 2004; Linke et al. 2007). Distribution models are imperfect, and the type of distribution model used can impact the final conservation area network due to false positive or false negative errors in the predictions of species' ranges (Loiselle et al. 2003; Wilson et al. 2005).

A second metric used to prioritize sites for conservation is "condition". Condition refers to the landscape or instream habitat quality. GIS-based analyses (Linke et al. 2007) or field-collected habitat data (Boon et al. 1998) can be used to score sites along a habitat gradient. Examples of specific variables related to condition include human population density, water quality, catchment land use, nutrient load, acidification, or road density (Boon et al. 1998; Linke et al. 2007; Walker et al. 2006). The addition of condition to conservation plans allows managers to separate those areas most in need of habitat protection and those that would benefit from habitat restoration.

The final widely used dimension in conservation planning is "vulnerability", which has been defined as the potential of each area to experience degraded condition in the future (Linke et al. 2007). This metric can identify future threats to habitat or the essential habitat of a species (Benayas and de la Montaña 2003) in order to select conservation areas which have the greatest chances of long-term success and species persistence. While conservationists lack a clear knowledge of the future, they do have data that can help them make informed prediction including land capability classes that capture the suitability of lands for human uses that may compete with conservation objectives (Linke et al. 2007). The predicted future conservation status of species may also be incorporated. Population viability analysis where detailed demographic or metapopulation data exists to predict population trajectories (Akçakaya and Sjögren-Gulve 2000).

In addition to future habitat conditions, managers also must consider the costs and practicality of future conservation in each catchment. Opportunism has led to the protection of marginally valuable conservation reserves across the country, and therefore opportunity should not be the foremost factor in deciding which lands are protected (Pressey et al. 1993). "Informed opportunism" which seeks to defensibly balance biological goals with opportunities for success can increase conservation efficiency (Noss et al. 2002; Pressey and Bottrill 2008). Stakeholders, who can be defined as any person who may be positively or negatively, and directly or indirectly affected by a management decision, ultimately will decide the outcome of conservation effort. Both Russell and Tazewell counties have poverty rates well above the Virginia state average of 11.5% with 18.5% and 19.5% of residents living below the poverty line ($12,071 for a single individual). Pressey and Bottrill (2008) argued that gathering and considering socioeconomic and political data, such as stakeholder involvement and cost analyses, should be the first steps of the conservation planning process.

Limited resources available for Clinch Dace conservation will require managers to implement "informed opportunism" when undertaking stream habitat restoration and cooperative land management projects. As such, systematic conservation planning can be a valuable tool for fostering recovery of

Clinch Dace populations. Data from chapters 1 and 2 can provide information on the population size and

habitat condition useful for ranking of CCAs by habitat irreplaceability and condition. Conservation of

Clinch Dace should begin in watersheds already containing robust populations and quality habitat

characterized by forested watersheds, low dissolved solids, and high habitat connectivity. Conservation in

degraded watersheds where surface mines or logging operations have impaired instream habitat and water

quality would require far more resources to achieve similar benefits. Moreover, land ownership

differences across watersheds will help managers tailor specific actions that will be most effective with

local stakeholder groups.

The goal of my proposed systematic conservation plan is to promote the existence of as many

demographically independent, self-sustaining Clinch Dace populations as possible given limited resources

for conservation. In this chapter, I use a four-step process (Table 3.1) based on the multi-species

systematic conservation planning literature to: 1) Develop a framework to prioritize conservation action

to specific Clinch Dace populations based on the metrics of irreplaceability and condition, 2) Describe

how such a framework would provide benefits to other species in the Upper Clinch River fish assemblage

(complementarity), 3) Examine the land ownership and land-use patterns within the prioritized

populations to highlight issues facing future implementation of recovery actions (opportunity and

feasibility), and 4) Pinpoint potential restoration actions that could meet recovery goals for the species.

Methods

Planning Units and Conservation Goals

I defined Candidate Conservation Areas (CCAs) as grouped occurrences of Clinch Dace that I

regard as demographically independent from all other populations. Therefore, I used the stream reaches

occupied by Clinch Dace outlined in Chapter 2 as my CCAs. The 15 CCAs that I consider are: Big Lick

Creek, Hart Creek, Hess Creek, Hurricane Fork, Jackson Fork, Greasy Creek, Indian Creek, Left Fork

Coal Creek, Laurel Fork, Lewis Creek, Middle Creek, Mudlick Creek, Pine Creek, Town Hill Creek, and

West Fork Big Creek. Grassy Branch was included with Hurricane Fork, Little Town Hill Creek with

Town Hill Creek, and Zeke Creek with Mudlick Creek because of their proximity to one another and likely interaction of Clinch Dace demes as metapopulations.

This framework does not envision the development of protected reserves, but rather presupposes the presence of humans and looks for opportunities for Clinch Dace habitat conservation alongside economic, residential, and other services that these landscapes provide. I did not set a minimum number of CCAs to conserve, instead I ranked the 15 CCAs based on the irreplaceability and condition indices. I also attempted to estimate the impact of habitat conservation in the headwaters on protection of other fish species downstream.

I did not include future threats defined by an index of CCA vulnerability as is used in many other frameworks. I took an alternate view of the future where I switched the negative perspective of vulnerability into a positive outlook for conservation opportunity (Knight and Cowling 2007). I also examine feasibility in order to predict where conservation actions might be most successful.

Step 1: Irreplaceability and Condition

I derived the definitions of irreplaceability and condition from Linke et al. (2007), who defined the irreplaceability of a CCA as the "conservation value of a river and its catchment." Unlike Linke et al. (2007) my assessments of irreplaceability are not based on species community complementarity because of my single species focus. Instead, irreplaceability was assessed as a measure of population demographic strength and thus measured the value of the CCA to range-wide conservation efforts of Clinch Dace. I also adopted Linke's definition for condition, which relates to instream or catchment level habitat conditions. I defined the variables and associated scoring scales for irreplaceability and condition based on the Clinch Dace habitat association research I conducted in Chapter 1. I assigned a 1 to 5 score for each variable, with 5 being the optimal condition (the highest irreplaceability or condition) (Table 3.2). Scoring ranges encompassed the measured range for each variable among the 15 populations. I assigned binomial variables such as presence or absence of centrarchids a 5 or a 1 score. The measures of

irreplaceability and condition may not be independent. CCAs with high condition may also have strong populations of Clinch Dace and consequently high irreplaceability scores as well.

I based irreplaceability on Clinch Dace distribution and relative abundance data from multiple surveys collected since 2007 using the following four variables: the percentage of surveys within an occupied stream in which Clinch Dace were detected, the relative abundance of Clinch Dace within each CCA (see chapter 2), connectedness to other populations as measured by the stream distance to the closest population, and the length of stream from the furthest upstream to the furthest downstream records of occurrence. I opted to use only records of confirmed presence for Clinch Dace instead of modeling the species' distribution in my calculation of irreplaceability. Developing predictive models was not a goal of the occupancy models developed in chapter 1. Models are likely to produce false-positive predictions of species occurrence (Loiselle et al. 2003) and wasting conservation effort on areas that were predicted to but do not actually contain Clinch Dace is unacceptable.

The condition index consisted of the following eight metrics: proportion of the CCA watershed that is forested, presence of centrarchid predators, proportion of the CCA watershed that has active mine permits, number of road crossings, conductivity, canopy cover, percent streambed covered by fine sediments, and occurrence of publicly owned land in the watershed. Table 3.2 provides a detailed description of the scoring system for all 12 metrics. Some authors have used multivariate statistical ordination methods to find correlations among habitat variables across sites so that variation can be captured in reduced dimensionality in a technique called stress gradient analysis (Linke et al. 2007). However, potentially stressful land-use or habitat variables may spread across multiple principal component axes or be positioned at opposite ends of the same axis, limiting the usefulness of stress gradient analysis to these data. As a result, I preferred a framework that scored CCAs for each condition variable independently.

I averaged all of the irreplaceability and condition metric scores with equal weight so that each population had a single score for each index. I plotted each population's scores for the two indices in two-

dimensional space to facilitate comparisons of irreplaceability and condition. Next, I summed

irreplaceability and condition scores to produce a composite score for each CCA, which determined its

conservation priority rank (CPR). I divided prioritized conservation areas into four tiers: "best", "above

average", "below average", and "marginal".

Step 2: Complementarity

The complementarity of fish communities present within each CCA deserves independent

attention due to my single-species focus. Metrics of complementarity should assess how well CCAs

protect the overall fish assemblage in 2nd-and 3rd-order streams within the study area. I expected little

variation between species communities among CCAs because my study area included streams of a similar

size in just a small area of three HUC 10-level watersheds. Much of the variation in fish communities in

the Clinch basin in Virginia is based on stream size, with the main-stem Clinch River and its largest

tributaries harboring large and distinct fish assemblages (Angermeier and Winston 1999). Many fish

species in the Clinch basin may indirectly benefit from conservation in headwater watersheds through

water quality improvement and reduction in sediment loads.

I compiled fish assemblage data from 70 sites sampled with backpack electrofishing and minnow

trapping in 2014 and 2015. I plotted the cumulative assemblage coverage, defined as the cumulative

proportion of the total species assemblage encountered at all sites in the upper Clinch basin, over the 15

occupied streams in order of increasing CPR (Figure 3.3). I examined the plot for the point of saturation,

where the curve leveled off and all members of the headwater fish assemblage had been represented

within given candidate conservation areas.

Step 3: Opportunity and Feasibility

I looked at two dimensions of opportunity and one dimension of feasibility of conservation action

for Clinch Dace CCAs. Focusing conservation actions in areas of existing opportunity improves

efficiency (Noss et al. 2002). The Surface Mining Control and Reclamation Act of 1977 (SMCRA)

already mandates restoration of surface mines and promotes the restoration of mines that were abandoned prior to its enactment (30 U.S.C. §§1201-1211, 1231-1251, 1252-1328). Managers may be able to reclaim watersheds with active permits in a way that benefits Clinch Dace. I calculated the area of overlap of the CCAs with two GIS shapefiles, reclamation status and designated post-mining land use obtained from the Virginia Department of Mines, Minerals and Energy (VADMME). Each permitted mining site is classified under one of three stages of reclamation: disturbed, regraded, and vegetated. Before mining permits are approved, companies must propose a post-mining land use (PML) to which they will attempt to restore the site to. Thirteen categories of PML exist, including agriculture-hay land, agriculture-grazing land, agriculture-managed forest, commercial, fish and wildlife habitat-wetlands, fish and wildlife habitat-species management, industrial gas wells or pipelines, industrial utilities, industrial manufacturing, public use-buildings and facilities, public use- public roads, residential, or undeveloped unmanaged lands. I calculated the proportion of each occupied watershed that overlaps with these PML categories in order to envision future land cover in these areas.

Reclamation on mined lands that were abandoned before 1977 is handled by the Office of Surface Mining Reclamation and Enforcement under the U.S. Department of the Interior. This agency maintains the (Abandoned Mine Land Inventory System Database https://amlis.osmre.gov/Default.aspx), which contains information on all priority 1 and 2 and some priority 3 abandoned mine sites. Priority 1 and 2 abandoned mine sites threaten human safety, while priority 3 sites threaten the environment. The database also includes information on the specific nature of the problems at each site and the estimated cost of their reclamation.

I selected all abandoned mine lands in all three priority levels that had problems that were potentially related to aquatic habitat degradation. I selected the following problem types: under priority classes 1 and 2— clogged streams, clogged stream lands, dangerous impoundments, industrial or residential waste, polluted water: human consumption. Under priority class 3— hillside benches, industrial or residential waste dumps, processing or transport equipment and facilities, gob piles, exposed

high-wall mines, haul roads, pits, spoils, slurry, slumps, water environmental impacts, and other environmental impacts.

One way to approach feasibility is through an assessment of land ownership patterns and potential number of stakeholders. I accessed land ownership records from plat maps at the Russell and Tazewell County government offices. I was not able to identify catchment boundaries on the paper maps, and instead recorded the landowner name, contact information (where possible), and acreage of all tracts adjacent to reaches with documented Clinch Dace presence. I compiled this information into a database for future use in contacting landowners for site access and management consultation (Appendix D).

Step 4: Possible Actions

I developed a list of possible management actions with characteristics of the Clinch watershed landscape and stakeholder base in mind. I also identifying existing conservation alliances and other possible approaches for fostering collaboration. I consider this step in the discussion section of this chapter.

Results

Step 1: Irreplaceability and Condition

Considerably more variation existed in scores of irreplaceability than condition among the 15 Clinch Dace populations, from a high score 4.75 in Pine Creek to a low score of 1 in Lewis Creek (Figure 3.1). Within all occupied streams, researchers have detected Clinch Dace at >40% of the site visits except for Lewis Creek. I discovered Clinch Dace in Lewis Creek in 2014 and repeated surveys since then have only detected Clinch Dace in only a few pools within a ~700 m reach of the stream. In 9 of 15 populations, estimated densities of Clinch Dace were very low, with <10 individuals/100 m. Density estimates exceeded 30 individuals/100 m at only four sites, Hart, Middle, and Pine creeks and Hurricane

Fork. Connectedness to other populations was generally low, highlighting the isolated nature of most populations. Exceptions were Pine and Big Lick creeks, which were separated by less than two miles of unoccupied stream habitat, and sites within the upper Indian Creek watershed (Greasy Creek, Indian Creek, and Jackson Fork). Stream length occupied was >4 km in Hurricane Fork, Mudlick/Zeke creeks, and Pine Creek.

These index scores can be expressed in terms of narrative site descriptions. Pine Creek and Hurricane Fork are the only streams in which Clinch Dace occupy a long stream length at relatively high densities. Mudlick Creek has a long occupied length, but low Clinch Dace density. Hart and Middle Creeks have high population densities over moderate distances. Big Lick Creek has moderately dense populations over moderate distances. Lewis Creek, Laurel Fork, Hess Creek, Left Fork Coal Creek, West Fork Big Creek, and Jackson Fork received the lowest scores for both length occupied and relative abundance.

Condition scores were less variable among CCAs and ranged from a high score of 3.88 in Mudlick Creek to 2.50 in Lewis Creek (Figure 3.1). Populations that received a high score for one condition metric often scored low for another. No publicly owned land existed in any of the catchments occupied by Clinch Dace. Forest cover in watersheds has been one of the most consistent predictors of Clinch Dace occupancy. Within watersheds occupied by Clinch Dace populations, forest cover ranged from just over 90% in Laurel Fork, Indian Creek, Jackson Fork, Mudlick Creek, and West Fork Big Creek to less than 70% in Hess Creek, Lewis Creek, and Left Fork Coal Creek. Scores for conductivity were the highest in Big Lick Creek and lowest in Hurricane Fork, Hess Creek and Greasy Creek. Only three populations had documented co-occurrences of Clinch Dace and centrarchid predators. Centrarchids were mostly Rock Bass or *Lepomis sp*. and always occurred at low densities. These three sites were also on the downstream margins of Clinch Dace CCAs. Human residential density showed a weak positive relationship with road crossing density (adjusted R^2=0.22, P=0.07). Pine Creek and Hart creeks had the

most road crossings. Town Hill Creek had far and away the most active mining, with 21% of its watershed under active coal permit.

Summed composite scores for irreplaceability and condition resulted in the assignment of populations to four conservation priority rank tiers as follows. Best: Pine Creek, Big Lick Creek, Hurricane Fork, and Mudlick Creek. Above Average: Middle Creek, Hart Creek, Indian Creek, Jackson Creek. Below Average: Greasy Creek, West Fork Big Creek, Town Hill Creek, Left Fork Coal Creek Marginal: Hess Creek, Laurel Fork, Lewis Creek (See color coding in Figure 3.2 A). CCAs will be examined in the following steps according to their composite score ranking.

Step 2: Complementarity

Sixteen out of the total of 28 species that were detected at 2nd-and 3rd-order streams within my study region co-occurred with Clinch Dace at least one survey site. Therefore, a conservative estimate of the proportion of the headwater fish assemblage that would directly benefit from conservation actions at all 15 CCAs would be 0.57 (Figure 3.3). An additional nine species occurred downstream of CCAs in 2nd- and 3rd- order streams and would thus benefit from stream restoration and watershed protection within catchments occupied by Clinch Dace. The cumulative proportion of the total fish assemblage of Upper Clinch tributaries within ranked CCAs quickly reached near-saturation within the four CCAs in the "best" tier (Figure 3.3). The only three species that I detected in 2nd- and 3rd-order streams, but not downstream of Clinch Dace populations were Largemouth Bass (*Micropterus salmoides*), Pumpkinseed (*Lepomis gibbosus*), and Saffron Shiner (*Notropis rubricroceus*).

Step 3: Opportunity and Feasibility

Opportunity

CCAs with the most land in a "disturbed" reclamation status land which could be candidates for restoration opportunities under SMCRA are Mudlick Creek and Pine Creek in the "best" tier and Town Hill Creek in the "above average" tier (Figure 3.2 C and 3.4). The proposed PLMs across occupied catchments were mostly undeveloped or unmanaged forestry (69.4%). Substantial portions of permitted lands were also designated as agriculture/grazing (17.2%) and industrial gas wells/pipeline (13.2%). A very small proportion of permitted land (<0.2%) is intended to be restored to fish and wildlife habitat following mining (Figure 3.5).

I identified 47 priority- 1, 2, or 3 mine sites with potential environmental impact in Russell and Tazewell counties, VA with a total of $22,169,818 (Abandoned Mine Land Inventory System Database https://amlis.osmre.gov/Default.aspx) in unfunded costs. Only ten of these abandoned mine sites occur within Clinch Dace CCAs with a total of $12,482,999 in unfunded costs. Abandoned mine sites were distributed evenly among candidate conservation areas. Lewis Creek had the most abandoned mine sites with two. Eight CCAs had one abandoned mine site and 6 CCAs had no abandoned mine sites (Figure 3.2 D).

Feasibility

Definite patterns emerged in land ownership among Clinch-dace-bearing watersheds that will affect restoration efforts for these populations (Figures 3.6 and 3.2 B). The CCAs with the largest number of unique property parcels were Town Hill Creek and Pine Creek, with over 50 potential stakeholders. In contrast, Indian Creek, West Fork Big Creek, and Hess Creek all had less than 10 properties adjacent to reaches occupied by Clinch Dace. CCAs with fewer landowners will usually have larger average property sizes (Figure 3.6). For instance, in West Fork Big Creek, the average parcel size is 813 acres.

CCAs with low human population density and land use likely devoted to resource extraction such as forestry, mining, or gas drilling were Greasy Creek, Hurricane Fork, Mudlick Creek, Middle Creek,

Jackson Fork, West Fork Big Creek, Indian Creek, and Laurel Fork. Other CCAs have mixed land-use, including Pine Creek, Big Lick Creek, Hess Creek, Lewis Creek, and Town Hill Creek. These CCAs are primarily residential; all have >5% current land cover in mining as well. Left Fork Coal Creek is primarily residential, but does have degraded condition from a large surface mine not far downstream of Clinch Dace populations. CCAs in the best tier were mostly residential with many small landowners: Big Lick Creek, Hurricane Fork, and Pine Creek although Mudlick Creek had a few large landowners. However, a large portion of Hurricane Fork's watershed that was not included in our analysis is leased by a forestry management company.

Conclusions

The systematic conservation plan that I propose addresses all of the conservation planning considerations proposed by Nel et al. (2009) outlined in the introduction. In step one, I set quantitative conservation targets and identified current ecological conditions. In step two, I planned for representation of the entire biotic community. In step three, I framed conservation action in light of future opportunity. Finally, regarding step four I will discuss management options for persistence and long term success below. Top-ranked sites for immediate conservation action are Pine Creek, Big Lick Creek, Hurricane Fork and Mudlick Creek. Other populations that scored high and may be prioritized depending on conservation goals include Middle Creek, Hart Creek, Indian Creek, and Jackson Fork.

Step 1

Irreplaceability and condition scores can help managers decide which conservation strategies would provide the most benefit to individual CCAs. Within the "best" tier of CCAs, populations of Clinch Dace in Pine Creek, Hurricane Fork, and Big Lick Creek are demographically strong and have high irreplaceability, but all three CCAs only had average condition scores, which suggests that these

populations are candidates for active habitat restoration projects. In contrast, Mudlick Creek had the highest condition score, suggesting that efforts might be better spent working with landowners to maintain preserve existing watershed condition.

Objective conservation frameworks are not perfect. Managers must interpret the conservation priority rankings with context not included in the scoring framework. Jackson Fork, a site that graded in the "above average" tier, has only a single record of a single Clinch Dace from White (2012). Although the habitat is largely undisturbed and looks suitable for Clinch Dace, predator densities are high and it is unknown whether a permanent population of Clinch Dace exists in this stream. Low scores for irreplaceability in West Fork Big Creek and Laurel Fork might be influenced by limited stream access in these watersheds. Yet, penalizing a population for limited access recognizes an obstacle to management actions. There remains the potential for some bias in estimates of population demographic strength. Snapshots of relative abundance may not fully characterize these highly variable populations. I would contend that environmental conditions within my small study area should follow synchronous patterns so that inter-annual fluctuations among populations would be similar. Additionally, I made use of all of the available data spanning 10 years for some metrics.

Potentially misleading scores also exist for condition. The Clinch Dace population in Left Fork Coal Creek received the highest score for its low current mining activity. However, the adjacent community of Fork Ridge, VA was named one of the 50 communities at the highest risk of mountaintop mining by the publication *Appalachian Voices*. As of 2014, the website reports over a 100 acres of surface mines within a one-mile radius of the town (http://ilovemountains.org/communities-at-risk). In 2015, I measured conductivity levels well over 1000 µS/cm in Jamison Creek, a small tributary that drains the mined land and enters Left Fork Coal Creek downstream of the documented Clinch Dace population and thus outside of the CCA.

Finally, my framework assumes that measures of habitat condition span biologically significant gradients. For instance, scores assume a biologically meaningful difference between a conductivity of

>300 (score of 1) and 100-150 (score of 5). This threshold has the empirical support of the EPA's proposed benchmark for conductivity for impaired streams, which is 300µS/cm in the central Appalachian region (EPA 2011). Because I have not developed population response thresholds for these variables, rankings should be interpreted relative to other sites, and not as from overall good to bad but rather from most to least disturbed.

Step 2

Complementarity proved insignificant in my prioritization framework due to the relatively homogeneous fish communities among study watersheds. Linke et al. (2008) recognized the importance of headwater catchments to downstream habitat quality and incorporated procedures that mandated protection of all catchments upstream of high priority reaches in their analysis. Protection of Clinch Dace habitats will undoubtedly provide benefits for diverse aquatic communities downstream, but the impact of location of conservation emphasis is unclear. None of the species that I captured in 2nd- and 3rd-order streams that did not occur sympatrically with Clinch Dace are of conservation concern. These species, Largemouth Bass and Pumpkinseed, require lentic habitats or slow pools in larger streams. I captured each species at one site, and both were likely introduced by anglers. Saffron Shiners occurred only in the Thompson Creek system. This is one of three tributaries within our study area along with Mill Creek and North Fork Clinch River that does not contain a known population of Clinch Dace. Thompson Creek and North Fork Clinch River are the only tributaries in my study reach that are located entirely within the Ridge and Valley eco-region. Streams here drain primarily agricultural lands and may have unique geological and thermal attributes that may favor Saffron Shiners, but not Clinch Dace. Clinch Dace do not fit the mold of a typical "umbrella species" (Hitt and Frissell 2004) and in the context of Clinch Dace conservation planning, conservation agencies should base justification of population prioritization solely on the Clinch Dace's populations they support.

Distribution of host species may affect the distribution of nest-associates (Peoples and Frimpong 2016). Therefore, managers must also consider the importance of host species — Creek Chubs and Stonerollers — in conservation of Clinch Dace. Without the clean gravel spawning substrate that these hosts provide, Clinch Dace may not be able to successfully spawn. Fortunately, Creek Chubs are generalists that are present in most headwater streams in the region and actually become increasingly dominant in watersheds affected by coal surface mining (Hitt and Chambers 2014).

Conservation of co-occurring mussel populations in these tributaries may prove more informative than fish populations. Lower Indian Creek contains the only known reproducing population of the endangered tan-riffleshell (*Epioblasma walkeri*) left in the world, which incentivizes conservation of upstream Clinch Dace CCAs in the Indian Creek watershed.

Step 3

Managers may find opportunity for conservation on mined lands where reclamation is already scheduled. The analysis of surface mines in the disturbed reclamation status category highlights ample opportunity for landscape reclamation and revegetation in watersheds containing priority Clinch Dace populations, such as Pine, Mudlick, and Town Hill creeks. However, the analysis of designated post-mining land uses for these watersheds indicates that reclamation standards in these watersheds may be set too low. Clinch Dace populations occurred in watersheds with large proportions of mining permits in undeveloped/unmanaged forestry, agriculture/grazing, and industrial gas wells/pipeline as post-mining land-uses. These post-mining land uses may be more of an obstacle than an opportunity to conservation efforts to the species. Lands designated for agriculture and grazing likely will not be returned to forest, which is the natural land cover for the region and is associated with Clinch Dace presence. Undeveloped or unmanaged forestry likely means that little restoration effort will be invested in the land as long as some form of vegetation is restored to meet bond requirements. The effect of gas drilling on Clinch Dace

is unknown, but with drilling comes the threat of more road installations, which would contribute sediments to the stream and could result in siltation and impassable barriers to migration. Wells also might use water that may be withdrawn from the creeks or underground sources that feed the some streams. Hopefully, more land will be returned to sustainably managed forestry and fish and wildlife habitat in the future. Until then, fisheries managers should work with the appropriate agencies and companies to restore mined lands to some semblance of pre-mining conditions.

Abandoned mine lands are infrequent in Clinch Dace watersheds, but their reclamation could provide water quality and habitat benefits for Clinch Dace populations. Yet, the abandoned mine lands that are primarily listed for their environmental impacts are a lower reclamation priority in the federal reclamation program than those impacting human safety. Reclaiming mined lands inside all Clinch Dace CCAs would cost millions of dollars. The trust fund that pays for abandoned mine land reclamation is administered by the Secretary of the Interior. In February 2016, bipartisan legislation called the RECLAIM Act (Revitalizing the Economy of Coal Communities by Leveraging Local Activities and Investing More Act of 2016) was proposed to release one billion dollars of reclamation funds for abandoned mine lands to be dispersed to states by the Department of the Interior over a five-year period to promote economic development in Appalachia. It is possible that areas of Southwest Virginia could benefit from reclamation funds made available by this or similar legislation.

It is unlikely that any of the CCAs are highly suitable for agriculture or urban development. For instance, 17% of the variation in forest cover on private lands in north-central Indiana was related to biophysical attributes such as slope and surficial geology (Frimpong et al. 2006). Catchments occupied by Clinch Dace are steep with mean slopes of 32.54% (95% conf. interval 30.87-34.20) and have a rocky surficial geology with a mean of 99% sandstone colluvium (NHDPlus V. 1). Future land cover conversion is driven by unpredictable coal and timber markets.

Step 4: Suggested Actions

While it is unlikely that any of these CCAs will ever receive the protection of "true" aquatic biodiversity reserves, through cooperation with stakeholders, we can improve conditions in the most critical habitats for Clinch Dace conservation. There is the potential for many stakeholder groups with varying interests to become involved in Clinch Dace conservation. Stakeholders may be broadly grouped in three categories: those that are directly impacted by management plans; those that are indirectly impacted, but have a declared moral or philosophical interest; and those that have little interest one way or another, but can help serve as bridge builders to resolve conflicts (Hirsch and Dukes 2014). Lists of stakeholders tied to the Clinch Dace are more extensive than might be readily apparent. Direct stakeholders include: multiple coal mining companies, power companies, residents (those employed in mines, farmers, and home owners), and local business owners. Indirect stakeholders may include: environmental organizations, activists, academic institutions, government agencies such as the Virginia Department of Game and Inland Fisheries, the Environmental Protection Agency, Virginia Department of Environmental Quality, the U.S. Fish and Wildlife Service, and the U.S. Army Corps of Engineers. Bridge building stakeholders could include church leaders, community leaders, teachers, and the Appalachian Research Initiative for Environmental Sustainability (ARIES), which provides coal mining company funding and assistance for environmental research. Furthermore, towns such as Cedar Bluff, Cleveland, and St. Paul, Virginia are beginning to embrace the prospect of ecotourism around the aquatic biodiversity in the Clinch River. Many indirect stakeholders may be viewed as outsiders by direct stakeholders and bridge builders can help build trust between these two groups. In my experience, levels of trust have been mixed, with some representatives of large landholding companies being apprehensive about granting stream access. Other companies have been very supportive of our research. Very few private landowners have denied access to streams for fish sampling.

Community meetings where stakeholders could express their opinions, learn about conservation efforts, and engage in dialogue with other stakeholders may be beneficial and could take place at

churches, schools, or other convenient locations. These meetings can lead to adoption of non-controversial actions that meet the values of most stakeholder groups. A good model for stakeholder consensus building is the Clinch River Valley Initiative (http://www.clinchriverva.com/). This program along with the Nature Conservancy's Clinch Powell-Clean Rivers Initiative (http://cpcri.net/) has already built stakeholder networks and may be able to bring crucial parties together. These conservation alliances have a much broader mission than species protection. They also hope to develop a sustainable economy to assist human residents as well. An economy that is based on outdoor recreation and ecotourism in the Clinch River will increase the river's value and create an environment that is friendlier to small businesses. Proposed actions such as creating a Clinch River State Park provides increased incentive to protect the Clinch River, its water quality, physical habitat, and native biota throughout the watershed.

Through this collaborative process, it may be possible to get stakeholders to agree on agency management responses in priority conservation areas, such as direct fee acquisition, conservation easements, management agreements, stewardship assistance to landowners, agency designations of special areas (e.g., research natural areas), congressional wilderness designations, and administrative actions such as national monument designations (Noss et al. 2002). Retrofitting road crossings with passable culverts or bridges that preserve the natural stream bed should be targeted in high priority populations where they may restrict Clinch Dace colonization and population connectivity.

Some small-scale habitat restoration projects can proceed on individual properties without total consensus. The Clinch-Powell Clean Rivers Initiative (2016) provides guides to landowners on their website regarding small steps that they can take to improve aquatic habitat. These include maintaining septic systems, planting native riparian grasses, herbs, and trees, installing rain gardens, using fewer pesticides and herbicides, testing soil to ensure proper application of fertilizers, disposing of trash properly, maintaining forest buffers along streams, fencing cows out of the stream, and creating conservation easements. Extension agents are available to assist landowners in these efforts. Environmental education is correlated with environmentally responsible behaviors (Ostman and Parker

1987), and education and outreach for Clinch Dace at schools and community events should continue. Because the sense of community and family is strong in rural areas, landowners may be willing to adopt positive management practices in which they observe their neighbor engage. These actions would address all of the factors that underlie the Theory of Planned Behavior (Azjen 1985), namely, that individuals see value in positive conservation actions, feel capable to perform positive conservation actions, and feel social pressures to perform conservation actions.

A long-term management plan must navigate regulatory and environmental uncertainty. Few proposed management actions that were conceived to increase species resiliency to climate change were designed to work at scales as small as the Clinch Dace's distribution. These include protecting refugia (e.g., creation of pools, shaded stream channels, and natural flow regimes), enhancing connectivity between and within populations (remove culverts and predators), and upholding ecosystem processes (large woody debris input, sediment transport reduction, etc.) (Groves et al. 2012). Changes in the enforcement and interpretation of laws regulating coal mining permitting will also affect Clinch Dace populations. Large surface mines — sometimes referred to as mountaintop removal mines or mountaintop mines — often bury headwater streams with waste materials that overlay deposits. Temperature, flow, and ionic composition of the water downstream may be altered downstream of valley fills. The political administration and appointees to the environmental agencies ultimately will affect the interpretation of key issues related to mining, such as the use of the Nationwide Permit that allows mines to dispose of waste materials in streams and the definition of "fill" as it pertains to valley fills adjacent to surface mines (Hirsch and Dukes 2014). Water quality surveys using macroinvertebrate community indicators are available for some of the streams within my study area, and may provide additional insight into habitat suitability for Clinch Dace (S. Wolf, M. Moore and D. Orth, Virginia Polytechnic and State University, unpublished data). An adaptive management approach in which management becomes experimental by incorporating feedbacks from monitoring data to evaluate project success and periodically adjust actions (Walters 1986) would be appropriate in the face of such uncertainty.

The best tactics to ensure that conservation plans are implemented are not been well studied, although a lead agent would help maintain continuity over time (Nel et al. 2009). I would recommend that a biologist at the Virginia Department of Game and Inland Fisheries serve in this role. Should the species receive federal protection in the future, the responsibilities may be shared with the U.S. Fish and Wildlife Service. This agency has developed recovery plans for fish species in the upper Tennessee River basin, but it can act only for species that are listed or are candidates for listing under the Endangered Species Act (U.S. Fish and Wildlife Service 2014).

The systematic conservation plan for Clinch Dace is a novel adaptation of multispecies systematic conservation planning theory to a critically imperiled aquatic species. Hopefully, a place-based identification of conservation hot-spots will help spark recovery for this rare species.

Table 3.1 An outline of the four-step conservation prioritization process, including metrics, explanation, and variables and goals.

Metric	Step1		Step 2	Step 3	
	Condition	**Irreplaceability**	**Complementarity**	**Opportunity**	**Feasibility**
Verbal Explanation	Instream and watershed habitat condition which reflects past land use practices.	Importance of population to global conservation of species. Strength of Clinch Dace population.	Degree to which protecting Clinch Dace populations also protects total fish diversity in second and third order streams in the Upper Clinch basin.	Opportunity for mining reclamation within the watershed.	Description of landownership patterns.
Variables and (Goals)	% watershed forested (max), presence of Centrarchid predators (min), % watershed mined (min), density of road crossings (min), conductivity (min), canopy cover (max), % fines (min), public landownership (max).	% of surveys Clinch Dace collected (max), Estimated abundance (max), connectedness of populations (max), length of stream occupied (max).	Species accumulation curve for ranked sites from fish sampling data (maximize assemblage coverage in top-ranked sites).	Area of disturbed current mine land and abandoned mine sites (max opportunities for restoration).	Number of landowners adjacent to occupied stream reaches, average parcel size in acres (subjective).

Table 3.2: Description of the 1-5 scoring system for each metric of irreplaceability and condition used to rank all 15 candidate conservation areas.

	Irreplaceability				Condition						
Score	% Surveys Dace Present	Relative Abundance	Dist. to Nearest Popn.	Stream Length Occupied	% Forest	Centrarchids	% Active Mining	Avg. Dist. btw Rd Crossings	Conductivity	Canopy Cover	% Fines
1	0-20	0-10	>8	<1km	<60	Present	>20	0-0.5	>300	<10	>75%
2	20-40	10-20	6-8	1-2km	60-70		15-20	0.5-1	250-300	10-13	50-75
3	40-60	20-30	4-6	2-3km	70-80	Mix	10-15	1-1.5	200-250	13-16	25-50
4	60-80	30-40	2-4	3-4km	80-90		5-10	1.5-2	150-200	16-19	5-25
5	80-100	>40	<2	>4km	90-100	Absent	0-5	>2	100-150	>19	<5%
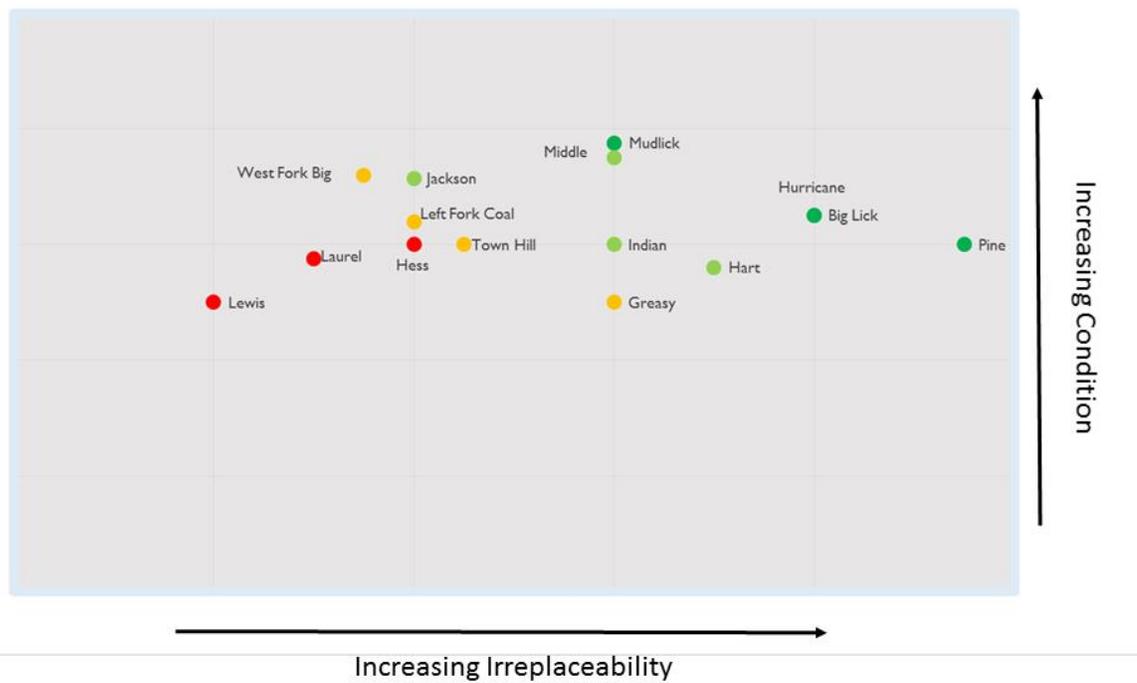
Figure 3.1: Ordination of Clinch Dace populations based on scores for condition and irreplaceability. The populations are color-coded into four tiers based on the ranking of their composite score. Best- dark green, above average- light green, below average- yellow, and marginal- red.
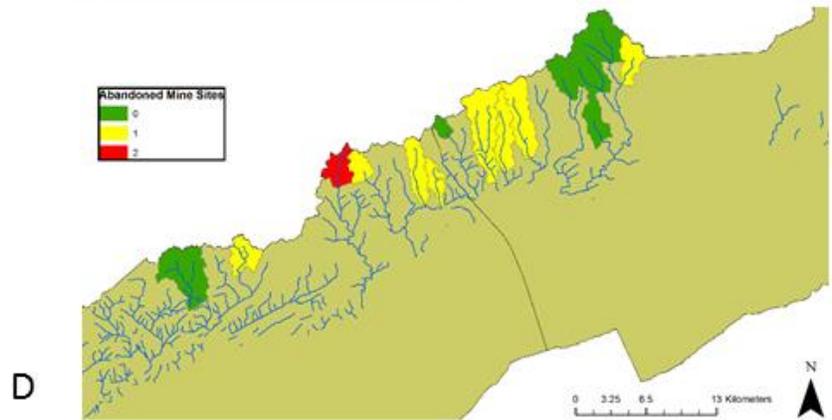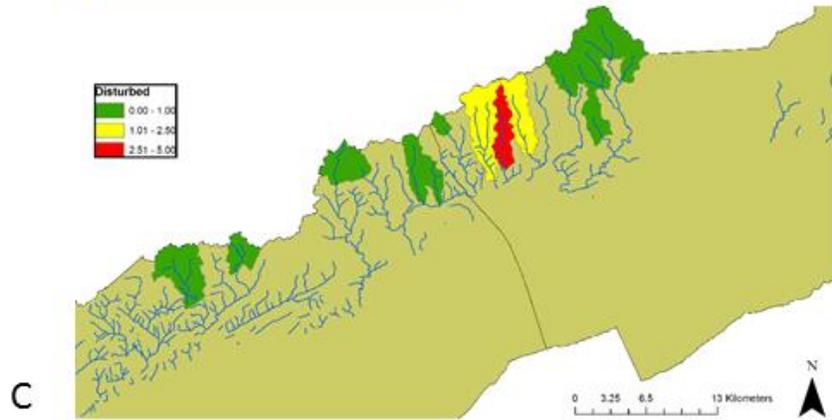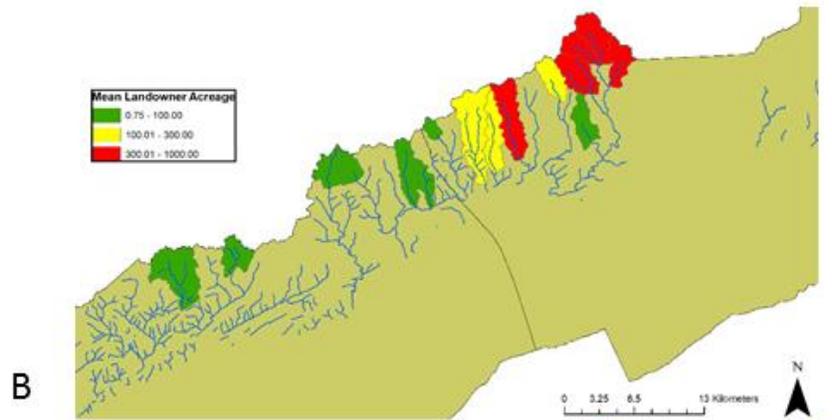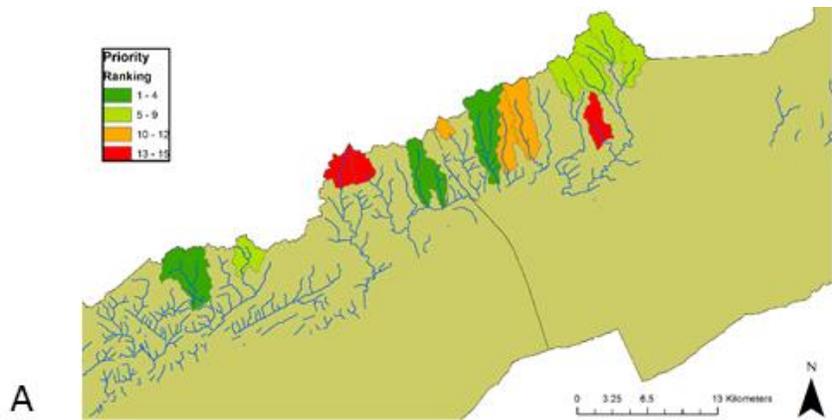
Figure 3.2: Map of Clinch Dace Candidate Conservation Areas (CCAs). A- color-coded based on the ranking of the composite scores for condition and irreplaceability for each CCA (optimal condition is dark-green), B- mean parcel acreage size adjacent to stream within each CCA (optimal condition is subjective), C- Percent of CCA in "disturbed" reclamation condition (optimal condition is dark green), D- Number of abandoned mine sites within each CCA (optimal condition is red because more opportunities exist for abandoned mine reclamation).
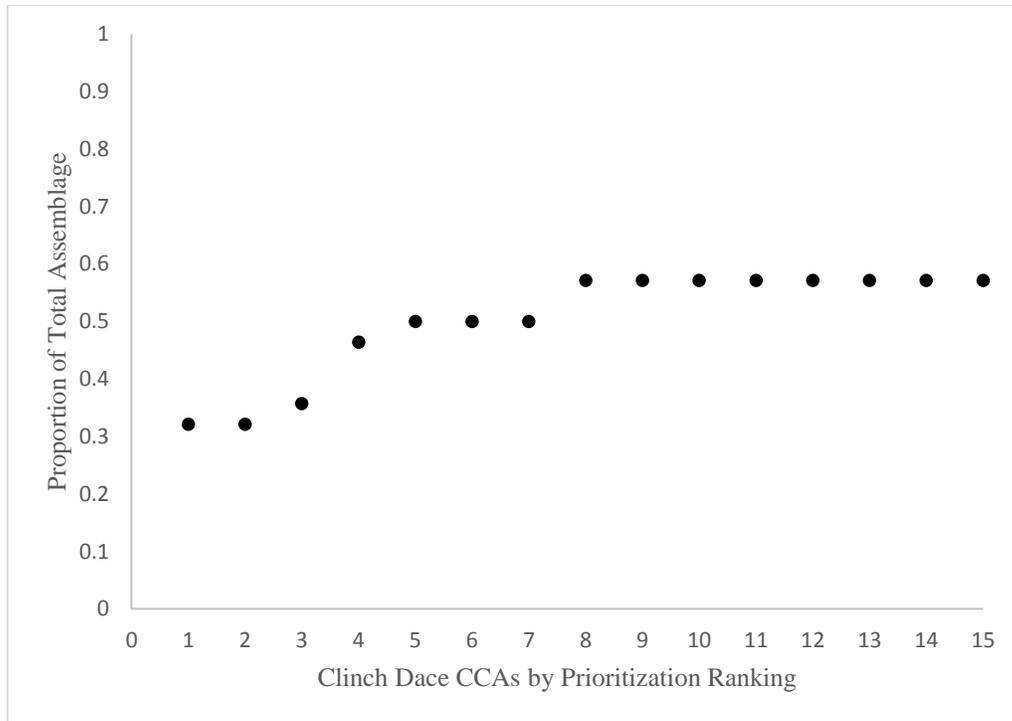
Figure 3.3: Species accumulation curve depicting the cumulative proportion of all species that were detected in 2nd- and 3rd-order streams where Clinch Dace were found. CCAs are identified on the *x*-axis by their composite ranking for irreplaceability and condition See table 3.2.
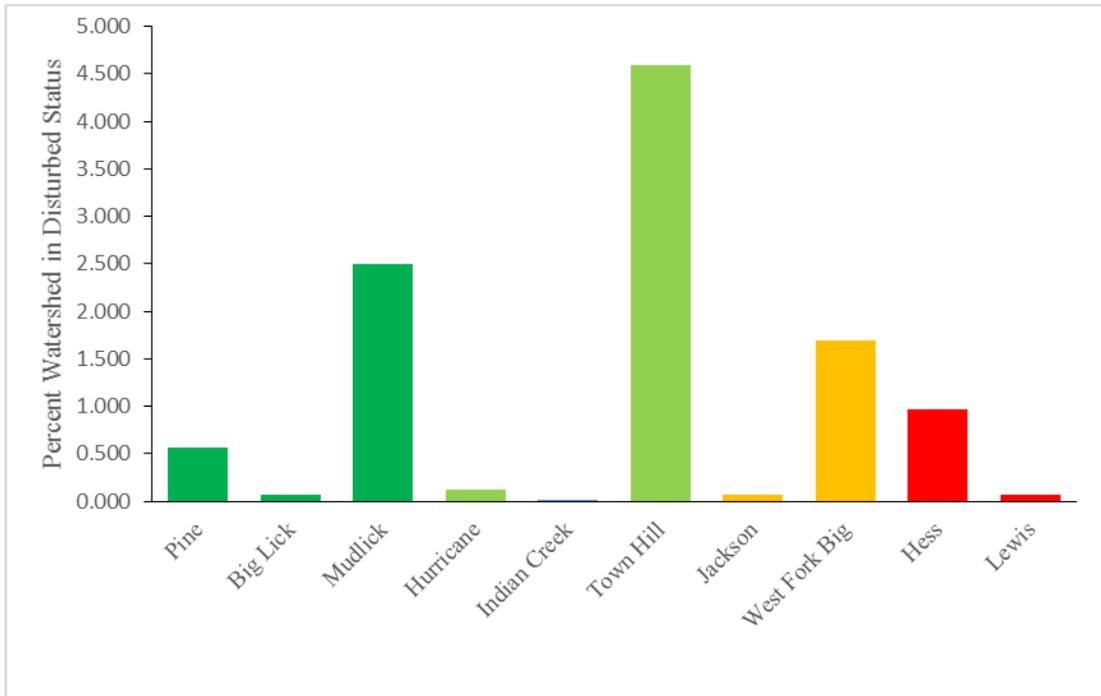
Figure 3.4: Percent of actively permitted mined lands within 15 watersheds occupied by Clinch Dace that have not yet been regraded or revegetated yet.
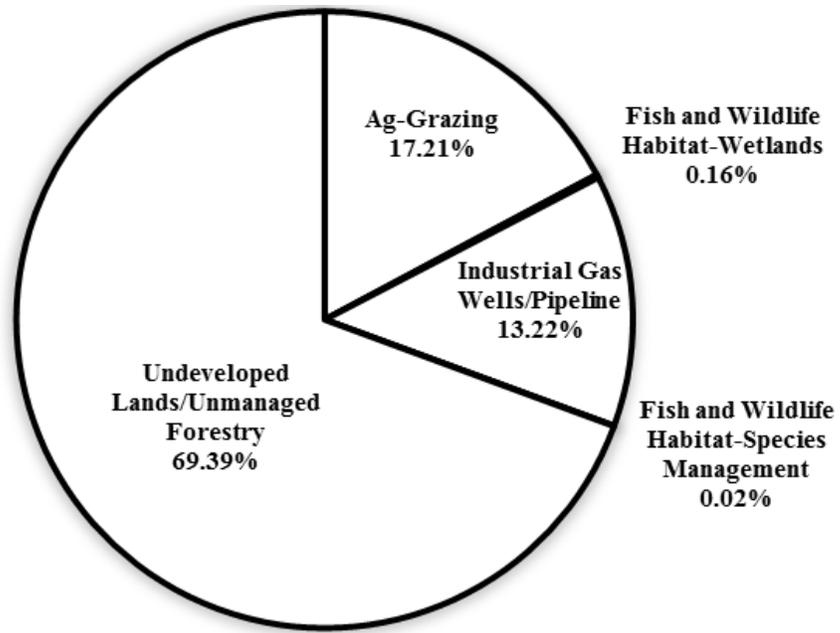
Figure 3.5: Designated post-mining land use for the total 6.54 km^2 of permitted mine lands in watersheds occupied by Clinch Dace.
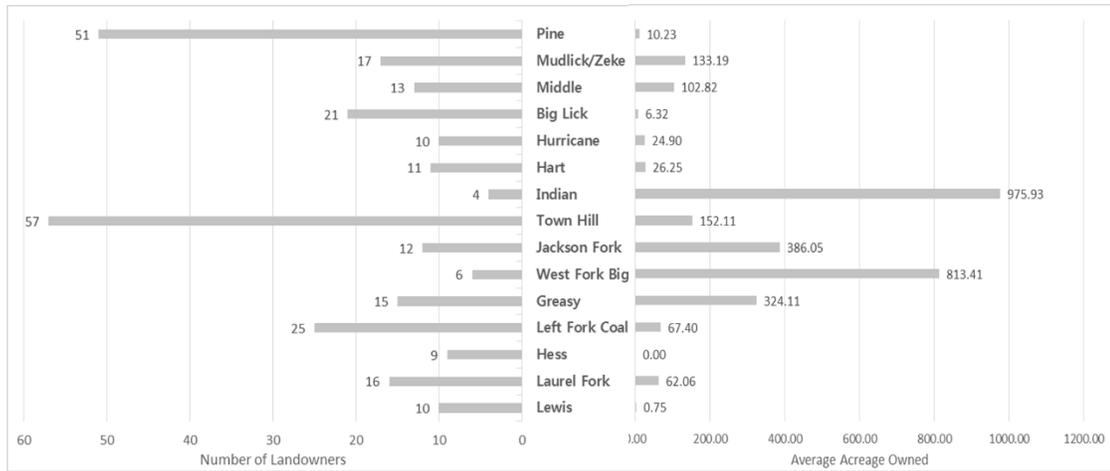
Figure 3.6: Land ownership patterns used in characterization of population feasibility for all 15 CCAs. Average landowner acreage and number of landowners adjacent to each stream.

Summary and Conclusions

This study reaffirms that Clinch Dace are patchily distributed in a small number of streams feeding the Clinch River in two counties in southwestern Virginia. I reported new records of Clinch Dace from Lewis Creek, Middle Creek, Zeke Creek, an unnamed tributary to Indian Creek, and Grassy Creek. Clinch Dace are now known from only 21 streams in 10 tributary networks.

Multiple gears may be used to effectively sample Clinch Dace in future monitoring frameworks. Electrofishing had higher species- and individual- level detection probabilities for Clinch Dace than minnow trapping. Minnow trapping may be more effective where multiple sampling replicates with electrofishing would risk stress or injury to Clinch Dace or in sampling deeper habitats such as beaver ponds where backpack electrofishing is not possible

Occupancy models using presence-absence data suggested that Clinch Dace presence was associated with higher substrate embeddedness and highly forested watersheds. Clinch Dace's apparent tolerance to some elevated levels of fine sediments in the presence of good water quality suggests that nest-building species such as Creek Chubs and Stonerollers are crucial in providing the clean substrate required for Clinch Dace reproduction. My data suggests that non-linear such as threshold relationships exist with other habitat variables such as conductivity. High levels of conductivity may deplete populations of the Clinch Dace's macroinvertebrate prey and it is unlikely that Clinch Dace populations persist at conductivity levels much higher than 600 µS/cm. Habitat models using count data did not support a positive relationship between substrate embeddedness and Clinch Dace abundance. Support still existed for a positive relationship with the proportion of the watershed forested and "spatially transformed" forested riparian cover.

Mark-recapture assessments revealed that Clinch Dace occur at low densities (<50 individuals/100 m). Abundance estimates suggest that many populations are very small and possibly

suffering from low genetic variability. Mean global estimates of abundance suggest that there may be fewer than 7,000 Clinch Dace presently living in these two counties.

I assessed the 15 known candidate conservation areas in order to prioritize conservation action for Clinch Dace using the metrics "irreplaceability" and "condition." The best candidate areas for conservation of Clinch Dace are: Pine Creek, Big Lick Creek, Mudlick Creek, and Hurricane Fork. Complementarity, as assessed by co-occurring fish assemblages at all CCAs, was not a useful planning metric. Opportunity for future mine land reclamation is greatest in Town Hill Creek and Lewis Creek although Lewis Creek was the lowest CCA in our priority rankings. Assessment of landownership patterns suggest that management should be tailored to the characteristics of stakeholders in each watershed. Some highly ranked CCAs such as Pine and Big Lick Creek consist of many small residential properties, whereas Indian Creek and West Fork Big Creek are owned by a few larger companies.

Habitat management should promote the conservation of forests in watersheds occupied by Clinch Dace. This includes preserving intact riparian buffers and ensuring rapid recovery of forests following logging. Large surface mines, which may elevate stream conductivity and prevent the future establishments of forests following mining, should not occur in close proximity to Clinch Dace populations. Additionally, managers should also work on smaller scales with landowners to implement habitat conservation measures on residential properties.

Many barriers to Clinch Dace dispersal exist in occupied streams that may limit access to important resources and colonization of new habitats. A population genetic assessment is needed to prioritize barriers for removal and in establishing genetically distinct management units. In the future resolving the taxonomic status of Clinch Dace will further motivate conservation action for this species.

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Appendix A: Counts of individuals by species captured with minnow trapping and single-pass backpack electrofishing at 70 standardized survey sites.

Site	Species	Trap	Shock
Alvy Creek			
	Rhinichthys obtusus	100	279
	Semotilus atromaculatus	0	2
Big Creek (1)			
	Campostoma anomalum	4	108
	Catostomus commersoni	1	9
	Cottus sp.	0	98
	Etheostoma blenniodes	0	4
	Etheostoma flabellare	1	99
	Etheostoma simoterum	0	22
	Hybopsis amblops	1	7
	Hypentelium nigricans	0	13
	Notropis telescopus	0	1
	Notropis leuciodus	0	3
	Rhinchthys obtusus	741	256
	Semotilus atromaculatus	4	7
Big Creek (2)			
	Campostoma anomalum	0	23
	Catostomus commersoni	0	2
	Rhinichthys obtusus	114	193
	Semotilus atromaculatus	0	5
Big Creek (3)	*Campostoma anomalum*	4	34
	Catostomus commersoni	0	6
	Rhinichthys obtusus	277	243
	Semotilus atromaculatus	46	16
Big Creek (4)			
	Campostoma anomalum	0	5
	Rhinichthys obtusus	0	3
	Semotilus atromaculatus	0	66
Big Lick Creek (1)			
	Campostoma anomalum	1	28
	Catostomus commersoni	1	21
	Etheostoma flabellare	2	53
	Etheostoma simoterum	1	1

	Hypentelium nigricans	0	2
	Lepomis auritus	0	2
	Luxilus chrysocephalus	0	2
	Moxostoma erythrurum	0	2
	Rhinichthys obtusus	32	35
	Semotilus atromaculatus	68	55
Big Lick Creek (2)			
	Campostoma anomalum	1	34
	Catostomus commersoni	0	8
	Chrosomus sp. cf. *saylori*	10	26
	Etheostoma flabellare	3	76
	Rhinichthys obtusus	61	115
	Semotilus atromaculatus	27	43
Breezers Branch			
	Campostoma anamolum	3	20
	Cottus sp.	1	32
	Etheostoma flabellare	5	16
	Etheostoma simoterum	0	9
	Luxilus chrysocephalus	0	19
	Luxilus coccogenis	0	2
	Nothonotus rufilineatum	0	3
	Notropis rubricroceous	3	8
	Notropis telescopus	0	2
	Rhinichthys obtusus	43	62
	Semotilus atromaculatus	10	5
	Unkown juvenile	1	1
Cavitts Creek			
	Ambloplites rupestris	0	6
	Campostoma anomalum	1	242
	Catostomus commersoni	0	1
	Cottus commersoni	0	18
	Etheostoma flabellare	1	33
	Etheostoma simoterum	0	10
	Hybopsis amblops	0	1
	Hypentelium nigricans	0	3
	Luxilus chrysocephalus	0	8
	Luxilus coccogenis	0	2
	Micropterus dolomieu	0	1
	Nothonotus rufilineatum	0	29
	Rhinichthys obtusus	5	81
	Semotilus atromaculatus	0	1

Claypool Branch

Lepomis macrochirus	0	4
Rhinichthys obtusus	38	98
Semotilus atromaculatus	12	38
Unknown juvenile	8	10

Coal Creek

Campostoma anomalum	0	11
Rhinichthys obtusus	60	165
Semotilus atromaculatus	1	2

Eagles Nest Branch

Campostoma anomalum	0	1
Rhinichthys obtusus	160	101
Semotilus atromaculatus	2	13

Grassy Branch

Campostoma anomalum	0	2
Catostomus commersoni	0	1
Chrosomus sp. cf. *saylori*	36	55
Etheostoma flabellare	2	22
Rhinchthys obtusus	50	60
Semotilus atromaculatus	56	62
Unknown Juvenile	0	5

Grassy Creek

Etheostoma flabellare	0	43
Rhinichthys obtusus	40	81
Semotilus atromaculatus	7	16

Gravel Lick Creek

Campostoma anomalum	1	25
Lepomis auritus	0	21
Lepomis cyanellus	0	2
Luxilus chrysocephalus	9	19
Rhinichthys obtusus	10	142
Semotilus atromaculatus	119	108
Unknown Juvenile	19	0

Greasy Creek (1)

Campostoma anomalum	14	56
Catostomus commersoni	17	43

Chrosomus sp. cf. *saylori*	6	1
Clinostomus funduloides	57	140
Etheostoma flabellare	0	53
Rhinichthys obtusus	70	191
Semotilus atromaculatus	82	113
Unknown Juvenile	0	1

Greasy Creek (2)

Campostoma anomalum	6	11
Catostomus commersoni	3	7
Chrosomus sp. cf. *saylori*	2	7
Clinostomus funduloides	29	25
Possible Chrosomus x Clinostomus	1	0
Etheostoma flabellare	2	24
Rhinichthys obtusus	131	91
Semotilus atromaculatus	161	42

Hamlin Branch

Campostoma anomalum	5	62
Catostomus commersoni	0	3
Etheostoma flabellare	1	55
Etheostoma simoterum	0	4
Hypentelium nigricans	0	1
Rhinichthys obtusus	41	169
Semotilus atromaculatus	2	19

Hess Creek

Campostoma anomalum	3	7
Chrosomus sp. cf. *saylori*	0	3
Etheostoma flabellare	0	10
Rhinichthys obtusus	80	192
Semotilus atromaculatus	16	17

Honey Branch

Ambloplites rupestris	0	2
Campostoma anomalum	0	52
Cottus sp.	0	36
Etheostoma simoterum	0	4
Hypentelium nigricans	0	2
Luxilus chrysocephalus	0	8
Luxilus coccogenis	0	12
Micropterus dolomieu	0	2

	Nothonotus rufilineatum	0	5
	Notropis telescopus	0	8
	Notropis volucellus	0	1
	Rhinichthys obtusus	36	84
	Semotilus atromaculatus	3	5
Hurricane Fork (1)			
	Campostoma anomalum	38	14
	Catostomus commersoni	0	1
	Etheostoma flabellare	2	43
	Hypentelium nigricans	0	4
	Lepomis macrochirus	0	1
	Nocomis micropogon	1	0
	Nothonotus rufilineatum	0	1
	Rhinichthys obtusus	63	91
	Semotilus atromaculatus	20	10
Hurricane Fork (2)	*Campostoma anomalum*	1	64
	Catostomus commersoni	0	9
	Chrosomus sp. cf. *saylori*	0	53
	Etheostoma flabellare	0	102
	Pimephales notatus	2	44
	Rhinichthys obtusus	83	151
	Semotilus atromaculatus	76	166
Indian Creek	*Ambloplites rupestris*	0	12
	Campostoma anomalum	0	30
	Catostomus commersoni	0	1
	Cottus sp.	0	32
	Cyprinella galactura	6	0
	Cyprinella spiloptera	1	0
	Etheostoma blennioides	0	13
	Etheostoma flabellare	0	7
	Etheostoma stigmaeum	0	8
	Etheostoma simoterum	0	4
	Etheostoma zonale	0	5
	Hybopsis amblops	0	7
	Hypentelium nigricans	0	8
	Ichthyomyzon greeleyi	0	1
	Luxilus chrysocephalus	6	3
	Luxilus coccogenis	0	3
	Lythrurus lirus	0	1

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	Nothonotus rufilineatum	0	10
	Notropis telescopus	0	2
	Percina evides	0	1
	Pimephales notatus	1	1
	Rhinichthys obtusus	0	1
	Unknown Juvenile	0	12
Jackson Fork	*Campostoma anomalum*	0	3
	Etheostoma flabellare	4	64
	Rhinichthys obtusus	23	109
	Semotilus atromaculatus	3	7
Laurel Branch			
	Rhinichthys obtusus	0	9
	Semotilus atromaculatus	0	7
Laurel Fork			
	Campostoma anomalum	3	1
	Catostomus commersoni	3	11
	Chrosomus sp. cf. *saylori*	4	0
	Clinostomus funduloides	9	5
	Possible Chrosomus x Clinostomus	2	0
	Etheostoma flabellare	9	19
	Rhinichthys obtusus	76	20
	Semotilus atromaculatus	23	15
Left Fork Lick Creek			
Lewis Creek (15)			
	Campostoma anomalum	2	19
	Etheostoma flabellare	1	59
	Etheostoma simoterum	0	2
	Hypentelium nigricans	0	1
	Rhinichthys obtusus	94	459
	Semotilus atromaculatus	6	1
Lewis Creek (1)			
	Ambloplites rupestris	0	18
	Campostoma anomalum	3	291
	Catostomus commersoni	0	8
	Cottus sp.	0	17
	Etheostoma flabellare	4	34

	Etheostoma simoterum	2	4
	Hypentelium nigricans	0	8
	Luxilus chrysocephalus	7	15
	Notropis telescopus	0	4
	Rhinichthys obtusus	178	203
	Semotilus atromaculatus	2	4
Lewis Creek (32)			
	Ambloplites rupestris	4	5
	Campostoma anomalum	4	65
	Etheostoma flabellare	0	74
	Etheostoma simoterum	0	11
	Hypentelium nigricans	0	5
	Luxilus chrysocephalus	62	23
	Notropis telescopus	0	1
	Rhinichthys obtusus	56	75
	Semotilus atromaculatus	10	11
Lewis Creek (5)			
	Campostoma anomalum	0	23
	Chrosomus sp. cf. *saylori*	5	0
	Etheostoma flabellare	0	15
	Luxilus chrysocephalus	0	2
	Rhinichthys obtusus	86	102
	Semotilus atromaculatus	18	29
Lewis Creek (34)			
	Ambloplites rupestris	20	20
	Campostoma anomalum	5	23
	Catostomus commersoni	2	4
	Etheostoma flabellare	0	35
	Etheostoma simoterum	0	17
	Hypentelium nigricans	0	6
	Luxilus chrysocephalus	2	6
	Rhinichthys obtusus	4	39
	Semotilus atromaculatus	11	8
Lick Creek			
	Ambloplites rupestris	1	20
	Campostoma anomalum	21	258
	Catostomus commersoni	0	7
	Etheostoma flabellare	0	1
	Etheostoma simoterum	0	50
	Hypentelium nigricans	0	14

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	Luxilus chrysocephalus	17	23
	Rhinichthys obtusus	30	166
	Semotilus atromaculatus	6	13
Lincolnshire Branch			
	Cottus sp.	0	13
	Rhinichthys obtusus	89	240
	Semotilus atromaculatus	2	23
Little Mill Creek			
	Campostoma anomalum	36	0
	Rhinichthys obtusus	29	0
	Semotilus atromaculatus	145	2
Long Branch			
	Ambloplites rupestris	3	25
	Campostoma anomalum	7	45
	Catostomus commersoni	2	1
	Etheostoma flabellare	0	20
	Etheostoma simoterum	0	13
	Hypentelium nigricans	0	11
	Lepomis macrochirus	0	1
	Rhinichthys obtusus	80	53
	Semotilus atromaculatus	17	11
Middle Creek Lower			
	Campostoma anomalum	0	72
	Catostomus commersoni	0	2
	Cottus sp.	0	31
	Etheostoma blenniodes	0	1
	Ehteostoma flabellare	5	94
	Etheostoma stigmaeum	0	2
	Etheostoma simoterum	0	12
	Hypentelium nigricans	0	3
	Luxilus chrysocephalus	2	2
	Rhinichthys obtusus	99	169
	Semotilus atromaculatus	7	11
Middle Creek Upper			
	Campostoma anomalum	0	15
	Catostomus commersoni	0	7
	Chrosomus sp. cf. *saylori*	7	38
	Clinostomus funduloides	8	137
	Rhinichthys obtusus	18	155

	Semotilus atromaculatus	7	173
Middle Fork Clinch River			
	Campostoma anomalum	0	17
	Etheostoma blennioides	1	2
	Lepomis cyanellus	2	0
	Lepomis macrochirus	2	0
	Luxilus chrysocephalus	51	12
	Nothonotus rufilineatum	0	1
	Semotilus atromaculatus	0	2
Mill Creek			
	Campostoma anomalum	8	10
	Rhinichthys obtusus	234	265
	Semotilus atromaculatus	27	19
Millstone Branch			
	Rhinichthys obtusus	13	18
	Semotilus atromaculatus	11	12
Mudlick Creek	*Campostoma anomalum*	0	14
	Chrosomus sp. cf. *saylori*	0	1
	Cottus sp.	0	5
	Etheostoma flabellare	2	21
	Lepomis macrochirus	0	1
	Luxilus chrysocephalus	0	3
	Rhinichthys obtusus	19	102
	Semotilus atromaculatus	4	10
North Fork Clinch River			
	Campostoma anomalum	0	3
	Cottus sp.	1	69
	Etheostoma flabellare	0	2
	Rhinichthys obtusus	27	173
	Semotilus atromaculatus	0	10
Pine Creek Lower			
	Campostoma anomalum	15	89
	Catostomus commersoni	0	8
	Chrosomus sp. cf. *saylori*	0	1
	Etheostoma flabellare	2	138
	Etheostoma simoterum	0	2

	Hypentelium nigricans	0	6
	Rhinichthys obtusus	186	297
	Semotilus atromaculatus	41	68
Pine Creek Upper			
	Campostoma anomalum	0	2
	Catostomus commersoni	0	2
	Chrosomus sp. cf. *saylori*	13	42
	Etheostoma flabellare	2	34
	Rhinichthys obtusus	69	114
	Semotilus atromaculatus	31	50
Right Fork Lick Creek			
	Campostoma anomalum	0	3
	Rhinichthys obtusus	19	317
Rock Lick Branch			
	Campostoma anomalum	0	105
	Etheostoma flabellare	0	101
	Rhinichthys obtusus	10	148
	Semotilus atromaculatus	19	65
Sample Branch			
	Campostoma anomalum	1	7
	Cottus sp.	0	2
	Lepomis cyanellus	1	0
	Luxilus chrysocephalus	8	1
	Rhinchthys obtusus	12	10
	Semotilus atromaculatus	5	17
	Unknown Juveniles	0	749
Smith Branch			
	No fish		
Stone Branch			
	Rhinichthys obtusus	21	211
	Semotilus atromaculatus	4	26
Stonecoal Creek			
	Campostoma anomalum	0	13
	Micropterus salmoides	0	3
	Rhinichthys obtusus	14	75
	Semotilus atromaculatus	0	4

Strow Creek

Campostoma anomalum	3	28
Catostomus commersoni	0	4
Etheostoma flabellare	0	20
Rhinichthys obtusus	180	104
Semotilus atromaculatus	34	53

Sulphur Spring Branch

Campostoma anomalum	2	12
Catostomus commersoni	0	7
Etheostoma flabellare	0	23
Rhinichthys obtusus	44	177
Semotilus atromaculatus	1	30
UK Juvenile	5	18

Swords Creek

Ambloplites rupestris	0	2
Campostoma anomalum	6	487
Catostomus commersoni	0	3
Cottus sp.	0	5
Etheostoma flabellare	1	103
Etheostoma simoterum	0	28
Hypentelium nigricans	0	16
Micropterus dolomieu	0	3
Notropis telescopus	9	9
Rhinichthys obtusus	79	334
Semotilus atromaculatus	17	6
Unknown Juvenile	1	1

Sykes Branch

Campostoma anomalum	0	3
Catostomus commersoni	0	4
Cottus sp.	0	23
Etheostoma flabellare	0	41
Etheostoma simoterum	0	2
Hypentelium nigricans	0	2
Luxilus chrysocephalus	0	27
Notropis rubricroceous	0	8
Pimephales notatus	0	1
Rhinichthys obtusus	59	194
Semotilus atromaculatus	0	14
Unknown juvenile	0	14

Thompson Creek (1)

Ambloplites rupestris	0	2
Campostoma anomalum	17	182
Catostomus commersoni	1	49
Cottus sp.	0	14
Etheostoma blennioides	0	8
Etheostoma flabellare	4	147
Etheostoma simoterum	0	45
Hybopsis amblops	1	10
Hypentelium nigricans	0	21
Luxilus chrysocephalus	14	69
Moxostoma duquesnei	0	2
Nothonotus rufilineatum	0	1
Notropis rubricroceous	0	12
Notropis sp. (sawfin shiner)	1	11
Notripis telescopus	2	5
Pimephales notatus	0	1
Rhinichthys obtusus	162	142
Semotilus atromaculatus	0	11
Unknown Juvenile	0	39

Thompson Creek (2)

Campostoma anomalum	2	40
Etheostoma flabellare	4	63
Hypentelium nigricans	0	1
Luxilus chrysocephalus	9	16
Notropis rubricroceous	0	31
Rhinichthys obtusus	40	114
Semotilus atromaculatus	6	18

Thompson Creek (3)

Campostoma anomalum	0	5
Cottus sp.	0	46
Etheostoma flabellare	0	1
Etheostoma simoterum	2	0
Lepomis auritus	0	1
Luxilus chrysocephalus	0	23
Rhinichthys obtusus	5	108
Semotilus atromaculatus	0	27
Unknown	0	3

Town Hill Creek Lower

Etheostoma flabellare	0	5
Rhinichthys obtusus	49	114

	Semotilus atromaculatus	3	4
Town Hill Creek Upper			
	Etheostoma flabellare	0	2
	Rhinichthys obtusus	5	41
	Semotilus atromaculatus	2	4
Unnamed Tributary to Chaney Creek			
	Rhinchthys obtusus	NA	4
Unnamed Tributary to Gravel Lick Creek			
	Campostoma anomalum	1	2
	Rhinichthys obtusus	5	4
	Semotilus atromaculatus	13	23
	Unknown Juvenile	1	0
Unnamed Tributary to Indian Creek			
	Campostoma anomalum	1	24
	Cottus sp.	0	2
	Rhinichthys obtusus	123	251
	Semotilus atromaculatus	35	58
Unnamed Tributary to Lowe Branch			
	Rhinchthys obtusus	94	119
Unnamed Tributary to the North Fork Clinch River (1)			
	Etheostoma flabellare	1	19
	Rhinichthys obtusus	0	60
Unnamed Tributary to the North Fork Clinch River (2)			
	Campostoma anomalum	0	4
	Catostomus commersoni	1	8
	Cottus sp.	0	11
	Etheostoma flabellare	2	88
	Etheostoma simoterum	7	15
	Rhinichthys obtusus	220	112
Unnamed Tributary to the North Fork Clinch River (3)			
	Ambloplites rupestris	2	18
	Campostoma anomalum	0	14
	Cottus sp.	0	1
	Etheostoma flabellare	0	13
	Etheostoma simoterum	0	5

Lepomis gibbosus	0	1
Luxilus chrysocephalus	0	1
Rhinichthys obtusus	25	98
Semotilus atromaculatus	35	19

Weaver Creek

Rhinichthys obtusus	114	157
Semotilus atromaculatus	24	0

Whitley Branch

Rhinichthys obtusus	1	31

Zeke Creek

Chrosomus sp. cf *saylori*	0	2
Etheostoma flabellare	0	7
Rhinicthys obtusus	19	47
Semotilus atromaculatus	3	23

Appendix B: Counts of individuals by species captured at 27 opportunistic (unstandardized) sampling sites using single-pass electrofishing.

Site	Species	Shock
Alvy Creek		
	Rhinichthys obtusus	112
	Semotilus atromaculatus	11
Beaver Pond at Confluence of Hess and Strow Creeks	*Campostoma anomalum*	1
	Catostomus commersoni	122
	Etheostoma flabellare	11
	Rhinichthys obtusus	158
	Semotilus atromaculatus	124
Benges Creek	*Campostoma anomalum*	N/A
	Cottus sp.	N/A
	Etheostoma simoterum	N/A
	Luxilus chrysocephalus	N/A
	Luxilus coccogenis	N/A
	Rhynicthys obtusus	N/A
Coal Creek	*Campostoma anomalum*	15
	Rhinichthys obtusus	31
	Semotilus atromaculatus	2
Coal Creek (2)	*Rhinichthys obtusus*	104
	Semotilus atromaculatus	27
Crooked Branch	*Rhinichthys obtusus*	15
	Semotilus atromaculatus	39
	Unknown Juvenile	1
Hamlin Branch	*Campostoma anomalum*	47
	Catostomus commersoni	3
	Cottus sp.	1
	Etheostoma flabellare	63
	Etheostoma simoterum	3
	Hypentelium nigricans	1

	Luxilus chrysocephalus	8
	Rhinichthys obtusus	91
	Semotilus atromaculatus	36
Hurricane Fork	*Camopstoma anomalum*	2
	Catostomus commersoni	5
	Chrosomus sp. cf. *saylori*	1
	Etheostoma flabellare	12
	Pimephales notatus	3
	Rhinichthys obtusus	70
	Semotilus atromaculatus	50
Hurricane Fork Downstream of Paul Artrip's House	*Ambloplites rupestris*	11
	Campostoma anomalum	4
	Catostomus commersoni	64
	Chrosomus sp. cf. *saylori*	3
	Etheostoma flabellare	36
	Lepomis auritus	10
	Pimephales notatus	79
	Rhinichthys obtusus	53
	Semotilus atromaculatus	118
Jackson Fork	*Ambloplites rupestris*	24
	Catostomus commersoni	34
	Clinostomus funduloides	38
	Etheostoma flabellare	12
	Lepomis auritus	8
	Luxilus chrysocephalus	1
	Rhinichthys obtusus	118
	Semotilus atromaculatus	104
Little Town Hill Creek	*Campostoma anomalum*	27
	Cottus sp.	4
	Etheostoma flabellare	20
	Rhinichthys obtusus	151
	Semotilus atromaulatus	26

Long Branch	*Campostoma anomalum*	2
	Etheostoma flabellare	2
	Rhinichthys obtusus	121
	Semotilus atromaculatus	52
Long Branch Direct Trib to Clinch	*Campostoma anomalum*	63
	Rhinichthys obtusus	54
	Semotilus atromaculatus	54
Middle Creek	*Campostoma anomalum*	4
	Catostomus commersoni	4
	Clinostomus funduloides	5
	Etheostoma flabellare	16
	Etheostoma simoterum	1
	Hypentelium nigricans	2
	Lepomis auritus	2
	Luxilus chrysocephalus	1
	Rhinicthys obtusus	38
	Semotilus atromaculatus	19
Raven's Nest Branch	*Lepomis auritus*	14
	Rhinichthys obtusus	131
Skeen Creek	*Campostoma anomalum*	1
	Etheostoma flabellare	2
	Rhinichthys obtusus	32
	Semotilus atromaculatus	10
Stillhouse Branch + Lower Thompson Creek	*Ambloplites rupestris*	2
	Campostoma anomalum	2
	Cottus sp.	7
	Etheostoma flabellare	1
	Hypentelium nigricans	3
	Luxilus chrysocephalus	4
	Luxilus coccogenis	1
	Notropis rubricroceous	7
	Notropis telescopus	1
	Rhinichthys obtusus	7
	Semotilus atromaculatus	2

Sykes Branch	*Campostoma anomalum*	1
	Cottus sp.	7
	Etheostoma flabellare	14
	Etheostoma simoterum	1
	Luxilus chrysocephalus	2
	Notropis rubricroceous	8
	Notropis telescopus	2
	Rhinichthys obtusus	79
	Semotilus atromaculatus	6
Town Hill Creek	*Etheostoma flabellare*	3
	Rhinichthys obtusus	27
	Semotilus atromaculatus	9
Unnamed Tributary to Greasy Creek	*Catostomus commersoni*	1
	Chrosomus sp. cf. *saylori*	2
	Clinostomus funduloides	1
	Etheostoma flabellare	22
	Rhinichthys obtusus	26
	Semotilus atromaculatus	17
Upper Mill Creek	*Rhinichthys obtusus*	25
	Semotilus atromaculatus	14
Upper Hurricane Fork	*Campostoma anomalum*	7
	Catostomus commersoni	4
	Chrosomus sp. cf. *saylori*	5
	Etheostoma flabellare	1
	Rhinicthys obtusus	55
	Semotilus atromaculatus	16
Upper Lewis Creek (1)		
	Campostoma anomalum	18
	Etheostoma flabellare	14
	Rhinichthys obtusus	43
	Semotilus atromaculatus	17
Upper Lewis Creek (2)	*Campostoma anomalum*	6
	Chrosomus sp. cf. *saylori*	1

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	Etheostoma flabellare	4
	Rhinichthys obtusus	26
	Semotilus atromaculatus	1
Upper Middle Creek	*Campostoma anomalum*	6
	Chrosomus sp. cf. *saylori*	26
	Clinostomus funduloides	42
	Etheostoma flabellare	2
	Rhinichthys obtusus	69
	Semotilus atromaculatus	41
	possible Chrosomus x Semotilus	1
West Fork Big Creek (1)	*Campostoma anomalum*	5
	Rhinichthys obtusus	209
	Semotilus atromaculatus	2
West Fork Big Creek (2)	*Chrosomus* sp. cf. *saylori*	6
	Catostomus commersoni	1
	Rhinichthys obtusus	93
	Semotilus atromaculatus	17
	Unknown Juvenile	6
Wolfpen Branch	*No fish*	

Appendix C: Habitat data collected at all 70 standardized sites.

Site	Lat	Long	Grad	Elev	Shed Area	Cond.	pH	Width	Depth	Width:Depth	Canopy Cover	>75% embed	B Rock
UN Trib to N Fk Clinch River	37.1374	-81.4814	1.14	744	2.1	256	8.54	1.0	15.4	14.9	32	8	1
UN Trib to N Fk Clinch River	37.1564	-81.4667	0.65	756	9.3	170	8.47	2.9	26.3	9.1	13	3	0
UN Trib to N Fk Clinch River	37.1691	-81.4828	1.23	774	5.6	355	8.44	1.8	13.9	7.8	70	2	0
Greasy Creek	37.1729	-81.7245	1.76	700	9.9	263	8.36	3.4	15.3	4.6	40	77	0
Cavitts Creek	37.1300	-81.5507	0.58	716	25.2	282	8.74	5.1	24.9	4.9	18	0	17
Indian Creek	37.0895	-81.7551	0.34	596	86.4	231	8.24	10.1	28.4	2.8	63	7	50
Lick Creek	36.9460	-82.2921	0	506	28.3	594	8.12	6.1	33.6	5.5	75	24	0
Unnamed Trib to Indian Creek	37.1019	-81.7194	2.55	635	2.2	497	8.27	1.1	9.6	8.8	37	22	0
Jackson Fork	37.1803	-81.7053	1.99	700	7.1	168	7.3	3.0	11.6	3.9	90	16	2
Lowe Branch	37.1155	-81.6924	2.12	666	2.5	432	7.28	0.9	12.1	13.1	61	43	0
Smith Branch	37.1086	-81.8639	13.32	671	1.0	150	7.86	0.8	3.3	4.0	81	6	0
Right Fork Lick Creek	36.9457	-82.2917	3.61	575	5.0	557	8.12	2.1	12.8	6.0	59	0	0
Big Creek	37.1393	-81.7837	1.08	679	12.9	570	8.5	3.7	24.5	6.6	64	16	2
Upper Big Creek	37.1575	-81.7870	0.19	688	8.4	467	7.83	1.0	6.8	7.0	55	17	3
Lewis Creek 1	37.0546	-81.9842	0.92	576	34.3	233	8.08	7.2	20.1	2.8	95	6	45
Lower Town Hill Creek	37.1003	-81.8245	3.19	596	10.4	242	8.12	2.8	13.2	4.8	75	14	0
Honey Branch	36.9155	-82.2993	3.06	456	14.1	315	8.4	4.2	23.3	5.5	65	3	25
Upper Hess Creek	37.1014	-81.9705	1.46	678	3.6	340	8.16	2.3	10.0	4.4	89	19	0
Left Fork Lick Creek	36.9790	-82.3126	2.92	559	6.0	450	8.08	2.7	13.9	5.2	48	12	0
Mudlick Creek	36.9318	-82.2152	1.9	594	18.9	184	8.12	4.0	20.6	5.1	91	8	6
Lower Pine Creek	37.0768	-81.9085	1.69	623	8.9	262	8.35	3.0	20.6	6.8	90	6	7
Little Mill Creek	37.0845	-81.8702	2.45	606	2.7	328	8.22	1.2	7.6	6.4	48	17	22
Sample Branch	37.0412	-81.9120	1.46	570	4.7	336	8.03	2.0	19.7	9.8	58	29	0
Swords Creek	37.0592	-81.9285	1.14	587	30.9	309	8.49	5.7	21.4	3.7	70	4	44
Big Lick Creek	37.0755	-81.9139	1.14	619	17.5	326	8.25	5.8	32.7	5.6	88	24	21
Upper Big Lick Creek	37.0875	-81.8943	1.52	659	4.1	143	8	2.5	13.2	5.3	56	10	1
Upper Pine Creek	37.1108	-81.9172	1.47	693	3.3	100	7.88	1.9	16.3	8.8	51	10	1

Long Branch	37.0680	-81.9455	0.86	629	18.2	313	8.37	5.0	41.2	8.3	37	64	0
Lewis Creek 2	37.0419	-81.9779	1.79	607	18.1	420	8.22	5.1	28.1	5.6	93	4	2
Thompson Creek	37.1041	-81.9555	3.25	571	4.7	260	8.44	2.6	13.4	5.1	88	8	4
Stone Branch	37.0467	-81.9716	2.22	612	3.1	120	7.63	1.5	10.7	7.3	88	24	0
Lewis Creek 3	37.0784	-81.9973	1.57	694	9.2	328	7.98	2.8	31.7	11.3	52	19	0
Lewis Creek 5	37.1045	-81.9994	0.92	709	2.8	275	7.93	1.4	13.9	9.7	62	87	0
Lewis Creek 4	37.0912	-81.9979	1.57	701	6.9	331	7.84	2.1	39.6	19.1	33	37	6
Millstone Branch	36.9670	-82.1698	2.37	494	1.9	135	7.64	1.4	3.9	2.8	93	15	2
Thompson Creek	36.9794	-82.2374	2.4	552	6.5	257	7.66	2.7	12.7	4.7	58	11	0
Breezer's Branch	37.0144	-82.0052	1.84	475	5.6	298	7.72	2.6	14.2	5.6	87	17	13
Alvy Creek	37.0062	-82.1063	2.45	532	10.8	60	8.41	4.6	12.6	2.7	100	8	33
Strow Creek	37.1041	-81.9955	0.94	670	4.3	350	8.16	1.9	16.1	8.5	88	37	0
Middle Fork	36.9510	-82.1961	3.71	465	3.2	882	7.59	2.5	5.4	2.1	97	3	0
Town Hill Creek	37.1082	-81.8245	3.19	629	9.8	200	8.13	2.7	12.3	4.5	87	10	6
Hurricane Fork	36.9465	-82.1490	1.31	578	16.6	516	8.21	3.0	12.4	4.1	55	19	15
Whitley Branch	37.1389	-81.5247	2.52	739	2.1	325	8.38	2.3	6.1	2.7	91	4	6
Eagle's Nest Branch	36.9299	-82.2155	5.97	458	3.9	473	8.39	2.5	11.2	4.6	54	11	47
UN Trib to Chaney Creek	36.9793	-82.2367	9.48	580	1.2	477	8.12	2.1	7.1	3.4	97	0	0
Stonecoal Creek	36.9528	-82.1964	3.79	520	4.3	900	8.35	2.7	7.8	2.9	85	0	0
UN Trib to Gravel Lick Creek	36.9348	-82.2622	5.81	558	1.1	126	8.12	0.7	3.7	5.0	25	53	0
Gravel Lick Creek	36.9348	-82.2622	1.26	532	5.4	148	8.12	1.6	13.4	8.2	83	29	0
Zeke Creek	37.1240	-81.8440	1.72	671	7.2	119	7.47	4.8	22.8	4.8	99	9	1
Weaver Creek	37.0243	-82.0734	2.36	571	4.5	160	8.21	2.7	12.1	4.5	74	16	2
Claypool Branch	37.0251	-82.0270	3.13	597	4.2	479	8.16	2.1	13.2	6.4	63	21	31
Mill Creek	37.1034	-81.8814	1.9	660	8.1	209	8.14	2.7	14.0	5.2	70	21	1
Lincolnshire Branch	37.1532	-81.4976	10.29	783	2.5	376	8.18	2.1	17.6	8.3	47	15	9
North Fork Clinch River	37.1888	-81.4562	1.47	795	3.9	261	8.1	3.6	20.0	5.6	98	23	17
Sulphur Spring Branch	37.0795	-81.9206	1.26	627	9.0	208	8.03	3.1	7.5	2.4	89	23	7
Laurel Fork	37.1332	-81.7258	1.33	668	7.2	242	8.13	3.4	34.0	10.1	85	55	8
Sykes Branch	36.9996	-82.0439	3.34	507	5.7	430	8.53	3.1	13.1	4.3	95	12	3
Grassy Creek	37.0663	-81.9967	3.18	658	4.6	92	7.92	3.0	12.6	4.2	77	10	3

Greasy Creek	37.1794	-81.7392	1.84	707	4.9	453	8.14	2.0	25.6	13.1	55	72	0
Laurel Branch	36.9902	-82.1724	3.59	546	3.5	353	8.23	2.3	12.0	5.1	91	14	10
Hurricane Fork	36.9802	-82.1850	1.8	520	28.1	422	8.42	6.4	21.7	3.4	97	12	2
Upper Middle Creek	37.1686	-81.9578	1.06	730	7.9	189	7.83	2.4	10.0	4.2	55	22	2
Lower Big Creek	37.1335	-81.7830	1.19	675	36.8	713	8.56	4.1	20.2	5.0	74	10.5	0
Hamlin Branch	36.9347	-82.2814	1.11	507	9.5	500	8.3	4.5	14.3	3.2	93	20	20
Lower Middle Creek	37.0978	-81.7610	0.61	655	27.7	485	8.68	4.1	30.1	7.3	50	12	44
Coal Creek	37.1113	-81.8722	2.91	659	12.0	929	8.6	4.0	14.6	3.6	60	18	0
Rock Lick Branch	37.0678	-81.9456	5.33	627	1.6	159	8.29	1.0	9.0	8.8	65	8	7
Thompson Creek	36.9915	-82.0576	0.44	483	36.0	509	8.55	4.0	31.8	8.0	76	21	0
Lowest Big Creek	37.1006	-81.7968	1.2	589	36.8	411	8.84	6.4	34.4	5.4	47	1	39
Grassy Branch	36.9991	-82.1501	12	584	2.9	323	8.16	2.8	8.9	3.2	94	38	9

Appendix D: Watershed land cover for all 70 sites.

Site	Decid. Forest	Everg. Forest	Mixed Forest	Forest	Hist. Mining
UN Trib to N Fk Clinch River	43%	1%	1%	45%	0%
UN Trib to N Fk Clinch River	47%	6%	1%	54%	0%
UN Trib to N Fk Clinch River	49%	2%	1%	52%	0%
Greasy Creek	84%	5%	0%	89%	1%
Cavitts Creek	60%	1%	1%	62%	0%
Indian Creek	70%	9%	0%	80%	1%
Lick Creek	77%	2%	0%	79%	13%
Unnamed Trib to Indian Creek	61%	9%	0%	70%	0%
Jackson Fork	75%	17%	1%	93%	4%
Lowe Branch	74%	6%	0%	80%	0%
Smith Branch	86%	0%	0%	86%	4%
Right Fork Lick Creek	69%	2%	0%	70%	14%
Big Creek	78%	5%	1%	84%	4%
Upper Big Creek	78%	6%	1%	84%	4%
Lewis Creek 1	74%	1%	0%	75%	6%
Lower Town Hill Creek	81%	4%	1%	87%	6%
Honey Branch	87%	1%	2%	91%	7%
Upper Hess Creek	61%	0%	0%	61%	11%
Left Fork Lick Creek	75%	5%	0%	79%	17%
Mudlick Creek	78%	12%	1%	90%	6%
Lower Pine Creek	75%	3%	0%	78%	4%
Little Mill Creek	56%	7%	0%	63%	1%
Sample Branch	53%	0%	0%	53%	0%
Swords Creek	74%	3%	0%	77%	4%
Big Lick Creek	71%	4%	0%	75%	4%
Upper Big Lick Creek	78%	1%	0%	80%	2%
Upper Pine Creek	82%	4%	0%	85%	3%
Long Branch	67%	4%	1%	71%	18%
Lewis Creek 2	80%	1%	0%	82%	6%
Thompson Creek	37%	1%	0%	38%	0%
Stone Branch	84%	1%	6%	90%	4%
Lewis Creek 3	70%	1%	0%	71%	10%
Lewis Creek 5	67%	0%	0%	68%	8%
Lewis Creek 4	68%	1%	0%	69%	10%
Millstone Branch	81%	1%	6%	89%	0%
Thompson Creek	30%	1%	0%	31%	0%
Breezer's Branch	55%	2%	0%	57%	0%
Alvy Creek	85%	0%	6%	92%	7%
Strow Creek	62%	4%	0%	66%	26%

Middle Fork	48%	0%	0%	48%	56%
Town Hill Creek	81%	4%	1%	87%	6%
Hurricane Fork	77%	4%	2%	83%	14%
Whitley Branch	57%	0%	0%	57%	0%
Eagle's Nest Branch	57%	2%	0%	58%	0%
UN Trib to Chaney Creek	83%	0%	0%	83%	9%
Stonecoal Creek	62%	0%	0%	62%	38%
UN Trib to Gravel Lick Creek	84%	0%	2%	86%	0%
Gravel Lick Creek	66%	1%	1%	68%	0%
Zeke Creek	79%	17%	1%	96%	5%
Weaver Creek	88%	4%	0%	92%	0%
Claypool Branch	49%	1%	0%	50%	0%
Mill Creek	76%	3%	0%	79%	2%
Lincolnshire Branch	50%	2%	1%	53%	0%
North Fork Clinch River	68%	1%	1%	70%	0%
Sulphur Spring Branch	81%	1%	0%	82%	3%
Laurel Fork	86%	6%	0%	92%	0%
Sykes Branch	48%	1%	0%	49%	0%
Grassy Creek	93%	1%	0%	95%	3%
Greasy Creek	86%	2%	0%	88%	1%
Laurel Branch	78%	11%	0%	88%	20%
Hurricane Fork	77%	6%	2%	85%	14%
Upper Middle Creek	80%	10%	0%	90%	1%
Lower Big Creek	78%	6%	1%	85%	4%
Hamlin Branch	88%	0%	1%	89%	17%
Lower Middle Creek	80%	9%	0%	89%	1%
Coal Creek	63%	2%	0%	65%	3%
Rock Lick Branch	92%	0%	1%	93%	6%
Thompson Creek	47%	1%	0%	48%	0%
Lowest Big Creek	78%	6%	1%	85%	4%
Grassy Branch	73%	7%	1%	82%	10%