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Life History, Diet and Habitat of the Federally Endangered Laurel Dace (Chrosomus saylori)

Shawna M. Fix
University of Tennessee, Knoxville, smitch55@vols.utk.edu

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Augustin C. Engman, Major Professor

We have read this thesis and recommend its acceptance:

Bernard R. Kuhajda, John B. Alford, Benjamin P. Keck

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

Life History, Diet and Habitat of the Federally Endangered Laurel Dace (*Chrosomus saylori*)

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DEDICATION

I dedicate this thesis to my parents, Misti and Todd Mitchell. Through good times and bad times and from 1000 miles away they never stopped supporting my dreams.

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ABSTRACT

Laurel Dace (*Chrosomus saylori*) is a small, freshwater minnow that is endemic to headwater streams on Walden Ridge, Tennessee. The species was listed as federally endangered in 2011 and the U.S. Fish and Wildlife Service Recovery Plan indicates a need for life history, diet and habitat studies. A literature review, samples of archived specimens, and a surrogate species, the Tennessee Dace (*Chrosomus tennesseensis*), were used to describe the life history and diet of the Laurel Dace. A total of 370 Tennessee Dace were collected from Laurel Ford Branch, a stream 8.6 km from the Laurel Dace type-locality, over an 18-month period. The standard length of 370 Tennessee Dace was measured and the age of 72 individuals was determined with otolith microstructure analysis. We examined patterns of growth with a von Bertalanffy model and length relationships. Mortality estimates were derived from model parameters. Tennessee Dace exhibit positive allometric growth, 4+ age groups and estimated annual natural mortality of 73%. Tennessee Dace reproductive biology was determined by identifying and weighing gonads and counting mature ova in all sexually-mature females. The female-to-male sex-ratio was 1.3:1, reproductive maturity began at age-2, and mature ova counts ranged from 64 to 143. Variation in ova maturity between individuals during the March-to-July spawning season indicated that Tennessee Dace are multiple-clutch spawners. Morphometric analysis revealed pectoral fin length-to-standard length ratio as a sexually-dimorphic characteristic. Tennessee Dace and Laurel Dace diets were quantified through multiple season; both species were opportunistic feeders that consumed aquatic and terrestrial invertebrates, plant matter, and algae. The habitat of the northern population of Laurel Dace was characterized in pools in four segments of three streams. Pool volume, water depth, silt depth, canopy cover, and substrate were measured in the field and the sub-watershed land cover of each stream was quantified with ArcGIS. Laurel Dace inhabit streams that are characterized by large pools with predominantly bedrock substrate, dense riparian vegetation and heavily-forested watersheds. The results of this study indicate that to conserve Laurel Dace populations managers may encourage responsible land use on Walden Ridge and change federal permitting to protect individuals during the spawning season.

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CHAPTER 1

A REVIEW OF LIFE HISTORIES OF DACE SPECIES WITHIN THE GENUS *CHROSOMUS* (CYPRINIFORMES: CYPRINIDAE)

Abstract

The North American dace in the genus *Chrosomus* (Family: Leuciscidae, formerly Cyprinidae) is comprised of seven described species in two subgenera and one undescribed species. A literature review was conducted for all species of *Chrosomus* in the subgenus *Chrosomus* to provide an overview of life histories. Their reproductive biology and life history strategies are one of the most studied of all the cyprinids. Most dace in this complex inhabit small to medium headwater streams and spawn over gravel nests of nest-building minnows. Average minimum ova count per clutch for the genus is 306 (min=8, max=724, SD=236) and average maximum ova count is 960 (min=432, max=2872, SD=944). The shortest estimated lifespan is that of the Clinch Dace (2+ years) and the longest are those of Laurel Dace and Tennessee Dace (4+ years). All are known or presumed multiple clutch spawners and spawn between March and August. While most of the species in this genus are well studied, the Laurel and Northern Redbelly Dace are the most understudied and are in need of additional research.

Introduction

The North American dace in the genus *Chrosomus* (Family: Leuciscidae, formerly Cyprinidae, Schönhuth et al. 2018) are medium-sized minnows (70-120 mm total length (TL)) that have very small scales, relative to more derived genera (Etnier and Starnes 1993; Stasiak 2006). The lateral line scales usually number greater than 80, and the pores of the lateral line canal only extend about halfway from the head to the tail (Stasiak 2006). In general, *Chrosomus* species thrive in clear water with gravel, cobble, and boulder substrate. All species are lotic except for the Northern Redbelly Dace which can be found in both lotic and lentic environments (Massicotte et al. 2008). Six out of the seven species reviewed here are found within the Southeast United States, a fish biodiversity hotspot, which is vastly understudied and where a large proportion of native fishes are threatened with extinction (Warren et al. 2000).

Previously, *Phoxinus* was considered the only cyprinid genus with a Holarctic distribution and consisted of three Eurasian and seven North American species. In the revision of the genus

Chrosomus, Strange and Mayden (2009) removed seven species from the genus *Phoxinus* based on phylogenetic studies using the cytochrome *b* gene which demonstrated that these species were not monophyletic with *Phoxinus*. Instead, the subgenera *Chrosomus* and *Pfrille* were considered to be a monophyletic group (Strange and Mayden 2009). Consequently, the subgenus *Chrosomus* now includes the Tennessee Dace (*Chrosomus tennesseensis*), Mountain Redbelly Dace (*Chrosomsus oreas*), Laurel Dace (*Chrosomus saylori*), Blackside Dace (*Chrosomus cumberlandesis*), Northern Redbelly Dace (*Chrosomus eos*), Southern Redbelly Dace (*Chrosomus erythrogaster*) and the undescribed Clinch Dace (*Chrosomus* sp. cf. *saylori*).

All species in the subgenus *Chrosomus* have similar pharyngeal tooth formulations (0,5-5,0 or 0,5-4,0). The Finescale Dace, (*Chrosomus neogaeus*) is classified under the subgenus *Pfrille* and was excluded from this evaluation because it has different pharyngeal tooth formulations (2,5-4,2) and shares few morphological characteristics with the other North American *Chrosomus* species (Strange and Mayden 2009).

Chrosomus species share nuptial pigmentation patterns that include bright red ventral coloration, yellow fins, and a pearly-silver spot at the fin origins (Etnier and Starnes 1993; Strange and Mayden 2009). Most of the species have two uninterrupted black stripes. Mountain Redbelly Dace and Tennessee Dace share an interrupted and ventrally decurved lower lateral stripe.

Phylogeographic patterns vary among *Chrosomus* species. The species are mostly allopatric except where Blackside Dace and Southern Redbelly Dace co-occur in the upper Cumberland River drainage. Mountain Redbelly Dace and Tennessee Dace have adjacent distributions in the Central Appalachians and Mountain Redbelly Dace also occur in tributaries to the New River and several streams on the Atlantic Slope of the Appalachians. There are also introduced populations of Mountain Redbelly Dace in tributaries to the upper Tennessee River (Etnier and Starnes 1993; Strange and Mayden 2009). Blackside Dace and Laurel Dace have very restricted distributions in the lower Ohio River System. Blackside Dace are found in the upper Cumberland River drainage on the Cumberland Plateau in southeast Kentucky and northeast Tennessee and have been introduced into a few headwater streams in the upper Tennessee River drainage in Virginia (Skelton 2013). Laurel Dace occur only in a few headwater streams on the Walden Ridge portion of the Cumberland Plateau in the Tennessee River drainage in east-central Tennessee (Strange and Mayden 2009; Skelton 2013; Floyd 2016).

The Clinch Dace, *Chrosomus* sp. cf. *saylori*, is currently the only undescribed species in the genus *Chrosomus*. It was identified in Virginia in 1999 and is now known from only eight tributaries in the Upper Clinch River. When discovered, it was thought to be a disjointed population of Laurel Dace, but White and Orth (2013) confirmed that they were morphologically distinct from the Laurel Dace. However, a genetic study and a more rigorous morphological comparison is needed to determine the phylogenetic distance from Laurel Dace (White and Orth 2013). It is recognized as a species in need of conservation on both the state (Virginia and Tennessee) and federal level (White and Orth 2014a). Mountain Redbelly Dace and Clinch Dace are the only two species in the genus *Chrosomus* that are not listed as threatened or endangered under any state or federal rule (Table 1.1). However, the federal status of the Clinch Dace is currently under review. Threats to the *Chrosomus* species include excessive sedimentation from poor agriculture and development practices, land use changes, climate change and collection for bait or the aquarium trade (Trautman 1981; U.S. Fish and Wildlife Service 2016). Conservation efforts for these minnow species will require an understanding of their ecology, behavior, life history, population dynamics, population genetics and habitat use (Johnston 1999).

Life History Descriptions of *Chrosomus* Species

Tennessee Dace – Chrosomus tennesseensis

Tennessee Dace occur sporadically in the Ridge and Valley physiographic province, and on the margins of the Blue Ridge and Cumberland Plateau provinces in the upper Tennessee River drainage (Etnier and Starnes 1993). They often inhabit spring fed, first order perennial streams with silt and fine gravel pools that contain undercut banks and coarse woody debris (Hamed and Alsop III 2005). Their diet is likely similar to the other *Chrosomus* species, but has not been documented. Populations typically include four size classes, and maximum standard length (SL) is reported to be 65 mm (Table 1.2) (Hamed and Alsop III 2005). Hamed et al., (2008) found that age-2 fish made up the majority of spawning individuals.

Male and female Tennessee Dace will reach breeding colors by early April and will spawn over gravel mounds constructed by nest-building minnows (Leuciscidae) such as Creek Chubs (*Semotilus atromaculatus*) until July. Breeding males have sulfur (yellow) pectoral, pelvic and anal fins and geranium (red) flanks and opercula. Males achieved spawning coloration more rapidly than females and spawning occurred when water temperature reached 21°C. Blacknose Dace (*Rhinichthys atratulus*) often co-occurred within the spawning mesohabitat, while Creek Chubs were found in the same pool microhabitat. Hamed et al., (2008) described their spawning behavior in Timbertree Branch and Trinkle Creek, in the South Holston River Drainage in northeastern Tennessee. They observed that spawning occurred when two males stopped and held their positions over a nest while females swam through the nest repeatedly for about 30 seconds. After 30 minutes of the above behavior, a female stopped over the nest and two male Tennessee Dace swam around her in a barrel roll pattern. At that time, up to 28 males joined the group and formed a large spawning aggregation. After another 11 seconds, the group dispersed while two Tennessee Dace remained over the nest to chase away Blacknose Dace for a period of about two hours. Tennessee Dace were also observed spawning over active and inactive nests of Stonerollers (*Campostoma* spp.) and pushing females into the substrate of the Stoneroller nests. As Tennessee Dace entered the spawning group, many of them jumped out of the water to catch insect prey. Females produce multiple clutches and total mature ova counts range between 398 and 721 per clutch with mature egg diameters of 0.9-1.5 mm (Table 1.2). Male Tennessee Dace over 50-mm SL appear to have longer and rounder pectoral fins than females of the same size, which may be the only sexually dimorphic characteristic of the species (Hamed et al. 2008).

Mountain Redbelly Dace - Chrosomus oreas

The Mountain Redbelly Dace inhabits upland streams of the Atlantic Slope from the Potomac River to the Pee Dee River drainages of Virginia and North Carolina, the New River drainage of North Carolina, Virginia, and West Virginia and is found sporadically in a few tributaries to the Tennessee and Big Sandy River drainages in Virginia. This species is most often found in deeper, slower pools and has been found to be both a herbivore and detritivore (Thompson et al. 2017). The Mountain Redbelly Dace lives to 3 years of age, and the largest specimen recorded was a 65-mm SL female (Table 1.2) (Thompson et al. 2017).

Elevated gonadosomatic index (GSI) occurred from April to June then declined in July, which suggested spawning occurred in spring and early summer when the water temperatures were between 14-18°C. Spawning activity declined when temperatures reached 27°C in July (Thompson et al. 2017). Thompson et al., (2017) did not observe spawning behavior for the Mountain Redbelly Dace, however they are known minnow nest associate spawners (Jenkins and Burkhead 1994). Mean GSI values for females was highest in June (14.70, SD=11.81) and lowest in March (0.99, SD=1.00). Mean GSI values for males was highest in July (2.09, SD=0.78) and lowest in October (0.26, SD=0.53). Elevated GSI persisted in the spring months for both males and females. All males ≥ age-2 were sexually mature, and 25% of age-1 males were sexually mature. Females did not have mature ova until age-2, and only 54.2% of females ≥ age-2 had mature ova during the spring spawning season. Mature ova counts were 8-610 per clutch, and maximum diameters were 1.61 mm (Table 1.2) (Thompson et al. 2017).

Blackside Dace - Chrosomus cumberlandensis

Blackside Dace is a federally threatened species that historically inhabited only small streams in the upper Cumberland River drainage on the Cumberland Plateau in southeastern Kentucky and northeastern Tennessee. Recent surveys have discovered populations in the Clinch and Powell River drainages in Virginia, which may have been bait bucket introductions (Skelton 2013; Floyd 2016). Blackside Dace inhabit pools in small, cool, upland streams with instream cover such as root wads, undercut banks and large rocks (Floyd 2016). Blackside Dace graze on algae, and in the winter, when algae is scarce, they will consume aquatic insects. This species ingests sand grains to aid in the digestion of diatoms and algal cells. The lifespan of Blackside Dace is 3 years and maximum length is 76 mm TL (Table 1.2) (Etnier and Starnes 1993).

During the breeding season males display an intense black stripe, bright scarlet coloration on the belly, head, and mouth, and bright yellow fins (Starnes and Starnes 1981; Floyd 2016). Blackside Dace spawn between April and July over silt-free gravel mounds built by nest-building minnow species such as Central Stoneroller (*Campostoma anomalum*), River Chub (*Nocomis micropogon*) and Creek Chub (Etnier and Starnes 1993; Floyd 2016). Starnes and Starnes (1981) observed spawning over a Stoneroller nest in a small upland stream in Kentucky. Large groups of 10-12 nuptial males hovered over a nest and waited for a female to enter the nest area. Three males then pinned a female down and the rest of the males swarmed around the group, all violently shaking for less than 3 seconds then dispersed. Once the male Stoneroller returned to its nest, the Blackside Dace scattered and did not attempt to spawn again. Additionally, Mattingly and Black (2014) observed Blackside Dace spawning behavior over Creek Chub nests in May and June in southeastern Kentucky. Rakes et al., (1999) spawned Blackside Dace in captivity. Captive spawning observations suggested that a minimum of eight reproductively active male Blackside Dace are necessary to induce spawning in females. In captive conditions many infertile ova were found in the nest. These “dummy” ova likely decreased the probability of predation on the few fertile ova. When natural spawning ceased, the dace resumed spawning after milt from nest-building minnow species were introduced into the spawning tanks. Female Blackside Dace produced 724-2872 mature ova that are between 0.82-1.36 mm in size (Table 1.2). Their ova are negatively buoyant and non-adhesive; therefore, they will sink into gravel crevices. Although the crevices may protect developing ova from predators, they may also be highly susceptible to being smothered by fine sediments like silt and clay (Rakes et al. 1999).

Blackside Dace populations have been greatly reduced by surface mining runoff which warranted its federally threatened status in 1987. This species is known to leave reaches were water quality and habitat is degraded, but will return when conditions improve (Rakes et al. 1999).

Northern Redbelly Dace - Chrosomus eos

The Northern Redbelly Dace is found from Canada and the northern portions of the Great Lakes and St. Lawrence River to the Mississippi River and Missouri River drainages. They are most often found in slow, sluggish, spring fed streams with abundant aquatic vegetation and woody debris. They also will inhabit beaver ponds that have cool groundwater influence as well as bog lakes and small lakes (Becker 1983; Stasiak 2006). Northern Redbelly Dace are omnivores that will feed on algae, zooplankton and small aquatic invertebrates (Becker 1983; Stasiak 2006). Studies have also shown that they feed on small fish fry (Stasiak 2006). Depending on location, Northern Redbelly Dace can live from 3-4 years and reach a maximum length of 80 mm TL (Table 1.2) (Stasiak 2006).

Northern Redbelly Dace spawning occurs between May and August (Becker 1983). The exact spawning period depended on a combination of photoperiod and temperature because some populations in Northern Canada have been found to spawn in late August. Spawning occurred when several male Northern Redbelly Dace chased a single female into filamentous algae along the littoral zone and the female deposited between 5 and 30 non-adhesive eggs (Becker 1983; Stasiak 2006). Sexual dimorphism in this species is very apparent. Males have red bellies, bright yellow fins or both and are described as having larger, scoop-shaped pectoral fins compared to females, and the males developed tubercles during spawning season. Females are larger than males and do not display the characteristic red bellies or yellow fins. (Becker 1983; Stasiak 2006). Ova hatched in 8 to 10 days in water temperature from 21°C to 26°C. Ripe females had between 410 and 435 mature ova per clutch that averaged 0.9-1.0 mm in diameter (Table 1.2) (Becker 1983; Stasiak 2006).

In many areas throughout their range, Northern Redbelly Dace are syntopic with the Finescale Dace, and these two species will hybridize. Finescale Dace can be differentiated by their much larger size and one large band on their sides (Stasiak 2006).

Southern Redbelly Dace – Chrosomus erythrogaster

The Southern Redbelly Dace was first described from specimens collected from the Kentucky River drainage as early as 1820 (Settles and Hoyt 1978). The range of the Southern Redbelly Dace consists of upland portions of the Mississippi River drainage and tributaries to Lake Michigan and Lake Erie with isolated populations occurring in west Tennessee, western Mississippi, east-central Arkansas, northeastern Kansas and northeastern New Mexico (Etnier and Starnes 1993). They inhabit pools in small streams with clear, cool water, gravel substrate, and undercut banks (Trautman 1981; Etnier and Starnes 1993). Southern Redbelly Dace feed on algae and aquatic invertebrates and have a 1:1 sex ratio (Settles and Hoyt 1978). Their lifespan is 3 years, and maximum length is 90 mm TL (Table 1.2) (Etnier and Starnes 1993).

Coloration differences between males and females were most obvious during breeding season. Males had characteristic scarlet abdomens and bright yellow fins. Breeding tubercles develop on their heads, backs, and fins. Mature females had flecks of red on their abdomens, but not to the extent of the males. The pectoral fins of males were longer and distally rounder than those of females, which were shorter and pointier (Settles and Hoyt 1978). Possible spawning behavior has been observed, during which Southern Redbelly Dace were very active over a well-defined, minnow gravel nest. Smith (1908) describes schools of Southern Redbelly Dace so thick that the surface of the water was violently agitated by the spawning aggregation. The aggregation, made up mostly of males, performed multiple upstream passes through a riffle over a nest of fine gravel. Occasionally, two males and one female would separate from the aggregation. The males pressed themselves on either side of the female, all facing upstream, and the males rapidly vibrated against the females. The behavior happened within seconds because it was difficult for the dace to maintain their position in the riffles. Immediately after spawning, other individuals in the aggregation were observed poking their snouts into the gravel presumably eating the recently extruded ova. Smith (1908) also observed, in very shallow water, that males and females were able to break from the group and spend time motionless, seemingly lying still in the pebbles. These spawning events were presumably more successful because ova were deposited directly into the substrate and more time was available for the milt to fertilize the ova before being washed away by the current. Settles and Hoyt (1978) found that age-1 (37-60 mm SL) and age-2 (56-65 mm SL) females reached maximum ovarian development during April (mean gonadosomatic ratio of 12.7%, n=4), age-0 females reached their peak in June (mean gonadosomatic ratio 5.96%, n=17), and development in all females progressively declined through July. Male Southern Redbelly Dace reached sexual maturity during April of their first year while females were not sexually mature until May or June. Young-of-the-year (14-15 mm) were first collected in July. Gonadal development in the species reached maximum condition just prior to spawning. Mature ova diameter averaged between 0.67 mm and 1.05 mm and mature ova counts were between 140 and 681 per clutch (Table 1.2). Mature ova size in all females decreased as a function of standard length (Settles and Hoyt 1978).

Clinch Dace- Chrosomus sp*.* cf*. saylori*

Clinch Dace currently persists in eight small tributaries in the upper Clinch River watershed in Russell and Tazwell Counties in southwestern Virginia (White and Orth 2013; Moore et al. 2017). These streams are typically narrow and forested with low pool volumes (White and Orth 2014a). Clinch Dace consume a diet of mostly macroinvertebrates and have a shorter gut than other *Chrosomus* species (White and Orth 2013). Clinch Dace likely live 2 years and reach a maximum size of 65 mm SL (Table 1.2). It is estimated that the average effective population size of Clinch Dace populations is less than 50 individuals. Clinch Dace have a 3:1 female-biased sex ratio (White and Orth 2014b).

White and Orth (2014b) reported that Clinch Dace spawn between May and July based on breeding color and GSI values. Breeding coloration included neon-yellow fins, bright red abdomens and two uninterrupted black lateral stripes running the entire length of the body. Clinch Dace were observed spawning over a gravel pit built by Central Stonerollers when the average water temperature was 15.4°C. Five to seven Clinch Dace were observed briefly pausing over the pit, violently vibrating then swimming off. The event lasted less than 30 seconds and occurred every 5-10 minutes for an hour. Central Stoneroller, Creek Chub and

Western Blacknose Dace (*Rhinichthys obtusus*) were observed burrowing in the gravel pits, possibly feeding on the expelled ova. Post-spawning, Clinch Dace were seen increasingly farther away from the pool in which they had spawned, and breeding colors were noticeably muted by the end of May. White and Orth (2014b) found that breeding coloration was a poor predictor of sex because the most vibrantly colored fish were often females and that age-2 individuals were the only ones that displayed breeding colors. They also found that age-2 males had tubercles across the dorsal and lateral axes of the body. Pectoral fin length was reportedly a sexually dimorphic character, with males having significantly longer pectoral fins than females (White and Orth 2014b). Only age-2 Clinch Dace had mature ova that averaged 1.0 mm diameter and mature ova per female averaged 267 per clutch (SE= 4.20, min= 153, max = 442, n= 12). Age-2 female GSI peaked in early July at 7.25 and was significantly higher than age-1 (1.99) and young-of-the- year (0.25) female GSI. Age-2 female GSI rapidly declined to around 4.00 after its peak. Age-2 males reached maximum GSI (2.00) in April. GSI then rapidly declined to 1.00 by the middle of May and remained low through July. Maximum GSI for age-1 males in July was 1.10, but on average it was below 0.75. The reported GSI for YOY males was 0.32 (White and Orth 2014b).

Laurel Dace – Chrosomus saylori

The Laurel Dace is endemic to three watersheds on the Walden Ridge portion of the Cumberland Plateau in east-central Tennessee: the Soddy Creek, Sale Creek and Piney River systems of the upper Tennessee River drainage (Strange and Skelton 2005). One adult and three juvenile Laurel Dace were found misidentified in a jar of preserved specimens from Grassy Cove, an endorheic basin in Cumberland County, TN. These specimens were collected in 1954 and surveys in 2013 did not find any live individuals (U.S. Fish and Wildlife Service 2016). Laurel Dace are likely extirpated from Soddy Creek as well as two streams in the Sale Creek system: Laurel Branch and Cupp Creek (U.S. Fish and Wildlife Service 2016). Laurel Dace are found in first and second order headwater streams characterized by thick riparian vegetation of mostly Mountain Laurel (*Kalmia* spp.) with cobble, boulder, and bedrock bottoms with water that is typically cool and clear, but has often been inundated with silt from local vegetable crop farms, logging roads and other private land practices (Skelton 2001; U.S. Fish and Wildlife Service 2016). Gut content analysis of preserved specimens indicate that Laurel Dace are omnivores with a wide diet, feeding on an array of terrestrial and aquatic insects and plant/algae matter (Chapter 2). Mitochondrial cytochrome *b* data suggest that there are two Evolutionary Significant Units (ESU) of Laurel Dace, the Piney River (northern population) and Sale and Soddy Creeks (southern population) (Strange and Skelton 2005). Four year groups have been observed including young-of-the-year.

Skelton (2001) observed nuptial pigmentation in Laurel Dace from March to mid-June (Table 1.2). While the act of spawning was not directly observed, Cronnon et al., (2019) recorded behaviors associated with spawning in a captive setting. Fish were observed during the breeding season and the most prominent behaviors included chasing, attacking and sigmoid display (Cronnon et al. 2019). In the wild, Laurel Dace were observed displaying spawning behavior while swimming over a Stoneroller nest in Soddy Creek, however, the actual act of spawning was not witnessed. Laurel Dace were also observed forcing their snouts into the gravel, presumably to eat the ova. Soddy Creek is the only creek where Laurel Dace were sympatric with a nest-building species (i.e., Largescale Stoneroller, *Campostoma oligolepis*) (Skelton 2001). Spawning behavior was also observed in Horn Branch and Bumbee Creek where fish were observed swimming repeatedly between a pool and shallow riffle, but again the act of spawning was not observed (Skelton 2001). A detailed life history study still needs to be conducted for this species. The Laurel Dace appears to have similar life history strategies to other species in its genus.

Patterns in Life History Strategy Among *Chrosomus* Species

North America is home to approximately 43% of the world's freshwater fish diversity (Jelks et al. 2008). Freshwater fish are incredibly diverse and exhibit some of the most diverse reproductive strategies in the world (Mims et al. 2010). For obligate freshwater biota, especially stream-dwelling fishes, rivers, and lakes are essentially "islands" embedded in the terrestrial landscape that limit dispersal. These isolated habitats have varied environmental drivers that exert differential selection pressures, resulting in taxonomic diversity and an array of life history attributes between species (Mims et al. 2010). Life history trait variation can vary widely across species in the same family. Species in the family Leuciscidae have some of the highest variation of functional life history traits with species exhibiting opportunistic, periodic and equilibrium traits (Winemiller and Rose 1992; Mims et al. 2010). In the southeast region of North America, fish in the family Leuciscidae are mostly dominated by opportunistic life history strategies (Mims et al. 2010). Six of the seven species of *Chrosomus* reviewed here are found in the southeast United States (Figure 1.1). Pleistocene glaciations did not extend to the southeast US, but during those times, this region was subjected to habitat desiccation and variable hydrologic regimes which isolated fish populations and constantly changed community assemblages. Species with an opportunistic life history strategy tend to thrive in these randomly changing environments (Mims et al. 2010).

Chrosomus species can be found in the pools of first to second order streams made up of undercut banks, woody debris and gravel, cobble, boulder, bedrock substrate. They are reported to feed on a wide variety of food items including aquatic macroinvertebrates and algae, therefore are considered to have opportunistic life styles.

Many of the *Chrosomus* species share similar reproductive strategies. All *Chrosomus* spawn in the spring-summer and all are likely nest associate spawners except for Northern Redbelly Dace. Size classes range between two and four. *Chrosomus* mature ova counts range anywhere from 8 to 2872 with ova diameters between 0.67 to 1.61mm and it appears that all are multiple clutch spawners, but this life history trait has not been confirmed in Mountain Redbelly Dace or Laurel Dace (Table 1.2). These traits allow these species multiple opportunities to reproduce during one spawning season. The reproductive strategies of these species align with Winemiller and Rose's "opportunistic strategy" i.e. frequent reproduction over an extended spawning season, small eggs and small clutches (Winemiller and Rose 1992). All of these species attain bright red and vibrant yellow coloration during the spawning season with males in most species becoming the most pronounced to attract females and increase spawning success.

In terms of habitat preference, range, size, and spawning behaviors, the least similar of all the *Chrosomus* species is the Northern Redbelly Dace. They live everywhere from sluggish spring fed springs and beaver ponds to the littoral zone of large lakes (Stasiak 2006) and will spawn in filamentous algae. They also have the largest range and are known to hybridize with Finescale Dace. Northern Redbelly Dace obtains the largest maximum size compared to all the others (Table 1.2). Many of these features could be a product of its more northern range because of differences in climate between habitats occupied by Finescale Dace and the rest of the *Chrosomus* species.

North American dace species are threatened by anthropogenic activities including habitat fragmentation and degradation and the introduction of species outside of their native ranges. These threats strain many of the narrow endemic dace species found in North America (Jelks et al. 2008). Five of the seven *Chrosomus* species discussed are either state or federally listed and one is currently under federal review for listing (Table 1.1). Wide ranging species such as the Northern Redbelly and Southern Redbelly Dace are only classified as threatened or endangered in the outermost extent of their range (Figure 1.1, Table 1.1). Mountain Redbelly Dace have the subsequent largest range, and they are the only *Chrosomus* species not listed at the state or the federal level (Figure 1.1, Table 1.1). Narrow endemic species are more likely to be affected by anthropogenic threats such as habitat destruction and introductions of non-native species (Jelks et al. 2008; Burkhead 2012). Species' habitats are often fragmented through the building of roads, dams, and poor commercial and agriculture practices (Jelks et al. 2008). These practices can further isolate and genetically bottleneck a narrow endemic species, putting them at greater risk for extinction than if their populations were able to disperse freely. Species in the *Chrosomus* genus that rely on other nest-building species for reproduction risk losing those nest associates to anthropogenic impacts which could drastically limit spawning and strain recruitment.

Up to 86 additional freshwater fish species could become extinct in North America by 2050 and frequently it is the lack of life history knowledge that impedes the conservation or recovery of a species (Warren et al. 2000; Burkhead 2012). Of all *Chrosomus* species, the least amount of life history research has been conducted on Laurel Dace. Laurel Dace have undergone an estimated >50% decline in range in the last 15 years (B. Kuhajda, Tennessee Aquarium Conservation Institute, pers. comm.) and is in serious threat of becoming extinct from poor agriculture practices and invasive species such as Green Sunfish (*Lepomis cyanellus*) and Bluegill (*L. macrochirus*). Part of the strategy in the Recovery Plan for the Laurel Dace states that a life history study needs to be conducted in order to meet the plan’s goals for recovery (U.S. Fish and Wildlife Service 2016).

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Appendix A

Table 1.1. Threatened and endangered status of minnows (Family Leuciscidae) in the subgenus *Chrosomus*; T= Threatened, E= Endangered and UR= Under Review (federal only).

	Tennessee Dace	Mountain Redbelly Dace	Blackside Dace	Laurel Dace	Clinch Dace	Northern Redbelly Dace	Southern Redbelly Dace
Federal			T	E	UR		
Colorado						E	E
Georgia	E						
Kentucky			T				
Massachusetts						E	
Michigan							E
Mississippi							E
Nebraska						T	
New Mexico							E
Pennsylvania						E	T
South Dakota						T	
Tennessee			T	E			
Virginia	E		T				

Table 1.2. Comparison of life history traits of six described and one undescribed *Chrosomus* species (Family Leuciscidae).

Species	Ova Counts	Mature Ova Diameter (mm)	Number of Size Groups	Multiple Clutch?	Spawning Behavior	Spawning Season	Max Size (mm) (SL or TL)	Reference
Tennessee Dace	398-721	0.9-1.5	4	Yes	Nest associate	April-July	65 (SL)	Hamed et al. 2008
Mountain Redbelly Dace	8-610	up to 1.61	3	Not Specified	Nest associate	April-July	65 (SL)	Jenkins and Burkhead 1994; Thompson et al. 2017
Blackside Dace	724-2872	0.82-1.36	3	Yes	Nest associate	April-July	76 (TL)	Starnes and Starnes 1981
Northern Redbelly Dace	410-435	0.9-1.0	3-4	Yes	Filament-ous algae, littoral zones	May-August	80 (TL)	Stasiak 2006
Southern Redbelly Dace	140-681	0.67-1.05	3	Yes	Nest associate	April-June	90 (TL)	Settles and Hoyt 1978
Clinch Dace	153-442	1.00	2	Yes	Nest associate	May-July	65 (SL)	White and Orth 2013, 2014b
Laurel Dace	Un-known	Unknown	4	Unknown	Unknown	March-mid June?	Un-known	Skelton 2001; Cronnon et al. 2019

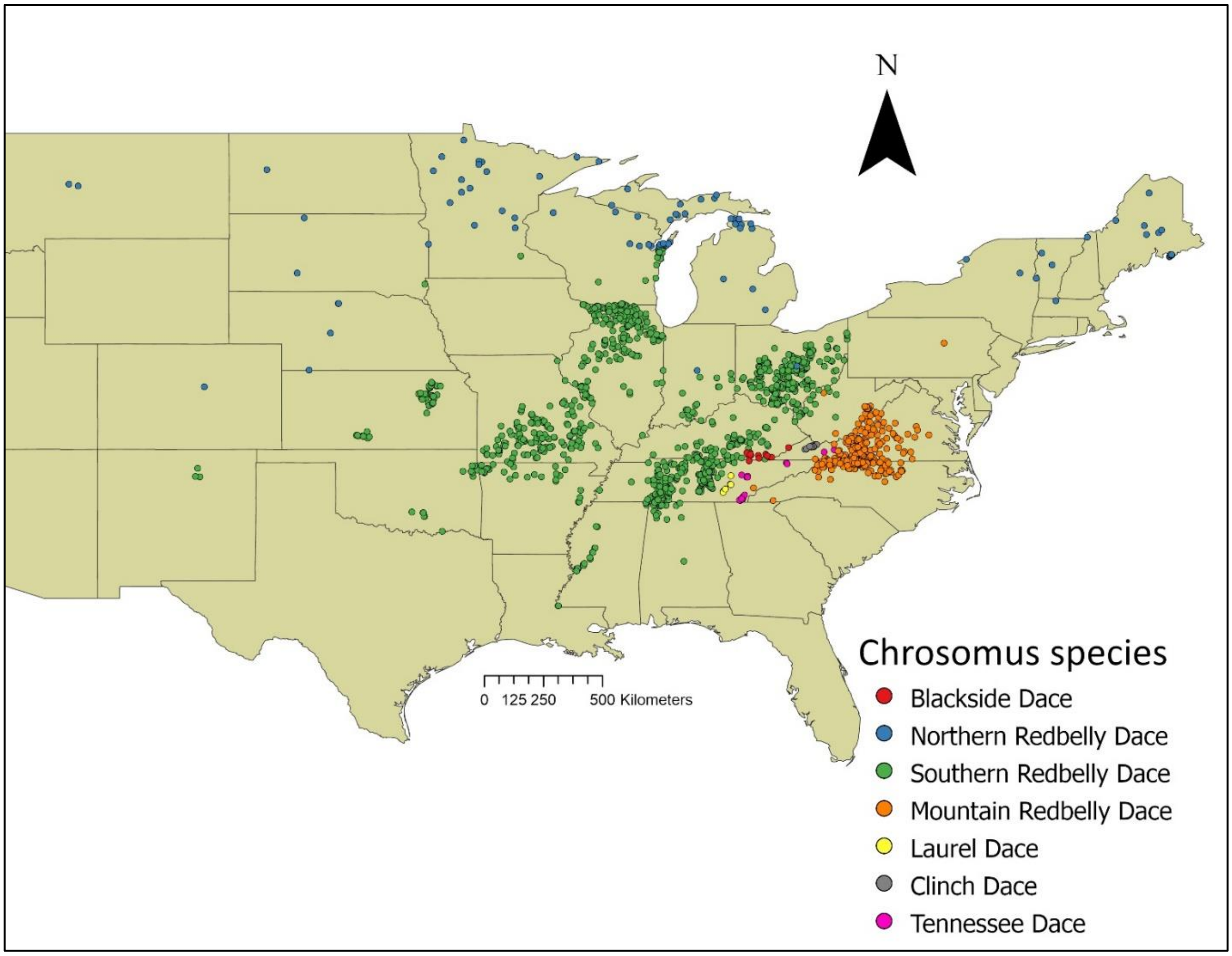

Figure 1.1. United State distribution map of seven minnow species (Family Leuciscidae) in the genus *Chrosomus*.

CHAPTER 2

LIFE HISTORY AND DIET OF THE LAUREL DACE (*CHROSOMUS SAYLORI*) UTILIZING A SURROGATE SPECIES, THE TENNESSEE DACE (*C. TENNESSEENSIS*)

Abstract

The Laurel Dace (*Chrosomus saylori*) is a federally endangered minnow found only on Walden Ridge within the Cumberland Plateau in the upper Tennessee River drainage in east-central Tennessee. The species' recovery plan calls for a life history study to be conducted to better manage the species. Due to the federal endangered status of the Laurel Dace, the Tennessee Dace (*C. tennesseensis*) was used as a surrogate species to describe the life history characteristics of the Laurel Dace. The Tennessee Dace population used in this study occurs 8.6-km from Bumbee Creek, the type-locality for Laurel Dace. Twenty Tennessee Dace per-month were collected from Laurel Ford Branch for 18 months. Data collected included standard length (mm), weight (g), gonadosomatic index (GSI), clutch size and age interpreted from annual growth rings in whole otoliths. Diet data were collected from Tennessee Dace sampled in the present study and from preserved specimens of Laurel Dace from a historic collection. The sex ratio of females to males was 1.3:1 and the mean standard length for all specimens was 33.8 mm (SD=10.6 mm). Length-frequency analysis suggested that there were 3+ age groups and otolith age data suggested 4+ age groups. A von Bertalanffy individual growth model on mean length-at-age data was estimated to be $L_t = 50.63\ mm*(1- e^{-0.38\ (t-1.84)})$. Percent annual natural mortality estimates range from 16% to 95%. Pectoral fin lengths were significantly longer in males than females. Female GSI slowly increased from October 2018 until it reached a peak the following April and May. Male GSI slowly increased in January, peaked in May, and returned to zero in June. Mature ova counts ranged from 64-143 and average ova diameters ranged between 0.83-1.37 mm. Both Tennessee Dace and Laurel Dace consumed a diverse array of aquatic and terrestrial invertebrates as well as algae and plant matter. Based on the data from the surrogate Tennessee Dace, it is projected that Laurel Dace have low mature ova counts, small eggs compared to other *Chrosomus* species, 4+ age groups, reach sexual maturity at age two, are multiple clutch spawners, and are opportunistic feeders. This information will help guide managers to make informed decisions on the recovery of the Laurel Dace.

Introduction

The surrogate species approach for determining life history is an invaluable tool in research to inform the conservation and management of threatened or endangered species. Due to restrictions from the Endangered Species Act (ESA) of 1973, it is often impossible to collect and sacrifice threatened or endangered species to obtain data on maturity, age and diet, which are needed for a life history analysis (Banks et al. 2010). Furthermore, time constraints on listing decisions often preclude collection on the life histories of species in the periods leading up to their listing decisions (Woods and Morey 2008). The ESA requires that recovery plans are developed for all species listed as threatened or endangered. Recovery plans guide managerial decisions on protecting and restore their populations and often require information on life history of the species in question (Boersma et al. 2001).

The Laurel Dace is a small, freshwater minnow that is endemic to headwater streams on the Walden Ridge portion of the Cumberland Plateau in east-central Tennessee that was listed as federally endangered in 2011, with critical habitat designated in 2012 (Skelton 2001;U.S. Fish and Wildlife Service 2016). The recovery plan for the species, which was published in 2016, recognizes two Evolutionary Significant Units (ESUs) of Laurel Dace (U.S. Fish and Wildlife Service 2016). The northern population occurs in Bumbee Creek, Youngs Creek, and Moccasin Creek, and the southern population occurs in Soddy Creek, Horn Branch, and Cupp Creek (Strange and Skelton 2005), all of which drain into the upper Tennessee River drainage. These streams are characterized by clean, clear water with pebble, cobble, boulder, and bedrock substrate and dense riparian vegetation consisting primarily of Mountain Laurel (*Kalmia latifolia*) and Eastern Hemlock (*Tsuga canadensis*) (Skelton 2001; U.S. Fish and Wildlife Service 2016). Eastern Hemlock in this area is threatened by the invasive hemlock wooly adelgid. Threats to the Laurel Dace include sediment and chemical runoff from intensive agricultural practices and watershed development, invasive species interactions, and climate change (U.S. Fish and Wildlife Service 2016). Laurel Dace are presumed to be tolerant of some siltation and other non-point source pollutants from agriculture, poor culvert replacement, and forestry practices; however increased sediment load in Laurel Dace habitat, observed since 2001, is a conservation concern (Skelton 2001; U.S. Fish and Wildlife Service 2016).

Laurel Dace life history is largely unknown, and this information is critical for this species' conservation and management. The one prior study, by Skelton (2001) provides some descriptive observations of life history characteristics including a mostly animal-based diet and some spawning behavior. It is assumed that Laurel Dace have spawning habitat requirements that are similar to other *Chrosomus* species, which need clean, clear gravel to spawn. Other *Chrosomus* species are also known minnow nest-associate spawners, however the only stream that Laurel Dace co-occurred with a nest associate minnow is Soddy Creek, suggesting that Laurel Dace may not be obligate nest-associate spawners (Skelton, 2001). The recovery plan for the federally endangered Laurel Dace (*Chrosomus saylori*) lists determining its life history as essential for the species' recovery and that the best approach to would be to utilize a surrogate species (Khudamrongsawat and Kuhajda 2007; U.S. Fish and Wildlife Service 2016).

Like the Laurel Dace, the Tennessee Dace is a small minnow in the family Leuciscidae, but this species has a broader distribution and is not listed as threatened or endangered by the Endangered Species Act. Tennessee Dace is native to the upper Tennessee River drainage from southwest Virginia to northern Georgia. They inhabit spring fed, first and second order streams with silt and fine gravel substrates, and pools that contain undercut banks and coarse woody debris (Etnier and Starnes 1993; Hamed and Alsop III 2005). Tennessee Dace diet is likely similar to the other *Chrosomus* species, but no data exist to validate this assumption. Age and growth are understudied in this species, but studied populations included four size classes. Tennessee Dace are nest associates that spawn in temperatures around 21°C and are multiple clutch spawners with females producing between 398 and 721 mature eggs per clutch (Hamed et al. 2008).

The Tennessee Dace is the best available surrogate species for the Laurel Dace. It is the most taxonomically and ecologically similar species to Laurel Dace that is not a threatened or endangered. Though the closest taxonomic relative to the Laurel Dace is the Blackside Dace

(*Chrosomus cumberlandensis*), this species is federally threatened and is found in southeastern Kentucky and northeastern Tennessee (Strange and Mayden 2009). Tennessee Dace habitat is adjacent and similar to that of Laurel Dace, they are found in Laurel Ford Branch in Spring City, TN, a stream approximately 8.6-km and 250 meters lower in elevation from Bumbee Creek, the type-locality for Laurel Dace. Both streams are within the upper Tennessee River drainage and due to their proximity, are subject to identical climate conditions. Therefore, the Tennessee Dace population in Laurel Ford Branch is the best surrogate for a life history study for the Laurel Dace. They have large enough population levels to sacrifice individuals without hurting their population levels for this study. This study provides the first detailed comparative study on the life history these two species (Khudamrongsawat and Kuhajda 2007).

Materials and Methods

Life history analysis

A sample of Tennessee Dace was collected monthly from Laurel Ford Branch in Spring City, TN over an 18-month period for life history and diet analyses. From May 2018 to October 2019, a 10x4’, 3/16” mesh seine was used to sample the population. The first 20 Tennessee Dace collected were humanely euthanized with a 50-ppm solution of MS-222, then preserved in a 10% sodium bicarbonate-buffered formalin solution. Specimens were left in the buffered formalin for 1-2 weeks then were rinsed, soaked in fresh water overnight and transferred to 70% ethanol for permanent storage.

Otolith microstructure analysis was used to measure length-at-age of Tennessee Dace. The standard length (SL) of each Tennessee Dace specimen was measured to the nearest 0.5 mm with a ruler. Each specimen was then lightly blotted with a paper towel and weighed to the nearest 0.01 g. Up to five specimens within multiple size classes were selected at random from each monthly collection (n=72) for otolith analysis. The January 2019 collection was excluded from this analysis because fish were left in formalin for too long and otoliths were too fragile to analyze. Sagittal otoliths were removed by cutting off the head of the specimen then cutting the head in half parallel to the length of the body (between the eyes). The otoliths were removed from under the brain tissue on the right side of the specimen's head with a pair of forceps and placed into a small glass vial. Whole otoliths were mounted using Devcon© brand 5-minute epoxy on a microscope slide and the epoxy was given 48 hours to dry. A small piece of 600 grit sandpaper was used to sand the otolith down to the central transverse plane. Two separate observers interpreted the number of otolith annuli, including the outermost edge, and recorded their observations separately. If there was a discrepancy in rings counted, a third observer would make the final decision of the number of annuli. Tennessee Dace age was determined by assuming that their birth month was in May. The age of each fish was then calculated down to month. For example, if an otolith from July had two rings including the outermost edge, then the age was calculated as 1.16 years old.

The reproductive biology of Tennessee Dace was determined by dissection and examination of 257 specimens. Each specimen ≥ 25 mm SL was dissected, and all internal organs were removed, and specimens were sexed as male or female and if there were no gonads they were classified as juvenile. Gonads were lightly blotted with a paper towel and weighed to the nearest 0.01 g. If the specimen was a female with mature ova, the mature ova were counted and the diameter of 10 randomly selected mature ova were measured with digital calipers to the nearest 0.01 mm under a dissecting microscope. Gonadosomatic index (GSI) was calculated for all adult male and female Tennessee Dace for all months sampled.

$$GSI = (\text{gonad weight} / \text{total body weight}) \times 100$$

Once the sex of each specimen was determined, a morphometric analysis was used to evaluate if pectoral fin length is a sexual dimorphic character. The length of the left pectoral fin was measured of all adult Tennessee Dace (n=257) with digital calipers at its widest point to the nearest 0.01 mm. If the left fin was damaged, then the right fin was measured.

Seasonal stream temperature was determined using HOBO© temperature loggers. One logger was placed in Laurel Ford Branch and the other one was placed in Youngs Creek, where there is an extant population of Laurel Dace, from March 2019 to February 2020. HOBO© loggers were placed inside of a 150 mm piece of sch40 PVC that had small holes randomly drilled in it. The piece of PVC was attached with a 0.5 m piece of wire to a sandbag that was filled with sand and gravel. The sandbags were placed in a pool that did not dry out during the summer months.

Statistical analyses were performed using Microsoft Excel to determine significant differences of life history aspects of the Tennessee Dace. A chi-square test was used to determine if the males-to-female ratio was significantly different from one. Regression analysis and t-tests were used to evaluate relationships between standard length and weight, pectoral fin length and standard length, clutch size and gonad weight and clutch size and standard length. A length-frequency histogram along with the otolith measurements was used to determine the number of age groups.

A von Bertalanffy individual growth model was fit to the length-at-age data to estimate the theoretical maximum size (L_{inf}) of individual Tennessee Dace as well as the rate (*k*) at which an individual reaches the maximum size.

$$L_t = L_{inf}(1 - e^{-K(t-t_0)})$$

Utilizing L_{inf} and *k* from the von Bertalanffy growth model, as well as t_{max} (i.e., longevity in years) and mean water temperature, seven different annual mortality estimates were calculated for Tennessee Dace. The mortality model from Gulland (1976) estimated total instantaneous mortality in exploited recreational fisheries with length limit regulations.

$$Z = -K\,(L_{inf} - l_x)/l_x - l_r$$

The mortality model from Quinn and Deriso (1999) estimated instantaneous natural mortality (M) where P_s is the proportion of the stock that survives to t_{max}, where t_{max} is the longevity of the population in years. P_s was estimated at 0.01 and 0.05 as the authors suggest as reasonable proportion for most fish populations.

$$M = -l_n(P_s)/t_{max}$$

Hoenig (1983) created a model that estimated instantaneous natural mortality (M) based off of the work by Quinn and Deriso (1999) for stocks that are either lightly exploited or unexploited.

$$l_n(M) = 1.46 - 1.01 * l_n(t_{max})$$

The mortality model from Jensen (1996) estimated instantaneous natural mortality (M) considering the theoretical relations of age-at-maturity, survival rates to reach maturity, growth in weight, and K from the von Bertalanffy growth model.

$$M = 1.5 * K$$

The mortality model from Pauly (1980) estimated instantaneous natural mortality (M) utilizing mean water temperature as one of the main factors influencing natural mortality.

$$\log_{10}(M) = -0.0066 - 0.279 * \log_{10}(L_{inf}) + 0.643 * \log_{10}(K) + 0.4634 * \log10(TEMP)$$

Chen and Watanabe (1989) created a mortality model that estimated instantaneous natural mortality (M) using growth, longevity, and survivorship curves.

$$M(t_i \text{ to } t_f) = (1/t_f - t_i) * \ln(e^{K * t_f} - e^{K * t_0})/(e^{K * t_i} - e^{K * t_0})$$

Diet analysis

Tennessee Dace diet was quantified by calculating the frequency (*%F*) of occurrence of each diet item through multiple seasons where J_i is the number of fish containing prey item *i* and *P* is the number of fish with food in their stomachs.

$$\%F = J_i / P * 100$$

Five specimens from each month during the 18 months that Tennessee Dace were collected were randomly chosen for diet analysis (n=90). Only the upper 1/3 of the gut was considered in the analysis because it was determined that the bottom 2/3 of the guts were completely digested and unidentifiable. The items consumed by each specimen were identified to the lowest taxonomic level possible, while the presence or absence of plant matter and algae was noted.

In 1991 and 1993, Dr. Chris Skelton collected and preserved Laurel Dace from both Horn Branch and Soddy Creek. The specimens were deposited at the University of Tennessee fish collection for future analysis. Twenty-one Laurel Dace specimens from these collections were examined for frequency of occurrence of diet items. From these collections, I used nine specimens from Horn Branch from 1991, seven specimens from Horn Branch from 1993, and four specimens from Soddy Creek from 1993. The entire gut lengths were examined, and items consumed by each specimen were identified to the lowest taxonomic level possible while the presence or absence of algae and plant matter were noted. All contents were preserved in a labeled glass vial with 70% ethanol to preserve them in the historical collection.

Laurel Dace and Tennessee Dace prey availability was evaluated in their respective habitats. Samples of aquatic invertebrates were collected in 11 pools in Laurel Ford Branch, where Tennessee Dace occur, as well as three stream segments where Laurel Dace occur, upstream Bumbee Creek (n=11), downstream Bumbee Creek (n=9) and Lick Branch (n=7). Eleven pools were randomly selected for aquatic invertebrate collection and only pools that had water were sampled. Samples were collected with a D-net by placing the net in the center of the pool and disturbing the substrate approximately $0.3m^2$ in front of the net. The entire sample was preserved in 70% ethanol and invertebrates were separated from detritus and sediments, identified to order, and counted.

Results

Life history analysis

Our sample of Tennessee Dace was adequate to describe the life history of this species. A total of 370 Tennessee Dace specimens were collected for the life history analysis (males=110, females=147, juveniles=113). All mature females collected from May-June 2018 and April-June 2019 (n=21) were examined to determine the number and size of ova per-clutch. Ninety randomly selected otoliths were chosen for age analysis from all months. Out of 90 otoliths, 18 could not be read including five from the month of January 2019 because the specimens were left in formalin for too long (>2 weeks). Most otolith reads were found to be age-3 (n=32), while age-5 represented the fewest number of otoliths (n=2). The observed sex ratio of females to males was 1.3:1, but no evidence was found for a significant difference from a 1:1 ratio (X^2=0.05, 1, n=257, P>0.05). Pectoral fin lengths were found to be a sexually dimorphic characteristic because they were significantly longer (P<0.001) in males (n=110, R^2=0.80) than females (n=147, R^2=0.68) (Figure 2.4).

Length-frequency, length-weight relationships and the von Bertalanffy model describe growth and mortality estimates for the Tennessee Dace. The mean standard length for all specimens was 33.8 mm (SD=10.6, min=13, max=53). There was a strong relationship between log standard length (mm) and log body weight (g) (R^2=0.98, P<0.001, Figure 2.1). The slope of the regression equation (3.1268) from the log standard length (mm) and log body weight (g) was >3 indicating Tennessee Dace have positive allometric growth. The length-frequency histogram suggested 3+ age groups (Figure 2.2) and the otolith age assignments suggested 4+ age groups. The von Bertalanffy model showed the observed length-at-age data was a good fit (R^2=0.94, L_{inf}=50.63, K=0.38, t_0= -1.84, Figure 2.3). The annual mortality estimates ranged from 16% to 95%, depending on which mortality model was used. If the mortality estimates designated for exploited fisheries are excluded, then the annual mortality rates for Tennessee Dace are estimated to be between 38% and 95% (Table 2.1).

Tennessee Dace exhibited seasonal variability in reproductive maturity. Female GSI slowly increased from October 2018 until it reached a peak the following April and May, then rapidly declined until July. In 2018, male GSI peaked in June and steadily declined until August; in 2019, it started to slowly increase in January, peaked in May, and was back to zero in June (Figure 2.5). An increase in male and female GSI from March to May 2019 followed an increase in stream temperature in both Laurel Ford Branch and Youngs Creek during the same time period. GSI was highest for male and female Tennessee Dace in Laurel Ford Branch when the stream temperature was between 13.3°C and 16.9°C. (Figure 2.6). Females were found to have both mature and immature ova. Mature ova counts representing one clutch for the season ranged from 64-143 (mean=154) and average ova diameters ranged between 0.83-1.37 mm (mean=1.06). Gonad weight and clutch size were positively correlated (R^2=0.50, P<0.001, Figure 2.7) and clutch size increased with standard length (R^2=0.39, P<0.001, Figure 2.8).

Diet analysis

Tennessee Dace are omnivorous with a diet dominated by aquatic invertebrates. Calculations and summaries of percent frequency of occurrence of diet items revealed that Tennessee Dace consumed mostly aquatic invertebrates in the spring (47.9%) and in the winter (40.9%). They consumed terrestrial invertebrates at a higher rate in the summer (24.5%) and fall (23.4%). Algae and plant items were consumed throughout all months but were most commonly consumed in summer (50.8%), fall (46.8%), and winter (50%) (Figure 2.9). The aquatic prey taxa consumed by Tennessee Dace most frequently were larval insects in the orders Trichoptera, Plecoptera and Ephemeroptera and the family Chironomidae, and the most consumed terrestrial prey taxon consumed were adult invertebrates in the order Diptera (Table 2.2).

Similar to the Tennessee Dace, Laurel Dace are also omnivores whose diets were dominated by aquatic invertebrates. Laurel Dace only consumed terrestrial invertebrates in the summer (24.4%) (Figure 2.9). In the fall, only two different prey items were consumed, Trichoptera larvae and algae/plant matter (Table 2.2). Overall, the aquatic invertebrates consumed by Laurel Dace the most were Diptera and Hydrchnidae and the most consumed terrestrial diet item were in the orders Hymenoptera and Aranae (Table 2.2).

Nine aquatic insect taxa and one terrestrial taxon were found to be available prey for Tennessee Dace and Laurel Dace. Five of these taxa overlap between Tennessee Dace and Laurel Dace habitats: Lepidoptera, Chironomidae, Coleoptera, Gerridae and Plecoptera (Table 2.3).

Discussion

The life history data from this study illustrated few similarities with the only other life history study of Tennessee Dace (Hamed et al. 2008), which was based on data from specimens collected in northeast Tennessee. Hamed et al.'s (2008) mature ova counts were 398-721 with diameters of 0.90-1.5 mm. My study showed mature ova to be smaller (0.83-1.37 mm) and mature ova counts to be much lower (64-143). While the length-frequency analysis only suggested 3+ age groups (Figure 2.2), the otolith data suggested 4+ age groups. This is more in line with Hamed et al. (2008), whose length-frequency analysis suggested four age groups. The largest individual in the Hamed et al. (2008) study was a 65 mm SL female while the largest in this study was a 53 mm male found in December. Both Hamed et al. (2008) and this study showed pectoral fin length to be a sexually dimorphic characteristic that can be used to non-lethally distinguish adult male and female Tennessee Dace. The regression lines in Figure 2.4 cross and diverge around 31 mm SL, indicating that this characteristic can only be used to determine the sex of fish greater than 31 mm SL. Differences of these life history traits could be attributed to local adaptations. Slight differences in spatial and temporal scale as well as climatic variables can alter genetic and morphological traits of fish which can alter their life history traits (Collin and Fumagalli 2011).

Applying the von Bertalanffy model to a non-game minnow species was a novel approach to model growth and estimate mortality using the resulting parameters for Tennessee Dace. The K value from the von Bertalanffy model for the Tennessee Dace was 0.38 and the L_{inf} was 50.63, indicating a relatively fast growth rate (Figure 2.3). Estimates of annual mortality range from 16% to 95%. The Hoeing et al. (1998) annual mortality model was built off of the work of Quinn and Deriso (1999) and was based off of unexploited fish stocks. Since the Tennessee Dace population is unexploited, the most appropriate annual mortality rate is likely to be nearest to 73%. The Gulland (1976) and Pauly (1980) mortality models were inappropriate annual mortality models for the Tennessee Dace because they are meant for exploited fisheries (Table 2.1). Minnow species have opportunistic life history strategies which categorizes them as being small, rapidly maturing, and short lived, therefore we would expect the Tennessee Dace to have a relatively low *K* value as well as a high annual mortality rate (Winemiller and Rose 1992). The *K* values and mortality rates will be important to monitor within these populations in the future as we see climatic shifts occurring with climate change. As the planet warms and precipitation patterns change, there could potentially be a shift in how quickly this species grows, how long it lives, and how big it gets.

All *Chrosomus* species spawn at either age-1 or age-2 and our data suggest that Tennessee Dace spawn at age-2. The von Bertalanffy model was used to predict the age of all mature spawning females, only one female Tennessee Dace in Laurel Ford Branch that had mature eggs was estimated to be age-1 (Figure 2.3). A majority (n=20) of mature spawning females (n=21) were 2+ years old and all males that had a GSI higher than zero (n=22) were 2+ years of age. Other *Chrosomus* species reportedly spawn at age-1 or age-0, including Southern Redbelly Dace (*Chrosomus erythrogaster*) (Settles and Hoyt 1978) and Mountain Redbelly Dace (*Chrosomus oreas*) (males only) (Thompson et al. 2017). Clinch Dace (*Chrosomus* sp. cf. *saylori*) were also found to begin spawning at age-2 (White and Orth 2013).

The clutch size, maximum number of ova, mean ova diameters and body-size of the Tennessee Dace in this study were less than have been measured in any previous study of a *Chrosomus* species (Chapter 1). Low mature ova counts could be related to the small body-size of the Tennessee Dace. Tennessee Dace are similar to Blackside Dace because they both have small individual ova, but Blackside Dace has the highest ova counts of any of the *Chrosomus* species (Starnes and Starnes 1981). The maximum size of the Tennessee Dace in this study is also the smallest compared to other *Chrosomus* species. Like the rest of the *Chrosomus* species, Tennessee Dace are multiple clutch spawners and both mature and immature eggs were present in each female ovary (Chapter 1).

The spawning season of Tennessee Dace in Laurel Ford Branch starts in March and ends in July and the beginning and end of the season appears to be influenced by stream temperatures. In 2018, Tennessee Dace had elevated GSI through July, but in 2019, GSI had gone back down to zero by July. The stream temperature data was taken in 2019, however stream temperatures were potentially cooler in July in 2018, allowing for an extended spawning season. Laurel Ford Branch was on average 0.9°C warmer than Youngs Creek (Figure 2.6), where there is an extant population of Laurel Dace. Tennessee Dace peak spawning period was in April when the temperatures were 13.3°C. In April, the average stream temperature in Youngs Creek was 12.4°C and in May was 16.6°C therefore, Laurel Dace peak spawning period would presumably be between the end of April and beginning of May when water temperatures get closer to 13.3°C. Since Youngs Creek is approximately a degree cooler than Laurel Ford Branch, this could also indicate that Laurel Dace spawning season extends into the end of June or early July.

Tennessee Dace exhibit an opportunistic life strategy (Winemiller and Rose 1992). They live for a short period of time and have high annual mortality rates. They also mature early in life and although they have some of the smallest clutch sizes and small eggs, their ability to continuously spawn over a long period of time allows them to make up for their high annual mortality rate. These traits allow Tennessee Dace to continue to repopulate themselves despite the unpredictable flow regimes of the first and second order streams that they live in. However, when their habitat becomes predictable and constant, especially with flow and discharge, opportunistic species like the Tennessee Dace will struggle to survive. If Tennessee Dace habitats become impounded or if stream substrates become uniform (i.e., stream bottom is filled in with silt), this habitat becomes more suitable for equilibrium species like sunfish (family Centrachidae). These anthropogenic disturbances open a pathway for non-native species invasions which could further interrupt the life history strategies of the Tennessee Dace (Rahel et al. 2008).

The diet analysis indicated that Tennessee Dace and Laurel Dace are opportunistic feeders with small differences in their diets. Both species consumed a diversity of aquatic and terrestrial invertebrates, plant matter, and algae. Tennessee Dace and Laurel Dace consumed approximately 25% terrestrial invertebrate prey during the summer months. Emergence rates of aquatic invertebrate rates vary, however species in the order Diptera are known to emerge year round, but in higher abundance in the Spring (Reynolds and Benke 2006). Both larval and adult Diptera were some of the most consumed aquatic and terrestrial taxa by both Tennessee Dace and Laurel Dace in all seasons except for winter (Table 2.2). Based on the prey availability and prey consumed, Laurel Dace seemed to be more dependent on larval aquatic invertebrates as a food source (Table 2.2, Table 2.3). The sample size of Laurel Dace diets in the fall months was low (n=4), however the only thing that Laurel Dace consumed in the fall were aquatic Trichoptera larvae. Trichoptera adults are known to emerge and lay their eggs during the summer and larvae will then hatch in late summer (Jannot et al. 2007), leading to an abundance of aquatic larvae as prey in the fall.

Aquatic macroinvertebrate sampling in Laurel Dace and Tennessee Dace habitats do not match completely with the prey items found in the stomachs of the fishes. Differences in rates of digestion of different macroinvertebrate taxa could skew the prey items that are able to be identified in the stomach contents (Baker et al. 2014). Both Tennessee Dace and Laurel Dace consumed terrestrial invertebrates. The prey availability results do not accurately reflect the allochthonous terrestrial prey since the survey was only conducted for benthic aquatic taxa, even though the survey picked up one terrestrial taxon. For the Laurel Dace, one reason for these differences could be because the specimens examined were from 1991 and 1993, and the habitat in these streams might have changed due to anthropogenic influences (U.S. Fish and Wildlife Service 2016). Another reason could be that the aquatic macroinvertebrates were sampled in different streams than where the Laurel Dace were collected. Aquatic macroinvertebrates were sampled in upper and lower Bumbee Creek and Lick Branch, while the Laurel Dace specimens whose gut contents were analyzed were from Horn Branch and Soddy Creek.

Given the close geographic proximity between Laurel Dace populations and Laurel Ford Branch, and the relatedness of these two species, life history traits from the suggorate species, Tennessee Dace, can be applied to the federally endangered Laurel Dace to make better management decisions for the species. The results demonstrate that Laurel Dace have low mature ova counts, small eggs compared to other *Chrosomus* species, have 4+ age groups, spawn at age-2 between March and July, are multiple clutch spawners, and are opportunistic feeders. Laurel Dace are classified as an opportunistic-type species given their small size, short life-span, high annual mortality, long spawning season, and low batch fecundity based on the Winemiller triangular life history model (Winemiller 2005). The life history traits of these species make them tolerant of environmental variability, but susceptable to anthropogenic influences. Heavy siltation from poor development and agriculture practices currently affect Laurel Dace populations and these stressors are assumed to be causing the decline of the species, especially the southern population. Heavy siltation is creating a homogenous habitat of silt substrate. Siltation combined with the removal of riparian vegetation create habitats that are more suitable for tolerant species. Laurel Dace have severely declined where habitat alteration has occurred (U.S. Fish and Wildlife Service 2016). Using this life history information, managers can make decisions that will protect and restore Laurel Dace habitats in order to prevent further decline of the species.

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Appendix B

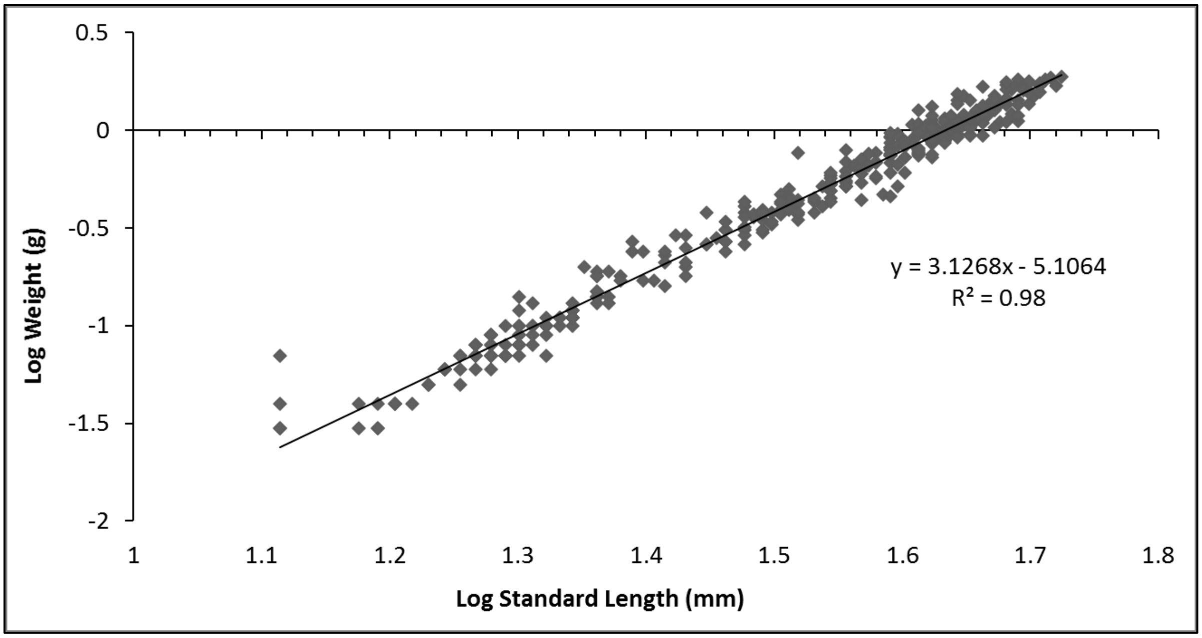

Figure 2.1. Length-weight relationship of Tennessee Dace (*Chrosomus tennesseensis*) collected in Laurel Ford Branch, TN (n=370).

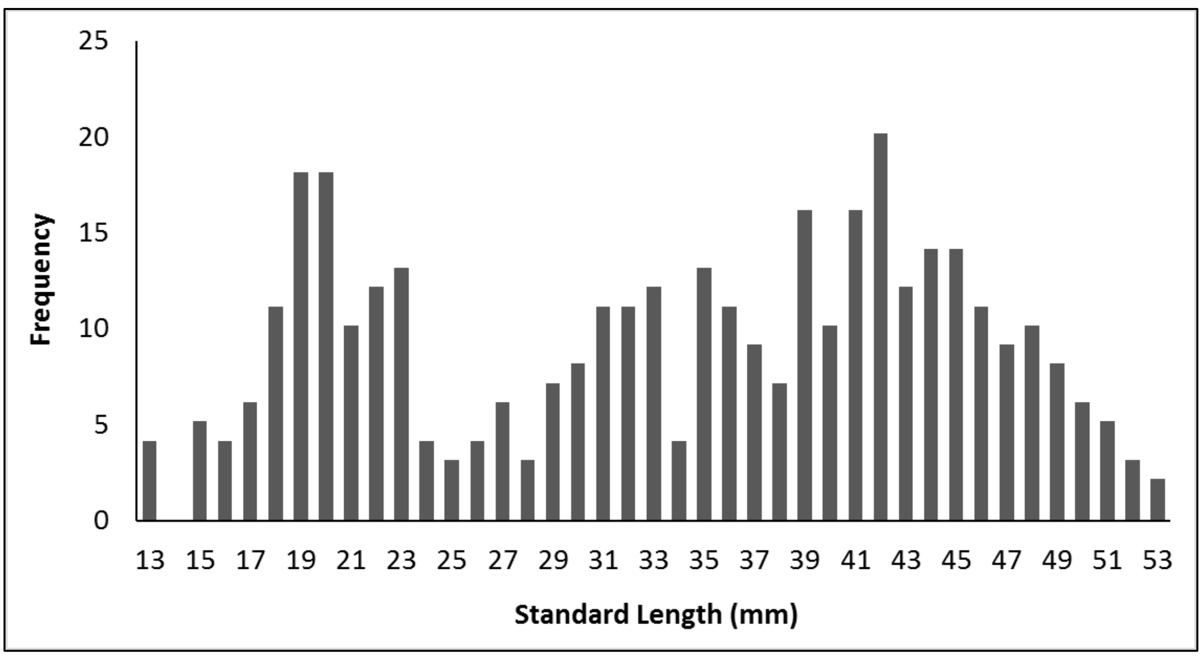

Figure 2.2. Length frequency distribution of all Tennessee Dace (*Chrosomus tennesseensis*) collected in Laurel Ford Branch, TN from May 2018 to October 2019 (n=370).

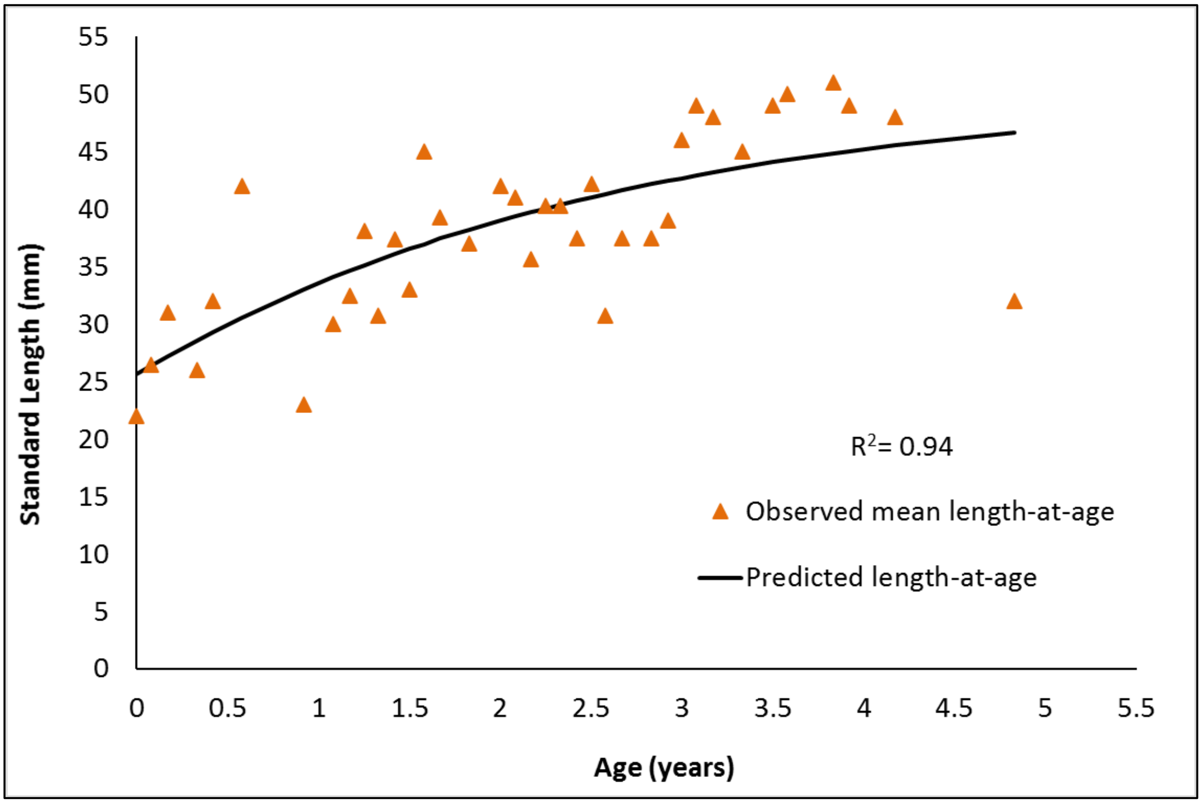

Figure 2.3. Von Bertalanffy growth model (L_{inf}=50.63, k=0.38, t_0= -1.84) utilizing Tennessee Dace (*Chrosomus tennesseensis*) otoliths for observed length-at-age (n=72). Specimens collected in Laurel Ford Branch, TN.

Table 2.1. Percent mortality and survival estimates for Tennessee Dace (*Chrosomus tennesseensis*) collected in Laurel Ford Branch, TN using parameterizations of the von Bertalanffy growth model (*meant for exploited fisheries only).

Citation	Mortality %	Survival %
Hoenig 1983	73	27
Chen and Watanabe 1989	38	62
Jensen 1996	57	43
Quinn and Deriso 1999 (P_s=0.01)	95	5
Quinn and Deriso 1999 (P_s=0.05)	62	38
Pauly 1980*	61	39
Gulland 1976*	16	84

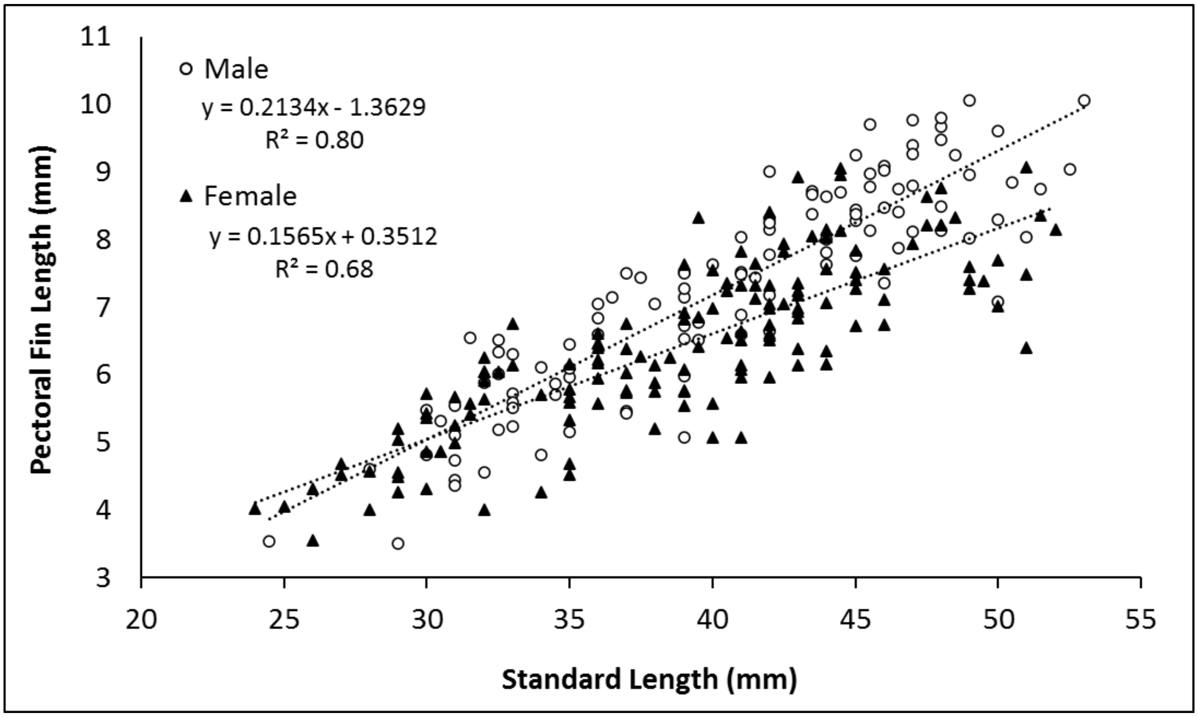

Figure 2.4. Regression of pectoral fin length on standard length of Tennessee Dace (*Chrosomus tennesseensis*) males (open circles, n=110) and females (black triangles, n=147) collected in Laurel Ford Branch, TN. Male pectoral fins are significantly larger than female pectoral fins ($P<0.001$).

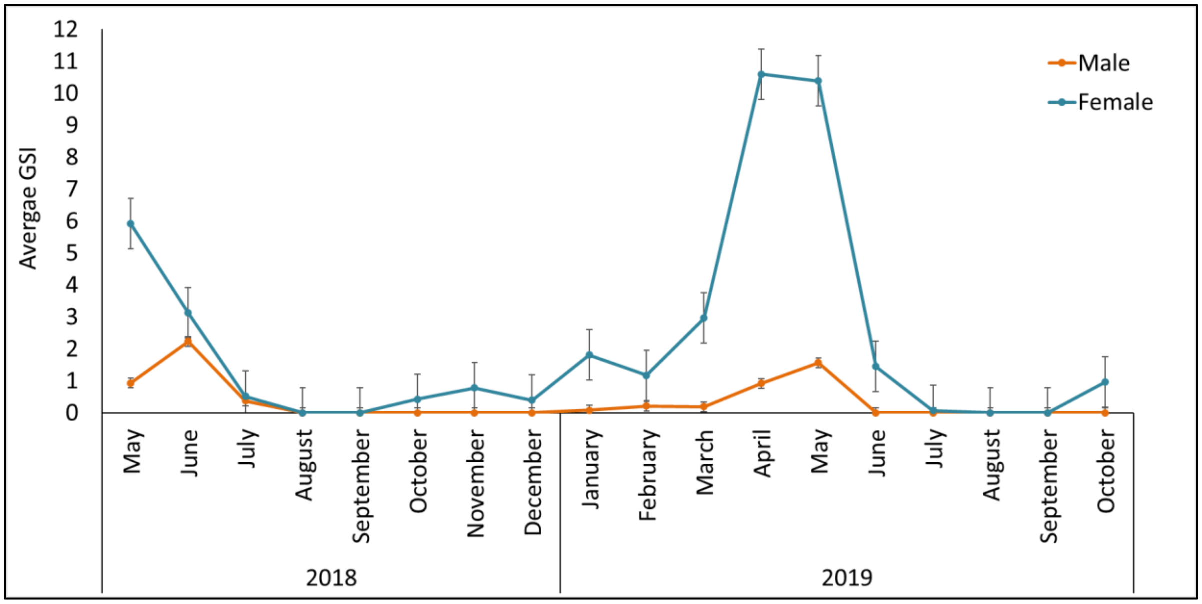

Figure 2.5. Average GSI for male and female Tennessee Dace (*Chrosomus tennesseensis*) collected in Laurel Ford Branch, TN from May 2018- October 2019 (n=257).

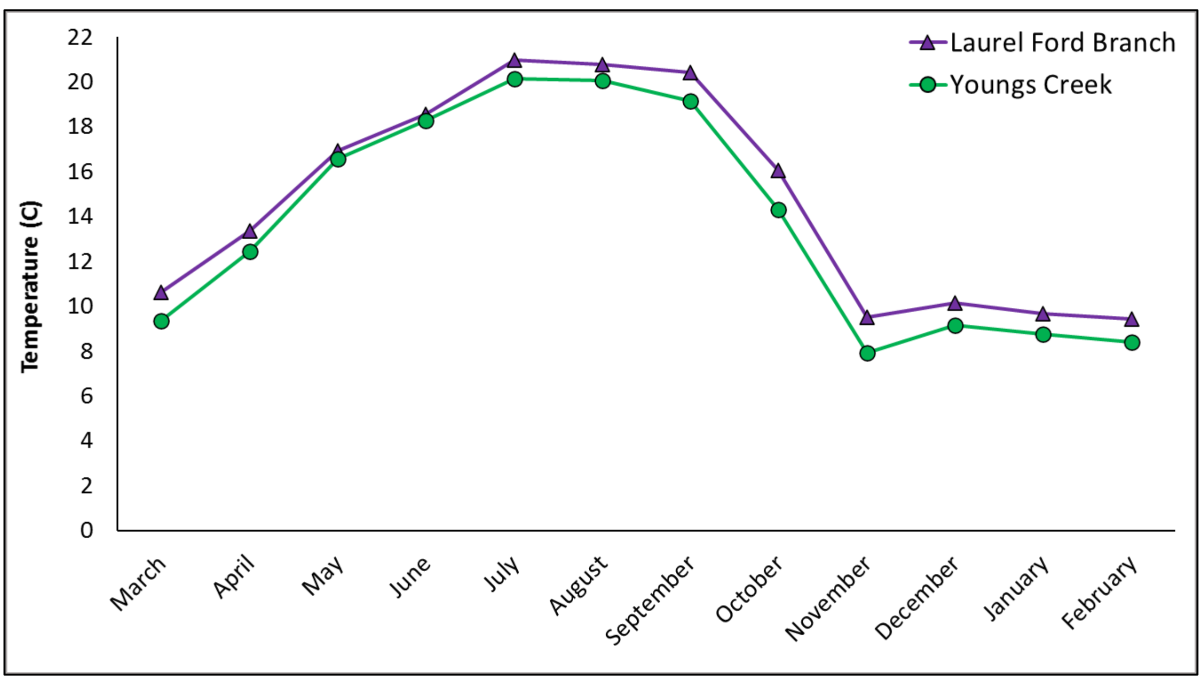

Figure 2.6. Seasonal temperatures (°C) of Laurel Ford Branch in Rhea county, TN and Youngs Creek in Bledsoe and Rhea counties, Tennessee from March 2019 to February 2020.

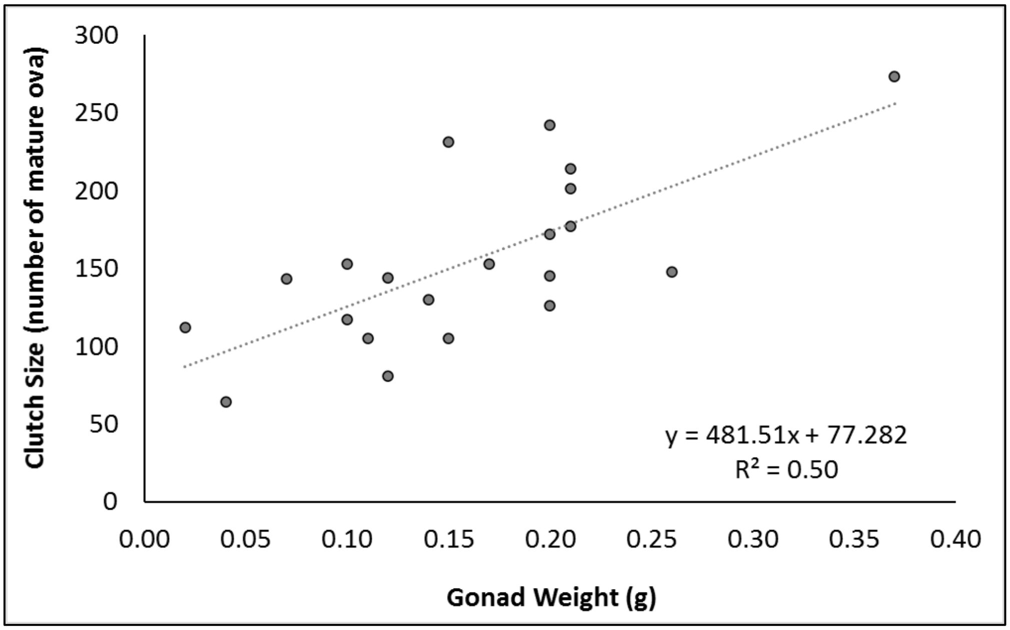

Figure 2.7. Gonad weight (g) versus clutch size of all mature female Tennessee Dace (*Chrosomus tennesseensis*) (n=21) collected in Laurel Ford Branch, TN during the reproductive season. Clutch size increased with gonad weight (P<0.001).

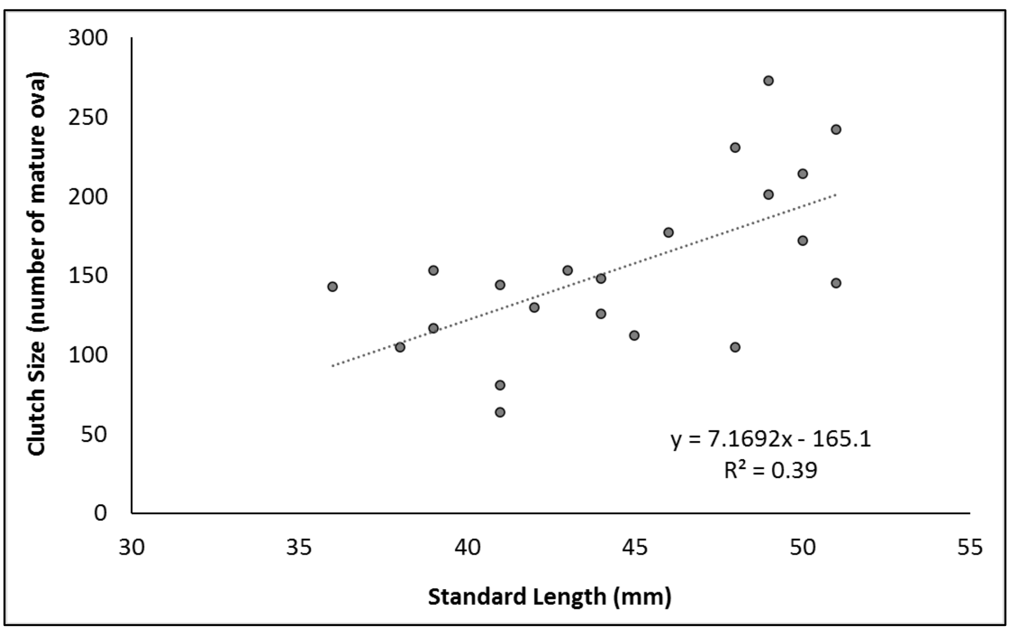

Figure 2.8. Clutch size versus standard length (mm) of all mature female Tennessee Dace (*Chrosomus tennesseensis*) (n=21) collected in Laurel Ford Branch, TN during the reproductive season. Clutch size increased with standard length (P<0.001).

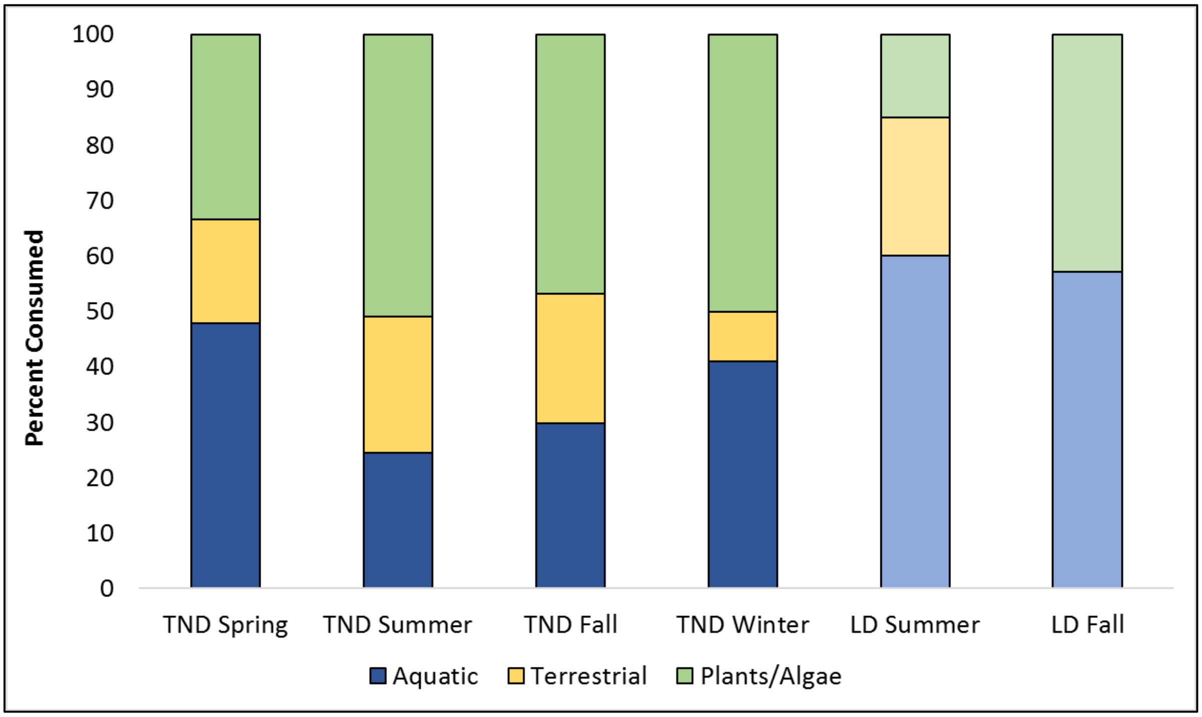

Figure 2.9. Percent of individual Tennessee Dace (*Chrosomus tennesseensis*) (TND, n=90) and Laurel Dace (*C. saylori*) (LD, n=20) that consumed aquatic invertebrates, terrestrial invertebrates, and plants/algae by season. Tennessee Dace were collected in Laurel Ford Branch, TN and Laurel Dace were collected in Horn Branch and Soddy Creek, TN.

Table 2.2. Percent frequency of occurrence of diet types consumed by Tennessee Dace (*Chrosomus tennesseensis*) collected in Laurel Ford Branch, TN and Laurel Dace (*C. saylori*) diets (n=21) collected in Horn Branch and Soddy Creek, TN. Cells shaded grey if percent frequency of occurrence is ≥ 30.

			Tennessee Dace				Laurel Dace	
	Scientific Classification	**Common Name**	Spring (n=20)	Summer (n=30)	Fall (n=25)	Winter (n=15)	Summer (n=16)	Fall (n=4)
Terrestrial	Araneae	Spiders	0	3	4	0	19	0
	Diptera	Flies	35	37	36	7	13	0
	Hemiptera	True insects	5	0	4	0	0	0
	Hymenoptera	Wasps, bees, ants	5	7	0	7	31	0
Aquatic	Cladocera	Water fleas	0	0	0	0	13	0
	Coleoptera	Beetle larvae	0	7	4	0	6	0
	Copepoda	Copepods	5	0	4	0	6	0
	Diptera	Fly larvae	30	13	12	33	50	0
	Ephemeroptera	Mayfly larvae	15	13	8	13	13	0
	Hydrachnidae	Water mites	10	7	8	0	38	0
	Odonata	Dragonfly larvae	10	0	0	0	0	0
	Plecoptera	Stonefly larvae	15	3	0	0	6	0
	Trichoptera	Caddisfly larvae	30	3	20	13	19	100
Algae/plant matter			80	97	88	73	38	75

Table 2.3. Prey availability for Tennessee Dace (*Chrosomus tennesseensis*) and Laurel Dace (*C. saylori*). Samples for Tennessee Dace were taken in Laurel Ford Branch (n=11) and samples for Laurel Dace were taken from upper and lower Bumbee Creek and Lick Branch (n=27).

	Scientific Classification	**Common Name**	**Tennessee Dace**	**Laurel Dace**
Terrestrial	Lepidoptera	Caterpillar	Yes	Yes
Aquatic	Anostraca	Fairy Shrimp		Yes
	Chironomidae	Midge Larvae	Yes	Yes
	Coleoptera	Beetle Larvae	Yes	Yes
	Decapoda	Crayfish	Yes	
	Ephemeroptera	Mayfly Larvae	Yes	
	Gerridae	Water Strider	Yes	Yes
	Odonata	Dragonfly Larvae		Yes
	Plecoptera	Stonefly Larvae	Yes	Yes
	Sphaeriidae	Fingernail Clam		Yes

CHAPTER 3

HABITAT OF THE LAUREL DACE (*CHROSOMUS SAYLORI*)

Abstract

The Laurel Dace (*Chrosomus saylori*) is a federally endangered minnow (Family Leuciscidae, formerly Cyprinidae) native to Walden Ridge in Tennessee. They currently occupy only three streams out of the seven where they were once known to occur. Laurel Dace streams are threatened by intense agriculture, removal of riparian vegetation and in-stream barriers such as hanging culverts. To better understand the habitat needs of the Laurel Dace we conducted a study of four stream segments containing extant populations of the northern Evolutionary Significant Unit of Laurel Dace. We measured eleven habitat variables: wetted length (m), wetted width (m), water depth, (cm), silt depth (mm), canopy cover (%), sand/silt/detritus (%), pebble/gravel (%), cobble (%), boulder (%), bedrock (%), and vegetation (%), as well as land cover in the upstream watersheds of each stream. Forty-four seine hauls (22 pools) in each stream segment were sampled for the presence and abundance of Laurel Dace. Univariate analysis was used to determine relationships between abundance and all individual habitat variables. Although there were no differences in habitat data, Laurel Dace occupied pools with an average depth of 16.1 cm (SD=8.6), average pool volume of 5.4 m^3 (SD=9.4), and average canopy cover of 93% (SD= 5). More than 75% of the land cover in all three watersheds was forest, which provides some protection from sediment and chemical runoff for the northern populations of Laurel Dace. It will be imperative for management actions to provide protection of this forested land to ensure a future for the Laurel Dace.

Introduction

The Laurel Dace (*Chrosomus saylori*) is a minnow (Family Leuciscidae, formerly Cyprinidae, Schönhuth et al. 2018) with a limited distribution in three stream systems in east-central Tennessee. When described as a unique species in 2001, Laurel Dace were only known to occur in seven streams on the Walden Ridge portion of the Cumberland Plateau: Soddy Creek in the Soddy Creek system, Horn Branch, Cupp Creek and Laurel Branch in the Sale Creek system, and Youngs Creek, Bumbee Creek and Moccasin Creek in the Piney River system. At the time of the species description, Laurel Dace was presumed to be extirpated from Laurel Branch because it had not been observed there since a Tennessee Valley Authority (TVA) survey conducted in 1976 (Skelton 2001). After its description, museum specimens of Laurel Dace were discovered from another stream system on Walden Ridge, Grassy Cove, an endorheic basin in Cumberland County, TN. This population is considered extirpated. Laurel Dace was listed as federally endangered in 2011, and Soddy Creek, Horn Branch, Cupp Creek, Youngs Creek, Bumbee Creek, and Moccasin Creek were designated as the critical habitat for the species in 2012 (U.S. Fish and Wildlife Service 2016). Based on a mitochondrial DNA study, two Evolutionary Significant Units (ESUs) exist for the Laurel Dace: the northern population in Bumbee Creek, Youngs Creek and Moccasin Creek and the southern population in Soddy Creek, Horn Branch and Cupp Creek (Strange and Skelton 2005).

Thorough surveys, conducted annually since 2011, indicate possible extirpations of Laurel Dace from its critical habitat. Laurel Dace are now presumed to be extirpated from Soddy Creek, where they have not been collected since 2004 and from Cupp Creek, where they have not been collected since 2014 (B. Kuhajda, Tennessee Aquarium Conservation Institute, pers. comm.). They have also not been collected in Moccasin Creek since 2013, but recently multiple individuals were found in one of its tributaries, Lick Branch. However, recent droughts likely extirpated them from Lick Branch as they have not been collected there since 2017. Recent surveys have not been conducted in Moccasin Creek due a lack of permission for access, therefore, it is undetermined if they still occur there. As of 2020, Laurel Dace are only known to occur in three streams: Youngs Creek and Bumbee Creek (northern population) with relatively stable populations, and Horn Branch (southern population) where only three juveniles were collected in 2020 after not being found since 2014 (B. Kuhajda, Tennessee Aquarium Conservation Institute, pers. comm).

USFWS and the study that resulted in the species description provide qualitative and general descriptions of Laurel Dace habitat. It is assumed that Laurel Dace require clean gravel/cobble in shallow riffles to spawn (Skelton 2001; U.S. Fish and Wildlife Service 2016). While most other *Chrosomus* species are known minnow nest-associate spawners, Laurel Dace have only been known to cohabitate with a nest- associate, Largescale Stoneroller (*Campostoma oligolepis*), in Soddy Creek (Skelton 2001), where they are now likely extirpated. Laurel Dace are usually found in pools or slow runs from undercut banks or underneath slabs of boulders or bedrock. The streams often have a dense riparian zone comprised of Mountain Laurel (*Kalmia latifolia*), Rhododendron (*Rhododendron* sp.), and Eastern Hemlocks (*Tsuga canadensis*) which provide ample canopy cover (Skelton 2001; U.S. Fish and Wildlife Service 2016).

Threats to Laurel Dace habitats include siltation, non-point source pollutants, riparian vegetation removal, and barriers created by road crossings (Skelton 2001; Strange and Skelton 2005; U.S. Fish and Wildlife Service 2016). One of the main causes of siltation in Laurel Dace streams is from row crop agriculture. Soil from these farms, often containing herbicides and pesticides, is washed into surrounding streams via surface runoff or ephemeral stream channels that carry soil into perennial streams occupied by fishes (U.S. Fish and Wildlife Service 2016). Removal of riparian vegetation and the subsequent decline in stream shading increases stream temperatures as well as algal and macrophyte production (Roth et al. 2010). Many landowners find removing riparian vegetation to be aesthetically pleasing and riparian vegetation is often removed at road crossings. No studies have been conducted yet on the thermal tolerance of Laurel Dace, but the maximum recorded stream temperature within Laurel Dace habitats was 26°C (Skelton 2001; Strange and Skelton 2005; U.S. Fish and Wildlife Service 2016). Improperly sized culverts at road crossings can prevent Laurel Dace recolonization and dispersal in reaches where they have suffered a localized extirpation (U.S. Fish and Wildlife Service 2016). Conversion of native forests for increasing residential use, pastures, crops, and clearcutting for Pine (*Pinus spp.*) monoculture have also changed the hydrology of the catchments of Laurel Dace streams (Skelton 2001; Strange and Skelton 2005; U.S. Fish and Wildlife Service 2016). Climate change is altering hydrology, specifically it is causing more intense droughts and floods to occur in the southeastern United States (Reidmiller et al. 2018). Climate change, in combination with other stressors, such as decreased riparian vegetation and increased siltation, poses a serious cause for concern for the future of the Laurel Dace.

Understanding habitat requirements can be crucial for the conservation of rare, non-game, and endemic species (Midway et al. 2010). Habitat loss has been found to be one of the primary factors that put species at risk of extinction and preventing habitat loss of endangered species is going to become more difficult has human populations continue to grow (Boersma et al. 2001). Human activity has influenced about 83% of the land surface of the Earth and the number of species at risk of extinction is rapidly increasing (Kerr and Deguise 2004). Detailed information on Laurel Dace habitat could help determine the vulnerability of the species to various threats and guide management programs to prevent extinction through future habitat restoration (U.S. Fish and Wildlife Service 2016).

Materials and Methods

Habitat characteristics were determined in four segments of three streams where the northern population of Laurel Dace occurs. These were first or second order, spring fed streams found on the eastern rim of the Cumberland Plateau in Bledsoe and Rhea counties, Tennessee (Figure 3.1). Habitat variables were characterized at four stream segments of three different streams: Lick Branch, Bumbee Creek above Pine Creek Rd, Bumbee Creek below Pine Creek Rd and upper Youngs Creek. These streams were chosen because they represented the only known extant populations of Laurel Dace at the time of this study. Examining habitat conditions in streams with extant populations is necessary to understand the habitat needs of the Laurel Dace.

The relative abundance and occurrence of Laurel Dace was sampled with a seine in pools of the four stream segments. A pool was defined as having little to no flow (< 10 cm/s) and were habitats between riffles, which is defined as faster moving and turbulent water. Each sample of Laurel Dace consisted of two seine-hauls, one in the upstream direction and one in the downstream direction, with a 10’ x 6’ seine with 3/8” mesh. After the first seine haul, all Laurel Dace were positively identified, counted, and placed into a black 5-gallon bucket with a battery-operated aerator in order to not recapture individuals on the second seine haul. A total of 44 seine hauls were made in each stream segment. If the total area of a pool was > 40 m^2 and was not able to be effectively sampled with two seine hauls, then the number of seine hauls was doubled to four. These “large” pools were considered two pools when determining habitat characteristics of the stream segments, therefore twice the amount of habitat characteristic data was measured in “large” pools. Habitat data collected for the “large” pools was then combined to represent one pool in the data analysis. The upstream end of each pool was marked with numbered flagging tape and habitat characteristics were later taken at these flagged points. The relative abundance of Laurel Dace in each pool was expressed as the number of Laurel Dace caught in two seine hauls.

Eleven habitat variables were measured in all pools (Table 3.1). The wetted length (m) and width (m) of each pool were quantified with a measuring tape. Three equidistant transects that were perpendicular to stream flow were established for measuring the remaining habitat variables in each pool. Along each transect, depth (cm) was measured with a meter stick at 10 equidistant points. The substrate composition on a modified Wentworth scale was measured with a $0.06m^2$ PVC quadrat classified at three equidistant points along each transect (n=9 per pool). Photos of each $0.06m^2$ area of substrate were taken with a GoPro Hero 5 in both sections of Bumbee Creek and in Lick Branch. Percent substrate types were analyzed using ImageJ software. From each photo taken of the $0.06m^2$ quadrat, the area (mm^2) inside the quadrat was measured using the area tool in ImageJ. The modified Wentworth scale was then used to identify different substrate types within the $0.06m^2$ quadrat and the area tool in ImageJ was used to calculate the sum total area of each substrate type. Percent of each substrate type was calculated for each quadrat and the average for each pool was determined. Substrate measurements at Youngs Creek were not taken with the GoPro because of technical difficulties and instead were estimated by eye to the nearest 1%. In each $0.06m^2$ along each transect, the deepest section of silt was measured with a meter stick (mm). In the middle of each pool canopy cover was measured using a spherical crown densiometer.

Univariate analysis was used to evaluate potential relationships between Laurel Dace abundance in each pool and habitat variables. Linear regressions were calculated of Laurel Dace abundance and average silt depth (mm), average pool depth (m), pool volume (m^3), average canopy cover (%), percent bedrock, percent boulder, percent cobble, percent pebble/gravel, percent sand/silt/detritus, and percent vegetation. T-tests were also used to determine if there were differences between means of habitat variables in pools where Laurel Dace were present and where they were absent.

To describe land cover in Laurel Dace streams, watershed boundaries were created from the most downstream point where samples were collected using the Watershed tool in the Ready To Use toolbox in ArcPro. Using the Reclassify tool, six different classifications from the 2016 National Land Cover Data (NLCD) dataset were classified: pasture/hay fields, herbaceous shrubbery, forest, barren/open land, developed land and open water. Percent land cover was then calculated for each watershed using the Zonal Statistics tool.

Results

Out of all four stream segments, Laurel Dace were found in more pools in Bumbee Creek below Pine Creek road. Eighteen total pools were sampled in Bumbee Creek below Pine Creek rd and in Youngs Creek, and 16 total pools were sampled in Bumbee Creek above Pine Creek rd and in Lick Branch. Fourty-four seine hauls were performed in each stream segment regardless of the number of pools. Laurel Dace occupied 13 out of the 18 total pools in Bumbee Creek below Pine Creek Rd and was the only segment where Laurel Dace were present in more pools than they were absent (Figure 3.2). Lick Branch had the lowest amount of pools with Laurel Dace present (n=4), while Bumbee Creek upstream of Pine Creek Rd and Youngs Creek had the same amount of pools with Laurel Dace present (n=5) (Figure 3.2).

There were no relationships found between Laurel Dace abundance and habitat characteristics except with pool volume, and pool depth. All R^2 values for the linear regressions of Laurel Dace abundance and habitat characteristics were below 0.20, except for pool volume which had an R^2 of 0.36 (Figure 3.3) and pool depth which had an R^2 of 0.22 (Figure 3.4). T-tests reveal there were no differences in the means of habitat characteristics of pools where Laurel Dace were present and where they were absent.

Five substrate types were present at all four sites. The most dominant substrate type across all sites was bedrock followed by sand/silt/detritis, boulder, pebble/gravel and then cobble. Vegetation was present at all four sites, but very minimally. In Bumbee Creek below Pine Creek Rd, where the most Laurel Dace were found, bedrock was the most prominnt substrate followed by boulder, cobble, sand/silt/detritis, and pebble/gravel. This stream segment also had the lowest percentage of sand/silt/detritus out of the four stream segments (Figure 3.5). Laurel Dace occupied pools with an average depth of 16.1 cm (SD=8.6), pool volume of 5.4 m^3 (SD=9.4), and canopy cover of 93% (SD= 5).

Forested land dominated the land cover in the watersheds of Bumbee Creek, Lick Branch and Youngs Creek. All three watersheds had > 76% forested land cover. Bumbee Creek had the highest levels of pasture/hay land cover (15.2%). Developed land cover was low in all three watersheds, with the highest being Lick Branch at 6.2%. Creeks in this watershed are very small and mostly surrounded by forest, therefore the water cover percentages are very low (<0.05%) (Figure 3.6).

Discussion

Understanding habitat factors that determine the distribution of a species is important to understand that species' response to a change in the environment (Peoples and Frimpong 2016). Laurel Dace are a rare, narrowly-endemic, federally endangered species that thrive in watersheds that are mostly forested, with little agricultural inputs and little developed land (Figure 3.7). The northern population of Laurel Dace has continued to exist within these watersheds, likely because of the protection that they have from large agriculture operations. Dense forests and riparian vegetation keep these streams cool and protected from major sedimentation events caused by poor practices at large agriculture operations (U.S. Fish and Wildlife Service 2016).

Similarities and differences from what Skelton (2001) described about Laurel Dace habitat were found. Skelton (2001) described Laurel Dace inhabiting first or second order, clear and cool streams with a maximum water temperature about 26°C. This study showed that Laurel Dace occupy both first order (Lick Branch) and second order streams (Bumbee Creek and Youngs Creek), however the maximum observed stream temperature was much lower at 20.1°C (Chapter 2). Climatic events such as droughts could explain the difference in maximum stream temperatures. The frequency of multi-year droughts is increasing and this combined with groundwater withdrawals could increase stream temperatures (Archdeacon et al. 2020). Laurel Dace are presumably tolerant of drought conditions since they have been able to persist in higher stream temperatures in the past. Skelton (2001) describes stream substrates consisting of three substrate types: cobble, rubble, and boulder. This study described five substrate types with bedrock being the most dominant substrate type, followed by boulder, cobble, pebble/gravel, and lastly sand/silt/detritis. Laurel Dace were also decribed in Skelton (2001) as occuring under slabs of bedrock. Most Laurel Dace individuals were collected from under slabs of bedrock, especially in Lick Branch. Pebble/gravel, which is presumably needed for Laurel Dace to successfully spawn, is most abundant in Lick Branch and Youngs Creek (Figure 3.5).

The Laurel Dace's closest relatives, the Blackside Dace (*Chrosomus cumberlandensis*) and the Clinch Dace (*Chrosomus* sp. cf. *saylori*), share similar habitat requirements (Starnes and Starnes 1981; Strange and Mayden 2009). Blackside Dace are found in the upper Cumberland River drainage and Laurel Dace are found in the upper Tennessee River drainage (Chapter 1). Both species inhabit first to second order cool, clear, upland streams with predominately bedrock substrates surrounded by dense forests and riparian vegetation (Starnes and Starnes 1981). Clinch Dace were previously believe to be a disjointed population of Laurel Dace, however based on morphology, they were determined to be a new undescribed species in the genus *Chrosomus*. Clinch Dace occupy small streams with predominately gravel substrates in watersheds with little development. Similar to this study, White and Orth (2013) did not find differences in the habitat variables where Clinch Dace were present and where they were absent. The average pool volume that Clinch Dace occupied was 2.5 m^3 (White and Orth 2013), while Laurel Dace occupied pools that were on average 5.4 m^3.

This study yields the most comprehensive description of the habitat of the northern population of Laurel Dace to date. The northern population of Laurel Dace is considered more stable than the southern population and this study revealed that the watersheds of streams containing the northern population were covered by greater than 76% forest cover. Laurel Dace were observed in greater abundances in larger pools with greater depths and they occurred in pools that had high levels of silt, but other substrate types were also available. The presence of pebble/gravel at all sites, which is presumed to be what they need to spawn successfully, could be a determining factor in what allows these populations to continue to exist (Skelton 2001; Cronnon et al. 2019). Laurel Dace and their habitat will need to continue to be monitored and protected in order to prevent this species from extinction.

There are future studies that need to be conducted in order to further investigate the habitat needs of the Laurel Dace. This study investigated whether there were differences in habitat characteristics between pools occupied by Laurel Dace and pools unoccupied by Laurel Dace as well as if there were differences in abundances of Laurel Dace determined by different habitat characteristics. This study found no differences in these factors, indicating that something other than habitat is controlling distribution of the northern population of Laurel Dace. Future studies should include streams that contain the southern population of Laurel Dace in order to understand if there are habitat differences between the two ESUs. There is likely a maximum tolerance threshold of habitat characteristics like silt depth, and pool volume that could be investigated by including measurements of habitat characteristics from the southern population. The seining methods that was used in this study was biased towards collecting Laurel Dace >20 mm. The habitat needs of young-of-the-year and juvenile Laurel Dace could also be a beneficial future study to understand if they have different habitat needs than the adults as it cannot be assumed that young-of-the-year and juvenile Laurel Dace have the same habitat needs of adult Laurel Dace.

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Appendix C

Table 3.1. Eleven habitat characteristics measured in four stream segments: Lick Branch, Youngs Creek, Bumbee Creek above Pine Creek Rd and Bumbee Creek below Pine Creek rd in Bledsoe and Rhea counties, TN. *Modified Wentworth Scale

Habitat Characteristic	Unit of Measurement
Wetted Length	Meters (m)
Wetted Width	Meters (m)
Water Depth	Centimeters (cm)
Silt Depth	Millimeters (mm)
Canopy Cover	Percent (%)
Sand/Silt/Detritus	0.004-2.0mm plus organic debris*
Pebble/Gravel	2.0-32.0 mm*
Cobble	32.0-256.0 mm*
Boulder	256.0+ mm*
Bedrock	Solid substrate*
Vegetation	Submerged vegetation

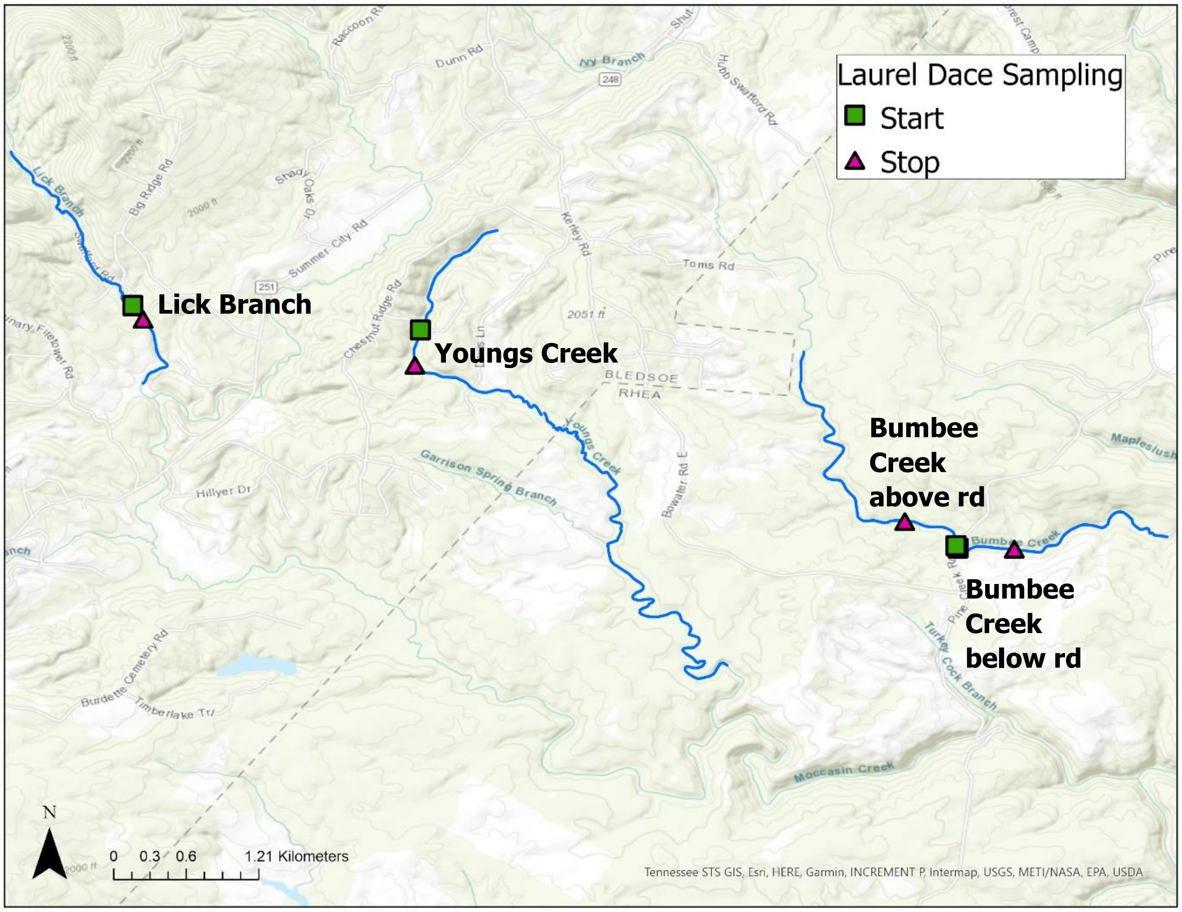

Figure 3.1. Sites where Laurel Dace (*Chrosomus saylori*) sampling and habitat parameter collection occurred on the Walden Ridge portion of the Cumberland Plateau in east-central Tennessee, Bledsoe and Rhea counties. Habitat parameters were collected between the start and stop points at each of the four sites.

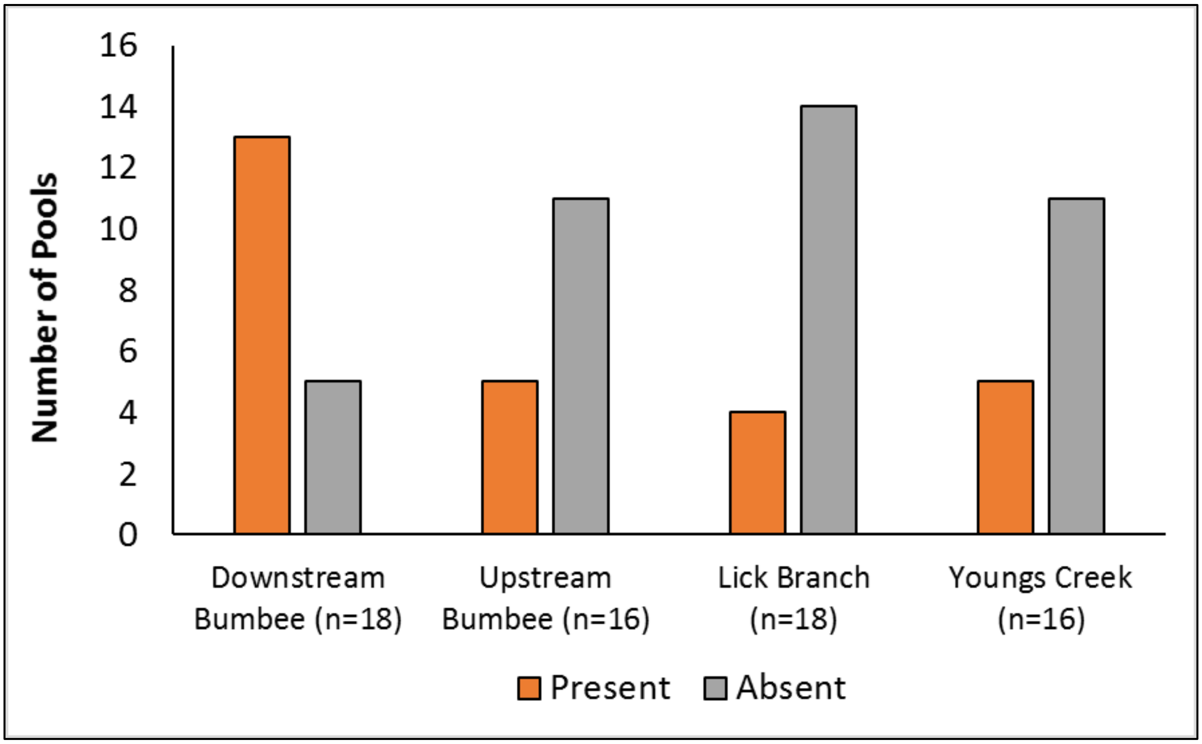

Figure 3.2. Presence and absence of Laurel Dace (*Chrosomus saylori*) in four sites in Bledsoe and Rhea counties, Tennessee where habitat parameters were collected.

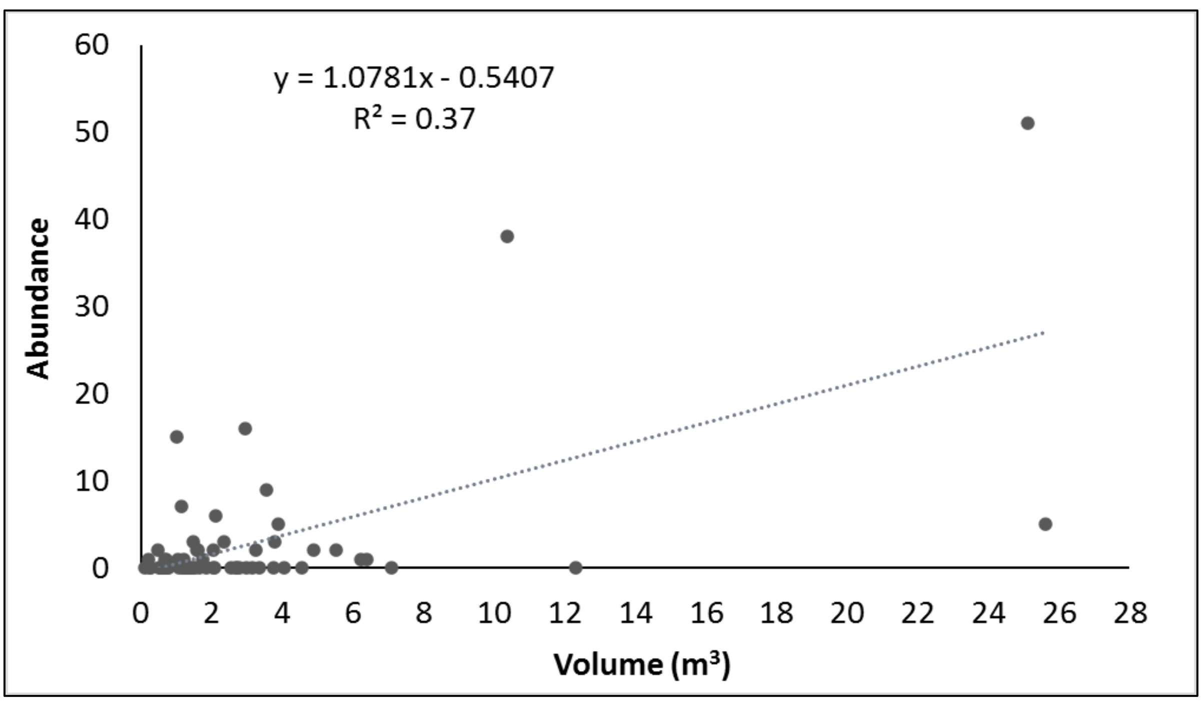

Figure 3.3. Linear regression of pool volume (m^3) in relation to Laurel Dace (*Chrosomus saylori*) abundance in Bledsoe and Rhea counties, Tennessee.

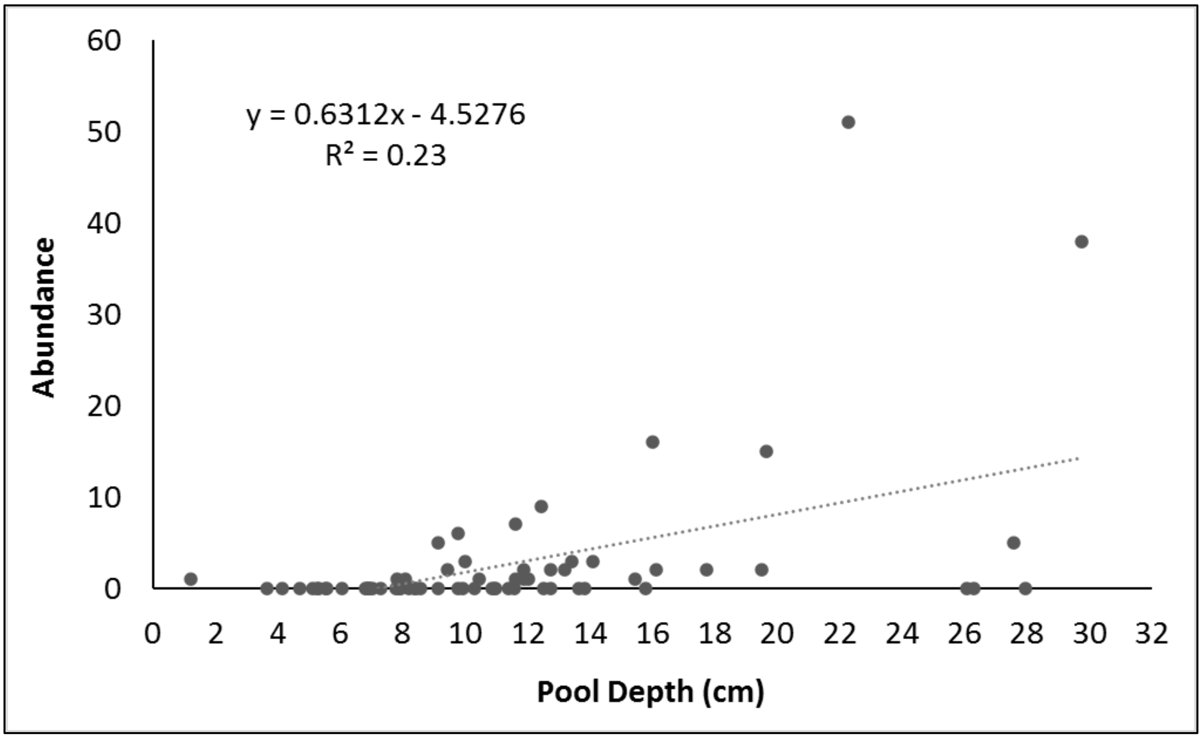

Figure 3.4. Linear regression of pool depth (cm) in relation to Laurel Dace (*Chrosomus saylori*) abundance in Bledsoe and Rhea counties, Tennessee.

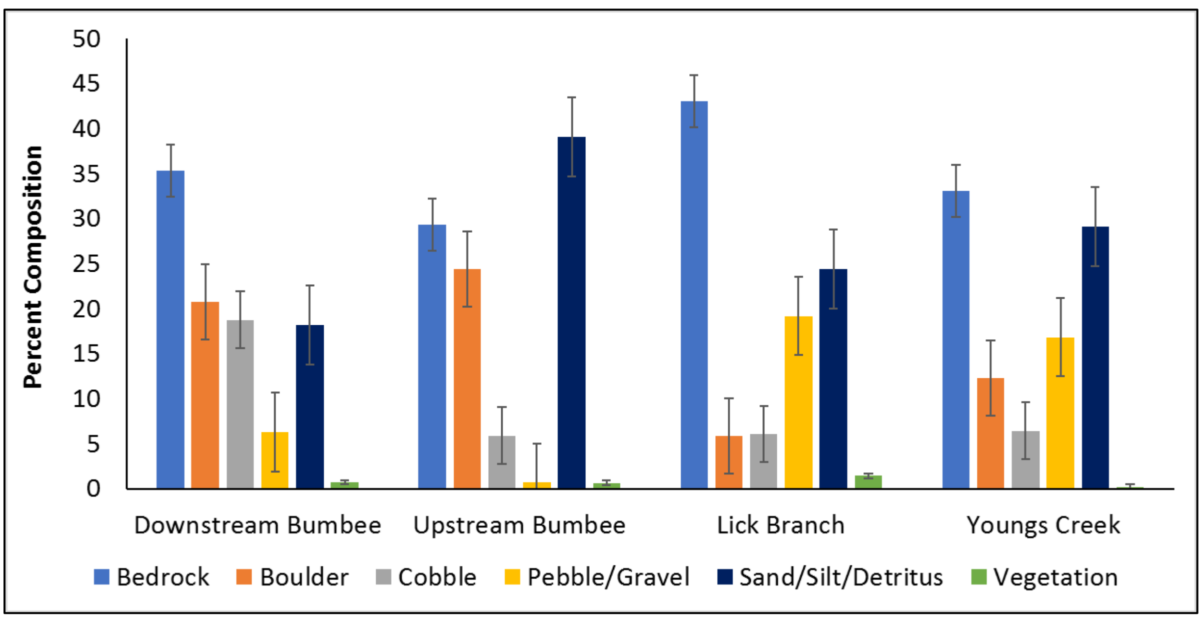

Figure 3.5. Percent composition of substrate types at all four sites in Bledsoe and Rhea counties, Tennessee.

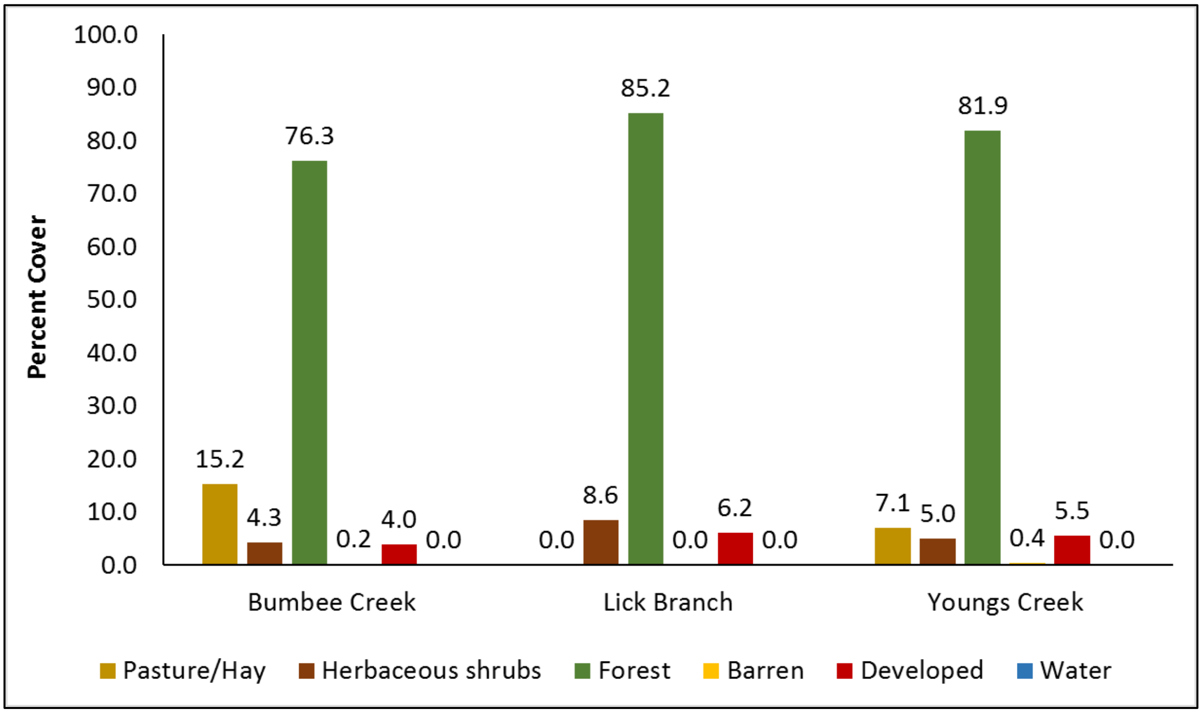

Figure 3.6. Percent land cover of the upstream watersheds of the four sites in Bledsoe and Rhea counties, Tennessee where habitat parameters were collected.

CHAPTER 4

SUMMARY AND CONCLUDING REMARKS

The Recovery Plan for the federally endangered Laurel Dace (*Chrosomus saylori*) stated information on life history, diet and habitat was necessary for the species' recovery and this study provides that information. Laurel Dace have an opportunistic life history and diet strategies and are tolerant of unpredictable changes in their environment, i.e., stream flow and discharge. Alterations of Laurel Dace habitat including siltation, and riparian vegetation removal change their habitat to a more constant and predictable environment. These predictable environments may allow species with equilibrium life history strategies like bass and sunfish to invade these streams and compete for resources with the Laurel Dace. Laurel Dace is tolerant of some silt, but excessive siltation could create a uniform stream bottom with less substrate variability and reduce the amount of available spawning substrate. Clear-cutting and removal of riparian vegetation increases stream temperatures as well as reduces the amount of terrestrial prey available for the Laurel Dace. Laurel Dace spawn at age-2 and can live to age 4+ but have a high annual mortality rate. While fecundity is unknown, Laurel Dace do have a long spawning season and even though their clutches are small, they are multiple clutch spawners. Laurel Dace have developed this life history strategy in order to persist in an unpredictable environment.

Management recommendations for the Laurel Dace are to encourage responsible land use on Walden Ridge and changes to the federal permitting that allows for sampling. Managers should work with foresters, farmers, and residential developers to implement best management practices (BMPs) that minimize stream siltation and avoid the loss of riparian vegetation and canopy cover. While this thesis does not extensively discuss the issue of invasive sunfish and bass species, yearly invasive species removals should be conducted to reduce their numbers to a level where they are no longer a burden to Laurel Dace or any other native fish species. Laurel Dace spawning season should be fully protected from human disturbance. The current United States Fish and Wildlife permit allows sampling for the Laurel Dace to start on the first day of June each year. It is recommended that the permit protects the entire Laurel Dace spawning season by moving the first date to sample for Laurel Dace to the first day of July, the likely end of their spawning season.

These recommendations will allow for future Laurel Dace recovery in streams where they have been extirpated. An improvement of habitat conditions along with the removal of invasive species will be the first steps towards recovery of the Laurel Dace. Once habitat conditions are suitable for natural recruitment and the correct protections are put in place, managers can start to consider how to repopulate the streams where Laurel Dace have been extirpated. The Laurel Dace is a rare endemic species to the Southeastern United States that deserves our time, attention, and protection and this study has helped further the knowledge recommended for their recovery.

VITA

Shawna Mitchell Fix grew up in Hillsdale, NY and received her A.A.S in Fisheries and Wildlife in 2014 and her B.T. in Fisheries and Aquaculture in 2015 from the State University of New York at Cobleskill. Shawna came to Chattanooga, TN to work for the Tennessee Aquarium Conservation Institute as a Science Coordinator soon after graduation and began working with threatened and endangered freshwater fishes. She is inspired every day by the work she gets to do in the field and by the wonderful people she has had the pleasure to meet and work with. She decided that in order to further her career, she would pursue her M.S. at the University of Tennessee studying a species that is near and dear to her heart, the Laurel Dace. Shawna lives in Chattanooga with her husband Kevin and their dog Roxy.