ABSTRACT

IVASAUSKAS, TOMAS JOHN. Early Life History of Suckers (Catostomidae) in a Southern Appalachian River System. (Under the direction of Dr. Thomas J. Kwak).

Suckers (Catostomidae) are a fish family that is of high conservation concern but has received relatively little attention from the scientific community. The knowledge gap in the early life history of suckers is related to the lack of efficient or standardized sampling methods and difficulty in identifying sampled fishes. The upper Hiwassee River system supports seven sympatric species of native suckers, including six species of redhorses (*Moxostoma*), which are vulnerable to effects of introduced species and habitat degradation. One of these species, the Sicklefin Redhorse *Moxostoma* sp., is imperiled with a restricted distribution and is of high conservation concern.

Efficiency of three techniques (drift netting, light trapping, and visual surveys) to sample larval fish assemblages was evaluated. The annual mean catch-per-unit-effort (CPUE) for drift nets was 1.64-1.70 suckers/100 m^3. The annual mean CPUE for light traps was 2.99-3.57 suckers/trap-night. Larval and juvenile suckers were visually observed at a mean density of 5.52 fish/m^2 and at quadrat densities up to 100 fish/m^2. The probability of catching suckers in light traps was negatively related to water velocity. Patch occupancy and abundance in visual surveys were also negatively related to water velocity, and no interspecific differences in habitat were detected.

By implementing a novel index of larval fish ontogeny and using genetic barcoding for species identification, phenology, ontogeny, and growth were estimated and compared among six sucker species. Emergence phenology paralleled previously described spawning phenology. Species with overlapping emergence periods exhibited significantly different mean ontogeny rates. Larval growth rates were similar among species, and high intraspecific size variability was observed. Differences in emergence timing, ontogeny, and size may mediate competition for food resources among sympatric larval suckers. Small innate differences in early life history among species likely facilitated the current sympatric diversity of suckers in the upper Hiwassee River system, or these differences may represent local adaptations that evolved as a result of specific competition pressures among species.

An understanding of the swimming ability of larval and juvenile fishes is important for identifying environmental influences during critical periods of early development. Swimming ability was studied via laboratory experiments, where water velocity was controlled and swimming behavior observed. A series of fixed velocity swimming tests was used to assess prolonged swimming speeds of larval and juvenile captive-reared Sicklefin Redhorse and four species of wild-caught suckers. The probability of prolonged swimming was negatively related to water velocity, positively related to fish total length, and differed slightly among species. The transition from pelagic to epibenthic swimming occurred at approximately the mid-point of the metalarva stage. Given the relative rarity of water velocity less than prolonged swimming speed for these fishes in riffles, short upstream migration may be possible, but is probably atypical.

Nonnative species introductions have been implicated as a major threat to the conservation of imperiled species. Blueback Herring *Alosa aestivalis* was introduced to the Hiwassee River system and became established in Hiwassee Lake by 1999. To evaluate possible predatory interactions of Blueback Herring with native sucker species, Blueback Herring were sampled from the Hiwassee River and Valley River using electrofishing. Stomach contents were examined visually and via genetic barcoding coupled with next-generation sequencing. Visual diet examinations detected 12 orders of aquatic invertebrates, as well as fish ova, fins, and larvae. Barcoding PCR allowed for identification of six sucker species in the Blueback Herring diet, but Sicklefin Redhorse was not detected.

The methods employed in this study addressed sampling considerations and demonstrated the effectiveness of genetics-based techniques for identifying cryptic samples. These findings may be applied to guide the conservation of suckers in the Hiwassee River and may be more broadly applied to other systems.

Early Life History of Suckers (Catostomidae) in a Southern Appalachian River System

by
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DEDICATION

This dissertation is dedicated to the mentors who have positively influenced my life.

BIOGRAPHY

I became fascinated by fisheries science during high school, when I participated in the American Fisheries Society's Hutton Program. Through the program, I was involved in various fisheries management activities and aquatic research projects conducted in Virginia. I obtained my Bachelor of Science degree in Fisheries Science from Virginia Tech in 2007. During that time, I also worked for the U.S. Forest Service Center for Aquatic Technology Transfer, where I conducted aquatic monitoring in National Forests throughout the southeastern USA. I obtained my Master of Science degree from Tennessee Tech University in 2009, under the guidance of Dr. Phil Bettoli. Subsequently, I worked for Tennessee Tech University, as Project Coordinator, on an assessment of the fish assemblage, recreational fishery, and water quality in the Caney Fork River. I began my doctoral education at North Carolina State University in Fall 2012, under the guidance of Dr. Tom Kwak. I have served as both Treasurer and President of the NCSU Student Fisheries Society. I promoted conservation of imperiled fishes by presenting preliminary reports of this research to a variety of audiences. Also during my tenure at NCSU, I mentored an undergraduate student who conducted research on the human dimensions of NCSU's on-campus fishery. I appreciate my experiences at NCSU and value the wealth of knowledge that I accrued from classes and by interacting with faculty, students, and collaborators. As I continue in my career, these lessons and experiences will be very useful. I look forward to influencing native fish conservation and contributing to the understanding of fisheries management.

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This project benefitted greatly from the input of several passionate conservationists, whom I would like to thank. Dr. Bob E. Jenkins spent many hours sharing his wisdom and passion for Sicklefin Redhorse, and through the process has become a friend. Scott D. Favrot provided his expertise and assistance in familiarizing me with the upper Hiwassee River system and Redhorse spawning habits. Bob Wallus tutored me in larval fish identification. Dr. Wayne C. Starnes provided useful suggestions and his assistance in identifying adult suckers. My father, Jonas A. Ivasauskas, helped extensively in designing and fabricating the swim tunnel apparatus used in this project. Steve J. Fraley, Mark A. Cantrell, and Jason Mays provided technical and logistical support. Thank you!

This project would not have been possible without the expertise of several professional collaborators. Dr. Morgan E. Raley (HydroGENomics) helped me plan the genetic component of this project, walked me through the genetic barcoding procedure, and assumed the role of an advice-giver. Dr. Heather K. Evans and the staff at North Carolina Museum of Natural Sciences facilitated the barcoding of numerous samples. Patrick L. Rakes, J. R. Shute, and Crystal Ruble (Conservation Fishes, Inc.) reared Sicklefin Redhorse in captivity and granted access to their facility and fish for swimming tests. Dr. Travis Glenn and W. Glenn Ballard (University of Georgia) completed the next-generation sequencing to

identify Blueback Herring stomach contents and aided in interpreting the results. Finally, the Sicklefin Redhorse Conservation Committee has provided me with continued insights and direction and outlet, where my research has already influenced the conservation of this species.

I would like to acknowledge my field assistants. In 2013, Jimmy Jenkins worked diligently on this project, and together we learned the skills required for conducting research on larval suckers. In 2014, Micah S. Qubeck worked industriously and had keen insights into refining sampling techniques. These gentlemen each lived with me for four months in beautiful Murphy, North Carolina. I thoroughly enjoyed their company, our experiences together, and most of all, working with them on this project. In 2014, Christopher Ackerman aided with fieldwork and lab-work as part of his undergraduate internship experience through NCSU; I am very grateful for his assistance. Spencer T. Gardner and James D. Wehbie assisted with fieldwork in 2015 and with lab-work, as needed. I would also like to thank all volunteers who assisted with the various components of this project.

Most importantly, I would like to thank my family and loved ones for their continued commitment, support, and confidence in me.

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CHAPTER 1: Research background and justification

Introduction

Effective conservation and management of inland fish populations requires a thorough understanding of stressors and other factors affecting survival at all life stages. Fish mortality is usually highest during the early life stages (Hjort 1914). As such, slight changes in the survival of larvae and juveniles can drastically influence recruitment dynamics (Chambers and Trippel 1997). Survival during early life stages has been linked to a number of factors, including the availability of appropriate nursery habitat (Scheidegger and Bain 1995), flow regime (Freeman et al. 2001), synchronous emergence with appropriate prey species (Cushing 1990), interspecific competition (Nunn et al. 2007), and predation (Fuiman and Magurran 1994). Due to growth and ontogeny, the specific requirements of fish during their early life stages are dynamic over time (Litvak and Leggett 1992). These requirements also differ among species because of interspecific differences in life history strategy, physiology, and morphology. Because these traits are most similar among closely related species, the specific requirements of larval and juvenile fishes tend to be most similar among closely related taxa (Fuiman 1985; Nunn et al. 2007). This dissertation outlines the early life history of suckers (family Catostomidae) in the Hiwassee River system. The sucker assemblage in the Hiwassee River system is composed of seven sympatric species in the tribe Moxostomatini, including six species of redhorse (*Moxostoma* spp.). Thus, the Hiwassee River system provided a model system for investigating and comparing early life history characteristics of suckers. The results of this study characterize traits that enable the match and mismatch of several sucker species with their prey and elucidate mechanisms involved in resource partitioning during early life stages. These findings can be applied to gain a deeper understanding of sucker evolution and distributional patterns and identify potential risks to species persistence.

Differences in spawning timing among adult redhorse have been documented and are considered an important reproductive isolating mechanism (Kwak and Skelly 1992; Grabowski and Isely 2007; Favrot 2009). These differences also serve to reduce interspecific

competition for similar food resources among larvae. The timing of reproduction in fishes has evolved to coincide with optimal food availability for offspring (Cushing 1990). A primary source of mortality during early life stages is starvation that may be density-dependent. Ontogenetic shifts in diet are common during early life stages, and larger individuals are more adept at locating, capturing, and competing for food (Miller et al. 1988; Sogard 1997). Furthermore, suckers shift from planktonic to benthic prey as they transition to juveniles (Markle and Clauson 2006). Thus, the staggered spawning and emergence sequence of suckers permits the dietary shift of some species before other species emerge. Spawning phenology has been described for several river systems in which the relative temporal sequences among species tended to correspond, despite discrepancies in absolute timing (e.g., Curry and Spacie 1984; Reid 2006; Favrot 2009; Catalano and Bozek 2015). One objective of this dissertation was to demonstrate that emergence phenology also follows a similar sequence across systems.

Resource partitioning occurs among larvae of distantly related species, where differences in microhabitat use are drastic, but partitioning among species occupying similar habitats is difficult to directly detect (Floyd et al. 1984; Childs et al. 1998; Markle and Clauson 2006). Slight differences in ontogeny rates (Chapter 3), and swimming ability (Chapter 4) indicate that some degree of resource partitioning among larval suckers is likely, despite high overlap in measured microhabitat parameters (Chapter 2). A wide variety of life history traits are at least moderately heritable in fishes (Carvalho 1993). Morphology, growth, and swimming performance of offspring have been linked to parental phenotypes (Powers et al. 1991; Langerhans et al. 2004). Thus, the interspecific differences in phenotypic traits examined in this dissertation may be the result of directional selection and local adaptation to competitive pressures. Although there was high interspecific overlap in all of the parameters that were measured, significant differences in the means and extremes of these parameters indicate that some of the larvae of each species are better able to utilize specific niches (e.g., food resources, nursery habitats) than most of the larvae of other species. Furthermore, these slight differences in early life history traits can manifest themselves as differences in recruitment, because interannual variation and stochastic events are likely to affect each of the species differently.

Community composition is determined by spatial, biotic, and abiotic factors (Jackson et al. 2001). The high diversity of suckers in the fish assemblage of the Hiwassee River can be explained by the interaction of these three factors. The Hiwassee River is geographically situated in the southern Appalachian Mountains and is a tributary to the Tennessee River. During the Pleistocene era, this region was free of glaciation, and the Tennessee River was reconfigured by river capture and a change in course of the mainstem. This resulted in a high diversity of native fish fauna and stimulated speciation within novel habitats and unique competition pressures (Etnier and Starnes 1993). The suckers comprising the Hiwassee River assemblage are all well-adapted to the local habitat of the Hiwassee River and its tributaries (Jenkins and Burkhead 1994; Jenkins 1999). According to the river continuum concept, the upper Hiwassee River and its tributaries tend to have low to moderate instream productivity and relatively high allochthonous inputs (Vannote 1980; Miranda et al. 2015). Such conditions promote colonization by macroinvertebrates, thereby providing an ample food resource for suckers (Grubaugh et al. 1997). Furthermore, the Hiwassee River is situated in a temperate climate and local fishes are adapted to typical flooding, drought conditions, and other stochastic events.

Of the seven species of sucker that inhabit the upper Hiwassee River system, six have relatively wide geographic ranges, but the Sicklefin Redhorse *Moxostoma* sp. is endemic to only the Hiwassee and Little Tennessee river systems. Both are tributaries to the Tennessee River, which historically facilitated migration between the two river systems and colonization of both. The Sicklefin Redhorse may have arisen from a hybrid origin, most likely between the River Redhorse *Moxostoma carinatum* and Smallmouth Redhorse *Moxostoma breviceps* (Jenkins 1999). Alternatively, it may have speciated sympatrically as a sister- or daughter-species to one of the extant forms of redhorse in the system. Its persistence indicates ample niche-space to support this diversity.

The Hiwassee River system has been strongly influenced by anthropogenic disturbances that have had profound effects on resident fish assemblages (Miranda et al. 2015). For example, the construction of dams extirpated Sicklefin Redhorse from an estimated 58% of its historic range (Jenkins 1999). In addition to ongoing concerns related

to construction and management of impoundments in the Hiwassee system, contemporary threats to the persistence of suckers and other fishes in the system include physical habitat degradation, nutrient loading, and nonnative species introductions. This dissertation demonstrates that larval and juvenile suckers require relatively slow moving nursery habitat (Chapters 2 and 4). Channelization and similar activities reduce the abundance of such habitat; likewise, changes in flow regime (i.e., frequency, speed, and duration) can impede a fish from accessing such habitat. More intensive land use within the Hiwassee River basin has resulted in increased nutrient runoff (Miranda et al. 2015). An increase in nutrient input can change the composition of invertebrate communities, thereby altering prey availability for suckers and other fishes at all life stages. Nonnative species introductions have been implicated as a cause of decline in native biodiversity and a major threat to conservation. The Blueback Herring *Alosa aestivalis* has been widely introduced into inland river systems, including the Hiwassee River. The potential for it to prey on larval fishes and fish ova is important for predicting its adverse effects on resident assemblages (Chapter 5). Thus, an understanding of early life stages is important for recognizing and mitigating the effects of anthropogenic disturbance.

Successful management of any fish population relies on an understanding of how it interacts with its environment during all life stages. This dissertation demonstrates disparities in size, swimming ability, and predation risk of larval suckers. Estimates of these characters were markedly different from values reported in existing literature for adults of the same species, which underscores the need to consider all life stages in conservation planning. Among the species studied for this project, the Sicklefin Redhorse is a candidate for federal protection by the Endangered Species Act and is currently protected by a Candidate Conservation Agreement. The conservation status of the other sucker species varies by jurisdiction, as a reflection of political boundaries and range limitations (Cooke et al. 2005). Despite conspicuous differences in various parameters within each species of sucker included in this project, interspecific differences were small. Thus, the findings of this research may be widely applied and indicate the utility of using a surrogate approach to provide information on fish larvae that cannot be studied directly (although such an approach should always be employed with caution, as differences may not always be minor). The findings

and associated methodologies presented herein can be widely applied by resource managers to gain a more thorough understanding of fish during the early life history critical period, to identify stressors affecting this stage, and to enhance conservation and management.

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CHAPTER 2: Efficiency of sampling techniques for larval suckers and other fishes in a Southern Appalachian river system

Abstract

Suckers (Catostomidae) are a fish family that is of high conservation concern but has received relatively little attention from the scientific community. The knowledge gap in the early life history of suckers is related to the lack of efficient or standardized sampling methods and difficulty in identifying sampled fishes. Efficiency of three techniques (drift netting, light trapping, and visual surveys) to sample larval fish assemblages was evaluated in the Hiwassee River system of North Carolina during spring 2013-2014. A random subset of suckers was selected from each sample for analysis and identification by a genetic barcoding procedure. Five species were represented in drift net samples and six species were in light trap and visual survey samples. The mean catch-per-unit-effort (CPUE) for drift nets was 1.70 suckers/100 m^3 in 2013 and 1.64 suckers/100 m^3 in 2014. The mean CPUE for light traps was 3.57 suckers/trap-night in 2013 and 2.99 suckers/trap-night in 2014. The likelihood of sucker catch in light traps was negatively related to water velocity, and floating traps had a higher proportion of a catch than sinking traps. Larval suckers were visually observed at a mean density, across samples, of 5.52 fish/m^2 and up to 100 fish/m^2 among quadrat densities. Patch occupancy and abundance of visual surveys were negatively related to water velocity, and no interspecific differences in habitat were detected. Drift net protocols did not sample any larval suckers on 30% of the occasions that other techniques did. The median size of suckers sampled in drift nets was slightly smaller than for light traps, and neither technique differed in fish size from that of visual surveys. The results of this study will guide future monitoring efforts for fish early life stages and the conservation of suckers in the Hiwassee River and other lotic ecosystems.

Introduction

Freshwater fishes represent one of the most imperiled groups of animals in North America and throughout the world (Jelks et al. 2008; Strayer and Dudgeon 2010). The cause for imperilment of many fish species can be linked to extensive anthropogenic alterations of freshwater ecosystems, including habitat modification, degradation, and fragmentation, and nonnative species introductions (Dudgeon et al. 2006; Cooke et al. 2012). Certain taxonomic and functional groups are particularly vulnerable to such modifications. Attributes that increase vulnerability include limited physiographic range, migratory behavior, ecological specialization, and long generation time (Angermeier 1995; Winemiller 2005; Olden et al. 2007). Many of the most imperiled freshwater fish species lack economic or recreational value, public awareness, and scientific understanding (Reynolds et al. 2002; Hogan 2011; Cooke et al. 2013). Suckers (Catostomidae), and especially redhorse (genus *Moxostoma*), comprise a taxon that is of high conservation concern but has received relatively little attention from the scientific community (Cooke et al. 2005).

Because fish mortality is usually highest during the early life stages (i.e., a life history bottleneck), it is important to develop an understanding of factors affecting fish during their early development (Hjort 1914). However, such information is lacking for the majority of freshwater fishes and for all sucker species (Cooke et al. 2005). Much of the knowledge gap in the early life history of suckers and other fishes is related to the lack of efficient or standardized sampling methods and difficulty in identifying sampled fishes. Common larval fish sampling techniques deployed in lotic systems include light trapping, drift netting, and dip netting (Kelso and Rutherford 1996).

Light trapping is a passive capture technique that is commonly used to sample photopositive larval fishes. Light traps are deployed nocturnally and utilize an artificial light source to attract fishes. Light traps are considered semi-quantitative sampling devices, because phototactic behavior can differ among species and developmental stages (Floyd et al. 1984; Doherty 1987; Miller and Shanks 2005). The efficiency of light traps in streams may depend on the location where the trap is set, current velocity, water clarity, and the locomotive ability of sampled fishes (Doherty 1987; Marchetti et al. 2004; Lindquist and

Shaw 2005). For most species, catch rates in stationary light traps are expected to be highest at minimal water velocities or increase slightly with moderate current (i.e., linear velocities < 0.3 m/sec) as more water is sampled; however, a linear decrease in catch rate is expected as faster current interferes with catchability (Doherty 1987; Lindquist and Shaw 2005). Light trap samples are particularly useful for estimating the occurrence of a species at a site (Kelso and Rutherford 1996; Niles and Hartman 2007; Neal et al. 2012). Sucker species encountered in light traps include June Sucker *Chasmistes liorus* (Billman 2008), Northern Hog Sucker *Hypentelium nigricans* (Floyd et al. 1984), Razorback Sucker *Xyrauchen texanus* (Mueller et al. 1993), Flannelmouth Sucker *Catostomus latipinnis* (Muth and Haynes 1984), buffalo species *Ictiobus* spp., and other unidentified sucker species (Dibble et al. 1995; Knight and Bain 1996; Marchetti et al. 2004; Niles and Hartman 2009).

Drift netting is a passive sampling technique that is used in flowing water and is effective for sampling drifting larval fish. Larval drift has been documented for many riverine fish species (Brown and Armstrong 1985). Larval drift is an important means of dispersal for young-of-year fish and may represent an evolutionary adaptation that enables early life stages to develop in productive nursery areas downstream of spawning sites. Larval drift tends to follow a diel pattern, and most species tend to drift nocturnally, although some species are diurnal or crepuscular (Gale and Mohr 1978; Muth and Schmulbach 1984; Reeves and Galat 2010). Larval drift has been documented for unidentified redhorses (D'Amours et al. 2001), Shorthead Redhorse *Moxostoma macrolepidotum* (Gale and Mohr 1978; Bednarski et al. 2008), White Sucker C*atostomus commersonii* (Corbett and Powles 1986), and four sucker species in Colorado (Snyder et al. 2004).

Dip netting, or sweep netting, is an active sampling technique that can be used to collect larval fishes in shallow and structurally complex habitats (Kelso and Rutherford 1996). This method is suitable for collecting many species of larval fish, including suckers (Childs et al. 1998; Billman 2008; Bunt et al. 2013). Parameters describing microhabitat can be measured at the precise point of capture for samples collected in this manner. Because it is difficult to standardize and quantify effort using dip netting, analysis of data obtained with this gear is typically limited to presence-absence or abundance categorization (Childs et al.

1998; Falke et al. 2010). However, relative densities and habitat associations can be estimated using standardizing protocols such as point abundance sampling (Copp 2010).

Larval fish ecology is rarely studied on a population level because it is difficult to visually identify larval fishes to lower taxonomic ranks with any degree of certainty, especially when sampled from a system with multiple confamilials that are meristically and morphometrically similar (Fuiman 1979; Kelso and Rutherford 1996). Fishes in the family Catostomidae are especially difficult to visually distinguish at early life stages, and there are no criteria available to visually distinguish young suckers (Kay et al. 1994). Larval fishes in the genus *Hypentelium* may occasionally be distinguished from those of *Moxostoma* based on slight differences in pigmentation, especially during later stages of the larval interval; however, they are physically similar to other suckers with significant overlap in meristic characters (Buynak and Mohr 1979; Fuiman 1979). Differentiating among juvenile congeners is also difficult and problematic, as external characters may not be well-formed and positive identifications may only be possible by comparing internal features such as the pharyngeal arch (Vachon 2003; R.E. Jenkins, Roanoke College, personal communication). Genetic methods have been previously used to differentiate between young suckers; electrophoresis was used to distinguish Golden Redhorse *Moxostoma erythrurum* from Silver Redhorse *Moxostoma anisurum* (Morgan et al. 1983), and analyses using mitochondrial DNA have been used to discriminate among multiple sympatric redhorse species (Branchaud 1996; Wirgin et al. 2004). Monitoring larval Robust Redhorse *Moxostoma robustum* relied on genetic procedures for the identification of sampled individuals (Peterson et al. 2008). Recent advances in molecular genetic procedures (i.e., genetic barcoding) have enabled researchers to identify larval and juvenile fishes relatively rapidly and with an exceptionally high degree of accuracy (Ward et al. 2009; Ko et al. 2013; Pereira 2013).

The Sicklefin Redhorse *Moxostoma* sp. was first recognized in 1992 by R.E. Jenkins and is restricted to the Hiwassee and Little Tennessee river systems (Jenkins 1999). It is currently a high priority candidate for listing under the Endangered Species Act and is protected by a Candidate Conservation Agreement. The Sicklefin Redhorse has been previously subjected to misclassification; the earliest known cataloged specimen was

collected in 1937 and misidentified as Black Redhorse *Moxostoma duquesnei* (Jenkins 1999), and in the Freshwater Fishes of North Carolina, Menhinick (1991) presented a drawing of a Sicklefin Redhorse that was misidentified and cataloged as a River Redhorse *Moxostoma carinatum*. Like other redhorses, the Sicklefin Redhorse has a fusiform body shape, somewhat enlarged pelvic fins, and a subterminal mouth. In contrast to congeners, Sicklefin Redhorse has a moderately sized head, well-rounded snout, and a highly falcate dorsal fin (Jenkins 1999). Since the species' discovery, identifying characteristics, range, habitat, life history, and reproductive habits of adults have been investigated and compared to other sympatric redhorse species (Jenkins 1999; Favrot 2009). However, the early life history of the species remains virtually unknown.

In the Hiwassee River system, the Sicklefin Redhorse co-occurs with five other species of redhorse upstream of Hiwassee Lake and Dam, including Black Redhorse, Golden Redhorse, River Redhorse, Silver Redhorse, and Smallmouth Redhorse *Moxostoma breviceps*. Interbreeding and hybridization among these sympatric congeners is rarely observed, due to highly specific and effective reproductive isolating mechanisms (e.g., timing, habitat, behavior, and morphology; Kwak and Skelly 1992; Grabowski and Isely 2007; Favrot 2009). These species all demonstrate a potamodromous migratory strategy (i.e., they migrate entirely in freshwater; Myers 1949). In the Valley River, a tributary to the Hiwassee River that is used for spawning by all species, Silver Redhorse tends to spawn earliest in the season, followed by Smallmouth Redhorse, Black Redhorse, Sicklefin Redhorse, Golden Redhorse, and River Redhorse, with considerable interspecific temporal overlap. Smallmouth Redhorse tends to spawn the farthest downstream in the Valley River, followed upstream by Silver Redhorse, River Redhorse, Sicklefin Redhorse, Golden Redhorse, and Black Redhorse, with considerable interspecific spatial overlap (Favrot 2009). Within the current extent of the species' range, adult Sicklefin Redhorse and other sympatric suckers have been sampled using boat electrofishing, prepositioned areal electrofishers, fish weirs, and large seines (Jenkins 1999; Favrot and Kwak 2016; J. G. Davis, Young Harris College, personal communication). Unlike adults, however, young-of-year suckers in the Hiwassee River have not previously been studied.

The lack of information on sucker larvae, in general, and the absence of information on those in the Hiwassee River system in particular, prompted an intensive study with field components carried out in 2013 and 2014. The objectives of this study were to (1) evaluate the efficacy of three common techniques (drift netting, light trapping, and visual surveys) for sampling larval and juvenile suckers, (2) apply genetic barcoding to estimate the species composition among sampled suckers, (3) compare size- and taxon-selectivity among gear-types, and (4) identify key attributes of stream nursery habitat.

Methods

Study Site

This study was primarily conducted on the Valley River, a major tributary to the Hiwassee River (Figure 1). The Valley River drains 303 km^2 in the southern Appalachian Mountains (Dobson and Wallus 2004). The Valley River was selected because it has previously been identified as the primary spawning tributary for Sicklefin Redhorse in the Hiwassee drainage basin (Favrot 2009). It originates in the Snowbird Mountains in western North Carolina and flows southwest, through the towns of Andrews, Marble, and Murphy to its confluence with the Hiwassee River. The confluence and downstream 2-km portion of the Valley River is inundated by Hiwassee Lake when reservoir levels are near full (summer) pool. During 2013, the reservoir filled rapidly as a result of several intensive rainfalls, and attained full summer pool in early-May. During 2014, the reservoir attained full summer pool by the end of May.

The Hiwassee River of the Tennessee River drainage flows through northern Georgia, western North Carolina, and southeastern Tennessee. Its mainstem channel is fragmented by (in descending order) Chatuge Dam, Mission Dam, Hiwassee Dam, and Appalachia Dam. The range of Sicklefin Redhorse is restricted to the Hiwassee River upstream of Hiwassee Dam and downstream of Mission Dam, and tributaries within this reach (Jenkins 1999). Hiwassee Lake is impounded by Hiwassee Dam, which was completed in 1940 for recreation, hydroelectric power generation, and flood control (TVA 2004). The reservoir is

oligotrophic and has a variable surface area that is approximately 2,400 ha at full summer pool, and water levels fluctuate approximately 13 m from winter to summer.

Five standard sampling sites were established for this study (Figure 1). Standard sampling sites were established at Hiwassee River rkm 153 (hereafter referred to as the Hiwassee Site, immediately downstream of the confluence, near the boat ramp on Payne Road), Valley River rkm 1.5 (Site 1, adjacent to Konehete Park in Murphy, North Carolina), Valley River rkm 9.5 (Site 2, near the privately-maintained hanging bridge and river access adjacent to Wells Road SR 1554), Valley River rkm 21 (Site 3, near the Forrest Hargett access-point and Pacesetters Camp adjacent to Fairview Road SR 1515), and Valley River rkm 29 (Site 4, adjacent to the U.S. Highway 19 Rest Area near Andrews, North Carolina). Sampling at these sites included an approximately 2-km reach centered around the nominal river distance (rkm).

Temperature (°C) was continuously monitored at each sampling site in 2014 using HOBO temperature loggers (Model U22 Pro; Onset Computer, Bourne, Massachussetts). Temperature loggers were weighted using cinder blocks. Those in the Valley River were set at points that were not in the thalweg but received constant flow and were perpetually submersed. The temperature logger in the Hiwassee River was repositioned as the reservoir filled, such that it was at a depth of 0.5-2.0 m for all readings.

Laboratory Procedures

All sampled fish were returned alive to the laboratory for analysis. Fish were transferred from sampling gears to 1.9 L containers filled with river water, which were transported in a cooler and frozen gel packs, as needed. A random subset of fish that were visually identified as suckers was selected from each sample for inclusion in a genetic barcoding procedure. A Leica EZ-4 HD microscope (Leica Microsystems, Heerbrugg, Switzerland) was used to inspect and photograph these specimens at 8x magnification. Fish were sedated and euthanized using MS-222 (tricaine methanesulfonate). Fish total length (TL) was measured to the nearest 0.01 mm using computer software (Leica Microsystems;

LAS EZ v. 2.1.0). Larval fish were preserved whole, in individual 2-mL polypropylene vials filled with molecular grade ethanol (Fisher Scientific, Waltham, Massachusetts; BP2818). For juvenile fish, upper lobes of caudal fins were preserved in 2-mL vials filled with molecular grade ethanol and bodies were preserved in a 10% buffered formalin solution and retained as vouchers. To avoid cross-contamination between samples, microscopy tools and surfaces were cleaned with chlorine bleach and rinsed between samples. The fish that were not selected for inclusion in genetic barcoding procedures were euthanized in MS-222 and preserved in the formalin solution. They were later counted, and those that were visually identified as suckers were measured for TL using a digital caliper.

Larval and juvenile suckers that were classified to species were typically identified using genetic barcoding, but older larval and juvenile Northern Hog Suckers could occasionally be positively identified based on pigmentation and distinguishing characteristics. Budgetary considerations precluded using this technique on every larval fish sampled, but subsampling provided a coarse estimate of the species composing samples obtained in this study.

Genetic barcoding was carried out by the North Carolina Museum of Natural Sciences following standard laboratory procedures (NCMNS, Raleigh, North Carolina). Samples were stored at -20^{o}C until analysis. Tissues were prepared by cutting them each to a suitable size and loading them into a 96-well plate. A solution composed of Proteinase K and Buffer T1 was added to each well to lyse cell membranes, a centrifuge was used to pull tissue into the lysis solution, and a nutator mixer circulated the solution as cells were digested for at least 12 hours. Next, the genetic material was isolated by adding a buffer (BQ1) that established conditions that caused DNA to bind to the filter plate, rinsing proteins from the silica membrane using vacuum suction and buffers B5 and BW, and finally, releasing the genetic material from the filter plate into solution by washing with the elution buffer (BE). The concentration of nucleic acid in each resulting sample solution was determined using a nanodrop spectrophotometer and was diluted to achieve a normalized concentration of approximately 40 ng/µL of genetic material. Next, a master-mix consisting of primers, taq (enzymes, buffers, and fluorescent dideoxynucleotides [fluorophores]), and MgCh was added

and placed in a thermocycler to facilitate a polymerase chain reaction (PCR). A final cleaning process using exo-sap-IT digested any remaining single-stranded or short strands of DNA and unincorporated fluorophores. Ethanol, sodium acetate, and EDTA were used to precipitate DNA from the sample. An Applied Biosystems 3130xl genetic analyzer machine (Foster City, California) carried out a Sanger di-deoxy sequencing procedure, which determined the order of dideoxynucleotides based on relative positions of fluorophores. Any sequences that did not adhere to the standards of the Barcodes of Life (Ward et al. 2009) were omitted, and corresponding samples were subsequently reanalyzed.

Analysis of genetics data was conducted by HydroGENomics (Raleigh, North Carolina). Basic Local Alignment Search Tool software (BLAST; U.S. National Laboratory of Medicine, Bethesda, Maryland) was used to compare larval fish sequences with reference sequences. For these references, I sequenced a total of 22 adult suckers, including at least two individuals belonging to each of the seven species present. These fish were collected on 17 April 2013, from the mainstem of the Hiwassee River immediately downstream of its confluence with the Valley River, using boat-mounted electrofishing. They were initially identified using morphological features (Menhinick 1991; Jenkins and Burkhead 1994). Tissue samples were obtained from the right pectoral fins and preserved in molecular grade ethanol. Fish were euthanized in MS-222, and bodies were preserved in a buffered formalin solution. Voucher specimens and associated tissue were catalogued in the NCMNS reference collection (Appendix).

Drift Nets

Sampling using drift nets was conducted 15 May-2 July 2013 (13 evenings) and 2 April-1 July 2014 (31 evenings). Drift netting was not conducted at Hiwassee Site or Site 1 after those sites became inundated. On each sampling occasion, two drift nets were simultaneously deployed. Drift nets used in this study were manufactured by Aquatic Research Instruments (Hope, Idaho). Each net was constructed on a rectangular steel frame (46 x 28 cm), measured 1 m in length, with 0.5-mm mesh, with a removable cod-end. Drift nets were anchored by driving steel stakes into the substrate.

Sampling commenced 30 min after sunset, with two drift nets that were each set for two 30-min intervals. Drift nets were set in riffle and run macrohabitats. They were each fished at the water surface, with the lower edge of the upper lip positioned even with the water's surface, which minimized turbulence in front of the net. Nets were only deployed in areas in greater depth than the net (28 cm). The mesh was manually brushed clean every 10-min, or more frequently if needed. Catch-per-unit-effort (CPUE) was calculated for each 30-min set and was expressed both in terms of number of larval suckers per 30-min set, and per 100 m^3 of filtered water (D'Amours et al. 2001; Bednarski et al. 2008; Reeves and Galat 2010). The quantity of water filtered through a net was calculated based on the velocity at the center-point for each net, which was measured prior to positioning the net using a Marsh McBirney Flo-Mate 2000 flow meter. Other ancillary data were recorded at the point of each deployment and included macrohabitat type (i.e., fast- or slow-water), distance to the nearest bank, water depth, and dominant substrate material.

After each 30-min set, all collected contents (fish, organic matter, particulates) were removed from the cod-end, transferred into a shallow sorting tray, and immersed in water. Contents were then sorted on-site, in the dark, using headlamps. Sorting samples in such a manner was advantageous, as photo-positive fish tended to swim toward the light, and researchers utilized light reflected from the tapetum lucidum, shadows, and contrasting shades to discern fish from extraneous matter. Fish were separated from other extraneous matter by agitating the material and pipetting swimming fish, and picking through the material and removing dead or immobile fish using forceps or a pipette.

Mean catch-per-unit-effort of suckers (CPUE; fish/30-min set and fish/100 m^3) and associated standard deviation were calculated for each driftnet sampling occasion (i.e., date x site). The median, 25th, and 75th percentiles of sucker TL were reported in lieu of the mean because catch rates varied with size. The species composition of drift net samples was quantified by randomly selecting five sampled fish from each drift net that caught at least five fish, or all fish from each net that caught less than fish suckers, and calculating the overall percent composition of each species in these randomly selected subsamples.

Light Traps

Sampling using light traps was conducted over 36 nights in 2013 (8 April-1 July) and 43 nights in 2014 (2 April-3 July). On each occasion, four to six light traps were simultaneously deployed. The light trap used in this study was similar to that deployed in another study (Bettoli and Goldsworthy 2011; Figure 2). This trap design was shallow enough to be deployed in most sections of the Valley River and has one opening, which was positioned perpendicular to the current or facing slightly upstream. The light source for each trap was a small waterproof LED flashlight that operated on one AAA battery and emitted up to 13 lumens of white light (Fenix Lighting, Lone Tree, Colorado, E01). Batteries were replaced prior to every set. Previous research found that white light was effective for attracting phototactic species (Gyekis et al. 2006; Pierce et al. 2007; Neal et al. 2012). During each sampling event, two floating and two benthic traps were deployed; floating traps had a piece of Styrofoam affixed to the top and were anchored in place by a system of ropes and anchors, whereas benthic-set traps were held in position by placing a weight on top of the trap. Traps were set immediately following sunset and retrieved shortly after sunrise. Effort was expressed as trap-nights. Any trap that was not still properly set when retrieved (i.e., tampered with, displaced by current, or otherwise altered) was excluded. The date associated with any given light trap event refers to the date that traps were set.

Traps were deployed without lights on four dates in May 2015 to assess whether larval suckers were attracted to the lights in light traps or if the traps were functioning mainly as activity traps. One unlit floating and one unlit sinking trap were deployed on each date, simultaneous with two lighted floating light traps and two lighted sinking light traps. The proportion of unlit traps with no catch of suckers was compared to the proportion of lighted traps with zero catch of suckers, on dates when both were set, by using a χ^2 contingency table. Unlit traps were excluded from all other analyses.

For each date and site, the proportion of lighted traps with catch was reported. The mean, minimum, and maximum number of suckers and other species (unidentified, grouped) caught among light traps was also reported. Species composition was estimated by first performing genetic barcoding on all fish from traps that caught less than five fish, and at least

five fish from every trap that caught more than five fish, and visually identifying any juvenile Northern Hog Suckers; next, the proportion of fish belonging to each species was estimated based on genetics data; finally, a weighted mean was calculated among traps, and the weights depended on the total catch of suckers in the trap. The median, 25th, and 75th percentiles of sucker TL were reported in lieu of the mean because catch rates varied with size.

Logistic regression modeling was used to relate catch of suckers at sites in the Valley River to several variables. Traps that did not catch any suckers were modeled as 0, and those that captured one or more suckers as 1. Continuous variables recorded for each trap included distance to the nearest bank, water depth, and water velocity at the opening of the trap. Categorical variables included macrohabitat type, dominant and subdominant substrate materials, presence of macrophytes within a 1.5-m radius, presence of woody debris within a 1.5-m radius, and presence of boulders within a 1.5-m radius. Using data from both years, beginning on the date after the first sucker was encountered in light traps, a stepwise selection procedure was employed to test the significance of these variables and the effect of trap orientation (i.e., sinking or floating) on light trap catch, where variable entry into and elimination from the model was contingent on $P \leq 0.10$. Because interannual differences in factors related to light trap efficiency were not expected, data were pooled across the two years. Models were developed using Proc Logistic in SAS 9.4 (SAS Institute, Inc., Cary, North Carolina)

Visual Surveys

During 2013, larval fish were found by wading or walking along the bank, and visually searching for larval fish for a variable period of time. Surveys were conducted 15 May-10 July 2013 during the day. At points where fish were found, the following habitat parameters were measured: macrohabitat type, distance to closest bank (m), depth (m), mean-column velocity (m/s), and presence of woody debris within a 1-m radius, presence of boulders within a 1-m radius. Fish were collected using dip nets, which were fabricated from large aquarium nets (frame opening: 25 cm x 18 cm, 1.6 mm mesh) mounted to a 1.5-m long wooden handle. At least one fish collected from each point was subjected to genetic

barcoding for species identification, and was assigned to one of three ontogeny classes (i.e., mesolarva, metalarva, or juvenile; Snyder 1976). These data were used to compare habitat use among larval sucker species by applying a principal components analysis in Primer v. 6.1.16 (Clarke and Gorley 2006).

During 2014, a more formal visual survey design, designed to quantify near-shore larval and juvenile fish abundance, was employed. Surveys were conducted 29 April-1 July 2014 after dark. These surveys were conducted four times at each site on the Valley River and twice on the Hiwassee River. For each survey, an approximately 0.5-km transect was walked, along the stream-bank, to search for larval and juvenile fishes. Observations were taken at 10 evenly-spaced points along the transect. The researcher then focused on a 1 x 1 m square quadrat adjacent to the bank, using a headlamp and hand-held flashlight for illumination. The number of fish in each quadrat was counted, indicating the abundance within the quadrat when it was initially encountered. The position of each counted fish in the water column (i.e., pelagic or epibenthic) was noted. At each quadrat, the adjacent macrohabitat type, depth (m), and mean-column velocity (m/s; absolute value, taken at the center point of the quadrat) were measured. During each survey, fish were dip netted from at least 3 of the quadrats. From these, at least five were selected, at random, to describe the composition of species observed.

For each quantitative visual survey, the mean number of suckers per quadrat was calculated. Among surveys conducted in the Valley River, patch occupancy (i.e., presence of at least one sucker) and trends in abundance were related to one categorical variable (adjacent habitat type) and two continuous variables (water velocity and depth). Logistic regression was used to relate patch occupancy (suckers present or absent) to these variables. A Poisson generalized linear model (GLM) was applied to model abundance. Logistic regression modeling was performed using Proc Logistic, and the Poison GLM was compiled using Proc GenMod (SAS 9.4).

Efficiency Comparison

Simultaneously employing multiple techniques at the same site on the same date provided a means for comparisons of catch rate among the techniques. Drift nets and light traps were paired on 12 dates in 2013 and 15 dates in 2014 where at least one of the techniques sampled larval fish. Visual surveys were paired with drift nets on 14 dates and with light traps on 17 dates in 2014. Relationships among drift net CPUE, light trap CPUE, and mean visual survey counts were assessed using correlation coefficients (r). Extreme outlying observations (i.e., those that were more than five times greater than the mean) were excluded from calculations because they were probably indicative of an encounter with a large aggregation rather than mean abundance.

Because catch rates varied with size, a non-parametric procedure was used to assess size-differences between light traps and drift nets. The median size of suckers observed in light traps and drift nets was compared using a Cochran-Mantel-Haenszel test, implemented in Proc Freq (SAS 9.4). Median TL was compared between the two gear-types while controlling for sample event. Only occasions that resulted in non-zero catches were included in this analysis.

Results

Median monthly discharge in the Valley River was higher in 2013 than in 2014 (USGS gauge 03550000; Figure 3). In general, temperature in the Valley River and Hiwassee River increased between April and July, was slightly higher in Hiwassee River and Hiwassee Lake, and increased slightly along the downstream gradient of the Valley River (Figure 4).

A total of 962 sucker specimens were identified using genetic barcoding. The technique was useful for positively identifying and differentiating between the species of sucker collected in this study. However, the cost and labor associated with employing this technique precluded its use on more than a subsample of fish. Smallmouth Redhorse was the

only sucker species known to be present in the Hiwassee River system that was absent from samples identified using genetic barcoding,

Larvae from many other fish species known to inhabit Hiwassee Lake and the Valley River were collected in samples. In Hiwassee Lake, commonly collected larvae (in addition to suckers) included Common Carp *Cyprinus carpio*, black bass *Micropterus* spp., and other sunfishes *Lepomis* spp. In the Valley River, commonly collected larvae included Central Stoneroller *Campostoma anomalum*, Longnose Dace *Rhinichthys atratulus*, shiners (Cyprinidae), and darters *Etheostoma* spp. and *Percina* spp.

Drift Nets

Drift nets were deployed in riffles and runs in depth 29-101 cm (mean = 49.8, SD = 15.4), in water velocity ranging 0.05-1.01 m/s (mean = 0.50, SD = 0.20), and 0.5-13.7 m (mean = 6.0, SD = 3.4) from the nearest bank. During 2013, larval suckers and other fish were first sampled on 15 May, and the mean catch rate of that and successive samples was 1.73 suckers/set (SD = 1.76) or 1.70 suckers/100 m^3 (SD = 1.79; Table 1). During 2014, larval suckers were first sampled on 5 May, and the mean catch rate of that and successive samples was 2.15 suckers/set (SD = 2.71) or 1.64 suckers/100 m^3 (SD = 1.97; Table 2). Calculation of this mean excluded one extremely high catch rate observed on 9 June 2014, which was atypical of drift net samples and likely the result of an encounter with a drifting aggregation. Across samples, and excluding the outlier, the two representations of catch rate (suckers/set and suckers/100 m^3) were highly correlated (r = 0.95).

The median size of suckers in drift nets in May 2013 (16.2 mm, 25th percentile = 15.4, 75th percentile = 17.4) was larger than in June 2013 (15.4 mm, 25th percentile = 14.6, 75th percentile = 17.1). Similarly, the median size of suckers in drift nets in May 2014 (17.3 mm, 25th percentile = 15.9, 75th percentile = 18.6) was larger than in June 2014 (15.5 mm, 25th percentile = 15.0, 75th percentile = 16.2). Five sucker species were collected in drift net samples each year (Tables 1 and 2).

Light Traps

Light traps in the Valley River were deployed at points where depths were 7-100 cm (mean = 35.9, SD = 19.4), in water velocities 0.00-0.39 m/s (mean = 0.06, SD = 0.08), and 0.0-10.0 m (mean = 1.1, SD = 1.3) from the bank. Among all light traps, 34% were within 1 m of woody debris, 10% were within 1 m of macrophytes, and 18% were within 1 m of boulders. They were most frequently deployed in habitats where the dominant substrate was sand (33%), cobble (25%), or silt (16%). Suckers were caught in traps deployed at depths 7-100 cm (mean = 35.2, SD = 19.3), with water velocities 0-0.32 m/s (mean = 0.04, SD = 0.09), and 0.0-10.0 m from the bank (mean = 1.1, SD = 1.2). Among traps with suckers, 37% were within 1 m of woody debris, 11% were within 1 m of aquatic macrophytes, and 15% were within 1 m of boulders. Suckers were most typically sampled from habitats where the dominant substrate was sand (35%), cobble (23%), or silt (16%).

During 2013, larval suckers were first sampled using light traps on 12 May, and the mean catch rate of that and successive samples taken in the Valley River was 3.57 suckers/trap-night (SD = 2.81; Table 3). During 2014, larval suckers were first sampled in the Valley River on April 29, and the mean catch rate of that and successive samples was 2.99 suckers/trap-night (SD = 2.77; Table 4). Calculation of this mean excluded one extremely high catch rate observed on 27 May 2014, which likely resulted from a chance encounter with an aggregation.

The stepwise logistic model indicated that light trap sucker catch (as presence) was negatively related to water velocity (Wald $\chi^2 = 4.3186$, df = 1, $P = 0.0377$). It also indicated that floating traps had a higher proportion of catch than sinking traps (Wald $\chi^2 = 3.7929$, df = 1, $P = 0.0515$). Accounting for these variables, no other measured variable was significant in determining light trap sucker catch. Based on the model, at least one sucker was caught in 50% of floating light traps set in 0.20 m/s water velocity, and at least one sucker was caught in 50% of sinking traps set in 0.10 m/s water velocity (Figure 5). Light trap catch did not differ among sites in the Valley River (Wald $\chi^2 = 2.0989$, df = 3, $P = 0.5521$) or with any other variable tested.

The median size of suckers in light traps in May 2013 (16.4 mm, 25th percentile = 15.7, 75th percentile = 17.0) was smaller than the median size in June 2013 was (18.1 mm, 25th percentile = 16.3, 75th percentile = 19.4). Conversely, the median size of suckers in light traps in May 2014 (17.7 mm, 25th percentile = 16.2, 75th percentile = 18.5) was larger than the median size in June 2014 (16.5 mm, 25th percentile = 15.4, 75th percentile = 19.1). Six species of sucker were represented in light trap samples during both years (Tables 3 and 4).

On the four dates in 2014 that lighted and unlighted light traps were set simultaneously, none of the 8 unlit traps caught any suckers (or any larval fish of any species), while 13 of the 16 lighted traps caught suckers (and other larval fish species). This indicated a significant difference in efficiency that favored lighted traps ($\chi^2 = 14.1818$, df = 1, $P = 0.0002$).

Visual Surveys

During 2013, visual surveys were effective at obtaining qualitative samples of species presence at exact points. Six species of sucker were sampled from these surveys, representing the mesolarval, metalarval, and juvenile life-stages (Table 5). Among the species, no interspecific differences in habitat (in terms of mean-column velocity, depth, or distance from bank) were apparent from the PCA (Figure 6). Suckers were encountered in the Valley River in depths ranging 8-75 cm (mean = 31.5, SD = 15.1), water velocities ranging 0.00-0.14 m/s (mean = 0.05, SD = 0.04), and at distances from the bank up to 7 m (mean = 1.4, SD = 1.7).

During 2014, larval suckers were visually observed within 1 m of the bank during the night at all sites, at a mean density of 5.52 fish/m^2 and up to an estimated 100 fish/m^2 (Table 6). Among all surveys conducted in lotic habitat, the mean column velocity was 0.07 m/s (standard deviation = 0.11, range = 0.00-0.70 m) and mean depth was 16.9 (standard deviation = 11.6, range = 3-68 m). Among all quadrats where suckers were present, the mean column velocity was 0.06 m/s (standard deviation = 0.07, range = 0.00-0.41 m) and

mean depth was 16.2 (standard deviation = 12.4, range = 3-68 m). Pelagic larvae were less abundant at all sites in the late-June survey, as compared to preceding surveys. A large proportion of patches occupied by pelagic larval suckers had ≥ 5 suckers/m^2 (67%), which reflected clumped distributions associated with aggregating behavior. As expected, the count-data approximated a Poisson distribution (Figure 7). Patch occupancy by larval and juvenile suckers was not related to depth (Wald χ^2= 1.0206, df = 1, P = 0.2810), but was negatively related to water velocity (Wald χ^2 = 4.1808, df = 1, P = 0.0409) and was greater in quadrats adjacent to fast-water habitats (Wald χ^2 = 4.9153, df = 1, P = 0.0266). Correspondingly, abundance was not related to depth (Wald χ^2 = 0.3149, df = 1, P = 0.2810), but was negatively related to water velocity (Wald χ^2= 50.65, df = 1, P < 0.0001) and was greater in quadrats adjacent to fast-water habitats (Wald χ^2 = 6.30, df = 1, P = 0.0121).

Comparison and Synthesis

Among dates, estimates of CPUE for each gear type were highly variable and followed similar trends, but did not fluctuate consistently (Figure 8). Drift net protocols did not sample any larval suckers on 30% of the occasions that light traps did; conversely, light traps always sampled suckers on occasions that drift nets did. Drift nets did not sample any larval suckers on 29% of occasions that systematic visual surveys did; conversely, visual surveys always sampled larval suckers when drift nets did. Light traps did not sample any suckers on 6% of occasions that systematic visual surveys did; conversely, visual surveys did not sample any larval suckers on 6% of occasions that light traps did. Indices of abundance obtained from the various sampling techniques weakly positively correlated with one another (Figure 9).

The length-frequencies of suckers sampled with each of the three techniques increased abruptly and decreased gradually, and the smallest fish were sampled with drift nets (Figure 10). Accounting for sampling event, the median size of suckers was significantly larger for light traps than drift nets (R_{CMH} = 399.404, df = 311, P = 0.0005). No differences were detected for visual surveys and drift nets (R_{CMH} = 191.274, df = 177, P =

0.2194) or light traps (R_{CMH} = 342.590, df = 331, P = 0.3188). The median size of suckers in light traps was larger than in drift nets on 72% of occasions, by an average of 1.5 mm.

Black Redhorse, Golden Redhorse, River Redhorse, Sicklefin Redhorse, Silver Redhorse, and Northern Hogsucker were all sampled via light traps and visual surveys. All except River Redhorse were sampled via drift nets. Smallmouth Redhorse was never sampled. Black Redhorse and Sicklefin Redhorse were most frequently encountered at upstream sites, whereas River Redhorse and Silver Redhorse were most frequently encountered at downstream sites, and Golden Redhorse and Northern Hog Sucker were distributed ubiquitously in the Valley River. In all gear types, Northern Hog Sucker was the most commonly collected species, and persisted in Valley River samples across dates. All species that were sampled in Valley River, except Sicklefin Redhorse, were also sampled in Hiwassee River or Lake.

Discussion

Despite its potential, genetic barcoding has been previously underutilized for investigating the early life history and ecology of freshwater larval fishes (Peoples et al. 2017). I am unaware of any previous study that uses genetic barcoding to identify freshwater larval fish as a means to clarify species compositions in sampling gears or to compare habitat use among sympatric species. However, the ability to accurately identify sampled larval fishes is essential for understanding their early life histories. The genetic barcoding procedure employed in this study was effective at assigning species-level identifications to larval suckers and at successfully differentiating among the seven species in the Hiwassee River system. As expected for closely related species, there was low genetic variation among the suckers (e.g., the Sicklefin Redhorse differs from River Redhorse by approximately 2%). This necessitated high quality genetic data that adhered to the standards set forth by the Barcodes of Life (Ward et al. 2009), and thus, there was certainty in species identities (Pereira 2013). Budgetary considerations precluded using this technique on every larval fish that was sampled, and although subsampling reduced the resolution of estimated species composition, it provided a coarse estimate of the species composing the various samples

obtained in this study. This information is valuable in guiding future studies on suckers, in terms of allocating sampling effort and refining the apportionment of subsamples for genetic barcoding.

Due to the staggered spawning schedule of suckers in the system, sucker larvae were present throughout May and June of 2013 and 2014. However, the species composition fluctuated as each respective cohort grew, developed, and experienced high mortality typically associated with the larval period (Hjort 1914). Despite the occasional observation of juveniles, all of the gear types tested in this study were most suited for sampling fish during their larval life stage (Kelso and Rutherford 1996).

Occupancy of the four sites on the Valley River was related to the spawning locations of adults. Black Redhorse and Golden Redhorse have been observed spawning near Site 2 and up to the headwaters of the Valley River (Favrot 2009). Black Redhorse and Golden Redhorse larvae were distributed ubiquitously across sites. River Redhorse has been observed spawning downstream of Site 2 and upstream nearly to Site 4. Although rarely sampled, River Redhorse larvae were present at downstream sites. Sicklefin Redhorse have been observed spawning upstream of Site 2 upstream to beyond Site 3. Sicklefin Redhorse larvae were present at Site 2 but more abundant at the two most upstream sites. Silver Redhorse were observed spawning at Site 1 and upstream beyond Site 2. Silver Redhorse larvae were typically sampled at downstream sites. In previous studies, which documented many observations of spawning among the various redhorse species in the Valley River, spawning among Northern Hog Suckers was never observed (R. E. Jenkins, Roanoke College, personal communication). In other systems, Northern Hog Sucker spawns in habitat consisting of shallow gravel riffles, which are abundant in all reaches of the Valley River (Jenkins and Burkhead 1994). Northern Hog Sucker larvae were likewise ubiquitous across sites. The absence of Smallmouth Redhorse in samples was due to its tendency to spawn downstream of all sites located on the Valley River.

Drift nets yielded periodically high catch rates of suckers, particularly on dates when Black Redhorse and Golden Redhorse were encountered. Although this may indicate a greater tendency of these species to engage in larval drift, the higher catch rate of suckers on

these dates was also influenced by the simultaneous emergence of later-emerging species (Favrot 2009; Chapter 3). Pilot sampling indicated that drift net sampling during the daytime was ineffective. Like other species of larval fish, the suckers that were studied in this research typically enter the drift after dark (Gale and Mohr 1978; Muth and Schmulbach 1984; Reeves and Galat 2010). The mesh size on drift nets (0.5 mm) was suitable for capturing drifting sucker larvae, but a larger mesh would reduce the need to clean the net during samples and the amount of particulate matter in the cod end. Because of the need to both tend the drift net and sort through particulate matter collected, this passive sampling technique required constant activity. It is also noteworthy that the churning of materials in the cod end during sampling resulted in high mortality and disfigurement of samples.

Light traps were effective for sampling six species of larval sucker. The small LED flashlight in the trap was essential for attracting larval suckers, as demonstrated by the lack of catch by unlighted traps set concurrently in equally suitable habitat. I personally observed the reaction of larval suckers to light traps on several occasions. They tended to be drawn into the vicinity of the light trap, where they formed loose aggregations, and occasionally entered the trap. This behavior indicated that they were either slightly photopositive or that they were attracted to photopositive prey (i.e., zooplankton and other microcrustaceans and small invertebrates). Previous studies characterized light trap samples as semi-quantitative (Floyd et al. 1984; Bettoli and Goldsworthy 2011). However, comparisons among and between sites were complicated by the high rate of occurrence of no catch, the high variability in catch among traps, and significant covariation of catch with microhabitat variables (e.g., water velocity). Additionally, the present study demonstrated that light traps are size-biased, which must be considered when interpreting results from this gear-type.

Dip netting samples were not directly quantified because extremely large sample sizes could be quickly obtained if researchers focused efforts on suitable habitats. Sample sizes may be more indicative of effort in such habitats, rather than true larval fish abundance. Consistent with other studies (e.g., Falke et al. 2010), dip netting was less labor intensive and far more effective than light traps or drift nets for obtaining large samples of larvae. Using the methods established in this study, only the near-shore larval fish assemblage was

included in visual surveys in 2014. Because water velocity is typically lower near stream banks, quadrats were inherently placed in superior habitat. Larval fish were encountered farther than 1 m from the bank in light traps, drift nets, and visual surveys collected in 2013. Depending on relative abundance of fish in habitats farther than 1 m from the bank, the index of abundance produced by the visual survey protocol may be biased, and any extrapolation to the entire stream area would be inaccurate.

Numerically, Northern Hog Suckers dominated the larval catch. This was due to their presence at all sites, persistence through the sampling period, and overall high abundance. The high abundance of Northern Hog Sucker larvae reflected differences in adult abundance and possibly in life history strategy. Sampling using prepositioned areal electrofishing in 2006 and 2007 indicated higher abundances of adult Northern Hog Sucker in all habitats than any of the Redhorse species (Favrot and Kwak 2016). The high abundance of Northern Hog Sucker larvae and juveniles, relative to the redhorses, may reflect differences in life history strategy between genera. Northern Hog Suckers grow to a smaller maximum size (up to 507 mm), attain maturity faster (age-2 for most males and age-3 for most females), and have a shorter lifespan (up to 11 years) than most of the redhorse species in this study (Jenkins and Burkhead 1994; Jenkins 1999). Thus, it is rational that the Northern Hog Sucker is more *r*-selected than the redhorses on the *r*-*K* continuum, with higher reproductive output and natural mortality through life (Gunderson 1980).

River Redhorse and Sicklefin Redhorse were notably less abundant in samples than other species, and Smallmouth Redhorse was absent from all samples. River Redhorse and Sicklefin Redhorse are both known to spawn concurrently with the Golden Redhorse and later than the other species (Favrot 2009). Thus, fewer larvae of these species were encountered because they only became available for sampling later in the season. Furthermore, River Redhorse larvae were only sampled at downstream sites, whereas Sicklefin Redhorse larvae were typically sampled at upstream sites. Smallmouth Redhorse was absent from samples because it exhibits minimal upstream migration for spawning (Favrot 2009).

Light traps and visual surveys indicated that point occupancy by suckers was negatively related to water velocity. This was likely related to the swimming ability of larval suckers (Chapter 4). On occasions when discharge was high, sucker larvae were observed utilizing current-breaks or were in lower-velocity habitats on the floodplain. Furthermore, suckers were more abundant at points adjacent to swift-water habitat; if threatened by a predator or disturbance, this swift water may provide a route for escape and dispersal. Analysis of microhabitat measurements showed that the six species occupied similar habitats. In fact, some of the aggregations that were sampled in 2013 included at least two species of sucker, and sometimes incorporated larvae of other families. Although larval suckers oriented themselves with other fish in the aggregation, they did not demonstrate classic schooling behavior, in terms of highly synchronized movements (Pitcher 1993). This behavior likely conferred little protection from predators on its own (Johnson et al. 1993). Although results of the present study suggest a lack of habitat partitioning among larval suckers, differences in ontogeny and swimming ability may facilitate partitioning of other resources (Childs et al. 1998; Markle and Clauson 2006; Chapters 3 and 4).

The weak correlation among sampling techniques indicated that each was independently suited for sampling a portion of the sucker assemblage with variable biases. Relative to the other two protocols, drift nets had highly variable catch rates, and the highest proportion of samples without suckers when other concurrently deployed methods indicated sucker presence. This is related to the drift behavior of larval fish, which is non-constant across time, and is influenced by ontogeny and a variety of abiotic factors (Pavlov et al. 2008). The slightly smaller fish that were collected in drift nets indicated that larval drift is more prevalent at earlier life stages. In shallow lotic habitats of the Valley River, visual surveys and light traps both confirmed the presence of suckers on nights that they were present. However, more suckers were encountered using the visual survey protocols and data associated with the visual survey was more directly interpretable because it was free of those biases commonly associated with light traps (Doherty 1987; Lindquist and Shaw 2005). Due to depth, lack of flow, and habitat heterogeneity, lentic sites were most effectively sampled using light traps. For example, light traps sampled suckers at Site 1, after it was inundated by the downstream lake, when drift netting was not practical and visual surveys did not sample

any suckers. Because of the high variation in catch rates among all protocols, robust estimates of larval abundance would require more replication and a high amount of sampling effort.

Occupation of Hiwassee Lake was confirmed for larval Black Redhorse, Golden Redhorse, River Redhorse, Silver Redhorse, and Northern Hog Sucker. These fishes may have been transported via drift from the Valley River, from a different tributary, or may have been spawned in the mainstem of the Hiwassee River. Because Hiwassee Lake is a recent anthropogenic modification, the life histories of suckers in the Hiwassee River system evolved in the context of a free-flowing mainstem, rather than a lentic impoundment. Involuntary displacement of larvae into inferior downstream habitats has previously been suggested as a factor limiting recruitment for some suckers (Kennedy and Vinyard 1997; Peterson et al. 2008). Although surface temperature at the site on Hiwassee Lake was generally warmer than any of the sites on the Valley River, the mean temperature difference during July 2014 between the Hiwassee Site and the coolest (i.e., most upstream) Valley River site was only 1.6°C. Therefore, temperature in the reservoir is not an obvious limiting factor to sucker survival. However, survival and subsequent recruitment may be affected by the physical habitat of the reservoir, which lacked current and shallow water. Survival might also be affected by higher rates of predation in the reservoir, exacerbated by the lack of refuge nursery habitats (Pringle 1997; Franssen and Tobler 2013). Blueback Herring *Alosa aestivalis*, an introduced fish in Hiwassee Lake, has been shown to prey on larval fishes, including suckers (Davis and Foltz 1991; Wheeler et al. 2004; Chapter 5). Finally, recruitment in the reservoir may be adversely affected by a mismatch between suckers and their prey due to the radically altered invertebrate community in lentic surroundings (Cushing 1990).

By developing effective sampling techniques and protocols, combined with modern technology to identify larval suckers and other juvenile fishes, a better understanding of their early life histories can be elucidated. This knowledge is crucial for designing monitoring protocols and implementing management and recovery strategies, but is lacking for most species. The results of this study may guide the conservation of Sicklefin Redhorse and other

suckers in the Hiwassee River system and be more broadly applied to gain a better understanding of the early life stages of suckers and other fishes in lotic ecosystems.

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Tables

Table 1. Summary of 2013 drift net sampling at four sites. Mean catch-per-unit-effort (CPUE) for suckers is expressed in terms of fish/30-min set and fish/100 m^3 of filtered water. Mean CPUE for all other fishes is expressed in terms of fish/30-min set. Species composition is reported as proportions (BR = Black Redhorse, GR = Golden Redhorse, SFR = Sicklefin Redhorse, SR = Silver Redhorse, NHS = Northern Hog Sucker). Dashes indicate no genetic analysis performed.

		Sucker CPUE				Others CPUE		Species Composition				
Site	Date	Fish/set	SD	Fish/100 m^3	SD	Fish/set	SD	BR	GR	SFR	SR	NHS
Site 1	16 May	0.00	0.00	0.00	0.00	0.00	0.00					
Site 2	21 May	0.00	0.00	0.00	0.00	0.00	0.00					
	16 June	3.25	3.95	2.17	2.42	11.75	15.2		0.60			0.40
	26 June	3.00	5.35	2.01	3.50	9.75	10.5	–	–	–	–	–
	2 July	0.00	0.00	0.00	0.00	2.50	3.11					
Site 3	15 May	0.50	0.58	0.76	0.88	2.25	3.86				0.33	0.67
	29 May	1.75	2.36	1.52	2.02	7.25	8.54	0.50				0.50
	4 June	0.00	0.00	0.00	0.00	1.00	0.00					
Site 4	23 May	3.00	3.16	3.86	4.16	1.50	1.22				0.14	0.86
	30 May	2.25	1.89	3.03	2.72	3.75	4.50					1.00
	11 June	3.75	2.63	3.37	2.54	4.00	1.41	0.38	0.13	0.25		0.25
	19 June	5.00	2.83	5.37	2.66	7.25	6.13	0.67		0.22		0.11
	1 July	0.00	0.00	0.00	0.00	7.00	2.16					

Table 2. Summary of 2014 drift net sampling at five sites. Mean CPUE for suckers is expressed in terms of fish/30-min set and fish/100 m^3 of filtered water. Mean CPUE for all other fishes is expressed in terms of fish/30-min set. The species composition is reported as proportions (BR = Black Redhorse, GR = Golden Redhorse, SFR = Sicklefin Redhorse, SR = Silver Redhorse, NHS = Northern Hog Sucker).

		Sucker CPUE				Others CPUE		Species Composition				
Site	Date	Fish/set	SD	Fish/100m^3	SD	Fish/set	SD	BR	GR	SFR	SR	NHS
Hiwassee	3 April	0.00	0.00	0.00	0.00	0.00	0.00					
	11 April	0.00	0.00	0.00	0.00	0.00	0.00					
	22 April	0.00	0.00	0.00	0.00	0.00	0.00					
Site 1	2 April	0.00	0.00	0.00	0.00	0.00	0.00					
	16 April	0.00	0.00	0.00	0.00	0.00	0.00					
	24 April	0.00	0.00	0.00	0.00	0.00	0.00					
	29 April	0.00	0.00	0.00	0.00	0.00	0.00					
	8 May	0.00	0.00	0.00	0.00	0.00	0.00					
	20 May	0.50	1.00	0.35	0.71	1.00	0.00				0.50	0.50
Site 2	10 April	0.00	0.00	0.00	0.00	0.00	0.00					
	14 April	0.00	0.00	0.00	0.00	0.00	0.00					
	28 April	0.00	0.00	0.00	0.00	0.00	0.00					
	5 May	2.25	1.50	1.57	1.07	0.75	0.50				0.11	0.89
	3 June	8.25	3.20	4.65	1.80	0.25	0.50		1.00			
	26 June	0.25	0.50	0.13	0.26	0.25	0.50		1.00			
Site 3	7 April	0.00	0.00	0.00	0.00	0.00	0.00					
	9 April	0.00	0.00	0.00	0.00	0.00	0.00					
	21 April	0.00	0.00	0.00	0.00	0.00	0.00					
	27 April	0.00	0.00	0.00	0.00	4.00	3.10					
	6 May	0.25	0.50	0.17	0.33	0.00	0.00					1.00
	15 May	0.00	0.00	0.00	0.00	0.00	0.00					
	27 May	5.75	1.50	4.03	0.78	1.25	0.96	0.83		0.17		
	9 June	17.50	8.39	13.41	6.28	4.75	4.11		0.90			0.10
	18 June	4.50	1.29	4.06	1.34	0.75	1.50		1.00			
	23 June	1.00	1.41	0.95	1.27	2.75	4.86		0.33			0.67
Site 4	8 April	0.00	0.00	0.00	0.00	0.00	0.00					
	13 April	0.00	0.00	0.00	0.00	0.00	0.00					
	25 April	0.00	0.00	0.00	0.00	0.00	0.00					
	12 May	0.75	0.96	0.59	0.79	0.50	0.58					1.00
	16 June	4.50	2.38	4.84	2.56	1.25	1.26		0.80	0.20		
	1 July	0.00	0.00	0.00	0.00	1.00	1.41					

Table 3. Summary of 2013 light trap sampling at 5 sites. Proportions of traps that caught ≥ 1 of any species and that caught ≥ 1 sucker are reported as proportion with catch. Mean CPUE (fish/trap-night) is indicated separately for suckers and all other fishes. Species composition is reported as proportions (BR = Black Redhorse, GR = Golden Redhorse, RR = River Redhorse, SFR = Sicklefin Redhorse, SR = Silver Redhorse, NHS = Northern Hog Sucker). Dashes indicate no genetic analysis performed.

		Proportion with catch		Mean CPUE		Species Composition					
Site	Date	Any Fish	Suckers	Suckers	Others	BR	GR	RR	SFR	SR	NHS
Hiwassee	12 May	1.00	1.00	3.67	1.67					1.00	
	3 June	1.00	0.50	1.00	4.00	–	–	–	–	–	–
	18 June	0.50	0.00	0.00	1.00						
	24 June	1.00	0.00	0.00	1.00						
Site 1	8 April	0.00	0.00	0.00	0.00						
	27 April	0.00	0.00	0.00	0.00						
	7 May	0.00	0.00	0.00	0.00						
	12 May	0.67	0.33	1.00	5.33					1.00	
	27 May	1.00	1.00	2.00	14.00					1.00	
	25 June	1.00	0.00	0.00	1.50						
Site 2	15 April	0.00	0.00	0.00	0.00						
	22 April	0.00	0.00	0.00	0.00						
	30 April	0.67	0.00	0.00	1.67						
	1 May	0.00	0.00	0.00	0.00						
	2 May	0.20	0.00	0.00	0.60						
	8 May	0.00	0.00	0.00	0.00						
	13 May	0.75	0.25	11.00	1.75						1.00
	19 May	0.50	0.25	1.50	1.75					0.80	0.20
	21 May	0.50	0.50	1.50	0.50						1.00
	28 May	0.67	0.67	5.00	4.00						1.00
	1 June	1.00	1.00	2.50	5.50	0.80					0.20
	16 June	0.75	0.75	6.00	1.25	0.16	0.33		0.07		0.45
	26 June	0.67	0.67	3.33	4.00	0.10	0.48	0.43			
Site 3	18 April	0.00	0.00	0.00	0.00						
	26 April	1.00	0.00	0.00	1.00						
	8 May	0.67	0.00	0.00	0.67						
	15 May	1.00	0.50	2.25	3.75						1.00
	29 May	0.75	0.75	7.00	10.75						1.00
	4 June	1.00	1.00	6.50	3.50	0.83	0.17				
Site 4	22 April	0.00	0.00	0.00	0.00						
	15 May	1.00	0.50	0.50	11.00						
	23 May	1.00	0.80	1.80	10.80						1.00
	30 May	0.67	0.33	5.67	13.67						1.00
	6 June	1.00	1.00	4.75	1.25	0.36					0.64
	19 June	1.00	1.00	4.50	7.50		1.00				
	1 July	1.00	1.00	1.00	1.50						1.00

Table 4. Summary of 2014 light trap sampling at 5 sites. Proportions of traps that caught ≥ 1 of any species and that caught ≥ 1 sucker are reported as proportion with catch. Mean CPUE (fish/trap-night) is indicated separately for suckers and other fishes. Species composition is reported as proportions (BR = Black Redhorse, GR = Golden Redhorse, RR = River Redhorse, SFR = Sicklefin Redhorse, SR = Silver Redhorse, NHS = Northern Hog Sucker).

		Proportion with catch		Mean CPUE		Species Composition					
Site	Date	Any Fish	Suckers	Suckers	Others	BR	GR	RR	SFR	SR	NHS
Hiwassee	3 April	0.00	0.00	0.00	0.00						
	22 April	0.00	0.00	0.00	0.00						
	28 April	0.75	0.75	1.67	2.33						1.00
	7 May	1.00	1.00	15.67	1.00					1.00	
	21 May	0.50	0.50	7.50	1.25		0.33			0.51	0.16
	5 June	0.75	0.00	0.00	1.50						
	11 June	0.75	0.50	3.00	2.00		1.00				
	17 June	1.00	0.60	15.00	4.60	0.12	0.88				
	25 June	1.00	0.50	1.00	5.25		0.75	0.25			
	2 July	0.50	0.00	0.00	1.00						
	3 July	1.00	0.50	0.67	19.6	0.25	0.75				
Site 1	2 April	0.00	0.00	0.00	0.00						
	16 April	0.00	0.00	0.00	0.00						
	24 April	0.25	0.00	0.00	0.25						
	29 April	0.75	0.50	1.75	1.00					0.18	0.82
	8 May	0.50	0.50	1.00	2.50					0.50	0.50
	20 May	1.00	1.00	8.25	0.50					0.35	0.65
	2 June	0.50	0.50	5.33	3.33		0.63			0.34	0.03
	23 June	0.67	0.00	0.00	1.00						
Site 2	10 April	0.00	0.00	0.00	0.00						
	14 April	0.00	0.00	0.00	0.00						
	28 April	0.50	0.00	0.00	3.00						
	5 May	0.50	0.50	1.25	0.25					0.40	0.60
	22 May	1.00	1.00	6.25	7.25	0.27				0.13	0.59
	3 June	1.00	1.00	5.25	0.75	0.38	0.63				
	19 June	0.00	0.00	0.00	0.00						
	26 June	0.50	0.25	1.00	0.50						1.00
Site 3	9 April	0.00	0.00	0.00	0.00						
	21 April	0.00	0.00	0.00	0.00						
	30 April	0.75	0.50	1.25	1.25						1.00
	6 May	1.00	0.25	0.75	3.75						1.00
	15 May	0.50	0.50	3.25	0.00					0.33	0.67
	27 May	0.67	0.67	52.17	4.33	0.63					0.37
	9 June	1.00	1.00	10.00	8.75	0.84	0.03		0.09		0.06
	18 June	1.00	0.75	1.75	6.00		0.64				0.36
	23 June	0.67	0.67	2.33	15.67		0.43		0.29		0.29
Site 4	8 April	0.00	0.00	0.00	0.00						
	13 April	0.00	0.00	0.00	0.00						
	25 April	0.25	0.00	0.00	0.25						
	12 May	0.75	0.75	3.25	2.00						1.00
	28 May	0.40	0.20	1.20	3.40	0.25					0.75
	16 June	1.00	0.75	4.50	1.50	0.88	0.07				0.06
	1 July	1.00	0.50	1.50	5.75		0.50		0.50		

Table 5. Species of sucker collected from the Valley River, using a dipnet, in 2013 and subsequently identified using a genetic barcoding procedure and classified according to ontogeny. Presence of mesolarvae (Ms), metalarvae (Mt), and juvenile (J) stages is indicated for each species. (BR = Black Redhorse, GR = Golden Redhorse, RR = River Redhorse, SFR = Sicklefin Redhorse, SR = Silver Redhorse, NHS = Northern Hog Sucker).

	Date	BR	GR	RR	SFR	SR	NHS
Site 1	22 May					Ms	Ms
	13 June	Ms, Mt	Ms				
	14 June		Ms	Ms, Mt			
	18 June		Ms				
Site 2	20 May					Ms	Ms
	28 May						Ms
	29 May						Ms
	10 June		Ms, Mt				Mt
	11 June		Ms		Ms		Ms, Mt
	12 June						Mt
	13 June						Mt
	18 June		Ms				
	19 June		Ms	Ms			J
	24 June						J
	25 June						J
	3 July						Mt, J
	10 July	J	Ms,Mt				J
Site 3	15 May						Ms
	20 May						Ms
	29 May						Ms
	30 May						Ms
	10 June						Ms
	18 June						Ms
	25 June						Mt
	9 July				Mt		Mt, J
Site 4	20 May						Ms
	23 May						Ms
	31 May						Ms
	10 June						Ms, Mt
	11 June						Ms
	14 June	J					Ms, Mt
	24 June						Ms
	28 June						Mt
	8 July						Mt, J

Table 6. Summary of visual quadrat count surveys conducted for larval and juvenile suckers during 2014. The percent of 1 m quadrats occupied is indicated (% Occ). The mean and maximum count, among surveyed quadrats, is reported separately for fish that were observed swimming in a pelagic or epibenthic orientation. The species composition is reported as proportions (BR = Black Redhorse, GR = Golden Redhorse, SR = Silver Redhorse, SFR = Sicklefin Redhorse, NHS = Northern Hog Sucker). Dashes indicate that genetic analysis was performed but not accompanied by a visual survey, or that the survey was performed but not accompanied by genetic analysis.

			Pelagic		Epibenthic		Species Composition				
Site	Date	% Occupied	Mean	Max	Mean	Max	BR	GR	SFR	SR	NHS
Hiwassee	14 May	60	4.2	35	0	0				0.86	0.14
	5 June	10	1.1	11	0	0		1.00			
Site 1	29 April	40	4.8	30	0	0					1.00
	8 May	40	4.5	35	0	0					1.00
	20 May	90	9.1	37	0	0				0.38	1.00
	2 June	0	0	0	0	0					
	23 June	10	0	0	0.1	1					
Site 2	5 May	–	–	–	–	–				0.11	0.89
	12 May	60	5.9	30	0	0					1.00
	22 May	80	6.3	25	0	0					1.00
	3 June	80	6.2	40	0.1	1		0.63			0.37
	26 June	30	0	0	0.4	2	–	-	-	-	-
Site 3	15 May	60	6.4	40	0	0					1.00
	27 May	100	6.4	20	0	0	0.68		0.04		0.28
	9 June	90	12.6	50	0.4	2		0.46	0.27		0.27
	18 June	50	12.5	100	0.7	6		0.75			0.25
	23 June	30	0.1	1	0.8	6		1.00			
Site 4	12 May	90	9.6	43	0	0					1.00
	29 May	70	13.4	40	0	0			0.08		0.92
	16 Jun	60	2.3	10	1.3	7					1.00
	1 July	40	0	0	1.1	7					1.00

Figures

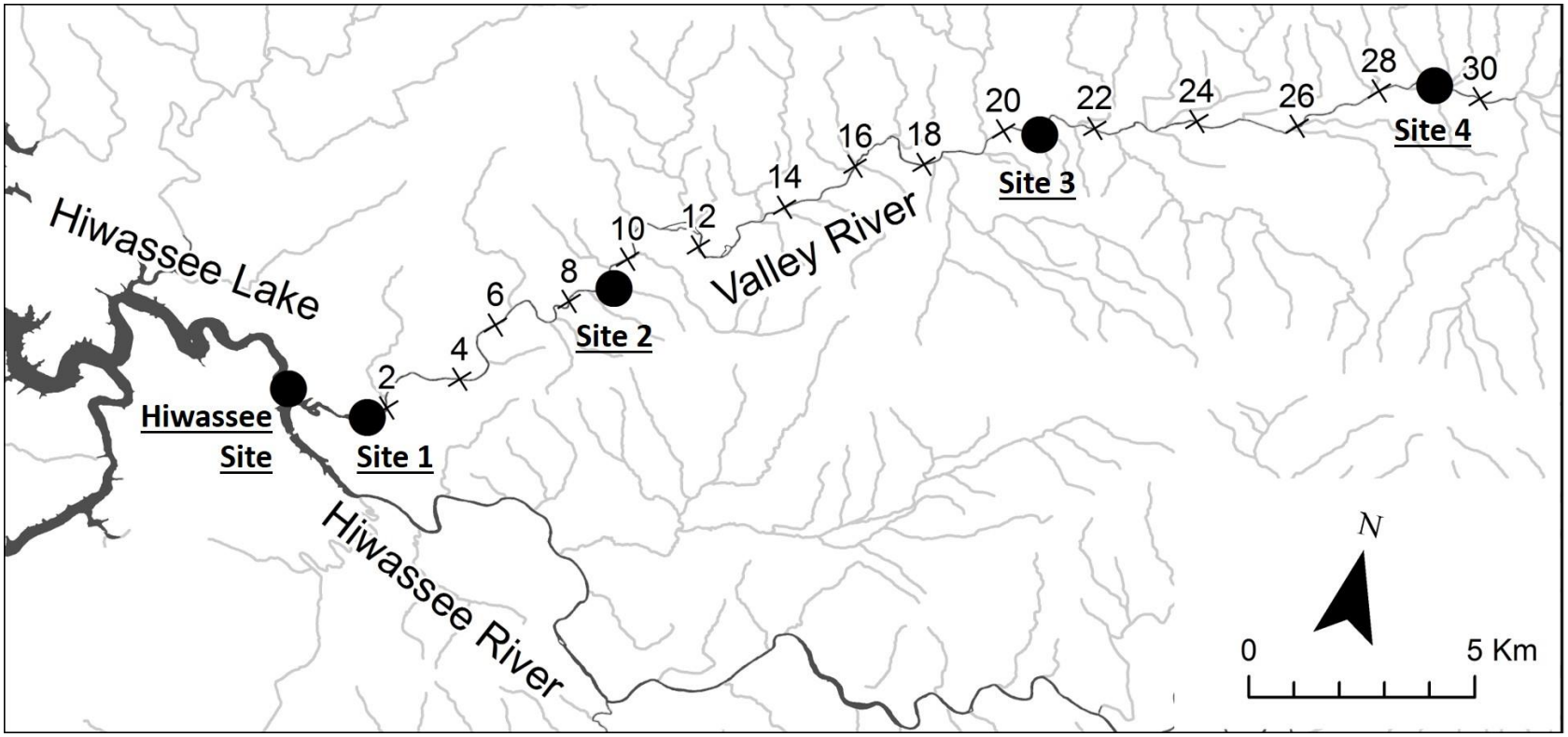

Figure 1. Map of the Hiwassee River and Valley River upstream of Hiwassee Lake, Cherokee County, North Carolina. Points with underlined labels indicate sites where sampling was conducted. Each × and associated number indicates Valley River rkm.

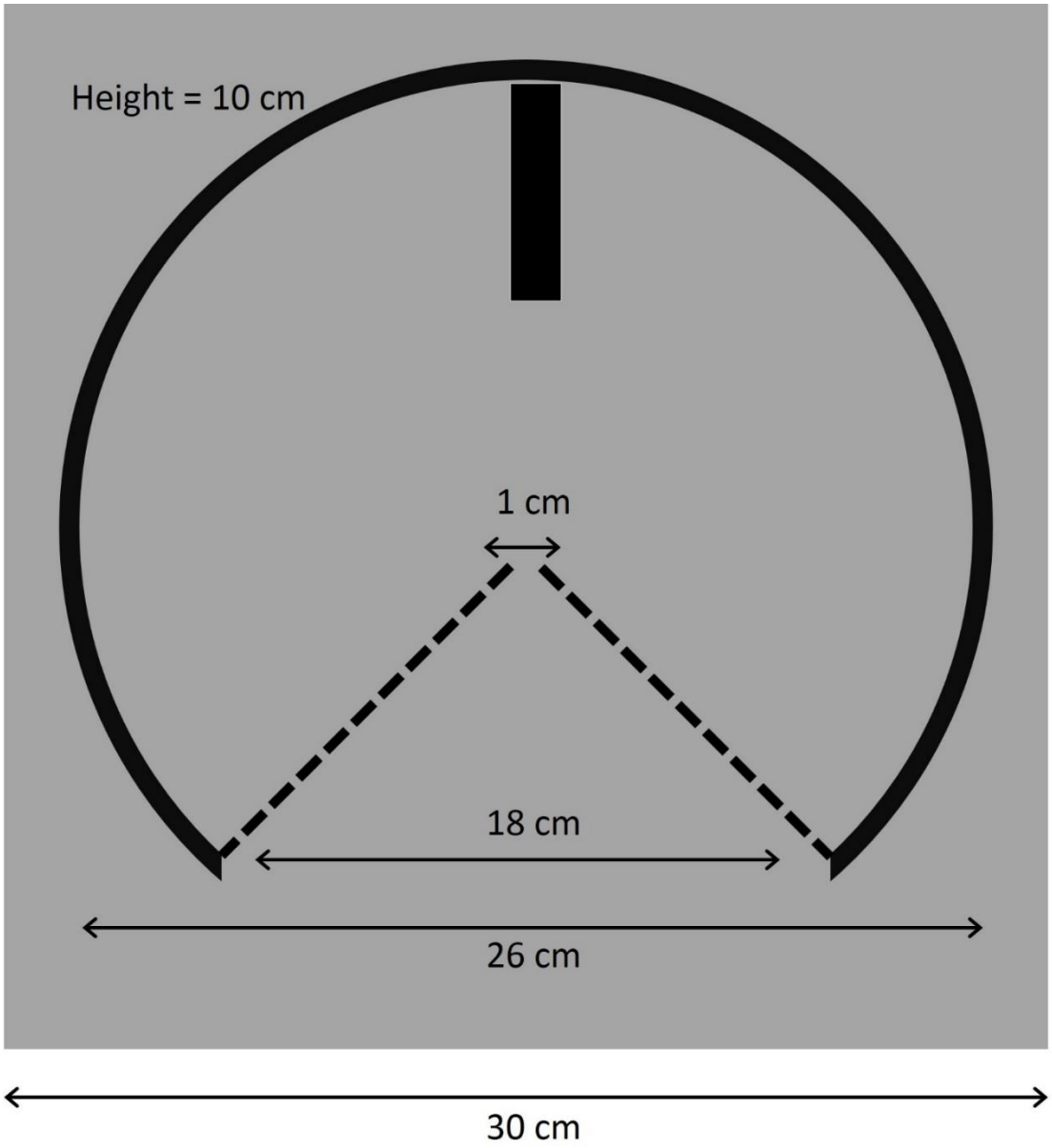

Figure 2. Schematic diagram of light trap used to sample larval fish in the upper Hiwassee River basin. Trap was constructed using a square of opaque sheet acrylic for the top and bottom (gray square), a modified piece of PVC pipe for the body of the trap (round 3/4-circle), and transparent acrylic (dotted lines) for the window. Light was provided by a small LED flashlight (black rectangle). Diagram drawn to scale, from the top-down perspective.

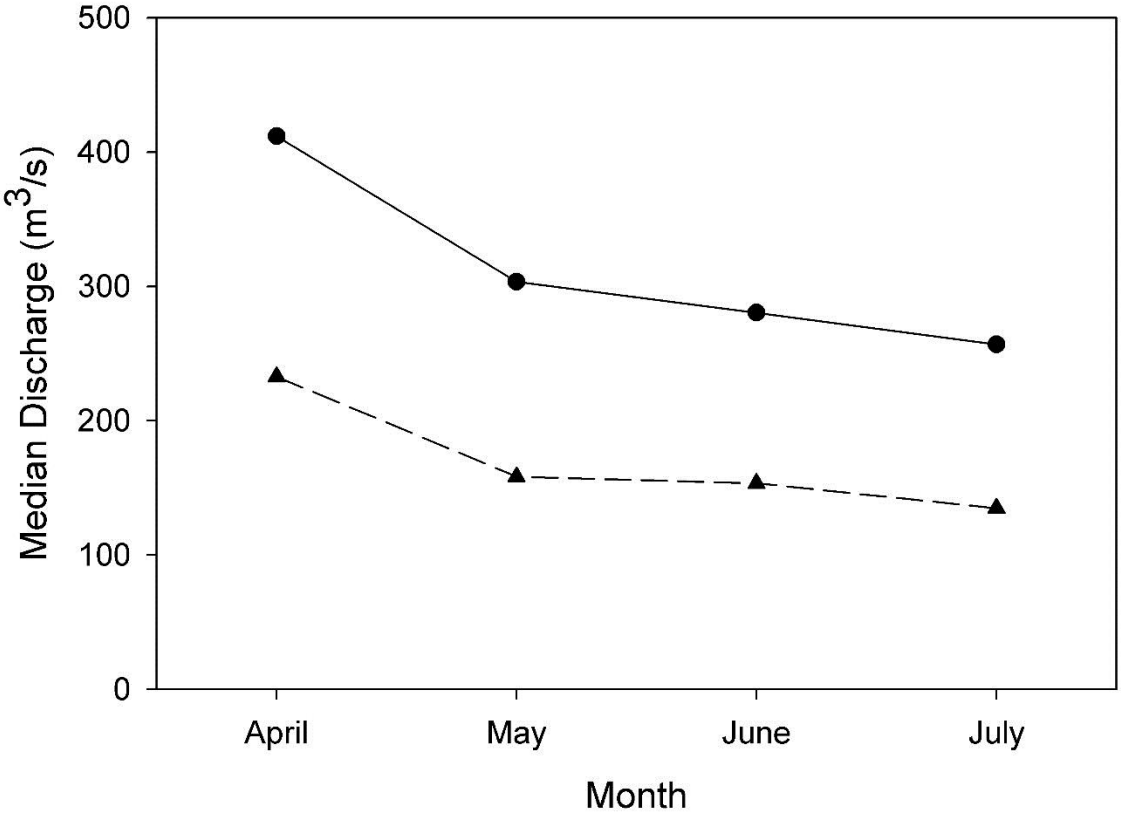

Figure 3. Monthly median discharge (m^3/s) for the Valley River during April-July, 2013 (circles, solid line) and 2014 (triangles, dashed line).

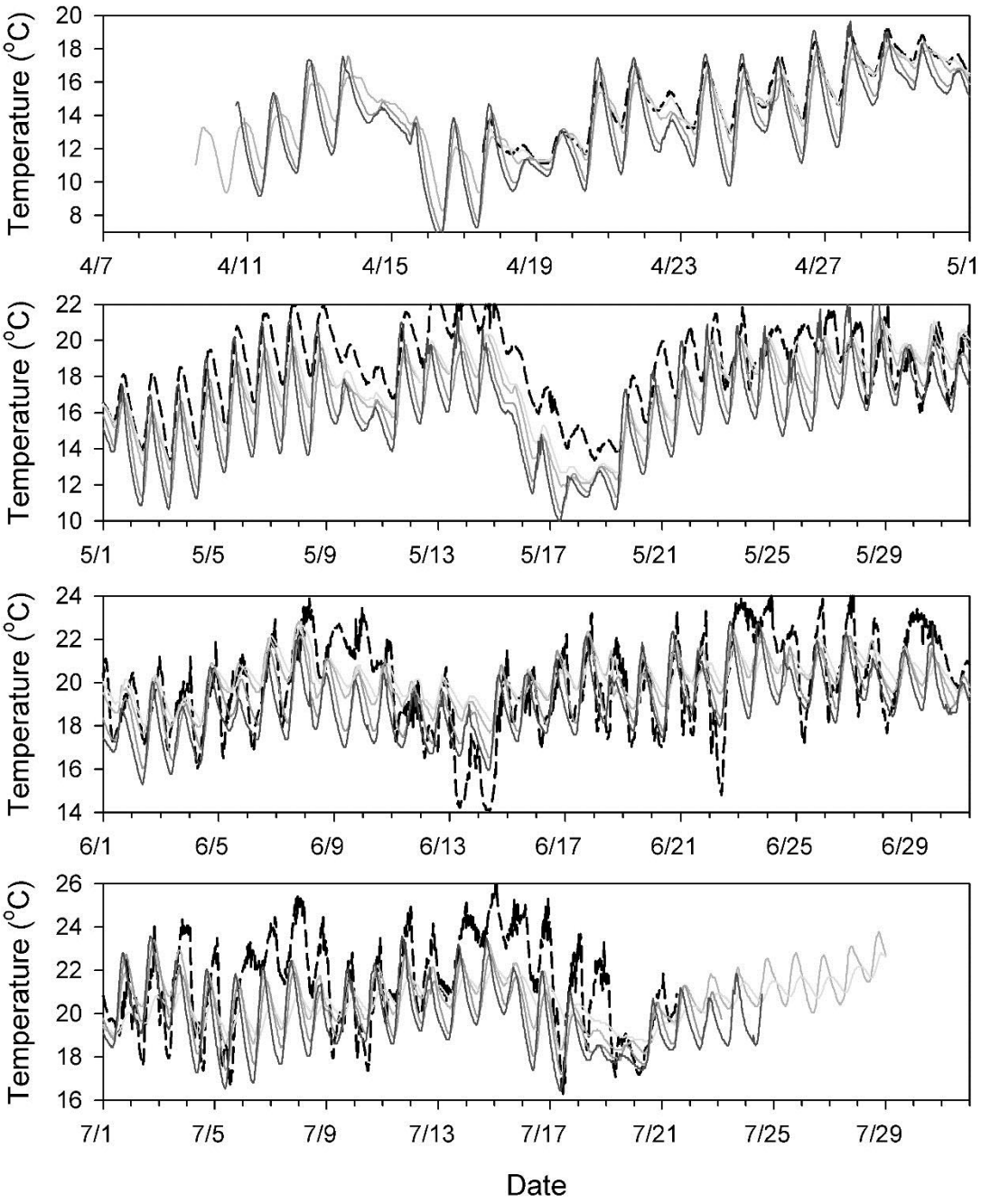

Figure 4. Temperatures recorded in 2014 by temperature loggers set at each of five sites. The site in the Hiwassee River is indicated by the black dashed line, and sites in the Valley River are indicated by solid gray lines and position indicated by shade; the lightest line is the farthest downstream site (Site 1) and the darkest line is the farthest upstream site (Site 4).

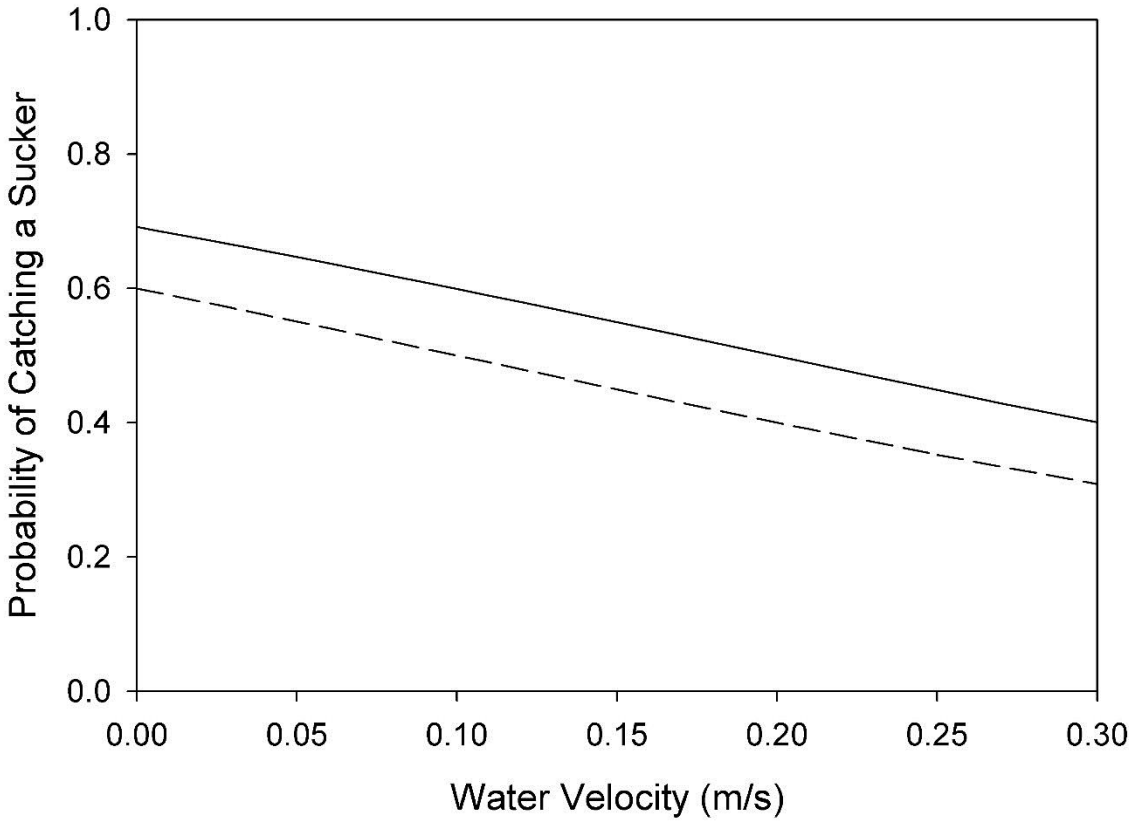

Figure 5. Estimated probabilities, across water velocities, of catching at least one sucker in floating (solid line) and sinking (dashed line) light traps.

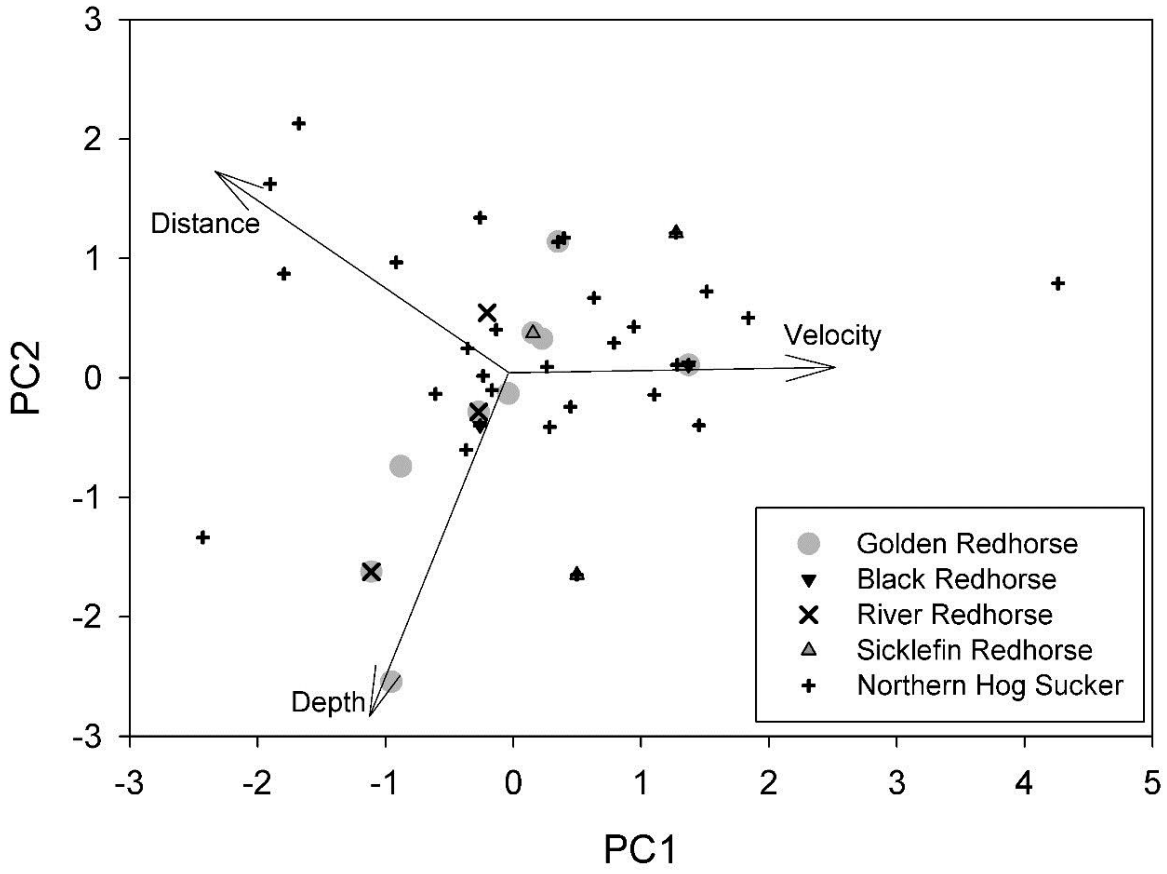

Figure 6. Principal Components Analysis relating distance to bank, depth, and mean-column velocity to the species of larvae collected in visual surveys in 2013.

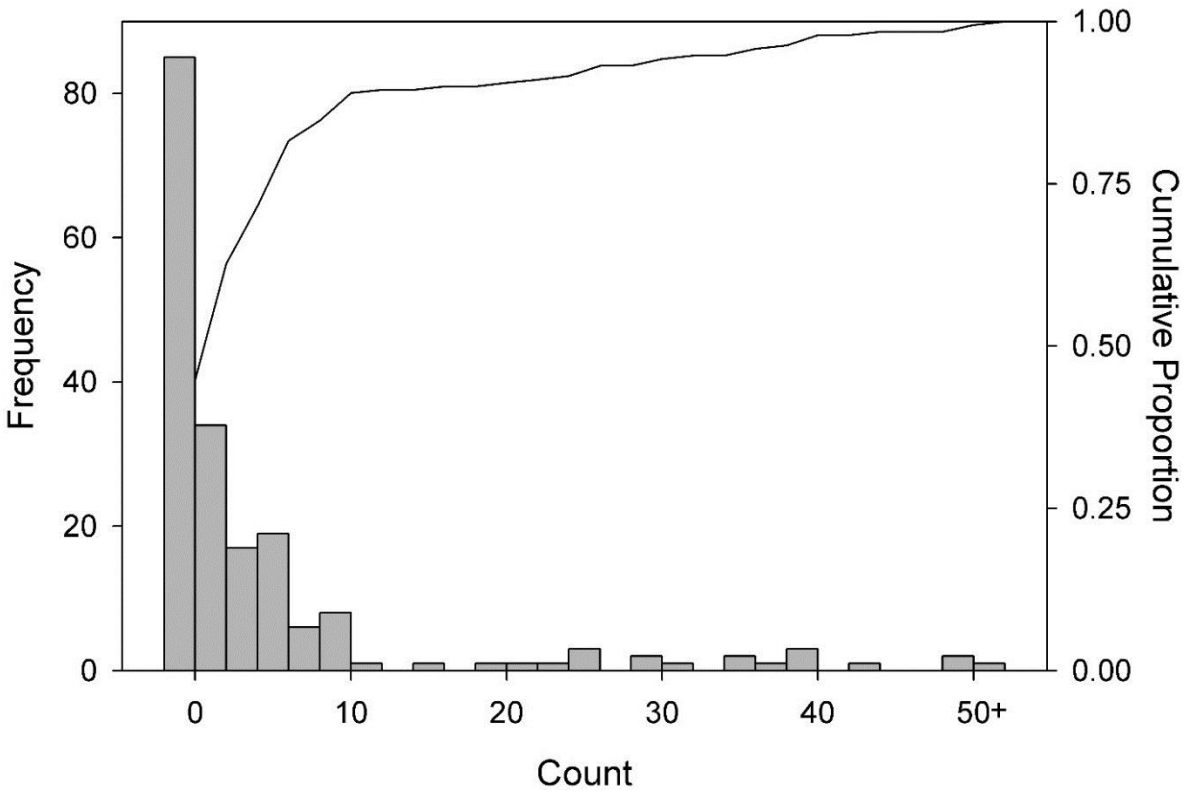

Figure 7. Frequency of larval and juvenile sucker counts in 0.5 x 0.5 m quadrats. Bars represent frequencies of counts (left y-axis), and the line represents the cumulative proportion of counts (right y-axis).

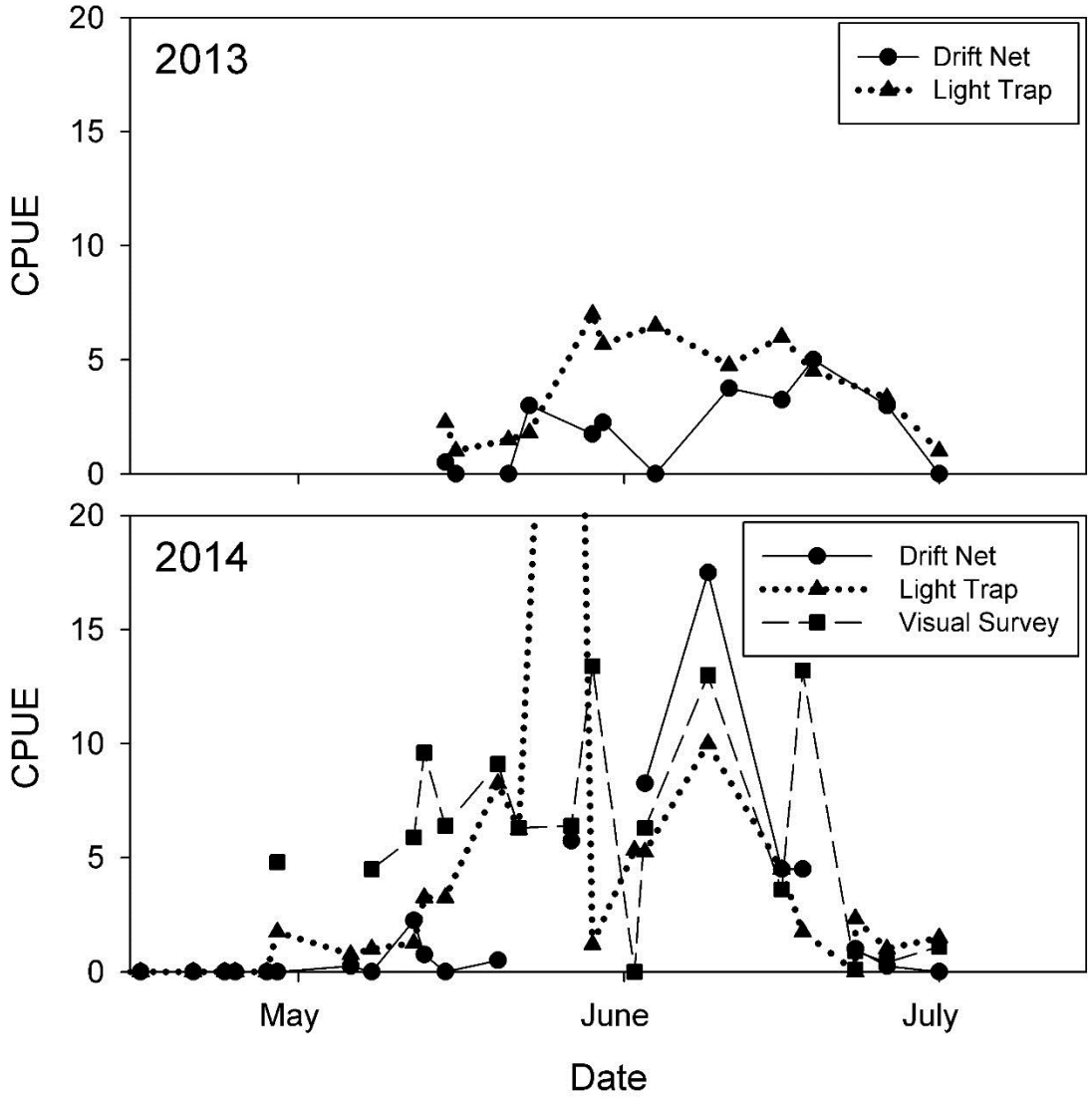

Figure 8. Catch-per-unit-effort (CPUE) for drift nets (suckers/30-min set), light traps (suckers/trap-night), and visual surveys (suckers/m^2) across dates in 2013 and 2014 when two or more techniques were simultaneously deployed.

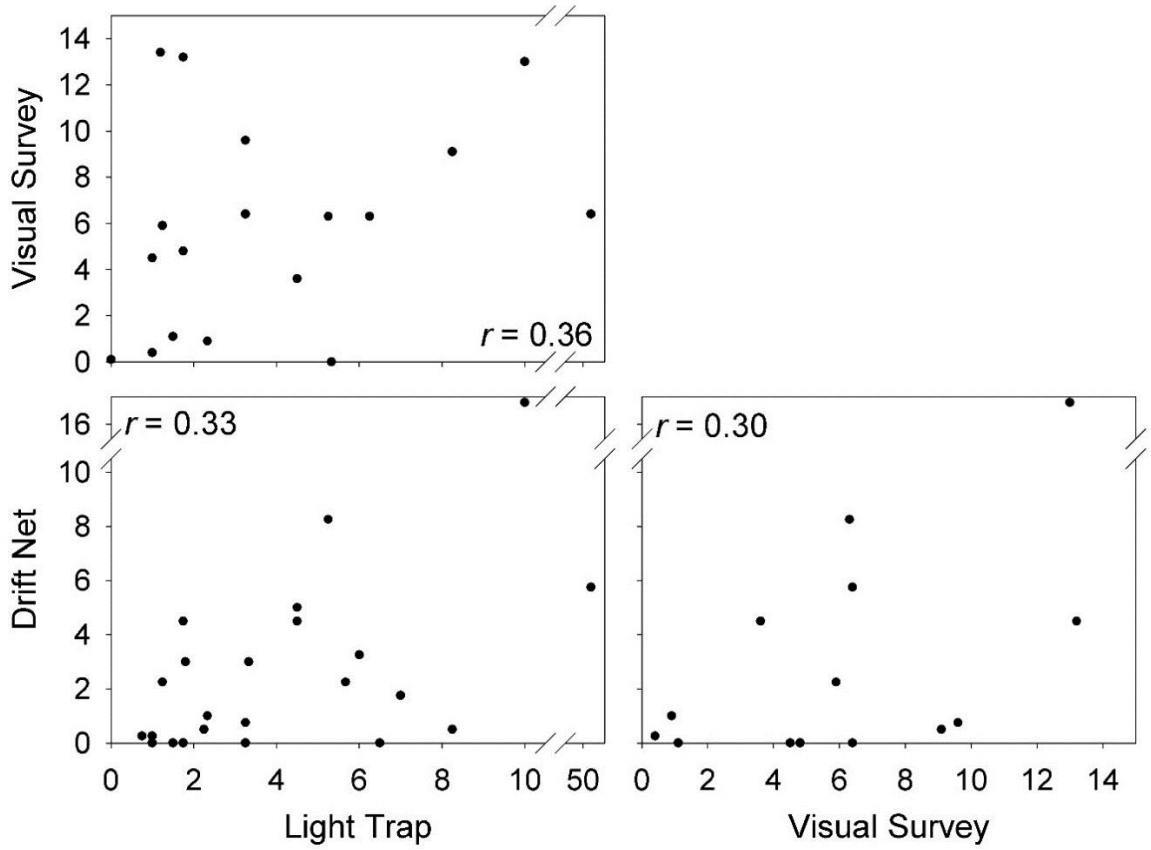

Figure 9. Correlations among drift net CPUE (suckers/30-min set), light trap CPUE (suckers/trap-night), and mean visual survey counts (suckers/m^2). Correlations exclude outlying observations.

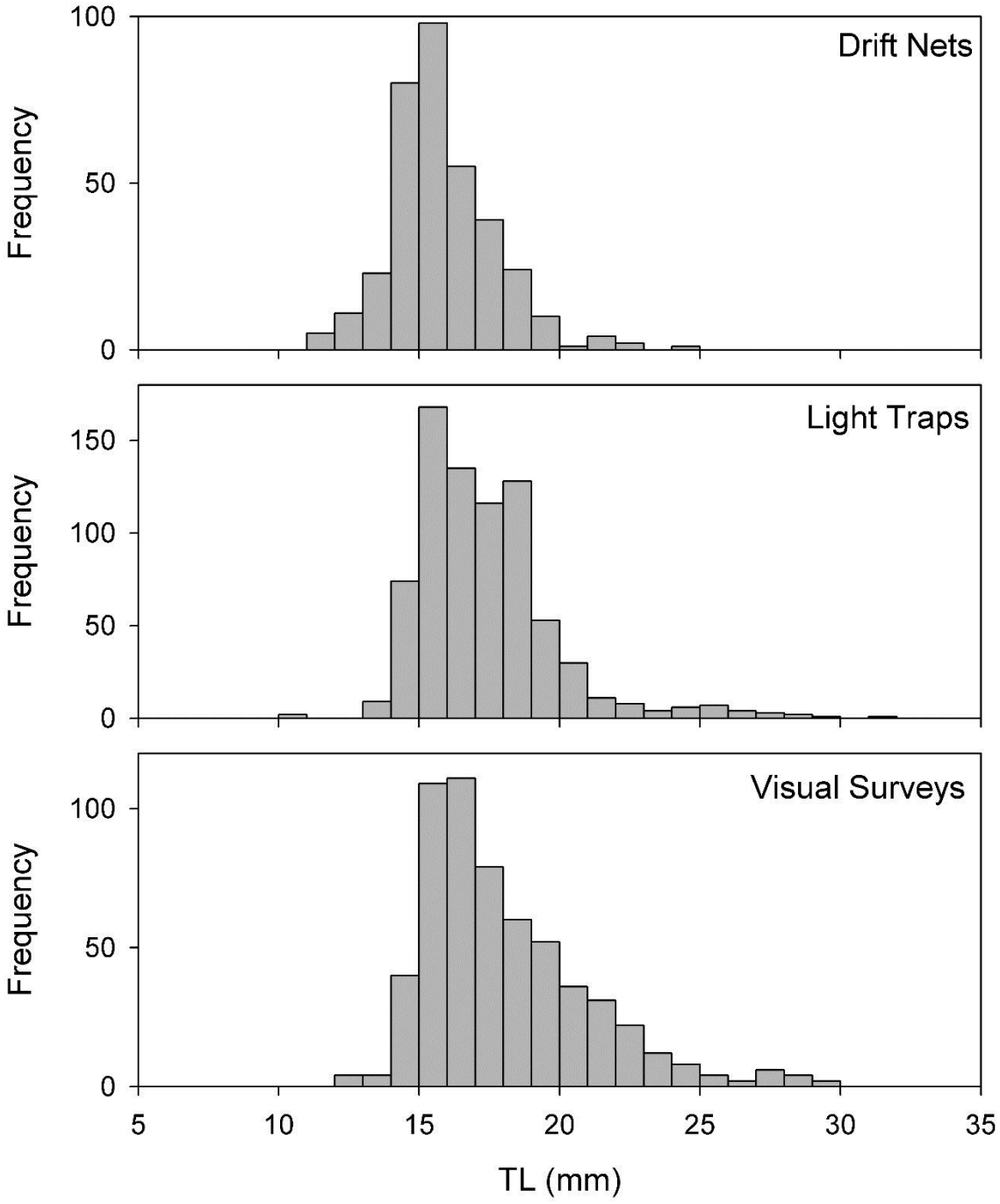

Figure 10. Length-frequency distributions of larval and juvenile suckers sampled using each of three techniques.

CHAPTER 3: Phenology, ontogeny, and growth of six sympatric suckers

Abstract

Suckers (Catostomidae) are a family of fishes that is widespread among freshwaters in North America and is of high conservation concern, but has received relatively little attention from the scientific community. Larval fish ecology is rarely studied on a species-level basis, because it is difficult to visually identify fish at early life stages, and there are no criteria available to visually distinguish larval suckers. Development of suckers during early life history encompasses the processes of ontogeny and growth. By implementing a novel index of larval fish ontogeny and using genetic barcoding for species identification, I estimated and compared phenology, ontogeny, and growth among six sympatric sucker species that occur in the upper Hiwassee River system of North Carolina. The new index of ontogeny was comparable to a commonly-applied classification, but provided finer resolution and may be incorporated into quantitative analyses. The emergence phenology of suckers paralleled previously described spawning phenology in the system. Species with overlapping emergence periods exhibited significantly different mean ontogeny rates. Larval growth rates were similar among sucker species, mean sizes on specific dates depended on emergence dates, and high intraspecific size variability was observed. Larvae of a given species tended to diverge in size over time, with larger individuals growing faster than smaller individuals, and slower growing individuals likely contributed to the high rate of mortality known to occur during this critical recruitment period. Differences in emergence timing, ontogeny, and size can mediate competition for food resources among sympatric larval suckers. Small innate differences in early life histories among species likely facilitated the current sympatric diversity of suckers in the upper Hiwassee River system, or these differences may represent local adaptations that evolved as a result of specific competition pressures among species.

Introduction

Suckers (Catostomidae) are a family of fishes that is widespread among freshwater ecosystems in North America and is of high conservation concern, but has received relatively little attention from the scientific community (Jenkins and Burkhead 1994; Cooke et al. 2005). Over 75 species comprise the taxon, which is named for the common feature of a fleshy, typically sub-terminal mouth, with protrusible lips, specialized for feeding on benthic plants and invertebrates. The sucker family has been subdivided into three subfamilies: Ictiobinae; Cycleptinae; and Catostominae, which is further subdivided into the tribes Catostomini and Moxostomatini (Smith 1992). Distribution patterns of suckers tend to reflect biogeographical processes and general habitat affinities, and there is a high degree of endemism within the family (Wiley and Mayden 1985; Smith, 1992; Harris and Mayden 2001). The distributional ranges of some sucker species overlap, resulting in diverse assemblages throughout North America that can encompass several sympatric species.

Where several species of sucker exist in sympatry, adults demonstrate interspecific differences in spawning behavior. Among the redhorses (tribe Moxostomatini, genus *Moxostoma*), spawning occurs during the spring and early-summer in clean gravel substrates (Jenkins and Burkhead 1994). However, sympatric redhorses demonstrate interspecific differences in specific reproductive timing and spawning microhabitat (Kwak and Skelly 1992; Grabowski and Isely 2007, Favrot 2009). Spawning phenology has been described for several river systems in which the relative temporal sequences among species that were common among them tended to correspond, despite discrepancies in absolute timing (e.g., Curry and Spacie 1984; Reid 2006; Favrot 2009; Catalano and Bozek 2015). Spawning timing has been linked to various environmental cues (e.g., photoperiod, stream discharge, water temperature) that differ spatially, temporally, and inter-annually (Reid 2006; Catalano and Bozek 2015; Straight et al. 2015). Interspecific differences in spawning timing and microhabitat selection have been implicated as important reproductive isolating mechanisms among sympatric redhorse species (Kwak and Skelly 1992; Grabowski and Isely 2007).

Development of suckers during early life history concurrently includes the processes of ontogeny and growth (Fuiman and Higgs 1997). Upon hatching, post-embryonic suckers

acquiesce in the interstitial spaces of the substrate while absorbing the yolk sac for a period of approximately 1-3 weeks, depending on species and incubation temperature (Buynack and Mohr 1978; Buynack and Mohr 1979; Bunt et al. 2013). Larval fish then emerge from the substrate and transition to exogenous feeding as remaining yolk reserves are depleted. Subsequent development encompasses profound physiological and morphological changes, including refinement of the feeding apparatus, development of paired and median fins, and somatic growth.

The ontogeny of larval fishes has been described in terms of various interval-based classification schemes formed on the appearance of ontogenetic landmarks (e.g., Balon 1975; Snyder 1976; Kendall et al. 1984). Literature describing the ontogeny of suckers, in particular, tends to rely on the terminology and intervals of ontogeny proposed by Snyder (1976). There are a number of limitations to superimposing saltatory categories (intervals of ontogeny) on the continuous process of ontogeny, including imprecision, ambiguity in terminology, species-specific discrepancies, and restrictions on analysis (Fuiman and Higgs 1997; Urho 2002). Acknowledging these limitations, ontogeny among larval fishes has occasionally been indexed based on size (Fuiman and Higgs 1997), assigned an ordinal scale that encompasses a suite of well-defined characters (Balinsky 1948), or summarized using a multivariate multi-character approach (Nikolioudakis et al. 2010). Growth of fishes during early life history tends to follow a Gompertz- or logistic-type curve (Zweifel and Lasker 1976; Barkalow et al. 2016). Growth rates and the onset of various intervals of ontogeny differ among some species (Kay et al. 1994), are correlated with age (Fuiman and Higgs 1997), and facilitate ecologically relevant ontogenetic shifts (Ruetz and Jennings 2000; Markle and Clauson 2006).

Among most fishes, mortality is highest during the critical period coinciding with the early life history transition to exogenous feeding (Hjort 1914). A second potential critical period among suckers coincides with the transition from planktonic to benthic prey (Markle and Clauson 2006). A primary source of mortality during early life stages is starvation that may be density-dependent. The match-mismatch hypothesis states that the survival of a larval fish is dependent on the synchronous availability of appropriate prey items during such

critical periods of ontogeny (Cushing 1990). Another important source of mortality during early life stages is predation. Because of their small size, lack of defense mechanisms, and poor swimming ability, larval fish are highly vulnerable to predators (Miller et al. 1988; Litvak and Leggett 1992). Given these ecological concepts, an understanding of larval life history is crucial for developing comprehensive fisheries management or conservation plans.

Larval fish ecology is rarely studied on a species-level basis because it is difficult to visually identify larval fishes to lower taxonomic ranks with any degree of certainty, especially when sampled from a system with multiple confamilials that are meristically and morphometrically similar (Fuiman 1979; Kelso and Rutherford 1996). Fishes in the family Catostomidae, in general, are especially difficult to visually distinguish at early life stages (Kay et al. 1994). Researchers have been unable to identify any characteristics for distinguishing among Moxostomatini larvae with reasonable certainty (Kay et al. 1994). Larval fishes in the genus *Hypentelium* may occasionally be distinguished from those in *Moxostoma* based on slight differences in pigmentation, especially during later stages of the larval interval; however, they are physically similar to other suckers with significant overlap in meristic characters (Buynak and Mohr 1979; Fuiman 1979). Differentiating among juvenile congeners is also difficult and problematic, as external characters may not be well-formed, and positive identifications may only be possible by comparing internal features such as the pharyngeal arch (Vachon 2003; R.E. Jenkins, Roanoke College, personal communication). Genetic techniques have been previously used to differentiate between young suckers; electrophoresis was used to distinguish Golden Redhorse *Moxostoma erythrurum* from Silver Redhorse *Moxostoma anisurum* (Morgan et al. 1983), and analyses using mitochondrial DNA have been used to discriminate among multiple sympatric redhorse species (Branchaud 1996). Monitoring larval Robust Redhorse *Moxostoma robustum* relied on genetic procedures for the identification of sampled individuals (Wirgin et al. 2004; Peterson et al. 2008). Recent advances in molecular genetic techniques (i.e., genetic barcoding) have enabled researchers to identify larval and juvenile fishes relatively rapidly and with an exceptionally high degree of accuracy (Ward et al. 2009; Ko et al. 2013).

One of the highest known diversities of redhorses (six species) occurs in the upper Hiwassee River system (Jenkins 1999). The upper Hiwassee River system is situated in the southern Appalachian Mountains of northern Georgia and western North Carolina. The lower portion of the Hiwassee River system is situated in southeast Tennessee. The river drains 6,993 km^2 and is a major tributary to the Little Tennessee River (Miranda et al. 2015). The six species of redhorses that occur in the system are Black Redhorse *Moxostoma duquesnei*, Golden Redhorse, River Redhorse *M. carinatum*, Sicklefin Redhorse *M.* sp., Silver Redhorse, and Smallmouth Redhorse *M. breviceps*. Additionally, the Northern Hog Sucker *Hypentelium nigricans* occurs sympatrically, also in Moxostomatini.

Because of its high diversity of redhorses, the upper Hiwassee River is a model system for studying this clade. Previous research in this system identified potamodromous spawning migrations common to redhorse, characterized and compared spawning habitats among species, and documented spawning phenology (Jenkins 1999; Favrot 2009). The Valley River, a major tributary to the Hiwassee River, has also been identified as a primary spawning area for redhorse (Favrot 2009). Observations of captive-reared larval and juvenile specimens from various river systems have yielded general descriptions of larval development for Black Redhorse (Bunt et al. 2013), Golden Redhorse (Fuiman and Whitman 1979), River Redhorse (Auer 1982), Silver Redhorse (Kay et al. 1994), Shorthead Redhorse *Moxostoma macrolepidotum* (a sister-species to the Smallmouth Redhorse; Buynak and Mohr 1979), and Northern Hog Sucker (Buynak and Mohr 1978). No description of the early life history of Sicklefin Redhorse is available in the primary literature. Further, larval and juvenile suckers are rarely studied in the wild, especially in the context of sympatry, and the early life history of suckers in the Hiwassee River system have not been studied.

By developing and implementing a novel index of larval fish ontogeny and employing genetic barcoding for species identification, I estimated and compared phenology, ontogeny, and growth among six sucker species. Specifically, my objectives were to (1) establish a chronology of emergence among the sucker species in the Valley River; (2) model and compare relationships between larval ontogeny and size among species; and (3) describe and compare size and growth rate of larval and juvenile suckers among species.

Methods

Larval and juvenile fish were collected from the upper Hiwassee River system, primarily from the Valley River, using standard sampling protocols (see Chapter 2). During 2013 and 2014, fish were sampled several times per week during April-July, and once in mid-September. During 2015, fish were sampled twice per month during April-June, and once in late-July. Valley River experienced higher flows throughout the spring of 2013, as compared to 2014 and 2015 (Table 1).

Sampled fish were returned, alive, to the laboratory. A Leica EZ-4 HD microscope (Leica Microsystems, Heerbrugg, Switzerland) was used to inspect and photograph specimens at 8x magnification. Fish were sedated and euthanized using MS-222 (tricaine methanesulfonate). Molecular biology grade ethanol (Fisher Scientific, Waltham, Massachusetts; BP2818) was added to slides to cause fins and median finfolds to become opaque. Fish total length (TL) was measured to the nearest 0.01 mm using computer software (Leica Microsystems; LAS EZ v. 2.1.0) calibrated to a 2.00-mm stage micrometer. Larval fish were preserved whole in individual 2-mL polypropylene vials filled with molecular grade ethanol. For juvenile fish, upper lobes of caudal fins were preserved in individual 2-mL vials filled with molecular grade ethanol, and bodies were preserved in a formalin solution and retained as vouchers. To avoid cross-contamination between samples, all microscopy tools and surfaces were cleaned with chlorine bleach and rinsed between samples.

The ontogeny of young fish was indexed on an ordinal scale of 0-5, with 20 possible scores. The ontogeny index score was based on the mean of four component scores. The component scores indexed the development of dorsal, anal, and pectoral fins, as well as yolk sac absorption, each on a scale of 0-5. For non-paired fins (dorsal and anal), a score of 0 was assigned for a total lack of development; a score of 1 was assigned if a condensation of mesenchyme resulted in a bulge or peak in the median finfold; a score of 2 indicated the presence of incipient fin rays proximal to the body; a score of 3 was assigned if incipient fin rays extended to the fin tips; a score of 4 was assigned if the fin was totally differentiated from the median finfold; and a score of 5 was assigned if fin rays exhibited segmentation and

the adjacent finfold was fully absorbed (Figures 1 and 2). For pelvic fins, a score of 0 was assigned if fins were not visible; a score of 1 was assigned if fin buds were barely visible; a score of 2 was assigned at the onset of incipient fin rays proximal to the body; a score of 3 was assigned if incipient fin rays extended to the tips of fins; a score of 4 was assigned if pelvic fins were mostly formed but incomplete in structure; a score of 5 was assigned if the fin was complete and fin rays exhibited segmentation (Figure 3). For the yolk sac, a score of 0 was assigned if yolk sac was complete and bulbous and a score of 5 was assigned if yolk sac was completely absent, with intermediate whole-number scores assigned inversely proportional to the degree of absorption (Figure 4). To standardize this index with existing descriptions of larval sucker ontogeny, larvae were also categorized using the nominal intervals of ontogeny outlined by Snyder (1976). Protolarvae were characterized by absence of dorsal-, anal-, and caudal- fin spines and rays. Mesolarvae were characterized by presence of at least on dorsal-, anal-, or caudal-fin spine or ray, but lacking the adult complement of principal soft rays in at least one median fin, or lacking pelvic fin buds. Metalarvae had developed the adult complement of principal soft rays on all median fins and had pelvic-fin buds. Juveniles had completely absorbed their fin folds and developed the full adult complement of rays and spines and segmentation in at least a few of the rays (Snyder 1976).

Larval and juvenile fish individuals were identified using genetic barcoding, but Northern Hog Sucker early life stages of some individuals could be positively identified based on pigmentation and morphometrics. Genetic barcoding was carried out by the North Carolina Museum of Natural Sciences following standard laboratory procedures (NCMNS, Raleigh, North Carolina). Samples were stored at -20^{o}C until analysis. Tissues were prepared by cutting them each to a suitable size and loading them into a 96-well plate. A solution composed of Proteinase K and Buffer T1 was added to each well to lyse cell membranes, a centrifuge was used to pull tissue into the lysis solution, and a nutator mixer circulated the solution as cells were digested for at least 12 h. Next, the genetic material was isolated by adding a buffer (BQ1) that established conditions that caused DNA to bind to the filter plate, rinsing proteins from the silica membrane using vacuum suction and buffers B5 and BW, and finally, releasing the genetic material from the filter plate into solution by washing with the elution buffer (BE). The concentration of nucleic acid in each resulting

sample solution was determined using a nanodrop spectrophotometer and was diluted to achieve a normalized concentration of approximately 40 ng/μL of genetic material. Next, a master-mix consisting of primers, taq (enzymes, buffers, and fluorescent dideoxynucleotides [fluorophores]), and MgCh was added and placed in a thermocycler to facilitate a polymerase chain reaction (PCR). A final cleaning process using exo-sap-IT digested any remaining single-stranded or short strands of DNA and unincorporated fluorophores. Ethanol, sodium acetate, and EDTA were used to precipitate DNA from the sample. An Applied Biosystems 3130xl genetic analyzer machine (Foster City, California) carried out a Sanger di-deoxy sequencing procedure, which determined the order of dideoxynucleotides based on relative positions of fluorophores. Any sequences that did not adhere to the standards of the Barcodes of Life (Ward et al. 2009) were omitted, and corresponding samples were subsequently reanalyzed.

Analysis of genetics data was conducted by HydroGENomics (Raleigh, North Carolina). Basic Local Alignment Search Tool software (BLAST; U.S. National Laboratory of Medicine, Bethesda, Maryland) was used to compare larval fish sequences with reference sequences. For these references, I sequenced a total of 22 adult suckers, including at least two individuals belonging to each of the seven species present. These fish were collected on 17 April, 2013, from the mainstem of the Hiwassee River immediately downstream of its confluence with the Valley River, using boat-mounted electrofishing. They were initially identified using morphological features (Menhinick 1991; Jenkins and Burkhead 1994). Tissue samples were obtained from the right pectoral fins and preserved in molecular grade ethanol. Fish were euthanized in MS-222 and bodies were preserved in a buffered formalin solution. Voucher specimens and associated tissue were catalogued in the NCMNS' reference collection (Appendix).

Emergence phenology was estimated using quantile regression and an inverse prediction procedure (Zar 1996; Falke et al. 2010). This procedure was necessary because the earliest-emerging fish from a cohort may not have been observed, due to imperfect detection, subsampling for identification, and gaps in the sampling schedule. Only fish collected from the Valley River were included in this procedure to avoid confounding that

might result from fish from other tributaries. Juvenile fish (ontogeny index score = 5.00) were omitted from these models because they represented the maximum indexed state and therefore did not conform to the regression (i.e., the slope across juvenile lengths was zero). Ontogeny index score was the dependent variable, day of the year was the independent variable, and year (2013, 2014, or 2015), species, and the interaction of species and day of year were included as covariates. Bounds on larval emergence were estimated from the models, as dates for which the ontogeny index score equaled 0.00 (the x-intercept). The lower bound on emergence was estimated from the modeled regression through the 90th percentile of observations. The 90th percentile was chosen following the assumption that the most developed individuals collected over time represent the oldest cohort (Falke et al. 2010). Similarly, the upper bound on emergence was predicted from the modeled regression through the 10th percentile of observations. Significant differences in emergence dates were evaluated among years, by specifying 2014 as the reference level and assessing for differences in intercept among years.

Patterns of larval ontogeny were modeled and compared among species using Analysis of Covariance (ANCOVA; Kutner et al. 2005). Juvenile fish (ontogeny index score = 5.00) were omitted from these models because they represented the maximum indexed state and did not conform to the regression. Ontogeny index score was the dependent variable, total length (mm) was the independent variable, and species and the interaction of species with total length were included as covariates. The significance of any interspecific differences in ontogeny were evaluated from this full model and from pairwise contrasts among the interaction terms for species and index score. For groups of species where the interaction term was similar, I created reduced models for each group that assumed a similar relationship of ontogeny index score with total length and assigned species-specific intercept terms. The fit of these reduced models was evaluated by the coefficient of determination (R^2). Parameter estimates and their standard error (SE) were reported, and 95% prediction intervals were calculated from these parameters. The typical sizes (TL) at which larvae transitioned between intervals of ontogeny (Snyder 1976) were determined using the final set of reduced models and the modal ontogeny index score at which transitions occurred. The mean size ranges associated with intervals of ontogeny were predicted from regression model

point estimates. The extreme size-range associated with each interval was estimated using the lower and upper prediction intervals corresponding to index scores that marked a transition to and from an interval, respectively (Snyder 1976).

Larval somatic growth rate was modeled using quantile regression. Differences in size were dependent upon both growth rate and emergence date, but the quantile procedure standardized estimation of growth rates to individuals with similar emergence timing. Juvenile fish were omitted from these models. Any species that did not demonstrate a significant increase in size over collection dates was omitted from growth models. These data were treated as a time series in which $\log_e$(total length) was the dependent variable, day of the year was the independent variable, and year, species, and, initially, the interaction of species and day number were included as covariates. Growth was modeled as the regression through the 90th percentile of observations, which was representative of the early-emerging fish within a cohort (Falke et al. 2010). Because the rate of somatic growth tends to increase exponentially during the larval phase, a $\log_e$ transformation was applied to total length (Zweifel and Lasker 1976; Barkalow et al. 2016). Significantly different growth rates among species were evaluated using the interaction term and pairwise contrasts. Where growth rates did not differ, reduced models were fit. Larval size ranges of each species were modeled over time using the previously described model and the 10th and 90th percentiles. To estimate the total size range of larvae, which was unlikely to be directly observed due to imperfect detection, models for each species were hind-cast to the previously predicted emergence dates and truncated at the modeled maximum length of metalarva.

Juvenile somatic growth was modeled using linear regression. To ensure similar observation durations, only species that were collected as juveniles during both July and September were included. Because critical periods occur prior to the juvenile interval, and modeled residuals were normally distributed, as indicated by a Shapiro-Wilk test, an ordinary least-squares regression model was appropriate for modeling growth during this ontogenetic interval. Furthermore, I assumed that growth was linear during the observed duration (Zweifel and Lasker 1976; Barkalow et al. 2016). Total length was modeled as the dependent variable, day of the year was the independent variable, and year, species, the

interaction of day of the year and species were included as covariates. Significant interspecific differences in growth rate were evaluated using the interaction term. If growth rate did not differ among species, reduced models assuming equal slopes with different intercepts were fit. The size range of juvenile fish over time was described using the linear regression parameters and the associated prediction intervals.

Statistical analyses were performed in SAS v. 9.4 (SAS Institute, Cary, North Carolina). Quantile regression was performed using Proc QuantReg. ANCOVA and linear regression models were specified in Proc GLM.

Results

A total of 917 larval and juvenile suckers were identified using the genetic barcoding procedure, and an additional 298 Northern Hog Suckers were identified based on pigmentation (16 metalarvae and 282 juveniles), resulting in a grand total of 1,213 fish, which included all occurring sucker species except for Smallmouth Redhorse (Table 2). The sizes of fish observed ranged 14.00-76.00 mm TL (Table 3). Sampled fish represented all potential ontogeny index scores, the most frequently assigned score assigned to larvae was 1.00 (Figure 5).

The numerical ontogeny index corresponded well with the commonly applied nominal classification system (Snyder 1976), but the numerical index was more precise. Mesolarva had scores 0.00-3.00; metalarva had scores 2.50-4.75; and juveniles were uniformly scored as 5.00 (Figure 6). The modal transitionary score for the mesolarva-metalarva metamorphosis (i.e., index score ranging from 2.50-3.00) was 2.75. Protolarva were absent from samples, but would have scored a 0.00 based on the component metrics. Among sucker species, the ontogeny index (and associated component scores) demonstrated a positive relationship with fish size during the larval phase, development of the dorsal fin initiated concurrent with yolk absorption, and anal and pelvic fins only began to appear when the yolk sac was nearly- or fully-absorbed (Figure 7).

The phenology of emergence resulted in a distinct sequence among species. Silver Redhorse and Northern Hog Sucker emerged earliest, followed by Black Redhorse, then by Golden Redhorse, Sicklefin Redhorse, and River Redhorse (Figure 8). The duration of emergence (i.e., presence of ontogeny index = 0) was approximately 1 week for Black Redhorse and Silver Redhorse; approximately 2 weeks for Golden Redhorse, Sicklefin Redhorse, and Northern Hog Sucker; and up to approximately 3 weeks for River Redhorse. The model indicated that larval emergence in 2013 (a year of relatively high stream discharge) was delayed by approximately 11 d relative to the other years (t = -9.65, df = 1, P < 0.0001) and that there was no significant difference in timing between 2014 and 2015 (t = -1.24, df = 1, P = 0.2021).

Larval sucker ontogeny occurred in conjunction with somatic growth and differed among species that emerged concurrently. The relationship of ontogeny and growth, was similar for Black Redhorse and Northern Hog Sucker (t = -1.79, df = 1, P = 0.0739). It was also similar for Golden Redhorse and Silver Redhorse (t = 1.33, df = 1, P = 0.1839). The rate of ontogeny of Sicklefin Redhorse was intermediate; it was significantly faster than Black Redhorse and Northern Hog Sucker (t = -2.07, df = 1, P = 0.0389) and significantly slower than Golden Redhorse and Silver Redhorse (t = 2.00, df = 1, P = 0.0459). Owing to low sample size, the ontogeny rate for River Redhorse was not significantly different from any other sucker species, but the point estimate was intermediate between Black Redhorse and Northern Hog Sucker, and I therefore assumed equal slopes with this group. All other contrasts indicated significant differences in rate. The resulting three models explained much of the variation in the relationship between size and ontogeny (R^2 = 0.774 for Black Redhorse, River Redhorse, and Northern Hog Sucker; R^2 = 0.759 for Golden and Silver Redhorse; and R^2 = 0.832 for Sicklefin Redhorse). On average, Black Redhorse, River Redhorse, and Northern Hog Sucker exhibited the slowest rate of ontogeny, Golden and Silver Redhorse exhibited the fastest rate of ontogeny, and Sicklefin Redhorse demonstrated an intermediate rate of ontogeny (Figure 9). The species covariate was significant in all models (P < 0.0001) indicating interspecific differences; estimates of standard error (SE) were relatively high indicating a high degree of intraspecific variability in ontogeny (Table

4). The modeled sizes at which suckers transitioned between intervals of ontogeny were comparable to those given in literature (Table 3).

Quantile regression estimated larval size ranges for Black Redhorse, Golden Redhorse, Sicklefin Redhorse, Silver Redhorse, and Northern Hog Sucker (Figure 10). For the largest 90th percentile of larval suckers, pairwise comparisons provided little evidence that growth rates differed among species ($t \geq 1.61$, df = 715, $P \geq 0.1074$). Therefore, a similar growth rate was assumed among all species for the final model, with species and year as covariates. Larval fish were smaller in 2013 than on corresponding dates in 2014 (t = -6.61, df = 719, $P < 0.0001$) or 2015 (t = -4.11, df = 719, $P < 0.0001$), whereas fish were similarly sized on corresponding dates in 2014 and 2015 (t = 0.71, df = 719, P = 0.4792). The slope (β_1) for $\log_e$(TL) of the 90th percentile was 0.0066, and intercepts (β_0) depended on the coefficients for species and year (Table 5). A similar model was fit for the $\log_e$(TL) of the 10th percentile of observations, which had a slope (β_1) of 0.0023, and intercepts (β_0) dependent on the coefficients for species and year (Table 5). Due to the small size range in the sample, River Redhorse did not demonstrate a significant increase in size over time (F = 0.22, df = 1,10, P = 0.6463) and was, therefore, excluded from the growth model.

Linear regression with 95% prediction intervals estimated juvenile size ranges for Black Redhorse, Golden Redhorse, Sicklefin Redhorse, and Northern Hog Sucker (Figure 11). Significant differences in slope ($t \geq 5.11$, df = 1, $P < 0.0001$) were detected among all species, and were thus modeled separately (Table 6). The model residuals were normally distributed, indicating that simple linear regression was appropriate (W = 0.9970; P = 0.7557) Compared to their sizes on specific dates in 2014, juvenile fish were considerably smaller in 2013 (t = -16.76, df = 1, $P < 0.0001$) and slightly larger in 2015 (t = 2.10, df = 1, P = 0.0363; Table 6). This model explained much of the variation within the data (R^2 = 0.8655). No River Redhorse juvenile was encountered during the course of this study, and Silver Redhorse juveniles were not encountered in September of 2013 or 2014; thus, these species were omitted from analysis.

Discussion

The ability to accurately identify larval and juvenile fish was key to elucidating patterns in phenology, growth, and ontogeny among six sympatric larval and juvenile sucker species. Given the lack of distinguishing characteristics among larval suckers, genetic barcoding was essential to achieving the objectives of this study. Although interspecific differences in emergence timing and mean ontogeny rate differed among species, high intraspecific variability resulted in substantial overlap. This, compounded with interannual variation, made it virtually impossible to develop criteria to confidently differentiate among the six species by timing of size, stage, or other characteristics.

Because the ontogeny index developed for this research was composed of separately-scored easy-to-distinguish criteria on an ordinal scale, it provided an objective, analyzable, and highly transferrable characterization of development. The final stages of yolk sac absorption during the early-mesolarva interval provided a convenient method of indexing ontogeny prior to initial onset of fin development. The index of ontogeny corresponded to the intervals of ontogeny typically referenced in sucker literature (Snyder 1976), where scores between 2.50 and 3.00 (typically 2.75) represented the transition from mesolarva to metalarva, and a score of 5.00 uniformly represented juveniles. This high degree of correspondence was expected, as both classification systems depended on the external anatomy and development of fins. However, the ordinal index provided a much finer resolution and, due to the ordinal nature of numerical index scores, could be incorporated into quantitative analyses. The modeling approach to analyzing patterns in sucker ontogeny in the upper Hiwasssee River system facilitated the estimation of relevant parameters from a wild population. These findings establish the first formal description of larval and juvenile Sicklefin Redhorse growth and ontogeny, and compliment previously reported descriptions of ontogeny for other suckers that were derived from captive-reared specimens (Buynak and Mohr 1978; Fuiman and Whitman 1979; Bunt et al 2013).

Prior to this study, the larval emergence phenology of sympatric suckers had never been estimated or compared to the spawning phenology among adults. In the Valley River, the emergence phenology of redhorse paralleled their previously described spawning

phenology (Favrot 2009). Northern Hog Sucker, which emerged early in the phenology, is known from other systems to spawn relatively early, in comparison to confamilials (Matheney and Rabeni 1995; Grabowski and Isely 2007). Larval Northern Hog Suckers and Silver Redhorse were among the earliest fishes to emerge in the Valley River, and were preceded approximately one week by Mottled Sculpin *Cottus bairdii*, Central Stoneroller *Campostoma anomalum*, and potentially several other species (pers. obs.). Despite interannual differences in spawning timing, the order of emergence among species was consistent. Interannual differences in emergence timing likely reflected interannual differences in spawning timing and conditions, because suckers tend to delay spawning due to high or erratic flows (Reid 2006; Favrot 2009; Catalano and Bozek 2015). Valley River experienced higher flows throughout the spring of 2013, as compared to 2014 and 2015, and resultantly, emergence occurred later during 2013.

For the model of emergence phenology, I assumed that emergence occurred when the index of larval fish ontogeny equaled 0.00. While this provided a convenient reference for standardization among species, it may not be an entirely biologically accurate assumption. The low relative frequency of scores equaling 0.00 in samples indicated that many individuals emerged slightly later in development, and the increasing frequency of fish in the sample from 0.00 to 1.00 indicated that swim up occurred during within this range. In a laboratory setting, suckers that were hatched on the same day emerged up to six days apart (Fisk et al. 2012). Thus, the actual emergence periods in the Hiwasssee River were probably slightly later and slightly protracted, as compared to the idealized model.

It is rarely appropriate to directly estimate phenology from sampling catch data, due to gaps in the sampling calendar, small sample size, and behavior of recently-emerged larvae that may make them less vulnerable to capture. Falke et al. (2010) modeled emergence phenology using length and a method similar to the one used in this study. However, using ontogeny to predict emergence is more appropriate in my study for three primary reasons. First, the high intraspecific variability in size at various ontogenetic landmarks can reduce the precision and interpretability of estimates. Second, interspecific differences in size at various ontogenetic landmarks complicates further analysis. And third, without a prior

understanding of larvae sizes in the assemblage, estimates of phenology hinge on the reported sizes at various ontogenetic landmarks from previous studies conducted in other systems.

Larval individuals of a given species tended to diverge in size over time, with larger individuals growing faster than the smaller individuals in the cohort. This trend was made apparent through the magnitude of differences in quantile coefficients associated with larval growth. The model describing the growth rate of the 90th percentile included the growth of the earliest-emerging and fastest growing larvae, whereas the 10th percentile exhibited a much slower growth rate that included both later-emerging and slower-growing larva. In a hatchery setting, a distinct subset of Sicklefin Redhorse exhibited a drastically slower growth rate than others from the same cohort (P. L. Rakes, Conservation Fisheries Incorporated, personal communication). Although this may have been influenced by captive-rearing conditions, it indicates inherent intraspecific differences in viability. Size is strongly positively correlated to swimming ability in larval and juvenile suckers (Ruetz and Jennings 2000; Chapter 4). Therefore, faster-growing individuals tend to be more adept at locating, capturing, and competing for food (Miller et al. 1988; Sogard 1997). Furthermore, predation risk tends to decrease with size, as larger fish are less available to gape limited predators and better able to evade predation (Miller et al. 1988; Litvak and Leggett 1992). Because of these factors, slower growing larvae likely had a higher likelihood of contributing to the high rate of mortality known to occur during this critical period (Hjort 1914; Miller et al. 1988; Markle and Clauson 2006).

Fluctuations in year-class strength have been noted for redhorse species (Kwak and Larimore 1987; Ely and Zimpfer 2012). Variations in the timing of emergence can potentially manifest as differences in recruitment of age-1 fish, due to resource limitations, size-related mortality, and biotic interactions. My results indicated that timing of emergence is dependent on abiotic factors. If abiotic factors affecting the timing of sucker spawning and emergence differentially influenced the phenology of prey (i.e., drifting and floating microinvertebrates; Markle and Clauson 2006), resulting predator-prey asynchronies could result in starvation among larvae (Cushing 1990). My results indicated that the sizes of

larvae and juveniles on specific dates through early-autumn were dependent upon emergence timing. This affects recruitment, because overwinter survival during the first year tends to be highly size-selective (Sogard 1997). Due to differences in emergence time, the various species of sucker inhabiting the upper Hiwassee River system might be differentially affected by stochastic events, which could further shape biotic interactions (e.g., by influencing competition dynamics). Other aspects of life history, such as female fecundity and population-level considerations, such as stock-recruitment dynamics, can further influence the recruitment dynamics of sucker populations. Therefore, an understanding of the larval critical period is essential for developing a basic understanding of recruitment and for developing sound management and conservation plans.

Competition for food resources can occur among riverine larval fishes occupying similar feeding niches (Welker et al. 1994; Einum and Nislow 2005; Skoglund et al. 2012). Asynchronous emergence timing reduces interspecific competition among sympatric larval fish species (Skoglund et al. 2012). An ontogenetic shift in sucker feeding ecology occurs during the larval stage (Bunt and Cooke 2004; Markle and Clauson 2006). Thus, asynchronous emergence minimizes diet overlap. In the present study, species with overlapping emergence periods exhibited slightly but significantly different mean ontogeny rates and slightly, but significantly, different mean sizes at any given time. Silver Redhorse and Northern Hog Sucker emerged earliest in the season; Silver Redhorse tended to develop slightly faster and became juveniles at a smaller mean size than Northern Hogsucker. Golden Redhorse, Sicklefin Redhorse, and River Redhorse emerged latest in the season. Golden Redhorse emerged at the smallest size, developed slightly faster, and became juveniles at the smallest mean size of all species studied. Although the small sample size of River Redhorse makes it difficult to comment on their early life history, they appear to emerge at a small size and develop at a slightly slower rate. The Sicklefin Redhorse emerges at a larger mean size than Golden Redhorse and River Redhorse, and exhibits a moderate rate of development. The Black Redhorse emerged intermediate to the other species, at the largest size, developed relatively slow, and became juveniles at the largest size. Because larval growth rates did not differ among species, these interspecific differences provide a mechanism for niche partitioning among sympatric suckers.

Small innate differences in early life histories among species, such as emergence timing, size, and ontogeny, likely facilitated the current sympatric diversity of suckers in the upper Hiwassee River system (Jackson et al. 2001). Alternatively, these differences can represent local adaptations that evolved through divergent selection and as a result of specific competition pressures among sympatric species (Conover and Schultz 1997). The findings presented on seven sympatric suckers expand the knowledge of the early life history dynamics of a poorly understood fish taxon and an imperiled species. These findings can be used to diagnose potential causes of variable recruitment among sympatric species, to explain interspecific differences in other early life history parameters, and to tailor conservation and management efforts to accommodate early life stages.

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Tables

Table 1. Median discharge (m^3/s) of the Valley River during three years of this study (2013-2015).

	Discharge (m^3/s)		
	2013	2014	2015
April	11.66	6.59	9.86
May	8.60	4.48	4.28
June	7.94	4.39	2.73
July	7.27	3.81	4.12

Table 2. Sample sizes of mesolarva, metalarva, and juveniles for each of seven sucker species of the upper Hiwassee River system.

	Number of fish sampled			
Species	Mesolarva	Metalarva	Juvenile	Total
Black Redhorse	67	13	83	163
Golden Redhorse	160	50	22	232
River Redhorse	7	5	0	12
Sicklefin Redhorse	24	8	12	44
Silver Redhorse	85	15	7	107
Smallmouth Redhorse	0	0	0	0
Northern Hog Sucker	262	87	306	655*
Total	605	178	430	1,213

*includes 16 metalarvae and 282 juveniles that were identified using pigmentation patterns

Table 3. Total length (mm) range of mesolarva, metalarva, and juveniles for each of six sampled sucker species. Intervals of ontogeny were described by Snyder (1976). Modeled mean TL ranges corresponding with ontogeny index thresholds between intervals (i.e., 0.00-2.75, 2.75-5.00, and 5.00+). Modeled extreme TL ranges are based on 95% prediction intervals.

		Total Length Range (mm)		
Species	Source	Mesolarva	Metalarva	Juvenile
Black Redhorse	Observed	17.10-20.28	19.00-23.28	25.69-76.00
	Model Mean	15.0-20.3	20.3-24.5	24.5+
	Model Extreme	12.7-22.6	18.1-26.8	22.3+
	Literature[a]	14.1-18.0	18.1-24.0	24.0+
Golden Redhorse	Observed	14.00-18.50	16.60-20.55	23.30-69.00
	Model Mean	13.6-17.5	17.5-20.5	20.5+
	Model Extreme	11.9-19.1	15.8-22.2	18.8+
	Literature[b]	12.0-14.4	12.0-19.9	21.3+
River Redhorse	Observed	14.76-17.50	16.50-17.20	-
	Model Mean	13.2-18.5	18.5-22.7	22.7+
	Model Extreme	10.8-20.9	16.2-25.0	20.4+
	Literature[d]	13.0-15.0	15.0-20.0	19.0+
Sicklefin Redhorse	Observed	14.26-19.60	18.19-22.17	28.00-62.9
	Model Mean	13.8-18.5	18.5-22.2	22.2+
	Model Extreme	11.4-20.6	16.5-24.5	20.1+
	Literature	-	-	-
Silver Redhorse	Observed	14.71-19.31	18.15-22.97	21.34-29.63
	Model Mean	15.0-18.9	18.9-21.9	21.9+
	Model Extreme	13.3-20.6	17.2-23.7	20.2+
	Literature[e]	13.0-17.0	17.0-23.0	19.5+
Northern Hog Sucker	Observed	14.12-20.47	15.10-20.6	22.00-49.22
	Model Mean	13.9-19.3	19.3-23.4	23.5+
	Model Extreme	11.6-21.4	17.0-25.7	21.2+
	Literature[c]	12.0-19.4	19.8-22.0	22.0+

[a]Bunt et al. 2013, [b]Fuiman and Whitman 1979, [c]Buynak and Mohr 1978, [d]Auer 1982, [e]Kay et al. 1994

Table 4. Estimated parameter coefficients and standard errors for the ANCOVA model of ontogeny. The independent variable was TL (mm) and the dependent variable was the ontogeny index score. Three similar slopes (β1) were fit, and separate intercepts (β0) were assigned for each species.

	Intercept(β_0)		Slope (β_1)	
Species	Estimate	SE	Estimate	SE
Black Redhorse	-7.6533	0.2646	0.5121	0.0136
Golden Redhorse	-9.5696	0.3950	0.7065	0.0241
River Redhorse	-6.7355	0.2813	0.5121	0.0136
Sicklefin Redhorse	-8.0308	0.8479	0.5826	0.0477
Silver Redhorse	-10.6130	0.4196	0.7065	0.0241
Northern Hog Sucker	-7.0967	0.2423	0.5121	0.0136

Table 5. Estimated parameter coefficients and standard errors for the quantile regression models describing larval growth among the 90th and 10th percentiles of TL. The independent variable was day of the year and the dependent variable was TL (mm). A common slope was fit, and species and year were modeled as covariates.

	90th percentile		10th percentile	
Parameter	Estimate	SE	Estimate	SE
Slope (β_1)	0.0066	0.0006	0.0023	0.0004
Species ($\beta_{Species}$)				
Black Redhorse	2.0097	0.0824	2.5194	0.0691
Golden Redhorse	1.8092	0.0919	2.3274	0.0727
Sicklefin Redhorse	1.8575	0.1125	2.3822	0.0815
Silver Redhorse	2.0272	0.0741	2.4744	0.0603
Northern Hog Sucker	2.0531	0.0801	2.4185	0.0640
Year (β_{Year})				
2013	-0.0705	0.0106	-0.0274	0.0097
2014	0.0000	0.0000	0.0000	0.0000
2015	-0.0106	0.0149	0.0091	0.0093

Table 6. Estimated parameter coefficients and standard errors for the linear regression models describing juvenile growth. The independent variable was day of the year and the dependent variable was TL (mm).

Parameter	Estimate	SE
Intercept (β_0)		
Black Redhorse	-55.5543	6.1097
Golden Redhorse	-94.7384	9.4845
Sicklefin Redhorse	-56.7723	20.4423
Northern Hog Sucker	-41.7257	2.4959
Slope (β_1)		
Black Redhorse	0.4917	0.0293
Golden Redhorse	0.6012	0.0411
Sicklefin Redhorse	0.4309	0.0843
Northern Hog Sucker	0.4134	0.0116
Year (β_{Year})		
2013	-10.8496	0.6474
2014	0.0000	0.0000
2015	2.2184	1.0554

Figures

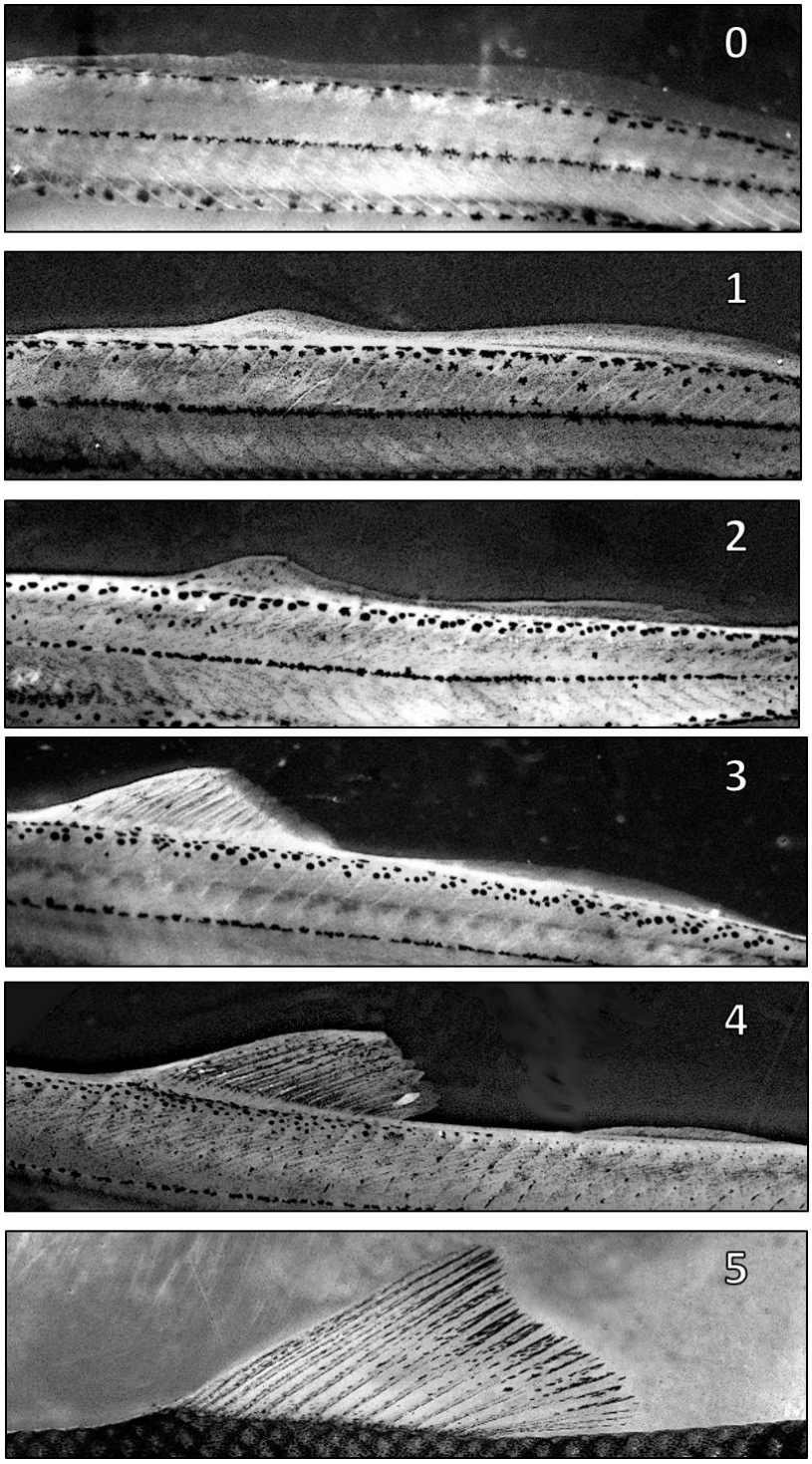

Figure 1. Development of the dorsal fin in larval and juvenile suckers and corresponding index scores.

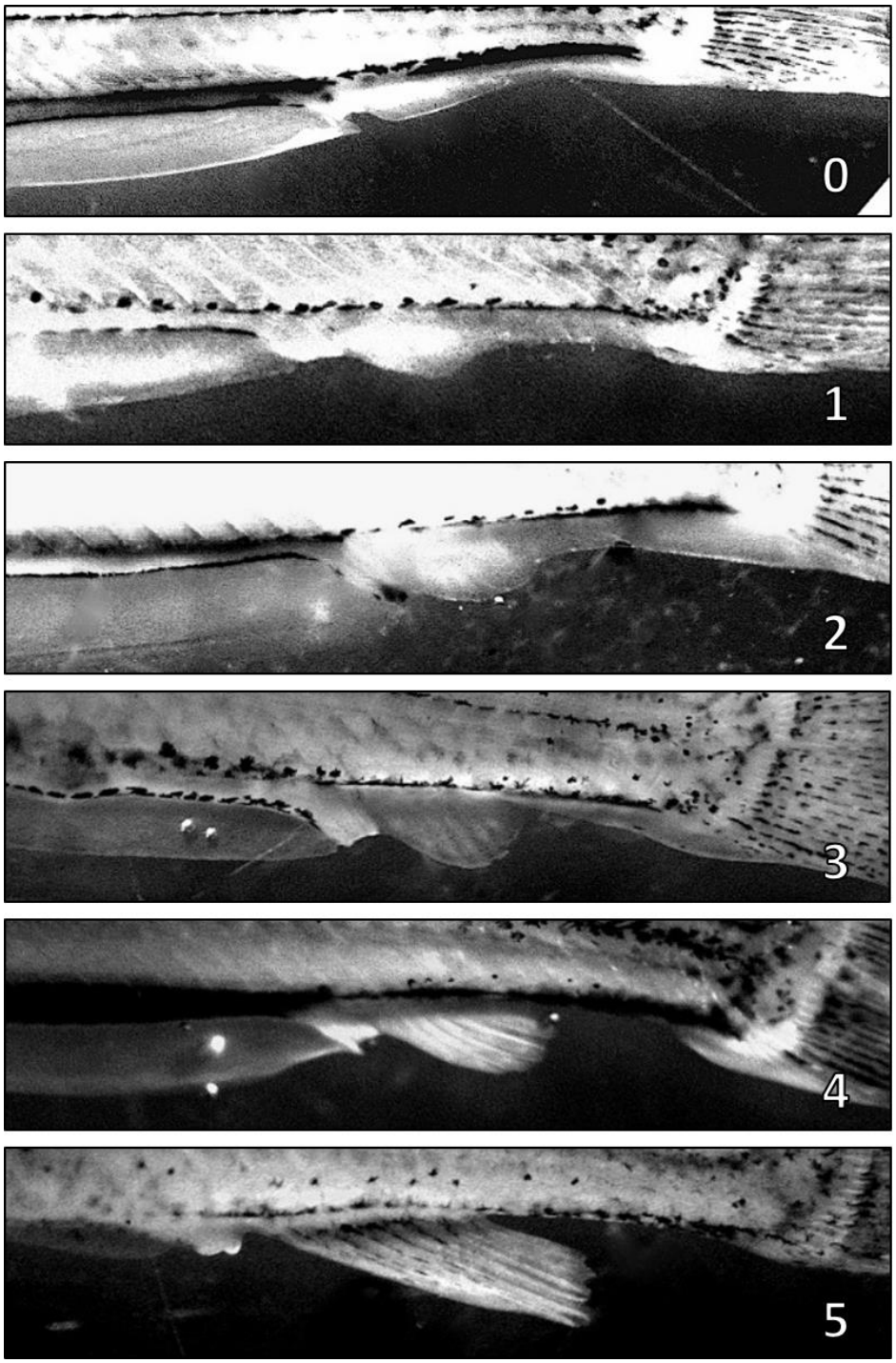

Figure 2. Development of the anal fin in larval and juvenile suckers and corresponding index scores.

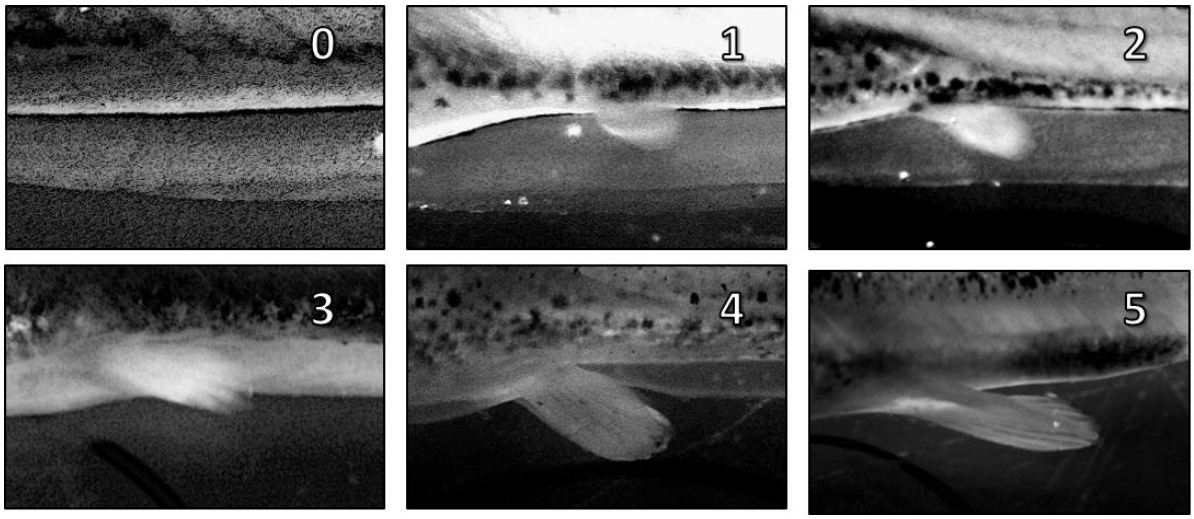

Figure 3. Development of the pelvic fin in larval and juvenile suckers and corresponding index scores.

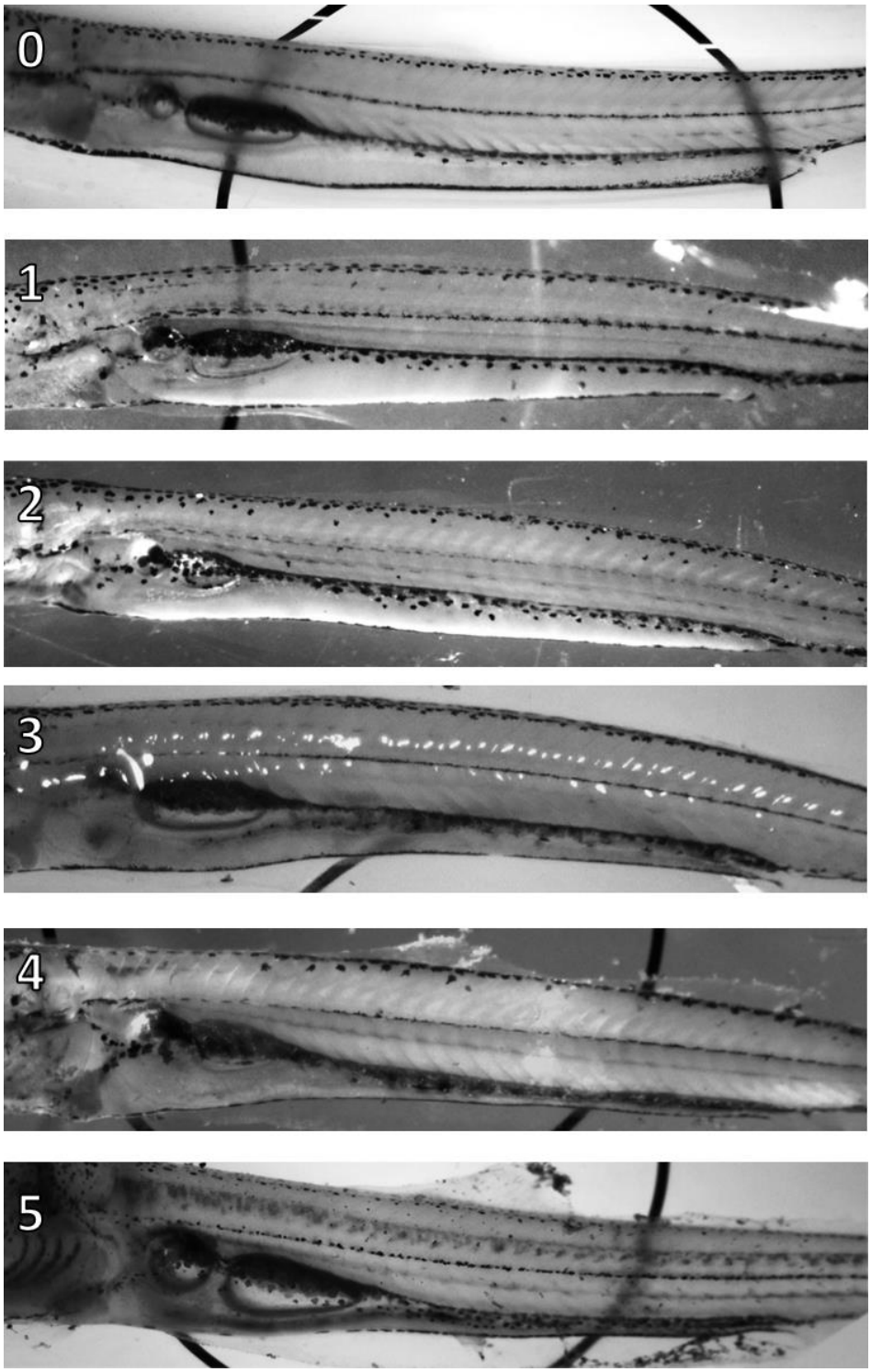

Figure 4. Yolk sack absorption in larval and juvenile suckers and corresponding index scores.

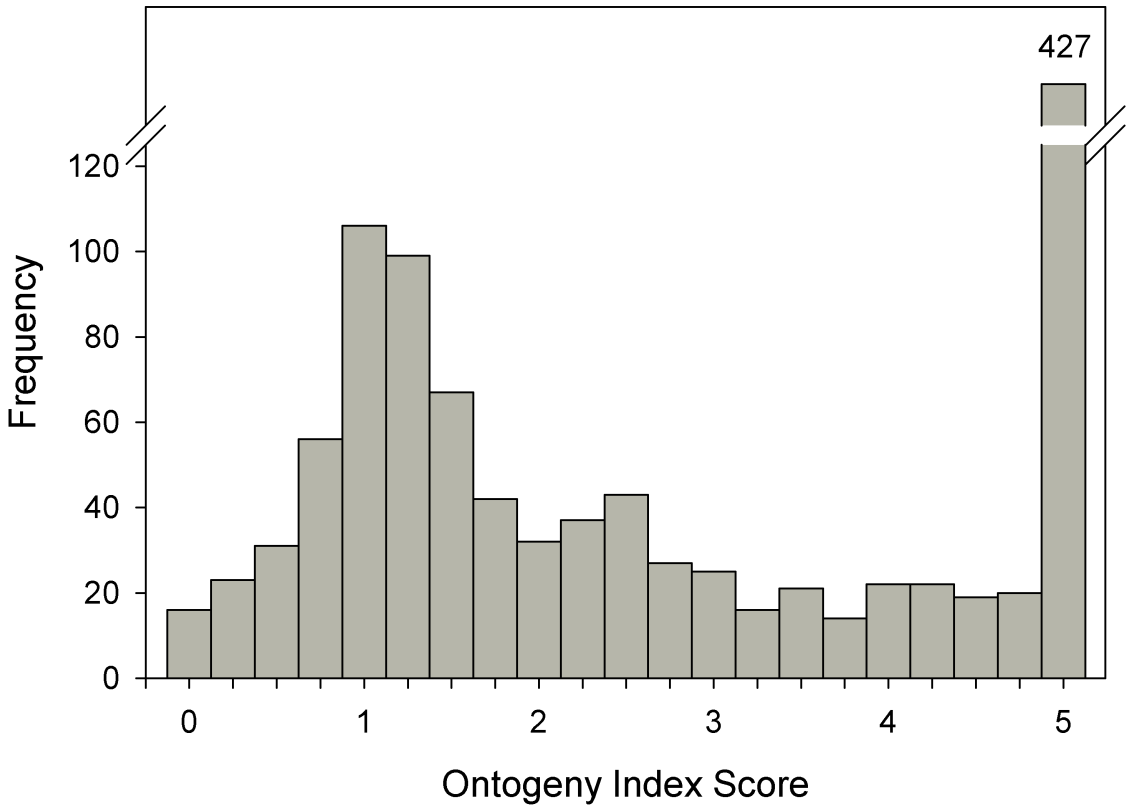

Figure 5. Histogram depicting frequency of ontogeny index scores of larval and juvenile suckers.

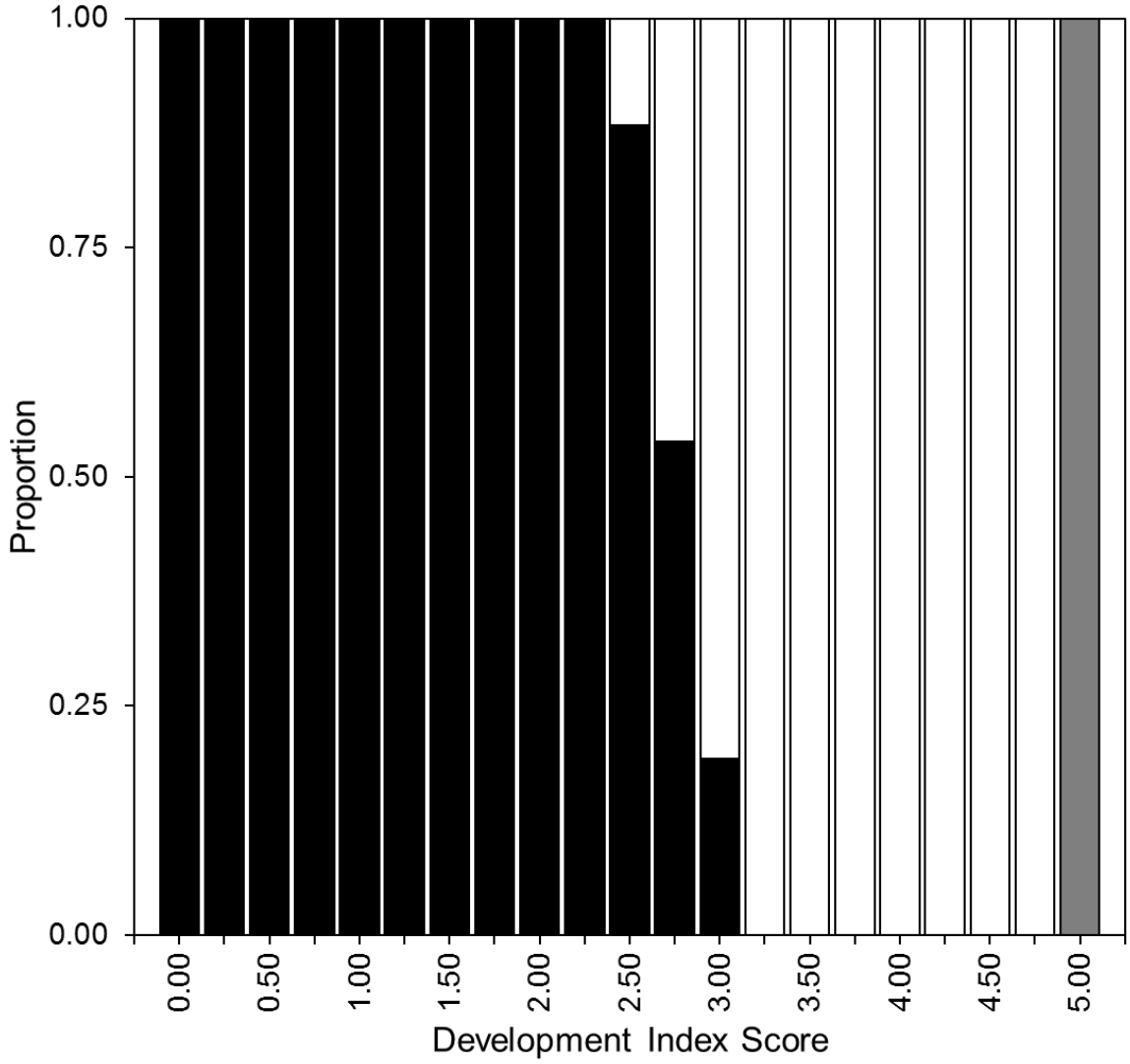

Figure 6. Proportions of development index scores corresponding to development classes mesolarva (black), metalarva (white), and juvenile (gray).

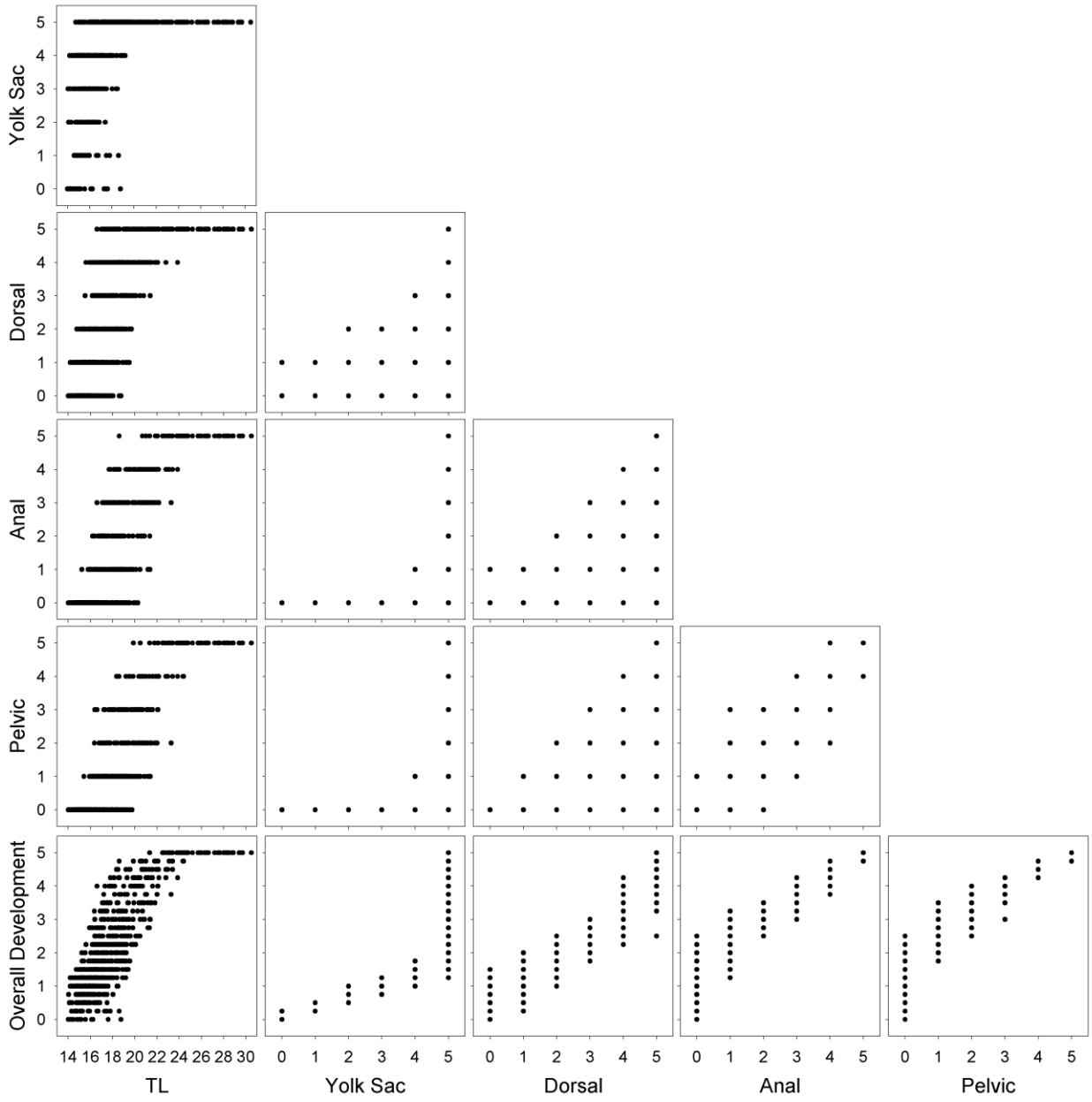

Figure 7. Relationships among overall development scores, four component development scores, and total length (mm) of larval and juvenile suckers. Points indicate one or more observations.

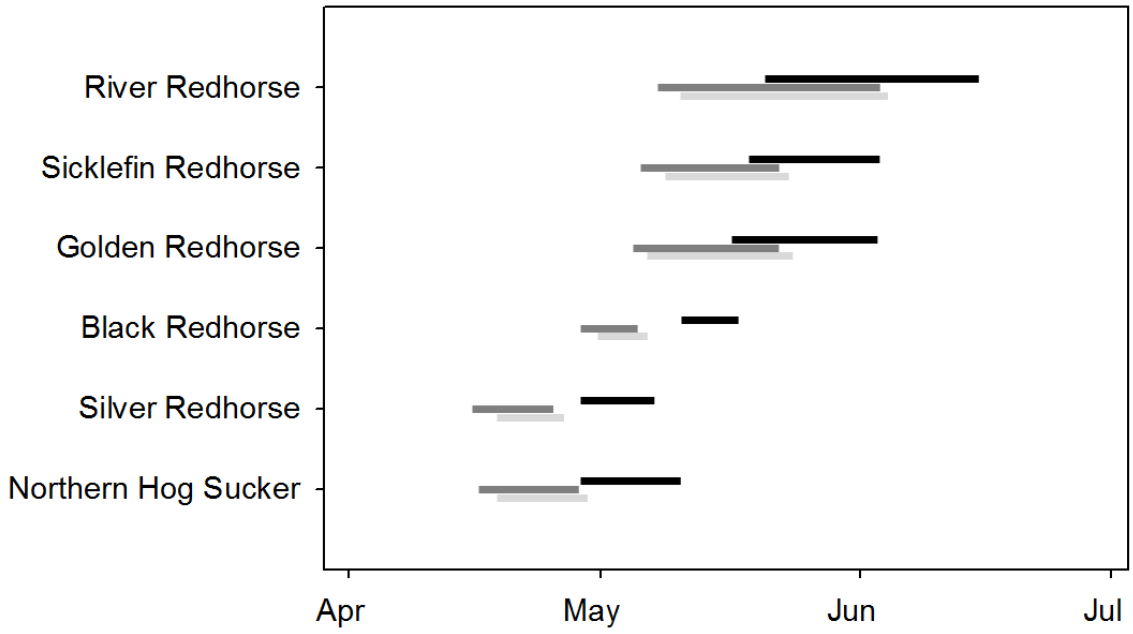

Figure 8. Emergence phenology of six sucker species in the Valley River. Shaded bars indicate the estimated duration of peak emergence for each species during 2013 (black), 2014 (dark gray), and 2015 (light gray).

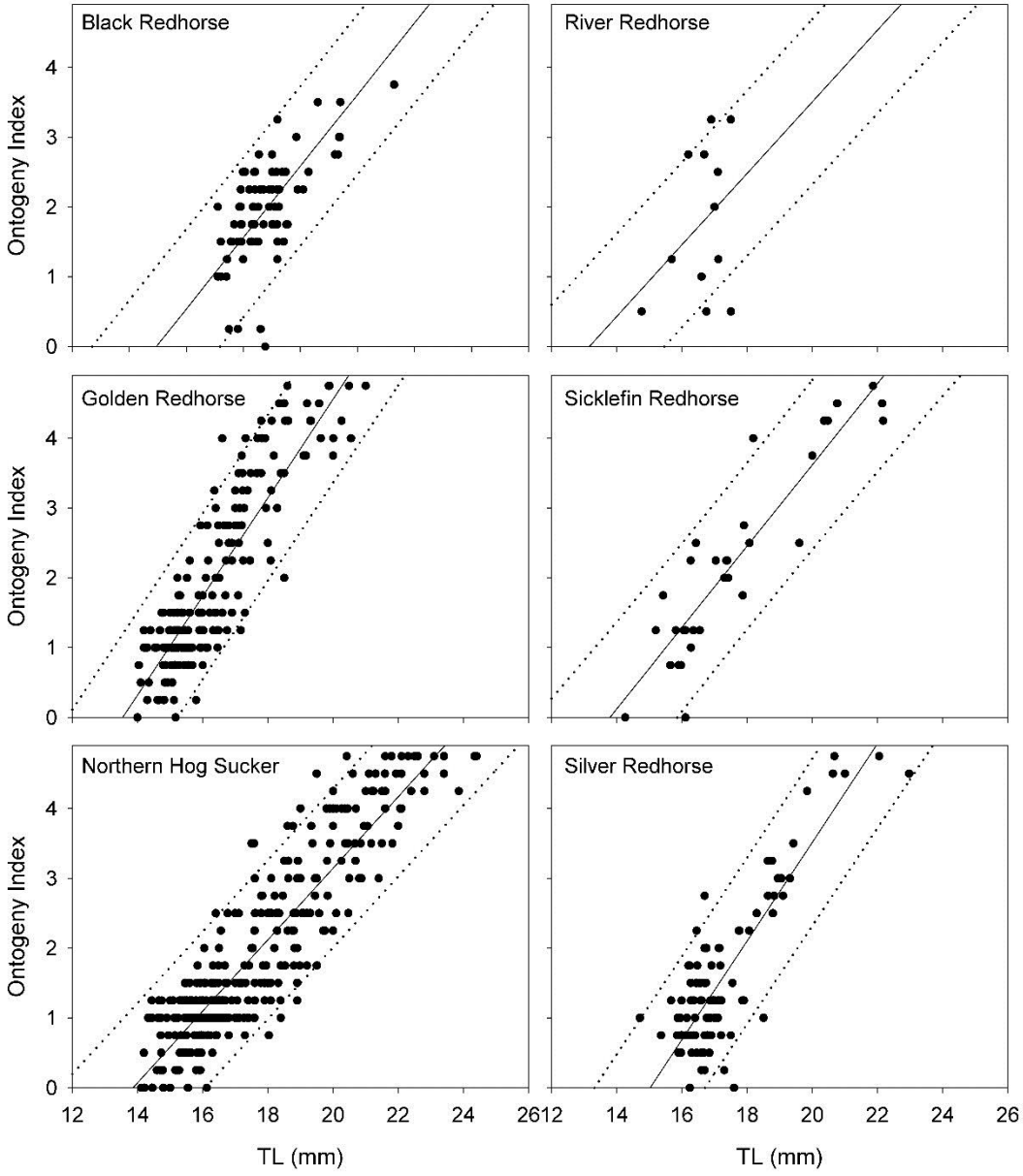

Figure 9. Ontogeny rates (index) of six larval sucker species. Solid regression lines represent the mean development rate, dotted lines represent the 95% prediction interval, and points represent specific observations.

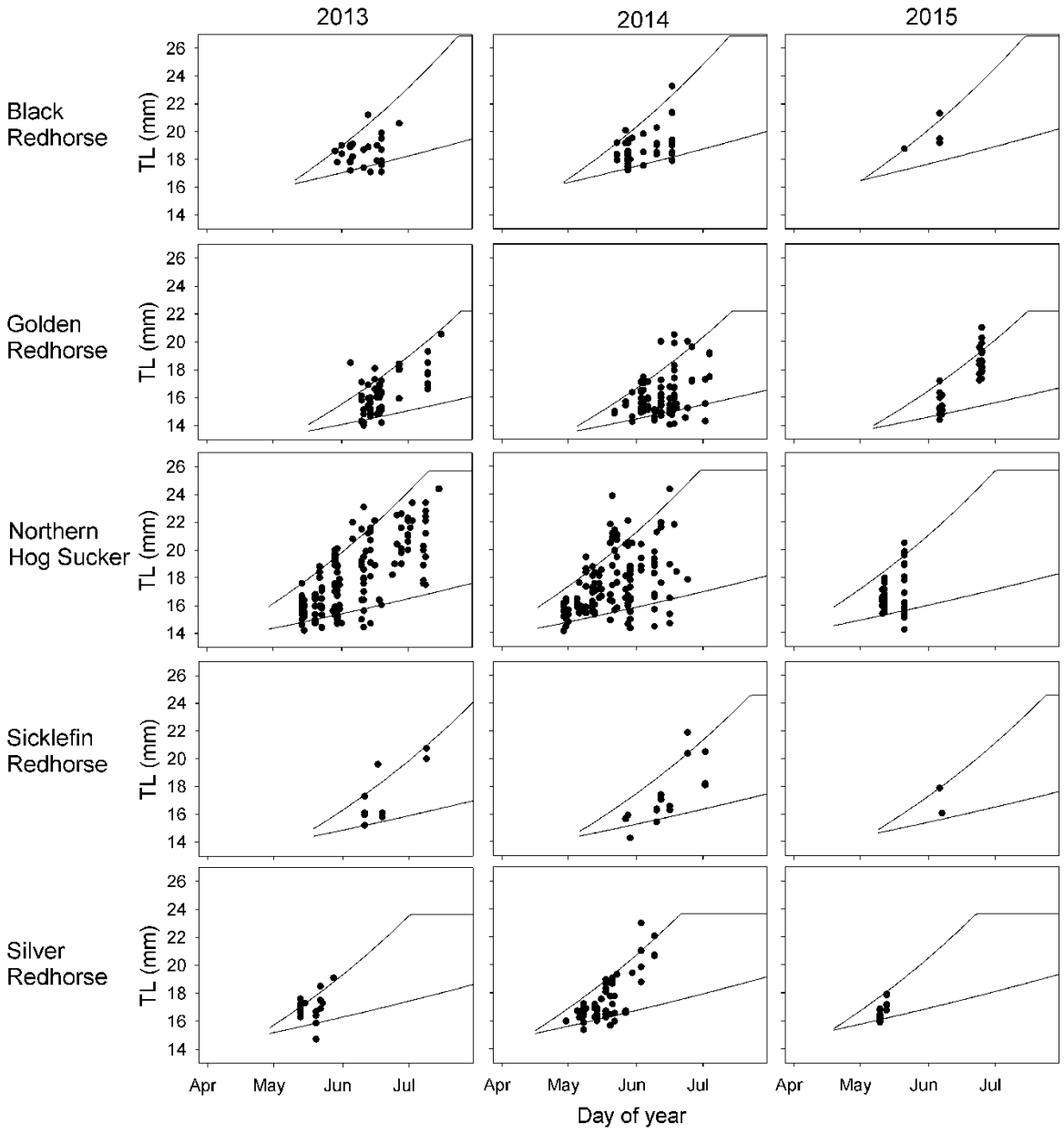

Figure 10. Total length (mm) of five larval sucker species in 2013, 2014, and 2015. Lines represent the 90th and 10th percentiles of modeled size, and points represent specific observations. Upper asymptotes represent the maximum size of larvae.

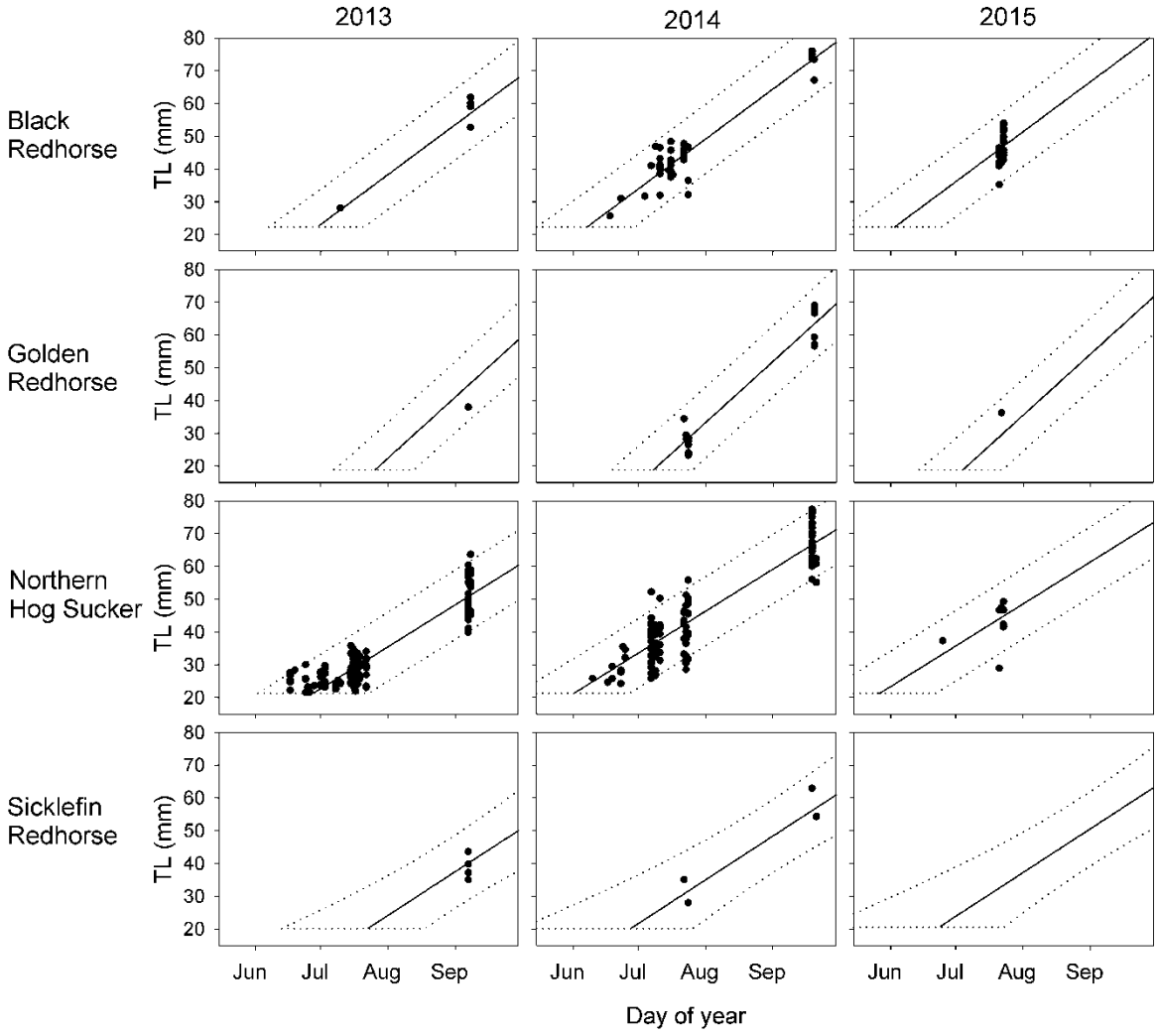

Figure 11. Total length (mm) of five juvenile sucker species sampled during 2013, 2014, and 2015. Solid lines represent modeled linear regressions, dotted lines represent 95% prediction intervals, and points represent specific observations. Lower bounds represent the minimum size of juveniles.

CHAPTER 4: Characterization and comparison of swimming ability among five species of larval and juvenile sucker

Abstract

An understanding of the swimming ability of larval and juvenile fishes is important for identifying environmental influences during critical periods of early-development. Swimming ability is studied via laboratory experiments, where water velocity can be controlled and swimming behavior observed. The early development of fishes encompasses dramatic ontogenetic changes and somatic growth, which affects swimming ability. Swimming performance regulates habitat selection by larval and juvenile fish and may determine dispersal within a system. A series of fixed velocity swimming tests was used to assess prolonged swimming speeds of larval and juvenile captive-reared Sicklefin Redhorse *Moxostoma* sp. and wild-caught Black Redhorse *M. duquesnei*, Golden Redhorse *M. erythrurum*, Silver Redhorse *M. anisurum*, and Northern Hog Sucker *Hypentelium nigricans*. The probability of prolonged swimming among Sicklefin Redhorse was negatively related to water velocity and positively related to fish total length (TL). The velocity at which 50% of fish failed to maintain position over a 30-min interval (FV50) was 0.118 m/s for 18.0 mm fish and 0.145 m/s for 25.0 mm fish. The probability of prolonged swimming among wild-caught suckers was negatively related to water velocity, positively related to TL, and differed slightly among species. FV50 was highest for Northern Hog Sucker (0.110 m/s at 18.0 mm, 0.142 m/s at 25.0 mm) and lowest for Black Redhorse (0.094 m/s at 18.0 mm, 0.126 m/s at 25.0 mm). Tailbeat frequency was positively related to water velocity and negatively related to TL. The transition to epibenthic swimming occurred at the mid-point of the metalarva stage (19.0-21.0 mm TL). Given the relative rarity of water velocity less than prolonged swimming speed for these fishes in riffles, short upstream migration may be possible, but is probably atypical. The findings of this study may be applied to enhance the conservation and management of these and other suckers, throughout their ranges.

Introduction

Fish typically rely on active swimming for locomotion and transport in the aquatic environment. By undulating the body and using fins to exert force on the surrounding water, fish generate thrust. The swimming ability of fish may be studied via laboratory experiments using swim tunnels, chambers, or flumes, where flow can be precisely controlled and fish swimming behavior can be observed (Beamish 1966; Bell and Terhune 1970; Ward et al. 2002a). Apparatuses, protocols, and associated metrics have been devised to describe burst-swimming, prolonged-swimming, and sustained-swimming ability. Burst swimming refers to the fastest speeds that a fish can achieve, depends on anaerobic energy-generating processes, and can be maintained for only a short duration (< 20 s). Prolonged and sustained swimming depend primarily on aerobic processes and can be maintained for durations of 2-200 min or >200 min, respectively (Brett 1964; Beamish 1978; Hammer 1995).

The early-development of fishes is a period of dramatic ontogenetic changes and rapid somatic growth, which affects swimming ability. Growth is particularly important when considering the swimming ability of larval and juvenile fish, because the effects of viscous drag are exacerbated at smaller sizes (Dabrowski et al. 1988; Muller et al. 2000). The fin structures of altricial fishes change substantially through development, as dorsal-, anal-, and caudal-fin spines and rays develop from the median finfold and pelvic and pectoral fins arise, which results in a more efficient propulsion system (Affleck 1950; Kay et al. 1994; Childs and Clarkson 1996). The body shape of larval fish also changes with development, typically becoming more hydrodynamic, and aerobic capacity increases commensurate with an increase red muscle fibers (Batty 1984; Hale 1999). Compared to adult fish, larvae typically employ a more anguilliform-like swimming style, with a large body wave amplitude that is more energetically advantageous, given the physiological constraints of most larvae (Batty 1984; Webb and Weihs 1986). Because of their small size, the propulsive wave of young fish generates less thrust, which must be compensated through higher tailbeat frequencies (Bainbridge 1957; van Weerden et al. 2014).

Swimming performance is an important regulator of local habitat selection by larval and juvenile fish and may determine larval fish dispersal within a system (Wolter and

Arlinghaus 2003; Pavlov et al. 2008). Larval fish nursery habitat may be defined as areas with water velocity less than the estimated prolonged swimming speed of fish that size (Scheidegger and Bain 1995). Due to the energetic demands of maintaining position in swifter water (e.g., higher tailbeat frequency), larvae in slower nursery habitats can exhibit higher rates of growth and survival (Weyers et al. 2003). Larval fish may occasionally become entrained in water velocity exceeding their swimming ability, or they may actively enter the drift (e.g., to locate better habitat, for predator avoidance, or to feed). The frequency and duration of larval drift is, therefore, related to a larva's ability to avoid displacement or to maintain control of its spatial position within the drift (Brown and Armstrong 1985; Kennedy and Vinyard 1997; Pavlov et al. 2008). Retention of larval fish in lotic systems has been linked to the upstream availability of low-velocity habitats (Wolter and Sukhodolov 2008; Schludermann et al. 2012). Exceedingly high flows may accelerate dispersal, distribute fish into suboptimal habitats, impede deliberate movement, and may result in fish mortality (Robinson et al. 1998; Ward et al. 2003).

Fixed velocity swimming tests may be used to assess prolonged swimming speeds of larval and juvenile fish (Hammer 1995). The fixed velocity study design involves a series of swimming tests that are akin to a bioassay. That is, fish swimming ability is tested at a number of static water velocities, and the prolonged swimming speed is indexed as the velocity at which 50% of fish fail to maintain their position over a prescribed time interval. This metric is referred to as the FV50 or the "fatigue value-50%" (Childs and Clarkson 1996; Ruetz and Jennings 2000; Ward et al. 2002b). The fixed velocity protocol and FV50 metric have been successfully applied among studies involving different species and sizes of fish (e.g., Houde 1969; Meng 1993; Childs and Clarkson 1996), including larval and juvenile suckers (family Catostomidae; Ruetz and Jennings 2000; Ward et al. 2002b). The FV50 metric can be incorporated into comparisons among treatments or experiments, and can aid in explaining the *in situ* biology and physiology of fishes.

Swim tunnels designed for studying larval and juvenile fish swimming ability must be appropriately-sized to provide minimal turbulence, a cross-sectional area where blocking forces caused by the fish are negligible, and should have sufficient space to accommodate

full-amplitude tailbeats (Bell and Terhune 1970). The swim tunnel must also be able to simulate a variety of water velocity rates. Previously developed swim tunnels for larval and juvenile fish have employed gravity-driven flow systems to provide velocities up to 0.16 m/s (e.g., Ruetz and Jennings 2000) or have utilized small electric pumps to generate higher water velocities (e.g., Ward et al. 2002a).

Suckers comprise a taxon that is of high conservation concern but has received relatively little attention from the scientific community (Cooke et al. 2005). Over seventy-five species of sucker are native to a variety of freshwaters environments in North and Central America (Harris and Mayden 2001). In some aquatic systems, suckers comprise a higher proportion of the biomass than any other taxon (Becker 1983). There is a high rate of endemism within the family, and many sucker species, particularly those with a limited distributional range, have experienced population declines due to habitat modification and other anthropogenic causes (Weyers et al. 2003; Cooke et al. 2005; Grabowski et al. 2012). Sympatry is typical where distributional ranges of several species overlap and local habitat parameters are mutually conducive, however hybridization is typically precluded by species-specific reproductive isolating mechanisms (Favrot 2009).

Seven species of sucker, including six redhorse (*Moxostoma*) species, inhabit and spawn in the Valley River, North Carolina, a tributary to the Hiwassee River (Table 1). This includes the Sicklefin Redhorse, which is of particularly high conservation concern, is endemic to only two southern Appalachian river systems (the Hiwassee and Little Tennessee), and has been extirpated from an estimated 58% of its historic range (Jenkins 1999). A Candidate Conservation Agreement was established for the Sicklefin Redhorse in February 2016, which instituted a formal arrangement between state and federal agencies, the Eastern Band of Cherokee Indians, and utility companies to conserve the Sicklefin Redhorse and its habitat. Furthermore, a captive-propagation program is in place for Sicklefin Redhorse, including release of cultured individuals into native waters (Petty et al. 2010). Suitable physical habitat, including water velocity distributions for spawning and adult foraging Sicklefin Redhorse have been previously identified from field observations of fish habitat use and availability (Favrot 2009). However, the swimming ability of larval and

juvenile Sicklefin Redhorse have never been studied or measured. The successful management of a species requires the availability of suitable microhabitats along the ontogenetic niche profile (Schiemer et al. 2003). Thus, the bounds of lotic habitat suitability for larval and juvenile Sicklefin Redhorse and other sucker species remain unknown, impeding effective conservation and management planning.

To develop a more thorough, comparative, and mechanistic understanding of nursery habitat requirements and dispersal of suckers, I conducted fixed velocity tests that incorporated wild-caught larval and juvenile suckers, as well as captive-reared larval and juvenile Sicklefin Redhorse. The goals of this research were to (1) determine and compare prolonged swimming speed among larval and juvenile captive-reared and wild-caught suckers, (2) model tailbeat frequency as a function of water velocity and fish size, (3) determine the lengths at which suckers transition from pelagic to epibenthic swimming, and (4) evaluate relevant water velocities *in situ* and assess the potential for upstream movement.

Methods

Fish Subjects

Captive-reared larval Sicklefin Redhorse were obtained from Conservation Fisheries Incorporated (CFI; Petty et al. 2010), and swimming tests were performed on-site at their rearing facility at Joe Johnson Animal Research and Teaching Unit, University of Tennessee, Knoxville. Sicklefin Redhorse were artificially propagated on 28 April 2014 using broodstock from the Little Tennessee River, near Franklin, North Carolina. Eggs were initially incubated in hatching jars, and were subsequently moved to trays set in oval vats (113 L; 86 cm x 61 cm x 33 cm; opaque black plastic) for hatching and early larval development. Most eggs had hatched and larvae were active by 9 May 2014 (11 d post-spawn). Larvae demonstrated swim-up behavior as early as 13 May 2014 (15 d post-spawn), and all larvae had exhibited swim-up behavior by 16 May 2014 (18 d post-spawn). Upon swim-up and feeding, larvae were moved into the oval vats for grow out. Although water in each vat exhibited some turbulence, directional flow was negligible. Vats were part of a

recirculating aquaculture system. Water filtration was provided by a common 570 L sump, which had provided mechanical and biological filtration via fiber filter pads and plastic bead media. The vats had lateral overflow drains (with screened standpipes) that drained through hoses to the sump. During the course of this study, larvae were fed twice daily with fine powdered or pelleted commercial fish larvae food (Ziegler Bros., Gardners, PA), live brine shrimp *Artemia spp.* nauplii. frozen zooplankton, and finely chopped frozen bloodworms *Chironimidae spp.*

A total of 100 captive-reared Sicklefin Redhorse were used in this experiment, ranging in size 15.6-35.8 mm total length (TL; Table 2). Captive-reared Sicklefin Redhorse were tested on 16 and 22 May, 06 and 19 June, and 17 July 2014, which represented ages of 1-, 2-, 4-, 6-, and 10-week(s) post-swim up, respectively. After each test, TL (mm) was measured using calipers or from photographs and software (Leica Microsystems, Heerbrugg, Switzerland; LAS EZ v. 2.1.0). Subjects were either retained as vouchers or returned to a separate rearing vat; no fish was tested more than once.

Wild larval suckers were collected from the Valley River and the Hiwassee River upstream of Hiwassee Lake (Cherokee County, North Carolina). Larval suckers were captured using hand-held dip-nets and stationary light traps, and juvenile suckers were collected using backpack electrofishing (see Chapter 2). Capture, holding, and handling stress was minimized by careful transfer from sampling devices to holding containers and swim tunnel, and by providing clean, oxygenated water at acclimatization temperatures within 1.5^{o}C of those observed at the points and times of capture. Fish that were apparently injured or deformed were excluded from this experiment. All fish test subjects were only used once. After each test, wild-caught fish were photographed at 20x magnification using a Leica EZ4HD dissecting microscope, measured, and transferred to vials filled with 100% ethanol. Wild-caught fish were subsequently identified to species using a genetic barcoding procedure (Ward et al. 2009; Chapter 2). A total of 220 wild-caught fish, representing six species of sucker, were used in this experiment, and they ranged in size 14.1-54.0 mm (Figure 1). However, due to the lack of a representative sample of fish larger than 25 mm for all species except Black Redhorse, analyses only incorporated wild-caught fish $\leq$ 25.0 mm.

Wild-caught fish were tested on 28 dates across two annual cohorts (29 Apr-11 July 2014 and 10 May-23 July 2015).

Swim Tunnel Apparatus

The swim tunnel apparatus (Figure 2) used in this study was generally based on a design presented by Ward et al. (2002a), but modified to include two in-line test chambers, rather than one. The apparatus was designed to fit in a 100-L cooler. Water-flow through the apparatus was provided by a DC-powered bilge pump (Rule Industries, Gloucester, Massachusetts; Model 800GPH) connected to a 12-V battery. The inside diameter of the swim tunnel was 3.8 cm, designed to accommodate typical larval and juvenile suckers with a dorsoventral height up to 4.5 mm (Bell and Terhune 1970). This system produced accurately measured water velocities up to 0.25 m/s. Two ball valves were incorporated into this design; one valve was used to precisely adjust the water velocity before fish were introduced to the system, and the other valve was used to gradually increase velocity to the final test velocity after fish were introduced. Fish were introduced into the system by decoupling the two chambers and pouring larval fish and water from the holding container into the open ends. Both ends of each chamber were bound by 1-mm nitex mesh. Water velocity was monitored using an in-line digital flow meter (Great Plains Industries, Wichita, Kansas; GPI TM050-N). This meter measured the volume of water circulating through the system as gal/min (3.79 L/min). Volumetric rates were converted to linear velocities in the swim chambers by calculating the cross-sectional area of the swim-chamber (in this system, 11.35 cm^2), applying appropriate conversion factors to volumetric rates (i.e., convert gal/min to m^3/s), and dividing the volumetric rate by the cross-sectional area. For this system, linear velocity (m/s) could be obtained by multiplying the volumetric rate (in gal/min) by 0.0555.

Fixed Velocity Tests

Fixed velocity swimming tests were conducted to determine larval and juvenile sucker swimming performance. In such tests, fish swimming ability is tested at a number of

water velocities, and the prolonged swimming speed is estimated as the velocity where 50% of fish fail to maintain their position in the swim tunnel during a prescribed time interval. The fatigue value (FV50) is estimated using logistic regression, assuming a sigmoidal relationship between prolonged swimming success and water velocity. Prolonged swimming was observed for up to 60 min for captive-reared Sicklefin Redhorse and up to 30-min for wild suckers.

In this experiment, fish were exposed to velocities ranging 0.057-0.248 m/s, and each individual was used for only one test at one experimental velocity. The swim tunnel was maintained within 1.5^{o}C of water temperatures observed at the points and times of capture (for wild fish) or rearing temperatures (for captive reared fish). This ensured that fish were acclimated and acclimatized to the temperature at which they were being tested. It also ensured that the indexed swimming ability was ecologically relevant to the temperatures encountered in their natural environment. Water was aerated between tests to maintain dissolved oxygen (DO) > 6.0 ppm for the duration of all tests. Water quality parameters were monitored continuously using a YSI-96 meter (Xylem Analytics, Yellow Springs, Ohio). Once introduced to the swim tunnel, fish were given approximately 5 min to become accustomed to the chamber at a gentle velocity (< 0.05 m/s), which was then increased gradually, over a 15-20 s period, to the final test velocity. Observations were censored from analysis for any fish that refused to swim or failed to establish a normal swimming gait at the test velocity before failing the test. The velocity treatments assigned to captive-reared and wild-caught suckers were selected based on the size of the fish and expected performance, to reduce variance in logistic regression modeling.

Logistic regression modeling was carried out using Proc Logistic in SAS 9.4 (SAS Institute, Cary, North Carolina), and was conducted separately for captive-reared and wild fish. For captive-reared Sicklefin Redhorse, which were observed for up to 60 min, separate models were built using observations at 30 min and 60 min. For wild suckers, FV50 estimates were obtained using observations at 30 min, only. Any fish that successfully swam for the duration of a test was modeled as ‘1’ and any fish that became impinged on the screen at the downstream end of the swim tunnel was modeled as ‘0’. The probability of prolonged

swimming was related to water velocity, the independent variable. For captive-reared Sicklefin Redhorse, TL was included as a covariate. For wild suckers, TL and species were included as covariates. The fit of models to the data was assessed using the Hosmer-Lemeshow test (Hosmer and Lemeshow 1989), and covariate significance was assessed. Prolonged swimming speed, across a range of sizes, was indexed as FV50, the modeled velocity at which 50% of fish failed to swim for the duration of a test. Associated 95% confidence intervals were also derived from logistic regression models.

Tailbeat Frequency and Swimming Position

A minimum of 1 min of video footage was recorded for each of 107 tests involving fish $\leq$ 25 mm (23 captive Sicklefin Redhorse and 84 wild suckers). Tests were video-recorded at 60 or 120 frames/s using a hand-held digital camera (GoPro, San Mateo, California; Hero 3 Silver). Videos were recorded from above, looking down on the fish's dorsum, and were only obtained after fish established a steady gait for prolonged swimming. Video footage was examined frame-by-frame using QuickTime video player (Apple, Cupertino, California; v. 7.7.9).

To obtain an estimated average tailbeat frequency (tailbeats/s) for a given fish swimming against a given water velocity, five individual periods where the fish remained stationary against the current for greater than 2 s were identified. For each period, the number of tailbeats were counted, where one tailbeat was counted as a complete oscillation from one side, to the other side, and back again (Ohlberger et al. 2007). For each of the five observations, the tailbeats frequency was calculated by dividing the number of tailbeats by the time elapsed (s). The arithmetic mean of the five observations was then calculated. Linear regression was used to model mean tailbeat frequency as a function of water velocity and the previously identified best measure of development (i.e., TL, ontogeny index, or age), including species as a classification variable. Significant relationships among variables were identified from the model, and Tukey's test was used to detect significant differences in tailbeat frequencies among the species.

The transition from a pelagic to epibenthic swimming position was also ascertained from video footage. For each fish, it was noted whether swimming was predominantly pelagic (at the top and sides of the swim tunnel) or predominantly epibenthic (along the bottom of the swim tunnel). These observations were incorporated into a logistic regression model, which related the probability of pelagic swimming to TL (mm) and incorporated species as a class covariate. The mean TL, and associated 95% confidence limits, at which 50% of fish had assumed an epibenthic swimming position was determined for each species.

Upstream Movement Assessment

I assessed the potential for sucker larvae and juveniles to move upstream by measuring water velocity along cross sectional transects at four riffle crests in the Valley River and one riffle crest in the Hiwassee River (Figure 3). The most downstream riffle in the Valley River (rkm 2.5) was surveyed on 22 May 2015 and represented the first swift-water habitat upstream of the impounded water of Hiwassee Lake. The three other riffles on the Valley River (rkm 8.8, 21.1, and 29.5) were surveyed on 26 June 2015 and were selected for their proximity to fish sampling sites. The most downstream riffle in the Hiwassee River (rkm 156.6) was surveyed on 22 May 2015, represented the first swift-water habitat upstream of Hiwassee Lake, and was 2.7 km upstream of the confluence of the Hiwassee and Valley rivers.

Instream water velocity was measured using a Marsh-McBirney Model 2000 digital flow meter (Hach, Loveland, Colorado). River discharge was less than the 8-yr median daily discharge for both systems on the dates examined, and reservoir elevations were near full-summer-pool (USGS gauge 03550000; TVA 2015). Water velocity was measured at transect points that were 0.25 m from each bank and every 2 m (Valley River) or 5 m (Hiwassee River) along the riffle crest at each site. At each station, mean-column velocity was measured at 60% of the total depth and at the streambed; these two measurements were summarized individually, as mean velocity (i.e., 60% depth) is relevant to pelagic larvae, whereas streambed velocity is more relevant to benthic juveniles. Data for each transect were summarized as the frequency of observed water velocity within 0.05 m/s intervals (i.e.,

0-0.05 m/s, 0.06-0.10 m/s, … and >0.5 m/s), and as the cumulative percentage less than each 0.05 m/s interval. The potential for upstream movement by larval and juvenile suckers was assessed by simply comparing these field-measured point velocities to prolonged swimming speeds.

Results

Fixed Velocity Tests

For captive Sicklefin Redhorse, prolonged swimming success over 30 min depended on water velocity (Wald $\chi^2 = 25.4332$, df = 1, $P < 0.0001$) and TL (Wald $\chi^2 = 15.6522$, df = 1, $P < 0.0001$). This model was a good fit to the data (Hosmer-Lemeshow $\chi^2 = 10.1685$, df = 8, $P = 0.2534$). It indicated that the probability of prolonged swimming was negatively related to velocity and positively related to TL (Table 3). Based on this model, FV50 increased with TL at a rate of 0.0038 m/s per mm, and was 0.118 m/s for 18.0 mm fish and 0.145 m/s for 25.0 mm fish (Figure 4).

The majority (76.6%) of captive Sicklefin Redhorse that successfully completed a 30-min test continued to swim successfully to 60 min. Prolonged swimming success over 60 min depended on water velocity (Wald $\chi^2 = 24.2463$, df = 1, $P < 0.0001$) and TL (Wald $\chi^2 = 14.8135$, df = 1, $P < 0.0001$), and the associated model was also a good fit to the data (Hosmer-Lemeshow $\chi^2 = 14.5640$, df = 8, $P = 0.0682$). Based on this model, FV50 increased with TL at a rate of 0.0036 m/s per mm, and was 0.108 m/s for 18.0 mm fish and 0.133 m/s for 25.0 mm fish. Thus, point estimates of FV50 over a 60-min duration were lower than those for a 30-min duration, but the two estimates were statistically similar across all sizes and water velocities that were assessed (Figure 4). The mean water temperature during fixed velocity tests conducted on captive-reared Sicklefin Redhorse ranged from 19.8°C to 21.5°C (Figure 5).

Logistic models were developed for wild, Black Redhorse, Golden Redhorse, Silver Redhorse, and Northern Hog Sucker. Due to low sample sizes, logistic models for wild Sicklefin Redhorse and River Redhorse did not converge and were, therefore, omitted from

the modeling procedure. A total of 36 observations of suckers larger than 25 mm (23 Black Redhorse, 2 Silver Redhorse, and 11 Northern Hog Suckers) that were collected in late-June and July of both years were excluded from analyses because of the low sample size of fish larger than 25 mm. Prolonged swimming success for these species depended on water velocity (Wald $\chi^2 = 35.6428$, df = 1, $P < 0.0001$) and TL (Wald $\chi^2 = 14.4157$, df = 1, $P < 0.0001$). There was also weak evidence suggesting that prolonged swimming ability differed among some species (Wald $\chi^2 = 6.7991$, df = 3, $P = 0.0786$). This model was a good fit to the data (Hosmer-Lemeshow $\chi^2 = 8.9227$, df = 8, $P = 0.3489$). It indicated that the probability of prolonged swimming was negatively related to velocity, was dependent on species, and was positively related to TL (Table 4). Across wild sucker species, FV50 increased with TL at a rate of 0.0045 m/s per mm. Point estimates of FV50 were highest for Northern Hog Sucker (0.110 m/s at 18.0 mm, 0.142 m/s at 25.0 mm), followed by Golden Redhorse (0.107 m/s at 18.0 mm, 0.139 m/s at 25.0 mm), Silver Redhorse (0.097 m/s at 18.0 mm, 0.129 m/s at 25.0 mm), and Black Redhorse (0.094 m/s at 18.0 mm, 0.126 m/s at 25.0 mm) (Figure 6). The mean water temperature during fixed velocity tests conducted on wild-caught suckers ≤ 25 mm ranged from 17.0°C to 24.8°C (Figure 5).

Tailbeat Frequency and Swimming Position

Tailbeat frequency was assessed for fish swimming at velocities 0.057-0.167 m/s. The model relating tailbeat frequency to water velocity and TL explained 48.7% of variance in the data. The tailbeat frequency was positively related to velocity ($F = 51.42$, $P < 0.0001$), negatively related to TL ($F = 19.73$, $P < 0.0001$), and depended on species ($F = 39.12$, $P < 0.0001$; Table 5). At a similar size and velocity, Golden Redhorse had a significantly higher mean tailbeat frequency than Sicklefin Redhorse and Silver Redhorse, and Silver Redhorse had a significantly lower mean tailbeat frequency than all other species (Tukey's test, $\alpha \leq 0.05$; Figure 7). The transition from pelagic to epibenthic swimming typically occurred at mean sizes (with 95% confidence intervals) of 19.4 mm (±1 mm) for Sicklefin Redhorse, 20.9 mm (±1.5 mm) for Black Redhorse, 19.0 mm (±0.8 mm) for Golden Redhorse, 20.9 mm (±1.7 mm) for Silver Redhorse, and 21.0 mm (±1.5 mm) for Northern Hog Sucker.

Upstream Movement Assessment

Water velocities measured in the most downstream riffle on the Hiwassee River ranged 0.08-0.49 m/s for mean-column velocity and 0.03-0.49 m/s at the streambed. Velocities among four riffles on the Valley River ranged 0.01 m/s-0.89 m/s for mean-column velocity and 0.01-0.93 m/s at the streambed (Figure 8). In general, the lowest velocity measurements were along the stream margins, with swifter currents occurring mid-channel. Based on results of the fixed velocity swimming tests, small larvae (< 20 mm) can sustain swimming in velocities < 0.10 m/s, whereas juveniles around 30 mm can sustain swimming in velocities < 0.15 m/s. At the riffle on the Hiwassee River, 5% of mean-column and 5% of streambed measurements were < 0.10 m/s, and 5% of mean-column and 16% of streambed measurements were < 0.15 m/s. Riffles on the Valley River averaged 9% velocities < 0.10 m/s and 11% velocities < 0.15 m/s, both mean-column and streambed; however, one riffle (Rkm 8.8) had no water velocity < 0.15 m/s along the cross-sectional transect measured. Based on these observations, short upstream migrations of larvae and juveniles may be technically possible, but are likely atypical, given physiological swimming constraints and habitat considerations.

Discussion

The swim tunnel design employed in this study included several advantages over previously employed systems. This swim tunnel achieved a wider range of test velocities, which were more precisely measured and regulated, relative to gravity-driven systems (Houde 1969; Ruetz and Jennings 2000). This design simultaneously accommodated two independent tests at the same velocity. Although previous studies have simultaneously tested multiple fish in one chamber (e.g., Childs and Clarkson 1996; Ward et al. 2003), the effects of doing so have not been assessed. Segregating subjects ensured independence and the avoidance of confounding factors, such as social interactions among test subjects (e.g., aggregating behavior, as is common in larval and juvenile suckers; Chapter 2), physical interference due to crowding, and hydrodynamic effects of swimming in proximity to other fish (Killen 2012). Based on the behavior and movement of swimming fish in the swim

tunnel, flows appeared laminar at the velocities tested, and velocity was uniform across the cross-section of the swim tunnel and longitudinally in both chambers. Furthermore, the swim tunnel design was highly portable, which made it possible to conduct experiments on-site at the rearing facility and in the field streamside.

The inclusion of fish length as a covariate was necessary for describing swimming ability, both in experiments involving captive-reared Sicklefin Redhorse and in experiments involving wild suckers from the upper Hiwassee River system. This was consistent with prior studies of swimming performance among larval and juvenile suckers (Childs and Clarkson 1996; Ruetz and Jennings 2000; Ward et al. 2002b). In general, swimming ability tends to increase during the larval and juvenile stages due to dramatic ontogenetic changes and rapid somatic growth (Beamish 1978; Batty 1984; Muller et al. 2000).

Previous researchers have variously assessed prolonged swimming ability over 30-min (e.g., Childs and Clarkson 1996; Ward et al. 2002b) and 60-min intervals (e.g., Houde 1969; Meng 1996; Ruetz and Jennings 2000). A shorter test duration can enable more rapid data collection, and both methods are accepted, as prolonged swimming activity is defined as lasting 2-200 min (Brett 1964; Hammer 1995). However, the effect of test duration on the FV50 metric has not been previously tested and is rarely discussed in literature. Childs and Clarkson (1996) speculate, citing loosely related studies, that point estimates of FV50 based on 30-min tests may be up to 20% higher than if they were based on 60-min tests. For captive-reared Sicklefin Redhorse in the present study, 30-min tests resulted in point estimates of FV50 that were less than 10% higher, across TL, than those derived from 60-min tests. This difference was not significant and the resulting slope relating FV50 to TL was nearly identical. The standard errors associated with 30-min tests were smaller than those associated with 60-min tests, indicating a higher amount of variability during the second 30 min of prolonged swimming. The majority of failures occurred during the first 30 min of tests, and failures during the second 30 min may have reflected random error (e.g., due to a momentary loss of swimming gait) that is not relevant to prolonged swimming ability. Thus, researchers must be aware of the testing protocols when comparing results among studies.

The FV50 values calculated in this study provided a convenient index for comparing swimming ability of Sicklefin Redhorse with the results of other experiments that utilized different sucker species. The FV50 values estimated for captive-reared larval Sicklefin Redhorse, over 60 min, at 16.2 mm (0.101 m/s, 95% CI = 0.089-0.111) and 20.4 mm (0.116 m/s, 95% CI = 0.106-0.123) were generally similar to those of similar-sized captive-reared larval Robust Redhorse *Moxostoma robustum* (0.106 m/s, 95% CI = 0.99-0.114, and 0.117 m/s, 95% CI = 0.107-0.126, respectively; Ruetz and Jennings 2000). The FV50 values estimated for captive-reared juvenile Sicklefin Redhorse, over 30 min, at 25.0 mm (0.145 m/s, 95% CI = 0.135-0.0.152) and at 35.0 mm (0.182 m/s, 95% CI = 0.163-0.198) were similar to those of similar-sized Flannelmouth Sucker *Catostomus latipinnis* held at 10˚C (0.157 m/s, 95% CI = 0.143-0.172, and approximately 0.190 m/s, respectively), but were lower than those of similar-sized Flannelmouth Sucker held at 14˚C (0.221 m/s, 95% CI = 0.209-0.233, and approximately 0.25 m/s, respectively; Ward et al. 2002b). Interspecific differences in swimming ability among these species are likely due to differences in ontogeny, body shape, and other physiological characteristics that evolved allopatrically in response to locally unique selection pressures. On average, FV50 increased with TL at a rate of 0.0063 m/s per mm for larval Robust Redhorse (Ruetz and Jennings 2000), which was slightly higher than the rate of increase for Sicklefin Redhorse, and at a rate of 0.0024 m/s per mm for juvenile Flannelmouth Sucker (Ward et al. 2002b), which was slightly lower than that for Sicklefin Redhorse. This difference in rates may be confounded by differences in the size ranges of fish tested in each experiment; Robust Redhorse was 12.5-21.5 mm, Flannelmouth Sucker was 25-114 mm, and Sicklefin Redhorse was 14.1-54.0 mm.

The FV50 values also provided a metric for comparing swimming ability among the various species of wild suckers tested in this study. Interspecific differences in swimming ability at comparable sizes were related to interspecific differences in ontogeny. For example, FV50 was lowest for Black Redhorse across sizes and, correspondingly, tends to be less ontogenetically advanced than other species at a given size (Chapter 3). Similarly, Golden Redhorse exhibited the highest FV50 among wild redhorse species and tends to be more ontogenetically advanced than other species at a given size (Chapter 3). Although they were slight, differences in swimming ability may facilitate resource partitioning and can lead

to differential survival and recruitment among sympatric species. Northern Hog Sucker and Silver Redhorse, which emerge at similar times in the phenological sequence and exhibit similar growth rates, differed significantly (albeit, slightly) in their swimming ability at similar sizes. Given their greater swimming ability, larval Northern Hog Suckers can inhabit a wider niche of nursery habitats than Silver Redhorse and may be more resistant to displacement. The emergence timing of Black Redhorse minimally overlaps with other species, and competition for appropriately-sized food resources is therefore minimized. Therefore, Black Redhorse may have faced fewer selection pressures for faster swimming at smaller sizes. Because body shapes, sizes, and general patterns of ontogeny do not differ appreciably among the sucker species examined in this experiment, substantial differences in swimming ability were not expected. It is possible that the differences observed among the species in this experiment represented local adaptations and divergent selection acting at the family-level. Such concepts may be further elucidated by additional testing among a broader range of fish sizes and taxa.

Across sizes, FV50 values were lower for all species of wild-caught suckers than for captive-reared Sicklefin Redhorse. Sicklefin Redhorse emergence coincides with that of both Golden Redhorse and River Redhorse, and therefore, evolutionary pressures for higher prolonged swimming ability may be high. Adult Sicklefin Redhorse are known to preferentially inhabit and spawn in faster current velocities than other species and are adapted to swift water (Jenkins 1999; Favrot 2009). In contrast to the findings in this study, others have demonstrated inferior swimming ability in captive-reared juvenile and adult fish, as compared to their wild counterparts, presumably due to lack of exercise and conditioning in the captive environment (Hammer 1995; Ward et al. 2002b). The fish in this study were reared in vats that did not exhibit directional flow but provided ample space for fish to swim. Fish were fed a variety of nutritious foods twice per day. Larvae and early-juveniles may benefit more from sustained high-quality rations and captive conditions than from exercise in a stochastic instream environment. Although efforts were made to minimize stress associated with the capture, handling, and holding of wild specimens, they were subjected to considerably more disturbance than captive-reared specimens, and the swimming ability may have been negatively affected by stress-related physiological reactions.

Tailbeat frequency decreased with size at corresponding velocity rates. This was due to somatic growth, which reduced viscous drag on the fish and increased the length of the propulsive wave, as well as ontogenetic changes, which improved swimming efficiency (Muller et al. 2000; van Weerden et al. 2014). Tailbeat frequency is related to metabolic rate and aerobic capacity, which can affect schooling behavior, interspecific interactions, movement dynamics, and habitat selection (McLaughlin and Noakes 1998; Ohlberger et al. 2007; Killen 2012). Slight interspecific differences detected in tailbeat frequencies did not coincide with differences in prolonged swimming ability. In comparison to previous studies that related the swimming ability of adult fish to water velocity, accounting for the length of test subjects, the linear model describing tailbeat frequencies among larval and juvenile suckers explained a relatively low proportion of variance in the data (Bainbridge 1957; Webb et al. 1984; Ohlberger et al. 2007). This may be due, in part, to the profound developmental changes that occur during early life history, which affect fish hydrodynamics and other swimming kinematics (Bainbridge 1957; Webb and Weihs 1986; van Weerden et al. 2014). The relatively high variability of tailbeat frequency estimates in this study may also be reflect variability in swimming kinematics among individuals, as these estimates were derived from direct observations of multiple individuals, each swimming at a constant velocity.

The transition from pelagic to epibenthic swimming occurred at sizes corresponding to approximately the mid-point of the metalarva stage for all species (Chapter 3). This finding was consistent with existing literature (Kay et al. 1994; Markle and Clauson 2006). This transition may have somewhat affected the energy required for fish to swim against various velocity rates, due friction with the acrylic tube and lower velocity in the boundary layer. Juvenile suckers that were larger and more developed than those considered in the analyses extensively utilized pectoral fins to deflect water and generate a downward force that increased friction with the bottom of the swim tunnel. The combination of more efficient swimming and a benthic orientation allows suckers to inhabit a greater variety of potential habitats as they grow.

The effects of temperature were not assessed in this experiment; however, over the relatively narrow range of temperatures under which fixed velocity tests were conducted, the

influence of temperature on swimming ability were expected to be minimal. In published studies where a temperature effect was statistically detected, temperatures varied by at least 4°C (Childs and Clarkson 1996; Ward et al. 2002b). The temperatures tested for wild-caught fish in this experiment matched those at the point of capture and were therefore ecologically relevant. The highest temperature of swimming tests was 24.8°C, on 9 June 2014; of the eight experiments performed on that date, seven were on Silver Redhorse. Because the FV50 for Silver Redhorse was lowest overall, temperature did not appear to bias results appreciably. Furthermore, due to their emergence timing, Northern Hog Sucker were typically tested at colder temperatures than other species at comparable sizes, but exhibited a higher FV50 across sizes, further suggesting a lack of thermal influence on swimming ability among tests. The temperatures under which captive-reared Sicklefin Redhorse were tested did not differ appreciably from those of wild fish tests, but were generally slightly higher (mean difference = 1.1°C), which may have minimally biased swimming ability comparisons between captive-reared and wild fish.

Water velocity in riffles in the Valley River and in the most downstream riffle on the upper Hiwassee River represent major impediments to upstream movement by larval and juvenile suckers, as most occurring velocities exceed their swimming capability. However, some areas in riffles, particularly those adjacent to the river bank, provided velocities lower than the fish prolonged swimming speeds. Additionally, microhabitats among primarily cobble (Valley River) or boulder (Hiwassee River) substrates also likely created velocities less than prolonged swimming speeds, but could not be measured by the instrument used in this study. These low-velocity microhabitats are disconnected along the lengths of riffles, and swimming by larval and early-juvenile suckers is probably not coordinated enough to navigate upstream through and among such habitats. It is well understood that some species of larval suckers actively drift in stream currents (Brown and Armstrong 1985; Kennedy and Vinyard 1997; Chapter 2); given the habitats in the Hiwassee River and Valley River, drift of larval suckers results in their downstream displacement until they achieve a size and swimming ability suitable for swimming against riffle currents. Advanced juvenile (i.e., age-1 and older) Sicklefin Redhorse have never been observed in reaches as far upstream as adults are known to spawn in tributaries (Jenkins 1999), which is likely due to larval drift and

restricted upstream movement in early juveniles, due to swimming ability. This aspect of their early life history, therefore, plays an important role in determining the longitudinal distribution and, potentially, survival of suckers during their early life history.

An understanding of the swimming ability of larval and juvenile fishes is important for identifying factors that influence their distribution and ecology during the critical period of early-development. This study represents a major advance in ascertaining prolonged swimming speeds and swimming characteristics of larval and juvenile suckers. Furthermore, this is the first study known to utilize a genetic barcoding procedure to identify wild fish larvae tested in swimming experiments, which allowed their identification to the species level to more accurately and precisely document and compare fish swimming ability at early life stages. The findings of this study are highly applicable to enhancing the conservation and management of suckers, including the imperiled Sicklefin Redhorse, in the Hiwassee River basin and may also be applied throughout their ranges in lotic ecosystems. An understanding of the swimming ability of early life stages can inform instream flow regulation, influence habitat rehabilitation design and implementation, and refine the employment of stocking protocols.

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Tables

Table 1. Seven species of sucker (Catostomidae) that inhabit the upper Hiwassee River system.

Common Name	Scientific Name
Black Redhorse	*Moxostoma duquesnei*
Golden Redhorse	*Moxostoma erythrurum*
River Redhorse	*Moxostoma carinatum*
Sicklefin Redhorse	*Moxostoma* sp.
Silver Redhorse	*Moxostoma anisurum*
Smallmouth Redhorse	*Moxostoma breviceps*
Northern Hog Sucker	*Hypentelium nigricans*

Table 2. Sample sizes (*N*), total lengths (TL, mm), and the typical development-stages of captive-reared Sicklefin Redhorse test subjects at various ages (number of weeks post-swim-up).

Age	*N*	Mean TL (mm)	Min TL (mm)	Max TL (mm)	Development stage
1 week	20	16.02	15.56	16.29	Early-mesolarva
2 weeks	25	16.12	15.86	16.72	Mesolarva
4 weeks	16	20.29	15.76	22.00	Metalarva
6 weeks	20	26.26	24.93	28.70	Juvenile
10 weeks	19	33.32	31.5	35.8	Juvenile

Table 3. Estimated parameter coefficients and associated standard errors (SE) for the logistic models describing captive Sicklefin Redhorse sustained swimming success over 30- and 60-min trial durations.

	30 min		60 min	
Parameter	Coefficient	SE	Coefficient	SE
Water velocity (m/s)	-81.6028	16.1810	-68.6373	13.9392
TL (mm)	0.3060	0.0774	0.2469	0.0642
Intercept	4.1432	1.2072	2.9448	1.1363

Table 4. Estimated parameter coefficients and associated standard error (SE) for the logistic model describing wild fish sustained swimming success. Silver Redhorse was modeled as the reference category, where the parameter coefficient and SE of other species represent the difference from Silver Redhorse.

Parameter	Coefficient	SE
Water velocity (m/s)	-82.5372	13.8250
TL (mm)	0.3722	0.0980
Species: Black Redhorse	-0.6696	0.4690
Species: Golden Redhorse	0.4280	0.3117
Species: Northern Hog Sucker	0.6622	0.3109
Species: Silver Redhorse	0.0000	-
Intercept	1.7447	1.7679

Table 5. Estimated parameter coefficients and associated standard error (SE) for the linear regression model describing tailbeat frequency.

Parameter	Coefficient	SE
Water velocity (m/s)	51.7919	7.1432
TL (mm)	-0.2494	0.0574
Species: Sicklefin Redhorse	11.3533	1.1559
Species: Black Redhorse	12.0360	1.2420
Species: Golden Redhorse	12.6429	1.0579
Species: Silver Redhorse	10.7340	1.1179
Species: Northern Hog Sucker	11.7835	1.0310

Figures

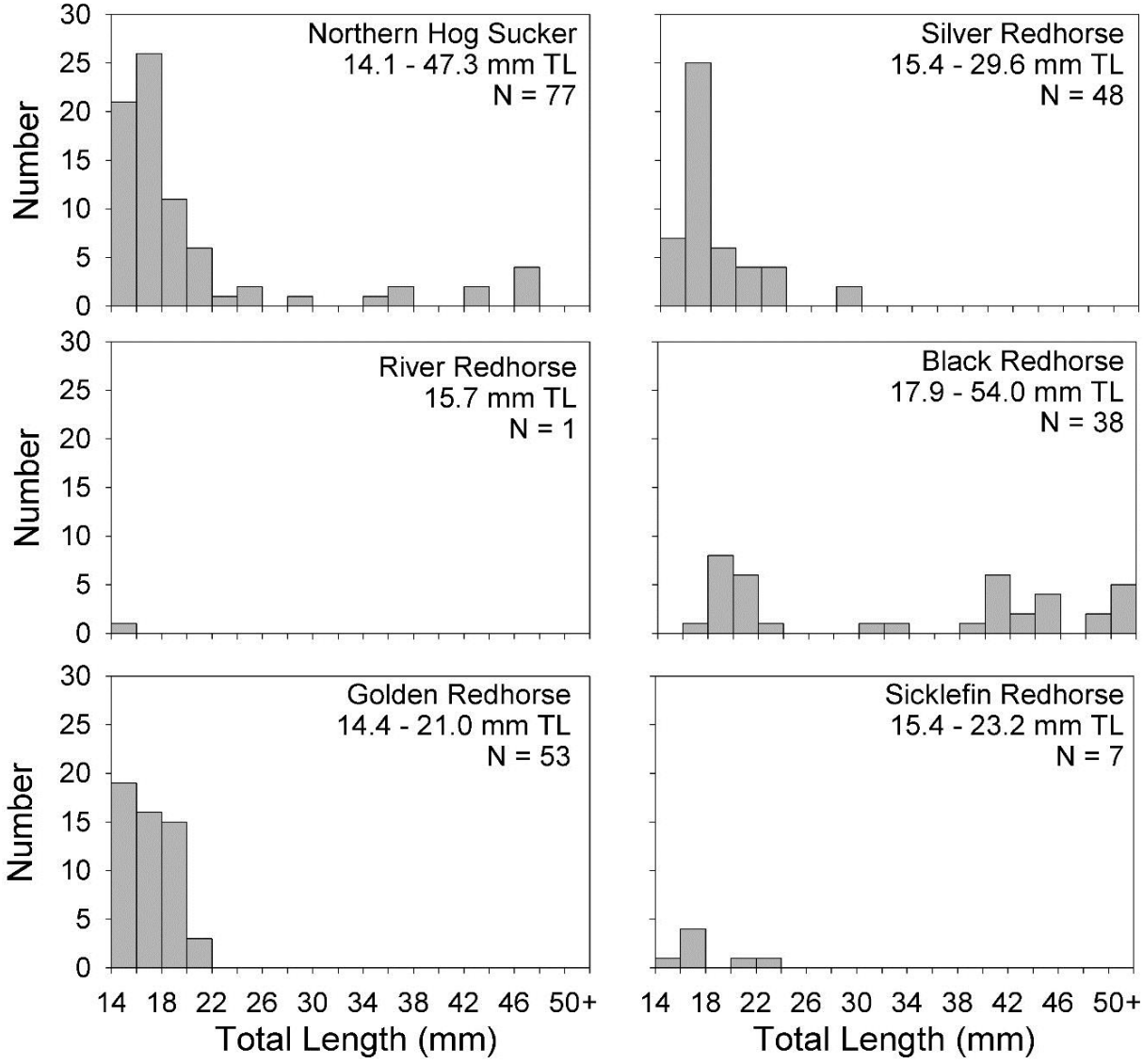

Figure 1. Length-frequency histograms for six species of wild-caught suckers that were subjected to fixed velocity swimming tests. Exact size ranges (mm, TL) and total numbers of test subjects (N) are indicated according to species.

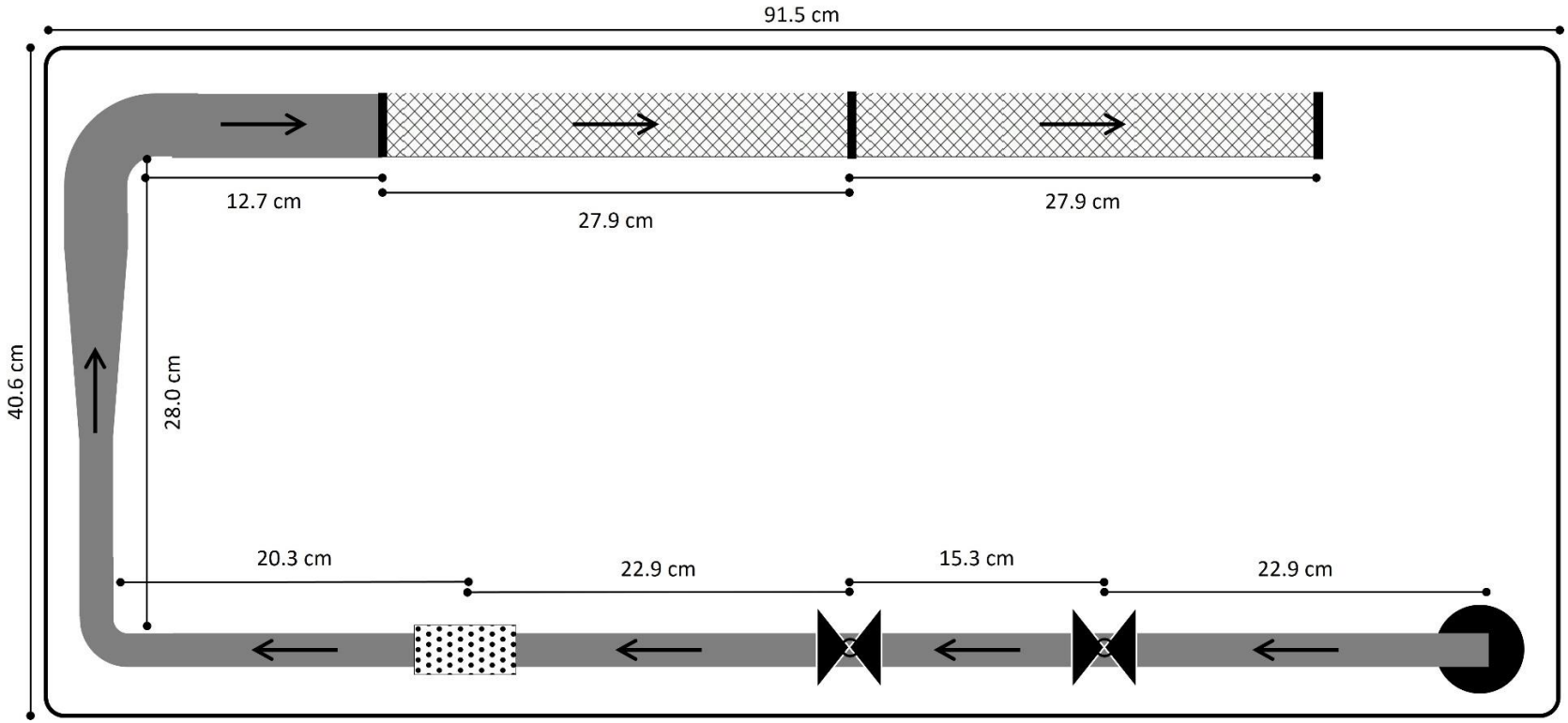

Figure 2. Swim tunnel apparatus used for tests in this study. The apparatus was slightly submerged in a 100-L cooler (black outline). Flow was provided by a bilge pump (black circle), regulated by two ball valves (black valve symbols), and monitored via an inline flow meter (stippled rectangle). The apparatus was constructed using PVC pipe (gray) with an internal diameter of 1.9 cm expanded to 3.8 cm, and the chambers were constructed of transparent acrylic pipe (cross-hatched) with an internal diameter of 3.8 cm. The two chambers were bound on both ends by 1-mm nitex mesh (black vertical bars). Flow direction is indicated by arrows. Diagram drawn to scale.

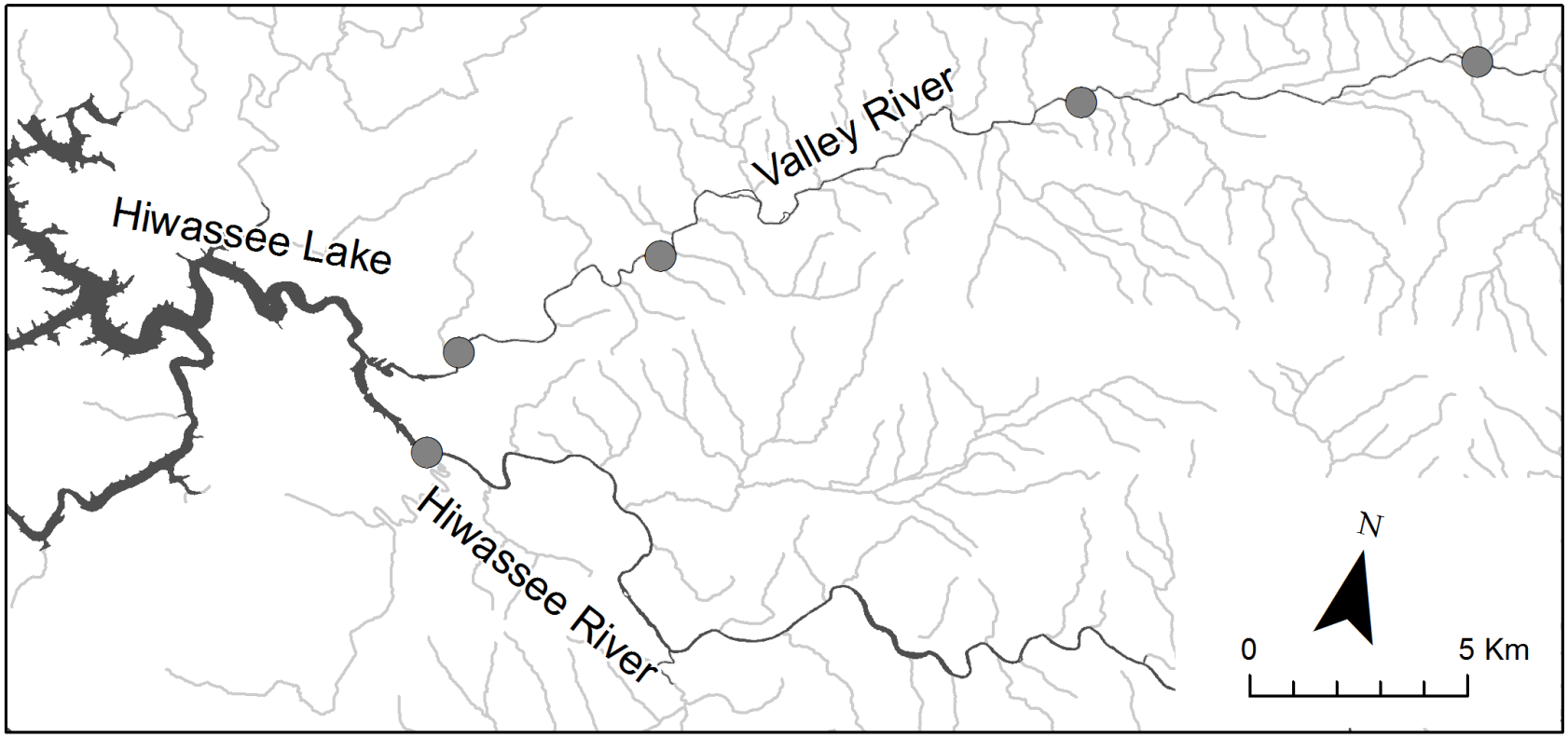

Figure 3. Map of the Hiwassee River and Valley River upstream of Hiwassee Lake, Cherokee County, North Carolina. Points indicate riffles where water velocities were measured.

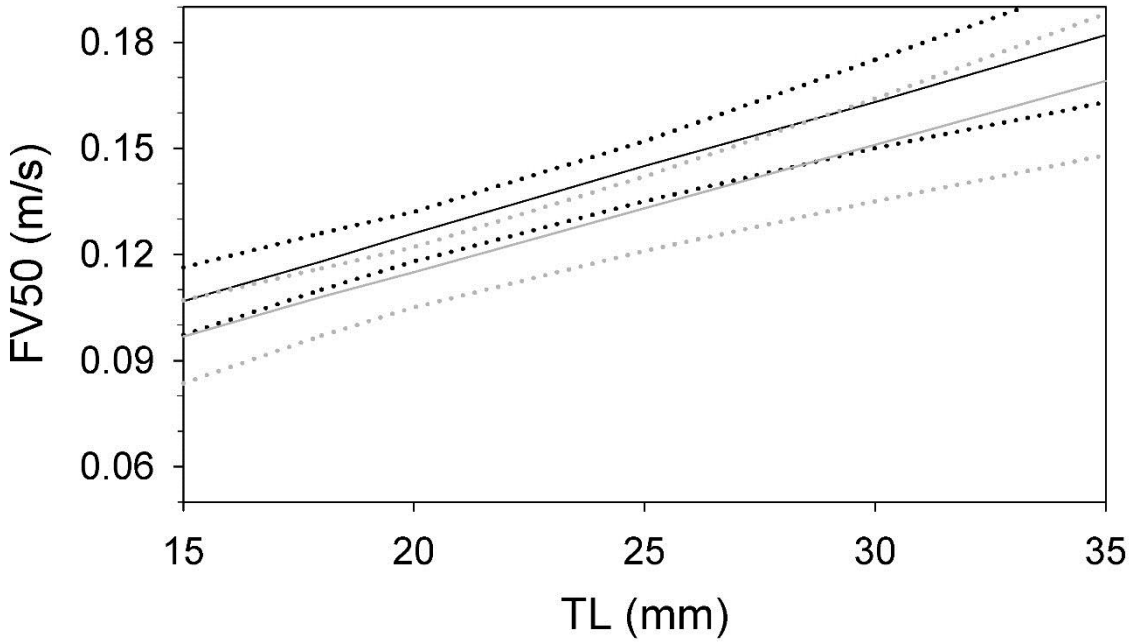

Figure 4. Modeled FV50 values by fish total length for captive-reared Sicklefin Redhorse, based on 30-min and 60-min fixed velocity tests. Solid lines indicate point estimates of FV50, and dotted lines represent 95% confidence intervals; black symbols indicate estimates derived from 30-min tests, whereas gray symbols indicate estimates derived from 60-min tests.

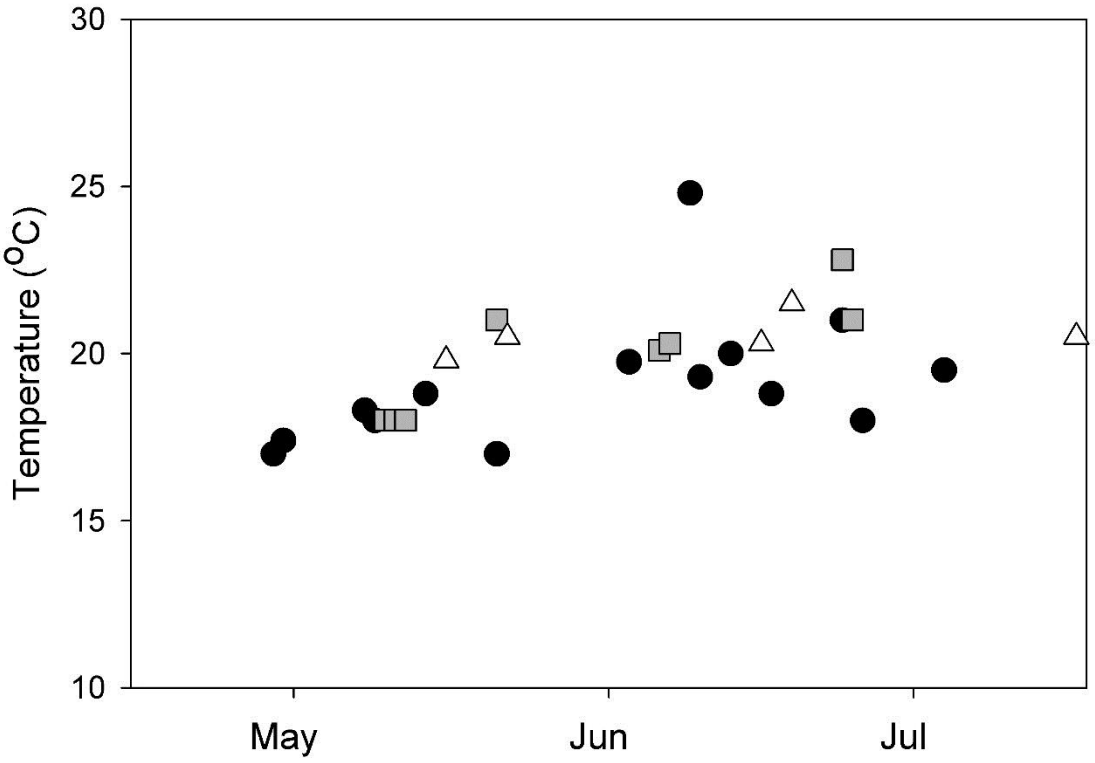

Figure 5. Mean water temperature of fixed velocity swimming tests on dates during May-July. Mean water temperatures for tests on captive-reared Sicklefin Redhorse in 2014 are indicated by white triangles; those for wild-caught suckers in 2014 and 2015 are indicated by black circles and gray squares, respectively.

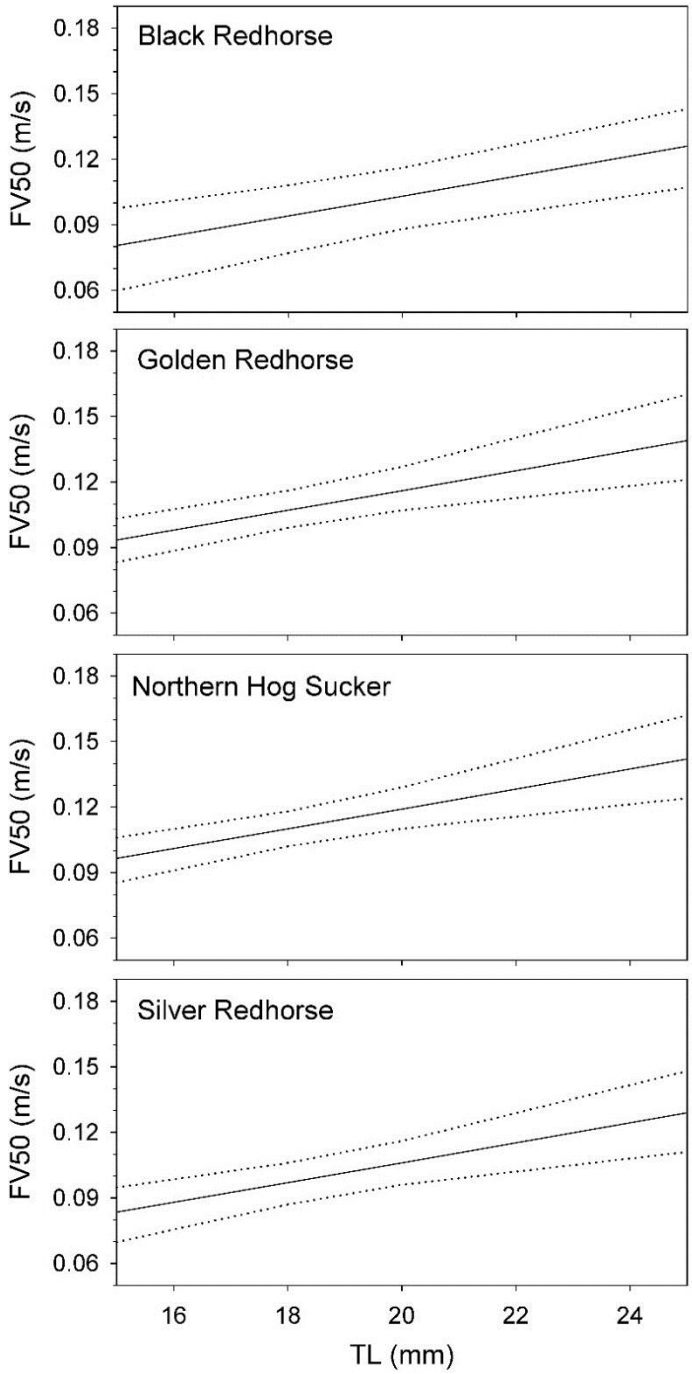

Figure 6. Modeled FV50 values by fish total length for four species of sucker, based on 30-min fixed velocity tests performed on wild-caught fish. Solid lines indicate point estimates of FV50, and dotted lines represent 95% confidence intervals.

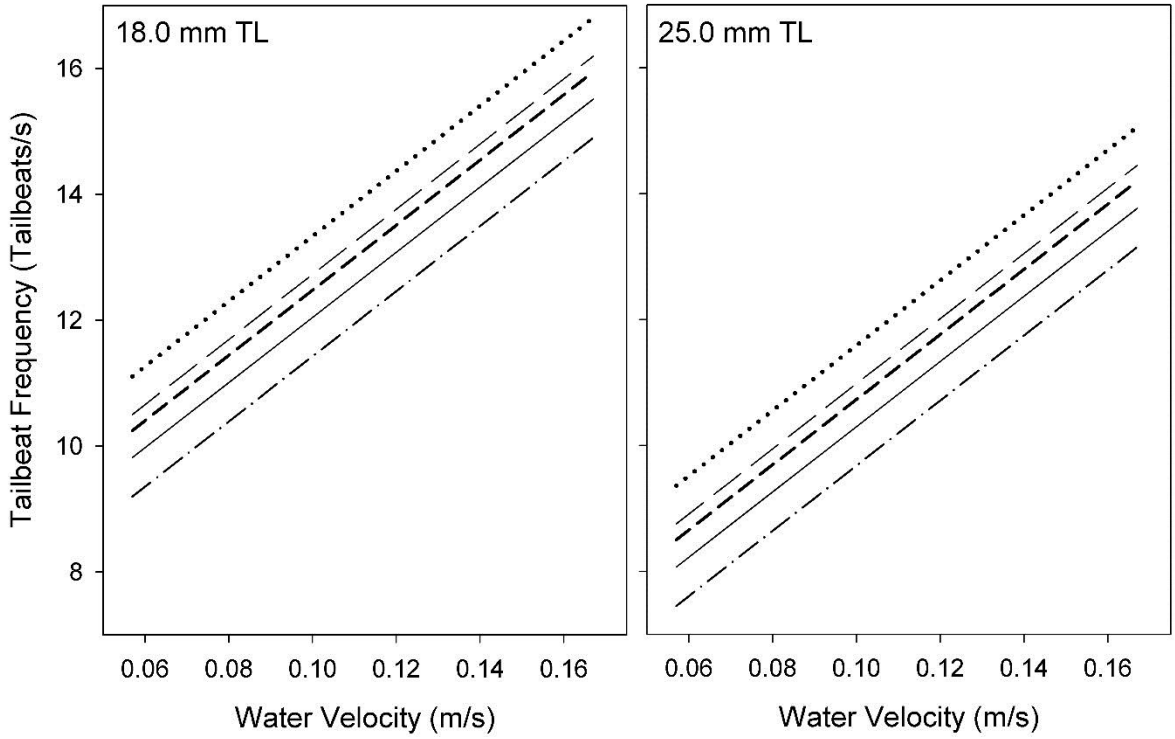

Figure 7. Predicted mean tailbeat frequency across water velocities for five sucker species, at two sizes (18.0 and 25.0 mm TL). Sicklefin Redhorse = solid line; Black Redhorse = long dashes; Golden Redhorse = dotted line; Silver Redhorse = dashes and dots; Northern Hog Sucker = short heavy dashes.

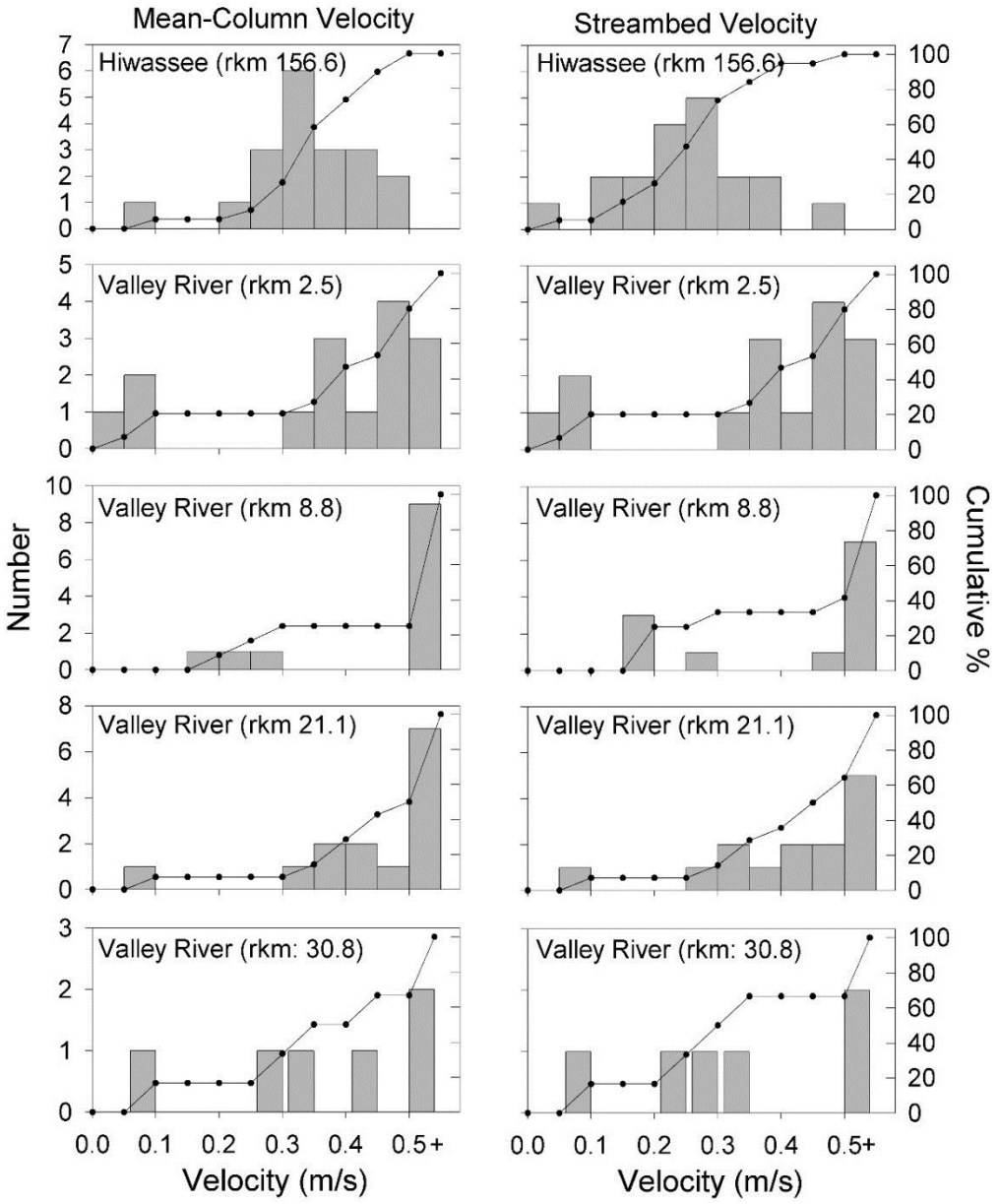

Figure 8. Water velocity frequencies (mean-column and streambed) measured along riffle crests at the most downstream swift-water habitat on the Hiwassee River and four riffles on the Valley River. Mean-column velocity was measured at 60% of the total depth, and streambed velocity was measured at the stream bottom. Velocity ranges are binned by 0.05-m/s increments, the frequency of measuring each binned category is indicated on the left-axis and bars, and cumulative percentages of velocities less than each bin are indicated on the right-axis and by lines and points.

CHAPTER 5: Using next-generation sequencing to detect the predation impact of introduced Blueback Herring on larval native fishes, including the imperiled Sicklefin Redhorse

Abstract

Nonnative species introductions have been implicated as a cause of decline in native biodiversity and a major threat to the conservation of imperiled species. Blueback Herring *Alosa aestivalis*, an anadromous fish native to the Atlantic Ocean and its coastal rivers, was introduced to the Hiwassee River system of the Interior Basin and became established in Hiwassee Lake, North Carolina, by 1999. The Hiwassee River supports six species of native redhorses (*Moxostoma*) of the sucker family (Catostomidae), which are vulnerable to effects of introduced species and habitat degradation. One of these species, the Sicklefin Redhorse *Moxostoma* sp., is imperiled with a restricted distribution and is of high conservation concern. To evaluate possible predatory interactions of Blueback Herring with native sucker species, Blueback Herring were sampled from the Hiwassee River and a major tributary, Valley River, using electrofishing during April-June, 2014 and 2015. Stomach contents of 232 Blueback Herring were examined visually and via genetic barcoding coupled with next-generation sequencing. Visual diet examinations detected 12 orders of aquatic invertebrates, as well as fish ova, fins, and larvae. Limitations on visual analyses included an inability to precisely identify prey items, especially fish tissue, and a large proportion of diets examined (36%) were completely unidentifiable. Barcoding PCR was successful for 67% of samples, allowing identification of five species of redhorses and the Northern Hog Sucker *Hypentelium nigricans* in the Blueback Herring diet, but Sicklefin Redhorse was not detected. Next-generation sequencing enabled species-level identification for 25 of 26 visually observed larval fish and for 5 samples with fish ova. These findings enhance understanding of trophic dynamics between introduced and native fishes and demonstrate the utility of an innovative method for investigating predatory interactions.

Introduction

Nonnative species introductions have been widely implicated as a cause of decline in native biodiversity and as a major threat to the conservation of imperiled species (Wilcove et al. 1998; Mooney and Cleland 2001; Jelks et al. 2008). Over 500 nonindigenous fish taxa have been documented in the United States including exotics and over 300 translocated domestic species that have become established outside of their native ranges (Nico and Fuller 1999; Copp et al. 2005). Although many introduced aquatic species fail to become established or have little impact upon introduction, the environmental and economic tolls of some invasive aquatic species are very high (Pimentel et al. 2005; Gozlan et al. 2010). The impact of invasive fishes on native fish assemblages varies widely and depends on complex ecological interactions, including predator-prey relationships (Mills et al. 2004).

River herring (i.e., Blueback Herring *Alosa aestivalis*, Alewife *Alosa pseudoharengus*) are anadromous fishes that are native to the Atlantic Ocean and coastal rivers along the east coast of North America but have been widely introduced into inland lentic ecosystems (Prince and Barwick 1981; Loesch 1987; Owens et al. 1998). Despite increases in the abundance of potential forage fish, negative effects of nonnative river herring introductions on resident sport fish stocks have been found in freshwater systems in North Carolina (Wheeler et al. 2004), Tennessee (Honeyfield et al. 2007), Virginia (Kohler 1984), and the Great Lakes (Madenjian et al. 2008). The relationship between river herring and reduced sportfish recruitment may be caused by combinations of several mechanisms and may vary by system and by species affected. Factors suggested for such declines include the inhibition of successful egg formation among species that utilize herring as prey (i.e., thiaminase I concentrations; Honeyfield et al. 2007; Rinchard et al. 2011), interspecific competition between herring and other fish species at early life stages (Strange et al. 1985; Davis and Foltz 1991), community-level interactions (Guest and Drenner 1991; Winkelman and Van Den Avyle 2002), and direct predation on larvae and embryos by herring (Brooking et al. 1998;Goodrich 2002; Wheeler et al. 2004).

Although Blueback Herring is generally classified as an obligatory zooplanktivore (Janssen 1982), predation on young fish (e.g., conspecifics, Largemouth Bass *Micropterus*

salmoides, and suckers Catostomidae) and piscine ova has been observed and is considered occasional and opportunistic (Davis and Foltz 1991; Goodrich 2002; Wheeler et al. 2004). In freshwater ecosystems, the diet of introduced Blueback Herring is comprised of mostly planktonic copepods and cladocerans, but small benthic invertebrates may also be common (Guest and Drenner 1991; Goodrich 2002; Winkelman and Van Den Avyle 2002). Blueback herring have a strongly oblique mouth, are gape-limited (maxillary measurements: 9.0-13.2% of standard length), and have 41-52 closely spaced gill rakers (Hildebrand et al. 1963). Feeding activity occurs primarily during daylight hours and is highest at dawn and dusk (Jessop 1990). Blueback Herring is a schooling species that employs a swim-search method of foraging and exhibits size-selective feeding (Brooks and Dodson 1965; Janssen 1982).

Rare, imperiled, and species of conservation concern that co-occur with introduced populations of river herring may be negatively impacted. The Sicklefin Redhorse *Moxostoma* sp. is such a fish in the sucker family (Catostomidae), that is geographically restricted to two southern Appalachian Mountains river basins (Hiwassee and Little Tennessee) and is of particularly high conservation concern (Cooke et al. 2005). Its current distribution has been estimated at about 42% of the species' historical range, reduced by dams, sedimentation, and pollution (Jenkins 1999). It follows a potamodromous life history strategy where spawning, egg incubation, and early development occur in river tributaries (Jenkins 1999; Favrot 2009). In the Hiwassee River system, the Sicklefin Redhorse is sympatric with five confamilials (Black Redhorse *Moxostoma duquesnei*, Golden Redhorse *M. erythrurum*, River Redhorse *M. carinatum*, Silver Redhorse *M. anisurum*, Smallmouth Redhorse *M. breviceps*, and Northern Hog Sucker *Hypentelium nigricans*), that may also be vulnerable to interactions with sympatric nonnative Blueback Herring.

Determining fish diet composition by sampling and inspecting digestive tract contents can yield valuable information about trophic interactions; however, limitations to traditional approaches of diet characterization are widely recognized. For instance, most zooplankton and macroinvertebrates consumed by Threadfin Shad *Dorosoma petenense* become digested beyond recognition within 3.5 h after consumption, and taxa are digested at variable rates (Gannon 1976). Sucker larvae are digested rapidly and may be unidentifiable within one

hour after consumption by a predator (Schooley et al. 2008). Furthermore, smaller larvae are digested faster than larger larvae (Kim and DeVries 2001). Larval fishes with similar morphological characteristics may be particularly difficult to differentiate in diet samples, as morphological characteristics that could be used to distinguish among prey species (e.g., fins, heads, pigmentation) are rapidly degraded in digestion (Legler et al. 2010). Identification of earlier life stages (e.g., ova and embryos) in the diet are virtually impossible to classify taxonomically. Because of the difficulty of sorting and identifying ova, embryos, and young fish in diet samples, genetic approaches have recently been applied to classify diet components of invasive predators and to assess the prevalence of predation on several species of larval suckers (Ley et al. 2014; Moran et al. 2015 Hereford et al. 2016; Ehlo et al. 2017). While research on the efficacy of genetic approaches to characterizing fish diet is recent and ongoing, they appear superior for detecting and identifying consumed larval fish and eggs, providing positive results where visual techniques fail to detect instances of predation (Hunter et al. 2012; Ley et al. 2014; Hereford et al. 2016).

Genetic barcoding coupled with next-generation sequencing (also known as massively parallel sequencing) is a powerful tool that can be used to address a variety of biological questions, including diet analysis (Valentini et al. 2009a; Pompanon et al. 2012). For genetic barcoding, researchers focus on specific portions of genes that occur in all species of large taxonomic groups and are variable in sequence among species, but contain little variation within species (Valentini et al. 2009b). In practice, these analyses rely on the polymerase chain reaction (PCR) to amplify small pieces of DNA from unknown samples so that they can be sequenced and compared to sequences from known samples in an existing database. Most previous work required that each sample to be analyzed was separated from all other samples, with the DNA extraction, PCR, and sequencing occurring separately for each sample. Mixed samples and even samples with trace contamination were problematic. MPS differs from traditional sequencing in that each input molecule is the source of an independent sequencing read; thus, mixed samples are easily sequenced on MPS instruments and costs are reduced by orders of magnitude (Valentini et al. 2009a; Pompanon et al. 2012). Previous studies have employed these techniques to successfully classify gut contents of fish

and allow researchers to make more informed conclusions about the diet and feeding habits of these species (Leray et al. 2013).

Blueback Herring was introduced by unsanctioned release (by the public) into the Hiwassee River system during the mid-1990s and became established in Hiwassee Lake by 1999 (Wheeler et al. 2004). The introduction of Blueback Herring coincided with a subsequent drastic decline in the abundance of Walleye *Sander vitreus* and White Bass *Morone chrysops*. In an effort to establish causality, diet samples of Blueback Herring from Hiwassee Lake were examined in 2003 (Wheeler et al. 2004). These samples indicated a high occurrence of ovivory, with 99% of ingested eggs identified as White Bass and 1% identified as redhorse. These samples also indicated a low rate of piscivory, but some ingested larvae were identified as suckers. Because of its restricted species range, the extirpation of Sicklefin Redhorse in the Hiwassee River system due to predation effects of Blueback Herring would be a major impediment to its conservation. An understanding of the predatory effects of Blueback Herring on larval Sicklefin Redhorse can also guide reintroduction efforts within its historical range in the Hiwassee River system, where Blueback Herring have been widely introduced. Blueback Herring have recently been detected in a reach of the Little Tennessee River that is inhabited by Sicklefin Redhorse and an understanding of their interactions can inform future management strategies as Blueback Herring encroach on reaches inhabited by Sicklefin Redhorse (S. J. Fraley, personal communication, North Carolina Wildlife Resources Commission). For these reasons, there was a need to reevaluate the feeding ecology of Blueback Herring in the Hiwassee River system with an emphasis on consumption of sucker species and the Sicklefin Redhorse.

Thus, research was initiated to evaluate predatory interactions of introduced Blueback Herring with native sucker species, including the Sicklefin Redhorse. The specific objectives were to (1) sample the Blueback Herring diet over time, (2) identify diet components using traditional visual techniques, (3) analyze diet tissue using next-generation sequencing techniques to classify the fish component of the diet, including ova, embryos, and larvae, and (4) compare the two approaches (visual and genetic) toward accurate diet characterization. I

then discuss the conservation implications of the trophic interactions between an introduced predator on a rare and imperiled fish species.

Methods

Study Area

Field sampling was conducted in the Hiwassee River basin of the southern Blue Ridge Province in the southern Appalachian Mountains of western North Carolina during the spring-summer seasons of 2014-2015. Specific water bodies sampled were the Hiwassee River, and a major tributary, the Valley River. The Hiwassee River originates in Georgia, flows north into North Carolina before turning west, where it reaches its confluence with the Tennessee River in Tennessee. The Hiwassee River drains 6,993 km^2 of mountainous, high-gradient watershed (Miranda et al. 2015). The river is impounded by four mainstem high dams. One of which, at river kilometer (rkm) 122, forms Hiwassee Lake, a 2,400-ha mainstem reservoir that extends upstream approximately 36 km southeast. Hiwassee Lake is managed by Tennessee Valley Authority (TVA) for flood control, power generation, and recreation. Water levels fluctuate approximately 13 m from winter to summer (TVA 2004). During both years of sampling, Hiwassee Lake elevations adhered closely to the flood guide curve, where lake filling commenced during late-March with full-summer-pool attained by mid-May (TVA 2015). Valley River is an unregulated, moderate-gradient stream that is approximately 47 km in length and drains 303 km^2 (Dobson and Wallus 2004). It is a known spawning tributary for six redhorse (*Moxostoma*) species, including the imperiled Sicklefin Redhorse (Favrot and Kwak 2016). The confluence of the Valley River with the Hiwassee River is at Hiwassee rkm 154. When reservoir levels are at or near full-summer-pool, the most downstream 2.3 km of the Valley River and its confluence with the Hiwassee River are impounded by Hiwassee Lake with lentic characteristics, where river and lake fish coexist seasonally.

Fish Sampling and Preservation

Blueback Herring were sampled from the Valley River during May-June 2014 and April-June 2015 and from the Hiwassee River or Hiwassee Lake during April-June 2014 and April 2015 (Table 1). Sampling was conducted using a small boat-mounted electrofisher equipped with a Smith-Root (Vancouver, Washington) Model 2.5GPP pulsed-DC power source, with one person netting stunned fish from the bow of the boat and one driver. Electrofishing was generally conducted in a downstream direction in a zig-zag pattern covering the entire width of the stream. Power was applied in approximately 10-s bursts at a frequency of 60 pulses/s. Sampling was conducted during the late-afternoon or shortly after sunset. Reservoir operations resulting in low water levels prohibited boat electrofishing in the Valley River during April of both years. Therefore, during 2015, one additional sample was conducted in the Valley River using a Smith-Root Model LR24 pulsed-DC backpack electrofisher. Upon capture, all Blueback Herring were immediately euthanized by overdosing with MS-222 (tricaine methanesulfonate), placed in a plastic bag, and buried in ice in a cooler.

All captured Blueback Herring were subjected to diet analysis, except for those collected on 4 June 2014, where a subset of 40 Blueback Herring was selected at random from the collected sample. Each Blueback Herring was measured for total length (TL, mm) and weighed (g). Within six hours of capture, the stomach, pyloric cecum, and esophagus of each Blueback Herring were excised using surgical scissors and forceps and placed in individual vials filled with approximately 20 mL of pure molecular grade ethanol (Fisher Scientific, Waltham, Massachusetts; BP2818). Between fish, all dissecting tools were cleaned in a chlorine bleach solution, rinsed in distilled water, and wiped dry. Sample vials were immediately refrigerated (2^{o}C) for approximately 72 h and were subsequently transferred to a freezer (-10^{o}C).

Diet Processing

Diet items were extracted from the preserved stomach, pyloric cecum, and esophagus through a longitudinal incision made using a scalpel, opened with forceps, and scraped into a petri dish. The percent fullness of each stomach was visually estimated (±5%; Hanson and Curry 2005; Simonin et al. 2007). Diet items were submersed in ethanol, coarsely sorted in the petri dish, and entire diets were examined and photographed at 20x power using a dissecting microscope (Leica Microsystems, Heerbrugg, Switzerland; EZ4 HD). Representative specimens of very small items were photographed at higher magnifications, as needed for identification from photographic records. Diet items were then transferred, using a pipette, to a 0.5 mL or 2.0 mL Eppendorf (Hamburg, Germany) centrifuge tube, depending on the volume of items. Any remaining space in the tube was filled with ethanol. Items that appeared to be piscine (e.g., ova, larvae, fin fragments) were preserved separately. The volume of each total diet sample (mL) was approximated based on graduations on the Eppendorf tube. To avoid cross-contaminating samples for molecular analysis, all scalpel blades, pipettes, Petri dishes, and Eppendorf tubes were used only once, whereas forceps were cleaned in a chlorine bleach solution, rinsed in distilled water, and wiped dry.

Visual identification of diet items was conducted using photographed samples. Items were identified to the lowest possible taxon and life-stage (e.g., larva, pupa, adult) using keys presented by Merritt and Cummins (1996) and Thorp and Covich (2009). Where feasible, individuals were enumerated according to taxon; however, counts may be less precise for samples containing over 200 individuals in a taxon. Any tissue fragments (e.g., fins, scales) of piscine diet items were also counted. When possible, sucker larvae in diets were measured and predigested TL was estimated. Any items that could not be visually identified (i.e., prey that were highly fragmented or digested) were not counted. The overall proportion of identifiable tissue in each sample was indexed as ‘unidentifiable’ (0-5% of total volume identifiable), ‘poorly identifiable’ (6-24% identifiable), ‘moderately identifiable’(25-74% identifiable), ‘highly identifiable’ (75-94% identifiable), or ‘perfectly identifiable’ (95-100% identifiable).

Molecular Techniques

Genetic analysis was carried out at the University of Georgia Genomic Facility in Athens, Georgia. Genetic barcoding was coupled with next-generation sequencing to analyze the entire sample of stomach contents. A Qiashredder Tissue and Cell Homogenizer kit (Quiagen, Hilden, Germany) was used to extract genetic material from stomach contents, and a Qiagen DNeasy Blood and Tissue kit was used to purify samples. Polymerase chain reaction (PCR) was used to amplify samples; the first round of PCR was conducted with site-specific fusion primers, followed by cleanup using Sera-Mag SpeedBeads (GE Healthcare, Little Chalfont, United Kingdom), a second round of limited-cycle PCR with i5/i7 primers, and pooling and cleaning of all samples using Sera-Mag SpeedBeads. Samples were quantified and sequenced using an Illumina (San Diego, California) MiSeq machine. Geneious and Qiime software were used to interpret results and Basic Local Allignment Search Tool (BLAST) was used to match obtained sequences to those in GenBank and produced from adult suckers collected from the Hiwassee River (Chapter 2). A 97% threshold was used to distinguish species, and the presence of a species in a diet was confirmed if PCR yielded at least 20 target sequences or if the target sequence comprised at least 10% of the total number of sequences detected. Specifically, samples were screened for the presence of DNA corresponding to any of the seven sucker species known to inhabit the Hiwassee River, 15 additional fish species known to inhabit the Hiwassee River, and three North Carolina fish species with ranges that do not extend into the Hiwassee River, which served as negative controls (Table 2).

Analysis

Catch rates of Blueback Herring on each date were tabulated as catch-per-unit-effort (CPUE; Hayes et al. 2012), where effort was quantified as the total time the water was electrified. Samples collected during a given month and year were combined and considered one sampling period for analysis. The mean TL of Blueback Herring collected during each sampling period were compared using one-way ANOVA, and Tukey's Post-Hoc test was applied when differences were detected. The length-weight relationship was estimated using

linear regression, where $\log_{10}$(TL) was the independent variable and $\log_{10}$(weight) was the dependent variable.

Stomach fullness was compared among sampling occasions using the Kruskal-Wallis test because those data did not meet a normality assumption. The proportions of empty stomachs were compared among sampling periods using the chi-squared test. Maximum stomach capacities (mL) at various sizes were estimated using a linear regression model. For each stomach sample that was at ≥ 50% fullness, the potential stomach capacity was estimated by multiplying the inverse of the percent fullness by the measured stomach content volume. Due to error associated with estimating of stomach fullness and the imprecision of volume measurements, a high amount of variability was expected; to reduce this, any observations with residuals > ±0.20 mL were excluded, and the linear regression model was compiled using remaining observations. Blueback Herring TL was modeled as the independent variable and estimated potential stomach capacity was modeled as the dependent variable.

Diet items that were identified visually were tabulated according to the lowest taxonomic level. For all subsequent analyses, zooplankton and aquatic macroinvertebrates were categorized according to order, winged insects were categorized as such, and any piscine items were categorized as 'larval fish,' 'fins,' 'scales,' or 'ova.' Diets were described as percent composition by number and as frequency of occurrence (Garvey and Chipps 2012). Samples with no content (empty stomachs) were excluded from these analyses. Differences in diet composition were compared among sampling periods using ANOSIM, which tests if there is a significant difference in the compositions of sampling groups using the Bray-Curtis similarity matrix of percent composition by number (Clarke 1993). The frequency of occurrence of any piscine item in diets was compared among sampling periods using a chi-squared test, and where differences were detected, 2x2 contingency tables were used for pairwise comparisons.

Species or genus identities of piscine prey items, as determined by genetic molecular techniques, were tabulated according to sampling period. The proportion of diets where genetic procedures confirmed piscine prey items not detected in visual sorting (false

negatives) was calculated. Occurrence frequencies of false negatives were compared to the index of diet identifiability using the Kruskall-Wallis test.

All univariate statistical procedures were conducted using SAS 9.4 (SAS Institute, Cary, North Carolina) and the ANOSIM procedure was conducted using Primer (Clarke and Gorley 2006; version 6.1.16). Tests were considered significant with $\alpha = 0.05$.

Results

Electrofishing samples were collected on six dates, each, in 2014 and 2015, with a total of 8.6 h of pedal-time (Table 1). A total of 483 Blueback Herring were captured. Catch rates were typically low (median CPUE = 0.10 fish/min) and were highly variable among sampling events (SD = 6.61). Blueback Herring analyzed for diet (N = 232) ranged in size 70-150 mm. Mean TL differed among sampling periods (F = 217.49, df = 5, 24, $P <$ 0.0001); sampled Blueback Herring were significantly larger, on average, during every month in 2015 than during any month in 2014 (Figure 1). The coefficients of the length-weight regression were equivalent to those previously reported in literature (i.e., Messieh 1977; Figure 2).

Stomach fullness did not differ among sampling periods (χ^2 = 7.3930, df = 5, P = 0.1930). The median fullness was 30%. The largest volume of digested material recovered from a single stomach was 0.8 mL, from a 110-mm Blueback Herring. Overall, 26 stomachs were empty (11.2% of samples). The proportion of diets that were empty did not differ significantly among sampling periods (χ^2 = 10.0762, df = 5, P = 0.0731), but point estimates, between years, were lower for May (3.9%) than April (13.21%) or June (13.51%). The majority of diet samples were quite digested; 32% were categorized as unidentifiable, 14% were poor, 22% were moderately identifiable, 20% were highly identifiable, and 12% were perfectly identifiable (Table 3). Stomach capacity was related to size (F = 67.83, df = 1, 56, $P <$ 0.0001; Figure 3).

Visual analysis of diet samples yielded 12 orders of aquatic invertebrates, as well as adult (winged) insects, ova, fish fins, and larval fish (Table 4). Diet analysis results reflected

opportunistic feeding habits, and Diptera larvae and pupae were a staple of the diet during all sampled periods (Table 5). Diet compositions differed among sampling periods ($R = 0.43$, $P = 0.001$), but pairwise comparisons did not detect differences during April 2014 and 2015 ($R = -0.102$, $P = 0.987$), May 2014 and 2015 ($R = -0.216$, $P = 0.969$), and May 2014 and April 2015 ($R = -0.139$, $P = 0.831$).

Remains of larval fish were visually discerned in the diet of 12 Blueback Herring ranging in size 105-150 mm. Diet samples containing larval remains typically consisted of 1 or 2 individuals, but the diet of one Blueback Herring (126 mm) consisted of 6 larvae (Figure 4). A total of 26 larvae were visually discerned among all samples. Of these, predigested length could be estimated for 14 larvae, whereas 12 were too digested or fragmented. The predigested sizes of larvae in the diet ranged in size from 11.9 mm to 18.1 mm (mean = 14.2 mm). Piscivory was detected through visual techniques in June 2014, May 2015, and June 2015; the frequency of occurrence of larval remains was lowest in June 2014 ($\chi^2 = 6.3906$, df = 1, $P \leq 0.0115$) and similarly high for May 2015 and June 2015 ($\chi^2 = 3.6991$, df = 1, $P = 0.0544$). Ova presumed to be piscine were visually discerned in the diets of 12 Blueback Herring that ranged in size from 105 mm to 132 mm, and was only observed in April 2015 and June 2015 at a similarly low frequency of occurrence ($\chi^2 = 0.4665$, df = 1, $P = 0.4946$). Items discerned as fragments of fish fins were present in 47 diet samples (23%) and during all sampling periods. The frequency of occurrence for fins differed among sampling periods ($\chi^2 = 33.8410$, df = 5, $P < 0.0001$). It was lowest and similar for April 2014 and April 2015 ($\chi^2 = 1.0656$, df = 1, $P = 0.3019$), and similarly highest for May 2015 and June 2015 ($\chi^2 = 0.0894$, df = 1, $P = 0.7649$). A total of 141 samples with contents did not contain items visually discerned as piscine.

Genetic analysis was conducted for 193 diet samples, which necessitated 244 separate barcoding polymerase chain reactions (PCR). Of these, 176 PCR procedures were successful. Thus, molecular analysis resulted in information on 130 diets. Barcoding PCR was successful for 11 of the diets containing visually detected larval fish remains (8% failure rate), and genetic material for suckers was detected in all 11 of these. Barcoding PCR was successful for all 12 samples containing visually detected ova (0% failure), and genetic

material from suckers was detected in 3 of these. Barcoding PCR was successful for 38 samples containing items visually discerned as fin fragments (19% failure), and genetic material from suckers was detected in 18 of these. PCR was successful for 75 samples that did not contain any items visually discerned as piscine (42% failure), and genetic material from suckers was detected in 18 of these. The occurrence of false negative visual identifications was related to the digestive state; a higher proportion of diets where no piscine items were visually identified, but genetic material was detected, were categorized as 'unidentifiable' than other categories (Table 3; $\chi^2 = 9.8724$, df = 4, $P = 0.0426$). Genetic material corresponding with suckers was found in a total of 45 of the 130 Blueback Herring diet samples successfully analyzed using next-generation sequencing.

Molecular analysis identified five species of sucker genetic materials in the diet of Blueback Herring, at varying frequencies of occurrence, including Northern Hog Sucker, Silver Redhorse, Smallmouth Redhorse, Black Redhorse, and Golden Redhorse (Table 6). A trace amount of genetic material from River Redhorse was detected in one sample, but it did not meet the threshold requirements. Genetic material from Sicklefin Redhorse was not detected in any sample, even in trace amounts. The frequency of occurrence for sucker genetic material was significantly different among sampling periods ($\chi^2 = 19.5425$, df = 5, $P = 0.0015$) and was highest in May 2015 and June 2015, which were similar ($\chi^2 = 0.4083$, df = 1, $P = 0.5228$). All visually discerned ova were identified as Golden Redhorse; visually discerned larvae were identified as Northern Hog Sucker, Silver Redhorse, Smallmouth Redhorse, or Golden Redhorse (Table 7).

Of the 15 other Hiwassee River fishes that were screened in genetic analysis, only four were present in any of the samples; they included Banded Darter, Common Carp, Gilt Darter, and Green Sunfish (Table 8). A trace amount of White Bass DNA was detected in one sample, but it did not meet the detection threshold requirements. As expected, no DNA corresponding with any of the three species serving as negative controls was detected in diet samples, even in trace amounts.

Discussion

The efficiency of boat electrofishing for Blueback Herring sampling was typically low and highly variable between sampling events. In general, boat electrofishing for Blueback Herring was consistently most efficient in April, when it was conducted in the lotic habitat of the Hiwassee River downstream of the confluence with the Valley River. This agreed with the findings of the 2003 study in the Hiwassee River basin, where the authors observed more difficulty in obtaining samples after reservoir filling (Wheeler et al. 2004). This was likely due to higher densities related to spawning behavior and lower sampling efficiency in deeper water. Although neither the present study nor the 2003 study was designed to reliably estimate or index population abundances, CPUEs in the present study were similar to, or slightly lower than, those observed in 2003. This may suggest that the population had reached or exceeded a carrying capacity by 2003, which may have subsequently declined due to the functional extirpation of White Bass or due to other variables.

The increasing size distributions observed over the two years of this study indicated that sampled Blueback Herring were primarily from the same cohort. It is possible that Blueback Herring in the Hiwassee River system exhibit periodically high and low recruitment. Weak and missing year classes of Blueback Herring were previously documented in Lake Theo, Texas (Schramm et al. 1992). Despite their presence and persistence, Blueback Herring have rarely been collected during routine sampling of Hiwassee Lake (Tennessee Valley Authority, unpublished data).

Movement patterns of Blueback Herring in the Hiwassee River system are also poorly understood. In Chatuge Lake, a tributary storage impoundment situated in the upper Hiwassee River system, Blueback Herring were primarily pelagic and resided in the mainstem of the reservoir (Goodrich 2002). During spawning, which occurs during spring, however, landlocked Blueback Herring may seek flowing water (Prince and Barwick 1981). This preference explained the relatively high abundance of Blueback Herring in the fluvial portion of Hiwassee Lake during April sampling. Although boat electrofishing in the Valley River was not possible during April and early-May, due to shallow water, the presence of

Blueback Herring near rkm 2.0 was confirmed via cast-netting on 9 May 2014 (unpublished data) and backpack electrofishing on 12 April 2015. Favrot (2009) observed Blueback Herring ranging upstream to rkm 22.4 in Valley River during the spring, although encounters were relatively rare. In an effort to document the longitudinal range of Blueback Herring in the Valley River, a canoe was used to navigate the farthest downstream 21.9 km and 9.7 km on 14 April 2013 and 27 April 2014, respectively, while visually searching for Blueback Herring; however, no Blueback Herring were observed on either date. Barge electrofishing gear or multiple backpack electrofishing units will be required for sampling Blueback Herring in the Valley River. Environmental DNA (eDNA) may also be used to determine their range (Jerde et al. 2011). A PIT-tag array could be used to determine course-scale movements of individual Blueback Herring past shallow sections of the Valley River. Biotelemetry could provide finer-scale information about movements of individual Blueback Herring throughout the upper Hiwassee River system.

Visual identification of dietary items was unreliable for nearly half of the Blueback Herring diet samples examined (those classified as unidentifiable or poorly identifiable), and only 12% of samples (those classified as perfectly identifiable) were free of a considerable portion of unidentifiable matter. Unidentifiable matter was composed of dark-colored particles that likely corresponded to invertebrate exoskeletons, light-colored fine particles that likely corresponded to partially-digested zooplankton or other invertebrates, and nondescript material that likely corresponded to partially-digested macroinvertebrates or fish larvae. Differential rates of digestion have previously been noted for various dietary items, and thus, it is likely that certain taxonomic groups are underrepresented in visual estimates of percent composition and frequency of occurrence. Consistent with other studies (Ley et al. 2014; Hereford et al. 2016), genetic analysis confirmed that visual observations of dietary material underestimated the prevalence of predation on sucker larvae and ova. The incidence of such false negatives was more prevalent among samples that were highly digested, but genetic material from suckers was also found in some diets that were perfectly identifiable, but suckers were not detected visually. Unlike the genetic procedure, visual analysis of dietary material provided numerical estimates across broad taxonomic groups of prey items. Because of the specificity of primers used, genetic analysis is limited in scope to specific

taxonomic groups (i.e., specific fishes). Because of inconsistencies in amplification and variable states of digestion, genetic analysis is also unreliable for obtaining precise percent compositions of dietary items. Furthermore, genetic analysis cannot distinguish between life stages, sizes, or recentness of consumption. Thus, these two methods of dietary analysis provide complementary data.

Genetic diet analyses have been previously applied to assess the impact of nonnatives on suckers in the Western United States and to guide associated recovery efforts (Ley et al. 2014; Hereford et al. 2016; Ehlo et al. 2017). The present study is unique compared to these, because genetic techniques were used to differentiate between seven closely related species. Additionally, this study paired genetic diet analysis with visual techniques in order to provide a more holistic understanding of the predatory impact of an invasive species.

Diets containing visually discernable larval sucker remains typically consisted of 1 or more individuals, which is likely related to the schooling behavior of larval suckers (Chapter 2). Predation likely occurred when a school of Blueback Herring momentarily encountered a larval sucker aggregation (Janssen 1982). The number of larvae consumed by each individual Blueback Herring in the school is limited by prey availability, prey escapement, competition with other Blueback Herring in the school, and physiological constraints (i.e., stomach capacity). The diet of one medium-sized Blueback Herring (126 mm) consisted of 6 larvae that were minimally digested, indicating that they were consumed during a single recent feeding bout, and that stomach contained no other visually identifiable items. The volume of that diet was 0.4 mL, and it was estimated at 100% of its stomach capacity. Given the relationship between Blueback Herring length and stomach capacity, it is plausible that larger fish can, therefore, ingest more than six larvae in one feeding bout.

Genetic material corresponding to five species of sucker was detected in the diet of Blueback Herring in the Hiwassee River basin. Genetic material corresponding to Black Redhorse, which emerged in early-May (Chapter 3), was detected at the highest frequency in May and was also detected in June. Golden Redhorse genetic material, which emerged in mid-May, was detected in April, May, and at the highest frequency in June. Northern Hog Sucker genetic material, which emerged in late-April, was detected in April, and at the

highest frequency in May, and in June. Silver Redhorse genetic material, which emerged in late-April, was detected in April, and at the highest frequency in May, and in June. Smallmouth Redhorse, which spawns in April (Jenkins 1999), was only observed in one diet sampled in May 2015. These results are intuitive, as predation on each of the various species depends on availability as ova or as appropriately-sized larvae. Sicklefin Redhorse and River Redhorse emergence times overlap with that of Golden Redhorse, which was consumed as both ova and larvae. However, genetic material corresponding to Sicklefin Redhorse and River Redhorse was absent from all Blueback Herring diets examined. This indicated that Sicklefin Redhorse and River Redhorse ova and larvae were spatially isolated from the Blueback Herring sampled for this study.

Among the 18 other fish species that were screened in diet samples, genetic material corresponding with four were present. Common Carp, which were observed spawning in late-April and early-May (personal observation), was detected in April 2014, at high frequencies in May 2014, and in June 2015. Common Carp larvae were very abundant in light trap samples collected from Hiwassee Reservoir, near the location where Blueback Herring were sampled and are small (Chapter 2). Thus, Common Carp larvae were highly available to Blueback Herring and digested rapidly in their diets. Banded Darter and Gilt Darter genetic material was detected in the Blueback Herring diet; these two prey species were abundant in the Valley River (Dobson and Wallus 2004). Green Sunfish, which was also detected in the Blueback Herring diet, are known to inhabit and spawn in the Valley River. Although the White Bass population in Hiwassee Lake persists at a very low density (e.g., one individual was observed via boat electrofishing), genetic analysis confirmed that eggs and larvae of that species were not a substantial part of the Blueback Herring diet (Wheeler 2004). These results further emphasize the opportunistic nature of the Blueback Herring feeding strategy.

Despite not observing any instance of Blueback Herring predation on Sicklefin Redhorse larvae or ova, this study provided evidence that such an interaction may be possible. In terms of morphology, size, and swimming ability, Sicklefin Redhorse larvae are generally similar to the other sucker species in the Hiwassee River system. Because

Blueback Herring preyed opportunistically on fish larvae, it is evident that they would indiscriminately prey on Sicklefin Redhorse larvae or ova, if encountered. Sicklefin Redhorse larvae were likely spatially isolated from the Blueback Herring that were sampled in this study. In concurrent larval fish sampling, Sicklefin Redhorse larvae were never observed downstream of Valley rkm 9.5 (Chapter 2). However, Blueback Herring are known to occasionally range upstream to reaches where Sicklefin Redhorse larvae have been detected (Favrot 2009). The extent to which predation by Blueback Herring may affect Sicklefin Redhorse larvae, therefore, depends on temporal aspects of its longitudinal distribution and abundance within the range that overlaps with larval Sicklefin Redhorse. Many regionally-native fishes prey on sucker ova and larvae (Jenkins and Jenkins 1980; Schooley et al. 2008; Hereford et al. 2016). However, if densities of Blueback Herring in the Valley River are high and range far upstream, predation by Blueback Herring may result in additive mortality. Population-level effects of Blueback Herring predation may also be exacerbated during years when Sicklefin Redhorse reproductive output is limited or recruitment is adversely affected by stochastic events.

Eradication of established nonnative fish species is impractical, if not impossible (Copp et al. 2005; Gozlan et al. 2010). However, gaining an understanding of predatory interactions of Blueback Herring and native fishes is important for guiding recovery efforts of potentially affected species, such as Sicklefin Redhorse and other confamilials. Population augmentation via hatchery-reared fish may be more effective if they are reared to a size that is less vulnerable to Blueback Herring predation, or if they are initially stocked in reaches that are not inhabited by Blueback Herring. Due to the potential for limited recruitment, the reintroduction of Sicklefin Redhorse to a system with an established Blueback Herring population may be dependent on a prolonged stocking regime. This study underscores the importance of continued monitoring of recruitment in fish populations that may be affected by a nonnative species introduction.

Throughout North America, nonnative species introductions have been widely implicated as a contributing factor in population declines of some sucker species (Cooke et al. 2005). The present study demonstrates the utility of genetic techniques for detecting

predation on multiple species of larval suckers in a southeastern United States river system and provides information that will guide the management and conservation of Sicklefin Redhorse and other fish species.

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Tables

Table 1. Electrofishing sampling effort and catch for Blueback Herring. Catch-per-unit-effort (CPUE) is calculated based on the total amount of time that electricity was applied.

Date	Water Body	Sampling Effort (sec)	Time of Day	Catch (no. fish)	CPUE (fish/min)
2014					
4/24/2014	Hiwassee River	907	Midday	27	1.79
5/7/2014	Hiwassee Lake	1,063	Afternoon	0	0.00
5/7/2014	Valley River	3,185	Afternoon	4	0.08
5/9/2014	Hiwassee Lake	1,200	Early-afternoon	0	0.00
5/9/2014	Valley River	1,200	Early-afternoon	2	0.10
5/14/2014	Hiwassee Lake	1,312	Mid-afternoon	0	0.00
5/14/2014	Valley River	2,328	Mid-afternoon	1	0.03
6/4/2014	Valley River	668	Late-afternoon	288	25.87
6/17/2014	Valley River	1,289	Late-afternoon	23	1.07
2015					
4/11/2015	Hiwassee River	1,168	Midday	74	3.80
4/12/2015	Valley River	1,400*	Midday	8	0.34
5/10/2015	Valley River	5,253	Early-evening	38	0.43
5/12/2015	Valley River	4,997	Early-evening	7	0.08
6/6/2015	Valley River	2,621	Early-evening	11	0.25
6/7/2015	Valley River	2,339	Early-evening	0	0.00

*Backpack electrofishing; all other sampling was conducted by boat-mounted electrofishing.

Table 2. Common and scientific names of fishes that were screened by genetic analysis in Blueback Herring diet samples.

Common Name	Scientific Name
Suckers	
Black Redhorse	*Moxostoma duquesnei*
Golden Redhorse	*Moxostoma erythrurum*
River Redhorse	*Moxostoma carinatum*
Sicklefin Redhorse	*Moxostoma* sp.
Silver Redhorse	*Moxostoma anisurum*
Smallmouth Redhorse	*Moxostoma breviceps*
Northern Hog Sucker	*Hypentelium nigricans*
Other Fishes	
Banded Darter	*Etheostoma zonale*
Bluegill	*Lepomis macrochirus*
Central Stoneroller	*Campostoma anomalum*
Common Carp	*Cyprinus carpio*
Gilt Darter	*Percina evides*
Green Sunfish	*Lepomis cyanellus*
Largemouth Bass	*Micropterus salmoides*
Redbreast Sunfish	*Lepomis auritus*
River Chub	*Nocomis micropogon*
Smallmouth Bass	*Micropterus dolomieu*
Spotted Bass	*Micropterus punctulatus*
Tennessee Shiner	*Notropis leuciodus*
Walleye	*Sander vitreus*
Warpaint Shiner	*Luxilus coccogenis*
White Bass	*Morone chrysops*
Outside of range (negative controls)	
Dollar Sunfish	*Lepomis marginatus*
Ironcolor Shiner	*Notropis chalybaeus*
Fantail Darter	*Etheostoma flabellare*

Table 3. Percent of samples classified in each digestive state for all visually assessed diet samples, for the subset of samples selected for genetic analysis, and among samples that returned false negatives for visual identification.

	All Samples	Genetics Samples	False Negatives
Perfectly identifiable	12%	11%	10%
Highly identifiable	20%	13%	15%
Moderately identifiable	22%	24%	10%
Poorly identifiable	14%	16%	20%
Unidentifiable	32%	36%	45%

Table 4. Frequency of occurrence of diet items, based on visual diet analysis. Frequency of occurrence was calculated among all stomachs with contents. N indicates the number of diet samples assessed.

	2014			2015		
Category	April (N = 26)	May (N = 6)	June (N = 55)	April (N = 66)	May (N = 44)	June (N = 9)
Larval fish	0	0	2%	0	16%	44%
Ova	0	0	0	12%	0	22%
Fins	12%	17%	41%	3%	39%	33%
Scales	8%	0	2%	0	0	0
Winged insects	4%	67%	0	41%	14%	0
Diptera*	100%	100%	11%	71%	64%	11%
Ephemeroptera**	20%	83%	0	15%	14%	0
Plecoptera	0	0	0	0	2%	0
Trichoptera	8%	50%	0	0	5%	0
Nematoda	56%	83%	2%	45%	5%	0
Coleoptera	8%	17%	2%	0	2%	0
Hemiptera***	4%	0	0	0	0	0
Oligochaeta	0	0	0	3%	5%	0
Hydracarina	4%	33%	9%	6%	14%	11%
Ostracoda	16%	0	2%	2%	18%	0
Copepoda	0	0	52%	0	9%	0
Cladocera	8%	0	69%	0	7%	0

*Includes larvae and pupae in the families Ceratopogonidae, Chironomidae, Ephydridae, Simuliidae, Therevidae, and Tipulidae.
**Includes nymphs in the families Baetidae and Heptageniidae.
***Family Corixidae.

Table 5. Percent composition by number of diet items, based on visual diet analysis. Values represent the mean among all stomachs with contents collected during a given sampling period, and values in parentheses represent the standard error of the mean. N indicates the number of diet samples assessed.

	2014			2015		
Category	April (N = 26)	May (N = 6)	June (N = 55)	April (N = 66)	May (N = 44)	June (N = 9)
Larval Fish	0 (0)	0 (0)	0.3% (0.3)	0 (0)	5.2% (2.7)	44.3% (8.0)
Ova	0 (0)	0 (0)	0 (0)	10.4% (3.6)	0 (0)	21.9% (6.6)
Fins	0.6% (0.5)	1.9% (1.9)	8.1% (3.2)	0.7% (0.5)	16.4%(0.05)	21.3% (5.8)
Scales	0.1% (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Winged insects	0.1% (0.1)	8.8% (3.8)	0 (0)	12.3% (2.9)	4.8% (2.3)	0 (0)
Diptera	92.5% (1.7)	68.5% (6.4)	3.9% (2.3)	58.4% (4.8)	34.1% (5.9)	10.0% (4.5)
Ephemeroptera	0.5% (0.3)	10.7% (7.0)	0 (0)	1.1% (0.4)	1.3% (0.6)	0 (0)
Plecoptera	0 (0)	0 (0)	0 (0)	0 (0)	2.0% (1.9)	0 (0)
Trichoptera	0.2% (0.1)	3.7% (2.2)	0 (0)	0 (0)	0.6% (0.5)	0 (0)
Nematoda	3.8% (1.0)	4.9% (1.4)	0.1% (0.1)	14.6% (3.3)	1.1% (0.8)	0 (0)
Coleoptera	0.1% (0.1)	0.3% (0.3)	0.1% (0.1)	0 (0)	0 (0)	0 (0)
Hemiptera	0.1% (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oligochaeta	0 (0)	0 (0)	0 (0)	1.5% (1.0)	5.3% (3.5)	0 (0)
Hydracarina	0.2% (0.2)	1.4% (1.1)	4.0% (2.4)	0.9% (0.5)	8.9% (3.7)	2.5% (1.1)
Ostracoda	1.8% (1.5)	0 (0)	0 (0)	0.1% (0.1)	6.8% (3.0)	0 (0)
Copepoda	0 (0)	0 (0)	29.1% (5.1)	0 (0)	9.7% (4.4)	0 (0)
Cladocera	0.1% (0.1)	0 (0)	54.5% (5.8)	0 (0)	4.1% (2.7)	0 (0)

Table 6. Frequency of occurrence of genetic material from catostomid species in diet samples of Blueback Herring collected in a given sampling month. N indicates the number of samples for which barcoding PCR produced reliable results.

	2014			2015		
Taxon	April (N = 15)	May (N = 6)	June (N = 30)	April (N = 30)	May (N = 40)	June (N = 9)
Sucker (any)	27%	17%	27%	13%	55%	67%
Black Redhorse	0	17%	3%	0	0	0
Golden Redhorse	20%	0	17%	13%	8%	67%
River Redhorse	0	0	0	0	0	0
Sicklefin Redhorse	0	0	0	0	0	0
Silver Redhorse	7%	0	3%	0	35%	11%
Smallmouth Redhorse	0	0	0	0	3%	0
Northern Hog Sucker	7%	0	3%	0	33%	0

Table 7. Identities of visually discerned piscine diet items identified as suckers using genetic procedures. NHS = Northern Hog Sucker, SRH = Silver Redhorse, SMRH = Smallmouth Redhorse, BRH = Black Redhorse, and GRH = Golden Redhorse.

	2014			2015		
Diet Item	April	May	June	April	May	June
Ova	-	-	-	GRH	-	GRH
Larva	-	-	-	-	GRH, SRH, SMRH, NHS	-
Fins	GRH	-	BRH, GRH, SRH	-	SRH, NHS	-
Nothing identified	SRH, NHS	BRH	SRH	GRH	GRH, SRH, NHS,	GRH

Table 8. Frequency of occurrence of genetic material from other fish species in diet samples of Blueback Herring collected in a given sampling month. N indicates the number of samples for which barcoding PCR produced reliable results.

	2014			2015		
Taxon	April (N = 15)	May (N = 6)	June (N = 30)	April (N = 30)	May (N = 40)	June (N = 9)
Banded Darter	0	0	0	0	8%	22%
Common Carp	0	0	47%	3%	45%	0
Gilt Darter	0	0	0	7%	0	0
Green Sunfish	0	0	0	0	0	11%

Figures

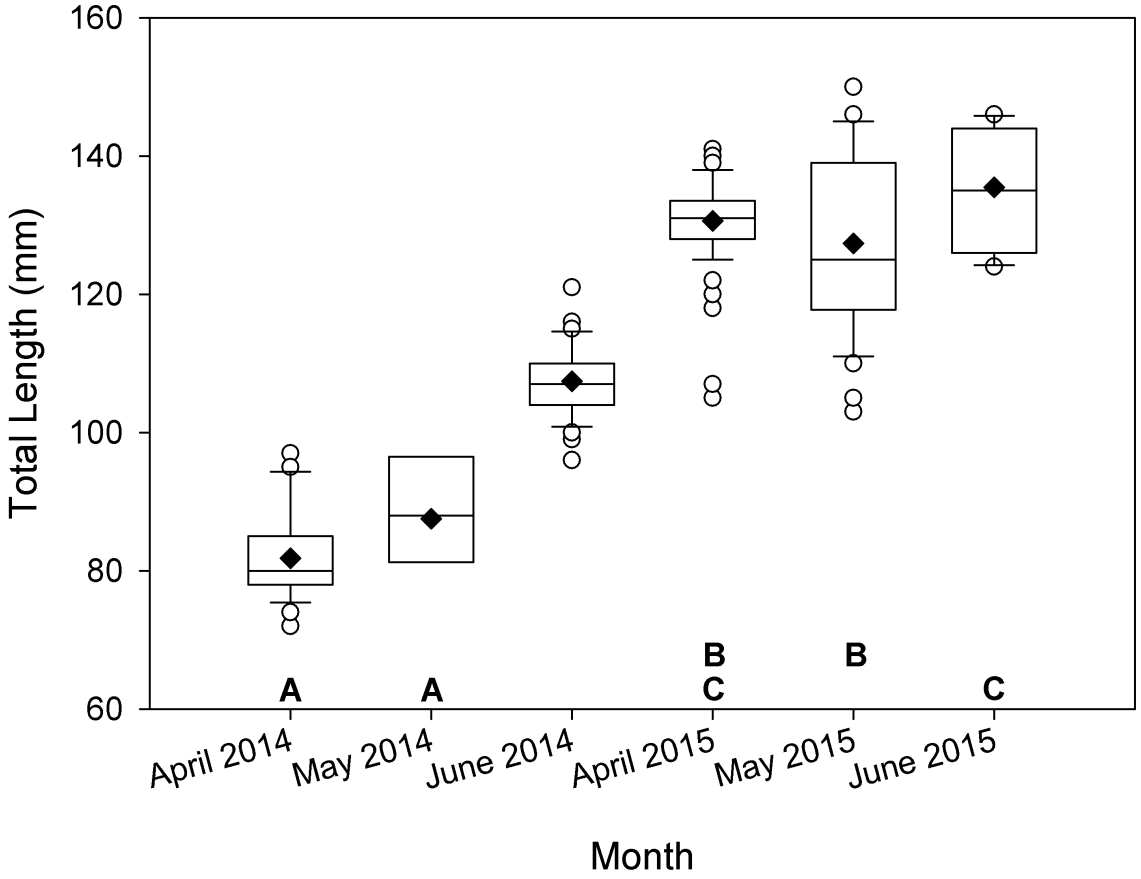

Figure 1. Total lengths of Blueback Herring according to sampling month. Boxplots represent the 10th, 25th, median, 75th, and 90th percentiles; open circles indicate outliers; and means are indicated by black diamonds. Months in which the mean total lengths were not signficantly different are indicated by common letters above the x-axis.

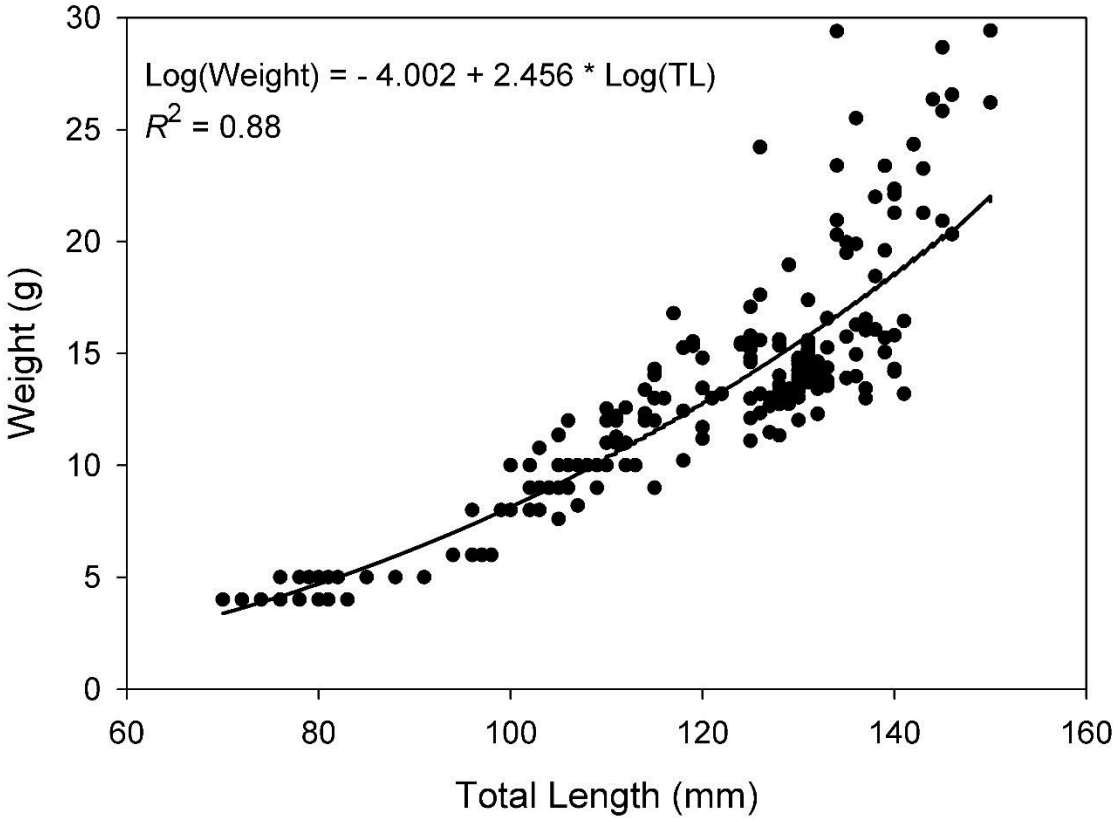

Figure 2. Length-weight relationship for Blueback Herring sampled from the Hiwassee and Valley rivers in 2014 and 2015.

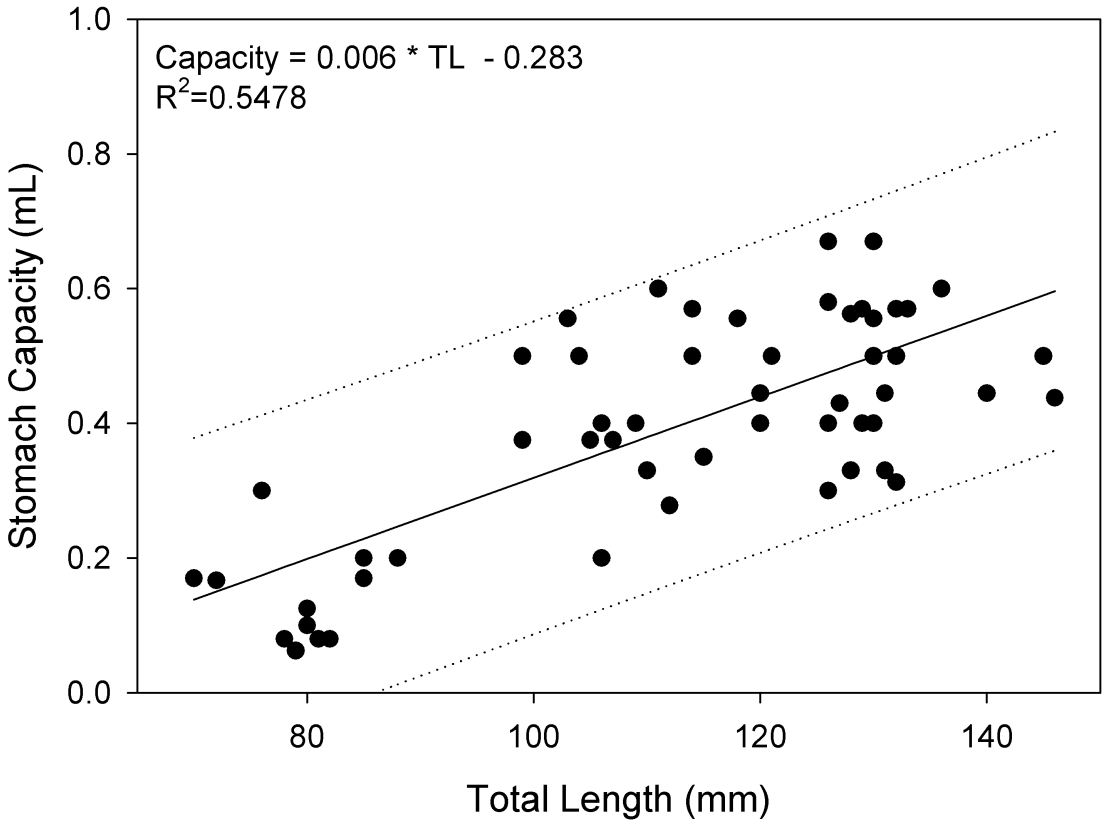

Figure 3. Stomach capacity (mL) of Blueback Herring at various sizes (TL, mm). Points represent individual estimates of stomach capacity; the solid regression line represents the mean stomach capacity, and dotted lines represent bounds on the 95% prediction interval.

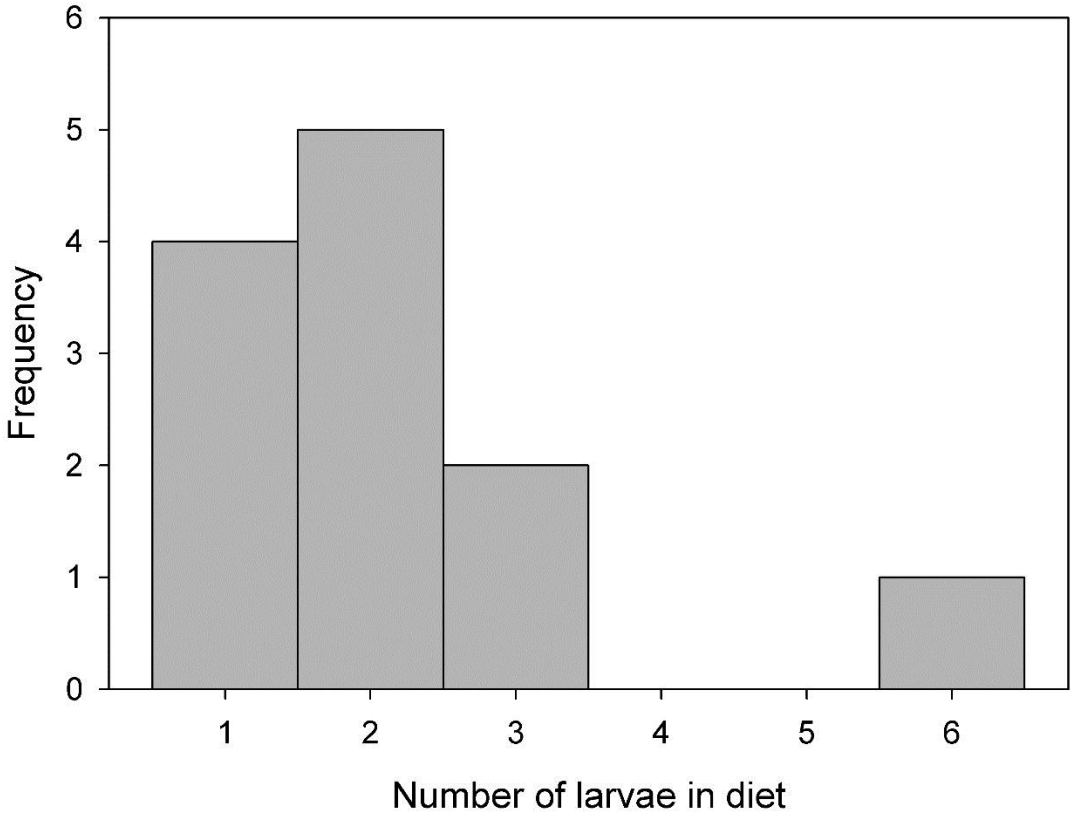

Figure 4. Frequency histogram of diet samples containing one or more visually discernible fish larvae, binned by the number of larvae in diet.

APPENDIX

Appendix. Genetic barcoding sequences of adult suckers that were used as references, and North Carolina Museum of Natural Sciences catalog numbers for associated voucher specimens.

NCMNS catalog no.	Species	Length (TL, mm)	Weight (g)	Sequence
74329	*Moxostoma erythrurum*	-	-	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCTTTAAGCCTTCTAATTCGAGCCGAATTAAG CCAACCTGGGTCACTTCTTGGTGACGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTTT CTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTTGTGCCATTAATGATTGGGGCCCCCGACAT AGCATTCCCCCGGATGAACAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTACTATTATTAGCTTCTTCCGG AGTTGAGGCCGGGGCCGGAACAGGATGAACAGTATATCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGCC TCTGTAGATCTAACCATCTTTTCTCTTCATCTGGCAGGAGTTTCATCAATTCTTGGGGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTTACAG CTGTTCTTCTTCTTTTATCGCTACCTGTCCTAGCTGCGGGTATTACCATGCTTTTAACAGACCGAAATCTAAATAC AACATTCTTTGACCCGGCAGGAGGTGGAGACCCAATTCTCTACCAACACTTA
74330.01	*Moxostoma sp*. (Sicklefin Redhorse)	508	1120	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74330.02	*Moxostoma sp*. (Sicklefin Redhorse)	425	728	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74330.03	*Moxostoma sp*. (Sicklefin Redhorse)	445	862	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA

NCMNS catalog no.	Species	Length (TL, mm)	Weight (g)	Sequence
74330.04	*Moxostoma sp.* (Sicklefin Redhorse)	445	758	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74330.05	*Moxostoma sp.* (Sicklefin Redhorse)	358	440	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCTATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74330.06	*Moxostoma sp.* (Sicklefin Redhorse)	439	796	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74330.07	*Moxostoma sp.* (Sicklefin Redhorse)	770	972	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATCGGGGGATTCGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGAC ATAGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTGTTATTATTAGCCTCTTCTG GAGTTGAAGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGC CTCTGTAGATTTAACCATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGGGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACA GCTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATCACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA

NCMNS catalog no.	Species	Length (TL, mm)	Weight (g)	Sequence
74331.01	*Moxostoma carinatum*	450	805	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATCTATAATGTTATTGTTACCGCTCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATCGGGGGATTTGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGACAT AGCATTTCCCCGAATAAATAATATAAGCTTCTGACTCCTGCCCCCCTCTTTCCTGCTATTATTAGCCTCTTCCGG AGTTGAGGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGCC TCTGTGGATTTAACTATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACAG CTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATTACCATGCTCTTAACAGACCGAAATCTAAATA CAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74331.02	*Moxostoma carinatum*	440	771	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATCTATAATGTTATTGTTACCGCTCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATCGGGGGATTTGGAAACTGACTTGTACCATTAATAATCGGAGCCCCTGACAT AGCATTTCCCCGAATAAATAATATAAGCTTCTGACTCCTGCCCCCCTCTTTCCTGCTATTATTAGCCTCTTCCGG AGTTGAGGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGCC TCTGTAGATTTAACTATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACAG CTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATTACCATGCTCTTAACAGACCGGAATCTAAATA CAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74331.03	*Moxostoma carinatum*	488	1038	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATCTATAATGTTATTGTTACCGCTCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATCGGGGGATTTGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGACAT AGCATTTCCCCGAATAAATAATATAAGCTTCTGACTCCTGCCCCCCTCTTTCCTGCTATTATTAGCCTCTTCCGG AGTTGAGGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGCC TCTGTGGATTTAACTATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACAG CTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATTACCATGCTCTTAACAGACCGAAATCTTAATA CAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74331.04	*Moxostoma carinatum*	455	792	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATCTATAATGTTATTGTTACCGCTCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATCGGGGGATTTGGAAACTGACTTGTACCATTAATGATCGGAGCCCCTGACAT AGCATTTCCCCGAATAAATAATATAAGCTTCTGACTCCTGCCCCCCTCTTTCCTGCTATTATTAGCCTCTTCCGG AGTTGAGGCCGGTGCCGGAACAGGATGAACAGTGTACCCACCCCTCGCGGGCAATCTTGCTCACGCCGGAGCC TCTGTAGATTTAACTATTTTTTCTCTTCATCTGGCAGGTGTCTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTAACAG CTGTTCTTCTTCTTTTATCACTACCCGTCCTAGCTGCAGGCATTACCATGCTCTTAACAGACCGGAATCTAAATA CAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA

NCMNS catalog no.	Species	Length (TL, mm)	Weight (g)	Sequence
74332.01	*Moxostoma duquesnei*	357	400	CCTATATCTTGTATTTGGTGCCTGAGCCGGGATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAACTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTCGTACCATTAATGATCGGAGCCCCAGAC ATGGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCTTCCTTCCTGCTACTATTAGCCTCTTCC GGGGTTGAGGCTGGAGCCGGAACAGGATGAACAGTATACCCGCCCCTTGCTGGCAATCTTGCTCATGCTGGAG CCTCTGTAGATCTAACCATTTTTTCTCTGCACCTAGCAGGTGTTTCATCAATTCTTGGAGCAATTAATTTCATTAC CACAACAATCAATATGAAACCCCCAGCCATCTCTCAATATCAAACTCCTCTGTTTGTCTGAGCTGTACTTGTAAC AGCTGTTCTTCTTCTTTTATCACTACCTGTCCTAGCTGCGGGCATCACCATGCTCTTAACAGACCGAAATCTAAA TACAACATTCTTTGACCCGGCAGGAGGAGGAGACCCAATTCTATACCAACACTTA
74332.02	*Moxostoma duquesnei*	360	422	CCTATATCTTGTATTTGGTGCCTGAGCCGGGATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAACTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTCGTACCATTAATGATCGGAGCCCCAGAC ATGGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCTTCCTTCCTGCTACTATTAGCCTCTTCC GGGGTTGAGGCTGGAGCCGGAACAGGATGAACAGTATACCCGCCCCTTGCTGGCAATCTTGCTCATGCTGGAG CCTCTGTAGATCTAACCATTTTTTCTCTGCACCTAGCAGGTGTTTCATCAATTCTTGGAGCAATTAATTTCATTAC CACAACAATCAATATGAAACCCCCAGCCATCTCTCAATATCAAACTCCTCTGTTTGTCTGAGCTGTACTTGTAAC AGCTGTTCTTCTTCTTTTATCACTACCTGTCCTAGCTGCGGGCATCACCATGCTCTTAACAGACCGAAATCTAAA TACAACATTCTTTGACCCGGCAGGAGGAGGAGACCCAATTCTATACCAACACTTA
74333.01	*Moxostoma anisurum*	522	1252	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTCCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTCGTACCACTAATGATTGGAGCCCCTGACAT AGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCCTTCCTGCTATTATTAGCCTCTTCTGG GGTTGAGGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGCC TCTGTAGATCTAACCATTTTTTCTCTACACCTGGCAGGTGTTTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATTTCTCAGTATCAAACTCCCCTGTTTGTCTGAGCTGTACTCGTAACAG CTGTTCTTCTTCTCTTATCACTACCTGTCCTAGCTGCGGGTATTACCATGCTCTTAACAGACCGAAATCTAAATA CAACATTCTTTGACCCGGCAGGAGGAGGAGACCCAATTCTTTACCAACACTTA
74333.02	*Moxostoma anisurum*	536	1312	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTCCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTCGTACCACTAATGATTGGAGCCCCTGACAT AGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCCTTCCTGCTATTATTAGCCTCTTCTGG GGTTGAGGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGCC TCTGTAGATCTAACCATTTTTTCTCTACACCTGGCAGGTGTTTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATTTCTCAGTATCAAACTCCCCTGTTTGTCTGAGCTGTACTCGTAACAG CTGTTCTTCTTCTCTTATCACTACCTGTCCTAGCTGCGGGTATTACCATGCTCTTAACAGACCGAAATCTAAATA CAACATTCTTTGACCCGGCAGGAGGAGGAGACCCAATTCTTTACCAACACTTA

NCMNS catalog no.	Species	Length (TL, mm)	Weight (g)	Sequence
74333.03	*Moxostoma anisurum*	490	1106	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTCCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTTGTTATAATTTT CTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTCGTACCACTAATGATTGGAGCCCCTGACAT AGCATTCCCCCGAATAAATAATATAAGCTTCTGACTCCTACCCCCCTCCTTCCTGCTATTATTAGCCTCTTCTGG GGTTGAGGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGCC TCTGTAGATCTAACCATTTTTTCTCTACACCTGGCAGGTGTTTCATCAATTCTTGGAGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATTTCTCAGTATCAAACTCCCCTGTTTGTCTGAGCTGTACTCGTAACAG CTGTTCTTCTTCTCTTATCACTACCTGTCCTAGCTGCGGGTATTACCATGCTCTTAACAGACCGAAATCTAAATA CAACATTCTTTGACCCGGCAGGAGGAGGAGACCCAATTCTTTACCAACACTTA
74334.01	*Moxostoma breviceps*	466	918	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTTGTACCATTAATGATTGGAGCCCCTGACA TAGCATTCCCCCGAATAAATAATATGAGCTTCTGACTCCTACCCCCCTCTTTCCTGCTACTATTAGCCTCTTCCG GGGTTGAGGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGC CTCTGTAGATCTAACCATTTTTTCTCTTCACTTAGCAGGTGTCTCATCAATTCTTGGAGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTATTTGTCTGAGCTGTACTTGTAACA GCTGTTCTCCTTCTTTTATCACTACCTGTCCTAGCTGCGGGTATTACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTCTACCAACACTTA
74334.02	*Moxostoma breviceps*	467	878	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCCTTAAGCCTTCTAATTCGAGCCGAATTAA GTCAACCTGGGTCACTCCTCGGTGATGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTTGTACCATTAATGATTGGAGCCCCTGACA TAGCATTCCCCCGAATAAATAATATGAGCTTCTGACTCCTACCCCCCTCTTTCCTGCTACTATTAGCCTCTTCCG GGGTTGAGGCCGGAGCCGGAACAGGATGAACAGTATACCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGC CTCTGTAGATCTAACCATTTTTTCTCTTCACTTAGCAGGTGTCTCATCAATTCTTGGAGCAATTAATTTTATTACC ACAACAATTAATATAAAACCCCCAGCCACCTCTCAATATCAAACTCCCCTATTTGTCTGAGCTGTACTTGTAACA GCTGTTCTCCTTCTTTTATCACTACCTGTCCTAGCTGCGGGTATTACCATGCTCTTAACAGACCGAAATCTAAAT ACAACATTCTTTGACCCAGCAGGAGGAGGAGACCCAATTCTTTACCAACACTTA
74335.01	*Moxostoma erythrurum*	366	488	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCTTTAAGCCTTCTAATTCGAGCCGAATTAAG CCAACCTGGGTCACTTCTTGGTGACGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTTT CTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTTGTGCCATTAATGATTGGGGCCCCCGACAT AGCATTCCCCCGGATGAACAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTACTATTATTAGCTTCTTCCGG AGTTGAGGCCGGGGCCGGAACAGGATGAACAGTATATCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGCC TCTGTAGATCTAACCATCTTTTCTCTTCATCTGGCAGGAGTTTCATCAATTCTTGGGGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTTACAG CTGTTCTTCTTCTTTTATCGCTACCTGTCCTAGCTGCGGGTATTACCATGCTTTTAACAGACCGAAATCTAAATAC AACATTCTTTGACCCGGCAGGAGGTGGAGACCCAATTCTCTACCAACACTTA

NCMNS catalog no.	Species	Length (TL, mm)	Weight (g)	Sequence
74335.02	*Moxostoma erythrurum*	413	775	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTAGGAACTGCTTTAAGCCTTCTAATTCGAGCCGAATTAAG CCAACCTGGGTCACTTCTTGGTGACGATCAAATTTATAATGTTATTGTTACCGCCCATGCCTTCGTCATAATTTT CTTTATAGTAATACCCATTTTAATTGGAGGATTTGGAAACTGACTTGTGCCATTAATGATTGGGGCCCCCGACAT AGCATTCCCCCGGATGAACAATATAAGCTTCTGACTCCTACCCCCCTCTTTCCTACTATTATTAGCTTCTTCCGG AGTTGAGGCCGGGGCCGGAACAGGATGAACAGTATATCCACCCCTCGCGGGCAATCTTGCTCATGCCGGAGCC TCTGTAGATCTAACCATCTTTTCTCTTCATCTGGCAGGAGTTTCATCAATTCTTGGGGCAATTAATTTTATTACCA CAACAATTAATATAAAACCCCCAGCCATCTCTCAATATCAAACTCCCCTGTTTGTCTGAGCTGTACTTGTTACAG CTGTTCTTCTTCTTTTATCGCTACCTGTCCTAGCTGCGGGTATTACCATGCTTTTAACAGACCGAAATCTAAATAC AACATTCTTTGACCCGGCAGGAGGTGGAGACCCAATTCTCTACCAACACTTA
74392	*Hypentelium nigricans*	211	100	CCTATATCTTGTATTTGGTGCCTGAGCCGGAATAGTGGGAACCGCCTTAAGCCTCCTAATTCGAGCCGAATTAA GTCAACCTGGATCACTTCTTGGTGATGACCAGATTTATAACGTTATTGTTACTGCCCATGCCTTCGTCATAATTT TCTTTATAGTAATACCCATCTTAATTGGAGGATTTGGAAACTGACTTGTCCCATTAATAATTGGGGCCCCTGACA TAGCATTTCCCCGAATGAACAACATAAGCTTCTGACTTCTACCCCCTTCCTTCCTGCTATTGTTGGCCTCTTCTGG AGTTGAAGCCGGGGCCGGAACAGGATGAACAGTATATCCCCCACTTGCAGGCAATCTTGCCCATGCTGGAGCTT CTGTTGATCTAACCATTTTTTCTCTACACCTGGCGGGTGTTTCATCAATTCTTGGGGCAATCAATTTCATTACCAC AACAATTAACATAAAACCCCCAGCCATCTCTCAATATCAAACCCCTCTATTTGTTTGAGCTGTGCTTGTAACAGC TGTACTTCTTCTCCTATCACTACCAGTATTAGCTGCGGGCATTACTATGCTCTTAACAGACCGTAATTTAAATAC AACATTTTTTGATCCAGCAGGAGGAGGAGACCCCATCCTTTACCAGCACTTA