## REPRODUCTION, MIGRATION, AND PROSPECTS FOR PERSISTENCE OF A REINTRODUCED POPULATION OF AN IMPERILED RIVERINE FISH, ROBUST REDHORSE (*MOXOSTOMA ROBUSTUM*)

by

CARRIE ALISON STRAIGHT

(Under the Direction of Mary C. Freeman)

#### ABSTRACT

Human modification of the world's landscapes and riverscapes have resulted in a high number of imperiled species worldwide. Nearly half of North American catostomid fishes are considered imperiled. The conservation of any imperiled species relies on understanding threats and requirements of the species at each life history stage. This study focused on assessing the reproductive biology of an imperiled large-bodied catostomid native to the southeastern United States, the Robust Redhorse (*Moxostoma robustum*). Robust Redhorse conservation has been hampered by failure to document recruitment. Therefore, gaining knowledge of the species' reproductive biology could provide managers with information critical for conservation. This study has documented (a) a new method using passive acoustic monitoring to assess spawning frequency of large-bodied catostomids when visual observations can not be made, (b) spawning frequencies and diel periodicity of Robust Redhorse in two river systems, (c) reproductive and migratory behavior of Robust Redhorse in a reintroduced population, compared to two wild, Coastal Plain populations in Georgia, and (d) evidence of recruitment in a reintroduced population of Robust Redhorse. These studies provide novel findings of Robust Redhorse behavior. I have documented Robust Redhorse use of reservoirs as wintering habitat as well as plasticity in use of river and reservoir as wintering habitat. I have also documented plasticity in use of spawning sites, tracking movements by three individuals between two spawning sites during a single spawning season. This study is the first to document nocturnal spawning; Robust Redhorse spawn at all hours of the day with a peak number of spawns after midnight and in the early hours of the morning. Robust Redhorse also display a range of numbers of individuals participating in spawning acts, in addition to the typical trio of two males and a female. I have also documented an alternate reproductive tactic of sneaking spawn attempts, rather than holding territories, by smaller, presumably younger Robust Redhorse males. These new findings and others in this study expand our understanding of reproductive behavior of this imperiled fish species and should provide valuable information for management of this species and future reintroductions.

INDEX WORDS:Robust Redhorse, Moxostoma robustum, River Redhorse, Moxostoma<br/>carinatum, Catostomid, Broad River, Oconee River, Savannah River,<br/>Reintroduction, Reproductive Behavior, Alternate Reproductive Tactic,<br/>Acoustic, Hydrophone, Recruitment, Conservation

# REPRODUCTION, MIGRATION, AND PROSPECTS FOR PERSISTENCE OF A REINTRODUCED POPULATION OF AN IMPERILED RIVERINE FISH, ROBUST REDHORSE (*MOXOSTOMA ROBUSTUM*)

by

### CARRIE ALISON STRAIGHT

B.A., Central College, 1993

M.S., University of Georgia, 2000

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial

Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

© 2014

Carrie A. Straight

All Rights Reserved

# REPRODUCTION, MIGRATION, AND PROSPECTS FOR PERSISTENCE OF A REINTRODUCED POPULATION OF AN IMPERILED RIVERINE FISH, ROBUST REDHORSE (*MOXOSTOMA ROBUSTUM*)

by

### CARRIE ALISON STRAIGHT

Major Professor:

Mary Freeman

Committee:

Byron Freeman Catherine Pringle Rhett Jackson Brett Albanese

Electronic Version Approved:

Maureen Grasso Dean of the Graduate School The University of Georgia May 2014

### DEDICATION

"To those devoid of imagination a blank place on the map is a useless waste; to others, the most valuable part."

- Aldo Leopold, A Sand County Almanac.

I dedicate this dissertation to my parents who, for as long as I can remember, encouraged me to visit the blank pages and appreciate them.

#### ACKNOWLEDGEMENTS

This project would not have been possible without the help and guidance from many people. First, I would like to thank my committee Mary Freeman, Bud Freeman, Brett Albanese, Rhett Jackson, and Cathy Pringle for their thoughtful insight and comments. Mary Freeman has provided direction and guidance since I started this endeavor and for that I will be eternally grateful. I would like to thank Bud Freeman for support throughout my time at the Odum School of Ecology and for introducing me to the Robust Redhorse. The members of the Freeman Lab, especially Megan Hagler, provided me with help with fieldwork and logistics with all of my research.

This research was funded in part by a Georgia Department of Natural Resources State Wildlife Grant and the USGS through the Piedmont South Atlantic Coast Cooperative Ecosystem Studies Unit (Warnell School of Forestry and Natural Resources, University of Georgia). The Georgia Ecological Services, U. S. Fish and Wildlife Service, provided acoustical equipment.

Lastly, I would like to thank my husband, Jason Lang, for support throughout my graduate career. He has been there through all of the field days, analyses, drafts, and to accompany me when I needed an extra hand in the field.

## TABLE OF CONTENTS

Pag	e
ACKNOWLEDGEMENTS	v
LIST OF TABLES	X
LIST OF FIGURES	ii
LIST OF APPENDICES	V
CHAPTER	
1 INTRODUCTION AND LITERATURE REVIEW	1
References1	1
2 PASSIVE ACOUSTIC MONITORING TO DETECT SPAWNING IN LARGE-	
BODIED CATOSTOMIDS	1
Abstract	2
Introduction2	3
Methods20	6
Results	2
Discussion	4
Acknowledgments4	0
References	0
3 DIEL PATTERNS AND SEASONAL TRENDS IN SPAWNING RATES OF	
ROBUST REDHORSE (MOXOSTOMA ROBUSTUM) AND RIVER REDHORSE	

	MONITORING	55
	Abstract	56
	Introduction	57
	Methods	59
	Results	66
	Discussion	70
	Acknowledgments	74
	References	75
4	COMPARISONS OF REPRODUCTIVE BIOLOGY OF AN IMPERILED	
	MIGRATORY FISH IN A PIEDMONT AND TWO COASTAL PLAIN RIV	/ERS99
	Abstract	100
	Introduction	101
	Methods	105
	Results	110
	Discussion	119
	Acknowledgments	128
	References	128
5	EVIDENCE FOR RECRUITMENT IN A REINTRODUCED POPUATION	OF AN
	IMPERILED CATOSTOMID, MOXOSTOMA ROBUSTUM	144
	Abstract	145
	Introduction	145
	Methods	148

## (M. CARINATUM) IN GEORGIA, ASSESSED USING PASSIVE ACOUSTIC

	Results	
	Discussion	
	Acknowledgments	
	References	
6	CONCLUSIONS	
	References	

## LIST OF TABLES

Page
Table 1.1: Number of Robust Redhorse (Moxostoma robustum) stocked within the Broad River
system from 1995-1998
Table 1.2: Estimated years of first reproduction by Robust Redhorse (Moxostoma robustum) in
the Broad River system
Table 2.1: Estimated depth, velocity at 60% of depth, and velocity at the bottom at locations of
spawning triads of Robust Redhorse (Moxostoma robustum) and River Redhorse (M.
carinatum) at three localities in the Broad River watershed (Broad / Hudson) and one
locality in the Coosawattee River used for passive acoustic monitoring in 2012
Table 2.2: Estimated number of Robust Redhorse and River Redhorse present at each recording
locality, the date of spawning (2012), and classification success for the best performing
automated assessment
Table 2.3: Mean and standard deviation (SD) of the dominant frequency, 95% frequency, peak
relative power, and duration of recorded spawning events by Robust Redhorse and River
Redhorse
Table 3.1: Dates of recorded spawning, and estimated depth, velocity at 60% of depth, and
velocity near the substrate at locations of spawning triads of Robust Redhorse in the
Broad River in 2012 and Savannah River in 2013, and River Redhorse in the
Coosawattee River in 2012
Table 3.2: Selected covariates used in linear and generalized least squares regression.         82

Table 3.3: Total time of subsampled acoustic recordings (Time), number of spawning events (N),
and the mean, standard deviation (SD), and range of duration of recorded spawning
events for Robust Redhorse in the Broad River in 2012 and Savannah River in 2013, and
River Redhorse in the Coosawattee River in 2012
Table 3.4: The number of hourly samples (N) and mean (SD) of continuous covariates in each
river system used for model analysis. NA indicates the variable was not available for that
study site
Table 3.5: Covariates, Akaike's information criterion corrected for small sample size (AICc),
AICc differences ( $\Delta$ AICc), and Akaike weights ( $w_i$ ) for models influencing spawning
duration of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in
the Coosawattee River
Table 3.6: Confidence model set, parameter estimates, standard error (SE) and 95% confidence
interval (CI) of parameter estimates and parameter/model specific p-values for spawning
duration (D) of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse
in the Coosawattee River
Table 3.7: Covariates, Akaike's information criterion corrected for small sample size(AICc),
AICc differences ( $\Delta$ AICc), and Akaike weights ( $w_i$ ) for models influencing spawning
rate (R) of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in the
Coosawattee River
Table 3.8: Confidence model set, parameter estimates, standard error (SE) and 95% confidence
interval (CI) of parameter estimates and parameter/model specific p-values for spawning
rate (R) of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in the
Coosawattee River

- Table 4.1: Characteristics (watershed area and river width) at spawning sites found in the Broad
   River system, Oconee River, and Savannah River

   135

- Table 5.2: Estimated years of first reproduction by Robust Redhorse (*Moxostoma robustum*) in

   the Broad River system
- Table 5.3: The probability that an individual fish with the given total length (mm) is less than 6,
- Table 5.4: Mean probability of Robust Redhorse captured in the Broad River in 2010 and 2011

   of being less than 12 years old.

   164

## LIST OF FIGURES

Figure 1.1: Robust Redhorse captured in the Oconee River, Georgia
Figure 1.2: Map of the historic presumed range of Robust Redhorse in the southeastern United
States from the Yadkin - Pee Dee River drainage (North Carolina/ South Carolina) south
to the Altamaha River drainage (Georgia)
Figure 2.1: Spawning triad (female flanked by one male on either side) of River Redhorse
Moxostoma carinatum, 10 May 2012, Coosawattee River, Georgia, downstream of
Carters Re-regulation Dam51
Figure 2.2: Map of the locations in north Georgia where we observed spawning and recorded
spawning acoustics (black dots) of River Redhorse in the Coosawattee River watershed
and Robust Redhorse in the Broad River watershed in 2012
Figure 2.3: A characteristic acoustic spawning signature (shown as the concentration of dark in
the middle of each view) of (A) Robust Redhorse Moxostoma robustum, and (B) River
Redhorse M. carinatum, (Hanning window, FFT length: 256 samples, bin resolution: 248
Hz)53
Figure 2.4: The estimated relationship between the duration of a spawning event and the
probability of detecting that event using automated analysis
Figure 3.1: Map of the locations in Georgia where we recorded spawning acoustics of Robust
Redhorse (circles) in the Broad River in 2012 and Savannah River in 2013 and River
Redhorse (square) in the Coosawattee River watershed in 2012

Figure 3.2: Robust Redhorse at the lower gravel bar on the Savannah River. Spawning
aggregation of males on the river right gravel lens (left panel) and a spawning triad, two
males flanking either side of a female (right panel)
Figure 3.3: Hydrophone in steel harness (left panel) and deployed with added weights and
attachment chain (right panel)
Figure 3.4: Location of the hydrophone placed at the downstream Savannah River spawning site
(small circle) and approximate range of detection (20 m radius; large circle)
Figure 3.5: Fraction of the moon illuminated (white in top bar of each graph) and spawning rates
of Robust Redhorse in the A. Broad River in 2012 and B. Savannah River in 2013, and C.
River Redhorse in the Coosawattee River in 2012 estimated from passive acoustic
recording devices
Figure 3.6: Trend of spawning rates of Robust Redhorse in the Broad and Savannah Rivers and
River Redhorse in the Coosawattee River estimated from time series decomposition93
Figure 3.7: Periodicity of spawning rates of Robust Redhorse in the Broad River and Savannah
River, and River Redhorse in the Coosawattee River estimated from time series
decomposition and illustrated for a 36-hour period94
Figure 3.8: Recorded water temperatures (dark line) and overall trend in spawning rate based on
time series decomposition (gray line) in the A. Broad River during 2012 and B. Savannah
River in 2013
Figure 4.1: Map of Robust Redhorse assumed historic range and known and presumed spawning
locations (circles) from the Yadkin-Pee Dee River, North Carolina to the Altamaha River
drainage, Georgia139

Figure 4.2: Map of Robust Redhorse spawning locations (solid circles), tagging / surgery
location (star), wintering receiver stations in Strom Thurmond Reservoir (hollow circles),
and release localities for captive-reared individuals (plus signs) in the Broad River,
Georgia140
Figure 4.3: Movements of (A) six Robust Redhorse captured in 2010 and (B) twelve Robust
Redhorse captured in 2011141
Figure 4.4: Spawning triad (female flanked by one male on either side) of Robust Redhorse in
the Savannah River, Georgia142
Figure 5.1: Map of the historic presumed range of Robust Redhorse in the southeastern United
States from the Yadkin - Pee Dee River drainage (North Carolina/ South Carolina) south
to the Altamaha River drainage (Georgia)165
Figure 5.2: Relationship of weight (triangles) and total length (circles) to age, based on known-
age Robust Redhorse recaptured after release to the wild
Figure 5.3: Total length (circles) and weight (triangles) of known age Robust Redhorse from 5 to
14 years old. Lines represent linear relationship between length or weight and age166
Figure 5.4: Mean probability and credible interval that an individual Robust Redhorse from 450 -
700 mm total length is A. less than 12 years old, B. less than 10 years old, C. less than 8
years old, and D. less than 6 years old167

## LIST OF APPENDICES

Appendix 3.A: The date, estimated number of spawning individuals (Ind.), number of spawning
events (N), average duration of spawning events (sec), and spawning rate (number of
spawns per hour) of Robust Redhorse at the Broad River based on sub-hourly samples of
acoustic recordings over 9 days in 2012
Appendix 3.B: The date, estimated number of spawning individuals (Ind.), number of spawning
events (N), average duration of spawning events (sec), and spawning rate (number of
spawns per hour) of Robust Redhorse based on hourly or sub-hourly samples of acoustic
recordings over 10 days in the Savannah River, Georgia / South Carolina in 201397
Appendix 3.C: The date, estimated number of spawning individuals (Ind.), number of spawning
events (N), average duration of spawning events (sec), and spawning rate (number of
spawns per hour) of River Redhorse based on sub-hourly samples of acoustic recordings
over 5 days at the Coosawattee River spawning site in 2012
Appendix 4.A: Description of physical characteristics of Robust Redhorse during the spawning
period143

#### CHAPTER 1

#### INTRODUCTION AND LITERATURE REVIEW

Aquatic systems worldwide have been modified directly and indirectly by humans, which has resulted in a general decline of aquatic species (Abramovitz 1996; Pringle et al. 2000; Dudgeon et al. 2006; Vaughn 2010; Vörösmarty et al. 2010). Humans have attempted to tame the world's rivers and contain their vast resources for human needs. Direct modifications to streams and rivers include damming, channelization, dredging, navigation improvements (snag, woody material, gravel, and bedrock removal), creating barriers, diversions, withdrawals, discharges, and sand and gravel mining. Indirect modifications have occurred through land use practices, and have altered water quality through sediment, chemical, and nutrient run-off. Flow and temperature alteration by dams have been implicated in declines of redhorse species (Moxostoma) and other native fishes (Bain et al. 1985; Travnichek and Maceina 1994; Humphries and Lake 2000; Freeman et al. 2001; Humphries et al. 2002; Weyers et al. 2003). A dramatic increase in the number of imperiled fishes in North America in the last two decades (with approximately 39% of described species considered imperiled) has lead to increased concern over causes of species decline (Richter et al. 1997; Jelks et al. 2008). Approximately 85% of the federally listed and candidate species have been listed because of habitat destruction (Wilcove et al. 1998).

*Decline of catostomid fishes* – Almost half of the species in the family Catostomidae (suckers) in the United States are considered imperiled and face multiple threats at all of stages of their life history (Cooke et al. 2005; Jelks et al. 2008). These threats include altered flows and

temperature from hydroelectric facilities, migration barriers (culverts and dams), increased fine sediments in spawning gravels, pollution, invasive species, and overharvest and removals as part of game fish programs (Travnichek and Maceina 1994; Cooke et al. 2005). Historically, catostomids composed a major part of riverine fish communities (Scott 1951; Hackney et al. 1967). Catostomids likely perform ecological functions including modifying bed substrates, providing nutrient subsidies during reproductive periods, and serving as hosts for native unionid mussel species (Hall 1972; Kwak and Skelly 1992; Freeman et al. 2003; Johnson et al. 2012; Quist and Spiegel 2012). Many *Moxostoma* species spawn in aggregations, and the potentially large numbers of spawners in an aggregation can supplement the local food web with eggs and larvae, as seen with salmonid species (Bilby et al. 1996; Montgomery et al. 1996; Holmlund and Hammer 1999; Naiman et al. 2002; Moore et al. 2007; Flecker et al. 2010). As of 2008, approximately 49% of catostomids were considered imperiled in North America, including at least three species in the southeastern United States (Jelks et al. 2008). One southeastern species of catostomid, the Robust Redhorse (Moxostoma robustum), has presumably undergone drastic population declines since its discovery in 1869 (Cope 1870; Bryant et al. 1996).

*Model species* – Robust Redhorse, *Moxostoma robustum* (Cope), is a large-bodied catostomid native to southeastern Atlantic slope drainages in the Piedmont and Coastal Plain, from the Yadkin-Pee Dee River system, North Carolina / South Carolina south to the Altamaha River system, Georgia (Figures 1.1 and 1.2). At present, however, there are only three known existing wild populations, which are restricted to the Coastal Plain of the Oconee River (Georgia) and Savannah River (Georgia / South Carolina) and the Coastal Plain and a very limited part of the Piedmont in the Pee Dee River drainage (North Carolina / South Carolina). Individuals are estimated to reach sexual maturity at 5-6 years of age, and the maximum age is

over 25 years (one individual has been estimated to be 26 years old; B. J. Freeman, personal communication).

After the Robust Redhorse was described as *Ptychostomus robustus* by E.D. Cope (Cope 1870), Cope's type specimen was lost and ichthyologists misapplied this scientific name to a different, smaller species (the "Smallfin Redhorse", now known as the undescribed Brassy Jumprock, *Moxostoma* sp.). The identity of Cope's original "Robust Redhorse" was essentially lost until the 1990's, although specimens had periodically been captured and misidentified. For example, biologists from the National Academy of Sciences collected a Robust Redhorse from the Savannah River in 1980, but failed to recognize the fish as the same species described by Cope from the upper reaches of the Pee Dee River system. However, during surveys for relicensing of Georgia Power Company's Sinclair Reservoir project in 1991, biologists collected five suckers, later identified as Robust Redhorse, in the Oconee River downstream of Sinclair Dam. Comparisons of these fish with specimens of Brassy Jumprock and to the original description revealed that these unidentified fish most appropriately fit Cope's description of Robust Redhorse. This century of misunderstanding and lack of collections resulted in limited research of the species, knowledge of life history requirements, and documentation of its decline.

When it was described, Robust Redhorse appeared abundant, with locals collecting "cart loads" of fish from traps (Cope 1970). Writing of this exploitation of spawning runs in North Carolina, Cope noted:

"But unfortunately, too many of the people with the improvidence characteristic of ignorance, erect traps, for the purpose of taking the fishes as they ascend the rivers in the spring to deposit their spawn. Cart loads have thus often been caught at once, so that the supply is at the present time reduced one half in many of the principal rivers of the State." Cope (1870)

In areas of the Yadkin River, in the Piedmont province, locals would erect spring traps to capture suckers migrating upstream to spawning aggregations (Cope 1870). This observation suggests that large numbers of individuals migrated upstream into the Piedmont portions of rivers to reach spawning habitats.

After its "rediscovery" in the early 1990's, fisheries personnel and researchers began intensive surveys to determine the extent and size of the population within the Oconee River and throughout its presumed historic range (Figure 1.2). A wild population of Robust Redhorse was discovered in the Savannah River in 1997, downstream from the downstream-most dam on the river. A single individual was collected in the Pee Dee River in 2000. Wild populations of Robust Redhorse have been estimated to be less than 200 individuals in each of the Oconee and Pee Dee River populations (Slaughter 2011; Fisk et al. 2014). As early as 1997, the Oconee River population appeared to be skewed to older age classes, indicating limited recruitment (DeMeo 1997). In recent years (2009-2013), no Robust Redhorse have been captured in the Oconee River downstream of Sinclair Dam, although GA Department of Natural Resources (GDNR) biologists have released over 4,200 captive-reared individuals in the river starting in 2000 (Slaughter 2011; RRCC 2013). Population size in the Savannah River has not been estimated, however adult spawning aggregation size in the river was estimated to be between 82-85 individuals in 2004 and 2005 (Grabowski and Isley 2008). Using genetic analyses of individuals collected for broodstock, researchers estimated the temporary effective population size of 80-160 individuals for the Savannah population and 10-20 in the Pee Dee River basin (Tanya Darden, RRCC 2011).

These estimates of limited populations of a presumably once abundant species imply that the species is drastically reduced from historical levels. The Robust Redhorse is also

substantially reduced in range, with the current distributions occupying approximately 30% of the presumed historic range. The known populations primarily exist only in the Coastal Plain portions of the large Atlantic Slope rivers within the species' native range. Only the population in the Pee Dee still has unobstructed access to a limited amount of Piedmont habitats, and all wild populations are downstream of large hydropower facilities that prevent upstream migrations to Piedmont headwaters and alter hydrology in potential spawning habitats. The species has thus lost not just range, but access to a different suite of riverine habitats. In Georgia, the Piedmont regions of the state are characterized by smaller floodplains, more stable substrates (gravel and larger), and steeper gradient than Coastal Plain reaches (Berndt et al. 1996).

Sampling in the 1990's suggested that the Robust Redhorse population in the Coastal Plain portion of the Oconee River was larger than in other systems (Savannah and Pee Dee), but that recruitment was extremely low. Because of insufficient knowledge of the threats to the species and life history needs, the U.S. Fish and Wildlife Service considers the Robust Redhorse as a species of special concern (Flebbe et al. 1996). Because the species only occurred downstream of large hydropower facilities, had limited population sizes, and appeared to have recruitment limitations, there were concerns by many that the species would be listed under the Endangered Species Act. To accommodate concerns of the stakeholders involved in conservation of the species, as well as in hydropower production where Robust Redhorse occurred, and specifically to avoid listing and begin recovery efforts, a Memorandum of Understanding (MOU) was developed involving federal, state, and private partners, including the Georgia Power Company, United States Fish and Wildlife Service, Georgia Department of Natural Resources, South Carolina Department of Natural Resources, North Carolina Wildlife Resources Commission, United States Geological Survey, and other utilities and conservation groups. In

1995 the Robust Redhorse Conservation Committee (RRCC) was formalized as part of the MOU.

The RRCC created a prelisting recovery plan with one of its goals being to have six selfsustaining populations occur throughout the species' historical range (Nichols 2003). The RRCC defined self-sustaining as "a population or all known populations are at a level where the natural recruitment rate is equal to or greater than its mortality" (RRCC 2002). By definition a population would need the presence of multiple year classes, which would at least signify successful recruitment into the breeding population. If the age to reproductive maturity were 5 years, a span of 25 years would allow for the documentation of up to five different generations of Robust Redhorse and allow for comparisons of recruitment as conditions vary from year to year.

The primary recovery tool used by the RRCC has been augmenting natural populations and reintroductions of additional populations into historically occupied watersheds. Conservation efforts have shown limited success in alleviating the suspected threats to Robust Redhorse, i.e. migration barriers, altered hydrology, and invasive species. Thus most recovery efforts have focused on stocking programs in Georgia and South Carolina. The goal of the RRCC stocking program was to identify potential river systems for reintroduction of Robust Redhorse and to implement releases utilizing the existing Oconee River population as the source. The first watershed identified for reintroduction was the Broad River watershed in Georgia. Other reintroductions have occurred in the Broad/Wateree system in South Carolina, and are under consideration for the Pee Dee River in North Carolina, and upper Oconee River in Georgia. One additional introduction has been conducted in the Ogeechee River to create a refugial population.

*Reintroduction* – The Broad River, a major Piedmont tributary to the Savannah River in Georgia, was prioritized as the first watershed for reintroduction of Robust Redhorse because of

the availability of habitat (gravel spawning shoals), relatively good water quality, limited development, lack of hydropower or major water development projects within the system. The propagation effort began with artificial spawning projects in 1993 and the first propagated Robust Redhorse were reintroduced into the Broad River watershed in 1995. This effort was followed by larger reintroductions in 1996, 1997, and 1998 (Table 1.1; see also Chapter 4 and Figure 4.2). Brood fish for these reintroductions were collected in the Oconee River. From 1995 to 1998, 33,743 fish representing 1993, 1995, 1997, and 1998-year classes of artificially propagated Oconee River-derived Robust Redhorse were released into the Broad River and its major tributaries in Franklin, Madison, and Oglethorpe Counties, Georgia (Table 1.1; Freeman et al. 2002). Before release, each stocked fish was injected with a binary coded wire tag in a year-specific location to facilitate aging of captively-reared fish recaptured after their release. The tag placement for each year class was unique: 1993 year class in the left cheek, 1995 in the right cheek, 1997 at the base of the dorsal fin, and 1998 at the base of the anal fin.

Reintroductions in the Broad River were discontinued in 1998 after two Robust Redhorse were captured in the Savannah River system downstream of Augusta, Georgia in late-1997. Six additional Robust Redhorse were captured during collections from October 1997 to October 1998 in the Savannah River. These collections documented the previously unknown but continued persistence of a wild population of Robust Redhorse within the Savannah River. A comparison of mtDNA from tissue samples from the Savannah and the Oconee River individuals provided evidence that populations in the two watersheds represented different Evolutionary Significant Units (Wirgin et al. 2001). The reintroductions to the Broad River (i.e., of juveniles originated from Oconee River parents into a Savannah River tributary) were halted before the planned five years of stocking was completed because of concerns that stocked individuals could

by-pass dams on the Savannah River (Thurmond, Stevens Creek, Augusta Canal Diversion, and the New Savannah Bluff Lock and Dam) and mix with the wild population occurring downstream in the Savannah River. This mixing could alter the genetic adaptations the Savannah population has made to their native river system. As of 2013, we are aware of no evidence that mixing of the stocked populations in the Broad River, Georgia and the wild Savannah population has occurred.

The Robust Redhorse originally stocked in the Broad River system (1998 to 1993 year classes) are now (in 2014) 18 years or older and are well within the known spawning age (minimum of five to six years). If the minimum spawning age is assumed to be five years, and those originally reintroduced individuals reproduced successfully, and young survived from the first reproductive year of each generation, the first generation of wild-spawned fish within the Broad River system should have entered the adult reproductive population between 2003 and 2008 and a possible second generation would enter the reproductive population between 2008 to 2013 (Table 1.2). Evidence of smaller individuals entering the reproductive population could provide support of successful recruitment into the breeding population.

Robust Redhorse conservation efforts have also involved research to learn more about the life history characteristics of the species. Through tagging and tracking studies conducted in the Oconee, Ocmulgee, Savannah, and Pee Dee Rivers (Freeman and Straight 2004; Grabowski 2006; Grabowski and Isley 2006; Fisk 2010; Ely 2012), Robust Redhorse were found to participate in long distance migrations between wintering and potential or known spawning locations. The redhorse participated in large spawning aggregations in the Oconee and Savannah Rivers (Freeman and Freeman 2001; Grabowski and Isley 2007). Studies also revealed lower survival of eggs and larval Robust Redhorse in conditions of increased sedimentation (Ruetz and

Jennings 2000) and reduced survival of larvae exposed to pulsed high flows (Weyers et al. 2003). However, no studies have succeeded in documenting recruitment or finding juvenile Robust Redhorse in any system except that a single juvenile Robust Redhorse was collected in the Savannah River by South Carolina Department of Natural Resources anadromous fish survey in 2012 (RRCC 2013). Recruitment is essential for persistence of Robust Redhorse, but is difficult to assess if juveniles cannot be located. Laboratory tests using juvenile Robust Redhorse suggest that this age class may use backwaters and eddies (Mosley and Jennings 2007). Survey efforts in these habitat types may yet document juvenile Robust Redhorse.

One of the major limitations for assessing the conservation success of Robust Redhorse has been the failure to document recruitment in reintroduction watersheds. Documenting recruitment could be limited by our inability to capture juvenile Robust Redhorse or could be caused by an actual limitation in recruitment. Recruitment failures could occur at multiple stages, i.e. in the reproductive efforts of adults, hatching of eggs, survival of larvae, or survival of juveniles. My dissertation has addressed questions about the reproductive biology and movements of Robust Redhorse with a particular focus on the reintroduced population in the Broad River watershed.

To assess the first critical step in recruitment, I focused on assessing spawning behavior. The only consistently used spawning site in the Oconee River has not been used since 2008. Spawning aggregations in all systems are difficult to document when water becomes deep, visibility is limited because of turbidity or turbulence, or conditions are not appropriate for observations. To circumvent these difficulties, I have developed a method using a passive acoustic recording device to verify spawning in Robust Redhorse and another large-bodied catostomid, River Redhorse *M. carinatum* (Chapter 2). The intent of Chapter 2 is to provide an

alternate, non-invasive method to assess the presence of spawning individuals and spawning frequency in relation to environmental variability. In Chapter 3, I apply the passive acoustic methods described in Chapter 2 to document spawning frequency of Robust Redhorse through the spawning season and over 24-hour cycles at spawning aggregations in the Savannah River and Broad River. I also compare spawning frequency patterns to those of spawning River Redhorse in the Coosawattee River. These data provide the first quantification of spawning frequency and of nocturnal spawning, and provide a baseline for comparison of spawning efforts under other environmental conditions.

Chapter 4 asks whether spawning habitat and behaviors in the Piedmont region (Broad River) differ from those in the Coastal Plain (wild populations in the Oconee and Savannah Rivers). I specifically document spawning migration by fish in the reintroduced Broad River population, overwinter use of a mainstem reservoir, and similarities in habitat characteristics at spawning locales between Piedmont and Coastal Plain populations.

Ultimately the success in any reintroduction occurs when the population can be selfsustaining. Although assessing the long-term viability of a population may be impossible and no research groups have been able to find young-of-year Robust Redhorse, documenting new individuals entering the reproductive population may be the only way to assess the effectiveness of Robust Redhorse reintroductions. In Chapter 5, I discuss evidence for recruitment in the Broad River population and alternate methods of aging individuals. Together, my studies used Robust Redhorse as a model species and developed a method to assess reproductive efforts in largebodied catostomids, documented reproductive behaviors in three different river systems, and assessed movements and recruitment in a reintroduction watershed.

### REFERENCES

- Abramovitz, J. N. 1996. Imperiled waters impoverished future the decline of freshwater ecosystems. Worldwatch Paper 128. Worldwatch Institute, Washington, D. C.
- Bain, M. B., J. T. Finn, and H. E. Booke. 1985. A quantitative method for sampling riverine microhabitats by electrofishing. North American Journal of Fisheries Management 5:489-493.
- Berndt, M.P., E.T. Oaksford, M.R. Darst, and R.L. Marella. 1996. Environmental setting and factors that affect water quality in the Georgia-Florida Coastal Plain: U.S. Geological Survey Water-Resources Investigations Report 95-4268.
- Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 53:164-173.
- Bryant, R. T., J. W. Evans, R. E. Jenkins, and B. J. Freeman. 1996. The mystery fish. Southern Wildlife 1:26-35.
- Cooke, S. J., C. M. Bunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and D. F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation 121:317-331.
- Cope, E. D. 1870. A partial synopsis of the fishes of the fresh waters of North Carolina. Proceedings of the American Philosophical Society 11(81):448-495.
- DeMeo, T. 1997. Report of the Robust Redhorse Conservation Committee Annual Meeting. Wildlife Resources Division, Social Circle, Georgia.

- Dudgeon D., A. H. Arthington, M. O. Gessner, Z-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006.
  Freshwater biodiversity: importance, threats, status and conservation challenges.
  Biological Reviews 81:163-182.
- Ely, P. C. 2012. Movement patterns, habitat use, and home range of adult Robust Redhorse *Moxostoma robustum* released into the Oconee River, Georgia. Prepared for the Georgia Department of Natural Resources, Georgia Power Company, and United States Fish and Wildlife Service.
- Fisk, J.M. 2010. Reproductive Ecology and Habitat Use of the Robust Redhorse in the Pee Dee River, North Carolina and South Carolina. M.S. Thesis, North Carolina State University, Raleigh, North Carolina.
- Fisk, L. M. II, T. J. Kwak, and R. J. Heise. 2014. Modeling riverine habitat for Robust Redhorse: assessment for reintroduction of an imperiled species. Fisheries Management and Ecology 21:57-67.
- Flebbe, P.A., J. Harrison, G. Kappesser, D. Melgaard, J. Riley, and L.W. Swift Jr. 1996. Status of Aquatic Resources. In Southern Appalachian Man and the Biosphere (SAMAB). The Southern Appalachian Assessment Aquatic Resources Technical Report. USDA Forest Service, Southern Region, Atlanta, Georgia.
- Flecker, A. S., P. B. McIntyre, J. W. Moore, J. T. Anderson, B. W. Taylor, and R. O. Hall, Jr.
  2010. Migratory fishes as material and process subsidies in riverine ecosystems.
  American Fisheries Society Symposium 73:559-592.
- Freeman, B. J. and M. C. Freeman. 2001. Criteria for suitable spawning habitat for the Robust Redhorse *Moxostoma robustum*. A report to the U.S. Fish and Wildlife Service.

- Freeman, B. J. and C. A. Straight. 2004 Tracking of Robust Redhorse (*Moxostoma robustum*) released on 19 March 2002 on the Ocmulgee River below Lloyd Shoals Dam. Report to National Fish and Wildlife Foundation.
- Freeman, B. J., C. A. Straight, J. R. Knight, and C. M. Storey. 2002. Evaluation of Robust Redhorse (*Moxostoma robustum*) introduction into the Broad River, GA spanning years 1995-2001. Report Submitted to Georgia Department of Natural Resources.
- Freeman, M. C., Z. H. Bowen, K. D. Bovee, and E. R. Irwin. 2001. Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. Ecological Applications 11:179-190.
- Freeman, M. C., C. M. Pringle, E. A. Greathouse, and B. J. Freeman. 2003. Ecosystem-level consequences of migratory faunal depletion caused by dams. American Fisheries Society Symposium 35:255-266.
- Grabowski, T. B. 2006. Reproductive ecology and seasonal migrations of Robust Redhorse (*Moxostoma robustum*) in the Savannah River, Georgia and South Carolina. PhD Dissertation, Clemson University, South Carolina.
- Grabowski, T. B. and J. J. Isely. 2006. Seasonal and diel movements and habitat use of Robust Redhorse in the Lower Savannah River, Georgia and South Carolina. Transactions of the American Fisheries Society 135:1145-1155.
- Grabowski, T. B. and J. J. Isley. 2007. Spatial and temporal segregation of spawning habitat by catostomids in the Savannah River, Georgia and South Carolina, U.S.A. Journal of Fish Biology 70:782-798.

- Grabowski, T. B. and J. J. Isely. 2008. Size of spawning population, residence time, and territory shifts of individuals in the spawning aggregation of a riverine catostomid. Southeastern Naturalist 7:475-482.
- Hackney, P. A., W. M. Tatum, and S. L. Spencer. 1967. Life history study of the River
  Redhorse, *Moxostoma carinatum* (Cope), in the Cahaba River, Alabama, with notes on
  the management of the species as a sport fish. Proceedings of the Annual Conference
  Southeastern Association of Fish and Wildlife Agencies 21(1967):324-332.
- Hall, C. A. S. 1972. Migration and metabolism in a temperate stream ecosystem. Ecology 53:585-604.
- Holmlund, C. M. and M. Hammer. 1999. Ecosystem services generated by fish populations. Ecological Economics 29:253-268.
- Humphries P. and P. S. Lake. 2000. Fish larvae and the management of regulated rivers. Regulated Rivers: Research and Management 16:421-432.
- Humphries, P., L. G. Serafini, and A. J. King. 2002. River regulation and fish larvae: variation through space and time. Freshwater Biology 47:1307-1331.
- Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A.
  Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A.
  Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren, Jr. 2008.
  Conservation status of imperiled North American freshwater and diadromous fishes.
  Fisheries 33:372-407.
- Johnson, J. A., J. M. Wisniewski, A. K. Fritts, and R. B. Bringolf. 2012. Host identification and glochidia morphology of freshwater mussels from the Altamaha River basin. Southeastern Naturalist 11:733-746.

- Kwak, T. J. and T. M. Skelly. 1992. Spawning habitat, behavior, and morphology as isolating mechanisms of the Golden Redhorse, *Moxostoma erythrurum*, and the Black Redhorse, *M. duquesnei*, two syntopic fishes. Environmental Biology of Fishes 34:127-137.
- Montgomery, D. R., J. M. Buffington, N. P. Peterson, D. Schuett-Hames, and T. P. Quinn. 1996. Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed surface mobility and embryo survival. Canadian Journal Fisheries Aquatic Sciences 53:1061-1070.
- Moore, J. W., D. E. Schindler, J. L. Carter, J. Fox, J. Griffiths, and G. W. Holtgrieve. 2007.
   Biotic control of stream fluxes: Spawning salmon drive nutrient and matter export.
   Ecology 88:1278-1291.
- Mosley, D. L. and C. A. Jennings. 2007. Flow preferences for juvenile Robust Redhorses in an experimental mesocosm: Implications for developing sampling protocols. North American Journal of Fisheries Management 27:1383-1392.
- Naiman, R. J., R. E. Bilby, D. E. Schindler, and J. M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5:399-417.
- Nichols, M. C. 2003. Conservation strategy for Robust Redhorse (*Moxostoma robustum*). Robust Redhorse Conservation Committee. Available at http://www.robustredhorse.com/
- Pringle, C. M., M. C. Freeman, and B. J. Freeman. 2000. Regional effects of hydrologic alteration on riverine macrobiota in the New World: tropical-temperate comparisons. BioScience 50:807-823.
- Quist, M. C. and J. R. Spiegel. 2012. Population demographics of catostomids in large river ecosystems: Effects of discharge and temperature on recruitment dynamics and growth. River Research and Applications 28:1567-1586.

- Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology. 11:1081-1093.
- Robust Redhorse Conservation Committee (RRCC). 2002. Robust Redhorse Conservation Committee Policies. Adopted October 18, 2002.
- Robust Redhorse Conservation Committee (RRCC). 2011. Annual meeting of the Robust Redhorse Conservation Committee. Morrow Mountain State Park, Albemarle, North Carolina. 3-5 October 2011.
- Robust Redhorse Conservation Committee (RRCC). 2013. Robust Redhorse Conservation Committee Annual Meeting. Charlie Elliott Wildlife Center, Mansfield, Georgia.
- Ruetz III, C. R. and C. A. Jennings. 2000. Swimming performance of larval Robust Redhorse
   *Moxostoma robustum* and low-velocity habitat modeling in the Oconee River, Georgia.
   Transactions of the American Fisheries Society 129:398-407.
- Scott, D. C. 1951. Sampling fish populations in the Coosa River, Alabama. Transactions of the American Fisheries Society 80:28-40.
- Slaughter, J.E. IV. 2011. Conservation and Restoration of the Robust Redhorse Moxostoma robustum in the Oconee River, Georgia. Volume 7. Report prepared for the Federal Energy Regulatory Commission by Georgia Power.
- Travnichek, V. H. and M. J. Maceina. 1994. Comparison of flow regulation effects on fish assemblages in shallow and deep water habitats in the Tallapoosa River, Alabama. Journal of Freshwater Ecology 9:207-216.
- Vaughn, C. C. 2010. Biodiversity losses and ecosystem function in freshwaters: Emerging conclusions and research directions. BioScience 60:25-35.

- Vörösmarty, C. J., P. B. McIntyre, M. O. Gessner, D. Dudgeon, A. Prusevich, P. Green, S. Glidden, S. E. Bunn, C. A. Sullivan, C. Reidy Liermann, and P. M. Davies. 2010. Global threats to human water security and river biodiversity. Nature 467:555-561.
- Weyers, R. S., C. A. Jennings, and M. C. Freeman. 2003. Effects of pulsed, high-velocity water flow on larval Robust Redhorse and V-lip Redhorse. Transactions of the American Fisheries Society 132:84-91.
- Wilcove D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607-615.
- Wirgin, I., T. Opperman, and J. Stabile. 2001. Genetic divergence of Robust Redhorse, *Moxostoma robustum* (Cypriniformes: Catostomidae), from the Oconee River and the Savannah River based on mitochondrial DNA control region sequences. Copeia: 526-530.

Stocking	Year	Number of
year	class	individuals
1995	1993	545
1996	1995	1424
1997	1997	25207
1998	1997	3841
1998	1998	2726

Table 1.1. Number of Robust Redhorse (*Moxostoma robustum*) stocked within the Broad River system from 1995-1998.

Table 1.2. Estimated years of first reproduction by Robust Redhorse (*Moxostoma robustum*) in the Broad River system. The first reproductive year is for reintroduced individuals, and the 1<sup>st</sup> and 2<sup>nd</sup> generations would be wild-born offspring of those reintroduced individuals. Estimates assume reproductive maturation at five years.

Year	First estimated	1 <sup>st</sup> Generation	2 <sup>nd</sup> Generation
class	reproductive year	reproductive year	reproductive year
1993	1998	2003	2008
1995	2000	2005	2010
1997	2002	2007	2012
1998	2003	2008	2013



Figure 1.1. Robust Redhorse captured in the Oconee River, Georgia. Photo by Byron J. Freeman.


Figure 1.2. Map of the presumed historic range of Robust Redhorse in the southeastern United States from the Yadkin – Pee Dee River drainage (North Carolina/ South Carolina) south to the Altamaha River drainage (Georgia). Known or presumed spawning locations are marked with squares and the locality where the original collection of Robust Redhorse occurred in the Yadkin River marked with a star.

## CHAPTER 2

# PASSIVE ACOUSTIC MONITORING TO DETECT SPAWNING IN LARGE-BODIED

 ${\bf CATOSTOMIDS}^1$ 

<sup>&</sup>lt;sup>1</sup> Straight, C.A., B.J. Freeman, and M.C. Freeman. 2014. Accepted by *Transactions of the American Fisheries Society*. Reprinted here with the permission of the publisher.

## ABSTRACT

Documenting timing, locations, and intensity of spawning can provide valuable information for conservation and management of imperiled fishes. However, deep, turbid or turbulent water, or occurrence of spawning at night, can severely limit direct observations. We have developed and tested the use of passive acoustics to detect distinctive acoustic signatures associated with spawning events of two large-bodied catostomid species (River Redhorse Moxostoma carinatum and Robust Redhorse M. robustum) in river systems in north Georgia, USA. We deployed a hydrophone with a recording unit at four different locations on four different dates when we could both record and observe spawning activity. Recordings captured 494 spawning events that we acoustically characterized using dominant frequency, 95% frequency, relative power and duration. We similarly characterized 46 randomly selected ambient river noises. Dominant frequency did not differ between redhorse species and ranged from 172.3-14987.1 Hz. Duration of spawning events ranged from 0.65-11.07 s, with River Redhorse having longer durations than Robust Redhorse. Observed spawning events had significantly higher dominant and 95% frequencies than ambient river noises. We additionally tested software designed to automate acoustic detection. The automated detection configurations correctly identified 80-82% of known spawning events, and falsely "identified" spawns 6-7% of the time when none occurred. These rates were combined over all recordings; rates were more variable among individual recordings. Longer spawning events were more likely to be detected. Combined with sufficient visual observations to ascertain species identities and to estimate detection error rates, passive acoustic recording provides a useful tool to study spawning frequency of large-bodied fishes that displace gravel during egg deposition, including several species of imperiled catostomids.

## **INTRODUCTION**

Documenting or quantifying animal behaviors in wild populations can be critical to understanding factors underlying changes in population abundances. In the case of riverine fishes, for example, documenting the arrival of reproductive individuals to spawning habitats, the intensity of spawning activity, and the length of the reproductive period, may provide useful predictors of reproductive success. However, deep, turbid or turbulent water, or occurrence of behaviors at night, can make direct observations of reproductive behaviors difficult or impossible. Remote sensing methods (including radio-tagging, passive-integrated transponders, LIDAR, sonar) have been developed to aid in detecting and tracking fishes through obscure habitats (Castro-Santos et al. 1996; Churnside and Wilson 2001; Eckert and Stewart 2001; Makris et al. 2006).

Acoustics (sonar) have been used in remote sensing applications using sound production to perform stock assessments and population surveys in fisheries (Hewitt et al. 1976). Another use of acoustics involves listening to sounds produced by fish to infer information about spawning activity, including spawning aggression, courtship, and communication between spawning individuals (Johnston and Johnson 2000; Johnston and Phillips 2003; Amorim and Neves 2008; Anderson et al. 2008; Luczkovich et al. 2008). The use of acoustic recordings can also apply to sounds made incidentally by fish, such as sounds associated with feeding behavior (Mallekh et al. 2003; Lagardère et al. 2004) or nest building (Holt and Johnston 2011). We have developed a method for using acoustic recordings and characteristic signatures of incidental sounds made during spawning to detect reproductive activity by large-bodied suckers.

Suckers (Catostomidae) face multiple stressors in many or all stages of their life history (Cooke et al. 2005). In the southeastern United States, many large-bodied catostomids

(*Moxostoma* spp. and *Minytrema melanops*) are affected by changes in flows and temperature from hydroelectric facilities, migration barriers, habitat degradation including siltation of spawning substrates from land-disturbing practices, contaminants, and general disregard as "nuisance" species (Travnichek and Maceina 1994; Cooke et al. 2005). Because they currently lack economic and recreational importance, research has been limited on these "non-game" species until they become imperiled (Cooke et al. 2005). At present, at least four of 16 redhorse species (*Moxostoma* spp.) are considered imperiled in the Southeast or throughout their range and one species is already considered extinct (*M. lacerum*; Warren et al. 2000).

Catostomids have historically been abundant in large-river systems (Scott 1951; Hackney et al. 1967) and although not well-documented, likely perform several ecological roles. For example, catostomids modify bed substrates and likely provide nutrient subsidies during reproductive periods as evidenced by egg consumption by schooling minnows (Cyprinidae), sunfish, and bass (Centrarchidae) at spawning shoals (C. A. S. and B. J. F. unpublished, Hall 1972; Kwak and Skelly 1992; Freeman et al. 2003; Quist and Spiegel 2012). The potentially large numbers (e.g., 80-100 individuals) of suckers spawning in a small area over multiple days can supplement the local food web with fry as well as eggs, as seen with salmonid species (Bilby et al. 1996; Montgomery et al. 1996; Holmlund and Hammer 1999; Naiman et al. 2002; Moore et al. 2007; Flecker et al. 2010). Additionally, catostomids may provide a critical life history role for native mussels. A recent study has shown the Robust Redhorse *Moxostoma robustum*, an imperiled large-bodied catostomid in the southeast, to be the only suitable host, of those studied, for glochidia of another imperiled endemic in the southeast, the Altamaha Arcmussel *Alasmidonta arcula* (Johnson et al. 2012).

Documenting population dynamics and reproductive activities provides important information to assess conservation activities and to inform management of imperiled redhorse species; however, life history characteristics of many species make them difficult to study. Many redhorses are migratory, some traveling long distances, as much as 100-195 river km between wintering and spawning localities (Jenkins and Burkhead, 1994; Grabowski and Isely 2006; Grabowski and Jennings 2009). Because of their migratory behavior, it is often difficult to reliably estimate population sizes and life history characteristics. Assessing populations and behavior at spawning sites may provide vital information about imperiled redhorse species. However, high discharge, depth, and turbidity in southeastern rivers can limit visual observations of individuals at spawning sites making it difficult to document activities. Where visibility is limited, spawning localities may be surveyed using electrofishing techniques to collect fish and assess their reproductive-readiness. However, shocking fish has the possibly undesirable potential to interfere with reproduction. Where water clarity and depth are not limiting, spawning activity can be estimated by observation, but direct observation is still limited by personnel-time and daylight hours (Bowman 1970; Kwak and Skelley 1992; Mellinger and Clark 2000). Our goal has been to develop a non-invasive technique for quantifying spawning by sucker species and to provide data on reproductive activity in conditions where direct and continual observations are not possible.

Passive acoustics may allow one to assess spawning activities by using the distinctive acoustic signature produced by many redhorse species, with minimal reliance on direct observation. Many species of catostomids spawn in aggregations associated with gravel shoals or other appropriate spawning substrate. The spawning act is typically characterized by a triad of fish, one female flanked by a male on either side (Figure 2.1; Page and Johnston 1990).

Spawning involves males and female quivering their bodies and fins as the eggs and sperm are discharged. This spawning process manipulates the substrate, frequently displacing fine materials from the gravel in which the eggs and larvae will develop (Bowman, 1970; Curry and Spacie, 1984). Disturbance of gravel also creates a distinctive sound that could be used to document spawning activities.

The purpose of this paper is to (1) describe a non-invasive, passive method of detecting spawning activities using acoustic recording, (2) examine the accuracy of an automated analysis of large acoustic datasets, and (3) describe and compare the acoustic signatures of spawning events observed for two species of large-bodied catostomids, River Redhorse, *Moxostoma carinatum*, and Robust Redhorse. Our observations and analyses illustrate the potential for using underwater acoustic recordings to investigate spawning activities of catostomids with minimal disturbance and under conditions that limit direct observation.

### **METHODS**

*Study Site* – We observed and recorded spawning activities by two species of *Moxostoma* during April and May of 2012, in north Georgia, USA (Figure 2.2). We observed spawning Robust Redhorse in the Broad River watershed, which is part of the Savannah River drainage in northeast Georgia. The Broad River is primarily free flowing until near its mouth where it enters the impoundment of Strom Thurmond Reservoir on the Savannah River. Three localities in the Broad River watershed, two in the Broad River mainstem and one in the Hudson River, a tributary to the Broad River, were used for this study. The Robust Redhorse natively occurs in rivers on the South Atlantic Slope, from the Pee Dee River drainage (North Carolina and South Carolina) to the Altamaha River drainage (Georgia), and is considered endangered by the

American Fisheries Society Endangered Species Committee (Jelks et al. 2008) and also is listed as "endangered" under the Georgia Endangered Wildlife Act.

We observed spawning River Redhorse, *Moxostoma carinatum*, in the lower Coosawattee River, which is part of the Coosa River system in northwest Georgia. Flows in the lower Coosawattee River are regulated by an upstream hydropower dam and reregulation structure; the one locality used for study was downstream of Carters Re-regulation Dam. The River Redhorse is native to the St Lawrence River – Great Lakes and Mississippi River basins, south to include the Gulf Slope drainages from the Escambia River to the Pearl River (Page and Burr 2011). The River Redhorse is listed as "rare" under the Georgia Endangered Wildlife Act.

The study sites ranged from 10-50 m in stream width. The gravel shoals used for spawning by both *Moxostoma* species had dominant substrates of 12.5-50 mm (coarse gravel). Water depths and velocities, measured at locations of spawning triads, were similar among sites (Table 2.1) and averaged 0.51 m in depth, with mean water velocities of 0.62 m/s at 60% of depth and 0.26 m/s immediately above the stream bottom. The River Redhorse spawning site was relatively homogenous in depth and velocity, and covered approximately 288 m<sup>2</sup>. The three localities used by Robust Redhorse in the Broad River watershed were more complex with bedrock ledges and mid-channel islands. The six distinct gravel patches used by spawning Robust Redhorse had more variability in depth and velocity (Table 2.1), and ranged in size from 20-104 m<sup>2</sup>. Visibility of spawning fish also differed between study systems. Visibility was good at the Coosawattee site, with turbidities ranging from 3.5 to 4.6 NTU. Turbidities ranged from 9.5-24.5 NTU during observations at the Broad River sites.

*Acoustic Recording* – To record spawning acoustics, we deployed a hydrophone upstream and within 5-15 m of spawning aggregations of the target species (either Robust Redhorse or

River Redhorse) on four dates when we could simultaneously observe spawning events while recording spawning acoustics (Table 2.2). On each date, we placed an omni-directional HTI-96-MIN hydrophone (sensitivity -165 dB re: 1 V/ $\mu$  Pa, frequency response: 0.002–40 kHz) in water at least 50 cm deep and recorded acoustic files (Song Meter Bioacoustics Recorder; Wildlife Acoustics, Inc.) using a 16-bit sampling rate of 44,100 Hz and an analog gain of +12 dB. We placed the hydrophone near the bank and in an area that minimized ambient acoustic noise. While observing spawning aggregations in the field, we noted the times of observed spawning events that corresponded to characteristic acoustic signatures. We also noted times of other, anthropogenic actions likely to generate recorded sounds, such as observers walking on gravel in the wetted channel.

*Acoustic Analysis* – All recordings were analyzed using Raven 1.4 (Laboratory of Ornithology, Cornell University, Ithaca, NY). We listened to each audio file and marked segments corresponding to observed spawning events (OBS for "observed"). We also marked any additional events that we recognized as having acoustic characteristics of spawning events (AUD for "auditory") during review of each audio file. We assumed that the AUD events represented spawns that we did not observe. For each event (OBS and AUD), we used Raven to generate duration, dominant frequency (the frequency at maximum power), 95% frequency (frequency at which 95% of the energy for a given selection occurs), and peak power (the maximum power within a selection) characteristics of each event (Hanning window, FFT length: 256 samples, bin resolution: 248 Hz). Peak power (kiloUnits (kU)), as represented in Raven software, is a measure of relative power of sound pressure rather than true dB levels. Because we used the same equipment and settings for each acoustic recording, the measure of relative power is consistent between all of our recordings. Therefore, we could use relative power

measurements to compare between species, between OBS and AUD events, and between spawning events and ambient noise. We hypothesized that the measurements of frequency and power would distinguish spawning events from background and non-target noise. We compared duration, dominant frequency, 95% frequency, and peak power between OBS and AUD events, OBS and ambient events, and between species using ANOVA (aov; R Development Core Team 2011). To compare between species, Raven measurements were taken over the complete range of available frequencies (0-22050 Hz). A sample video with an acoustic overlay of spawning River Redhorse and an example of an acoustic recording of spawning Robust Redhorse are available at http://fishesofgeorgia.uga.edu/index.php?page=suppl/acoustics.

To characterize ambient noise, we used a random number generator to select 46 ambient events that ranged in duration from 2.5 to 5 seconds (the  $25^{th}$  and  $75^{th}$  quartile duration of OBS spawning events). We selected 2-5 samples from each acoustic file based on the file length (2 samples from files < 1000 s in length, 3 from files 1000-2000 s, 4 from files 2000-3000 s, and 5 from files > 3000 s). If a random selection fell within a spawning event or anthropogenic noise, another random sample was chosen until the number of samples for each file was reached. Using an ANOVA, we compared dominant frequency and 95% frequency between ambient events and OBS events, using a selection ranging over all frequencies (0-22050 Hz). To estimate the strength of the acoustic signal created by a spawn relative to background noise, we also compared peak power and average power between the ambient noise segments and OBS events for the frequency range (1000-1100 Hz) that encompassed the dominant frequency of observed spawning events.

*Automated Analysis Assessment* – Manually identifying recorded spawning events may be impractical for, for example, quantifying temporal dynamics in spawning behavior over

extended time periods. Therefore, we assessed the accuracy of Raven's Band Limited Energy Detector (Mills 2000) for identifying spawning events within our audio files. We used the following detector configuration for all analyses: frequency 4000 - 8000 Hz, duration 1.00 -20.00 s, minimum occupancy 20%, threshold 10 dB, hop size 10.0 s, percentile 50 with no exclusion band and no bandwidth filter. The selected frequency range (4000-8000 Hz) excluded lower frequencies dominated by turbulent water at some sites and sounds created by movements of fish in shallow water (e.g., chases by territorial males). We could find no documentation for the minimum duration of a spawning event that successfully fertilizes and buries eggs. We chose a one second minimum duration for these automated configurations and assumed that events shorter than one second were unsuccessful at burying fertilized eggs. We varied two additional parameters: minimum separation and block size. Minimum separation is the minimum amount of time required between two consecutive events. Increasing minimum separation may combine events close together or that overlap or it could miss later events in consecutive events with little separation. Decreasing minimum separation may separate closely spaced events, but may also split events that have bi-modal peaks in frequency. Block size is the amount of time over which the detector is set to estimate background noise. Shorter block sizes allow the automated detector to account for higher variability in background noise. We tested one configuration using a minimum separation of 0.099 s and a block size of 40 s. Two additional configurations both used a shorter minimum separation of 0.089 s, but had block sizes of 40 and 45 s, respectively.

We assessed the performance of the three detector configurations at correctly detecting spawning events (either OBS or AUD). We divided each audio file (N=16) into 15-second segments. Next, we classified each real event (OBS and AUD) as either true positive (TP) if the automated assessment correctly detected the event, or false negative (FN) if the assessment failed

to detect a spawning event. Other possible event classifications were false positive (FP) when the assessment "detected" an event that did not occur, or true negative (TN) when the assessment did not detect any events in the 15-s segment and no events occurred. Using these classifications, we calculated omission (FN) and commission (FP) errors and the following classification rates: true positive rate (TPR), the rate of correctly classified spawning events (TPR = TP / (TP+FN)), false positive rate (FPR), the rate of incorrectly classified events (FPR = FP / (FP+TN)), and accuracy, the proportion of correct classifications within the dataset ((TP+TN) / (total classifications)). We compared differences in occurrences of TP, FN, and FP events between recordings for River Redhorse and Robust Redhorse using a Chi-square test of independence.

Automated analysis also estimated the duration for each identified spawning event. We performed a paired t-test (t.test; R Development Core Team 2011) to compare estimated durations with manually calculated durations for correctly classified spawning events using the best-performing assessment configuration (i.e., the configuration with the highest TPR relative to FPR). We also evaluated the effect of spawn duration, calculated manually, on the probability that the spawn was detected using the best-performing assessment configuration. Using 494 events, we related spawn detections to duration (standardized to 0 mean and unit SD) using logistic regression in OpenBUGS 3.2.1 (Lunn et al. 2009). We used uninformative priors with normal distributions and predicted probability of detecting a spawning event in relation to spawn duration from 200,000 Markov chain Monte Carlo (MCMC) iterations, after discarding a burn-in of 100,000.

## RESULTS

Acoustic Recording – We recorded 16 acoustic files (> 6 hours total) for which we simultaneously observed spawning fish while recording acoustic data for at least some portion of the file (Table 2.2; Figure 2.3). We observed 102 spawning events that were distinguishable in the audio files (OBS) and selected an additional 392 events detected while listening to the acoustic files (AUD). The maximum distance we recorded a known spawning event was 20 m from the hydrophone in the Coosawattee River site. Several spawning events occurred at this distance both downstream and to the side of the hydrophone (perpendicular to the flow). At the Broad River sites, detection distances when fish were present and spawning varied from 10 to 17 m, which spanned all available spawning gravel in the patches sampled. Ad hoc testing at these sites by manually disturbing bed sediments at measured distances from the hydrophone demonstrated a detection distance of at least 20 m.

Dominant frequencies of all spawning events ranged from 172.3 - 14987.1 Hz (mean = 483.98 Hz; SD = 1694.41). Durations of OBS events were longer (mean = 4.08 s; SD = 2.14) than AUD events (mean = 3.46 s; SD = 1.81; F = 8.77; df = 1,492; p = 0.003). Dominant frequencies were higher for OBS events (mean = 1029.56 Hz; SD = 2791.77) than AUD events (mean = 341.49 Hz; SD = 976.53; F = 16.21; df = 1,492; p < 0.001). Peak power was also higher for OBS events (mean = 100.59 kU; SD = 6.94) than AUD events (mean = 96.79 kU; SD = 6.59; F = 26.27; df = 1,492; p < 0.001).

River Redhorse spawning events were of significantly longer duration than Robust Redhorse (F = 85.21; df = 1,492; p <0.0001; Table 2.3). River Redhorse spawns had higher 95% frequencies (mean = 9599.65 Hz; SD = 4846.13) than Robust Redhorse (mean = 5015.50 Hz; SD = 5567.89; F = 95.68; df = 1,492; p<0.001). Analysis of Variance showed no difference in the dominant frequencies between River Redhorse and Robust Redhorse (F = 0.66; df = 1,492; p = 0.42). Robust Redhorse spawning events had a higher relative peak power than River Redhorse (F = 358.36; df = 1,492; p < 0.0001).

*Ambient Noise* – The 46 randomly selected ambient sound segments had a mean dominant frequency of 172.30 Hz (SD = 0.00), which was substantially lower than OBS events (mean = 1028.56 Hz; SD = 2791.77; F = 4.31; df = 1, 146; p = 0.04). The 95% frequency of ambient events was also substantially lower (mean = 295.87 Hz; SD = 496.17) than OBS events (mean = 9631.67 Hz; SD = 5955.78; F = 112.26; df = 1, 146; p <0.001). At the mean dominant frequency characterizing OBS events (i.e., 1000-1100 Hz), mean peak relative power was lower for ambient events (74.08 kU; SD = 12.40) compared to observed spawning events (100.59 kU; SD = 6.94); F = 275.82, df = 1,146; p < 0.001). Similarly, within the range of frequencies chosen for the automated analysis (4000-8000 Hz), relative peak power for ambient events (mean = 76.49 kU; SD = 9.21) was again lower than for observed spawning events (mean = 87.76 kU; SD = 5.63; F = 83.704; df = 1,146; p < 0.001).

*Automated Analysis Assessment* – The three automated analysis configurations for detecting spawning events performed similarly, with relatively high TPR (0.80-0.82) and low FPR (0.06-0.07) totaled for 494 known spawning events. The detector configuration with the highest TPR and the lowest FPR used a minimum separation of 0.089 s and a block size of 45 s and resulted in overall accuracy ranging from 0.81 to 0.96 across the 16 files (Table 2.2). Among individual files using the best-performing configuration, TPR and FPR were more variable, with TPR ranging from 0.36 to 1 and FPR ranging from 0 to 0.20. The analysis-derived durations for the best-performing configuration were slightly shorter than their corresponding known durations (mean difference = 0.1027 s, SD = 0.98; paired *t*-test; T = 2.1074; df = 402, p = 0.04).

Longer spawning events were more likely to be detected by automated analysis [Logit P = 1.948 + (1.365\*duration)]. For spawning events ranging from 1-10 seconds in duration, the mean posterior probability ranged from 0.52 to 1.00 (95% credible intervals = 0.42-0.62 to 0.99-1.00; Figure 2.4). Events with a duration of 4.5 s or more had a probability > 0.90 (mean = 0.93; 95% CI = 0.90 - 0.96) of being detected using the best-performing detector configuration. Events with a mean duration of 7 s or greater had a mean probability of being detected of 0.99 (CI = 0.97 - 1.00).

Relative occurrences of detected spawns (TP), false detections (FP), and spawns that were missed (FN) differed between the two species. For all files combined, automated analysis of River Redhorse activity resulted in relatively higher numbers of detected spawns (228) and lower numbers of false detections (36) and missed spawns (31) compared to Robust Redhorse (175, 65 and 60 true detections, false detections, and missed spawns, respectively;  $X^2 = 24.50$ , df = 2, p < 0.0001; Table 2.2). However, the number of spawning individuals within range of the acoustic recorder and spawning rates (number of OBS and AUD events per unit time) were generally greater in the Coosawattee River site (River Redhorse). The relatively fewer intervals without spawning activity resulted in higher false detection rates and somewhat lower overall accuracy (Table 2.2).

#### DISCUSSION

We found that a hydrophone and recorder could be used to detect spawning activity in coarse gravel substrates by two different catostomid species. By matching times of observed and recorded spawns, we were able to confirm the auditory signature made by spawning triads of suckers. Moreover, in most cases, the acoustic recordings documented spawns that were not

directly observable because of water depth, distance from an observation point, or turbidity. Although visibility ranged from good (in the Coosawattee River) to fair (in the Broad River) during this study, turbidity has in other (wetter) years exceeded 70 NTU at Broad River sites during the spawning season, making visual observation impossible.

Substrate roughness and water depth partially define how far sound travels in water. In shallow water and with rough surfaces low frequency sounds can attenuate rapidly (Urick 1983), decreasing the likelihood of being detected. At our sites, however, spawning sounds created by catostomids disturbing the substrate typically span large ranges of frequencies at higher power than the ambient river noises. Thus, even though the dominant frequency produced by spawning activity may fall below the cut-off frequency for transmission in shallow water, the large range of frequencies with relatively high power produced by spawning events allows detection as much as 20 m from the hydrophone in our study sites. Nonetheless, hydrophone placement and detection should be considered in light of the limited propagation of low-frequency sounds in shallow water especially with rough substrates.

Acoustic characteristics differed between visually identified spawning events (OBS) and events that were only identified from the field recordings (AUD). The observed spawning events (OBS) had longer durations and higher dominant frequencies and peak power. These differences most likely reflected the conditions under which we made spawning observations. Longer duration spawns were more likely to be visually verified in the large area of the Coosawattee study site as well as in the more turbid waters of the Broad River sites. Shorter events typically did not allow the viewer enough time to find the spawning individuals. Additionally, events with lower power (i.e. quieter events) could be obscured from the listener by extraneous noises in the surroundings, like wind, people talking, and water turbulence.

Differences in 95% frequency and duration between River Redhorse and Robust Redhorse may have resulted from differences in substrate type or size, time within the spawning season, or behavioral differences between the species. At this time, we are unable to determine what caused these differences between species. Because these sounds are incidental to spawning behavior, they lack the harmonics and distinctive patterning that distinguish purposeful sounds created by some fish species that could allow for differentiation between species (Hawkins 1993; Popper and Fay 1993; Rountree et al. 2006; Speares et al. 2011). The large overlap in duration, frequency, and power measurements suggests that acoustic recordings should be accompanied by visual confirmation of the species and behaviors to the extent possible. Multiple species of suckers may spawn in particular locations and may overlap in timing (Meyer 1962; Curry and Spacie 1984; Kwak and Skelly 1992; Grabowski and Isley 2007) reinforcing the need for visual confirmations of species recorded acoustically.

Even though observation is likely necessary for ascertaining identities of spawning catostomids, acoustic recording can be useful for documenting spawning activity. The differences between dominant frequency, 95% frequency, and peak power of ambient noise segments and observed spawning events showed that known events can be separated from ambient noise through these variables. Placement of the hydrophone relative to turbulent flows could alter this relationship. Ambient noise within the river changes throughout the year, for example with weather-related events, such as wind and rain (Amoser and Ladich 2010), making spawning events less distinguishable. To maximize the difference between spawning events and ambient noise, we suggest placing the hydrophone in quiet zones, such as eddies, pools, backwaters or areas away from areas with higher frequency ambient noise that could disguise "quieter" spawning events in the same frequency range with limited power levels (Lugli and Fine

2003; Tonolla et al. 2009). Testing the detection range for a specific hydrophone placement is essential to ensuring the targeted area is adequately covered. Hydrophone placement should be placed to minimize ambient noise, but not to sacrifice detection within the targeted area.

Ambient noise at the location of the hydrophone also likely affects the distance at which spawning events can be detected. At the Coosawattee River spawning site, our observations indicated that the hydrophone location and ambient river sounds allowed us to detect spawning events over the whole aggregation of 115 individuals and covering an area of approximately 288 m<sup>2</sup>. More turbulent flows at our Robust Redhorse spawning sites could limit the distance over which spawning events could be distinguished from ambient noise. However, within the patches used for this study, testing in the field revealed that events were detected throughout the available spawning habitat we targeted. The relative power also likely differed with differing distance to the hydrophone, substrate type and size, and possibly with size of the individuals participating in the spawning event. Therefore, we would expect the best detection for larger species burying eggs in coarser bed sediments.

The automated analysis could correctly differentiate as many as 80% of recorded spawning events (totaled over all observation files) from ambient noise, with correspondingly low false positive rates (<7%). Automated analysis could thus be useful for quantifying temporal variation in spawning frequency, at least in the area within detection range of the hydrophone. Variability in automated processing among files illustrates the need to verify portions of recordings, if possible, and estimate TPR and FPR for the species and location. As discussed earlier, detection is expected to vary in relation to site characteristics and possibly among species.

The behavior of the species being studied might also play a role in the accuracy of automated detections. For example, numbers of spawning events could be overestimated if the target species participates in "post-spawning" digging events. This behavior (of unknown use) involves additional quivering and disturbing of substrates by females after the spawning event has ceased, and has been observed in the Sicklefin Redhorse (*Moxostoma* sp.; Favrot 2009). Although we did not observe River Redhorse or Robust Redhorse digging after spawning, behaviors like these could alter interpretation of recorded events.

The assessment-derived durations of spawning events were shorter than our known, observed events; however, this difference averaged only 0.1 s of a mean spawn duration of 3.9 s (range: 1-11 s), which is likely not biologically meaningful for the species we studied. This result suggests that if duration of spawning is a research concern, a portion of events should be verified in the field, if possible. We also found that shorter spawns were more likely to be missed by automated analysis of the recordings. The significance of missing short events may depend on the minimum duration of a spawning event needed for a species to successfully bury fertilized eggs. Short events often result when a spawning triad is interrupted by other fish. Longer events may be more likely associated with completed spawns, but a basis for identifying successful egg fertilization and burial is needed. Visible sediment plumes, created when spawners displace fine sediment from the gravel, may indicate particularly deep or vigorous egg burial and perhaps successful spawns. In that case, the shortest "successful" spawn (accompanied by a sediment plume) we have observed is 2.9 s in duration (C. A. S. unpublished data). Because longer spawning events are more likely to be detected, using information on minimum duration for spawn success to set a threshold for counting detection could result in more accurate quantification of spawning frequency. Although fertilization cannot be verified, duration of

observed spawning events for other catostomid species range from 1-9 s for Sicklefin Redhorse (Jenkins 1999), 2-4 s for Black Redhorse (*M. duquesnei*; Bowman 1970), and 2-6 s for Spotted Suckers (*Minytrema melanops*; McSwain and Gennings 1972). We presume longer spawning events are more likely to successfully fertilize and bury eggs, and increasing the minimum threshold to a meaningful number for the species of interest could thus be used to minimize classification error.

Recent reviews have highlighted the usefulness of passive acoustics as a non-invasive method for studying behaviors and habitat use of many fishes (Rountree et al. 2006; Gannon 2008; Gammell and O'Brien 2013). We suggest that acoustic analysis of incidental sounds could be useful for assessing patterns in spawning behavior by a wide variety of catostomid and other fishes that manipulate substrate during spawning. Species of suckers known to mound gravel or disturb bed sediments during spawning include *Hypentelium nigricans, Minytrema melanops, Moxostoma carinatum, M. congestum, M. duquesnei, M. erythrurum, M. macrolepidotum, M. robustum, M. valenciennesi, Catostomus commersonii,* and *Erimyzon oblongus* (C. A. S. and B. J. F. personal observation, Bowman 1970; Burr and Morris 1977; Jenkins and Jenkins 1980; Curry and Spacie 1984; Martin 1986; Page and Johnston 1990). Additionally, acoustics could be useful for detecting spawning or nesting activities of other fishes that bury eggs or manipulate bed sediments if activities can be discerned from ambient noise, for example, salmon and trout (Salmonidae), and minnows (*Nocomis, Semotilus, Exoglossum*; Cyprinidae; Helfman et al. 2009).

Acoustic analysis of incidental sounds could be used to examine changes in spawning frequency, for example, as the spawning season progresses, relative to time of day, or in relation to spawner density, streamflow, or other habitat variables. Most basically, passive acoustic

recording could be used to verify use of expected spawning localities by large-bodied catostomids and other species of fishes that disturb bed sediments during spawning or courtship. Passive acoustic methods thus offer promise for providing difficult to obtain, behavioral information that may be critical to conservation and management.

## ACKNOWLEDGMENTS

This study was funded in part by the USGS through the Piedmont South Atlantic Coast Cooperative Ecosystem Studies Unit (hosted by the Warnell School of Forestry and Natural Resources, University of Georgia) and a Georgia Department of Natural Resources State Wildlife Grant. We thank the Georgia Ecological Services, U.S. Fish and Wildlife Service for funding for equipment. Landowners (Chris and Eric Wagoner, Keith Nix, and Jim Langford) generously provided access to the river along their properties. Tim Krein from Cornell Lab of Ornithology was helpful in setting up the Raven Band Limited Energy Detector. We thank our reviewers for valuable comments on this manuscript. Use of trade, product, or firm names does not imply endorsement by the U.S. Government.

#### REFERENCES

- Amorim, M. C. P. and A. S. M. Neves. 2008. Male painted gobies (*Pomatoschistus pictus*) vocalise to defend territories. Behaviour 145:1065-1083.
- Amoser, S. and F. Ladich. 2010. Year-round variability of ambient noise in temperate freshwater habitats and its implications for fishes. Aquatic Sciences 72:371-378.
- Anderson, K. A., R. A. Rountree, and F. Juanes. 2008. Soniferous fishes in the Hudson River. Transactions of the American Fisheries Society 137:616-626.

- Bilby, R. E., B. R. Fransen, and P. A. Bisson. 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 53:164-173.
- Bowman, M. L. 1970. Life history of the black redhorse, *Moxostoma duquesnei* (Lesueur), in Missouri. Transactions of the American Fisheries Society 99:546-559.
- Burr, B. M. and M. A. Morris. 1977. Spawning behavior of the shorthead redhorse, *Moxostoma macrolepidotum*, in Big Rock Creek, Illinois. Transactions of the American Fisheries Society 65:80-82.
- Castro-Santos, T., A. Haro, and S. Walk. 1996. A passive integrated transponder (PIT) tag system for monitoring fishways. Fisheries Research 28:253-261.
- Churnside, J. H. and J. J. Wilson. 2001. Airborne lidar for fisheries applications. Optical Engineering 40:406-414.
- Cooke, S. J., C. M. Bunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and D. F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation 121:317-331.
- Curry, K. D. and A. Spacie. 1984. Differential use of stream habitat by spawning catostomids. American Midland Naturalist 111:267-279.
- Eckert, S. A. and B. S. Stewart. 2001. Telemetry and satellite tracking of whale sharks, *Rhincodon typus*, in the Sea of Cortez, Mexico, and the north Pacific Ocean. Environmental Biology of Fishes 60:299-308.

- Favrot, S. D. 2009. Sicklefin redhorse reproductive and habitat ecology in the Upper Hiwassee River basin of the southern Appalachian Mountains. Masters Thesis, North Carolina State University, Raleigh.
- Flecker, A. S., P. B. McIntyre, J. W. Moore, J. T. Anderson, B. W. Taylor, and R. O. Hall, Jr.
  2010. Migratory fishes as material and process subsidies in riverine ecosystems.
  American Fisheries Society Symposium 73:559-592.
- Freeman, M. C., C. M. Pringle, E. A. Greathouse, and B. J. Freeman. 2003. Ecosystem-level consequences of migratory faunal depletion caused by dams. American Fisheries Society Symposium 35:255-266.
- Gammell M. P. and J. M. O'Brien. 2013. Acoustic communication in aquatic animals: all quiet on the freshwater front? Aquatic Conservation: Marine and Freshwater Ecosystems 23:363-365.
- Gannon, D. P. 2008. Passive acoustic techniques in fisheries science: a review and prospectus. Transactions of the American Fisheries Society 137:638-656.
- Grabowski, T. B. and J. J. Isely. 2006. Seasonal and diel movements and habitat use of robust redhorse in the Lower Savannah River, Georgia and South Carolina. Transactions of the American Fisheries Society 135:1145-1155.
- Grabowski, T. B. and J. J. Isley. 2007. Spatial and temporal segregation of spawning habitat by catostomids in the Savannah River, Georgia and South Carolina, U.S.A. Journal of Fish Biology 70:782-798.
- Grabowski, T. B. and C. A. Jennings. 2009. Post-release movements and habitat use of robust redhorse transplanted to the Ocmulgee River, Georgia. Aquatic Conservation: Marine and Freshwater Ecosystems 19:170-177.

- Hackney, P. A., W. M. Tatum, and S. L. Spencer. 1967. Life history study of the river redhorse, *Moxostoma carinatum* (Cope), in the Cahaba River, Alabama, with notes on the management of the species as a sport fish. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 21(1967):324-332.
- Hall, C. A. S. 1972. Migration and metabolism in a temperate stream ecosystem. Ecology 53:585-604.
- Hawkins, A. D. 1993. Underwater sound and fish behaviour. Pages 129-169 *in* T. J. Pitcher, editor. The behaviour of teleost fishes. Chapman and Hall, New York.
- Helfman, G. S., B. B. Collette, D. E. Facey, and B. W. Bowen. 2009. The Diversity of Fishes:Biology, Evolution, and Ecology. Wiley-Blackwell, Hoboken, New Jersey.
- Hewitt, R. P., P. E. Smith, and J. C. Brown. 1976. The development and use of sonar mapping for pelagic stock assessment in the California Current area. Fishery Bulletin 74:281-300.
- Holmlund, C. M. and M. Hammer. 1999. Ecosystem services generated by fish populations. Ecological Economics 29:253-268.
- Holt, D. E. and C. E. Johnston. 2011. Can you hear the dinner bell? Response of cyprinid fishes to environmental acoustic cues. Animal Behaviour 82:529-534.
- Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A.
  Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A.
  Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren, Jr. 2008.
  Conservation status of imperiled North American freshwater and diadromous fishes.
  Fisheries 33:372-389.
- Jenkins, R. E. 1999. Sicklefin redhorse *Moxostoma* sp., undescribed species of sucker (Pisces, Catostomidae) in the upper Tennessee River drainage, North Carolina and Georgia –

description, aspects of biology, habitat, distribution, and population status. Report to the U.S. Department of Interior, Fish and Wildlife Service, Asheville, North Carolina, and the North Carolina Wildlife Resources Commission, Raleigh, North Carolina.

- Jenkins, R. E. and N. M. Burkhead. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Maryland.
- Jenkins, R. E., and D. J. Jenkins. 1980. Reproductive behavior of the greater redhorse, *Moxostoma valenciennesi*, in the Thousand Islands region. Canadian Field-Naturalist 94:426-430.
- Johnson, J. A., J. M. Wisniewski, A. K. Fritts, and R. B. Bringolf. 2012. Host identification and glochidia morphology of freshwater mussels from the Altamaha River basin. Southeastern Naturalist 11:733-746.
- Johnston, C. E. and D. L. Johnson. 2000. Sound production in *Pimephales notatus* (Rafinesque) (Cyprinidae). Copeia 2000:567-571.
- Johnston, C. E. and C. T. Phillips. 2003. Sound production in sturgeon *Scaphirhynchus albus* and *S. platorynchus* (Acipenseridae). Environmental Biology of Fishes 68:59-64.
- Kwak, T. J. and T. M. Skelly. 1992. Spawning habitat, behavior, and morphology as isolating mechanisms of the golden redhorse, *Moxostoma erythrurum*, and the black redhorse, *M. duquesnei*, two syntopic fishes. Environmental Biology of Fishes 34:127-137.
- Lagardère, J. P., R. Mallekh, and A. Mariani. 2004. Acoustic characteristics of two feeding modes used by brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and turbot (*Scophthalmus maximus*). Aquaculture 240:607-616.
- Luczkovich, J. J., D. A. Mann, and R. A. Rountree. 2008. Passive acoustics as a tool in fisheries science. Transactions of the American Fisheries Society 137:533-541.

- Lugli, M. and M. L. Fine. 2003. Acoustic communication in two freshwater gobies: Ambient noise and short-range propagation in shallow streams. Journal of the Acoustical Society of America 114:512-521.
- Lunn, D. J., D. Spiegelhalter, A. Thomas, and N. Best. 2009. The BUGS project: Evolution, critique, and future directions. Statistics in Medicine 28:3049-3067.
- Mallekh, R., J. P. Lagardère, J. P. Eneau, and C. Cloutour. 2003. An acoustic detector of turbot feeding activity. Aquaculture 221:481-489.
- Makris, N. C., P. Ratilal, D. T. Symonds, S. Jagannathan, S. Lee, and R. W. Nero. 2006. Fish population and behavior revealed by instantaneous continental shelf-scale imaging. Science 311:660-663.
- Martin, R. F. 1986. Spawning behavior of the gray redhorse, *Moxostoma congestum* (Pisces: Catostomidae) in central Texas. The Southwestern Naturalist 31:399-401.
- McSwain, L. E. and R. M. Gennings. 1972. Spawning behavior of the spotted sucker *Minytrema melanops* (Rafinesque). Transactions of the American Fisheries Society 101:738-740.
- Mellinger, D. K. and C. W. Clark. 2000. Recognizing transient low-frequency whale sounds by spectrogram correlation. Journal of the Acoustical Society of America 107:3518-3529.
- Meyer, W. H. 1962. Life history of three species of redhorse (*Moxostoma*) in the Des Moines River, Iowa. Transactions of the American Fisheries Society 91:412-419.
- Mills, H. G. 2000. Geographically distributed acoustical monitoring of migrating birds. Journal of the Acoustical Society of America 108:2582.
- Montgomery, D. R., J. M. Buffington, N. P. Peterson, D. Schuett-Hames, and T. P. Quinn. 1996. Stream-bed scour, egg burial depths, and the influence of salmonid spawning on bed

surface mobility and embryo survival. Canadian Journal Fisheries Aquatic Sciences 53:1061-1070.

- Moore, J. W., D. E. Schindler, J. L. Carter, J. Fox, J. Griffiths, and G. W. Holtgrieve. 2007.
   Biotic control of stream fluxes: Spawning salmon drive nutrient and matter export.
   Ecology 88:1278-1291.
- Naiman, R. J., R. E. Bilby, D. E. Schindler, and J. M. Helfield. 2002. Pacific salmon, nutrients, and the dynamics of freshwater and riparian ecosystems. Ecosystems 5:399-417.
- Page, L. M. and B. M. Burr. 2011. Peterson field guide to freshwater fishes of North America and North Mexico, second edition. Houghton Mifflin Harcourt, New York.
- Page, L. M. and C. E. Johnston. 1990. Spawning in the creek chubsucker, *Erimyzon oblongus*, with a review of spawning behavior in suckers (Catostomidae). Environmental Biology of Fishes 27:265-272.
- Popper, A. N. and R. R. Fay. 1993. Sound detection and processing by fish: critical review and major research questions. Brain Behavior and Evolution 41:14-38.
- Quist, M. C. and J. R. Spiegel. 2012. Population demographics of catostomids in large river ecosystems: Effects of discharge and temperature on recruitment dynamics and growth. River Research and Applications 28:1567-1586.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, Available: http://www.R-project.org/ (May 2013).
- Rountree, R. A., R. G. Gilmore, C. A. Goudey, A. D. Hawkins, J. J. Luczkovich, and D. A. Mann. 2006. Listening to fish: Applications of passive acoustics to fisheries science. Fisheries 31:433-446.

- Scott, D. C. 1951. Sampling fish populations in the Coosa River, Alabama. Transactions of the American Fisheries Society 80:28-40.
- Speares, P., D. Holt, and C. Johnston. 2011. The relationship between ambient noise and dominant frequency of vocalizations in two species of darters (Percidae: *Etheostoma*).
   Environmental Biology of Fishes 90:103-110.
- Tonolla D., M. S. Lorang, K. Heutschi, and K. Tockner. 2009. A flume experiment to examine underwater sound generation by flowing water. Aquatic Sciences 71:449-462.
- Travnichek, V. H. and M. J., Maceina. 1994. Comparison of flow regulation effects on fish assemblages in shallow and deep water habitats in the Tallapoosa River, Alabama. Journal of Freshwater Ecology 9:207-216.
- Urick, R. J. 1983. Principles of underwater sound. McGraw-Hill, New York.
- Warren, M. L., B. M. Burr, S. J. Walsh, H. L. Bart, R. C. Cashner, D. A. Etnier, B. J. Freeman,
  B. R. Kuhajda, R. L. Mayden, H. W. Robison, S. T. Ross, and W. C. Starnes. 2000.
  Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries 25(10):7-31.

Table 2.1. Estimated depth, velocity at 60% of depth, and velocity at the bottom at locations of spawning triads of Robust Redhorse (*Moxostoma robustum*) and River Redhorse (*M. carinatum*) at three localities in the Broad River watershed (Broad / Hudson) and one locality in the Coosawattee River used for passive acoustic monitoring in 2012. Values are means and standard errors; N denotes the number of measurements at each site.

Site	Ν	Depth (m)	Velocity 60% (m/s)	Velocity bottom (m/s)
Moxostoma robustum				
Broad 1	32	0.47 (0.02)	0.59 (0.03)	0.25 (0.03)
Broad 2	5	0.46 (0.03)	0.53 (0.09)	0.26 (0.07)
Hudson	5	0.40 (0.02)	0.58 (0.05)	0.18 (0.04)
Moxostoma carinatum				
Coosawattee	17	0.65 (0.03)	0.70 (0.03)	0.32 (0.03)

Table 2.2. Estimated number of Robust Redhorse and River Redhorse present at each recording locality, the date of spawning (2012),
and classification success for the best performing automated assessment. Classification categories: True Positive (TP), True Negative
(TN), False Positive or commission errors (FP) and False Negatives or omission errors (FN). True positive rate (TPR) is defined as TP
/ (TP + FN), false positive rate (FPR) as FP / (FP + TN), and accuracy as (TP + TN) / total of all events, according to the best
automated assessment. Files recorded at the same site on the same day were recorded at different times with the earliest file listed first.

			File	Fish	OBS	AUD							
-	Date	File Name	length (s)	present	(N)	(N)	TP	TN	FP	FN	TPR	FPR	Accuracy
		Moxostoma robustum											
	4/29	Broad 1 - A	1192	13	5	6	4	92	0	7	0.3636	0	0.9320
	4/29	Broad 1 - B	1798	13	2	17	12	131	1	7	0.6316	0.0076	0.9470
	4/30	Hudson - A	358	10	2	0	2	20	1	0	1	0.0476	0.9565
	4/30	Hudson - B	1775	10	3	19	22	88	12	0	1	0.1200	0.9016
	4/30	Broad 1 - C	1066	20	1	13	9	66	2	5	0.6429	0.0294	0.9146
	5/3	Broad 1 - D	868	13	16	6	11	125	8	11	0.5	0.0602	0.8774
	5/3	Broad 1 - E	916	13	7	4	5	48	1	6	0.4545	0.0204	0.8833
	5/3	Broad 2 - A	91	26	2	2	2	11	1	2	0.5	0.0833	0.8125
	5/3	Broad 2 - B	1900	26	26	18	38	235	8	6	0.8636	0.0329	0.9512
	5/3	Broad 2 - C	1809	26	4	43	35	155	13	12	0.7447	0.0774	0.8837
	5/3	Broad 1 - F	1244	27	1	7	8	98	12	0	1	0.1091	0.8983
	5/3	Broad 1 - G	2069	9	2	10	11	123	4	1	0.9167	0.0315	0.9640
	5/3	Broad 1 - H	1831	7	2	17	16	105	2	3	0.8421	0.0187	0.9603
		Moxostoma carinatum											
	5/10	Coosawattee - A	1111	115	9	62	55	41	4	16	0.7746	0.0889	0.8276
	5/10	Coosawattee - B	720	115	1	22	22	41	10	1	0.9565	0.1961	0.8514
	5/10	Coosawattee - C	3599	115	19	146	151	148	22	14	0.9152	0.1294	0.8925
-													

Table 2.3. Mean and standard deviation (SD) of the dominant frequency, 95% frequency, peak relative power, and duration of recorded spawning events by Robust Redhorse and River Redhorse.

Species	Ν	Dominant frequency (Hz)	95% Frequency (Hz)	Peak power (kU)	Duration (s)
Moxostoma robustum	235	423.73 (1352.69)	5015.50 (5567.89)	102.22 (7.00)	2.83 (1.47)
M. carinatum	259	537.45 (1725.89)	9599.65 (4846.13)	93.36 (2.68)	4.29 (1.97)



Figure 2.1. Spawning triad (female flanked by one male on either side) of River Redhorse *Moxostoma carinatum*, 10 May 2012, Coosawattee River, Georgia, downstream of Carters Reregulation Dam.



Figure 2.2. Map of the locations in north Georgia where we observed spawning and recorded spawning acoustics (black dots) of River Redhorse in the Coosawattee River watershed and Robust Redhorse in the Broad River watershed in 2012.



Figure 2.3. A characteristic acoustic spawning signature (shown as the concentration of dark in the middle of each view) of **(A)** Robust Redhorse *Moxostoma robustum*, and **(B)** River Redhorse *M. carinatum*, (Hanning window, FFT length: 256 samples, bin resolution: 248 Hz). For each species, the waveform is shown in the upper panel and the spectrogram in the lower panel. Amplitude (kU) is represented by a relative scale.



Figure 2.4. The estimated relationship between the duration of a spawning event and the probability of detecting that event using automated analysis. The solid line represents the posterior means and the dashed lines represent the corresponding 95% credible intervals.

## CHAPTER 3

## DIEL PATTERNS AND SEASONAL TRENDS IN SPAWNING RATES OF ROBUST REDHORSE (*MOXOSTOMA ROBUSTUM*) AND RIVER REDHORSE (*M. CARINATUM*) IN GEORGIA, ASSESSED USING PASSIVE ACOUSTIC MONITORING<sup>1</sup>

<sup>&</sup>lt;sup>1</sup> Straight, C. A., C. R. Jackson, B. J. Freeman, and M. C. Freeman. To be submitted to *Transactions of the American Fisheries Society*.
# ABSTRACT

Imperiled species conservation is dependent upon understanding threats to the species at each stage of its life history. Recruitment failures, caused by human-modified riverscapes, are a problem faced by many imperiled migratory fishes and understanding reproductive biology could provide managers with information critical for species conservation. We used passive acoustic recorders to document spawning rates and intensity in relation to environmental variables for two large-bodied catostomids (Robust Redhorse, Moxostoma robustum, in the Savannah and Broad Rivers, Georgia, and River Redhorse (M. carinatum) in the Coosawattee River, Georgia). We also measured water temperature and time of day and obtained data on discharge, moonlight, and weather. This is the first study to show diel patterns in spawning rates over a 24 h period and for multiple days in a spawning period. The highest spawning rate recorded (168 spawns / h) was by River Redhorse in the Coosawattee River. The peak spawning rate of Robust Redhorse was 84 spawns / h in the Savannah River site. The overall trend of spawning rates for Robust Redhorse in the Savannah and Broad Rivers showed an increase to a peak from day 4 -7 or 8 and then declined. Spawning rates in the Savannah were highest in the early morning (0100-0400 h) and lowest near mid-day (1300 h). In the Broad River, spawning rates increased from their lowest around 1300 h throughout the day and into the morning when peak spawning rates occurred around 0800-1000 h. River Redhorse in the Coosawattee showed no discernable pattern in spawning rates. These patterns and relationships between the environment and spawning rates and intensity could provide important information for management of these species downstream of hydropower facilities.

# **INTRODUCTION**

Conservation of imperiled species involves understanding threats to each stage of a species' life history (Wilcove et al. 1998; Venter et al. 2006). Although threats to species persistence likely involve multiple life history stages, a major challenge in species recovery is understanding recruitment failures. Species imperilment commonly stems from inadequate recruitment, which managers have addressed through captive-breeding and stocking programs (California Condor, Gymnogyps californianus, Whooping Cranes, Grus americana, salmon stocks and many other species; Beck et al. 1994; NRCC 1996; Jones 2004). Commonly, however, recruitment failure and species imperilment are caused by human modification of natural systems. Modification of riverine conditions includes alteration of habitat, flows, temperatures, and sediments, resulting in the global decline of aquatic species (Dudgeon et al. 2006; Vaughn 2010). Catostomids are one group of fishes that have been heavily impacted by human modification of rivers (Travnichek and Maceina 1994; Cooke et al. 2005). The number of imperiled fishes in North America has dramatically increased in the last two decades with approximately 39% of described species considered imperiled (Jelks et al. 2008). An even higher percentage (49%) of catostomids are considered imperiled. A better understanding of how environmental variability may influence reproduction and recruitment is essential to recovering and conserving imperiled catostomids, especially in managed rivers.

The Robust Redhorse (*Moxostoma robustum*) exemplifies the need for a better understanding of reproductive biology in relation to environmental variables. The Robust Redhorse is an imperiled, long-lived, large-bodied catostomid fish native to the Piedmont and Upper Coastal Plain of southeastern Atlantic slope drainages. The species has experienced a severe reduction in range since its discovery in 1869 in the upper Yadkin River, in the North

Carolina Piedmont (Cope 1870; Bryant et al. 1996). Originally believed to have inhabited all major river systems from the Yadkin – Pee Dee River in North and South Carolina south to the Altamaha River system in Georgia, the species is presently known from only three extant wild populations. These populations, in the Oconee, Savannah, and Pee Dee Rivers, are all downstream from hydropower dams that prevent migrations to river habitat upstream from the Fall Line (the high gradient zone that separates Piedmont and Coastal Plain portions of Atlantic and Gulf slope rivers). Species recovery efforts have primarily focused on a propagation and stocking program that has allowed managers to introduce Robust Redhorse to locations within the species' native range and to supplement wild populations (Nichols 2003). Populations of introduced fish now occur in the Ocmulgee, Ogeechee, and Broad Rivers in Georgia, and the Broad-Wateree Rivers in South Carolina. However, juvenile recruitment has only been documented in one of the wild populations (the Savannah River, where a single wild-reared immature individual has been captured; RRCC 2013). The other wild populations appear small (RRCC 2013), and reproductive success is not known for the introduced populations.

The purpose of this study was to assess the influence of environmental variability on spawning behavior of Robust Redhorse and another large-bodied catostomid, River Redhorse (*M. carinatum*). The River Redhorse natively occurs over a broader geographic area (encompassing Great Lakes, central Mississippi and Gulf Slope drainages) than the Robust Redhorse, but has also declined in a substantial part of its range, especially along its periphery (COSEWIC 2006). Flow and temperature alteration by dams has been implicated in depressing reproductive success in populations of redhorse species and of other native fishes by reducing habitat availability (Bain et al. 1985; Travnichek and Maceina 1994; Freeman et al. 2001) and larval survival (Humphries and Lake 2000; Humphries et al. 2002; Weyers et al. 2003).

However, effects of flow and temperature variability on fish spawning behavior have not been as widely studied (Andress 2002; McMichael et al. 2005; Grabowski and Isley 2007b; Martin 2008; Tiffan et al. 2009). In particular, because most suckers spawn over a relatively short period in the spring, environmental changes that interrupt or curtail spawning could have a large deleterious effect on reproductive success.

We used acoustic recordings (Straight et al. in press) to assess the influence of environmental variables (e.g. temperature, discharge, time of day, moonlight, weather) on spawning of Robust Redhorse in an unregulated river and Robust Redhorse and River Redhorse downstream of hydropower dams. Environmental influences on spawning behavior of catostomids have not been extensively investigated (McSwain and Gennings 1972; Curry and Spacie 1984; Kwak and Skelly 1992; Cooke and Bunt 1999). Additionally, past studies have been based upon daytime spawning observations, because of the difficulty of quantifying spawning activity at night (Bowman 1970; Kwak and Skelly 1992). Specifically, our objectives were to 1) characterize spawning rates and intensity (measured as the length of individual spawning events) continuously over multiple days of the spawning season, 2) correlate spawning rates and intensity to various measures of environmental conditions through the spawning period, and 3) compare differences in responses between regulated and unregulated systems. These data represent the first quantification of nocturnal spawning in *Moxostoma*, and provide a baseline for future assessments of river management effects on spawning frequencies and duration.

## **METHODS**

*Study Sites* – In 2012, we recorded spawning activities of Robust Redhorse in the Broad River watershed and River Redhorse in the Coosawattee River, Georgia (Figure 3.1). We also

recorded spawning activities of Robust Redhorse in the Savannah River in 2013. The Broad River is a tributary to the Savannah River and is mostly unimpeded, except by some low-head dams in its tributaries and near its mouth where it enters the impoundment of Strom Thurmond Reservoir. The Broad River spawning site was a gravel shoal (approximately 150 m<sup>2</sup>) that extended across the river channel, in the upper portion of the watershed.

In the Savannah River, the only known Robust Redhorse spawning sites both occur near Augusta, Georgia, and are downstream of three large hydropower dams and one low-head lock and dam operated by the USACE. These dams and associated reservoirs are managed for flood control, hydropower, navigation, fish and wildlife, recreation, and water supply. We recorded redhorse spawning activities at the more downstream of the two sites, a mid-channel gravel bar approximately 16 river km downstream of the New Savannah Bluff Lock and Dam. The gravel bar is approximately 4,200 m<sup>2</sup> in size (Grabowski and Isley 2008), and includes a large area that is exposed at lower river flows and not used for spawning. Typically, Robust Redhorse spawn along the upstream edge of mid-channel gravel bars (C. A. S. personal observation). During the study, spawning Robust Redhorse were observed using an area of less than 300 m<sup>2</sup> with the majority of individuals using the upstream edge of the submerged gravel lens on river right.

We recorded River Redhorse spawning activity in the lower Coosawattee River (Coosa River system; Figure 3.1) at a spawning area that spanned most of the width of the river and covered approximately 288 m<sup>2</sup>. Flows in the study area were regulated by a hydropower dam and reregulation structure approximately 15.4 km upstream of the spawning site.

Adult Robust and River Redhorse migrate to spawning gravels in early spring (March-May). In Georgia, Robust Redhorse spawning occurs from mid-April to late-May when water temperatures are between 16 and 27 °C (Freeman and Freeman 2001; Grabowski and Isely

2007a; Straight et al. in prep.). Once spawning has started, the spawning period has been documented to last from 12 (Grabowski and Isely 2008) to 20 d (Straight et al. in prep.). During spawning, both Robust and River Redhorse males defend territories over appropriate spawning gravels. Females typically stage away from the males and in areas with cover and/or reduced water velocity. When ready to spawn, females move into the aggregation of males and choose a location for spawning by swimming between two males. Spawning in both species typically occurs in a triad of fish, one female to two males (Figure 3.2). In a quivering bout, the female and males release gametes that become fertilized and subsequently buried in the gravel disturbed by the spawning triad. We have measured similar average depths and velocities at spawning triads at the sites used for this study (Table 3.1). Additionally, spawning at all sites occurs over gravel having a dominant particle sizes of 12.5-50 mm.

*Acoustic Recording* – Large-bodied catostomids that disturb bed sediments during spawning activities create a distinctive acoustic signature (Straight et al. in press). To record spawning sounds of Robust Redhorse and River Redhorse, we used a passive acoustic recorder at each spawning site (Song Meter SM2M Marine Recorder or SM2 Recorder; Wildlife Acoustics, Inc.). Passive acoustic recorders can record spawning activity at times and in conditions where visible observations are not feasible or safe (i.e. at night, during bad weather, when water is too deep or turbid to see fish, and when discharge is high). To accommodate potential high flows and distance of the spawning locality to the bank in the Savannah and Coosawattee Rivers, we used SM2M recorders, which combine hydrophone and recorder in a single, deployable unit. We designed a 45 lb steel harness to counteract the buoyancy of the unit and to hold it in a horizontal position relative to the bottom (Figure 3.3). Because of concerns of high discharge moving the unit, we also attached 100 lbs of weight to the harness and secured the unit to an anchor (tree or

rebar stake) using chain. We placed the hydrophone near territorial males to maximize the number of spawning events within the area of detection (Figure 3.4). The SM2M recorder was powered by a bank of 32 D-cell batteries, and recorded data to SDHC flash cards (Wildlife Acoustics 2011; hydrophone sensitivity -165 dB re: 1 V/ $\mu$  Pa, frequency response: 2Hz - 48 kHz). In the Broad River, we used a SM2 acoustic recorder. The SM2 recording unit, which can not be submerged, was placed on the bank with a 6-volt sealed, lead-acid battery for power. It had a corded, external omni-directional HTI-96-MIN hydrophone (sensitivity -165 dB re: 1 V/ $\mu$  Pa, frequency response: 0.002–40 kHz) and used SDHC flash cards for recordings. We saved all recordings in 30-minute files and used a 16-bit sampling rate of 44,100 Hz and an analog gain of +12 dB. In all instances the hydrophone was placed in water at least 50 cm deep, in an area within 10-15 m from the largest visible density of territorial males and in areas that minimized ambient acoustic noise. If possible, we visually estimated the minimum number of redhorse individuals present in the spawning aggregation on days when we visited the site.

We made continuous recordings of redhorse spawning acoustics during the three periods (Table 3.1). Human activities (boaters, our measurements of velocity and replacement of the recording unit) also occasionally obscured recording sounds, although typically less than 60 seconds at a time (e.g. passing motor boats on the Savannah River). During these times, the corresponding portions of the recording were not included in analyses.

*Acoustic Analysis* – All recordings were analyzed using Raven 1.5 (Laboratory of Ornithology, Cornell University, Ithaca, NY). We used an automated-detector (Band Limited Energy Detector; Mills 2000) to select suspected spawning events from each recording following methods in Straight et al. (in press). Spectrogram parameters were: Hann size 256 samples; 3db Filter Bandwidth 248 Hz; overlap 50 percent; hop size 128 samples; DFT size 256 samples; grid

spacing 172 Hz. Within Raven, we visually and acoustically verified each spawning event within the sampled recordings and also included additional events not selected by the detector.

Although we recorded continually throughout the spawning period, we subsampled portions of most files for analyses. In the Savannah River, we sampled all recordings from 18 through 22 April and subsampled the first 30 minutes of each hour from 23 to 27 April. In the Broad River, we subsampled the first 30 minutes of each hour, and in the Coosawattee (where spawning rates were highest), we subsampled the first 15 minutes of each hour.

We discarded from analyses recorded events that we regarded as too brief to represent a successful spawn. During spawning, movements of the bed sediments by the fins of spawning individuals commonly dislodge fine sediment particles, creating a visible sediment plume. We used the shortest duration of a spawning event observed with a sediment plume (2.9 sec; C. A. S. personal observation) as the minimum duration (3 s) for spawning events to be included in subsequent analyses. We extracted two datasets from the recordings; one contained spawn duration and associated covariates for each spawning event and included all spawning events recorded in the Broad and Coosawattee Rivers and any event that occurred from 18 April until 2 May in the Savannah River. The second dataset contained spawning rate (number of spawns per hour) and covariates averaged over the measurement period (15, 30 or 60 min) within the hour and included all recording days in the Broad and Coosawattee Rivers, and from 18 April until 27 April in the Savannah River. Definitions and data sources for covariates are described below and in Table 3.2. All continuous covariates were standardized to a mean of zero mean and SD of one unless otherwise noted.

*Time of Day / Lunar Classification* – We used <u>http://aa.usno.navy.mil</u> and the locations of Augusta, Georgia, for the Savannah River site, Danielsville, Georgia, for the Broad River site,

and Oakman, Georgia, for the Coosawattee River site, to document sun altitude and moon altitude and illumination. We then classified each recording segment as dawn (-12 to 0° altitude), daylight (>0° altitude), dusk (0 to -12° altitude), or night (< -12° altitude). In the Coosawattee we used a binomial classification of night for the interaction with moon illumination, because the moon was never more than -12 degrees altitude during dusk. In this case, night was operationally defined as times when the sun was less than 0 degrees altitude (i.e. including dawn, dusk, and night). We did not standardize moon illumination. When the moon was at or above -12 degrees altitude, moon illumination could influence visibility at night. Therefore, we included a binomial variable to indicate when the moon was "up" (more than -12 degrees altitude, scored as "1") compared to when the moon was less than -12 degrees altitude (0). Because moon illumination could be influenced by its altitude, we also created a variable that was the product of illumination and altitude.

*Weather* – In the Savannah River basin, we used archived hourly weather data for the Augusta, Georgia, airport (KDNL) as covariates for weather (http://www.wunderground.com/). We calculated average air temperature and total precipitation per hour. We also created a binary variable to document the presence of clouds (1) or clear (0) time periods. There were no archived weather stations within 20 km of either the Broad River or Coosawattee spawning sites, so weather covariates were not analyzed.

*Discharge* – We used USGS gages upstream of the spawning sites to estimate discharge in the Coosawattee River and the Savannah River (gages 02382500 and 02197000, respectively). The lack of any nearby USGS gage in the Broad River made it impossible to estimate discharge at that locality without additional measurements. Based on additional discharge estimates at gages downstream from the Coosawattee and Savannah River sites, we estimated the

approximate time that events recorded at the gages upstream of the spawning sites took to travel downstream to the spawning site. We estimated minimum travel time from the gages to the spawning sites in both river systems as six hours during the period we used for this study. We thus applied a six-hour and a 10-hour (to allow for a delayed response by fish) lag time to discharge data from the upstream USGS gages as covariates on spawning activities.

*Water Temperature* – We recorded water temperature at 5-min increments at the Broad River locality. Because of equipment malfunction, we were unable to retrieve absolute water temperature data at the Savannah River and Coosawattee River sites. However, for the Savannah River site, we used 6-hour lagged temperatures measured at the New Savannah Bluff Lock and Dam recorded at 15-minute intervals by the Southeastern Natural Sciences Academy.

*Data Analysis – Spawn Duration –* In all analyses, we modeled sites separately, except to compare the duration of spawning events between sites and species, using Analysis of Variance (aov; R Development Core Team 2011). We modeled the relationship between spawning event duration and covariates of time of day, time-lagged standardized discharge, temperature, moon illumination and altitude, and weather using linear models (lm; R Development Core Team 2011; Table 3.2). We used an information theoretic measure, Akaike's information criterion corrected for small sample sizes (AIC<sub>c</sub>), to evaluate the best models relating spawning duration to the covariates. We calculated model weights to determine the relative support for each model (Burnham and Anderson 2002) and chose a confidence set of models that represented the best models of those tested. In the confidence set of models, we included any models with AIC<sub>c</sub> weights within 10% of the best model (the model with the lowest AIC<sub>c</sub>), which is comparable to the 1/8 cutoff suggested by Royall (1997).

*Spawning Rates* – We analyzed the difference in spawning rates between species using Analysis of Variance. Exploration of the dataset comprising hourly spawning rates as the response variable indicated serial autocorrelation of the response and an overall trend through the spawning period. We used a time series analysis and a series decomposition for each site to describe the periodicity (seasonality) and trend in spawning rate at each site (stl; R Development Core Team 2011). To account for autocorrelation for other analyses, we used a three-hour moving average of spawning rate (spawns / hour) for our response variable and used generalized least squares with a corARMA (p=3) correlation structure, fitted using maximum likelihood, which allows for correlated errors (GLS; R Development Core Team 2011; package nlme, Pinheiro et al. 2010). We used GLS to examine the relationship between spawning rate and temperature, discharge, moon phase, time of day, and weather and used the same informationtheoretic approach and covariates as the spawn duration models (Table 3.2).

## RESULTS

We documented 12,469 spawning events greater than three seconds in duration at the three sites, over 360 hours of samples spanning 28 days of recording (Table 3.3; Appendices 3.A, 3.B, and 3.C). For our analysis of spawning rates, we used 10,969 spawning events over approximately 300 h of sampling. In the Broad River, Robust Redhorse were recorded spawning for 9 days during which we sampled 101 hours of acoustic recordings (Table 3.3). Based on previous observations in the Broad River, spawning likely began one or more days prior to initiation of recording. The estimated number of spawning individuals at the spawning site ranged from seven individuals, documented on the last day of spawning, to 27 individuals recorded on 3 May 2012 (Appendix 3.A).

In the Savannah River, Robust Redhorse were observed spawning at the lower gravel bar on 18 April, 25 April, and on 13 May, indicating a spawning period spanning at least 26 days. We sampled 159 hours of recordings from the Savannah River from 18 April until 27 April. The mean 6 h time-lagged discharge was 5392.07 cfs (Table 3.4) and ranged from 3972.5 cfs to 11200 cfs during the period of study. Although we could not address variations in spawning rate after the 27 April because of interference with our equipment, the equipment continued to record and we documented and included spawning events for duration analyses when discharge was high enough (corresponding to 9000 cfs or greater at the upstream gage) to submerge the displaced recorder. Our first visit to the spawning shoal on 18 April was likely near the first day of spawning, with only seven individuals identified (all of which were males based on tubercles on their snouts and territory holding; Appendix 3.B). On the next visit on 25 April, we counted 65 individuals covering most of the visible area on the leading edges of the gravel bar. On 9 May 2013, no fish were visible, although acoustic recordings suggested spawning was still occurring. On 13 May 2013, personnel from South Carolina Department of Natural Resources visited the spawning site and observed an unknown number of spawning Robust Redhorse (F. Sessions, RRCC 2013).

In the Coosawattee River, spawning River Redhorse were first documented on 9 May and spawning finished, based on acoustic recordings, on 14 May 2012, a period spanning six days. We sampled approximately 37 hours of recording at the Coosawattee spawning site. Discharge was relatively stable (Table 3.4); the mean 6 h time-lagged discharge ranged from 605.5 to 638.75 cfs. The large number of spawning fish counted on 10 May (115 individuals) and 11 May (85 individuals) indicated we had likely arrived at the spawning shoal at or after the peak spawning period (Appendix 3.C).

Spawn Duration – Duration of individual spawning events varied significantly among sites (Table 3.3; F = 51.23; df = 2,12466; p < 0.001). Post hoc comparisons using Tukey HSD indicated that both Robust Redhorse sites had significantly lower mean duration of spawning events than the River Redhorse site (adjusted p <0.001). Duration of Robust Redhorse spawning events at the Broad River site also tended to be shorter in duration than those at the Savannah River site (adjusted p = 0.09).

No single variable explaining spawn duration occurred in the top models for all sites, however, water temperature was the explanatory variable in the top models for both Robust Redhorse sites. In the Broad River, the confidence set of models included five models plus the null model and the confidence intervals of all model parameters except the intercept encompassed zero (Tables 3.5 and 3.6). The best model to explain spawn duration was water temperature in the Savannah River, with average spawn duration increasing with increasing water temperature. In the Coosawattee River, the confidence model set included models with day of the spawning period and spawning rate. Spawn duration decreased with day of the spawning period and spawn duration increased with increasing 15-minute spawning rate.

*Spawning Rate* – Hourly spawning rate differed among sites (F = 94.26; df = 2,598; p < 0.001), likely corresponding to apparent differences in numbers of spawning individuals. Spawning rates were the lowest in the Broad River (mean = 16.9 spawns / h; SD = 10.6; range 0-48), intermediate in the Savannah (mean = 41.2 spawns / h; SD = 19.3; range 4-84), and highest for River Redhorse in the Coosawattee (mean = 55.5 spawns / h; SD = 49.7; range 0-168). Overall, the spawning rates at each site were variable within and between days (Figure 3.5), and showed both trends and periodicity based on time series decomposition (Figures 3.6 and 3.7, respectively). Spawning rates for the Broad River were relatively stable from the first day of recording (29 April) through the fifth day of recording (3 May) and then declined (Figure 3.6). The trend in spawning rate at the Savannah River site showed a gradual increase until day four (21 April), then a relatively stable rate until day seven or eight (24 or 25 of April), and a decline thereafter. The trend in the Coosawattee River showed a rapid decline throughout the recording period.

Periodicity in the time series data was most evident in the Savannah River data (Figure 3.7). Spawning rates were lowest around 1300 hours and increased in the evening to a peak in the early morning hours between 0100 and 0400. The Broad River showed a similar periodicity with the lowest rates around 1300 hours and a gradual increase in rates through the evening, overnight, and into the morning, and a rapid decline after 1000 hours. In the Coosawattee River, the periodicity was less defined with spawning peaks during the day and night.

Relations between spawning rate and covariates also varied among sites and partly reflected differences in trends and periodicity. In the Broad River, models showing Robust Redhorse spawning rate declining with increasing water temperature or day of the spawning period were equally well supported (Tables 3.7 and 3.8). In contrast, in the Savannah River, where diel periodicity was most evident, the confidence set of models projected higher spawning rate at night, at lower water temperature, or in relation to lower air temperature, or cloud cover (Tables 3.7 and 3.8). There also was evidence for higher spawning rates at night with increasing moon illumination (Table 3.8). The confidence set of models for the Coosawattee similarly projected higher spawning rates at night with increasing moon illumination. A second, equally supported model related increasing spawning rate to increasing discharge, however the total change in discharge in the Coosawattee during the observation period was < 10%.

Overall, the hypothesis that changes in river discharge would disrupt spawning was not strongly supported. The Savannah River experienced the largest variation in discharge, varying from 3973 to 5698 cfs over the first 11 days of observations. Spawning rate was not strongly associated with discharge during this period; models using either 10-h or 6-h lagged discharge estimates were less supported than the null model (Table 3.7). Displacement of the recorder prevented quantification of spawning rates during the highest flows following a rain event (11,200 cfs), which occurred after the recorder was moved up onto the gravel bar. However, we were able to identify spawn events in acoustic recordings made during those highest flows when the displaced instrument was re-inundated (i.e. when flows were > approximately 5,000 cfs). Although we could not estimate rates at higher flows, these observations provided evidence that spawning did not cease. Temperature effects in both the Broad and Savannah Rivers showed a relationship between increases in water temperature correlated with decreases in spawning rate.

#### DISCUSSION

Using acoustic recordings, this study provided the first quantification of spawning rates and intensity over a large portion of a reproductive season for riverine catostomids. The results showed that both Robust and River Redhorse spawn during day and night, with high spawning rates after midnight and into the early morning hours at Robust Redhorse sites. Of the environmental variables measured, time of day, water temperature, and moonlight influenced spawning rates the most. Water temperature influenced Robust Redhorse spawning rates at regulated and unregulated sites. The presence and/or illumination of the moon positively influenced spawning rates at both regulated sites.

All three sites showed trends in redhorse spawning rates during the period of recording and fit into a general model of spawning frequency (corresponding to density of fish) increasing during the initial period of spawning, reaching a peak period and then declining. This trend was most easily seen in the Savannah River and the difference in trends among sites likely resulted from not capturing the beginning of the spawning season in the Broad and Coosawattee systems. Overall peak spawning rates for each system differed in relation to apparent densities of spawners, but in all cases a majority of spawns occurred in just a few days. In the Broad and Savannah Rivers, four days of spawning accounted for 60% of all spawns documented over the 9 and 10 days of recordings. Although we did not capture the complete spawning period in the Coosawattee River, two days of the recordings accounted for over 70% of the documented spawns. By estimating changes in density of spawning individuals Jenkins and Jenkins (1980) similarly estimated the "peak run" for spawning Greater Redhorse, M. valenciennesi, to be approximately four days. We have not found references to peaks in spawning seasons for other Moxostoma species, although most species appear to spawn over a period of three weeks or less (River Redhorse: 5-8 d, Hackney et al. 1967; Black Redhorse, M. duquesnei: 7-8 d, Parker 1989; Robust Redhorse: 7-14 d, Grabowski 2006; Greater Redhorse: 12-14 d, Jenkins and Jenkins 1980; Sicklefin Redhorse, Moxostoma sp.: 21 d, Jenkins 1999). There are exceptions: Grabowski (2006) reported Notchlip Redhorse (*M. collapsum*) spawning for 57 days in the Savannah River, at the same site where we have documented similarly protracted spawning by Robust Redhorse. In most cases, however, maintaining appropriate conditions for spawning from initiation through the peak of spawning activity may maximize the potential of successful reproduction. This window, based on our study cases, consists of at least eight days for Robust Redhorse and at

least six days for River Redhorse. Disruption of spawning for any species with a limited period of reproductive readiness could result in reduced fecundity (Tsuda et al. 2006).

By using acoustic recorders, we were able to make novel observations documenting spawning Robust and River Redhorse during all 24 hours of the day throughout the spawning period. In both Robust Redhorse sites, spawning showed periodicity with the lowest rates occurring in the mid-afternoon. Although not quantitatively measured, Cooke and Bunt (1999) found the opposite, noting that Greater Redhorse spawned most actively during sunny afternoons. However, if we had not been able to use acoustic recordings, we likely would have concluded that spawning in the Savannah peaked in early evening, when in fact in the Savannah River, spawning rates were highest between 0100 and 0400 hrs. Acoustic monitoring also allowed us to document a lack of spawning periodicity by River Redhorse in the Coosawattee River. Observations for additional sites and species will be needed to know whether our results reflect differences among species or effects of differing environments.

Environmental variation clearly does influence spawning behavior. Robust Redhorse typically spawn within a specific window of water temperatures (15-27° C; Freeman and Freeman 2001; Grabowski 2006; Straight et al. in prep), and in the Broad and Savannah Rivers, spawning rates decline with increasing water temperature. We have also previously observed Robust Redhorse cease spawning efforts when water temperatures dropped to 16° C (Straight et al. in prep), indicating a minimum critical temperature for spawning. Interestingly, data in our current study indicate that temperature may drive spawning activity independently of day within the spawning season. Specifically, the Broad and Savannah data show differing trends in temperature across the recording periods (initially decreasing with time in the Savannah and increasing in the Broad, Figure 3.8), however spawning rates vary similarly with temperature.

Data from our flow-regulated sites suggested that nocturnal spawning activity was influenced by moonlight. Fraction of moon illumination likely had the largest influence on ambient light during the night, so that if *Moxostoma* species use visual cues for spawning, then variation in moon illumination could reasonably facilitate nocturnal spawning. In the Coosawattee and Savannah rivers, we observed that redhorse spawning rates increased with moon illumination at night. That we did not observe a similar effect in the Broad River, which had generally higher turbidity (> 10 NTU) and more canopy closure compared to both regulated systems during the study period (< 10 NTU) suggests that differences in water clarity or overhead cover could moderate influences of moon light on spawning rates between the regulated and unregulated systems.

Quantifying effects of streamflow variation on redhorse spawning activity presents a substantial challenge, but has direct implications for managing dam releases to avoid effects on downstream catostomid populations. We did not document an effect of discharge variation on spawning rates or intensity in the Savannah River, although we were able to detect spawning events when discharge was highest. River Redhorse showed an increase in spawn rate with increasing 10-hour lagged discharge in the Coosawattee. Although spawning rate increased with increasing discharge, discharge in the Coosawattee changed less than 40 cfs during the measured period; in comparison, the Savannah River fish experienced changes in excess of 1700 cfs. Conceivably, however, discharge over a critical level could make it difficult for males to continue to hold their position and defend territories and to properly cover eggs during the spawning act. Influences of higher discharge may also be selective, only allowing the largest fish or individuals that have more energy reserves (i.e. fish that have not spawned as much) to spawn during increased water velocity (Tiffan et al. 2009). Future work in regulated systems exploring

the relationship between discharge and spawning rate could help inform decisions about water management.

Understanding the patterns of and environmental influences on spawning should provide important information for management of regulated systems where spawning aggregations of imperiled catostomids occur downstream of hydropower facilities. By using acoustic recordings, we have been able to document spawning behavior over multiple days as well as at night. We believe this method could be a useful tool for future studies of catostomid spawning responses to environmental variability. The concentration of a large majority of spawning effort over a short time period suggests that these limited periods may be critical for reproductive success. Higher spawning rates at night and early morning may also indicate time periods, when undisturbed, for Robust Redhorse to increase their chances of reproductive success. Except for the Broad River watershed, all of the confirmed spawning locations of Robust Redhorse occur downstream of hydropower dams. Although we could not assess the influence of pulsed releases on spawning, temperature did influence spawning rates and could also be modified by management of hydropower facilities. Future work quantifying the optimal ranges of environmental variables that influence spawning rates may provide invaluable insight into management and conservation of species in regulated systems.

## ACKNOWLEDGMENTS

This research was funded in part by a Georgia Department of Natural Resources State Wildlife Grant and the USGS through the Piedmont South Atlantic Coast Cooperative Ecosystem Studies Unit (Warnell School of Forestry and Natural Resources, University of Georgia). Georgia Ecological Services, U.S. Fish and Wildlife Service, provided acoustical

equipment. Oscar Flite and the Southeastern Natural Sciences Academy generously provided temperature data for the Savannah River. We would like to thank the landowners for providing access along the river (Chris and Eric Wagoner and Jim Langford). Cornell Lab of Ornithology and Tim Krein was helpful in setting up the Raven Band Limited Energy Detector. We thank our reviewers for valuable comments on this manuscript. The use of trade, product, or firm names does not imply endorsement by the U.S. Government.

#### REFERENCES

- Andress, R. O. 2002. Nest survival of Lepomis species in regulated and unregulated rivers. MS. Auburn University, Auburn, Alabama.
- Bain, M. B., J. T. Finn, and H. E. Booke. 1985. A quantitative method for sampling riverine microhabitats by electrofishing. North American Journal of Fisheries Management 5:489-493.
- Beck, B. B., L. G. Rapaport, M. S. Price, and A. C. Wilson. 1994. Reintroduction of captive-born animals. Pages 265-286 in Creative conservation. Springer Netherlands.
- Bowman, M. L. 1970. Life history of the Black Redhorse, *Moxostoma duquesnei* (Lesueur), in Missouri. Transactions of the American Fisheries Society 99:546-559.
- Burnham, K. P. and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach, second edition. Springer-Verlag, New York.
- Bryant, R. T., J. W. Evans, R. E. Jenkins, and B. J. Freeman. 1996. The mystery fish. Southern Wildlife 1:26-35.

- Cooke, S. J., and C. M. Bunt. 1999. Spawning and reproductive biology of the Greater Redhorse, *Moxostoma valenciennesi*, in the Grand River, Ontario. Canadian Field-Naturalist 113: 497-502.
- Cooke, S. J., C. M. Bunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and D. F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation 121:317-331.
- Cope, E. D. 1870. A partial synopsis of the fishes of the fresh waters of North Carolina. Proceedings of the American Philosophical Society 11(81):448-495.
- COSEWIC. 2006. COSEWIC assessment and update status report on the River Redhorse Moxostoma carinatum in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa.
- Curry, K. D. and A. Spacie. 1984. Differential use of stream habitat by spawning catostomids. American Midland Naturalist 111:267–279.
- Dudgeon D., A. H. Arthington, M. O. Gessner, Z-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006.
  Freshwater biodiversity: importance, threats, status and conservation challenges.
  Biological Reviews 81:163–182.
- Freeman, B. J. and M. C. Freeman. 2001. Criteria for suitable spawning habitat for the Robust Redhorse *Moxostoma robustum*. A report to the U.S. Fish and Wildlife Service.
- Freeman, M. C., Z. H. Bowen, K. D. Bovee, and E. R. Irwin. 2001. Flow and habitat effects on juvenile fish abundance in natural and altered flow regimes. Ecological Applications 11:179-190.

- Grabowski, T. B. 2006. Reproductive ecology and seasonal migrations of Robust Redhorse (*Moxostoma robustum*) in the Savannah River, Georgia and South Carolina. PhD Dissertation, Clemson University, South Carolina.
- Grabowski, T. B. and J. J. Isely. 2007a. Spatial and temporal segregation of spawning habitat by catostomids in the Savannah River, Georgia and South Carolina, USA. Journal Fish Biology 70:782-798.
- Grabowski, T. B. and J. J. Isely. 2007b. Effects of flow fluctuations on the spawning habitat of a riverine fish. Southeastern Naturalist 6:471-478.
- Grabowski, T. B. and J. J. Isely. 2008. Size of spawning population, residence time, and territory shifts of individuals in the spawning aggregation of a riverine catostomid. Southeastern Naturalist 7:475-482.
- Hackney, P. A., W. M. Tatum, and S. L. Spencer. 1967. Life history study of the River
  Redhorse, *Moxostoma carinatum* (Cope), in the Cahaba River, Alabama, with notes on
  the management of the species as a sport fish. Proceedings of the Annual Conference
  Southeastern Association of Fish and Wildlife Agencies 21(1967):324-332.
- Humphries P. and P. S. Lake. 2000. Fish larvae and the management of regulated rivers. Regulated Rivers: Research and Management 16:421-432.
- Humphries, P., L. G. Serafini, and A. J. King. 2002. River regulation and fish larvae: variation through space and time. Freshwater Biology 47:1307-1331.
- Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren, Jr. 2008.

Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372-407.

- Jenkins, R. E. and D. J. Jenkins. 1980. Reproductive behavior of the Greater Redhorse, *Moxostoma valenciennesi*, in the Thousand Islands region. Canadian Field-Naturalist 94:426-430.
- Jenkins, R. E. 1999. Sicklefin Redhorse *Moxostoma* sp., undescribed species of sucker (Pisces, Catostomidae) in the upper Tennessee River drainage, North Carolina and Georgia description, aspects of biology, habitat, distribution, and population status. Report to the U.S. Department of Interior, Fish and Wildlife Service, Asheville, North Carolina, and the North Carolina Wildlife Resources Commission, Raleigh, North Carolina.
- Jones, C. G. 2004. Conservation management of endangered birds. Pages 269-301 in W. J. Sutherland, I. Newton, and R. E. Green, editors. Bird Ecology and Conservation: A Handbook of Techniques. Oxford University Press, New York.
- Kwak, T. J. and T. M. Skelly. 1992. Spawning habitat, behavior, and morphology as isolating mechanisms of the Golden Redhorse, *Moxostoma erythrurum*, and the Black Redhorse, *M. duquesnei*, two syntopic fishes. Environmental Biology of Fishes 34:127-137.
- Martin, B. M. 2008. Nest Survival, Nesting Behavior, and Bioenergetics of Redbreast Sunfish on the Tallapoosa River, Alabama. MS Thesis, Auburn University, Auburn, Alabama.
- McSwain, L. E. and R. M. Gennings. 1972. Spawning behavior of the Spotted Sucker *Minytrema melanops* (Rafinesque). Transactions of the American Fisheries Society 101:738-740.
- McMichael G. A., C. A. McKinstry, J.A. Vucelick, and J. A. Lukas. 2005. Fall Chinook Salmon spawning activity versus daylight and flow in the tailrace of a large hydroelectric dam.
   North American Journal of Fisheries Management 25:573-580.

- Mills, H. G. 2000. Geographically distributed acoustical monitoring of migrating birds. Journal of the Acoustical Society of America 108:2582.
- Nichols, M. C. 2003. Conservation strategy for Robust Redhorse (*Moxostoma robustum*). Robust Redhorse Conservation Committee.
- NRCC. 1996. Upstream: salmon and society in the Pacific Northwest. National Research Council Committee on Protection and Management of Pacific Northwest Anadromous Salmonids. National Academy Press, Washington, DC.
- Parker, B. J. 1989. Status of the Black Redhorse, Moxostoma duquesnei, in Canada. Canadian Field Naturalist 103:175-179.
- Pinheiro J., D. Bates, S. DebRoy, and D. Sarkar. 2010. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-97. R Development Core Team.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/.
- Robust Redhorse Conservation Committee (RRCC). 2013. Robust Redhorse Conservation Committee Annual Meeting. Charlie Elliott Wildlife Center, Mansfield, Georgia.
- Royall, R. M. 1997. Statistical evidence: a likelihood paradigm. Chapman and Hall, New York.
- Straight, C. A., B. J. Freeman, and M. C. Freeman. In press. Passive acoustic monitoring to detect spawning in large-bodied catostomids. Transactions of the American Fisheries Society.
- Straight, C. A., M. C. Freeman, and B. J. Freeman. In Preparation. Comparisons of reproductive biology of an imperiled migratory fish in a Piedmont and two Coastal Plain rivers.

- Tiffan, K. F., C. A. Haskell, and T. J. Kock. 2009. Quantifying the behavioral response of spawning Chum Salmon to elevated discharges from Bonneville Dam, Columbia River, USA. River Research and Applications 26:87-101.
- Travnichek, V. H. and M. J. Maceina. 1994. Comparison of flow regulation effects on fish assemblages in shallow and deep water habitats in the Tallapoosa River, Alabama. Journal of Freshwater Ecology 9:207-216.
- Tsuda Y., R. Kawabe, H. Tanaka, Y. Mitsunaga, T. Hiraishi, K. Yamamoto, and K. Nashimoto. 2006. Monitoring the spawning behaviour of chum salmon with an acceleration data logger. Ecology of Freshwater Fish 15:264-274.
- Vaughn, C. C. 2010. Biodiversity losses and ecosystem function in freshwaters: Emerging conclusions and research directions. BioScience 60:25-35.
- Venter, O., N. N. Brodeur, L. Nemiroff, B. Belland, I. J. Dolinsek, and J. W. Grant. 2006. Threats to endangered species in Canada. BioScience 56:903-910.
- Weyers, R. S., C. A. Jennings, and M. C. Freeman. 2003. Effects of pulsed, high-velocity water flow on larval Robust Redhorse and V-lip Redhorse. Transactions of the American Fisheries Society 132:84-91.
- Wilcove D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607-615.

Wildlife Acoustics, Inc. 2011. Song Meter SM2M Marine Recorder User Manual.

Table 3.1. Dates of recorded spawning, and estimated depth, velocity at 60% of depth, and velocity near the substrate at locations of spawning triads of Robust Redhorse in the Broad River in 2012 and Savannah River in 2013, and River Redhorse in the Coosawattee River in 2012. Values are means (standard errors); N denotes the number of habitat measurements at each site. Measurements were collected in the Savannah and Coosawattee River on one date and in the Broad River on three different days.

Site	Observation Period	Ν	Depth (m)	Velocity 60% (m/s)	Velocity Bottom (m/s)
Moxostoma robustum					
Broad	29 April - 7 May 2012	23	0.47 (0.02)	0.59 (0.03)	0.25 (0.03)
Savannah	18 - 27 April 2013 <sup>1</sup>	10	0.62 (0.07)	0.70 (0.07)	0.24 (0.06)
Moxostoma carinatum					
Coosawattee	10 - 14 May 2012	17	0.65 (0.03)	0.70 (0.03)	0.32 (0.03)

<sup>1</sup> The unit was deployed until 9 May, however vandals (or curious boaters) moved the recording unit onto the gravel bar at 1500 h on 27 April and prevented analysis of spawning rate after this date.

Variable	Abbreviation	Definition
Spawn day	Day	Approximate day within the spawning period for each species.
Time of day	Time	Time of day based on the sun's altitude. Classified into dawn (-12 to 0° altitude), daylight (>0° altitude), dusk (0 to - $12^{\circ}$ altitude), and night (< - $12^{\circ}$ altitude).
Hour	Hour	Hour of the day (0-23).
Night	Night	Time when the sun's altitude is less than 0 degrees $(1)$ or not $(0)$ .
Moon illumination	MoonIll	Fraction of the moon surface illuminated (1 = full moon; 0.5 = first or last quarter; 0 = new moon)
Moon altitude	Moon	Binomial variable when the moon is -12 degrees (0) or greater or not (1).
Moon altitude / illumination	MoonP	Product of Moon Illumination and Altitude.
Water temperature	H2OT	Water temperature °C in the Broad River and 6-hour time lagged water temperature in the Savannah River.
Discharge	Q6H or Q10H	Time-lagged discharge of 6 or 10 hours obtained from upstream USGS gages. No discharge data were available for the Broad River.
Spawning rate	Rate15	Number of spawning events per 15 minutes.
Air Temperature	AirT	The average air temperature during the measurement period.
Clouds	Clouds	Binomial classification for clouds (1) or no clouds (0) recorded in the measured period.
Precipitation	Precip	The amount of precipitation over the period measured.

Table 3.2. Selected covariates used in linear and generalized least squares regression.

Table 3.3. Total time of subsampled acoustic recordings (Time), number of spawning events (N), and the mean, standard deviation (SD), and range of duration of recorded spawning events for Robust Redhorse in the Broad River in 2012 and Savannah River in 2013, and River Redhorse in the Coosawattee River in 2012.

Species	Site	Time (h)	Ν	Mean (SD; Range)
Robust Redhorse	Broad	101	1709	4.30 (1.33; 3-13.33)
Robust Redhorse	Savannah	233.5	9218	4.37 (1.30; 3-12.50)
River Redhorse	Coosawattee	37	1542	4.71 (1.35; 3-13.48)

Table 3.4. The number of hourly samples (N) and mean (SD) of continuous covariates in each river system used for model analysis. NA indicates the variable was not available for that study site.

Covariate	Broad River	Savannah River	Coosawattee River
N	203	294	105
Spawning Rate (spawns / hour)	16.93 (10.06)	41.22 (19.26)	55.5 (49.72)
Water Temperature (°C)	22.45 (1.44)	17.30 (0.76)	NA
Moon Altitude (° from horizon)	24.05 (20.43)	23.84 (21.03)	21.83 (19.47)
Moon Illumination (fraction illuminated)	0.84 (0.16)	0.8 (0.16)	0.49 (0.12)
Discharge Lagged 6 H (cfs)	NA	5392.07 (1646.37)	621.62 (5.4)
Discharge Lagged 10 H (cfs)	NA	5381.01 (1649.1)	621.87 (6.19)

Table 3.5. Covariates, Akaike's information criterion corrected for small sample size (AICc), AICc differences ( $\Delta$  AICc), and Akaike weights ( $w_i$ ) for models influencing spawning duration of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in the Coosawattee River. The confidence set of models for each system are shown in bold. Definitions of covariate abbreviations are in Table 3.2.

Broad River:			
Models	AICc	$\Delta$ AICc	$W_i$
D ~ H2OT	5819.07	0	0.33
D ~ Moon	5819.32	0.25	0.29
Null (D ~ 1)	5820.40	1.34	0.17
D ~ Day	5821.93	2.86	0.08
D ~ Rate15	5822.07	3.01	0.07
D ~ Time	5822.32	3.26	0.06
D ~ MoonP * Ti	5827.21	8.15	0.01
Savannah River:			
Models	AICc	$\Delta$ AICc	$w_i$
D ~ H2OT	30895.55	0	1
D ~ Day	30910.37	14.82	0
$D \sim MoonP * Time$	30985.81	90.82	0
$D \sim Q6H$	30992.97	97.42	0
D~Q10H	30994.77	99.22	0
$D \sim Time$	30997.54	101.99	0
Null (D $\sim$ 1)	31010.99	115.44	0
$D \sim Rate15$	31012.26	116.71	0
D ~ Moon	31012.89	117.34	0
Coosawattee River:			
Models	AICc	$\Delta$ AICc	$\mathcal{W}_i$
D ~ Day	5291.26	0	0.63
D ~ Rate15	5292.41	1.15	0.36
$D \sim MoonP * Time$	5299.93	8.67	0.01
$D \sim Q6H$	5304.58	13.32	0
$D \sim Moon$	5307.43	16.17	0
$D \sim Time$	5309.93	18.67	0
$D \sim Q10H$	5311.73	20.57	0
Null (D ~ 1)	5311.86	20.60	0

Table 3.6. Confidence model set, parameter estimates, standard error (SE) and 95% confidence interval (CI) of parameter estimates and parameter/model specific p-values for spawning duration (D) of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in the Coosawattee River. Definitions of covariate abbreviations are in Table 3.2.

Broad River:			95% CI			
Models	Parameter	Parameter Estimate	SE	Lower	Upper	P(> t )
D ~ H2OT	Intercept	4.30	0.03	4.24	4.36	0.00
	H2OT	-0.06	0.03	-0.12	0.00	0.07
D ~ Moon	Intercept	4.36	0.05	4.27	4.45	0.00
	Moon	-0.11	0.06	-0.24	0.01	0.08
Null (D ~ 1)	Intercept	4.30	0.03	4.24	4.36	0.00
D ~ Dav	Intercept	4.38	0.12	4.15	4.60	0.00
	Day	-0.01	0.01	-0.04	0.02	0.49
	_					
D ~ Rate15	Intercept	4.34	0.08	4.19	4.49	0.00
	Rate15	-0.01	0.01	-0.03	0.02	0.56
D ~ Time	Intercept	4.32	0.13	4.06	4.58	0.00
	Day	0.04	0.14	-0.23	0.31	0.77
	Dusk	-0.10	0.22	-0.52	0.32	0.65
	Night	-0.09	0.14	-0.37	0.18	0.51
Savannah River:			95% CI			
Models	Parameter	Parameter Estimate	SE	Lower	Upper	P(> t )
D ~ H2OT	Intercept	4.39	0.01	4.37	4.42	0.00
	H2OT	0.15	0.01	0.12	0.18	0.00
Coosawattee River:			95% CI			
Models	Parameter	Parameter Estimate	SE	Lower	Upper	P(> t )
D ~ Day	Intercept	5.60	0.19	5.23	5.97	0.00
	Day	-0.17	0.03	-0.23	-0.10	0.00
D ~ Rate15	Intercept	4.35	0.08	4.19	4.52	0.00
	Rate15	0.02	0.00	0.01	0.02	0.00

Table 3.7. Covariates, Akaike's information criterion corrected for small sample size(AICc), AICc differences ( $\Delta$  AICc), and Akaike weights ( $w_i$ ) for models influencing spawning rate (R) of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in the Coosawattee River. The confidence set of models for each system are shown in bold. Definitions of covariate abbreviations are in Table 3.2.

Broad River:			
Models	AICc	$\Delta$ AICc	$W_i$
R ~ H2O T	992.65	0	0.60
R ~ Day	993.55	0.90	0.38
Null $(\mathbf{R} \sim 1)$	1001.46	8.82	0.01
$R \sim MoonP$	1002.63	9.98	0
$R \sim MoonIll$	1003.44	10.80	0
$R \sim Hour$	1003.54	10.90	0
$R \sim Time$	1003.83	11.18	0
$R \sim MoonIll * Time$	1010.51	17.86	0
Savannah River:			
Models	AICc	$\Delta$ AICc	Wi
R ~ Time	1311.90	0	0.36
R ~ AirT	1312.31	0.41	0.30
R ~ Clouds	1314.14	2.24	0.12
R~H2OT	1315.46	3.58	0.06
R ~ MoonIll * Time	1316.38	4.49	0.04
Null ( $\mathbf{R} \sim 1$ )	1316.92	5.02	0.03
$R \sim Q10H$	1317.65	5.76	0.02
$R \sim Day$	1318.35	6.46	0.02
R ~ Precip	1318.53	6.63	0.01
$R \sim MoonP$	1318.98	7.08	0.01
$R \sim MoonIll$	1319.02	7.12	0.01
$R \sim Q6H$	1319.04	7.14	0.01
$R \sim Hour$	1319.04	7.14	0.01
Coosawattee River:			
Models	AICc	$\Delta$ AICc	$W_i$
R ~ MoonIll * Night	693.40	0	0.46
R ~ Q10H	694.23	0.83	0.30
Null (R ~ 1)	697.16	3.76	0.07
$R \sim MoonIll$	697.53	4.13	0.06
$R \sim Hour$	698.86	5.46	0.03
$R \sim Day$	698.95	5.56	0.03
$R \sim Q6H$	699.03	5.63	0.03
$R \sim MoonP$	699.32	5.92	0.02
$R \sim Time$	701.92	8.52	0.01

Table 3.8. Confidence model set, parameter estimates, standard error (SE) and 95% confidence interval (CI) of parameter estimates and parameter/model specific p-values for spawning rate (R) of Robust Redhorse in the Broad and Savannah Rivers and River Redhorse in the Coosawattee River. Definitions of covariate abbreviations are in Table 3.2.

Broad River:			95% CI			
Models	Parameter	Parameter Estimate	SE	Lower	Upper	P(> t )
R ~ H2OT	Intercept	17.01	1.69	13.70	20.33	0.00
	H2OT	-3.92	1.06	-6.00	-1.84	0.00
R ~ Day	Intercept	30.87	3.98	23.07	38.66	0.00
	Day	-1.66	0.47	-2.58	-0.75	0.00
Savannah River:				95%	∕₀ CI	
Models	Parameter	Parameter Estimate	SE	Lower	Upper	P(> t )
R ~ Time	Intercept	47.28	3.72	39.99	54.57	0.00
	Day	-2.66	1.42	-5.44	0.12	0.06
	Dusk	1.66	1.54	-1.36	4.68	0.28
	Night	3.59	1.42	0.80	6.37	0.01
R ~ AirT	Intercept	47.80	2.53	42.84	52.76	0.00
	AirT	-5.29	1.74	-8.70	-1.89	0.00
R ~ Clouds	Intercept	45.65	3.82	38.17	53.13	0.00
	Clouds	2.69	1.20	0.35	5.04	0.03
R ~ H2OT	Intercept	48.16	2.31	43.63	52.69	0.00
	H2OT	-5.99	2.26	-10.41	-1.56	0.01
R ~ MoonIll * Time	Intercept	46.95	3.72	39.67	54.24	0.00
	MoonIll	-1.58	1.56	-4.64	1.48	0.31
	Day	-2.59	1.43	-5.40	0.22	0.07
	Dusk	2.57	1.97	-1.30	6.44	0.19
	Night	2.94	1.60	-0.19	6.08	0.06
	MoonIII * Day	0.77	1.32	-1.81	3.35	0.56
	MoonIll * Dusk	-0.11	2.40	-4.81	4.58	0.96
	MoonIll * Night	2.59	1.40	-0.14	5.33	0.06

# Table 3.8. Continued.

Coosawattee River:			95% CI			
Models	Parameter	Parameter Estimate	SE	Lower	Upper	P(> t )
R ~ MoonIll * Night	Intercept	68.79	57.59	-44.10	181.67	0.24
	MoonIll	-0.29	2.39	-4.97	4.40	0.91
	Night	-1.35	2.58	-6.40	3.71	0.60
	MoonIll * Night	5.84	2.05	1.82	9.85	0.01
R ~ Q10H	Intercept Q10H	64.64 3.39	52.59 1.50	-38.43 0.46	167.71 6.33	0.22 0.03



Figure 3.1. Map of the locations in Georgia where we recorded spawning acoustics of Robust Redhorse (circles) in the Broad River in 2012 and Savannah River in 2013 and River Redhorse (square) in the Coosawattee River watershed in 2012.



Figure 3.2. Robust Redhorse at the lower gravel bar on the Savannah River. Spawning aggregation of males on the river right gravel lens (left panel) and a spawning triad, two males flanking either side of a female (right panel).



Figure 3.3. Hydrophone in steel harness (left panel) and deployed with added weights and attachment chain (right panel).



Figure 3.4 Location of the hydrophone placed at the downstream Savannah River spawning site (small circle) and approximate range of detection (20 m radius; large circle).


Figure 3.5. Fraction of the moon illuminated (white in top bar of each graph) and spawning rates of Robust Redhorse in the A. Broad River in 2012 and B. Savannah River in 2013, and C. River Redhorse in the Coosawattee River in 2012 estimated from passive acoustic recording devices.



Figure 3.6. Trend of spawning rates of Robust Redhorse in the Broad and Savannah River and River Redhorse in the Coosawattee River estimated from time series decomposition.



Figure 3.7. Periodicity of spawning rates of Robust Redhorse in the Broad River and Savannah River, and River Redhorse in the Coosawattee River estimated from time series decomposition and illustrated for a 36-hour period.



Figure 3.8. Recorded water temperatures (dark line) and overall trend in spawning rate based on time series decomposition (gray line) in the A. Broad River during 2012 and B. Savannah River in 2013.

Appendix 3.A. The date, estimated number of spawning individuals (Ind.), number of spawning events (N), average duration of spawning events (sec), and spawning rate (number of spawns per hour) of Robust Redhorse at the Broad River based on sub-hourly samples of acoustic recordings over 9 days in 2012. NA indicates observations of number of individuals or acoustic recordings were not made during the listed time period. Dawn and dusk are counts for (or a portion of) 1 h per day and day and night are averages over several hours.

		Dawn				Daylight				Dusk			Night		
Date	Ind.	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate	1	N	Average (SE)	Rate	Ν	Average (SE)	Rate	
4/29	11		NA	NA	38	4.87 (0.23)	11.69		9	3.73 (0.23)	18	32	4.14 (0.22)	25.6	
4/30	20	14	4.89 (0.61)	28	129	4.39 (0.13)	19.11		5	3.59 (0.24)	10	99	4.15 (0.13)	23.29	
5/1	25	16	4.12 (0.3)	32	145	4.18 (0.1)	21.48	1	0	4.9 (0.57)	20	107	4.15 (0.1)	25.18	
5/2	NA	14	4.54 (0.54)	28	133	4.52 (0.12)	19.7	1	3	4.52 (0.39)	22	99	4.43 (0.14)	23.76	
5/3	27	20	4.12 (0.28)	40	158	4.33 (0.11)	22.96		8	4.74 (0.62)	22	117	4.13 (0.1)	27.53	
5/4	10	7	4.35 (0.37)	14	89	4.28 (0.14)	13.19		5	3.58 (0.25)	10	72	4.21 (0.16)	16.94	
5/5	NA	11	4.1 (0.34)	22	62	4.18 (0.18)	9.04		7	3.49 (0.09)	12	48	4.7 (0.24)	11.76	
5/6	NA	6	3.81 (0.19)	12	69	4.42 (0.17)	10.22		3	4.52 (0.95)	4	60	4.09 (0.13)	14.35	
5/7	7	14	4.41 (0.2)	28	54	4.33 (0.15)	8			NA	NA	36	4.08 (0.2)	8.47	

Appendix 3.B. The date, estimated number of spawning individuals (Ind.), number of spawning events (N), average duration of spawning events (sec), and spawning rate (number of spawns per hour) of Robust Redhorse based on hourly or sub-hourly samples of acoustic recordings over 10 days in the Savannah River, Georgia / South Carolina in 2013. NA indicates observations of number of individuals or acoustic recordings were not made during the listed time period. Dawn and dusk are counts for (or a portion of) 1 h per day and day and night are averages over several hours.

		Dawn			Daylight				Dusk			Night		
Date	Ind.	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate	
4/18	7		NA	NA	98	4.5 (0.17)	15.2	16	5 (0.45)	17	67	5.5 (0.24)	22	
4/19	NA	33	4.82 (0.27)	35	403	4.6 (0.07)	33.66	35	4.22 (0.17)	36.8	316	4.89 (0.09)	35.22	
4/20	NA	47	4.64 (0.2)	49	661	4.6 (0.06)	50.27	40	4.5 (0.17)	42	435	4.89 (0.08)	48.78	
4/21	NA	63	4.16 (0.12)	69	617	4.21 (0.05)	52.86	63	4.28 (0.15)	66	599	4.38 (0.05)	66.86	
4/22	NA	68	4.13 (0.12)	71	702	4.33 (0.05)	53.39	65	4.04 (0.13)	63	655	4.33 (0.05)	74.63	
4/23	NA	39	4.32 (0.21)	78	313	4.14 (0.07)	47.38	30	4.13 (0.15)	62	276	4.18 (0.07)	62.22	
4/24	NA	32	4.44 (0.25)	64	356	4.43 (0.07)	53.94	33	4.08 (0.19)	70	279	4.43 (0.08)	62.89	
4/25	65	27	4.12 (0.2)	54	220	4.33 (0.08)	35.86	25	4.42 (0.4)	54	245	4.27 (0.07)	55.33	
4/26	NA	20	3.61 (0.14)	40	274	4.2 (0.07)	41.23	17	4.25 (0.23)	36	246	4.19 (0.07)	55.78	
4/27	NA	21	4.44 (0.28)	42	119	4.29 (0.12)	29.75		NA	NA	163	4.21 (0.09)	54.33	

Appendix 3.C. The date, estimated number of spawning individuals (Ind.), number of spawning events (N), average duration of spawning events (sec), and spawning rate (number of spawns per hour) of River Redhorse based on sub-hourly samples of acoustic recordings over 5 days at the Coosawattee River spawning site in 2012. NA indicates observations of number of individuals or acoustic recordings were not made during the listed time period. Dawn and dusk are counts for (or a portion of) 1 h per day and day and night are averages over several hours.

			Dawn			Daylight			Dusk			Night	
Date	Ind.	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate	Ν	Average (SE)	Rate
5/10	115		NA	NA	243	4.86 (0.08)	138.86	30	4.59 (0.22)	120	51	4.78 (0.18)	102
5/11	85	30	5.31 (0.31)	120	317	4.81 (0.08)	90.57	33	4.46 (0.21)	132	240	4.88 (0.1)	120
5/12	NA	18	4.96 (0.37)	72	218	4.44 (0.08)	62.29	4	3.54 (0.13)	16	159	4.8 (0.11)	79.5
5/13	NA	12	4.43 (0.33)	24	113	4.49 (0.13)	16.14	6	3.86 (0.3)	12	43	4.32 (0.15)	15.64
5/14	NA	3	3.89 (0.51)	6	15	3.8 (0.16)	2.14		NA	NA	7	3.88 (0.2)	2

# CHAPTER 4

# COMPARISONS OF REPRODUCTIVE BIOLOGY OF AN IMPERILED MIGRATORY FISH IN A PIEDMONT AND TWO COASTAL PLAIN RIVERS<sup>1</sup>

<sup>&</sup>lt;sup>1</sup> Straight, C.A., B.J. Freeman, and M.C. Freeman. 2014. To be submitted to *Environmental Biology of Fishes*.

## ABSTRACT

Reintroductions and translocations are tools used in the conservation and recovery of many imperiled species. These actions provide immediate actions that can be conducted by recovery groups. However, in the past, reintroductions have commonly failed because of limited knowledge of threats and species' life history requirements and behavior. Many reintroductions occur in historical, but currently unoccupied portions of the species' range, based on observations of species habitat requirements in the currently occupied range. We studied the migratory and reproductive behaviors of a reintroduced population of Robust Redhorse, Moxostoma robustum, in a Piedmont river (Broad River), Georgia, and compared behaviors to wild populations in two Coastal Plain rivers (Oconee and Savannah Rivers). All life history information for Robust Redhorse prior to their reintroduction was based on observations of a single population in the Oconee River. This study was the first to document reproduction in the reintroduced population in the Broad River, and to detail reproductive behavior, including evidence of an alternative reproductive tactic of non-territorial males (sneakers). We also provide the first documentation of this migratory riverine fish using reservoirs as wintering habitat. Most reproductive behaviors were similar between Piedmont and Coastal Plain populations of Robust Redhorse. However, in the reintroduction watershed we documented three times the number of spawning sites compared to the Coastal Plain systems. Robust Redhorse in the reintroduction watershed also showed variability in migration patterns and movements among spawning sites, and used higher velocities than observed in the larger river, Coastal Plain populations. This study showed that reproductive behaviors of Robust Redhorse in the reintroduction watershed could only be partially explained by our observations in the Oconee and Savannah Rivers.

## **INTRODUCTION**

Reintroduction is a tool used by many conservation groups to assist in the recovery of imperiled species (Beck et al. 1994; NRCC 1996; Jones 2004). In some cases low population numbers may require immediate action. In the early 1980's, many reintroductions failed because of inadequate knowledge of the species' behavior and life history requirements, and the persistence of threats that contributed to or remained a cause of the species' decline (Kleiman 1989; Seddon et al. 2007; Fraser 2008). This experience prompted the recommendation that introductions be limited to areas where the conditions that caused the decline have been eliminated (Kleiman 1989; Snyder et al. 1996); this practice has not always been followed, however. Additionally, basing assumptions of species' requirements from observations of habitat species occupy in altered ecosystems may lead to misguided conservation and management actions. To avoid incorrect assumptions, feasibility studies should be conducted prior to reintroduction to determine a species' needs throughout all stages of its life history and to ensure that the reintroduction area meets these needs (Kleiman 1989). Understanding behavior is particularly critical for conservation success of any species, because behavior influences both survival and reproductive success (Shumway 1999).

Our knowledge of many species in need of conservation comes from observations in a reduced range, and potentially in highly modified environments. Examples include Black-footed Ferrets (Biggins and Schroeder 1987), multiple salmonid species (NRCC 1996), Red Wolves (Parker 1988), and Florida Panthers (Kautz 1994), among many other species that now persist in reduced ranges. Habitat destruction accounts for approximately 85% of species that are listed or proposed to be listed under the Endangered Species Act and the primary cause of habitat loss for aquatic species is water development (Wilcove et al. 1998). Humans have directly modified

freshwater habitats globally through creating barriers, diversions, withdrawals, discharges, dredging, channelization, and sand / gravel mining, and indirectly through land use modification and riparian buffer changes. Because natural environments are limited, aquatic species reintroductions almost always occur in a human-modified environment.

Many reintroductions target areas of historical ranges that are outside of currently occupied environments. The question remains of whether current conditions of the historically occupied environments will meet the life history needs of reintroduced species. This study investigates the reproductive biology of a species, the Robust Redhorse (Moxostoma robustum), reintroduced into a former part of its range. The Robust Redhorse is a high-profile imperiled fish species in the southeastern United States, where the primary recovery tool has been an intensive propagation and stocking (reintroduction and supplementation) program. We compare components of reproduction from wild Coastal Plain populations to those of a reintroduced Piedmont region population, to ask whether our understanding of this species' reproductive habitats, based on two populations, has been predictive of habitat use and behavior in a reintroduced population. The Robust Redhorse is a large-bodied catostomid species, native to the Piedmont and upper Coastal Plain rivers on the South Atlantic Slope from the Pee Dee River drainage, North Carolina and South Carolina, south to the Altamaha River drainage, Georgia (Figure 4.1). The species is listed as endangered under the Georgia Endangered Wildlife Act and is a high conservation priority in the state (The Georgia State Wildlife Action Plan http://www.georgiawildlife.com/conservation/wildlife-action-plan). The Robust Redhorse is thought to have been widely abundant historically within its native range, but to have undergone severe reductions in range and population sizes (Cope 1870; Bryant et al. 1996). Widespread dam construction for hydropower has likely contributed to this decline. Current wild populations

in the Oconee, Savannah, and Pee Dee Rivers are all downstream of large hydropower dams, which restrict most populations to the Coastal Plain, except a small portion of the Pee Dee River that is accessible in the Piedmont. Since its description in the 19<sup>th</sup> century (Cope 1870), the distribution of Robust Redhorse has declined to less than half and possibly as little as 30% of its historical range. The locality from which Cope (1870) collected and described the Robust Redhorse was in the Yadkin River at a spawning aggregation in the Piedmont region (Figure 4.1). This collection indicated that spawning aggregations occurred well above the Fall Line zone and into the Piedmont of North Carolina, providing potential insight for future reintroductions in historical portions of its range.

To help recovery and conservation of the Robust Redhorse, a coalition of federal and state resource management agencies, hydropower interests, and non-governmental organizations formed the Robust Redhorse Conservation Committee (RRCC) in 1995. According to the RRCC goals, restoration would be deemed successful when six self-sustaining populations occurred throughout the species' historical range (Nichols 2003). Only a single wild population, in the Coastal Plain portion of the Oconee River (Figure 4.1), was known to exist when the RRCC was formed; populations were subsequently discovered in the Savannah River in 1997 and in the Pee Dee River in 2000 (DeMeo 1998, 2000). Self-sustaining, as defined by the RRCC, meant that "a population or all known populations are at a level where the natural recruitment rate is equal to or greater than its mortality". Managers assumed it would take at least 22 years (to encompass at least one generation) to document variability in recruitment (RRCC 2002). The Broad River in the Piedmont of Georgia was chosen for the initial reintroduction effort because the river was believed to contain potential spawning habitat and to be within the historic range of Robust Redhorse. Reintroductions of Robust Redhorse occurred in the Broad River basin from 1995-

1998 with over 33,000 1-2 year old individuals (spawned from parental stock taken from the Oconee River) released at eight locations in the headwaters and tributaries of the Broad River (Figure 4.2).

The purpose of this paper is to document migratory and reproductive behavior of Robust Redhorse and assess differences between those behaviors of the reintroduced population in the Broad River watershed, and populations in Coastal Plain of the Oconee and Savannah Rivers. This paper provides the first detailed documentation of Robust Redhorse spawning behavior observed at three locations in Georgia. We also compare our work with other studies of movements in the Oconee River (Ely 2012), spawning habitat use and movements in the Savannah River (Grabowski 2006, 2007; Grabowski and Isley 2006, 2008), and spawning season habitat use and movements in the Pee Dee River (Fisk 2010; Fisk et al. 2014). Documenting the variability in spawning behavior will also provide a comparison for future reintroductions in the Piedmont region and to assess changes in Coastal Plain populations. All of our initial assumptions about reproduction and spawning habitat requirements for Robust Redhorse have derived from a restricted portion of the species range in the Coastal Plain of Georgia. The only known spawning locations prior to reintroduction in the Broad River occurred in the Oconee River, where flows and temperatures are modified by hydropower operation upstream of the spawning site.

Our specific objectives are to answer the following questions: 1) Do Robust Redhorse spawn in the reintroduction watershed? And 2) are Robust Redhorse migrations, winter habitats, spawning behaviors, and timing of reproduction similar between the reintroduction watershed in the Piedmont and populations in the Coastal Plain watersheds?

#### **METHODS**

Study Site - To understand differences and similarities between the Coastal Plain populations of Robust Redhorse and the reintroduction watershed in the Piedmont, we compared data collected in the Broad River (reintroduction) watershed to observations within the Coastal Plain populations in the Oconee and Savannah River systems. We observed spawning Robust Redhorse at six sites in the Broad River watershed (part of the Savannah River system; Figures 4.1 and 4.2), one site in the mainstem Savannah River, and one site in the Oconee River (Figure 4.1). Both the Savannah and Oconee spawning sites were mid-channel gravel bars downstream of hydropower dams located in the Coastal Plain. The spawning sites in the Broad River were free-flowing and in the Piedmont physiographic province. Another major difference between the Broad River watershed and the Oconee and Savannah River, is that Strom Thurmond Reservoir impounds the Broad River near its mouth. Although this reservoir does not alter hydrology at the upstream spawning sites, it prevents further downstream migration past the dam. Finally, the Piedmont and Coastal Plain sites differed in drainage areas. Of the six studied sites in the Broad River watershed, four were in the Broad River mainstem, one was in the North Fork Broad River (and downstream from a low-head dam), and one was in the Hudson River (Figure 4.2). Watershed area and river channel width varied considerably among these sites, however all had an order of magnitude smaller watershed area than the Coastal Plain spawning sites (Table 4.1).

*Movements* – To answer questions about movements, we collected Robust Redhorse in the Broad River system and implanted them with sonic transmitters. We also compared movements of Robust Redhorse in the Broad River to other studies in the Oconee, Savannah, and Pee Dee Rivers (Grabowski 2006; Grabowski and Isley 2006; 2008; Fisk 2010; Ely 2012; Fisk et al. 2014). In February and March 2010 and 2011, we used standard boat electrofishing

techniques, using a Smith-Root<sup>™</sup> 2.5 GPP unit, to capture twenty Robust Redhorse (5 females; 15 males) in the lower Broad River prior to their upstream spring spawning migrations (Figure 4.2). We chose to collect and tag fish before individuals completed their migration upstream to the spawning shoals, before reproductive organs were fully enlarged to avoid risk of nicking internal organs and to minimize the potential of added stress that may occur from handling closer to the time of spawning (Jepsen et al. 2002). Each Robust Redhorse was surgically implanted with a sonic transmitter that had an expected life of 650 days (V13T-1L; VEMCO Ltd, Halifax, Nova Scotia; diameter: 13mm; length: 41mm; weight: approximately 6 g). All fish surgeries were conducted by advanced veterinary school students and Dr. Stephen Divers or Dr. Jörg Mayer (Small Animal Medicine & Surgery, University of Georgia). Each captured fish was anesthetized for surgery using buffered tricaine methanesulonate (MS-222 at 150 mg/L). In the field, all surgical equipment was sterilized in 2% glutaraldehyde and rinsed in sterile water prior to each fish surgery. Each Robust Redhorse was given an injection of an anti-inflammatory, Meloxicam, at a dose of 0.2 mg/kg and an injection of a broad-spectrum antibiotic, Ceftazidime, at a dose of 22 mg/kg by intramuscular injection. The anti-inflammatory and antibiotic helped to decrease recovery time, minimize any discomfort, speed the fish's return to normal swimming and response behaviors, and minimize chances of infection. A gas-sterilized sonic transmitter was inserted through a 4 cm ventral midline skin incision and the incision was closed using a single layer closure with 2/0 Monocryl-Plus suture in a simple interrupted pattern. During surgery, heart rates were monitored and a dilute solution of anesthetization water or river water was washed over the head, mouth, eyes, and gills of the individual fish. Surgery times took 7-12 min, after which each individual was held in an aerated recovery tank with river water until it was able to maintain an upright position and responded to stimulus. The fish were then moved to

a flow-through enclosure placed in the river near the surgery locale and held prior to release for a minimum of 20 minutes or until responsive and behaving normally.

To track migration movements within the Broad River system, we placed stationary submerged receivers (VR2W; VEMCO Ltd, Halifax, Nova Scotia) at a minimum of six locations staggered along approximately 90-river km from Strom Thurmond Reservoir up the Broad River to the known spawning locations. These receivers were in place from February through August and downloaded at least once every two months to track pre- and post-spawning migratory movements as fish moved past the receivers. During the fall and winter six receivers were placed within the reservoir attached to U. S. Army Corps of Engineers mid-channel buoys (Figure 4.2). Using sonic transmitters allowed us to track fish within the depths of Strom Thurmond Reservoir, but made detections within some shallow spawning aggregations difficult because of transmission interference.

*Observations* – To answer questions about differences in timing of the start of the spawning season and spawning behavior, we observed spawning Robust Redhorse during daylight hours and when river stage and turbidity were low enough to allow observations, in the Oconee River from 1995-2008, in the Broad River watershed from 2007-2013, and the Savannah River in 2013 (Table 4.2). We recorded maximum number of individuals observed in each spawning area and noted behaviors. In 2012 and 2013, we also attempted to follow individual females to document the interval of time between spawning events. When conditions and time allowed, we observed an hour of spawning events, documenting male and female participation in each event. After observations were completed for the day, we measured depth and velocity at locations where spawning events had taken place.

In each of these river systems, many large-bodied catostomids used similar or the same gravel patches for spawning and spawned in a distinct sequence. At all sites, these species included, in sequential order from earliest spawner to latest, Spotted Sucker (*Minytrema melanops*), Notchlip Redhorse (*Moxostoma collapsum*), Brassy Jumprock (undescribed species, hereafter *Moxostoma* sp.), and Robust Redhorse. Robust Redhorse could be distinguished from the other species by size, coloration, markings, morphology, and behavioral differences.

*Data Collection* – To assess differences in microhabitat characteristics between spawning sites, we collected water temperatures and microhabitat characteristics at a subset of specific locations of spawning events. Measurements included depth, and velocity at 60% depth and near the bottom using a Marsh-McBirney Inc. Flo-Mate<sup>TM</sup> Model 2000 portable flow meter. We also measured depth and velocity in some locations where females were holding position while resting between spawning bouts. In the Broad River in 2011 and 2012, we additionally collected measurements at no less than one non-spawning location for each spawning location. These non-spawning locations were taken approximately 2 m perpendicular to the flow from the spawning location. If the location fell within the spawning aggregation, we continued to move perpendicular to the flow until we reached at least 1 m beyond the outside edge of the spawning patch.

To characterize substrate composition between sites where the fish were spawning, we used a freeze-coring device (Stocker and Williams 1972) in the Oconee River in 1997, 1998, and 2000. In the Broad River in 2010-2012, we collected four grab samples (each with an average dry mass of 1200 g) of bed sediments within each spawning patch each year, which were averaged for analyses (Wolcott and Church 1991). Samples were collected after spawning season was completed, but before large amounts of fine sediments started to fill in at the spawning site.

We collected the samples from within the downstream lens (tailspill) of gravel created by spawning suckers. Because these patches of gravel were moved during the spawning process, sampling at that location should provide an accurate measure of substrate chosen for spawning. Each substrate sample from the Broad River was dried and shaken using U.S. standard sieve sizes (<0.25mm (fines), 0.25-2.0 mm (sand), 2.0-4.75 mm (coarse sand, fine gravel), 4.75-12.5 mm (gravel), 12.5-50 mm (coarse gravel), and >50 mm (very coarse gravel, small cobble)). We weighed (g) the portion of substrate that passed through each size and created a proportion by dividing by the total sample weight. For the Oconee River, all samples larger than 4.75 mm mesh size were combined. Although grab-sampling may lose a small portion of fines relative to freeze-core methods, we believe the samples accurately reflect substrate conditions at the spawning sites, since a proportion of the fine sediments are winnowed out of the gravel during the spawning act.

*Data Analysis* – We used an ANOVA to assess differences in depth, velocity at 60% depth, and velocity at the bottom between spawning locations in Broad River and Oconee River and also between sites where spawns occurred and did not occur (aov; R Development Core Team 2011). We also compared depth and velocities between sites used by females holding positions (resting locations) and non-spawning locations. Limited habitat measurements in the Savannah, made on one day in one year, prevented us from including this site in some analyses.

Using beta regression, we compared the proportion of substrate that was measured as gravel or greater (>4.75 mm diameter) between the Oconee River and Broad River spawning sites (package betareg; Cribari-Neto and Zeileis 2010). Beta regression models account for heteroskedastic or skewed tendencies when the response variable is bounded by 0 and 1 (rates or

proportions). All samples within a year and spawning site were averaged and the averaged value was used as a response for these analyses.

We used data from the three river systems over 18 years to test for correspondence of spawning initiation to temperature and moon phase (Table 4.2). We calculated growing degree-days (cumulative maximum daily temperature difference above 50° F) as our surrogate for assessing temperature. Growing degree-days started on 1 January and were summed until the start of the spawning season for each year. These were downloaded from a growing degree-day calculator at weather.com for the Broad and Savannah Rivers and hand-calculated using the National Climatic Data Center's daily data for Macon, Georgia for the Oconee River sites (http://cdo.ncdc.noaa.gov/ulcd/ULCD). Because researchers suggested a correlation between spawning by Black Redhorse (*M. duquesnei*; Bowman 1959) and Greater Redhorse (*M. valenciennesi*; Jenkins and Jenkins 1980) and time of the first full moon, we also assessed days to the first quarter moon phase. We used data hosted by the U.S. Naval Observatory (http://aa.usno.navy.mil) to obtain date of the first quarter moon cycle for the months of April and May for each study year. We assessed differences between rivers and differences between years (Broad only) using ANOVA.

#### RESULTS

*Migration in the Broad River* – Captured, tagged Robust Redhorse ranged from 439-555 mm standard length, 535-690 mm total length, and 1890-4330 g in weight. Of the twenty-tagged individuals, one individual was not detected after tagging and one additional tag was stationary since the day after tagging indicating potential tag expulsion or mortality. Four additional males were not detected after the first spawning season (1-2 months post-tagging). We recorded over

93,500 detections from the 18 individuals that we detected moving, over 17,500 of which occurred during the spring migration period (from February to April). The remaining detections occurred during summer, fall, and winter within the river and reservoir (Figure 4.2). Based on documented times of fish passing receivers, males and females moved during the day and night during migrations, the spawning season, and in winter. All 18 of the detected transmittered fish travelled at least once between their wintering and spawning locations, covering distances of 55 to 98.5 rkm.

In general, most Robust Redhorse showed a pattern of wintering in or near Strom Thurmond Reservoir, migrating upstream to the spawning sites in spring, and returning downstream in late-spring through summer. All of the fish that wintered in the reservoir started their pre-spawning migration upstream from late-February to early-April (Figure 4.3). Prespawning migration upstream was more directed than post-spawning migrations for most individuals, taking 2-4 weeks to migrate upstream from the reservoir to the spawning shoals. Post-spawning migration typically took longer, lasting from June through September. All of the tagged fish detected in the fall or winter (September - January) migrated downstream of the downstream-most spawning site to their wintering location. Of the six fish captured and tagged in 2010, all individuals showed similarities in movements within the reservoir and river, spending winters in the reservoir and moving at least 71 km from the tagging location to the spawning sites (Figure 4.3 A). In the summer and fall of 2011, flows within the river were below normal, and movement patterns of the 12 fish tagged and detected in the spring of 2011 were less consistent than those fish tagged in 2010 (Figure 4.3 B). For the 2011 fish, the overall pattern of upstream migration to the spawning sites was similar to that of the fish in 2010, but downstream movements in a season of low flows were variable. One exception to the spring migration pattern was a male that moved between the tagging location and areas downstream in the reservoir two times before initiating upstream movements within the river (adding 40 km to its overall movements prior to upstream migration). Another exception to the normal downstream migration occurred when two individuals that wintered in the reservoir in 2010-2011 likely wintered in the lower reaches of the river in 2011-2012. Of the four fish that occurred near stationary receivers in the reservoir during the winter, all moved in and out of receiver range and were detected near a single receiver for the majority of the winter. In all instances where these four individuals were located within the main reservoir, they were detected in water 5-9 m in depth, bottom sediments were fines, and they were associated with woody material. One individual was recorded moving several times between two receiver stations in September and October (a distance of 3.1 km).

Most tagged fish arrived in the vicinity of the spawning shoals and likely remained in deeper waters prior to the start of the spawning season (where many were detected by stationary receivers). Individuals also showed some fidelity to spawning sites. In the vicinity of the spawning shoals, we began detecting tagged individuals almost a month prior to when we visually observed spawning. During the three years we tracked fish, the first tagged individuals arrived 19-25 days before the spawning season started. There was no evidence of one sex arriving in the vicinity of spawning areas before the other. During at least one spawning season, three individuals (two males, one female) moved between two known spawning sites 6.5 rkm apart (Hudson and Broad 4; Table 4.1). Of the eight individuals recorded at a known spawning site (other tagged fish were in the vicinity of spawning sites but not documented at a particular aggregation), four individuals showed some site fidelity arriving at the same site first in each year.

*Timing* – The spawning season documented at all three sites occurred from late-April through late-May (Table 4.2). Documented spawning in the Oconee River coincided with flows modified by Georgia Power Company from Sinclair Dam for Georgia Department of Natural Resources annual standardized sampling and broodstock collections. Typically, flows were reduced to 600 cfs for a period of 3 to 5 d a week (depending on inflows) from mid- to late-April until sampling was completed (usually 3-4 weeks). During low flow events, spawning Robust Redhorse could be observed. It is unknown if fish continued to spawn at times when flows were not modified.

Temperatures during documented spawning ranged from 16.3 to 26.8 °C (Table 4.3). At several sites we were able to record temperatures at 15-minute increments, which allowed us to record higher temperatures than those that we measured by hand while making behavioral observations. In the Broad River in 2011, we observed that when water temperatures dropped below 16.3° C after spawning had started, many males abandoned their territories and no spawning events were seen until the next day when temperatures had increased to above 16.5° C.

The start of the spawning season in the Broad, Oconee, and Savannah Rivers did not differ by day of the year (F = 0.44, df = 2,27, p = 0.65) or growing degree-days (F = 2.44, df = 2, 25, p = 0.11; Table 4.2). The beginning of the spawning season ranged from 11 days before to 12 days after the first quarter moon phase for all river systems. We found differences in days to the first quarter moon between rivers (F = 3.74, df = 2,25, p = 0.04). In the Broad River, the start of the spawning season averaged 4.91 days (SD = 4.06) before the moon reached its first quarter, which differed from the Oconee's average of 0.79 days (SD = 6.64) after the first quarter. In the Savannah River, days to first quarter moon averaged 0.2 days (SD = 8.6) after first quarter.

*Habitat* – Robust Redhorse spawning occurred at sites with a variety of river widths and watershed sizes (Table 4.1). The sites used for spawning with smallest watershed areas occurred in the Broad River, with the Oconee River and Savannah River sites having upstream watershed areas that were 5-times and 10-times larger than the largest Broad River sites.

Spawning sites in the Broad River system were typically characterized by multiple patches of gravel separated by areas of non-spawning substrates (fines, sand, and bedrock) in a short reach of river (< 200 m). Four of the six sites within the Broad River system contained at least two distinct spawning patches (Table 4.1). Patches spanned a majority of the channel width at most locations, and occurred in side channels alongside islands, or between widely-spaced bedrock ledges. Elevated winter discharge shifted some patches from year-to-year and low summer flows covered some patches in fine sediments. Some patches continued to be covered and were not used for spawning the following spring. The single gravel patches at two Broad River spawning sites were elongated, lateral bars along the river left margin of the river. At the largest Broad River site (Broad 4; containing seven spawning patches), males typically occupied territories in the largest patches first. As the spawning season progressed, and density of territorial males increased, males started to occupy the smaller patches. After the peak of spawning season and density began to decrease, the smaller patches were typically abandoned first, leaving the largest patches the last to be occupied.

The documented spawning areas in both the Oconee and Savannah Rivers during this study were single large patches of mid-channel gravel (both  $> 1500 \text{ m}^2$ ). Topographic variation on these bars provided a range of depths and velocities and at least two areas where males held territories (typically along two break points were flow moved around river right and river left of

the bar). These large expanses of gravel had flow directed around both sides of the most-elevated point of gravel, which was typically exposed during times we observed spawning.

Spawning groups used a variety of microhabitats with depths ranging from 0.15 to 0.88 m, velocity at 60% depth ranging from 0.2 to 1.4 m/s, and bottom velocities ranging from -0.14 to 0.85 m/s (Table 4.3). Depths at spawning locations were similar between the Broad and Oconee Rivers (F = 1.83, df = 1,198, p = 0.18), as were velocities at the bottom (F = 0.07, df = 1,175, p = 0.79). Measured velocities at 60% depth were lower in the Oconee River than the Broad River sites (Table 4.3; F = 23.42, df = 1,198, p < 0.001). Spawning locations had greater mean depths than non-spawning locations (Table 4.4; F = 92.66, df = 1,276, p < 0.001) and lower mean velocities at the bottom (F = 11.59, df = 1,238, p = 0.001). There were no differences in velocities at 60% of depth between spawning and non-spawning locations (F = 1.42, df = 1,276, p = 0.24).

Bed sediments within the spawning patches were loosely packed and easily moveable, dominated by particles > 4.75 mm (gravel, coarse gravel, and larger). The proportion of samples > 4.75 mm was greater in the Broad River than the Oconee River (Broad: mean = 0.82, SD = 0.11; Oconee: mean = 0.63, SD = 0.11; pseudo- $R^2 = 0.27$ , p < 0.001). When collecting freezecore samples in the Oconee River for substrate analysis, eggs were found in clumps ranging from near the surface up to 15 cm into the gravel substrate.

*Behavior* – Counts of spawning individuals varied considerably among sites and years in the Broad River and Oconee River. We observed as few as 5, and as many 73 spawning individuals at one time in the Broad River, with fewer individuals generally observed at smaller patches (Table 4.2). The smallest number of territorial males counted in a single patch was three, typically at patches only occupied when the number of individuals at the site was at or near its peak. Conversely, we counted as many as 31 territorial males in a single patch during the peak of spawning. At the Oconee River spawning site, the maximum number of fish counted was over 50 individuals, and the observed aggregation size has declined since original discovery of the site in 1995; no fish have been seen at the spawning site since 2008 (Table 4.2). In the Savannah River, we observed a maximum of 65 fish at the spawning site.

During 231 documented spawning attempts in the Oconee, Savannah, and Broad Rivers, 201 (87%) events were typical triads (1 female / 2 male), 4 were duos (1 female / 1 male), 16 (7%) were 1 female / 3 males, 5 (2%) were 1 female / 4 males, and 5 included 2 females and 2 or more males (Table 4.5). The proportions of occurrences were similar among sites. Spawning duos were typically seen very late in the season when only a few or a pair of individuals were seen at a site. Because more observations were made within the Broad River, the extra observation time likely allowed us to see a wider range of group combinations. When multiple females participated in a group-spawning event, they were separated by males, except on one occasion when two females were next to one another.

The most common spawning act occurs between a trio of fish when a female moves between two territorial males and the males approach her on either side (Table 4.5; Figure 4.4). The males press their bodies against the female and the trio begins to quiver as eggs and sperm are released. As the spawn progresses, the participants dig into the substrate with their posterior fins disturbing bed sediments into which they bury the fertilized gametes. During the spawning act, the dorsal fins of participants fan forward and in very shallow water extend above the surface. During prolonged spawning events, the spawning trio may move slightly upstream. Once a spawn is completed, the participating males commonly return to their territories and females move to resting locations. At all Robust Redhorse sites, spawning "pits" or depressions were not constructed prior to spawning, as in redds of salmon spp., but were an artifact of the spawning act. Repeated spawning events with the same individuals resulted in depressions that increased in size and had a tailspill downstream of the depression that is typically elevated relative to surrounding undisturbed gravel. Of 10 spawning depressions measured in the Broad River, they ranged from 1-3.5 m wide, 1.1-4.9 m long, and had a depth of 0.15-0.8 m below the normal bed elevation. As spawning pits increased in size, pits from neighboring territories joined creating larger crescentshaped depressions where several males held territories. These crescent-shaped depressions were visible for several weeks after spawning had ceased. We did not measure spawning depressions in the Oconee or Savannah Rivers.

For 15 different females, we were able to follow an individual and calculate the time between spawning events in the Broad and Oconee Rivers. Females averaged 217.5 s between spawns (N = 59 between spawn intervals; SD = 164.6). There was high variability in an individual's time between spawns. Limited samples from the Oconee River and none from the Savannah River prevented our analysis of differences between systems. However, females in the Oconee and Broad showed a similar range (17-540 and 12-693 sec, respectively). Shorter times between spawns mostly occurred after an interrupted spawning event, when the females did not return to a resting place before attempting to spawn again. It was easier to maintain sight of females for shorter periods of time, so the dataset is likely skewed toward the shorter intervals. Although we did not document duration between spawns for males, we did note that the shortest time between two presumably completed spawns observed was 20 s with the same 2 males and 2 different females.

Observations of male territorial behavior revealed alternative strategies. In all three systems males usually faced upstream and aligned in rows within the spawning patch. Larger males appeared to be central within the spawning aggregation and smaller males (many with reddish tinges to their fins) tended to be along the shallower, marginal areas of the spawning aggregation (For additional information on physical characteristics of spawning individuals, see Appendix 4.A). On many occasions, neighboring males would attempt to join a spawning trio as the spawn was initiated and were either chased away by the territorial male or joined the spawning group. On three occasions, once in the Oconee River and on two occasions in the Broad River, we observed males that did not maintain a territory, but followed the females to and from their resting locations. Individuals participating in this alternate reproductive tactic were typically smaller than the majority of territorial males. They usually attempted to join spawning events that females and territorial males had initiated by moving in between one of the territorial males and the female, "sneaking" spawns. One male was observed to successfully spawn three times in this manner. It is unknown whether this alternate tactic is a way for smaller individuals that cannot compete for prime territories to participate in spawning events, or if it is a genetically-based alternate reproductive strategy.

When not actively spawning, females spent most of their time in areas of flow refugia over fine sediments away from the males. These refugial areas were in pools or low-flow areas created by projections of point bars, large woody material, or bedrock ledges near the spawning aggregation. Numerous females were often seen together in the same resting areas away from the spawning aggregation. However, during peak spawning and late in the spawning season, females were seen resting within the spawning shoal. These females were found in areas immediately downstream of the tailspill where bottom velocities were usually 0 m/s or negative. Nearby

males would occasionally follow a female, nudging her as she moved to a resting location or visit and nudge females where they rested. Locations where females rested had greater mean depths (F = 22.94, df = 1,85, p < 0.001), lower velocities at 60% of depth (F = 5.98, df = 1,85, p = 0.02) and lower velocities at the bottom than non-spawning locations (Table 4.4; F = 14.22, df = 1,70, and p < 0.001). We were unable to evaluate differences between Broad River and Oconee or Savannah River resting locations, because females in the Oconee and Savannah Rivers typically moved into deeper water after spawning where they were no longer visible. On one occasion, a female Robust Redhorse was observed foraging within the spawning shoal. On a few other occasions, females were observed foraging away from the spawning shoals and near their resting areas.

Robust Redhorse commonly broke the water surface as males defended territories, after spawning events, and for unknown purposes. Chases and post-spawn events typically involved individuals breaking the surface with their heads and/or dorsal fins. Another behavior involved individuals jumping out of the water head-first typically oriented upstream. During these porpoises, half to three-quarters or more of an individual's body exited the water. Porpoises typically occurred in deeper water and upstream of the spawning aggregation. Although noted frequently in the Oconee River (up to 15 porpoises in an hour), this behavior was only occasionally noted in the Broad River watershed sites (a maximum of 1 or 2 times per hour).

#### DISCUSSION

We have documented the migratory and reproductive behavior of Robust Redhorse in the Broad River watershed, a reintroduced population, and reproductive behavior in Coastal Plain populations in the Oconee and Savannah Rivers. This study has allowed the first detailed

description of spawning behavior (excluding spawning habitat measurements by Grabowski and Isley 2007, Freeman and Freeman 2000, and Straight and Freeman 2013) and documented an alternative male reproductive strategy (sneaker males). We have documented similarities between reproductive behavior in the reintroduced, Piedmont population and the wild, Coastal Plain populations, and also several differences. Most behaviors were similar between the reintroduced and Coastal Plain populations including long distance migrations, spawning participation, and evidence of spawning and wintering site fidelity. The dates and air temperature at the start of spawning season, water temperatures during spawning, and habitat measurements including, depth, and bottom velocity of spawning groups were also similar. Differences among systems include variability in migration patterns and movements among spawning sites in the reintroduced population, and higher velocity than observed in the larger river, Coastal Plain populations. The timing of start of spawning season related to moon cycle and some spawning habitat measurements also differed.

*Movements* – In the Broad River, we recorded migrations of individuals between the spawning and wintering sites, as observed in the Coastal Plain populations in the Oconee and Savannah Rivers. All of our tagged fish travelled between 55-98.5 rkm between wintering and spawning locations. However, we found no evidence that subgroups of individuals were resident near the spawning sites as inferred for the Pee Dee River by Fisk (2010). We also found less winter and spawning site fidelity than other studies of Robust Redhorse movements (Grabowski and Isley 2006; Fisk 2010; Ely 2012). All of our tagged fish were captured within the pool of a large reservoir, where they likely spent the winter, and many returned to areas within the reservoir in the following year(s). This use of reservoir habitat was a novel finding for this species. Robust Redhorse were reintroduced in the Wateree River system, South Carolina, which

also has a downstream reservoir. Tagged Robust Redhorse in the Wateree system, however, have not been documented entering the reservoir of Lake Marion (S. Lamprecht, South Carolina Department of Natural Resources, personal communication). Possible differences in environmental conditions in the reservoir, relatively shallow and warmer water, could account for this variation in behavior.

In our study, at least two individuals that wintered in the reservoir the year they were tagged wintered the following year in the lower reaches of the river, showing a potential ability to alter migration patterns dependent on environmental conditions. Mean daily flows during each month from June through December 2011 fell below the 2.5 percentile of daily flows for the same months for the period 2000-2010. These low flows may have influenced downstream movements and wintering localities of some Robust Redhorse. Wild-reared Robust Redhorse within the Savannah and Pee Dee Rivers showed fidelity of spawning and wintering sites (Grabowski and Isely 2006; Fisk 2010), as was observed for at least one other species of redhorse, Sicklefin Redhorse (Moxostoma sp., Favrot 2009). Site fidelity was also observed in winter for Black Redhorse (Bowman 1959) and during spawning season for White Sucker (Catostomus commersoni; Olson and Scidmore 1963). Alternate wintering and migration patterns may be reflected in the "resident" and "migratory" subgroups in the Pee Dee River seen by Fisk (2010). Longer studies may show that some individuals, the "residents" in the Pee Dee River, may alter their migratory strategies during particular environmental conditions and that these behaviors may be plastic rather than fixed.

In the Broad River, tagged individuals stayed within the vicinity of a spawning shoal during the whole spawning season (typically > 10 d), except for those individuals moving between sites. This behavior differed from the average of residence of 3.6 days for males at the

lower Savannah River spawning site (Grabowski and Isley 2008). The individuals moving between sites in the Broad River resided at one site a minimum of 1-2 d and as many as 14 d before moving to another. The difference among systems in residence time at a spawning site could have reflected different spawning efforts by individuals during different years, or greater energetic costs associated with spawning or migration effort in larger rivers.

*Spawning Habitat: Macrohabitat* – Robust Redhorse in the Broad River used spawning sites within the main tributaries of the Broad River as well as the mainstem for spawning. The use of small rivers above confluences with mainstems has been observed in Notchlip Redhorse, Brassy Jumprocks, Shorthead Redhorse, *M. macrolepidotum*, Black Redhorse, Golden Redhorse, *M. erythrurum*, and Greater Redhorse (C. A. S. unpublished data; Curry and Spacie 1984; Sule and Skelly 1985; Kwak and Skelly 1992; Reid et al. 2008; Favrot 2009). In the Oconee, Savannah and Pee Dee Rivers, fish used only the mainstem for spawning (Grabowski and Isely 2006; Fisk 2010; Ely 2012).

One of the questions relative to success of the Robust Redhorse reintroduction was whether, upon maturity, individuals would aggregate at particular sites to spawn. The wild populations in the Coastal Plain of Georgia were only known to spawn at one or two areas in each river, however these were the largest known areas of gravel available to the fish. Similarly, Fisk (2014) noted that the gravel used by Robust Redhorse in the Pee Dee River may have been the best available habitat rather than the preferred habitat. Thus, the spawning site fidelity shown in the wild, Coastal Plain populations (Grabowski and Isely 2006; Fisk 2010) may have reflected limited spawning habitat availability in those systems. In contrast, the Piedmont context of the Broad River provided gravel substrates at multiple locations along the length of the river traversed by individuals moving upstream from the reservoir in the spring migration. For

example, tagged individuals moved upstream through an extensive shoal in the downstream-most portion of the Broad, where the river meets the reservoir. The return of individuals in sequential years to specific sites > 80 rkm upstream of the reservoir provided evidence of site choice. The documentation of at least two tagged individuals moving between spawning shoals also indicated a hierarchical preference for sites by those individuals. These observations suggest that Robust Redhorse select particular sites to spawn. The additional evidence that at least eight tagged fish passed locations used for spawning by Robust Redhorse during the same season indicates that there may be some preference in spawning gravels. At minimum, sites must provide sufficient gravel for egg burial and for spawning fish to create the elevated tailspill of gravel to enhance oxygen infiltration of eggs (Kondolf 2000).

*Spawning Habitat: Microhabitat* – Spawning habitat in the Broad River was characterized by depths and velocities that fell outside of those suggested by Freeman and Freeman (2000) in their criteria for suitable spawning habitat based on measurements in the Oconee River system. The range of depths measured in the Broad River (0.15 - 0.88 m) were shallower and water column velocity (60% velocity) had a wider range with a higher maximum than the recommendations (i.e., depths of 0.29 - 1.1 m, velocity of 0.26 - 0.67 m/s). The column velocity was also higher than the mean (0.24 m/s, SE = 0.01) measured in the Savannah River (Grabowski and Isley 2007). Although not measured by Freeman and Freeman (2000), velocity near the substrate may also be important to spawning microhabitat suitability. Bottom velocities at spawning sites ranged from -0.14 to 0.85 m/s with a single standard deviation ranging from 0.13 - 0.51 m/s and may be a good guide for future comparisons. Because spawning events change the topography of bottom sediments, measurements at the same location at beginning and end of the spawning season will likely differ, which could account for some of the variability in this measurement. In systems where spawning aggregations are not visible, such as the Pee Dee, or before spawning aggregations are found (EA 1994) estimates of suitable habitat are typically derived from the habitat conditions at the location of the capture of reproductively ready individuals (individuals that express gametes with slight pressure, have breeding tubercles, and have lost their mucus coat (see Appendix 4.A for descriptions)). These scenarios could easily lead to erroneous assumptions about spawning habitats, e.g. we observed individuals moving between spawning aggregations during the spawning season that would exhibit reproductive characteristics while not within a spawning aggregation. Additionally, we have observed that females and some males (i.e. sneakers) also hold in in resting areas outside of the actual spawning habitat. Thus, appropriate spawning habitat could be described as the juxtaposition of resting and spawning habitat, but failing to differentiate these could bias estimates of actual spawning habitat. In all of the spawning areas in the Broad River, there were deep areas along the margin of the river or behind bedrock or large woody material for resting females. These places provided areas where there was a low energetic cost (low velocities) for females to remain stationary and the females were away from territorial males (limiting harassment by males). This complexity of habitat was not included in descriptions of suitable spawning habitat in the Pee Dee River (Fisk et al. 2014).

In Georgia rivers, spawning aggregations of some species of large-bodied catostomids, including Spotted Suckers, River Redhorse, Robust Redhorse, can be identified from a distance by clean, crescent-shaped patches of gravel, although differentiation between gravel used by these species would be difficult after individuals vacate the spawning patch. The clean patches and depressions in the spawning gravel are created through movement of gravel and the winnowing of fines during the spawning act and results in typically lighter patches of gravel in a

darker, sediment-filled matrix. When excessive fines are removed during a spawning event, a sediment plume was visible downstream of the spawning group.

*Spawning Behavior* – The primary spawning behavior difference between the Oconee and the Broad River sites was the apparent frequency of porpoising by males. Although the purpose of porpoises is unknown, these may serve as a behavioral signal to potential mates. This behavior has also been seen in Greater Redhorse (Cooke and Bunt 1999), Black Redhorse (Bowman 1970), Shorthead Redhorse (Burr and Morris 1977), and Spotted Suckers (McSwain and Gennings 1972). In most cases porpoises are associated with pools or deeper water, and the deeper water and larger pools available in the Oconee River may have provided a more conducive environment for this type of behavior, or lower visibility in deeper waters may promote this alternate form of signaling.

*Conservation Implications* – The Broad and Savannah Rivers are the only places where large spawning aggregations of Robust Redhorse, most comparable to those described by Cope (1870), are currently known. Large aggregations had been documented in the Oconee in the early years of study, but no more than 9 individuals have been observed since 2005. We presume large aggregations, as opposed to smaller groups of spawners, to be the natural reproductive organization for most territorial *Moxostoma*. As is suggested with other aggregate spawning species (e.g. White Abalone, Malakoff 1977; Blackside Dace, Rakes et al. 1999; Atlantic Cod, Rowe et al. 2004; Rainbow Smelt, Purchase et al. 2007), breeding aggregation size may be important to the reproductive success of Robust Redhorse. The Broad River additionally provides an arena for site (i.e., multiple spawning sites within a distance that could be reached in the same day) and mate choice by males and females. By comparison, spawning had only been observed at a single site in most years in the Oconee, and the two Savannah spawning sites are

separated by 16 rkm, likely restricting site choice. Mate choice is evidenced by directed positioning of females, presumably when ready to spawn, near territorial males. Sexual selection as seen through mate choice provides a mechanism for natural selection to increase fitness of individuals (Hamilton and Zuk 1982; Maynard Smith 1991; Reynolds and Gross 1992; Rowe and Hutchings 2003).

Success of the Broad River reintroduction is still uncertain. Although we have documented three times the number of spawning sites in the reintroduction watershed and numbers of spawning individuals appear promising relative to the virtual disappearance of Robust Redhorse from the Oconee River basin, we have not observed juvenile recruitment to the reintroduced population. In fact, efforts targeted to find wild-reared juvenile Robust Redhorse in all populations have been unsuccessful (Slaughter 2011; RRCC 2012; Straight and Freeman 2013). Even assuming reproduction has been successful within the Broad River system, recruitment and survival are likely both variable from year-to-year. For species with a periodic reproductive strategy (Winemiller and Rose 1992), including late age to maturity (5 or 6 years for Robust Redhorse), spawning for a relatively short period (4 weeks or less), and individuals that forego spawning in some years (Grabowski and Isley 2006), protection of a minimum density of reproductive adults, reproductive and nursery habitat, and spawning conditions may be crucial to allow the species to take advantage of periodically favorable reproductive conditions (Pavlov et al. 2007).

Using reintroductions and supplementing existing populations of Robust Redhorse has provided managers an immediate conservation action, with the benefit of increasing public awareness of species imperilment with media-friendly events (such as releasing captively-raised juveniles; Seddon et al. 2007). Reintroduction, however, may only be a stop-gap to address the

drastic decline of the Robust Redhorse, given that two historical causes of decline (overharvest and sedimentation) had already impacted the species populations and at least two suspected current causes of decline (large hydropower dams and invasive catfish) are difficult to unmanaged. Given the long life-span of Robust Redhorse (> 20 years), stocking fish into other systems across the species native range could at least provide additional time to address causes of species decline. Successfully establishing new populations, however, may hinge on identifying where conditions can support the species.

So the final question remains: was using Coastal Plain populations of Robust Redhorse appropriate for making assumptions about reintroductions in a watershed in the Piedmont region? The answer is yes and no. Reintroduced juveniles have survived to maturity and now exhibit spawning behaviors that are similar to Coastal Plain populations. Together, information from the two types of systems reinforces the importance of migration to the reproductive biology of the species. The lack of Robust Redhorse records from smaller, primarily Coastal Plain watersheds like the Ogeechee and Edisto suggests that these watersheds, lacking access to Piedmont habitats, were inappropriate for Robust Redhorse in the past. Large interconnected expanses of habitat in the Piedmont and Coastal Plain may be required to support populations in the future. However, observations from the Coastal Plain would not have predicted use of sites far upstream in the Piedmont watershed where rivers are smaller and spawning season flows lower. The assumptions that more natural flows in the Broad River provides a better spawning environment for Robust Redhorse remains untested, lacking evidence of recruitment in the system. The Broad River population provides the largest number of documented spawning sites for Robust Redhorse in any system, and except for the Savannah River, the largest known
spawning aggregations. The Broad River reintroduced population may provide one of the best chances of persistence if undisturbed by human manipulation.

#### ACKNOWLEDGMENTS

We would like to thank Dr. Stephen Divers and Dr. Jörg Mayer and advanced veterinary students, Small Animal Medicine & Surgery, University of Georgia's College of Veterinary Medicine, who performed all surgeries. Landowners generously provided access to the river along their properties. We thank our reviewers for valuable comments on this manuscript. Use of trade, product, or firm names does not imply endorsement by the U.S. Government.

#### REFERENCES

- Beck, B. B., L. G. Rapaport, M. S. Price, and A. C. Wilson. 1994. Reintroduction of captive-born animals. Pages 265-286 in Creative conservation. Springer, Netherlands.
- Biggins, D. E. and M. H. Schroeder. 1987. Historical and Present Status of the Black-Footed Ferret. Great Plains Wildlife Damage Control Workshop Proceedings. Paper 50.
- Bowman, M. L. 1959. The life history of the Black Redhorse, *Moxostoma duquesnei* (Lesueur), in Missouri. Ph.D. dissertation, University of Missouri, Columbia, Missouri.
- Bowman, M. L. 1970. Life history of the Black Redhorse, *Moxostoma duquesnei* (Lesueur), in Missouri. Transactions of the American Fisheries Society 99:546-559.
- Burr, B. M. and M. A. Morris. 1977. Spawning behavior of the Shorthead Redhorse, *Moxostoma macrolepidotum*, in Big Rock Creek, Illinois. Transactions of the American Fisheries Society 65:80-82.

- Bryant, R. T., J. W. Evans, R. E. Jenkins, and B. J. Freeman. 1996. The mystery fish. Southern Wildlife 1:26-35.
- Cooke, S. J., and C. M. Bunt. 1999. Spawning and reproductive biology of the Greater Redhorse, *Moxostoma valenciennesi*, in the Grand River, Ontario. Canadian Field-Naturalist 113:497-502.
- Cribari-Neto, F. and A. Zeileis. 2010. Beta Regression in R. Journal of Statistical Software 34(2):1-24.
- Cope, E. D. 1870. A partial synopsis of the fishes of the fresh waters of North Carolina. Proceedings of the American Philosophical Society 11(81):448-495.
- Curry, K. D. and A. Spacie. 1984. Differential use of stream habitat by spawning catostomids. American Midland Naturalist 111:267-279.
- DeMeo, T. 1998. Report of the Robust Redhorse Conservation Committee Annual Meeting. Wildlife Resources Division, Social Circle, Georgia.
- DeMeo, T. 2000. Report of the Robust Redhorse Conservation Committee Annual Meeting. Charlie Elliott Wildlife Center, Mansfield, Georgia.
- EA Engineering, Science, and Technology, Inc. 1994. Sinclair Hydroelectric Project. Relicensing Technical Studies (FERC Project No. 1951) Oconee River Instream Flow Study.
   Prepared for Georgia Power Company.
- Ely, P. C. 2012. Movement patterns, habitat use, and home range of adult Robust Redhorse Moxostoma robustum released into the Oconee River, Georgia. Prepared for the Georgia
   Department of Natural Resources, Georgia Power Company, and United States Fish and Wildlife Service.

- Favrot, S. D. 2009. Sicklefin Redhorse reproductive and habitat ecology in the Upper Hiwassee River basin of the southern Appalachian Mountains. Masters Thesis, North Carolina State University, Raleigh, North Carolina.
- Fisk, J. M. 2010. Reproductive Ecology and habitat use of the Robust Redhorse in the Pee Dee River, North Carolina and South Carolina. M.S. Thesis, North Carolina State University, Raleigh, North Carolina.
- Fisk, L. M. II, T. J. Kwak, and R. J. Heise. 2014. Modeling riverine habitat for Robust Redhorse: assessment for reintroduction of an imperiled species. Fisheries Management and Ecology 21:57-67.
- Fraser, D. J. 2008. How well can captive breeding programs conserve biodiversity? A review of salmonids. Evolutionary Applications 1:535-586.
- Freeman, B. J. and M. C. Freeman. 2001. Criteria for suitable spawning habitat for the Robust Redhorse *Moxostoma robustum*. Report to the U. S. Fish and Wildlife Service, Athens, Georgia.
- Grabowski, T.B. 2006. Reproductive ecology and seasonal migrations of Robust Redhorse (*Moxostoma robustum*) in the Savannah River, Georgia and South Carolina. PhD Dissertation, Clemson University, South Carolina.
- Grabowski, T. B. and J. J. Isely. 2006. Seasonal and diel movements and habitat use of Robust Redhorse in the Lower Savannah River, Georgia and South Carolina. Transactions of the American Fisheries Society 135:1145-1155.
- Grabowski, T. B. and J. J. Isley. 2007. Spatial and temporal segregation of spawning habitat by catostomids in the Savannah River, Georgia and South Carolina, U.S.A. Journal of Fish Biology 70:782-798.

- Grabowski, T. B. and J. J. Isely. 2008. Size of spawning population, residence time, and territory shifts of individuals in the spawning aggregation of a riverine catostomid. Southeastern Naturalist 7:475-482.
- Hamilton, W. D. and M. Zuk. 1982. Heritable true fitness and bright birds A role for parasites. Science 218:384-387.
- Jenkins, R. E., and D. J. Jenkins. 1980. Reproductive behavior of the Greater Redhorse, *Moxostoma valenciennesi*, in the Thousand Islands region. Canadian Field-Naturalist 94:426-430.
- Jepsen, N., A. Koed, E. B. Thorstad, and E. Baras. 2002. Surgical implantation of telemetry transmitters in fish: how much have we learned? Hydrobiologia 483:239-248.
- Jones, C. G. 2004. Conservation management of endangered birds. Pages 269-301 in W. J. Sutherland, I. Newton, and R. E. Green, editors. Bird Ecology and Conservation: A Handbook of Techniques. Oxford University Press, New York
- Kautz, R. 1994. Historical trends within the range of the Florida Panther. Pages 285-296 in: D.B.Jordan, ed., Proceedings of the Florida panther conference. U.S. Fish and WildlifeService; Atlanta, Georgia.
- Kleiman, D. G. 1989. Reintroduction of captive mammals for conservation. BioScience 39:152-161.
- Kondolf, G. M. 2000. Assessing salmonid spawning gravel quality. Transactions of the American Fisheries Society 129:262-281.
- Kwak, T. J. and T. M. Skelly. 1992. Spawning habitat, behavior, and morphology as isolating mechanisms of the Golden Redhorse, *Moxostoma erythrurum*, and the Black Redhorse, *M. duquesnei*, two syntopic fishes. Environmental Biology of Fishes 34:127-137.

Malakoff, D. 1997. Extinction on the high seas. Science 277:486-488.

- Maynard Smith, J. 1991. Theories of sexual selection. Trends in Ecology and Evolution 6:146-151.
- McSwain, L. E. and R. M. Gennings. 1972. Spawning behavior of the Spotted Sucker *Minytrema melanops* (Rafinesque). Transactions of the American Fisheries Society 101:738-740.
- Nichols, M. 2003. Conservation strategy for Robust Redhorse (*Moxostoma robustum*). Report prepared for the Robust Redhorse Conservation Committee.
- NRCC. 1996. Upstream: salmon and society in the Pacific Northwest. National Research Council Committee on Protection and Management of Pacific Northwest Anadromous Salmonids. National Academy Press, Washington, DC.
- Olson, D. E. and W. J. Scidmore. 1963. Homing tendency of spawning White Suckers in Many Point Lake, Minnesota. Transactions of the American Fisheries Society 92:13-16.
- Parker, W. T. 1988. A Historic Perspective of *Canis rufus* and its Recovery Potential. Red Wolf Management Series, Technical Report No. 3.
- Pavlov, D. A., N. G. Emel'yanova, and G. G. Novikov. 2009. Reproductive Dynamics. In Fish Reproductive Biology: Implications for Assessment and Management. T. Jakobsen, M. J. Fogarty, B. A. Megrey, and E. Moksness, editors. Wiley-Blackwell, Ames, Iowa.
- Purchase, C. F., D. J. Hasselman, and L. K. Weir. 2007. Relationship between fertilization success and the number of milt donors in Rainbow Smelt *Osmerus mordax* (Mitchell): implications for population growth rates. Journal of Fish Biology 70:934-946.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/.

- Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered fishes. Environmental Biology of Fishes 55:31-42.
- Reid, S., N. Mandrak, L. Carl, and C. Wilson. 2008. Influence of dams and habitat condition on the distribution of redhorse (*Moxostoma*) species in the Grand River watershed, Ontario. Environmental Biology of Fishes 81:111-125.
- Reynolds, J. D. and M. R. Gross. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata*. Proceedings of the Royal Society B 250:57-62.
- Robust Redhorse Conservation Committee (RRCC). 2002. Robust Redhorse Conservation Committee Policies. Adopted October 18, 2002.
- Robust Redhorse Conservation Committee (RRCC). 2012. Annual meeting of the Robust Redhorse Conservation Committee. Morrow Mountain State Park, Albemarle, North Carolina.
- Rowe, S. and J. A. Hutchings. 2003. Mating systems and the conservation of commercially exploited marine fish. Trends in Ecology and Evolution 18(11):567-572.
- Rowe, S., J. A. Hutchings, D. Bekkevold, and A. Rakitin. 2004. Depensation, probability of fertilization, and the mating system of Atlantic Cod (*Gadus morhua* L.). ICES Journal of Marine Science 61:1144-1150.
- Seddon, N. J., D. P. Armstrong, and R. F. Maloney. 2007. Developing the science of reintroduction biology. Conservation Biology 21:303-312.
- Shumway, C. A. 1999. A neglected science: applying behavior to aquatic conservation 55:183-201.

- Slaughter, IV, J. E. 2011. Conservation and restoration of the robust redhorse Moxostoma robustum in the Oconee River, Georgia, Volume 7. Prepared for the Federal Energy Regulatory Commission. Washington, DC.
- Snyder, N. F. R., S. R. Derrickson, S. R. Beissinger, J. W. Wiley, T. B. Smith, W. D. Toone, andB. Miller. 1996. Limitations of captive breeding in endangered species recovery.Conservation Biology 10:338-348.
- Stocker Z. S. J. and D. D. Williams. 1972. A freezing core method for describing the vertical distribution of sediments in a stream bed. Limnology and Oceanography 17:136-139.
- Straight, C. A. and B. J. Freeman. 2013. Status and assessment of Robust Redhorse (Moxostoma robustum) in the Broad River System, Georgia. Final report prepared for the Georgia Department of Natural Resources.
- Sule, M. J. and T. M. Skelly. 1985. The life history of the Shorthead Redhorse, *Moxostoma macrolepidotum*, in the Kankakee River Drainage, Illinois. Illinois Natural History Survey, Champaign Illinois, Biological Notes No. 123
- Wilcove D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607-615.
- Winemiller, K. O. and K. A. Rose. 1992. Patterns of life-history diversification in North American Fishes: Implications for population regulation. Canadian Journal of Fisheries and Aquatic Science 49:2196-2218.
- Wolcott, J. and M. Church. 1991. Strategies for sampling spatially heterogeneous phenomena: the example of river gravels. Journal of Sedimentary Petrology 61:534-543.

Table 4.1. Characteristics (watershed area and river width) at spawning sites found in the Broad River system, Oconee River, and Savannah River. Patches refer to separated areas of gravel where spawning aggregations of Robust Redhorse spawned. Not every patch of spawning gravel was used in all years. Size of patches shows ranges among patches and years. Empty cells (.) indicate data were not available for that location.

Spawning Locations	Watershed area (km <sup>2</sup> )	River width $(m)^1$	Number of patches	Size of patches (m <sup>2</sup> )
Broad River				
Broad 1	2396	33	1	75-90
Broad 2	2380	60 (15-20)	2	
Broad 3	1129	33	1	
Broad 4	1128	60 (13-30)	7	3-120
North Fork	508	27 (5-15)	3	6-56
Hudson	1168	31	3	31-50
Oconee River	11876	26-45	2	
Savannah River	29498	120	1	300

<sup>1</sup> River width varied in the Broad River because of island complexes, (ranges indicate differences in channel width at spawning patches). River width in the Oconee River changed dramatically over the period of observation, with yearly widening at the spawning site caused by bank erosion.

Table 4.2. Dates of observed spawning, total days of the spawning season, days to the first quarter moon phase (positive values indicate the start date is before the first quarter), growing degree days (GDD), day of the year, and peak count of estimated spawning Robust Redhorse in the Broad River system, Oconee River, and Savannah River. Empty cells (.) indicate that data were not available.

River	Year	Dates	Total days	Days to 1st quarter	GDD (base 50)	Day of vear	Peak count
Broad	2010	9-23 May	15	11	744	129	29
	2010	11-19 May	9	9	766	131	26
	2010	10-22 May	13	10	752	130	31
	2011	3-23 May	21	7	758	123	73
	2011	3-17 May	15	7	758	123	10
	2011	7-21 May	15	3	788	127	24
	2012	26 Apr - 7 May	12	3	901	117	23
	2012	26 Apr - 7 May	12	3	901	117	66
	2012	30 Apr - 7 May	8	-1	993	121	14
	2013	17-31 May	15	1	783	137	27
	2013	17-30 May	14	1	793	137	5+
Oconee <sup>1</sup>	1995	30 Apr - 22 May	23	7		120	50+
	1996	20-May		5		141	
	1997	10-13 May	4	4	1087	130	
	1998	10-18 May	9	-7	900	130	
	1999	27 Apr - 18 May	22	-5	878	117	
	2000	23 Apr - 4 May	12	-12	746	114	30-50+
	2001	2-15 May	14	-2	835	122	15
	2002	28-Apr		-8	929	118	16
	2003	5-May		4	904	125	7
	2004	4-8 May	5	-7	918	125	9
	2005	8-22 May	15	8	939	128	25
	2006	2-12 May	10	2	958	123	9
	2007	30 Apr - 15 May	16	-6	836	120	9
	2008	6-7 May	2	6	881	127	8
Savannah	2004 <sup>2</sup>	2-15 May	14	-5	849	123	
	2004 <sup>2</sup>	9-15 May	7	-12	994	130	
	$2005^{2}$	7-18 May	12	9	903	127	
	2005 <sup>2</sup>	9-16 May	8	7	950	129	
	2013	18 Apr - 13 May	26	0	611	108	65

 $\frac{2013 \quad 18 \text{ Apr - 13 May}}{26} \quad \frac{26}{0} \quad \frac{611}{108} \quad \frac{108}{65}$ 

Site	Ν	Temperature	Depth	Velocity 60%	Velocity bottom
		(10)	(m)	(m/s)	(m/s)
Broad River					
Broad 1	5	18.6-23.9	0.46 (0.07)	0.53 (0.2)	0.26 (0.15)
Broad 2					
Broad 3	8		0.56 (0.11)	0.58 (0.11)	0.31 (0.19)
Broad 4	86	16.5-25.6	0.48 (0.11)	0.72 (0.23)	0.33 (0.21)
North Fork	31	16.3-22	0.53 (0.11)	0.74 (0.16)	0.31 (0.18)
Hudson	39	16.7-26.8	0.42 (0.17)	0.76 (0.19)	0.32 (0.17)
Oconee River	31	17-26.7	0.51 (0.15)	0.53 (0.12)	0.3 (0.2)
Savannah River	9	17-21	0.61 (0.25)	0.67 (0.23)	0.27 (0.17)

Table 4.3. Temperature range (°C), depth, velocity at 60% depth, velocity at the bottom, and samples (N) measured at spawning sites in the Broad River system, Oconee River, and Savannah River. Empty cells (.) indicate measurements or observations were not collected for those locations. Values are means and (standard deviations).

Table 4.4. Estimates of depth, velocity at 60% depth, velocity at the bottom, and samples (N) measured at spawning sites, non-spawning sites, and female resting sites, averaged between all samples in the Broad River system and Oconee River. Values are means and (standard deviations).

Variable	Ν	Depth (m)	Velocity 60% (m/s)	Velocity bottom (m/s)
Spawning	200	0.48 (0.13)	0.69 (0.21)	0.32 (0.19)
Female resting	9	0.53 (0.15)	0.45 (0.41)	0.10 (0.21)
Non-spawning	78	0.32 (0.12)	0.73 (0.32)	0.42 (0.25)

Table 4.5. Frequency and proportion (in parentheses) of female and male Robust Redhorse participating in spawning events in the Oconee River, Broad River system, and Savannah River. Years observations were made in each system are located in Table 4.2.

Particip	oation		Locations		
Female	Male	Oconee	Broad	Savannah	Total
1	1	0	3	1	4 (0.017)
1	2	61	125	15	201 (0.87)
1	3	2	12	2	16 (0.069)
1	4	3	2	0	5 (0.022)
2	2	0	3	0	3 (0.013)
2	3	0	1	0	1 (0.004)
2	4	1	0	0	1 (0.004)
	Totals	67	146	18	231



Figure 4.1. Map of Robust Redhorse assumed historic range and known and presumed spawning locations (circles) from the Yadkin-Pee Dee River, North Carolina to the Altamaha River drainage, Georgia. Star indicates the collection location from the species' description. Inset shows the HUC 8 watersheds in Georgia where observations were made.



Figure 4.2. Map of Robust Redhorse spawning locations (solid circles), tagging / surgery location (star), wintering receiver stations in Strom Thurmond Reservoir (hollow circles), and release localities for captive-reared individuals (plus signs) in the Broad River, Georgia. The inset shows the location of the Broad River watershed in Georgia.

#### A. 2010 Robust Redhorse



Figure 4.3. Movements of (A) six Robust Redhorse captured in 2010 and (B) twelve Robust Redhorse captured in 2011. River kilometer 0 was arbitrarily placed at the tagging location in the Broad River at the edge of Strom Thurmond Reservoir. Positive numbers are upstream of the tagging location in the Broad River and negative numbers indicate movements downstream into the reservoir. The dashed gray line at river km 71 represents the lowest known spawning location within the system.

Date



Figure 4.4. Spawning triad (female flanked by one male on either side) of Robust Redhorse in the Savannah River, Georgia.

Appendix 4.A. Description of physical characteristics of Robust Redhorse during the spawning period.

*Striping* - Spawning individuals had varying degrees of striping not typically seen during other times of year, and included a heavy, dark midlateral stripe, which sometimes extended onto the face creating a mask, a dorsal stripe which was more prominent anteriorly, dorsolateral stripe (occurs halfway between the midlateral stripe and dorsal stripe) and a dark stripe or saddle over the nape of the neck. A smaller number of individuals had an additional dark patch or saddle near the insertion of the dorsal fin. Striping was most common on territorial males. Presumably younger males (determined by size relative to other territorial males), had no or limited midlateral stripes and varying degrees of red coloration in the caudal and dorsal fins.

*Tubercles* - Male Robust Redhorse have well-developed tubercles on their snout, throat, caudal fin, and anal fins and smaller tubercles on their dorsal fins, snout along the sides of their face to and around the eyes and occasionally past the nape of the neck on the back, cheek, and opercle. Females have reduced numbers and much smaller to absent tubercles on the snout, head, caudal fin and anal fins. Tubercles develop at least two weeks before the spawning season.

*Mucus Coat* - Near the start of the spawning season, Robust Redhorse lose their mucus coat. In addition to loss of the mucus coat as the spawning season progresses, some individuals (males and females) in the spawning aggregations change coloration and appear paler on the posterior parts of their bodies. The scales in this area appeared to become thickened or "cornified" causing a difference in color. During the first few days of the spawning season this characteristic is rarely seen, and was seen most often in the Broad River system approximately 5-8 days after the spawning season started. Many individuals also had visible injuries during the spawning season.

## CHAPTER 5

# EVIDENCE FOR RECRUITMENT IN A REINTRODUCED POPUATION OF AN IMPERILED CATOSTOMID, *MOXOSTOMA ROBUSTUM*<sup>1</sup>

<sup>&</sup>lt;sup>1</sup> Straight, C. A., M. C. Freeman, and B. J. Freeman. To be submitted to *Southeastern Naturalist*.

### ABSTRACT

Conservation and recovery for many imperiled species include the goal of creating and maintaining sustainable populations. Natural recruitment is essential for reaching this goal. In the southeastern United States, efforts to recover an imperiled riverine fish, the Robust Redhorse (Moxostoma robustum), have been ongoing since 1992. One of the main concerns in the recovery effort of Robust Redhorse is the absence of documented natural recruitment in most of the populations throughout its range. In this study, we assessed age-size relationships of Robust Redhorse as a tool to estimate age of captured and observed Robust Redhorse. We found that growth rate was highest in the first 5 years, prior to maturity, and that variation in growth rates resulted in a relatively wide range of lengths at a given age. As a result, the age-length relationship could not be used to estimate exact ages, however the linear relationship of age to length after maturity could be used to document occurrences of age-classes with an estimated degree of certainty. At least one captured individual was estimated to have an 84% probability of being a wild-reared fish. Size assessments along with observations of coloration and mating tactics indicative of younger fish may provide noninvasive methods for monitoring populations for natural recruitment, in support of conservation efforts.

#### **INTRODUCTION**

The number of imperiled fish species in North America has increased dramatically in the last decade (Jelks et al. 2008). The diversity of fish in the southeastern United States is the highest in the United States, however approximately 28% of southern fish species are considered imperiled (Warren et al. 2000). The main goal of many conservation efforts for imperiled species is to preserve or establish self-sustaining populations. Past and current habitat modification may

limit the ability of managers to reach that goal. Habitat destruction and degradation are the leading threats to imperiled aquatic species (Dudgeon et al. 2006). Catostomids (suckers) are one family of fishes in the United States heavily impacted by habitat destruction and other threats (Cooke et al. 2005). Several catostomid species are of conservation concern, including the Robust Redhorse (*Moxostoma robustum*), found in the southeastern United States.

The Robust Redhorse is a large-bodied (to 750 mm total length) catostomid that occurred historically in Piedmont and Coastal Plain rivers from the Yadkin-Pee Dee River system in North and South Carolina south to the Altamaha River system in Georgia. The current, native range has been reduced to approximately 30% of the species' historic range. Wild populations are currently known to persist in the Oconee, Pee Dee and Savannah Rivers, although all populations appear to comprise fewer than 200 individuals (Tanya Darden, RRCC 2011; Slaughter 2011; Fisk et al 2014). Since 2008, there has been no documented spawning in the Oconee River populations (C.A.S. personal observation). Since 2009, there have been no captures of Robust Redhorse in the Oconee River downstream of Sinclair Dam (RRCC 2013).

Initial population studies of Robust Redhorse in the Oconee River in the 1990's revealed that the population was dominated by older age classes (DeMeo 1997). Concerns for the longterm survival of the species motivated an intensive propagation program using brood stock removed from the Oconee River, with juveniles reared in hatchery ponds (Slaughter 2011). As part of this conservation effort, over 150,000 captively-reared Robust Redhorse have been released into several systems in the southeast, including the Broad River (Table 5.1; Figure 5.1; Freeman et al. 2002; RRCC 2009; RRCC 2013).

Natural resource managers set a goal of establishing six self-sustaining populations of Robust Redhorse within the species' native range (Nichols 2003). However, biologists have been

unable to confirm recruitment in either the wild or reintroduced populations. Although spawning aggregations of Robust Redhorse have been positively documented in the Broad and Savannah Rivers, spawning has not been observed in the Oconee River since 2008. Low water clarity and high flows impede attempts to locate spawning Robust Redhorse within other river systems throughout its range. Although biologists have attempted to find juvenile Robust Redhorse in several river systems, only one wild-reared juvenile (< 400 mm total length) has been collected (in the Savannah River). Individuals reach sexual maturity at 5-6 years or around 400-450 mm total length (B.J.F.; RRCC Oconee River TWG 2010). Lacking capture of juvenile Robust Redhorse, it is unknown at what stage the recruitment bottleneck occurs. Appraising the success of conservation and reintroduction efforts for this species depends in part on documenting recruitment of individuals into the reproductive population.

We have studied the population of Robust Redhorse established by reintroductions in the Broad River. Our purpose here is to evaluate evidence for recruitment into the reproductive population, using observations of individuals captured during 2010 and 2011 as part of a study of migration and reproduction (Straight et al. in prep.). One line of evidence of recruitment would be the capture of Robust Redhorse lacking a coded-wire tag (CWT) or a passive integrated transponder (PIT). All released individuals have been given one of these tags (usually CWTs, in a position on the body indicating year class) at the time of release. However, there has been documented loss of both types of tags, with loss rate potentially as high as 30% (J. Evans, RRCC 2013), which creates difficulties in determining whether a captured individual is wild- or captively-reared. A second line of evidence applies to systems, including the Broad River, where captive release programs of Robust Redhorse have ceased, so that capture of individuals that are younger than the last released year class would document natural recruitment. In this study we have used size measurements of Robust Redhorse captured in the Broad River to assess evidence of recruitment. Generally, being able to age Robust Redhorse in systems where reintroductions or population augmentation has occurred could provide valuable information for species management. Age can reliably be estimated for many species of fish using scale annuli. However, aging catostomids with scale annuli has commonly been determined to underestimate age (Beamish 1973; Beamish and McFarlane 1983; Scoppettone 1988; Jenkins 1999; Sylvester and Berry 2006; Quist et al. 2007). Additionally, managers may wish to assess recruitment without sacrificing or harming individual fish (as would be necessary to age fish using internal or skeletal structures). Therefore, we have asked whether sizes of recaptured individuals provide evidence of recruitment within the Broad River population. Specifically, our objectives are to 1) assess the age-length and age-weight relationships of captured Robust Redhorse, 2) estimate the probability of age based on total length of knownaged individuals, and 3) discuss alternate methods for documenting recruitment.

#### **METHODS**

We obtained total lengths (mm) and weights (g) of known-aged recaptured Robust Redhorse from our previous studies and the Robust Redhorse Conservation Committee collection database from river systems in Georgia (Figure 5.1). Age was based on coded-wire tag position (indicating year-class of a propagated and stocked fish) and the river basin in which the individual was captured. Although captive-rearing may have influenced growth rates, most individuals were only in captivity one year or less, and almost all were in captivity 2 years or less. We removed four individuals from analyses that spent more than 2 years in hatchery ponds prior to their release. Because pre- and post-maturation growth rates typically differ for

catostomids (Bowman 1970; Meyer 1962; Wakefield and Beckman 2005; Bettinger and Crane 2011), we used individuals five or more years old for our analyses (based on assumed maturation at 5 years of age). We performed a linear regression relating age to length and weight to assess which measurement would be more likely to accurately predict age of an individual.

To evaluate the probability that a captured Robust Redhorse without a detectable codedwire tag could be a new recruit to the population, we used logistic regression to model the effects of total length on the odds of a fish being less than 6, 8, 10, or 12 years old. A fish less than 12 years old in 2010 in the Broad River would necessarily have been wild-spawned; a fish less than 6 years old could represent a second-generation recruit to the population (Table 5.2). Thus, in sequential analyses, we classified each individual in the dataset of known age fish as younger or older than the target age (6, 8, 10 or 12 years) and fit a logistic regression for the probability of being younger than the target age as a function of total length. We used a Bayesian analysis with uninformative, normally distributed priors for the intercept and length coefficient. For this analysis we standardized lengths by subtracting the grand mean and dividing by the standard deviation. Regression parameters, and the probability that an individual of length 450 to 700 mm (by 1 mm increments for creating probability curves and estimating probability of an individual being less than 12 years old for cross-validation analysis and 10 mm increments for summaries) was less than the target age were estimated using Open BUGS (Lunn et al. 2009), with 200,000 Markov chain Monte Carlo (MCMC) iterations, a 100,000 burn-in, and three chains. We inspected chains to assessed convergence using the Gelman-Rubin diagnostic (Gelman and Rubin 1992) and convergence occurred within the burn-in period.

We used 15 known-age (based on coded-wire tag position) individuals captured for a movement study in the Broad River (Straight et al. in prep) to assess the ability of our logistic

regression to correctly classify individuals as captively-reared (>12 years old). We tested the model accuracy by leaving one of the 15 individuals out of the analysis, re-running the model, and predicting the probability of the held individual of being less than 12 years old. We then used the regression parameters for the model fit to all individuals to estimate the posterior probability that each of 5 individuals captured in the Broad River without a detectable codedwire tag was wild-reared (<12 years old). These 5 individuals included the smallest individual we captured. All posterior probabilities were estimated using MCMC analyses as above.

#### RESULTS

We identified 191 fish that were collected from the Broad, Ocmulgee, Oconee, and Ogeechee River systems for which we could determine age based on coded-wire tag position. Eighty-nine fish were 5 or more years old and had measurements of total length (mm). These fish showed a slowing in the rate of growth (total length and weight) as age increased (Figure 5.2), however growth appeared approximately linear after age five. For fish five or more years old, age explained more variation in length ( $R^2 = 0.61$ ) than in weight ( $R^2 = 0.48$ ; Figure 5.3). Therefore, we used length for our Bayesian analysis (below). We noted, however, considerable variation in length at age even for fish over 5 years. For example, the dataset included an age 6 individual as large as 601 mm, and an age 14 fish as small as 555 mm.

The estimated probability that a Robust Redhorse captured in the Broad River could be wild-reared (i.e., less than 12 years old) declined with increasing total length (Table 5.3; Figure 5.4). Based on these regressions, individuals greater than about 600 mm would almost certainly be older than 12, whereas individuals less than about 520 mm would almost certainly be younger than 12. Estimated probabilities were less certain for individuals between 520 and 600 mm.

Twenty Robust Redhorse captured in the Broad River in 2010 and 2011 ranged from 535 to 690 mm total length (Table 5.4). The model based on known-aged fish correctly classified (i.e., as having a less than 50% mean probability of being younger than 12 years old) 9 out of 15 individuals with coded-wire tags. Actual ages were 13 or 14 years (Table 5.2). Six individuals (ages 13 and 14 y) had estimated mean probabilities of over 50% of being less than 12 years old, indicating they could have been incorrectly classified as wild-reared. Estimated probabilities that the five fish in which a coded-wire tag could not be detected were wild-reared ranged from 7% for a 620 mm individual, to 84% for the smallest individual captured (535 mm). For this individual and the next smallest fish (544 mm, Table 5.2) of unknown age, credible intervals for the probability of being less than 12 years old were entirely above 60%.

#### DISCUSSION

Using an analysis of total length versus age in known age, captively-reared Robust Redhorse that were recaptured after release to the wild, we have estimated the likelihood that individuals of unknown age captured in the Broad River, Georgia, could represent natural recruitment. We estimated that an individual smaller than about 520 mm at the time of this study would almost certainly have been wild-reared, and that at least one captured individual was highly likely to have been wild-reared.

There was substantial variation in both the age-length and age-weight relationships for Robust Redhorse. Most catostomids show variability in their length and weight as they develop, but typically grow more quickly prior to sexual maturation (Bowman 1970; Meyer 1962; Wakefield and Beckman 2005; Bettinger and Crane 2011). All of the Robust Redhorse used in this study were captively propagated, and differences in the amount of time spent in hatchery

ponds may have caused some of the variability in size at a given age. For example, four outliers with respect to weight at age spent at least twice as long in hatchery ponds as other individuals. Sex may also account for some variation, although we have not assessed differences between males and females because many individuals in the database were not identified by sex. Seasonal and yearly fluctuations in resources and differences in reproductive condition (Bettinger and Crane 2011) could also contribute to variation in size-age relationships. Although the best relationship was between age and length, there may be other measurements that could estimate age more accurately than total length. For example, the ratio of head length to body length has proven helpful for estimating age of Cui-ui (*Chasmistes cujus*; Scoppettone et al. 1986). Additional lengths and possibly other measurements for known-aged and sexed Robust Redhorse could reduce uncertainty in age-size relationships.

An accurate and precise age-size relationship would support current efforts to document recruitment in populations of Robust Redhorse. Aging catostomids using scale annuli has proven unreliable for mature fish (Reid 2007; Scoppettone 1988), including for Robust Redhorse and at least one other species of *Moxostoma* (Jenkins et al. 1998; Jenkins 1999). Aging using alternative structures, e.g. pectoral fin rays, may be more accurate than scale-aging (Scidmore and Glass 1953; Curry and Spacie 1984; Sylvester and Berry 2006; Labay et al. 2011), but requires a large sample of fin (2 cm) and may require sacrificing fish for validation purposes. When possible, developing genetic markers to distinguish second and later generation individuals from the stocked generation would be ideal. For example, developing and cataloging molecular markers for the Oconee River parents of the juveniles released in the Broad River could allow the positive identification of all natural recruitment within the system (T. Darden, South Carolina Department of Natural Resources pers. comm.). Presently, however, resource agency personnel are primarily

relying on size of recaptured individuals to evaluated evidence for recruitment (J. Evans, Georgia Department of Natural Resources, pers. comm.).

The easiest time to capture Robust Redhorse is when the fish are migrating to or aggregated at spawning shoals. Although electrofishing has been used to collect broodstock for conservation efforts, electrofishing within the spawning aggregation incurs a risk of harming eggs and larval Robust Redhorse and increasing stress of individuals already stressed by spawning activities, and reducing egg survival in gravid females (Bonga 1997; Muth and Ruppert 1997; Snyder 2003). There have been no long-term studies to estimate the potential effects of shocking and handling fish prior to and during spawning. To reduce impacts of electrofishing on spawning trout, for example, Alberta, Canada's, Fisheries Management Division limits electrofishing of spawning females when alternate methods can be used, and prohibits any shocking on shoals containing developing trout embryos (Alberta Government 2013). Because effects of electrofishing Robust Redhorse in spawning shoals has yet to be fully explored or considered, minimizing disturbance during the spawning season may be desirable.

The length at age analysis developed in this study, although variable, does provide a basis for estimating threshold lengths for documenting recruitment within the Broad River system. Our analysis can easily be updated to estimate the maximum length at which an individual would have a high likelihood of being wild-reared. When spawning areas have sufficient water visibility, a weighted scale (rebar with 10 cm segments marked) could be placed in the vicinity of smaller individuals suspected to be recruits. As suspected recruits move into range of the scale marker, a photograph could be taken to document approximate size of the individual. If needed the image could be placed into image analysis software to make more accurate estimates of size. Although water quality may be insufficient in every year or every system to make estimates this

way, of six years of study in the Broad River four years would have had sufficient water clarity to estimate size using this method.

There may be other ways to collect evidence of younger fish that do not require electrofishing or handling individuals. Based on observations in the Oconee and Savannah Rivers, smaller and presumably younger male Robust Redhorse have enhanced crimson coloration in the fins, although the exact age at which this coloration is lost is unknown (B.J. Freeman personal observation). Occurrence of particularly red-finned males in the Broad River could be taken as evidence of young fish in the population. Another method to document recruitment may be to identify behavioral differences between recently matured individuals into the spawning aggregation relative to older, typically larger fish in the aggregation. In particular, we have witnessed an alternate mating tactic by small, red-finned Robust Redhorse males on one and two occasions in the Oconee and Broad Rivers, respectively. This alternate mating tactic is for males to forego holding territories or competing within a spawning aggregation in favor of attempting to follow females and join in spawning events as they occur (referred to as sneakers or kleptogamy). In species that form spawning aggregations and when there is a large intrasexual size difference, there is a cost to holding and defending territories. Smaller, likely younger, males that attempt to hold a territory may not participate in many spawns because of an inability to compete for prime spawning habitat with larger males, and smaller male redhorse are commonly seen holding territories in the marginal areas of the spawning aggregation (C.A.S. and B.J. F. personal observations; Bowman 1970; Kwak and Skelly 1992). Smaller individuals, therefore, may choose an alternate tactic to increase the possibility of participating in spawning events and increase their reproductive fitness as a sneaker (Taborski 2008). Alternative reproductive strategies are common in salmonid species, and parasitic exploitation (participating in spawning

events by nearby males) occurs in many other species of fish including Robust Redhorse and other catostomids (Reighard 1920; Gross 1984; Taborsky 1994 and references therein; Straight et al. in prep.). Forms of alternate reproductive strategies, like sneakers, tend to occur in species that have large intrasex size difference or those that mature at a young age (Shuster and Wade 2003; Taborsky 2008). In salmonids, sneakers are commonly younger, 2-year old, males (Gross 1984). Observing kleptogamy may not prove recruitment of younger individuals to the spawning population, because for some species a sneaker tactic is a fixed strategy, where some individuals remain small or mimic female condition or coloration throughout their life (Oliveira et al. 2008). However, if it can be confirmed that this is a strategy for younger individuals in redhorses, documentation of this behavior could be support for younger individuals entering the reproductive population. Until it can be determined if the sneaker tactic in male Robust Redhorse is a strategy for smaller individuals rather than a genetic or fixed trait, caution should be used when estimating age based on length of individuals in these smaller size classes.

Although we cannot confirm that recruitment is occurring in the Broad River system, the presence of smaller, red-finned individuals at several spawning aggregations, and individuals participating in alternate reproductive tactics is evidence in support of recruitment within the system. The capture of at least one individual with a 0.84 probability of being wild-reared also provides evidence of recruitment. If mortality rates increase in the last years of life for Robust Redhorse as seen in White Suckers (*Catostomus commersonii*; Wakefield and Beckman 2005), we will begin to see a rapid decline in oldest year classes of released individuals in the next 5 to 10 years. If the maximum lifespan of Robust Redhorse is 25 years, then the majority of released individuals will be deceased by 2022, whereas if the average lifespan is only 20 years, most released individuals will be deceased in the next 3 years. Regardless of the average or maximum

life span, the Broad River system has appeared to provide a diverse assortment of habitat and multiple spawning aggregations for individuals to increase fitness through habitat choice, mate choice, and alternate reproductive strategies (Straight et al. in prep). Because of our limited knowledge of the mechanisms that may play a role in reproductive success, i.e. behavioral, aggregation size, stress, and recruitment, limited manipulation coupled with non-invasive observation of this population over the next 10 years could provide information on population processes as the numbers of released individuals decline through natural mortality.

#### ACKNOWLEDGMENTS

We would like to thank the Robust Redhorse Conservation Committee for use of the capture data for use in this study. We thank our reviewers for valuable comments on this manuscript. Use of trade, product, or firm names does not imply endorsement by the U.S. Government.

#### REFERENCES

- Alberta Government. 2013. Alberta Fisheries Management Division Electrofishing Policy Respecting Injuries to Fish. Copyright 2013 Government of Alberta, Canada.
- Beamish, R. J. 1973. Determination of age and growth of populations of the White Sucker (*Catostomus commersoni*) exhibiting a wide range in size at sexual maturity. Journal of the Fisheries Research Board of Canada 30:607-616.
- Beamish, R. J. and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112:735-743.

- Bettinger, J.M. and J.S. Crane. 2011. Validation of annulus formation in otoliths of Notchlip Redhorse (*Moxostoma collapsum*) and Brassy Jumprock (*Moxostoma* sp.) in Broad River, South Carolina, with observations on their growth and mortality. Southeastern Naturalist 10:443-458.
- Bonga, S. E. W. 1997. The stress response in fish. Physiological Reviews 77:591-625.
- Bowman, M. L. 1970. Life history of the Black Redhorse, *Moxostoma duquesnei* (Lesueur), in Missouri. Transactions of the American Fisheries Society 99:546-559.
- Cooke, S. J., C. M. Bunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and D. F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers (Catostomidae) in North America: insights from regional case studies of a diverse family of non-game fishes. Biological Conservation 121:317-331.
- Curry, K. D. and A. Spacie. 1984. Differential use of stream habitat by spawning catostomids. American Midland Naturalist 111:267-279.
- DeMeo, T. 1997. Report of the Robust Redhorse Conservation Committee Annual Meeting. Wildlife Resources Division, Social Circle, Georgia.
- Dudgeon D., A. H. Arthington, M. O. Gessner, Z-I. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006.
  Freshwater biodiversity: importance, threats, status and conservation challenges.
  Biological Reviews 81:163-182.
- Fisk, L. M. II, T. J. Kwak, and R. J. Heise. 2014. Modeling riverine habitat for Robust Redhorse: assessment for reintroduction of an imperiled species. Fisheries Management and Ecology 21:57-67.

- Freeman, B. J., C. A. Straight, J. R. Knight, and C. M. Storey. 2002. Evaluation of Robust Redhorse (*Moxostoma robustum*) introduction into the Broad River, GA spanning years 1995-2001. Report Submitted to Georgia Department of Natural Resources.
- Gelman, A. and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. Statistical Science 7:457-472.
- Gross, M. R. 1984. Sunfish, salmon, and the evolution of alternative reproductive strategies and tactics in fishes. In Fish reproduction: strategies and tactics. Edited by R.J. Wootton and G.W. Potts. Academic Press, London.
- Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A.
  Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A.
  Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren Jr. 2008.
  Conservation status of imperiled North American freshwater and diadromous fishes.
  Fisheries 33:372-407.
- Jenkins, R. E. 1999. Sicklefin Redhorse *Moxostoma* sp., undescribed species of sucker (Pisces, Catostomidae) in the upper Tennessee River drainage, North Carolina and Georgia – description, aspects of biology, habitat, distribution, and population status. Report to the U.S. Department of Interior, Fish and Wildlife Service, Asheville, North Carolina, and the North Carolina Wildlife Resources Commission, Raleigh, North Carolina.
- Jenkins, R. E., M. D. Clements, and S.C. Moore. Unpublished manuscript, 1998. Synopsis of age, growth, and maturation of the Robust Redhorse *Moxostoma robustum*.
- Kwak, T. J. and T. M. Skelly. 1992. Spawning habitat, behavior, and morphology as isolating mechanisms of the Golden Redhorse, *Moxostoma erythrurum*, and the Black Redhorse, *M. duquesnei*, two syntopic fishes. Environmental Biology of Fishes 34:127-137.

- Labay, S. R., J. G. Kral, and S. M. Stukel. 2011. Precision of age estimates derived from scales and pectoral fin rays of blue sucker. Fisheries and Management Ecology 18:424-430.
- Lunn, D., D. Spiegelhalter, A. Thomas, and N. Best. 2009. The BUGS project: Evolution, critique and future directions (with discussion). Statistics in Medicine 28:3049-3082.
- Meyer, W. H. 1962. Life history of three species of redhorse (*Moxostoma*) in the Des Moines River, Iowa. Transactions of the American Fisheries Society 91:412-419.
- Muth, R. T. and J. B. Ruppert. 1997. Effects of electrofishing fields on captive embryos and larvae of Razorback Sucker. North American Journal of Fisheries Management 17:160-166.
- Nichols, M. 2003. Conservation strategy for Robust Redhorse (*Moxostoma robustum*). Report prepared for the Robust Redhorse Conservation Committee.
- Oliveira, R. F., M. Taborsky, H. J. Brockmann, eds. 2008. Alternative Reproductive Tactics: an integrative approach. Cambridge University Press, NewYork.
- Quist, M. C., Z. J. Jackson, M. R. Bower, and W. A. Hubert. 2007. Precision of hard structures used to estimate age of riverine catostomids and cyprinids in the upper Colorado River Basin. North American Journal of Fisheries Management 27:643-649.
- Reid, S. M. 2007. Comparison of scales, pectoral fin rays and opercles for age estimation of Ontario Redhorse, *Moxostoma*, species. Canadian Field-Naturalist 121:29-34.
- Reighard, J. 1920. The breeding behaviors of the suckers and minnows. I. The suckers. Biological Bulletin 38:1-33.
- Robust Redhorse Conservation Committee (RRCC). 2009. Report of the Robust Redhorse Conservation Committee Annual Meeting. Compiled by J. Zelko. Web Wildlife Management Area, Garnett, South Carolina.

- Robust Redhorse Conservation Committee (RRCC). 2011. Annual meeting of the Robust Redhorse Conservation Committee. Morrow Mountain State Park, Albemarle, North Carolina.
- Robust Redhorse Conservation Committee (RRCC). 2013. Robust Redhorse Conservation Committee Annual Meeting. Charlie Elliott Wildlife Center, Mansfield, Georgia.
- Robust Redhorse Conservation Committee (RRCC) Oconee River Technical Working Group (TWG). 2010. Management Plan for the Oconee River Robust Redhorse Population.
- Scidmore, W. J. and A. W. Glass. 1953. Use of pectoral fin rays to determine age of the White Sucker. Progressive Fish-Cultutrist. 15:114-115.
- Scoppettone, G. G., M. Coleman, and G. A. Wedemeyer. 1986. Life history and status of the endangered Cui-ui of Pyramid Lake, Nevada. U.S. Fish and Wildlife Service, Fish and Wildlife Research.
- Scoppettone, G. G. 1988. Growth and longevity of the Cui-ui and longevity of other catostomids and cyprinids in western North America. Transactions of the American Fisheries Society 117:301-307.
- Shuster, S. M. and M. J. Wade. 2003. Mating Systems and Strategies. Princeton University Press, Princeton, New Jersey.
- Slaughter, J.E. IV. 2011. Conservation and Restoration of the Robust Redhorse Moxostoma robustum in the Oconee River, Georgia. Volume 7. Report prepared for the Federal Energy Regulatory Commission by Georgia Power.
- Snyder, D. E. 2003. Electrofishing and its harmful effects on fish. Information and Technology Report USGS/BRD/ITR--2003-0002: U.S. Government Printing Office, Denver, Colorado.

- Sylvester R. M., and C. R. Berry, Jr. 2006. Comparison of White Sucker age estimates from sales, pectoral fi n rays, and otoliths. North American Journal of Fisheries Management 26:24-31.
- Straight, C. A. and B. J. Freeman. 2013. Status and assessment of Robust Redhorse (*Moxostoma robustum*) in the Broad River System, Georgia. Final report prepared for the Georgia Department of Natural Resources.
- Straight, C. A., B. J. Freeman, and M. C. Freeman. In Preparation. Comparisons of reproductive biology of an imperiled migratory fish in a Piedmont and two Coastal Plain rivers.
- Taborsky, M. 1994. Sneakers, satellites, and helpers: parasitic and cooperative behavior in fish reproduction. Advancements in the Study of Behavior 23:1-100.
- Taborsky, M. 2008. Alternative Reproductive Tactics in Fish. In R. F. Oliveira, M. Taborsky, H.J. Brockmann, eds. Alternative Reproductive Tactics: an integrative approach.Cambridge University Press, New York.
- Wakefield, C. K. and D. W. Beckman. 2005. Life history attributes of White Sucker (*Catostomus commersoni*) in Lake Taneycomo and associated tributaries in southwestern Missouri.
   The Southwestern Naturalist 50:423-434.
- Warren, M. L., B. M. Burr, S. J. Walsh, H. L. Bart, R. C. Cashner, D. A. Etnier, B. J. Freeman,
  B. R. Kuhajda, R. L. Mayden, H. W. Robison, S. T. Ross, and W. C. Starnes. 2000.
  Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries 25(10):7-31.

asses released in t	the Broad Rive	er system.	
River syst	em	Year class	
	Year class	number	Total Number <sup>1</sup>
Broad Riv	er		33,743
	1993	545	
	1995	1424	

> 4,200

13,734

43,048

> 65,000

To be stocked in near future

Table 5.1. Number of Robust Redhorse (*Moxostoma robustum*) 1995-2011 with a break-down of year classes released in the Broad River system.

<sup>1</sup> Numbers for the Broad River taken from Straight and Freeman 2013; number for all other systems taken from Slaughter 2011.

29048

2726

1997

1998

Oconee

Ocmulgee

Ogeechee

Pee Dee

Broad / Wateree (SC)

Table 5.2. Estimated years of first reproduction by Robust Redhorse (*Moxostoma robustum*) in the Broad River system. The first reproductive year is for reintroduced individuals, and the 1<sup>st</sup> and 2<sup>nd</sup> generations would be wild-born offspring of those reintroduced individuals. Estimates assume reproductive maturation at five years.

Year	First estimated	1 <sup>st</sup> Generation	2 <sup>nd</sup> Generation
class	reproductive year	reproductive year	reproductive year
1993	1998	2003	2008
1995	2000	2005	2010
1997	2002	2007	2012
1998	2003	2008	2013

Total length	Probability < 12	Probability < 10	Probability < 8	Probability < 6
(mm)	years old	years old	years old	years old
450	1 (0.98-1)	1 (0.99-1)	0.98 (0.94-1)	0.91 (0.8-0.98)
460	0.99 (0.97-1)	1 (0.99-1)	0.97 (0.92-1)	0.86 (0.72-0.95)
470	0.99 (0.96-1)	1 (0.98-1)	0.96 (0.89-0.99)	0.78 (0.63-0.91)
480	0.99 (0.95-1)	0.99 (0.97-1)	0.94 (0.85-0.99)	0.68 (0.51-0.83)
490	0.98 (0.93-1)	0.99 (0.95-1)	0.9 (0.8-0.97)	0.55 (0.38-0.72)
500	0.97 (0.9-1)	0.98 (0.92-1)	0.85 (0.73-0.95)	0.42 (0.26-0.58)
510	0.95 (0.87-0.99)	0.96 (0.87-0.99)	0.78 (0.64-0.9)	0.29 (0.15-0.45)
520	0.92 (0.82-0.98)	0.92 (0.81-0.98)	0.69 (0.53-0.82)	0.2 (0.08-0.34)
530	0.87 (0.75-0.96)	0.85 (0.7-0.95)	0.57 (0.41-0.72)	0.13 (0.04-0.25)
540	0.81 (0.66-0.92)	0.74 (0.56-0.88)	0.44 (0.28-0.61)	0.08 (0.02-0.18)
550	0.71 (0.54-0.85)	0.58 (0.37-0.76)	0.33 (0.17-0.5)	0.05 (0.01-0.13)
560	0.6 (0.4-0.77)	0.4 (0.19-0.61)	0.23 (0.1-0.4)	0.03 (0-0.09)
570	0.47 (0.26-0.66)	0.25 (0.08-0.47)	0.16 (0.05-0.31)	0.02 (0-0.06)
580	0.35 (0.15-0.56)	0.15 (0.03-0.34)	0.1 (0.02-0.24)	0.01 (0-0.04)
590	0.24 (0.08-0.46)	0.08 (0.01-0.24)	0.07 (0.01-0.18)	0.01 (0-0.03)
600	0.17 (0.04-0.37)	0.05 (0-0.16)	0.05 (0.01-0.14)	0 (0-0.02)
610	0.11 (0.02-0.29)	0.03 (0-0.11)	0.03 (0-0.1)	0 (0-0.02)
620	0.07 (0.01-0.22)	0.01 (0-0.07)	0.02 (0-0.07)	0 (0-0.01)
630	0.05 (0-0.17)	0.01 (0-0.05)	0.01 (0-0.05)	0 (0-0.01)
640	0.03 (0-0.12)	0 (0-0.03)	0.01 (0-0.04)	0 (0-0)
650	0.02 (0-0.09)	0 (0-0.02)	0.01 (0-0.03)	0 (0-0)
660	0.01 (0-0.07)	0 (0-0.01)	0 (0-0.02)	0 (0-0)
670	0.01 (0-0.05)	0 (0-0.01)	0 (0-0.01)	0 (0-0)
680	0.01 (0-0.04)	0 (0-0)	0 (0-0.01)	0 (0-0)
690	0 (0-0.03)	0 (0-0)	0 (0-0.01)	0 (0-0)
700	0 (0-0.02)	0 (0-0)	0 (0-0.01)	0 (0-0)

Table 5.3. The probability that an individual fish with the given total length (mm) is less than 6, 8, 10, and 12 years old. Values are means (credible interval).
Table 5.4. Mean probability of Robust Redhorse captured in the Broad River in 2010 and 2011 of being less than 12 years old. Known age is based position of coded wire tag, when detected. NA indicates a coded wire tag was not detected. TL is total length (mm) at time of capture. ID is the serial code of implanted sonic transmitter for each individual.

ID	Known	TL	Mean probability of being < 12
	age		(95% credible interval)
53959	NA	535	0.84 (0.71-0.94)
53955	NA	544	0.77 (0.61-0.90)
53975	NA	563	0.55 (0.36-0.74)
53956	NA	566	0.52 (0.32-0.71)
53963	NA	620	0.07 (0.01-0.22)
53965	13	541	0.82 (0.68-0.93)
53958	14	555	0.68 (0.5-0.83)
53962	13	563	0.58 (0.38-0.76)
53972	14	564	0.57 (0.37-0.75)
53971	14	564	0.57 (0.37-0.75)
53966	14	566	0.54 (0.34-0.73)
53961	13	570	0.49 (0.28-0.69)
53951	14	579	0.38 (0.17-0.59)
53964	13	590	0.26 (0.08-0.48)
53949	13	608	0.13 (0.02-0.32)
53977	14	610	0.12 (0.02-0.3)
53974	14	615	0.1 (0.01-0.27)
53948	13	624	0.07 (0.01-0.21)
53967	14	662	0.01 (0-0.06)
53953	14	690	0 (0-0.03)



Figure 5.1. Map of the historic presumed range of Robust Redhorse in the southeastern United States from the Yadkin - Pee Dee River drainage (North Carolina/ South Carolina) south to the Altamaha River drainage (Georgia). Presumed spawning locations are marked with squares and the locality where the original collection of Robust Redhorse occurred in the Yadkin River marked with a star.



Figure 5.2. Relationship of weight (triangles) and total length (circles) to age, based on knownage Robust Redhorse recaptured after release to the wild. Age 1 fish were measured upon removal from a hatchery. The two points marked with asterisks are weights for four fish that spent three to six years at the hatchery prior to stocking and showed elevated weights for their age class. Lines represent logarithmic trendlines with age.



Figure 5.3. Total length (circles) and weight (triangles) of known age Robust Redhorse from 5 to 14 years old. Lines represent linear relationship between length or weight and age.



Figure 5.4 Mean probability and credible interval that an individual Robust Redhorse from 450 - 700 mm total length is A. less than 12 years old, B. less than 10 years old, C. less than 8 years old, and D. less than 6 years old.

## **CHAPTER 6**

## CONCLUSIONS

Causes of decline of many imperiled aquatic species, including Robust Redhorse, include multiple factors influencing all life history stages (Wilcove et al. 1998; Cooke et al. 2005; Dudgeon et al. 2006). The Robust Redhorse is just one species where conservation efforts have included reintroductions and releases to augment natural populations. In the original description, Cope (1870) notes that the migration runs of large-bodied catostomids in North Carolina rivers were likely already reduced by half as early as 1869 and laments the difficulty and costs associated in restoring a population after its decline rather than protecting it. In the 1900's, new threats to aquatic species emerged with construction of dams that impede migration upstream to spawning habitat and alter river hydrology, sediment regimes, flooding and access to floodplain habitats, water temperature, and chemistry downstream. Additionally, poor land use practices increased sediment loads in rivers across the southeast (Trimble 1974; Jackson et al. 2005) with deleterious effects on native biota dependent on coarse substrata free of fine sediments (Waters 1995; Kondolf 2000; Jennings et al. 2010). Introduced species have also been implicated in the decline of Robust Redhorse. In particular, non-native Flathead Catfish (*Pylodictis olivaris*) are hypothesized to prey on immature suckers. The conservation of Robust Redhorse, as stated in Cope's 1870 manuscript, "involves much time, attention and expense." Although multiple factors have likely contributed to decline of the Robust Redhorse, the apparent lack of recruitment in extant, wild populations suggests that reproductive failure presently limits recovery of the species. Increasing our knowledge of reproductive biology should provide

invaluable information for managers about potential variation in behavior and habitat use by reproductive individuals. My study has documented (a) a new method to assess spawning frequency in species of large-bodied catostomids when visual observations can not be made (Chapter 2), (b) spawning frequencies and diel periodicity of Robust Redhorse in two river systems (Chapter 3), (c) reproductive and migratory behavior of Robust Redhorse in a reintroduced population compared to two wild, Coastal Plain populations (Chapter 4), and (d) evidence of recruitment in a reintroduced population of Robust Redhorse (Chapter 5).

Known spawning locations for wild Robust Redhorse are limited to two primary sites in each of the Oconee, Savannah, and Pee Dee Rivers. Robust Redhorse have been reintroduced into the Broad River system, and "introduced" in the Ogeechee River system. The Ogeechee is not considered to be part of the species' original range and has limited spawning habitat (B. J. Freeman, personal communication; DeMeo 1997; Slaughter 2011). The population introduced into the Ogeechee River is thus considered a refugial population for research and for holding individuals for use in future augmentation elsewhere. In contrast, the reintroduction in the Broad River has been intended to establish a self-sustaining population in a river with limited hydrological alterations by dams and has allowed Robust Redhorse access to areas in the Piedmont region that likely included their historic spawning grounds in the Savannah River basin. This reintroduction has allowed study of a unique population of Robust Redhorse for comparison to other river systems.

Limitations in making visual observations of spawning large-bodied catostomids in southeastern rivers include poor water clarity, large variability in flows downstream of hydropower facilities, lack of personnel, or other constraints. Using a hydrophone and recorder, spawning activity of Robust Redhorse and River Redhorse could be detected with a high degree

of accuracy (Chapter 2). This method generates a large amount of data on the spawning frequency and duration, potentially useful for assessing effects of variable environmental conditions on spawning behavior when direct observations cannot be conducted. Although I have verified all spawning events recorded in this study, I also have shown that an automated detector using commercially available software can correctly identify at least 80% of known spawning events, with a corresponding low rate (6-7%) of falsely "identified" spawns. This method could provide manager and researcher with a way to document spawning using a device that requires minimal set-up time, minimal manipulation once deployed, and an automated method to define spawning events.

Using acoustic recordings, this study has provided the first documentation of spawning rates and intensity throughout a large portion of the spawning season and at all hours of the day for two species of redhorse species, Robust and River Redhorse (Chapter 3). Both species spawn actively at night, when observations are limited or not possible. At Robust Redhorse spawning sites in the Broad and Savannah Rivers, spawning rates were highest after midnight and into the early morning hours. In addition to time of day, water temperature and moonlight appeared to influence spawning rates the most. In systems with regulated hydrology, alterations of water temperature (e.g., by hypolemnetic reservoir releases) after the spawning season has commenced could influence the fecundity of individuals if spawning rates slow or cease during periods of low temperature, causing over maturation of eggs. Conversely, reduced hatching success and increased deformities could result if water temperatures increase over a critical limit (Walsh et al. 1998; Pavlov et al. 2009). Using data spanning the majority of the spawning season (9 d) in the Broad River, over 60% of the spawning events occurred in only four days. This finding indicates that there may be a peak or critical period over which most of the reproductive effort

occurs in a system. Minimizing temperature or flow disruptions to spawning in this critical period may increase reproductive success.

Robust Redhorse showed some similar behaviors when comparing a reintroduction watershed in the Piedmont, the Broad River, to observations of wild, Coastal Plain populations in the Oconee and Savannah Rivers (Chapter 4). Similar behaviors included long distance migrations, formation of spawning trios by males and females, and some evidence of wintering and spawning site fidelity. This study was the first to describe detailed spawning behavior of Robust Redhorse (except habitat measurements by Grabowski and Isley 2007 and Freeman and Freeman 2001) and to document an alternative male reproductive strategy (sneaker males). Individual Robust Redhorse in the Broad River were not as consistent in their use of spawning sites, with some individuals moving between spawning sites in two different rivers during the same spawning season. Additionally, fish showed variability in their post-spawning migratory behavior with some individuals switching between wintering in the reservoir to wintering in the river.

The main conservation efforts involved in the recovery efforts of Robust Redhorse include captive-propagation and releases of Robust Redhorse in several river systems throughout its range, and efforts to define the life history requirements of this species. To assess any population's persistence through time, a species needs to recruit new individuals into the reproductive population in at least the same rate as reproductive individuals leave the reproductive population through senescence or death. In Chapter 5, I assessed evidence for recruitment within the Broad River population based on documentation of the relationship between size and age, and occurrence of alternate mating tactics. Robust Redhorse become reproductively mature at 5-6 years and between 400-450 mm total length. Because only one

wild-reared individual less than 400 mm TL has been captured, assessing recruitment may be limited to noting new individuals as they enter the reproductive population and become accessible to documentation. Robust Redhorse growth is accelerated in its early years until reaching reproductive maturity. After maturity growth is linear, and therefore, should allow the ability to predict age based on total length. However, variability in growth among individuals results in relatively wide ranges of size at a particular age. I have therefore used data for known aged fish to probabilistically assess evidence that smaller fish represent recruitment to the population of new individuals. Using the relationship between age and length, and being able to visually estimate age without capture may provide a non-invasive way to evaluate recruitment. Because of the unknown mechanisms causing recruitment failure in the Oconee River and limited recruitment in other systems, limiting disturbance during reproduction may provide the best opportunity to allow Robust Redhorse to reproduce naturally.

The methods and results described in this study can be used to evaluate reproductive effort of large-bodied catostomids under a variety of conditions that would preclude visual observations, and to assess evidence of variability of reproductive behavior of Robust Redhorse at other times and places. Reproductive behavior has been underestimated in its importance in the conservation and recovery of imperiled species and interruption of behavioral mechanisms that increase reproductive fitness could prevent species recovery from declines (Helfman 1999; Shumway 1999; Pavlov et al. 2009). This research has documented a diversity of spawning habitat and diverse spawning behaviors in the Broad River. This complexity of habitat may provide the best arena for a variety of spawning behaviors to allow the species to persist into the future.

## REFERENCES

- Cooke, S. J., C. M. Bunt, S. J. Hamilton, C. A. Jennings, M. P. Pearson, M. S. Cooperman, and
  D. F. Markle. 2005. Threats, conservation strategies, and prognosis for suckers
  (Catostomidae) in North America: insights from regional case studies of a diverse family
  of non-game fishes. Biological Conservation 121:317-331.
- Cope, E. D. 1870. A partial synopsis of the fishes of the fresh waters of North Carolina. Proceedings of the American Philosophical Society 11(81):448-495.
- DeMeo, T. 1997. Report of the Robust Redhorse Conservation Committee Annual Meeting. Wildlife Resources Division, Social Circle, Georgia.
- Dudgeon D., A. H. Arthington, M. O. Gessner, Z-I. Kawabata, D. J. Knowler, C. Lévêque, R. J.
  Naiman, A-H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006.
  Freshwater biodiversity: importance, threats, status and conservation challenges.
  Biological Reviews 81:163-182.
- Freeman, B. J. and M. C. Freeman. 2001. Criteria for suitable spawning habitat for the Robust Redhorse *Moxostoma robustum*. A report to the U.S. Fish and Wildlife Service.
- Grabowski, T. B. and J. J. Isley. 2007. Spatial and temporal segregation of spawning habitat by catostomids in the Savannah River, Georgia and South Carolina, U.S.A. Journal of Fish Biology 70:782-798.
- Helfman, G. S. 1999. Behavior and fish conservation: introduction, motivation, and overview. Environmental Biology of Fishes 55:7-12.
- Jackson, C. R., J. K. Martin, D. S. Leigh, and L. T. West. 2005. A southeastern Piedmont watershed budget: Evidence for a multi-millennial agricultural legacy. Journal of Soil Water Conservation 60:298-310.

- Jennings, C. A., E. W. Dilts, J. L. Shelton Jr., R. Cull Peterson. 2010. Fine sediment affects on survival to emergence of Robust Redhorse. Environmental Biology of Fishes 87:43-53.
- Kondolf, G. M. 2000. Assessing salmonid spawning gravel quality. Transactions of the American Fisheries Society 129:262-281.
- Pavlov, D. A., N. G. Emel'yanova, and G. G. Novikov. 2009. Reproductive Dynamics. In Fish Reproductive Biology: Implications for Assessment and Management. T. Jakobsen, M. J. Fogarty, B. A. Megrey, and E. Moksness, editors. Wiley-Blackwell, Ames, Iowa.
- Shumway, C. A. 1999. A neglected science: applying behavior to aquatic conservation 55:183-201.
- Slaughter, IV, J. E. 2011. Conservation and restoration of the Robust Redhorse Moxostoma robustum in the Oconee River, Georgia, Volume 7. Prepared for the Federal Energy Regulatory Commission. Washington, DC.
- Trimble, S. W. 1974. Man induced soil erosion on the Southern Piedmont; 1700 1970. Soil Water Conservation Society, Ankeny, Iowa.
- Walsh, S. J., D. C. Haney, C. M. Timmerman, and R. M. Dorazio. 1998. Physiological tolerances of juvenile robust redhorse, Moxostoma robustum: conservation implications for an imperiled species. Environmental Biology of Fishes 51:429-444.
- Waters, T. F. 1995. Sediments in streams: Sources, biological effects and control. Monograph 7. American Fisheries Society, Bethesda, Maryland.
- Wilcove D. S., D. Rothstein, J. Dubow, A. Phillips, and E. Losos. 1998. Quantifying threats to imperiled species in the United States. BioScience 48:607-615.