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Habitat Use and Tributary Occupancy of the Threatened River Redhorse (*Moxostoma carinatum*) in the Grand River, MI, USA.

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Abstract

The resiliency of our aquatic ecosystems hinges on our ability to protect the native species that reside within them. The river redhorse (*Moxostoma carinatum*) is one such example and populations have become low enough to warrant listing by the State of Michigan. Causes of decline include overfishing, habitat alteration, and lack of knowledge of basic life-history attributes including their use of non-spawning habitat and spawning locations. In order to understand the river redhorse's habitat use we implanted 15 individuals with radio transmitters and tracked their locations over the course of a summer. Tagged river redhorse were found to move as far as 50 km down river after spawning and establish themselves in small home ranges between 0.04 and 0.12 km^2. The presence of mussels and snails, the river redhorse's preferred food source, was the primary habitat characteristic selected for by tagged individuals and was documented at 79 percent of all tracked locations. In order to locate the tributaries that the river redhorse use for spawning we developed a species-specific genetic test and used eDNA collection to examine their springtime occurrence in five tributaries of the Grand River. While no tributary samples amplified successfully it is possible to use our test in future studies to identify river redhorse spawning areas and to identify potential river redhorse specimens. The recovery of the river redhorse in the lower Grand River will depend on our ability to protect these newly discovered feeding areas and to ensure an accurate understanding of the river redhorse's current distribution. Future management should therefore focus on expanding our knowledge of the river redhorse's distribution, protecting native mussels and snails, and should attempt to maintain migration routes between spawning and summer habitats.

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Chapter 1

Background

Introduction

Freshwater fish are among the most imperiled groups of organisms, having the highest rate of extinction and extirpation in the twentieth century among vertebrates (Burkhead 2012). These extinctions and extirpations include a well-known population of Atlantic salmon (*Salmo salar*) in Lake Ontario as well as lesser known species like the harelip sucker (*Moxostoma lacerum*) in the southeastern United States. Many anthropogenic issues are associated with these declines including habitat loss, introduction of invasive species, and lack of informed management (Cooke et al. 2005, Jelks et al. 2008).

Fisheries management in the United States is primarily concerned with game species, which provide significant economic impacts for the country (Reynolds et al. 2008). As a result, non-game species receive far less attention and are often ignored until they either become imperiled enough to warrant state or federal listing (Ricciardi and Rasmussen 1999), or until they have gone extinct, as was the case with the harelip sucker (Burkhead 2012). Once listed, management agencies develop plans for the recovery of the species but with little prior research into their life history these plans can be difficult to form and may not cover the full breadth of the species' needs.

The river redhorse (*Moxostoma carinatum*) is one example of a non-game species brought into the focus of management agencies by its imperiled status. This species once occupied a large area of eastern North America including watersheds in Quebec, Ontario, Minnesota, Wisconsin, Michigan, New York, Pennsylvania, Ohio, Indiana, Illinois, Iowa, Kansas, Missouri, Kentucky, Tennessee, West Virginia, Virginia, North Carolina, South

Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, and Oklahoma (Lee et al. 1980, White and Trautman 1981, Becker 1983a, Yoder and Beaumier 1986). Following declines in the middle to late 1900's this range was severely restricted and the species was thought to be extirpated from Michigan, Iowa, and Indiana (Gerking 1945, Becker 1976, Roosa 1977). River redhorse are currently imperiled or critically imperiled in thirteen states and provinces and are listed as threatened by the State of Michigan (Michigan Natural Features Inventory 2007, NatureServe 2018). Threats facing the river redhorse include the presence of flow control structures, river fragmentation, river channelization, siltation, degraded water quality, and loss of native mollusks, which are its main food source (Lee et al. 1980, Becker 1983a, Reid 2003, Weyers et al. 2011). In addition to these physical threats river redhorse have not been well studied, which has prevented the effective management of the species (Parker 1988).

Our knowledge of river redhorse is primarily composed of observations of their spawning activities and the species' physical characteristics. They are known to spawn over gravel and cobble substrates in fast flowing water in both the main stem of large rivers and their tributaries (Hackney et al. 1968, Jenkins 1970, Parker 1988, Campbell 2002). In their northern range spawning begins in May when water temperatures rise above 15°C and concludes in early June (Campbell 2002, Reid 2003, Reid et al. 2006). Eggs hatch in three to four days but once hatched very little is known about their behavior or growth patterns. River redhorse mature in approximately four years, though this is likely variable and dependent on local conditions (Jenkins 1970, Becker 1983a, Beckman and Hutson 2012), and have been found to live up to age twenty-eight (Reid et al. 2006). As they mature they can grow over 75 centimeters and reach weights of over 4.5 kilograms. Mature river redhorse possess large pharyngeal teeth that enable them to feed on both native and invasive mollusks and have even been presented as a potential biological control for zebra mussels (*Dreissena polymorpha*; French 1993). However, little research has been done to explore this potential and their populations would likely need to rebound in order to effectively perform this ecosystem service.

Successful management of the river redhorse will depend on our ability to fill the gaps in our knowledge of the species. One of these gaps is knowledge of their habitat use outside of their spawning season. This includes habitat use by juveniles, observations of which are extremely rare, and by adults, which has been documented but with mixed results. Yoder and Beaumier (1986) suggested that river redhorse avoid slow flowing waters in favor of riffles and runs, while a study by Campbell (2002) found the species in a variety of habitats during the summer months including areas with slow current, soft substrate, and abundant vegetation. The use of slow flowing water was also suggested by Reid (2003), who found that river redhorse were absent from the shallow, fast flowing water during the fall they had used the previous spring. This suggested that the species was using some form of alternative habitat. A more recent study documented differential habitat use during different seasons with the species occupying shallow riffles with rapid flow during the spring, boulder filled runs during the summer, runs of moderate velocity and depth during the fall, and deeper runs during the winter (Butler and Wahl 2017). These apparent contradictions and mixed findings in habitat use may indicate that we have not yet identified the main characteristics that the river redhorse select for in their environment.

In Michigan, another key gap in our knowledge of river redhorse is the exact streams and rivers in which they spawn (Derosier et al. 2015). The identification of spawning locations is critical for the protection of any fish species but this has proven difficult with river redhorse as they are rare, difficult to capture, and difficult to identify (Becker 1983a, Parker 1988). They are one of six *Moxostoma* species in Michigan and are the only threatened species in that genus. They are often mistaken for other redhorse by both fisheries professionals and the fishing public as a result of their morphologic similarities with the other five *Moxostoma* species. This has been identified as one of the key problems facing the species and alleviating it is noted as a priority action in the Michigan Wildlife Action Plan (Parker and McKee 1984, O'Keefe 2002, Cooke et al. 2005, Reid et al. 2006, Reid and Wilson 2006, Derosier et al. 2015). Genetic techniques may provide solutions to both of these problems. Specifically, forensic genetics has been used to help differentiate redhorse species in the past (Reid and Wilson 2006), and eDNA may provide greater chances of detecting the species than traditional survey methods (Jerde et al. 2011, Janosik and Johnston 2015, Davison et al. 2016, Williams et al. 2017).

Purpose

The river redhorse is an important native species deserving our protection. It provides a number of ecosystem services including invasive mollusk control, prey for mammals, birds, and large fish, angling opportunities, nutrient and sediment redistribution during spawning and feeding, and improved food web connectivity (Hackney et al. 1968, French 1993, Holmlund and Hammer 1999, Cooke et al. 2005). Beyond these factors the river redhorse also exemplifies a troublesome trend in the way we manage our non-game species, one that could be reversed given the right insights and incentives.

Non-game species are often poorly understood and even despised by the community at large. Considered to be "… as useless and destructive in our productive waters as wolves and foxes formerly were in our pastures and poultry yards," species like gar, bowfin, and suckers have been historically persecuted despite their native status and potential benefits (Scarnecchia

1992, Cooke et al. 2005). Management of these species today no longer includes their purposeful removal from our waterways but the perception that they are harmful to some of the more celebrated species still persists despite evidence to the contrary (Holey et al. 1979, Marrin and Erman 1982). Further understanding of the river redhorse could not only help in its own protection but could also help change the current perception of many non-game species. By managing for river redhorse we can contribute to a paradigm shift that values biodiversity and native species over simple game fish production.

In order to effectively manage the river redhorse and effect change in the broader management of all native fish we must first fill the gaps in our knowledge of these species. For the river redhorse two chief gaps are knowledge of the habitat they select for and the specific locations in which they spawn. Both of these have been identified as areas of need by the Michigan DNR (Derosier et al. 2015, Hanshue and Harrington 2017). Filling these gaps will provide resource managers with the information they need to help protect this state threatened species and by taking action we will begin to alter the current perceptions toward non-game fish.

Scope

This study primarily explores the river redhorse's selection of summer habitat in the Grand River from Grand Rapids to Lake Michigan and their tributary occupancy during their spring spawning run. Because of this seasonal limitation and focus on adults of the species, conclusions and applications should be limited to summer habitat selection for adult river redhorse and tributary occupancy during spawning. The diversity of habitat types, land cover types, and main stem tributaries in and around the Grand River (Hanshue and Harrington 2017), suggest that our results should be applicable to large rivers throughout the river redhorse's range.

However, management decisions should be considered on a case by case basis as all findings will not be applicable in all situations.

Assumptions

The primary assumption of all telemetry projects is that the fish fitted with transmitters are a representative sample of the population as a whole. In order to meet this assumption, we took great care in choosing, tagging, and tracking our fish. Of the fifteen fish that were tagged, seven were noted as male based on the presence of tubercles or tubercle scars on their head and fins while eight lacked these indicators and were assumed to be female. Length at age relationships and the fact that many females were visibly gravid indicated that these non-tuberculate fish were likely not juvenile males, reinforcing our assumption (Beckman and Hutson 2012). Transmitter tags represented less than two percent of the fishes' overall mass in order to prevent overburdening the individual and tracking began two weeks following tagging to allow each fish to acclimate to the inserted tag (Winter 1996, Brown et al. 1999, Jepsen et al. 2002). Tracking was conducted by wading, walking the shoreline, and by boat. While fish tracked by boat were observed to move once the anchor was dropped over top of their position it is assumed that no tracking technique altered their behavior prior to locating their position.

Habitat selection was determined by comparing the habitat present at each fish's tracked location with the habitat available to them within their home range. This was done to avoid arbitrarily defining the habitat available to each fish but made it necessary to assume that all habitat within their home range was accessible to them.

Objectives

The primary goals for these projects were to (1) identify the habitat needs of river redhorse and (2) identify the tributaries in which they spawn. This information would provide resource managers with the knowledge necessary to develop plans for the species' recovery. The primary objective with regards to identifying their habitat needs was to isolate the habitat characteristics that are selected for by the species during the summer months. Secondary objectives included identifying movement and distribution patterns of the species in the Grand River and generating home range maps for tagged individuals. The primary objective for identifying tributary spawning locations was to develop a species-specific genetic test to identify the presence of river redhorse from collected water samples. A secondary objective was to use this test as a means of identifying possible River Redhorse specimens without visual cues.

A secondary goal for the project was and continues to be improving the perception of non-game species with specific focus on catostomids. This has been accomplished through outreach and further education of members of the public and through publication and presentation of our work.

Significance

While game species such as walleye (*Sander vitreus*), largemouth bass (*Micropterus salmoides*), and muskellunge (*Esox masquinongy*) are studied at length, the basic physiology, ecology, and life history of the river redhorse is less well known. Despite being imperiled throughout much of its range, few studies have attempted to explain how best to manage its populations and protect its habitat. My study took the first steps down this path by attempting to identify the characteristics of the summer habitat that river redhorse select for and the tributaries in which they spawn. Of these, the former will help aid management of the species throughout its range and the latter will help aid management of the species in Michigan. Furthermore, this work identified movement and distribution patterns of the fish in the Grand River allowing local agencies to better protect specific areas of importance for the species. The future of the river redhorse is unclear but with continued research and management there is the possibility for its recovery and de-listing in Michigan.

Definitions

eDNA – Environmental DNA. DNA released from an organism through waste, dead cells, and excretions that is preserved in the environment in which it is present.

Game fish – Fish species that are frequently sought after by anglers and so provide direct use benefits to state and local economies.

Non-game fish – Fish species that are not frequently sought after by anglers and so do not provide direct use benefits to state and local economies.

Press disturbance – A chronic disturbance lasting longer than the life-span of the longest lived species in a community.

Selection – The process by which an organism chooses to occupy a given habitat type.

Chapter 2

River Redhorse Telemetry

Abstract

The resiliency of our aquatic ecosystems hinges on our ability to protect the native species that reside within them. The river redhorse (*Moxostoma carinatum*) is one such example and populations have become low enough to warrant listing by the State of Michigan. Causes of decline include overfishing, habitat alteration, and lack of appropriate management stemming from an insufficient understanding of the species. In order to aid its recovery, we implanted 15 individuals with radio transmitters and tracked their locations over the course of a summer. Tagged river redhorse were found to move as far as 50 km down river after spawning and establish themselves in small home ranges between 0.04 and 0.12 km^2. The presence of mussels and snails, the river redhorse's preferred food source, was the primary habitat characteristic selected for by tagged individuals and was documented at 79 percent of all tracked locations. This selection presents a unique opportunity for future mussel restoration as river redhorse could lead resource managers to otherwise undocumented mussel beds. It also provides insight into river redhorse management, indicating that the recovery of the species will likely depend on our ability to protect these newly discovered feeding areas. Future management should therefore focus on the protection of native mussels and snails and should attempt to maintain migration routes between spawning and summer habitats.

Introduction

Freshwater fish have the highest rate of extinction and extirpation in the twentieth century among vertebrates (Burkhead 2012). These extinctions and extirpations include a well-known population of Atlantic salmon (*Salmo salar*) in Lake Ontario as well as lesser known species like the harelip sucker (*Moxostoma lacerum*) in the southeastern United States. These declines are associated with multiple anthropogenic issues including habitat loss, introduction of invasive species, and lack of informed management (Cooke et al. 2005, Jelks et al. 2008).

Fisheries management in the United States is primarily concerned with game species, which provide significant economic impacts for the country (Reynolds et al. 2008, U.S. Department of the Interior, U.S. Fish and Wildlife Service 2016). As a result, non-game species receive far less attention and are often ignored until they either become imperiled enough to warrant state or federal listing (Ricciardi and Rasmussen 1999), or until they have gone extinct, as was the case with the harelip sucker. Once listed, management agencies develop plans for the recovery of the species, but with little prior knowledge of their life history these plans can be difficult to form and may not cover the full breath of the species' needs.

The river redhorse (*Moxostoma carinatum*) is one example of a non-game species brought into the focus of management agencies by its imperiled status. This species once occupied a large area of eastern North America including watersheds in Quebec, Ontario, Minnesota, Wisconsin, Michigan, New York, Pennsylvania, Ohio, Indiana, Illinois, Iowa, Kansas, Missouri, Kentucky, Tennessee, West Virginia, Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, and Oklahoma (Lee et al. 1980, White and Trautman 1981, Becker 1983a, Yoder and Beaumier 1986; Figure 1). This range was severely restricted following declines in the middle to late 1900's, and the species was thought to be extirpated from Michigan, Iowa, and Indiana (Gerking 1945, Becker 1976, Roosa 1977). River redhorse are currently imperiled or critically imperiled in thirteen of the twenty six states and provinces in which they are present and are listed as threatened in the State of

Michigan (Michigan Natural Features Inventory 2007, NatureServe 2018). Threats facing the river redhorse include the presence of flow control structures, river fragmentation, river channelization, siltation, degraded water quality, and loss of native mollusks, which are its main food source (Lee et al. 1980, Becker 1983a, Reid 2003, Weyers et al. 2011). In addition to these physical threats river redhorse have not been well studied, which has prevented the effective management of the species (Parker 1988).

Our knowledge of river redhorse is primarily composed of observations of their spawning activities and the species' physical characteristics. They are known to spawn over gravel and cobble substrates in fast flowing water in both the main stem of large rivers and their tributaries (Hackney et al. 1968, Jenkins 1970, Parker 1988, Campbell 2002). In their northern range, spawning begins in May when water temperatures rise above 15°C and concludes in June (Campbell 2002, Reid 2003, Reid et al. 2006). Eggs hatch in three to four days but once hatched very little is known about their behavior or growth patterns. River redhorse mature in approximately four years, though this is likely variable and dependent on local conditions (Jenkins 1970, Becker 1983a, Beckman and Hutson 2012), and have been found to live up to age twenty-eight (Reid et al. 2006). As they mature they can grow to over 75 centimeters and reach weights of over 4.5 kilograms. Mature river redhorse possess large pharyngeal teeth that enable them to feed on both native and invasive mollusks and have even been presented as a potential biological control for zebra mussels (*Dreissena polymorpha*; French 1993). However, little research has been done to explore this potential and their populations would likely need to rebound in order to effectively perform this ecosystem service.

Successful management of the river redhorse will depend on our ability to fill the gaps in our knowledge of the species. One of the key gaps is their habitat use outside of their spawning season. This includes habitat use by juveniles, observations of which are extremely rare, and by adults, which has been documented but with mixed results. Yoder and Beaumier (1986) suggested that river redhorse avoid slow flowing waters in favor of riffles and runs, while a study by Campbell (2002) found the species in a variety of habitats during the summer months including areas with slow current, soft substrate, and abundant vegetation. The use of slow flowing water was also suggested by Reid (2003), who found that river redhorse were absent from the shallow, fast flowing water during the fall they had used during the previous spring to spawn, suggesting that the species was using some form of alternative habitat. A more recent study documented differential habitat use during different seasons with the species occupying shallow riffles with rapid flow during the spring, boulder filled runs during the summer, runs of moderate velocity and depth during the fall, and deeper runs during the winter (Butler and Wahl 2017). These apparent contradictions and mixed findings in habitat use may indicate that we have not yet identified the main characteristics that the river redhorse select for in their environment. To provide further insight into this issue, we sought to identify the key characteristic(s), rather than the generalized habitat types, that river redhorse select for during the summer months. This improved detail should provide a better understanding of necessary habitat and lead to more informed management of the species.

Methodology

Study Site – The Grand River watershed covers an area of 14,440 square kilometers in Michigan's lower peninsula and varies greatly along its course (Hanshue and Harrington 2017). Its upper river segments are characterized by steep gradients and relatively stable flows, while its lower river segments are predominantly flat with little channel slope. This study was conducted in these lower river segments covering approximately 65 river kilometers bounded by 6th Street Dam, a large run-of-river dam in the City of Grand Rapids, and Lake Michigan (Figure 2). While most of the river in this area has a nearly flat slope, dropping approximately 0.05 m/km, the stretch within the city of Grand Rapids experiences a much steeper gradient of 1.04 m/km. This section of river is also influenced by the 6th Street Dam, which accounts for eight feet of drop, and four smaller low head dams originally constructed for river beautification. The steep gradient and run-of-river dams in this stretch of the Grand River result in far different habitat characteristics than are found in the rest of the study area. These unique characteristics include faster flows, shallower water, and a lower proportion of fine sediment.

Fish Tagging and Tracking – River redhorse were collected and tagged during May of 2018 using a combination of backpack electrofishing and boom shocking. Individuals were deemed suitable for tagging if their body mass was at least 190 grams, at which point telemetry tags would constitute less than two percent of their overall mass and would not adversely affect swimming ability (Winter 1996, Brown et al. 1999, Jepsen et al. 2002). An approximately equal number of males and females were used for this study in order to identify any differences in habitat use by the two sexes. Seven individuals were determined to be male by the presence of tubercles or tubercle scars on their head and fins while eight lacked tubercles and were assumed to be female. Later analysis, and the fact that many females were visibly gravid, indicated that all tagged individuals were adults and that the possibility of juvenile, none-tuberculate males being misclassified as females was low.

River redhorse were anesthetized in an immersion bath of river water and AQUI-S® at a concentration of 20 mg/l. Once an individual lost equilibrium it was transferred from the bath to a v-shaped surgical board where an ATS model F1580 trailing whip tag was implanted into their body cavity via the shielded needle technique (Ross and Kleiner 1982). Following surgery, fish were allowed to recover in a flow through tank and released into the river downstream of their capture points in a slow-moving eddy to allow further recovery time. Collection and surgery took place in accordance with the Grand Valley State University Institutional Animal Care and Use Committee, study number 18-12-a, and with the Michigan Department of Natural Resources Threatened and Endangered Species permit number 2231.

Tagged individuals were located twice a week for the first two weeks via homing telemetry with an ATS model R410 radio receiver to ensure that they remained healthy and to maintain knowledge of their position. After this two week acclimatization period fish were tracked two to three times per week and data on their location and habitat use were documented. Number of tracked locations per fish ranged from 0 to 19 (Table 1). Tracking was conducted via wading, walking the shoreline, and by boat. Fish locations were recorded with a Garmin GPSmap 62 and later downloaded into ArcGIS for spatial analysis. All habitat characteristics were documented immediately after the location of a fish was determined.

Habitat Quantification – We collected data on the sediment, mollusks, macrophytes, water depth, and water velocity at each tracked location. Sediment samples were collected either with a Ponar grab sampler or by hand depending on the depth of the water and the tracking technique being used. Sediment samples were quantified using the Wentworth scale (Wentworth 1922). Presence or absence of mollusks and aquatic macrophytes were also documented at this time. Water depth was taken using an extendable survey pole and was recorded to the nearest millimeter. Water velocity was taken using a Marsh-McBirney model 2000 portable flow meter at six tenths depth and was recorded to the nearest millimeter per second. Water temperatures were taken from the USGS gauging station number 04119400 near Eastmanville, Michigan, which is located in the center of the tracking area (Figure 2, USGS 2019).

A grid was established within each home range in order to understand what habitat was available to each fish. These grids were scaled to the size of the given home range to ensure complete coverage of the available habitat and 150 random points were distributed along the grid (Marcum and Loftsgaarden 1980). The habitat characteristics at these points were quantified using the same methods used at the tracked locations to evaluate habitat selection.

Data Analysis – Spatial data were processed using ArcGIS version 10.4.1 (ESRI, Redlands, California). All tracked locations were downloaded as shapefiles and used to create 95% minimum convex polygons as estimates of each river redhorse's home range. Heat maps of these points were also developed and overlaid onto the minimum convex polygons to further understand the area used by each individual. Home ranges were only developed for individuals located at least ten times. Movement over the course of the summer was calculated by first merging the tracked locations with a line drawn through the center of the river. Position of each tracked location along the line was then compared to the release point of each fish. Distance from the release point was graphed with positive numbers indicating positions upstream of the release point and negative numbers indicating positions downstream of the release point.

We compared the microhabitat characteristics used by each individual with the characteristics available to them within their home range. By comparing the habitat within each individual's home range we eliminated the possibility of arbitrarily defining what was available to them (Aebischer et al. 1993). The proportional habitat use of each fish was used as the sample unit rather than point locations to alleviate issues with serial correlation. These proportions were subject to the unit sum constraint and so were numerically ranked before statistical comparisons took place (Alldredge and Ratti 1992). Categories were required to rank the data so water depth was divided into seven categories each representing a half-meter increment. Water velocity was similarly divided into five categories each representing quarter-meter per second increments. All habitat ranks had a non-normal distribution and so a non-parametric Kruskal-Wallis test was used for comparisons. Habitat use by males (n=7) and females (n=8) was compared using a Kruskal-Wallis test and no significant differences were found; data were then pooled between the sexes. A combination of Principle Component Analysis and Kruskal-Wallis tests were used to examine differences between habitat use and habitat availability. If a difference was found between the proportional use of a habitat characteristic and its proportional availability, that habitat type was said to be selected for or against depending on the values at hand. All statistical tests were performed in R version 3.3.2 (R Core Team 2016).

In addition to microhabitat, macrohabitat was examined to provide comparisons to past studies and to further document the importance of certain habitat types. Three regularly surveyed macrohabitats were delineated in GIS and their total area was documented alongside the total area of each within the river redhorses' recorded home ranges. The three macrohabitat categories used in this analysis were "rapids" (defined by gradient greater than the Grand River's average of 0.42 m/km and predominantly hard substrate), "main stem" (defined by gradient less than 0.42 m/km and predominantly soft substrate), and "attached waterbodies" (mouths of tributaries, permanently attached floodplain lakes, bayous, and slow moving side channels).

Results

Fifteen river redhorse were collected, tagged, and given identification numbers based on the last three digits of their radio transmitters (Table 1). Individuals ranged from 1.21 to 3.02 kilograms and from 49 to 63 centimeters total length. This is consistent with adult sizes of river redhorse reported in the literature (Jenkins 1970, Trautman 1981, Beckman and Hutson 2012), with the sizes of river redhorse caught during surveys in the Grand River earlier in the year (Figure 3), and indicates that individuals likely ranged from 7.5 to 12 years of age (Beckman and Hutson 2012). Three individuals, ID number 431, 271, and 291 were collected using backpack electrofishing and were released approximately one kilometer upstream of the other fish. The remaining individuals were captured while boom shocking and were released approximately one kilometer downstream of Grand Rapids. Of the fifteen fish that were tagged, fourteen were located at least once following tagging; fish 291 was never relocated. Twelve fish were located throughout the summer and so were used in movement analyses; fish 431 and 473 were not located following the conclusion of spawning. Nine fish, 152, 171, 201, 231, 271, 349, 372, 393, and 453, were tracked at least ten times and so were used in home range analyses.

Following release, eight individuals moved upstream into the spawning grounds where they had been captured, while four individuals left the spawning area and moved downstream (Figures 4a & 4b). Five fish remained within the spawning area over the course of the summer while the others established home ranges farther down river. Fish that transitioned downstream began to leave the spawning grounds three to ten days after tagging following a week long warming trend in which water temperatures rose from 16°C to 26°C (USGS 2019a). Fish were seen to travel as much as 47 kilometers in an eight-day period following spawning but remained in their established home ranges over the course of the summer. One individual, fish number

201, moved 30 kilometers downriver before returning upriver to its original home range, covering a distance of 68 kilometers in late June to early July.

River redhorse home ranges were small, between 0.042 and 0.123 square kilometers, and spread throughout the study area with the greatest concentration occurring within the city of Grand Rapids (Figure 5). Available habitat in this area was primarily composed of the rapids macrohabitat and was typically less than one meter in depth, composed of gravel/cobble substrates, and contained fast-moving water. The rapids macrohabitat made up less than 1 percent of the overall study area, but represented approximately 60 percent of river redhorse home range area (Table 2). Macrohabitat outside of downtown Grand Rapids was characterized mostly as main stem and attached waterbodies. These areas were deeper, possessed slower flowing water, and were predominantly sand substrates. The main stem and attached waterbody macrohabitats represented 39 and 60 percent of the overall surveyed area and 40 and less than 1 percent of river redhorse home range area, respectively.

Kruskal-Wallis tests detected no significant differences between habitat use and habitat availability for water depth, sediment type, macrophyte presence, and water velocity. However, a significant difference was found for the presence of mollusks ($p < 0.001$). Mollusks were found in just 12 percent of all the available habitat throughout the sampled area but were present in nearly 80 percent of tracked locations indicating selection favoring the presence of mollusks. A Principle Component Analysis indicated the same selection with used and available habitat separating along a vector of proportional mollusk presence (Figure 6).

Discussion

Dispersal can have profound effects on community structure. It enables a species to maintain gene flow, recolonize habitat patches where extirpation has occurred, and seek refuge when their occupied habitat becomes unacceptable for their present needs (Reeves et al. 1995, Neraas and Spruell 2001, Fausch et al. 2002, Yamamoto et al. 2004, Dingle 2014). River redhorse are no exception and appeared to rely on dispersal to access spawning, foraging, and refuge habitat. Tagged individuals displayed a broad range of post-spawn dispersal patterns with movement ranging from 1 to approximately 50 kilometers down river. If spawning site fidelity is assumed, this suggests significant gene flow through the un-fragmented sections of the Grand River, although a long-term genetic study would be required to confirm this hypothesis. Post-spawn movement patterns began following a 10° C warming trend that appeared to signal the end of the river redhorse's spawning season. Thermal cues have been noted as a primary driver for the species spawning activities and our findings are consistent with this idea (Hackney et al. 1968, Becker 1983a, Reid et al. 2006, Straight et al. 2015). Individuals were seen to travel considerable distances during the course of the summer. Whether this was a result of a predatory threat, a localized disturbance, competition for limiting resources or some other factor remains unclear but it emphasizes the need to allow the species access to multiple habitat patches broadly distributed throughout the river corridor.

While river redhorse spawning habitat has been well documented, data on habitat use during other seasons is contradictory. Studies have reported both avoidance (Campbell 2002) and selection for slow-flowing waters during the summer and fall (Reid 2003, Reid et al. 2006); their use of fast-flowing gravel-filled riffles and runs (Hackney et al. 1968, Scott and Crossman 1973, Becker 1983a), and differential habitat use during different seasons (Butler and Wahl 2017). In the Grand River, river redhorse used the same variety of habitat characteristics found in previous studies including both fast and slow flowing waters and hard and soft substrates. However, examination of macrohabitat indicated disproportionate use of fast flowing water and hard substrate. Approximately 60 percent of river redhorse home range area was composed of the rapids macrohabitat despite this macrohabitat representing less than 1 percent of the overall study area. This highlights the importance of high gradient areas for river redhorse summer habitat use and lends support to some previous studies (Hackney et al. 1968, White and Trautman 1981, Becker 1983a, Yoder and Beaumier 1986). However, microhabitat analyses indicated that macrohabitat use may not be as straightforward as it seems.

Our microhabitat analyses indicated that river redhorse will use many of the previously documented habitat characteristics (depth, sediment, macrophyte presence and water velocity) in proportion to their availability. However, while our river redhorse did not exhibit any obvious selection for depth, sediment, macrophyte presence, and water velocity, they strongly selected for habitat containing freshwater mollusks, the primary food source for the species. Clearly, they were seeking foraging habitat during this time period. Previous studies have noted this possibility when attempting to explain the summer habitat use of the river redhorse (Yoder and Beaumier 1986, Campbell 2002, Butler and Wahl 2017), but none have identified the presence of suitable food in their study areas. When you consider the commonality of post-spawn dispersal toward feeding habitat in other fish species such as in shortnose sturgeon *(Acipenser brevirostrum*) and razorback sucker (*Xyrauchen texanus*; Schlosser 1991, Hall et al. 1991, Mueller et al. 2000) it seems even more likely that the presence of suitable food is driving the river redhorse’s summer habitat use. This hypothesis is supported by the size of the home ranges calculated in our study.

River redhorse home ranges were small and use within the home range was often unequally distributed and associated with the presence of mollusks. As a result, home ranges appeared to follow the distribution patterns commonly seen in mollusk populations (Mulcrone and Rathbun 2018). Mollusk beds are typically small and their positions are difficult to predict. Their presence has been correlated with environmental parameters ranging from watershed wide characteristics, like slope and land use, to microhabitat characteristics like water chemistry, water depth, and water velocity (Hastie et al. 2000, Hardison and Layzer 2001, Arbuckle and Downing 2002). As with habitat use in river redhorse there is little consensus as to what factors influence the position and distribution of mollusk beds (Mulcrone and Rathbun 2018). However, due to issues associated with siltation, mollusk beds are often thought to be associated with high velocity areas, which flush fine sediment (Williams et al. 1993, Allen and Vaughn 2010, Freshwater Mollusk Conservation Society 2016). This association may explain the disproportionate use of the rapids macrohabitat by our river redhorse while the general stochasticity displayed by the mollusk community may explain the variability seen in reports of the river redhorse's habitat use in other studies. By establishing themselves over mollusk beds, river redhorse maintain easy access to their principal food source, but as a result they are seen to occupy habitat with a wide range of characteristics that may have little to no impact on their overall fitness. A feeding study would be needed to confirm this hypothesis but our findings suggest a strong link between mollusk distribution and river redhorse summer habitat use.

Mollusks are highly influential on their surrounding ecosystem. They provide bio-deposition of nutrients, physical habitat, and support for a number of other benthic invertebrates (Beckett et al. 1996, Aldridge et al. 2007, Vaughn et al. 2008, Strayer 2014). However, without properly defined habitat needs it can prove difficult to locate and manage for native mollusks.

For this reason, the link between the mollusk community and river redhorse may prove beneficial for malacologists. The apparent selection for the presence of mollusks seen in river redhorse could be used to locate patchily distributed mollusk beds. By tagging and tracking river redhorse, resource managers could gain a better understanding of the overall mollusk community, although tagging a threatened species to find threatened species raises some interesting ethical considerations. At any rate, creative problem solving is essential as nearly 70 percent of North American mussels are imperiled (Stein et al. 2000, Mulcrone and Rathbun 2018), and more complete information may be needed to help preserve the populations that remain. In the Grand River a specific issue may be lack of connectivity for host fish (Haag and Warren 1998, Stein et al. 2000, Mulcrone and Rathbun 2018). Small dams and even fish ladders can prevent host darters and minnows from spreading larval mussels to locally extinct patches, thus preventing colonization (Watters 1996, Schwalb et al. 2011). Protection and enhancement of river connectivity could help prevent these issues.

Protection of the river redhorse will likely require ecosystem-based management (Link 2002, Pikitch et al. 2004). This approach avoids ecosystem degradation and specifically accounts for the requirements of non-target ecosystem components in order to promote ecosystem structure and function (Pikitch et al. 2004). Our results documented both feeding and spawning areas used by river redhorse in the lower Grand River, both of which are necessary for the continued survival of the species. A focus on protecting and enhancing mollusk communities is particularly important. In order to manage for healthy mollusk communities, we must maintain adequate water quality, prevent excess sedimentation, and ensure the health of host fish species that have coevolved to carry mussel larvae. Host fish will have their own requirements for feeding and reproduction and may require additional considerations related to fish passage. With this complex web of interactions all influencing river redhorse populations, the best policy is to maintain the natural structure and function of a river-floodplain ecosystem (Ward 1989, Stanford and Ward 1993). This coincides with an ecosystem-based approach where maintaining an intact river ecosystem is a main priority. Examples of this include maintaining riparian buffers to prevent non-point source pollution and erosion and preventing direct degradation of river habitat through dredging and in-stream construction.

Two projects are currently being proposed for the Grand River that could impact river redhorse populations, the Grand Rapids Whitewater project and the Grand River Waterway project ("Grand Rapids Whitewater" 2019, "Grand River Waterway" 2019). The Grand Rapids Whitewater project seeks to create rapids within the city of Grand Rapids and mitigate for invasive species by removing four lowhead dams, lowering the level of one lowhead dam, building an adjustable dam, and installing whitewater features in the river. The Grand River Waterway project aims to dredge the Grand River's natural gravel and sand bar formations to allow large boats access further up river. Both of these projects are examples of press disturbances. They would alter the habitat for a duration longer than the life span of the longest lived species in the community (Detenbeck et al. 1992) and so would require an extensive recovery period. For example, fish communities affected by dredging and channelization have been shown to require over 52 years to recover (Bayless and Smith 1964, Congdon 1971, Detenbeck et al. 1992) and bottom dwelling species like river redhorse can be particularly affected (Padgett 1975). Concerns have been raised over both projects' potential to harm the Grand River's existing communities. Channelization projects such as the Grand River Waterway typically degrade fish and mollusk communities (Watters 1996, Lau et al. 2006, Freshwater Mollusk Conservation Society 2016), and river redhorse are likely to decline as a result.

Construction for habitat restoration projects like the Grand Rapids Whitewater project can harm fish populations in the short term, but if they are properly designed can also benefit fish over the long term (Detenbeck et al. 1992)

The dredging necessary to maintain the channel proposed by the Grand River Waterway project would cause lasting harm to the spawning and foraging habitat used by river redhorse both within the project area and further downstream. Dredging would directly impact the foraging habitat of three tagged river redhorse, fish 372, 349, and 271, as well as any individuals that they may be representing. It would also eliminate a large area of spawning habitat located near the home range of fish 372 (Figure 5). This is the only section of river containing the rapids macrohabitat outside of downtown Grand Rapids and it was observed to contain a large school of approximately 50 spawning river redhorse during spring surveys. This density is unheard of in the Grand River watershed and may represent the largest spawning congregation ever documented in Michigan (O'Keefe 2002, D. O'Keefe personal communication), emphasizing the importance of maintaining the integrity of the site.

The Grand Rapids Whitewater project would directly affect spawning habitat used by all of our study fish. Construction associated with the project could reduce spawning habitat or prevent access to spawning habitat by increasing water velocity above the swimming capability of the species (Stephens et al. 2015, Fox et al. 2016) or by eliminating interstitial spaces in a similar manner to that seen behind lowhead dams (Ligon et al. 1995). Foraging habitat could also be affected for those individuals that remain in the project area during the summer months. However, in addition to the potential negative consequences associated with this project it may also result in an improvement to the current habitat present in downtown Grand Rapids. This will depend on the final design and implementation of the project. Potential guidelines for maintaining and even benefiting river redhorse and the ecosystem as a whole include designing in-stream structures that protect natural structure in the river, designing impermanent structures that facilitate natural processes such as sediment deposition and erosion, and designing channels which mimic the natural geomorphology of the river (Colburn 2012).

Preventing the direct degradation of the river ecosystem is a key part of ecosystem-based management. The impacts associated with the Grand Rapids Whitewater and Grand River Waterway projects could have lasting consequences for the river redhorse and the lower Grand River ecosystem as a whole. To prevent negative consequences it is important to examine and plan each project with ecosystem-based management in mind. This is especially important with the Grand Rapids Whitewater project, which has the potential to be benefit the Grand River ecosystem. Re-examination of these and future projects with ecosystem-based management in mind could eventually be instrumental in the de-listing of the river redhorse while also preventing additional non-game species from becoming imperiled in the first place.

Tables

Table 1. River redhorse collected during May of 2018 in the Grand River in Grand Rapids, Michigan. Average distance from release includes all tracked locations. Positive numbers indicate distance upstream from the release site and negative numbers indicate distance downstream from the release site. Fish that were only located during the spring and not during the summer are listed as N/A. Home range size is not listed for fish located fewer than ten times during the summer.

Fish ID	Sex	Mass (Kg)	Length (cm)	Average Distance From Release (Km)	Home Range Size (Km^2)	Number of Times Located
372	f	1.90	54.0	-34.9	0.042	11
171	f	2.33	57.5	0.6	0.042	19
201	f	2.47	59.0	-15.4	0.045	13
291	f	2.63	62.0	N/A	N/A	0
349	f	2.71	62.5	-1.3	0.045	13
431	f	2.96	61.5	N/A	N/A	1
231	f	2.96	63.0	0.8	0.123	18
332	f	3.02	62.0	-7.4	N/A	2
312	m	1.21	49.0	-2.9	N/A	2
393	m	1.34	50.5	0.5	0.054	18
152	m	1.49	52.0	0.7	0.115	17
453	m	1.61	55.8	0.6	0.042	14
412	m	1.65	56.7	-6.6	N/A	7
473	m	1.81	57.5	N/A	N/A	3
271	m	1.92	58.5	-17.1	0.094	15

Table 2. Macrohabitat characteristics within the section of the Grand River used for tracking and their representation within the home ranges of tagged river redhorse. The rapids macrohabitat is defined by gradient greater than the Grand River's average of 0.42 m/km and predominantly hard substrate, main stem is defined by gradient less than 0.42 m/km and predominantly soft

substrate, and attached waterbodies include the mouths of tributaries, permanently attached floodplain lakes, bayous, and slow moving side channels.

Macrohabitat	Total Area (Km^2)	Home range area (Km^2)	Proportion of Total Area	Proportion of Home range Area
Rapids	0.19	0.36	0.01	0.59
Main stem	9.34	0.24	0.39	0.40
Attached waterbodies	14.52	<0.01	0.60	<0.01
Total	24.05	0.60	1	1

Figures

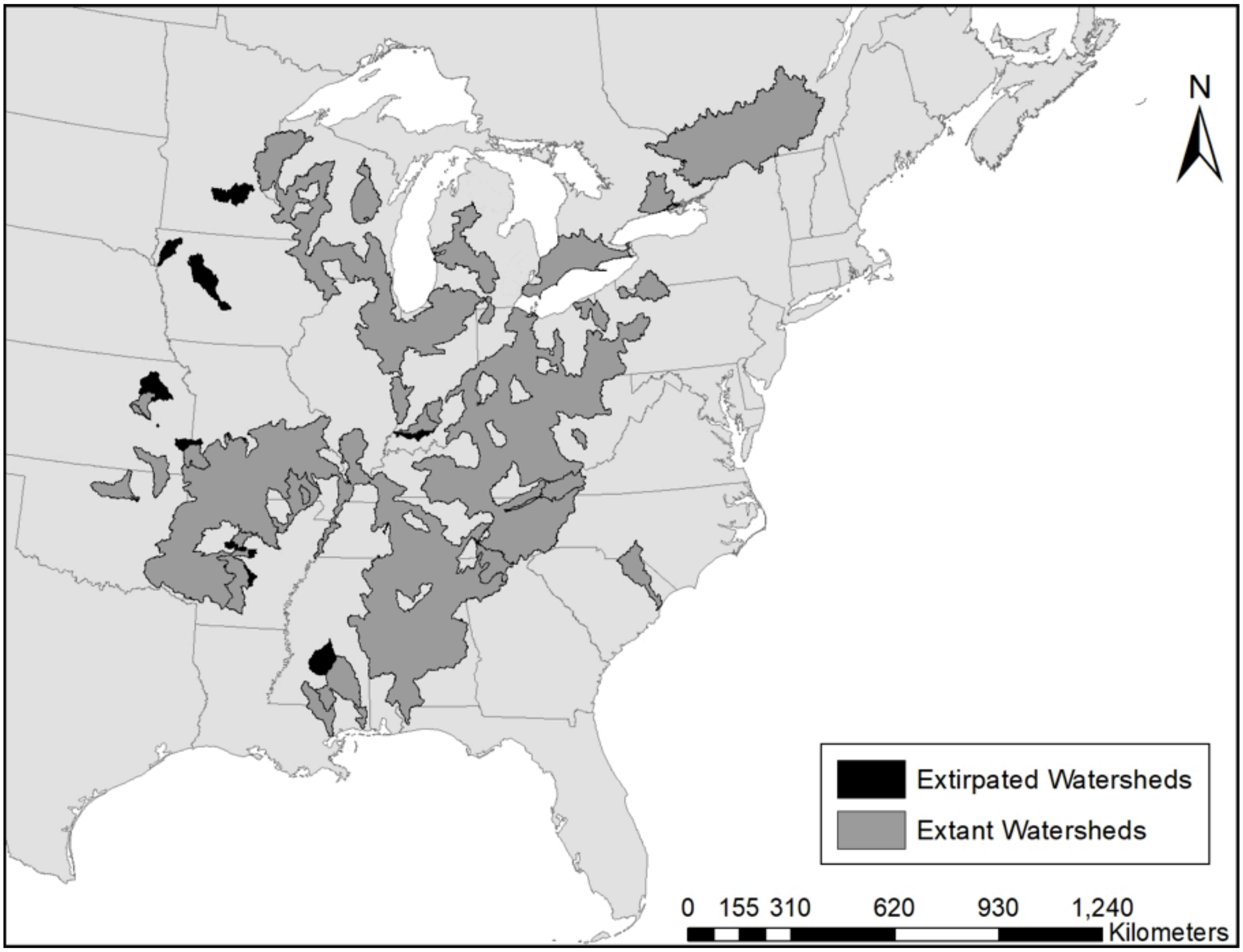

Figure 1. Current range of the river redhorse in the eastern United States. Data provided by the IUCN Red List (IUCN 2019) and edited to exclude Michigan's Detroit and Au Sable river watersheds, which were documented as a result of misidentification (O'Keefe 2002).

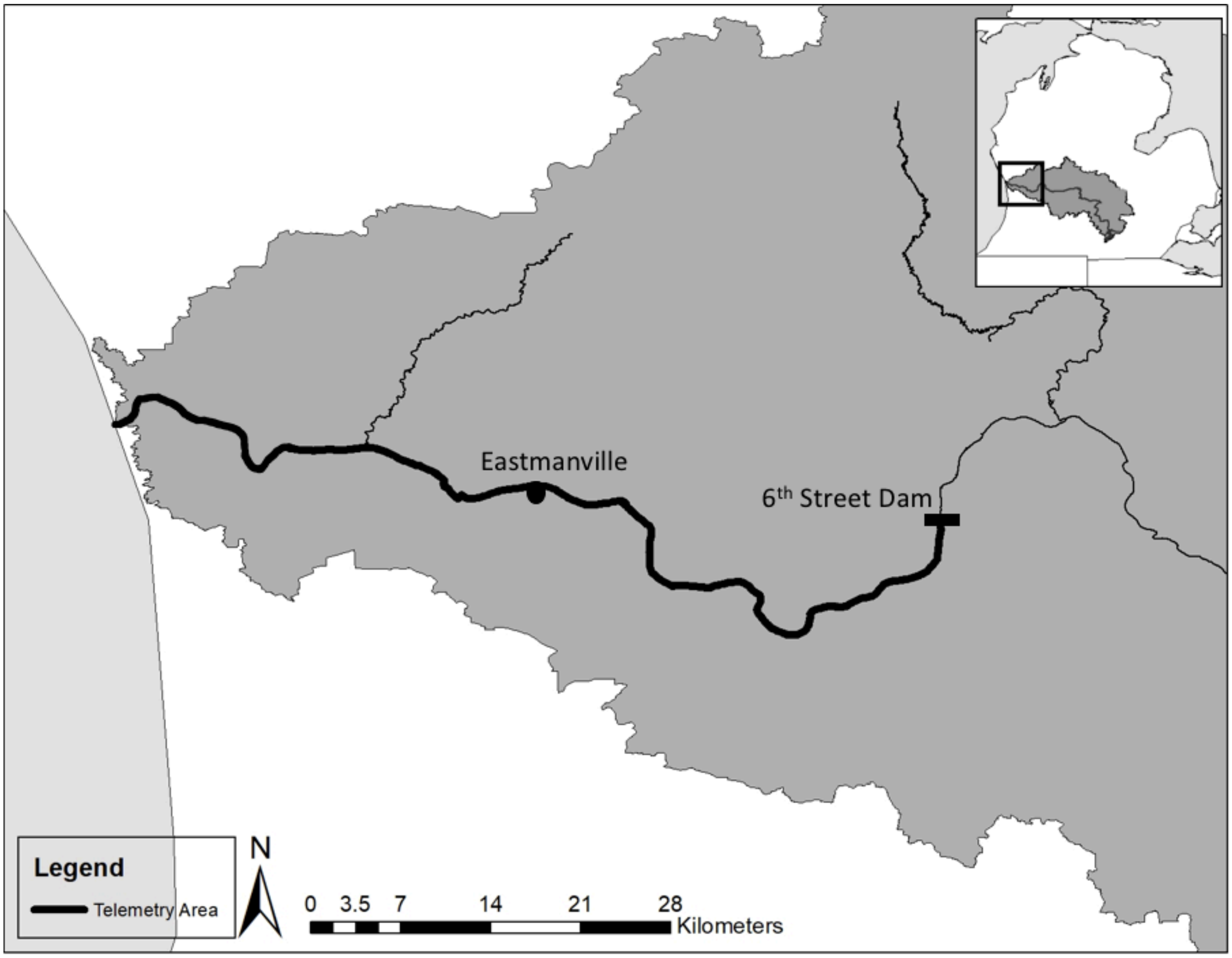

Figure 2. Section of the Grand River used for tracking river redhorse. Fish were tagged near 6th Street Dam and the telemetry area was bounded by the dam on the upstream end and Lake Michigan on the downstream end.

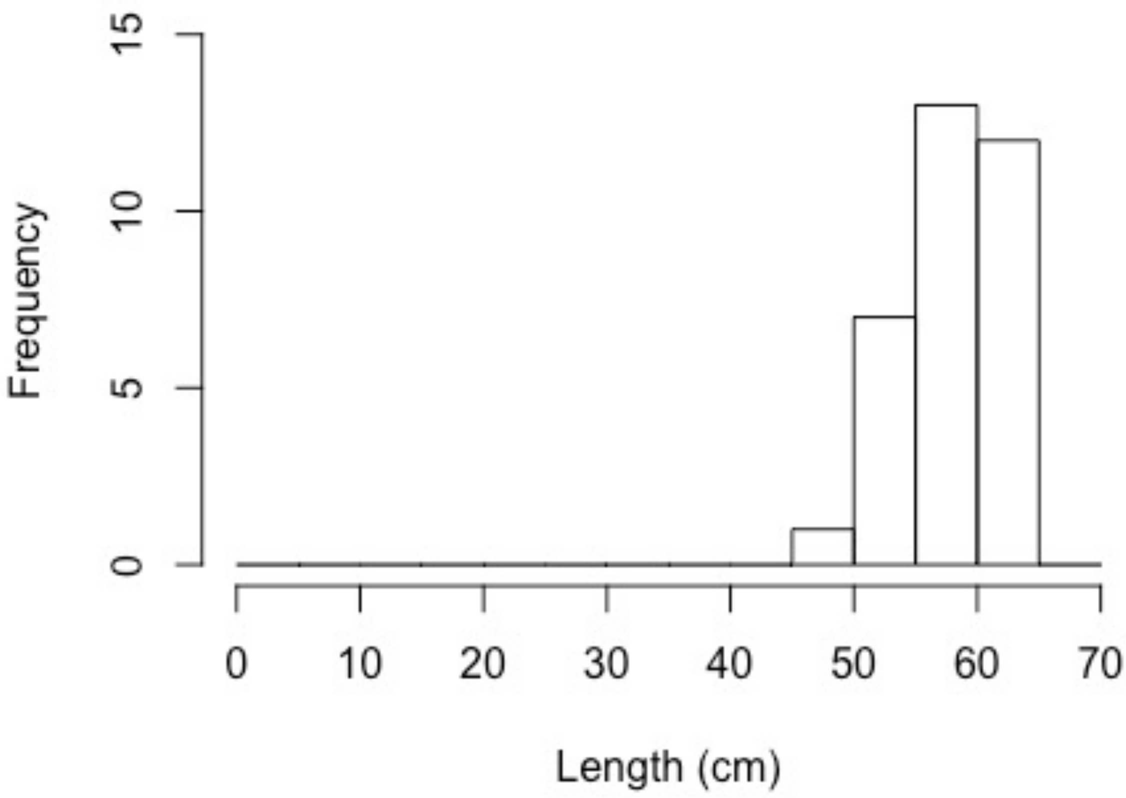

Figure 3. Histogram of river redhorse length (cm) distributions in the Grand River (n = 33). Collections were done via boom shock electrofishing and were conducted on 3-20-18 and 5-29-18. The histogram includes river redhorse collected alongside the Michigan DNR during their spring surveys and river redhorse that were tracked during this project.

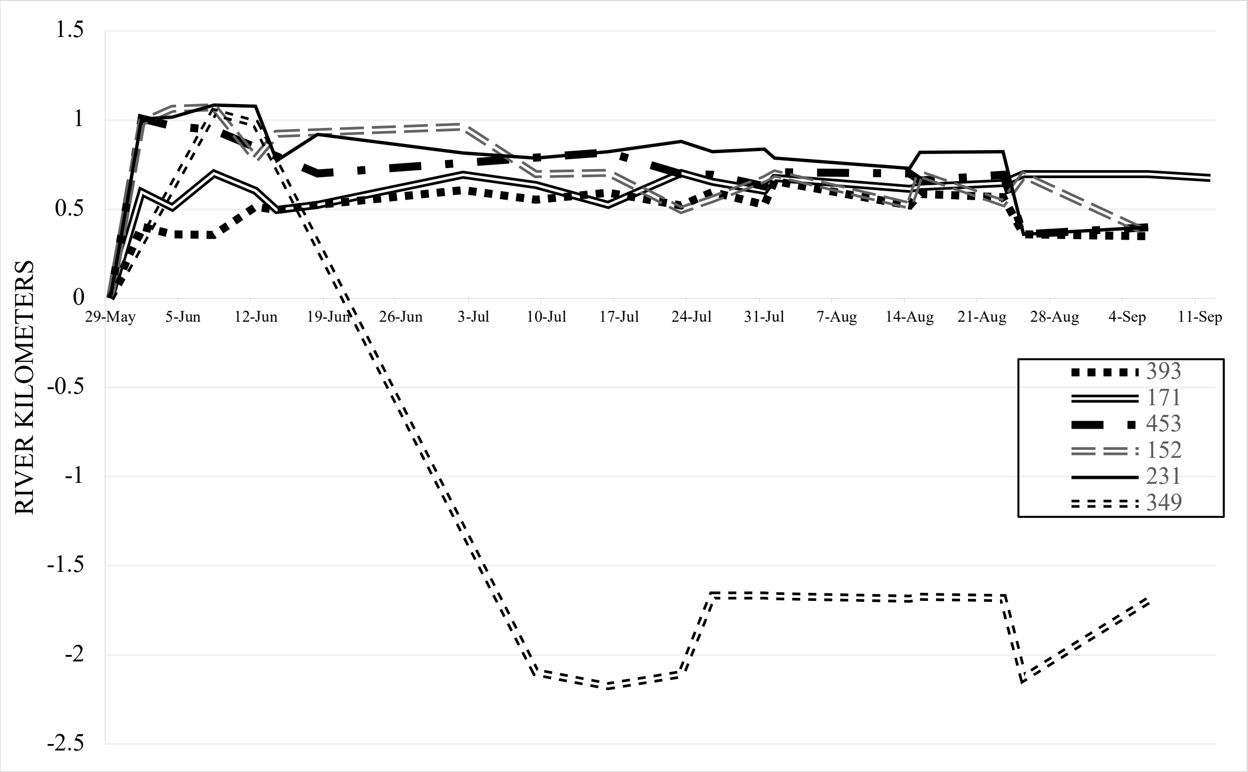

Figure 4a. Distance traveled by six river redhorse in the Grand River from release during their spawning season until early September. Positive numbers indicate tracked locations upstream of the release point. Individuals included in this graph traveled less than 10 km from the release site and were found at least once during the summer of 2018.

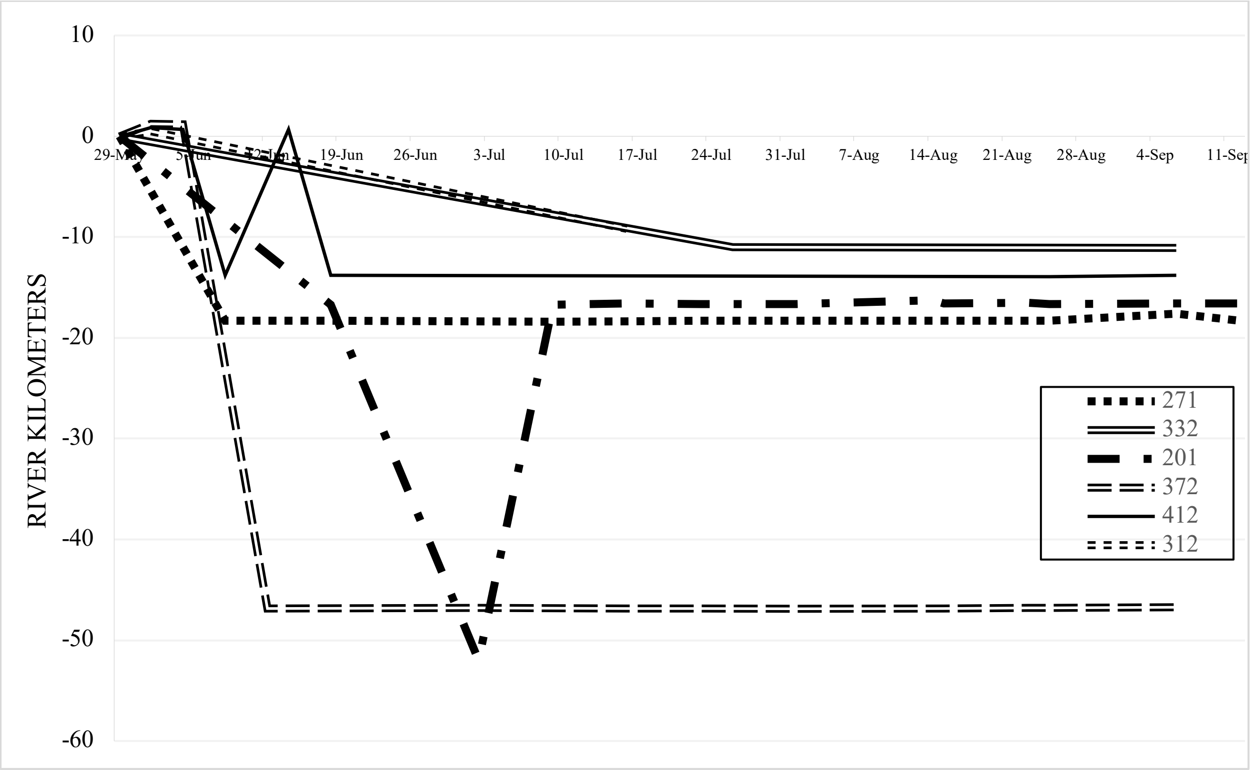

Figure 4b. Distance traveled by six river redhorse in the Grand River from release during their spawning season until early September. Positive numbers indicate tracked locations upstream of the release point. Individuals included in this graph traveled more than 10 km from the release site and were found at least once during the summer of 2018.

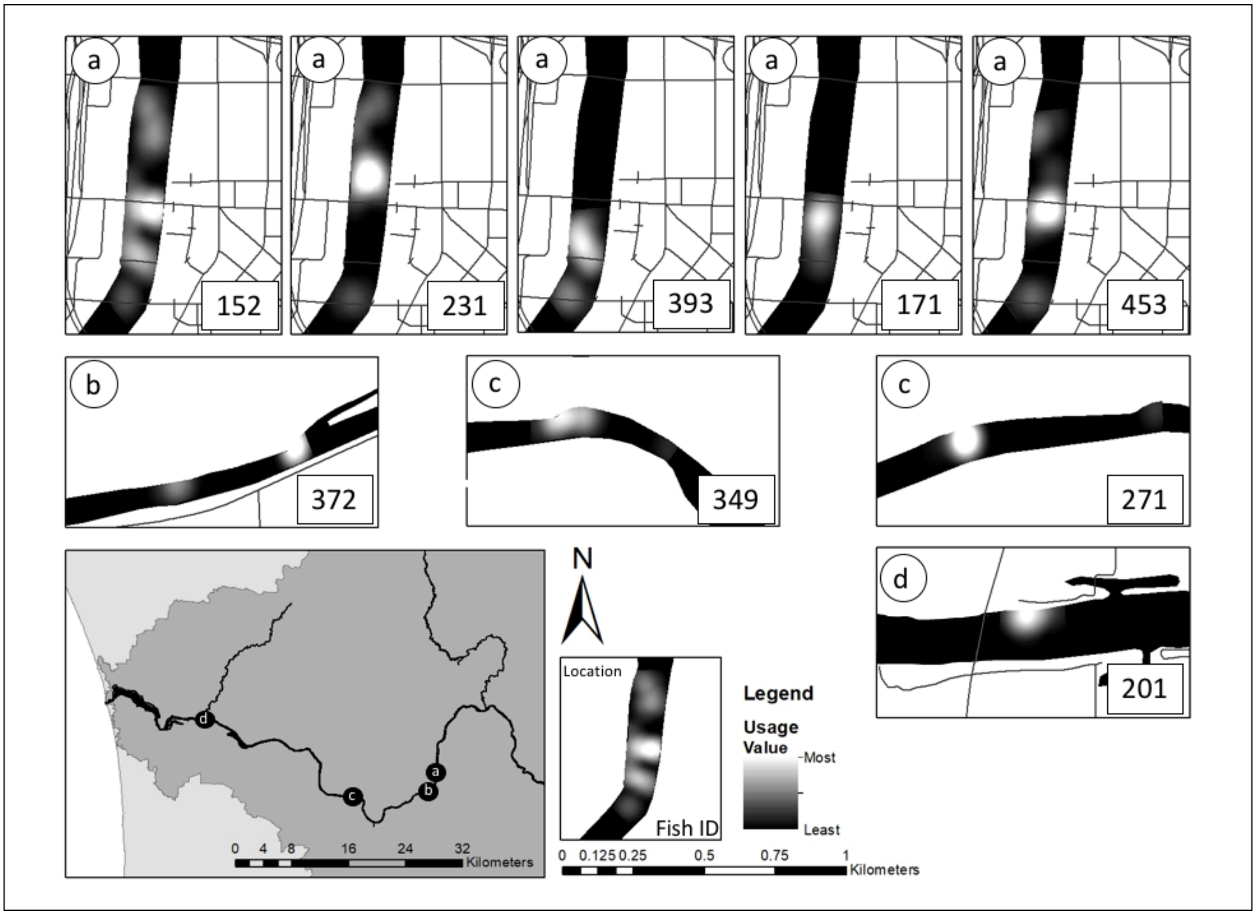

Figure 5. Home ranges of nine river redhorse tracked in the Grand River through the summer of 2018. Home ranges varied between 0.04 and 0.12 km^2 and were widely distributed. Five individuals established home ranges within the city of Grand Rapids (a), one approximately two kilometers downriver (b), two approximately fifteen kilometers downriver (c), and one nearly 46 kilometers downriver (d).

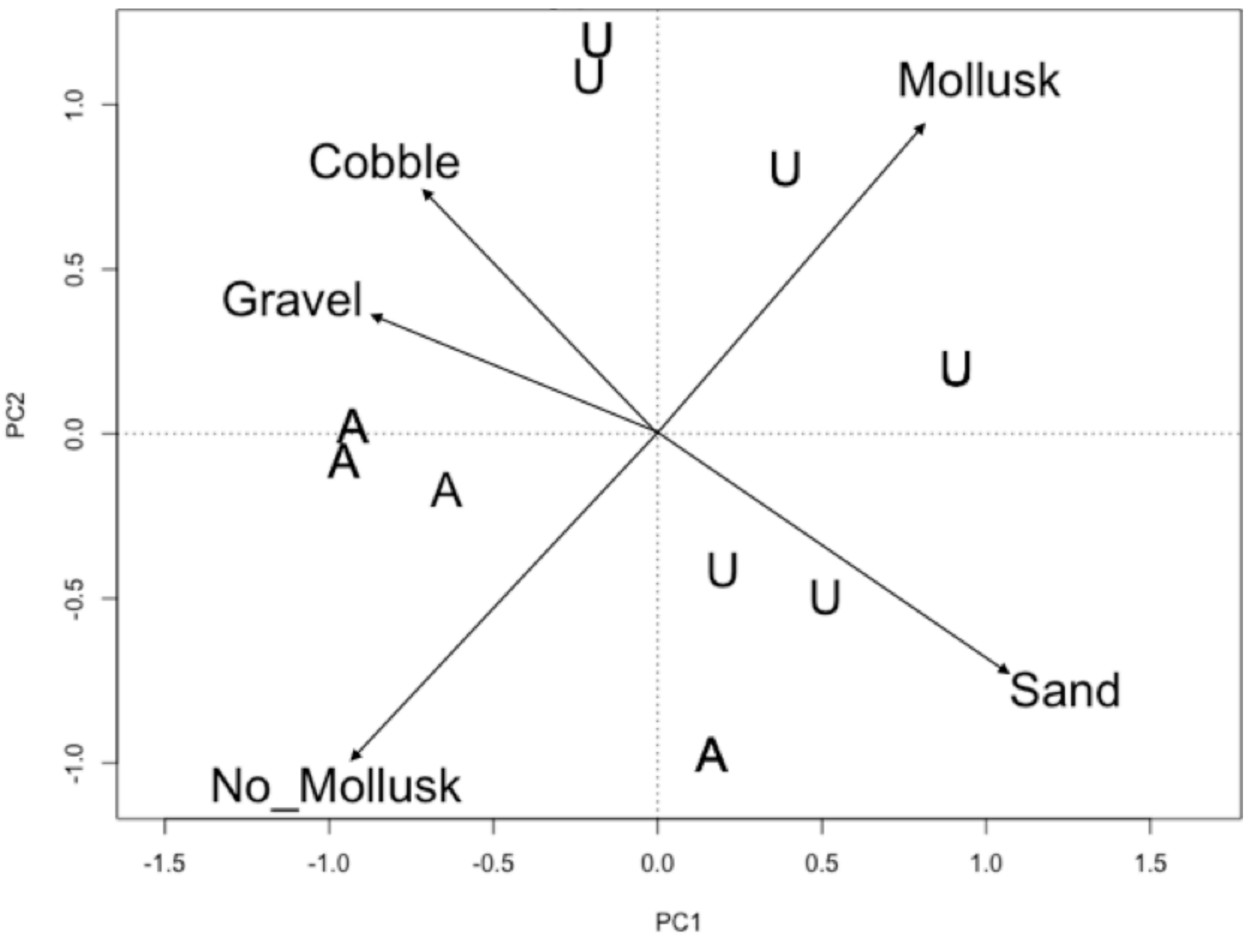

Figure 6. Principle Component Analysis of habitat used by river redhorse (U=used) and habitat available to them within their home range (A=available). PC1 = 50%, PC2 = 35% variation explained.

Chapter 3

River Redhorse Tributary Spawning

Introduction

The identification of spawning locations is critical for the protection of any fish species. However, this can prove difficult when working with rare, endangered, or cryptic populations as traditional survey methods require capture and visual identification of given individuals. These methods are often hindered by the environmental characteristics such as high water levels and turbidity and may cause undesirable effects on the study population. Environmental DNA or eDNA is an alternative survey method that has gained traction in recent years. Environmental DNA relies on the collection and amplification of genetic material released from a fish through its waste, slime, and scales. It has been used for the early detection of invasive species and their range expansions (Jerde et al. 2011, Davison et al. 2016), the detection of rare and imperiled species (Janosik and Johnston 2015), and for examining the biodiversity of freshwater ecosystems (Thomsen et al. 2012). Environmental DNA provides improved detection probabilities when compared to traditional survey techniques and can be used in situations where physical collection surveys may not be feasible (Jerde et al. 2011, Hinlo et al. 2017a, Deiner et al. 2017).

River redhorse (*Moxostoma carinatum*) are rare, difficult to capture, and difficult to identify (Becker 1983a, Parker 1988). They are one of six *Moxostoma* species in Michigan and are the only state threatened species in that genus. As a result of their morphological similarities they are often mistaken for other species of redhorse by both fisheries managers and the fishing public. This has been identified as one of the key problems facing the species and alleviating it is noted as a priority action in the Michigan Wildlife Action Plan (Parker and McKee 1984, O’Keefe 2002, Reid 2003, Cooke et al. 2005, Reid et al. 2006, Derosier et al. 2015). During a 2002 study, reports of the species’ presence in the Detroit, St. Clair, and Au Sable rivers were called into question as a result of misidentification. In addition, the lumping of the species into a group with the other fish in its genus was found to have resulted in the State of Michigan incentivizing bowfishing through its Master Angler Award program. This lead to multiple instances of accidental take in the Flat River near Lowell, Michigan (O’Keefe 2002). The distribution of the species in Ontario has also been called into question as a result of unreliable field identifications and subsequent genetic testing of collected specimens (Reid and Wilson 2006).

Because of these sampling issues and their distinction as a non-game species, river redhorse are remarkably understudied and management of the species has suffered as a result. We understand their general spawning habitat, typically in the mainstem of large rivers and the the lower reaches of their tributaries, but few spawning locations have been identified. A contributing factor to this lack of knowledge is the high water levels present during the river redhorse’s spring spawning run which limits the effectiveness of typical field survey methods. To alleviate this problem we created a species-specific DNA amplification procedure, which can be used to identify water samples potentially containing river redhorse eDNA.

Methods

Study site – Four tributaries were selected as potential river redhorse spawning sites and a fifth, the Flat River, was used as a positive control as river redhorse were known to spawn near its confluence with the Grand River (Figure 1, O’Keefe 2002). These five tributaries include a wide variety of physical, chemical, and biological factors that may influence the spawning activities of the species. Stony Creek is farthest upriver and is a major tributary to the Maple River. It has been extensively channelized but given its proximity to Fish Creek, another Maple River tributary and known river redhorse spawning location, was thought to be a potential spawning site (O'Keefe 2002). Prairie Creek is approximately ten kilometers down river of Stony Creek and has considerable habitat alterations in its headwaters. Despite this it remains a high quality cold water stream near its mouth. The Flat River joins the Grand approximately 25 kilometers downstream of Prairie Creek and is a known river redhorse spawning location (O'Keefe 2002). The Flat River maintains stable flows due to higher groundwater connectivity and an intact riparian zone, but has a large dam near its confluence with the Grand River limiting potential fish passage (Hanshue and Harrington 2017). The Rogue River is thirty kilometers further down river and includes many cold water tributaries. These cold water systems provide a diverse array of thermal regimes that may influence the timing of the river redhorse's spawning runs. Crockery Creek is a cold transitional stream (Lyons et al. 2009) and is the furthest downriver of our surveyed tributaries. It is the only surveyed tributary below Sixth Street Dam and may provide important river redhorse spawning habitat as few other large tributaries are present in this section of the Grand River.

Water Sampling – Two liters of river water were collected from each tributary near its confluence with Grand River using a modified version of the USGS eDNA collection guidelines (Laramie et al. 2015). Specifically, water samples were collected away from any potential sources of contamination, such as boat launches and bird nests, and at sections of the river that would accumulate potential eDNA like eddies and pools. Water samples were collected approximately two inches below the water's surface in a sterilized Nalgene collection bottle and held on ice alongside a cooler blank of sterilized water until being transported to a lab for filtration. Bottles were wiped with a ten percent bleach solution before entering or leaving the cooler. Eight water samples were collected from each tributary over a two week period starting May 15th and ending on the 31st to alleviate the potential for missed detections and in case river redhorse moved into different tributaries at different times (Ficetola et al. 2015).

Water samples were transported to a lab and processed within two hours of collection. A one liter equipment blank was first filtered onto 0.45µm nitrocellulose filter paper in a sterilized hood followed by one liter of water from each sample, each of which was filtered onto its own filter paper. Filter equipment was sterilized using DNA AWAY ™ (Thomas Scientific) between each water sample and was triple rinsed with sterilized water. Filter papers were stored in ethanol until DNA extraction using a Qiagen DNeasy Blood and Tissue kit. This combination of immediate filtering onto nitrocellulose filter paper, storage in ethanol, and extraction using a Blood and Tissue kit has been shown to yield the most DNA from water samples (Hinlo et al. 2017b).

DNA Processing - To test for the presence or absence of river redhorse eDNA in each water sample a species-specific protocol was created. We developed two primers, ForRRH (5'CAAGTCATAACGGCACAGCA3') and RevRRH (5'TTATCCAACCCCACCATCACG3') (Integrated DNA Technologies), which targeted a 245bp region of the mitochondrial ATP6 and ATP8 genes similar to those developed by Reid and Wilson (2006). This smaller fragment size provided a greater likelihood of its persistence in water samples and was examined for unintended templates using NCBI's Primer Blast. The potential for false positives from spurious amplification of other catostomid DNA was also examined using fin clips collected alongside the

Michigan DNR in the Grand River, Michigan. Specifically, amplicons of DNA from fin clips from shorthead redhorse (*Moxostoma macrolepidotum*), golden redhorse (*Moxostoma erythrurum*), silver redhorse *(Moxostoma anisurum*), white sucker (*Catostomus commersonii*), longnose sucker (*Catostomus catostomus*), greater redhorse (*Moxostoma valenciennesi*), and black redhorse (*Moxostoma duquesnei*) were compared to river redhorse amplicons. Spurious amplification was noted and to alleviate this a restriction enzyme, *Cac*8I (New England Biolabs), was used to create the species-specific protocol. The combination of positive amplification with the ForRRH and RevRRH primers and cutting the 245bp amplicon into 115 and 129bp fragments with the *Cac*8I restriction enzyme provided a species-specific test that was applied to all extracted water samples.

Amplified and digested water and tissue samples were visualized using gel electrophoresis with a 2% agarose gel. DNA fragments were sized using a 50bp to 10kb Fast DNA Ladder (New England Biolabs). Water samples were also run alongside a positive control containing known river redhorse DNA extracted from tissue, as well as a negative control containing no river redhorse DNA, an equipment blank, and a cooler blank.

Results

During screening for non-specific amplification of our primers using NCBI's Primer Blast two potential false positives were noted, coho salmon (*Oncorhynchus kisutch*) yielding a 481bp fragment and wild carrot (*Daucus carota*) yielding a 3650bp fragment. Theoretical amplicons from these false positives were considerably larger than the amplicon of River Redhorse, 245bp, and so were not of concern in our tests. Fin clip DNA tests found identical 245bp fragment amplification from river redhorse, shorthead redhorse, greater redhorse, golden redhorse, silver redhorse, white sucker, and longnose sucker DNA. Black redhorse DNA was not amplified by the listed primers. To resolve the similar amplification seen in the other catostomid species noted above, a restriction enzyme, *Cac*8I, was used to cut the amplified DNA in a species-specific fashion. *Cac*8I restriction digests resulted in a 116bp fragment and a 129bp fragment in river redhorse samples, but did not cut the DNA from other amplified species. This result provided a species specific test for water sample analyses.

None of the forty collected water samples exhibited any DNA amplification. Negative controls, cooler blanks, and equipment blanks also came back negative while positive controls containing known river redhorse DNA extracted from tissue exhibited the expected banding pattern.

Discussion

River redhorse have experienced significant management problems as a result of misidentification (Parker and McKee 1984, O'Keefe 2002, Cooke et al. 2005, Reid et al. 2006, Reid and Wilson 2006). Their similarities with five closely related species have caused anglers and managers issues for years and as a result there have been cases of accidental take and errors in range delimitation. The species-specific test developed here provides a simple means of alleviating some of these issues. By analyzing a small tissue sample a potential manager can identify if a species is a river redhorse or not. A similar method was used on a broader range of redhorse species in Reid and Wilson (2006). However, this does not address issues with angler misidentification. To alleviate this issue fisheries managers should provide greater emphasis on public education projects focused on identifying closely related species. A good example of this can be seen in O'Keefe (2002), which provides easily identifiable characteristics for redhorse species in Michigan.

Environmental DNA detection can be hindered by a number of factors (Nielsen et al. 2007, Barnes et al. 2014, Matheson et al. 2014, McKee et al. 2015, Ficetola et al. 2015, Williams et al. 2017). Environmental characteristics like temperature, pH, and turbidity can affect the longevity of eDNA fragments (Barnes et al. 2014), soils can bind to eDNA preventing its degradation but also limiting its collectability (McKee et al. 2015, Williams et al. 2017), and plant constituents like humic acid can inhibit PCR amplification (Matheson et al. 2014). These factors are likely most problematic during high water sampling as excess runoff can reduce the residency time of eDNA and increase the number of PCR inhibitors present in water samples. There are several methods that can be used to limit these effects (Laramie et al. 2015, McKee et al. 2015, Williams et al. 2017), but these can increase the cost of an otherwise affordable method.

Some of the primary advantages of eDNA analysis are the reduced sampling costs, the reduced effort needed for field sampling, and the higher detection probabilities for rare species. There is evidence that many of these factors hold true in low turbidity systems (Williams et al. 2017), but in more turbid water bodies DNA processing costs and lack of experienced personnel may prove prohibitive. We would advise anyone considering eDNA analysis to examine the environmental factors of their study site in relation to eDNA collection and processing to ensure that it is a viable sampling option. In addition, if eDNA analysis is attempted in a highly turbid system then researchers should be prepared to remove any potential PCR inhibitors.

Figures

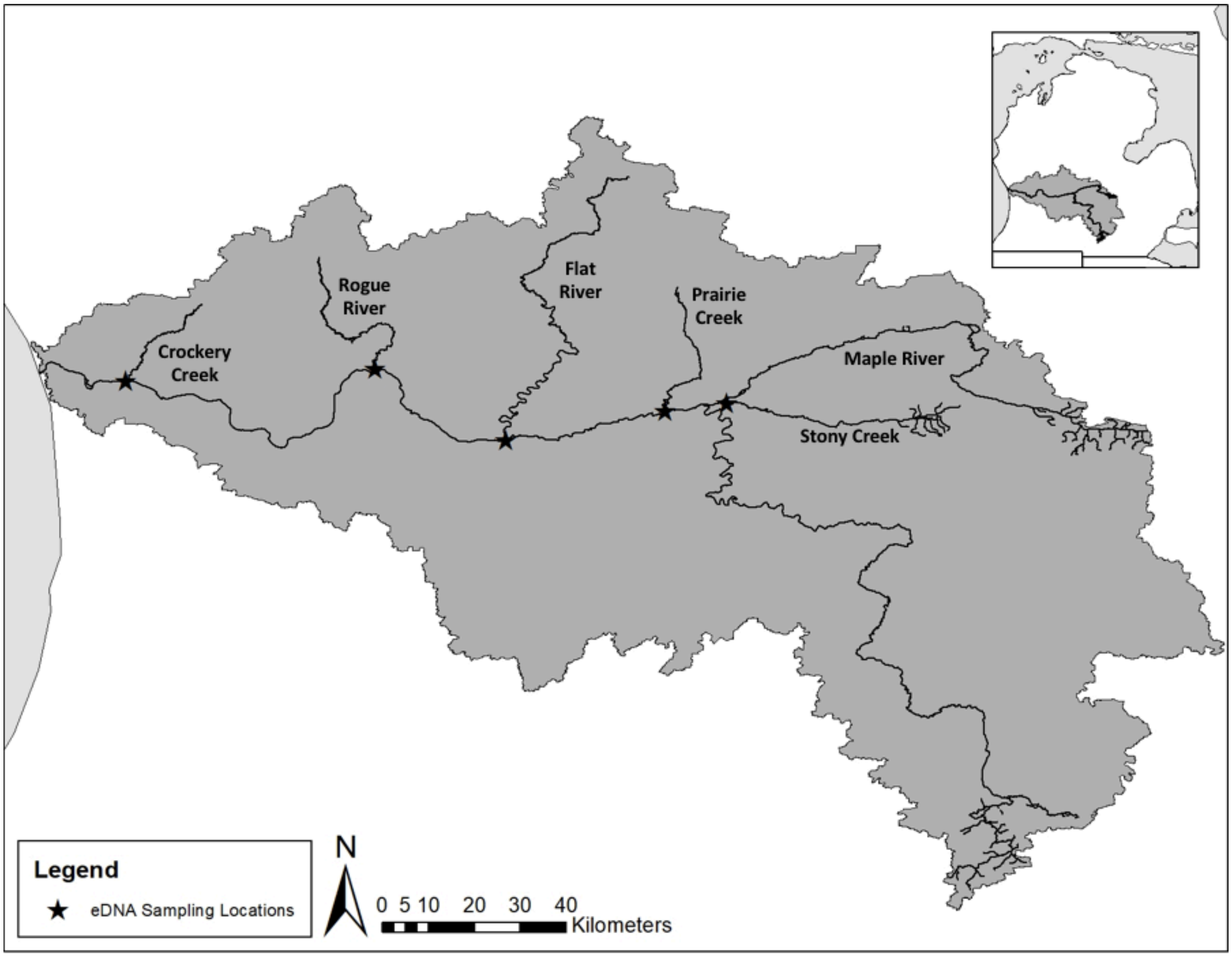

Figure 1. Tributaries of the Grand River targeted for eDNA sample collections. Four tributaries were targeted as possible spawning locations and one tributary, the Flat River, was used as a positive control as River Redhorse were known to spawn near its confluence with the Grand River.

Chapter 4

Grand River Surveys

Introduction

Our understanding of the resources and services that natural rivers can provide has grown immensely in the last few decades, and as a result interest in stream and river restoration has grown as well. Throughout the country an estimated $15 billion was spent on stream and river restoration from 1990 to 2005 (Bernhardt et al. 2005), though this was considered an underestimate. Of this, the Midwest has spent approximately 440 million dollars predominantly on riparian management and bank stabilization (Bernhardt et al. 2005, Alexander and Allan 2006). Despite the large amounts of money involved in these restoration projects, only 10 percent of country-wide projects were ever monitored or assessed following completion. This figure was found to be even lower in Michigan where only 1.9 percent of projects indicated that any post-project monitoring took place (Bernhardt et al. 2005). Lack of post-project monitoring is not only a wasted opportunity, as it can provide valuable information on the causes of success or failure for future restoration projects, but is also a flaw in our current methods of restoration as success cannot be evaluated without proper assessment and cannot be compared with other projects without an agreed upon set of criteria (Palmer et al. 2005)

Lack of post-project monitoring may be ubiquitous but it is also a paradigm that can be shifted through proper education and successful examples. By providing a procedure for the assessment of the fish communities in the Grand River before a large scale dam removal and river restoration project we hope not only to better understand the effects of dam removal and habitat restoration but also to provide an example of a pre-project to post-project monitoring plan. The effectiveness of the monitoring protocol, and the example that it will set, will depend on the underlying standards that they are based around. Palmer et al. (2005) provide a detailed list of five ecologically effective standards for river restoration but here we will focus on the completion of ecological assessment, while two others, improvement of the ecosystem and increased ecosystem resiliency, will provide the theoretical approach by which we could evaluate success.

Ecologically speaking, dam removal is primarily concerned with the elimination of migration barriers, improvement of habitat above and below the dam, and restoration of a natural biotic community. In the specific case of the Grand River restoration project, there is also concern over the health of a number of threatened and endangered species including the lake sturgeon (*Acipenser fulvescens*), river redhorse (*Moxostoma carinatum*), and snuffbox mussel (*Epioblasma triquetra*) as well as the potential spawning migration of the invasive sea lamprey (*Petromyzon marinus*; Hanshue and Harrington 2017, "Grand Rapids Whitewater" 2019). In order to provide a full assessment of the current impacts of the dams in downtown Grand Rapids and to provide baseline data on the fish community prior to any restoration work we conducted a series of easily replicable electrofishing surveys aimed at documenting the complete summer fish community in the river.

Methods

Study site- Fish surveys were conducted in the Grand River near Grand Rapids, Michigan, during July 2018. Discharge at this time ranged from 1400 to 1800 cubic feet per second (USGS 2019b). This section of the river has a steep slope, dropping 1.04 m/km as well as five run of river dams which significantly alter its flow regime. These factors are responsible for faster flows, shallower water, and a lower proportion of fine sediment than is typically seen in the

Grand River. This stretch of river is also scheduled for a restoration project that would remove the four small coffer dams and replace the larger 6th Street Dam with an adjustable barrier further up river.

Four study reaches were used to account for the habitat variability present in and around Grand Rapids and to examine any potential downstream impacts of the eventual restoration project (Figure 1). The first study reach was within the proposed restoration area and consisted of four electrofishing transects of approximately equal length (Figure 1d). These were located on the west bank of the Grand River and consisted primarily of riffle and run habitat. The second study reach was immediately downstream of the restoration area and consisted of three electrofishing transects of approximately equal length located along the west bank, east bank, and center of the river (Figure 1c). The third study reach was approximately 100 meters downstream of the second and included three equal length transects located along the west bank, east bank, and center of the river (Figure 1c). The fourth study reach included three areas of potentially high quality habitat further down river, one at the mouth of Plaster Creek, one at the City of Grand Rapids wastewater treatment outflow, and one at a large gravel bar near Johnson Park (Figure 1a & 1b).

Sampling Methods – We employed two electrofishing techniques to accommodate the different depths in our survey reaches and to provide a better estimation of the overall fish community. Catch per unit effort (CPUE) calculations were made to provide a standardized measure for comparisons with future studies. General abundance and species richness were also documented.

In the restoration transects we used tote-barge electrofishing which allowed access to the shallow riffles and shoreline habitat a boom shocking boat would not have and provided a more efficient sampling technique for smaller-bodied fish like minnows and darters. Fish were held in a flow through floating net pen until the completion of a transect at which time they were identified, measured to the nearest millimeter, and released. To facilitate rapid processing and because our primary concern was assessing the broader fish community we only measured the first ten individuals of a given species with the exception of logperch (*Percina caprodes*), which were of particular interest due to their relationship with snuffbox mussels.

The reaches immediately below the restoration area and further downriver were sampled using an electrofishing boat as the transects here were considerably deeper and it would have been unrealistic to sample them using a tote-barge. Fish were held in a large aerated trough until the completion of a transect and in a flow through net pen while awaiting processing. Following the completion of a transect fish were identified, measured to the nearest millimeter, and released.

Results

A total of 1,184 fish representing 45 species were captured during all electrofishing surveys and two species, muskellunge (*Esox masquinongy*), and longnose gar (*Lepisosteus osseus*), were seen but not captured. This represents just under half of the 108 fish species documented to be present in the Grand River and includes multiple species of note. In addition, as this study was conducted during the summer we failed to document the large number of migratory species that utilize the area including coho and chinook salmon (*Oncorhynchus kisutch* and *O. tshawytscha*), steelhead (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), longnose sucker (*Catostomus catostomus*), and lake sturgeon. Of the fish captured, black redhorse (*Moxostoma duquesnei*), river chub (*Nocomis micropogon*), and striped shiner (*Luxilus* chrysocephalus) are all listed as Species of

Greatest Conservation Need and river redhorse are listed as threatened. We also captured two non-native species, common carp (*Cyprinus carpio*) and round goby (*Neogobius melanostomus*) as well as a native species, the logperch, which is critical to the propagation of the federally endangered snuffbox mussel.

The greatest species diversity was found in the restoration transects, though this may have been due to methods used in the survey. Cyprinids represented nearly half of the 878 fish captured in this reach with the bulk of this coming from spottail and sand shiners (Table 1). This reach was also home to a number of logperch, which appeared to be from two distinct year classes with mean lengths around 60mm and 100mm (Figure 2). Other species of note in this reach were river chub, striped shiner, river redhorse, and round goby.

The transects immediately adjacent to the restoration area tended to contain fewer but larger fish and the community here was largely made up of redhorse species. Species of note in this reach included black redhorse and logperch. The next reach down river followed the same community pattern, being primarily made up of redhorse species, and included logperch and river redhorse. River redhorse were also found in the three transects further down river. In addition, a spawning school of over 50 individual river redhorse was documented at the Plaster Creek sampling transect earlier in the year. Males in this school exhibited nuptial stripes, indicating active spawning.

Discussion

The fish community in and around Grand Rapids is highly diverse and contains a number of species of concern. The electrofishing transects within the restoration area alone contained approximately one third of all species present in the Grand River. The biodiversity seen here is rare in the rest of the river as it includes species associated with both larger waterways, like river redhorse and black buffalo, and small streams, like rainbow darters (*Etheostoma caeruleum*). While this biodiversity should be valued for its own sake it also provides greater resistance to disease, invasion, and disturbance than would otherwise be present as well as greater resource use, productivity, and diversity in other communities (Tilman 1995, Holmlund and Hammer 1999, Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2006).

Biodiversity benefits are dependent on the roles played by the organisms in the overall ecosystem. River redhorse, for example, are unique in their ability to feed on large mollusks and so can pass energy and nutrients from an otherwise static state into the food web. River, golden, and black redhorse stir the bottom sediment during their feeding and increase the food available for drift feeding fish as a result. Longnose gar help maintain baitfish populations by preventing overpopulation, especially when other predators are over-harvested from a given waterway (Robinson and Buchanan T. M. 1988, Scarnecchia 1992). Logperch are unique in their ability to survive the forced infestation of snuffbox mussel glochidia into their gills and so are able to help propagate a species that would otherwise be extirpated from our rivers.

The interaction between logperch and snuffbox mussels is of particular interest in the Grand River as it is one of the few places with locally abundant populations of the endangered mussel. We documented two age classes of logperch in the Grand River; a young of the year group with an average length around 60 millimeters and an adult group with an average length around 100 millimeters. Logperch are short lived species and typically mature in two years (Winn 1958, Becker 1983b). Considerable overlap in the length of adults has been observed making it difficult to differentiate age based on length past the first year (Winn 1958, Becker 1983b). However, it is important for the persistence of the species to note the presence of both age classes in future studies as it will provide an early indication of potential issues with its reproduction.

There are many important linkages in the Grand River beyond those mentioned here but these begin to break down as anthropogenic changes alter the river's ecosystem. One such issue involves the competitive interaction between round gobies and logperch. Round gobies, an invasive species, have been seen to outcompete logperch for both food and space leading to their extirpation from certain sections of Great Lakes (French and Jude 2001, Balshine et al. 2005). This could prove problematic given the number of round goby found in the restoration area; therefore populations of the two species should be monitored closely. The dams present in this section of the river also likely alter the biotic community. Small bodied fish have limited migration potential and these barriers can limit their local distribution. This has consequences for the fish, the mussels they host, and the food web in general (Watters 1996). The fish ladder at the 6th Street Dam is even more problematic as smaller bodied fish have not been observed passing over this structure (Ryckman 1986). The last passage assessment was conducted in the 1980's and it is currently unclear if the passability of the barrier remains the same today. This is especially true given that the fish ladder was recently modified to reduce the upstream movement of adult sea lamprey. A follow up study should be conducted to better understand the fish community that is currently able to use the ladder.

The restoration project scheduled for this section of the Grand River will have far-reaching consequences on the fish community. Restoration projects have been shown to cause initial degradation in ecosystem health and in some cases these degradations can last for multiple years (Detenbeck et al. 1992, Pess et al. 2008, Foley et al. 2015). These changes can often follow predictable patterns but the manipulation of natural ecosystems tends to have unintended consequences. For example, a dam removal project in the Elwha River, Washington resulted in a large sediment plume moving down river. This was easily predicted but the drastic water quality change seen in the mouth of the river that resulted from the increased sedimentation was not (Foley et al. 2015). In the Grand River potential issues include loss of spawning habitat for river redhorse, lake sturgeon, and a number of other species, lack of recolonization following restoration, and alterations to the sediment present both in the restoration reach and further downstream. These factors will require further monitoring as the project proceeds and for several years after its completion. Potential impacts of the project, both positive and negative, will depend on its final design and implementation. Guidelines for maintaining and even benefiting the fish community in the Grand River include designing in-stream structures that protect natural structure in the river, designing impermanent structures that guide natural processes and which allow the natural dynamics, including sediment deposition and erosion, of the river to continue, and designing channels which mimic the natural geomorphology of the river (Colburn 2012).

Lack of post-project monitoring is all too common and prevents us from understanding the causes of success and failure during restoration. This project offers an opportunity to examine the river ecosystem before and after restoration and to evaluate the success of that restoration. Here we have outlined the current fish community in the Grand River and provide a baseline for future researchers to compare to the community following the completion of the project. Comparisons should include assessments of the improved resiliency and composition of the ecosystem as outlined in Palmer et al. (2005). Dam removal and river restoration has grown alongside our understanding of the resources and services that natural rivers can provide. The continued growth of these two fields will depend on improving our understanding of the impacts of these projects and this study is one step toward that understanding.

Tables

Table 1. Fish community present in each of the four electrofishing reaches during July 2018 listed from most downstream to most upstream. The sites furthest downriver are included separately as they are too far apart to warrant grouping. Total abundance is broken into six categories; Catostomidae, *Moxostoma*, Centrachidae, Percidae, Cyprinidae, and Ictaluridae. *Moxostoma* was included as a separate category of Catostomidae to highlight the abundance of redhorse species. River redhorse were present at the Restoration, Adj. Down, Plaster Creek, and Wastewater samples reaches.

Site	Sample Date	Abundance	Species	CPUE (Fish/Min)	Cato.	Mox.	Centr.	Perc.	Cypr.	Icta.
Johnson Park	4-11	17	10	2.5	3	3	9	2	2	0
Wastewater	4-11	28	12	2.48	13	13	6	1	0	5
Plaster Creek	4-10	37	10	2.08	18	18	14	2	0	0
Adj. Down	4-11	113	21	3.82	55	51	37	4	5	9
Adjacent	4-10	106	25	3.02	54	54	17	5	8	12
Restoration	4-12 & 4-19	883	30	9.6	128	122	174	78	387	0

Figures

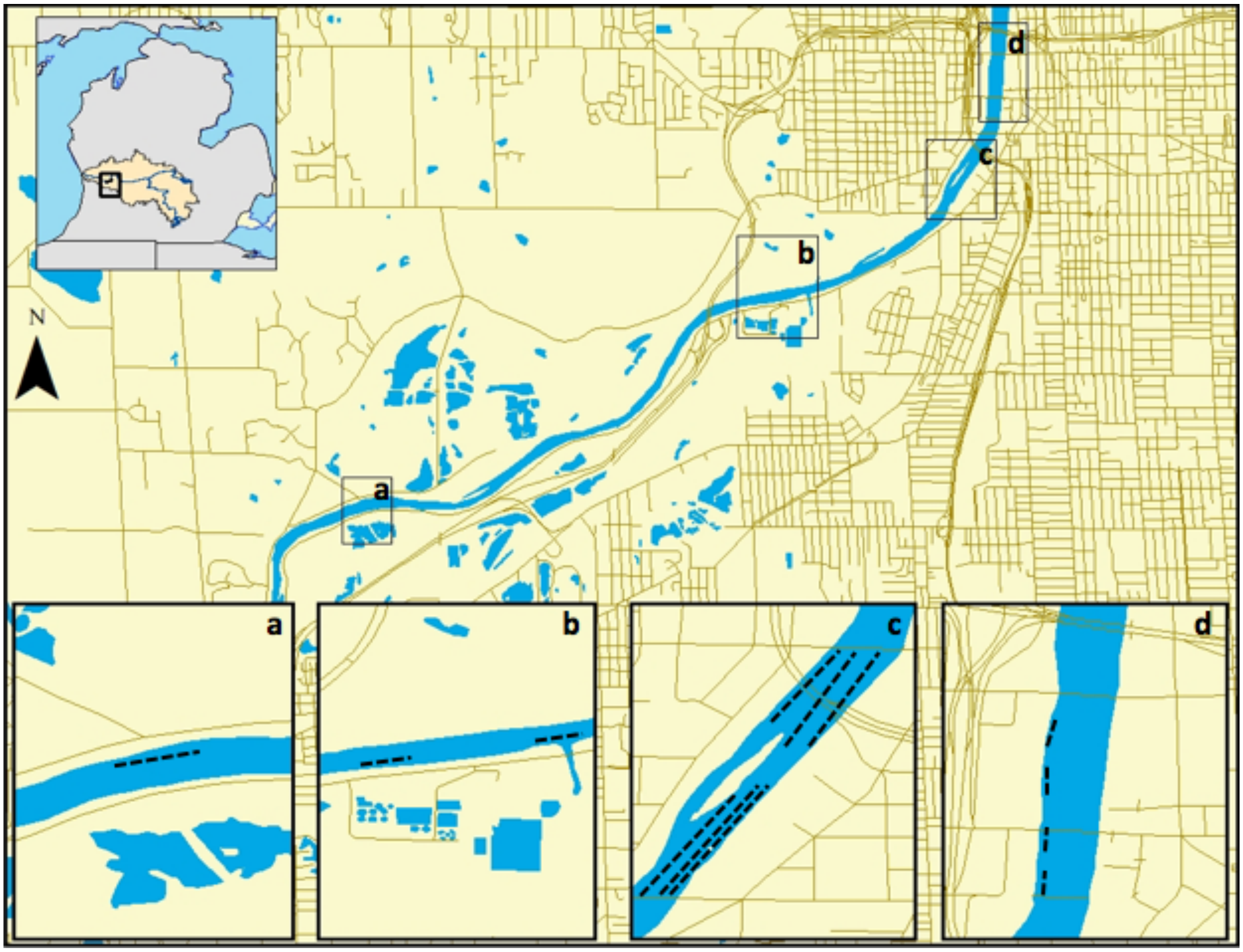

Figure 1. Electrofishing transects within the four study reaches of the Grand River. From most downstream to most upstream; box a shows the area of high quality habitat at Johnson Park, box b shows the areas of high quality habitat at the wastewater treatment outflow and Plaster Creek, box c represents the reaches adjacent to the restoration area (top right of the box) and those just downriver (bottom left of the box), box d shows the four transects within the proposed restoration area.

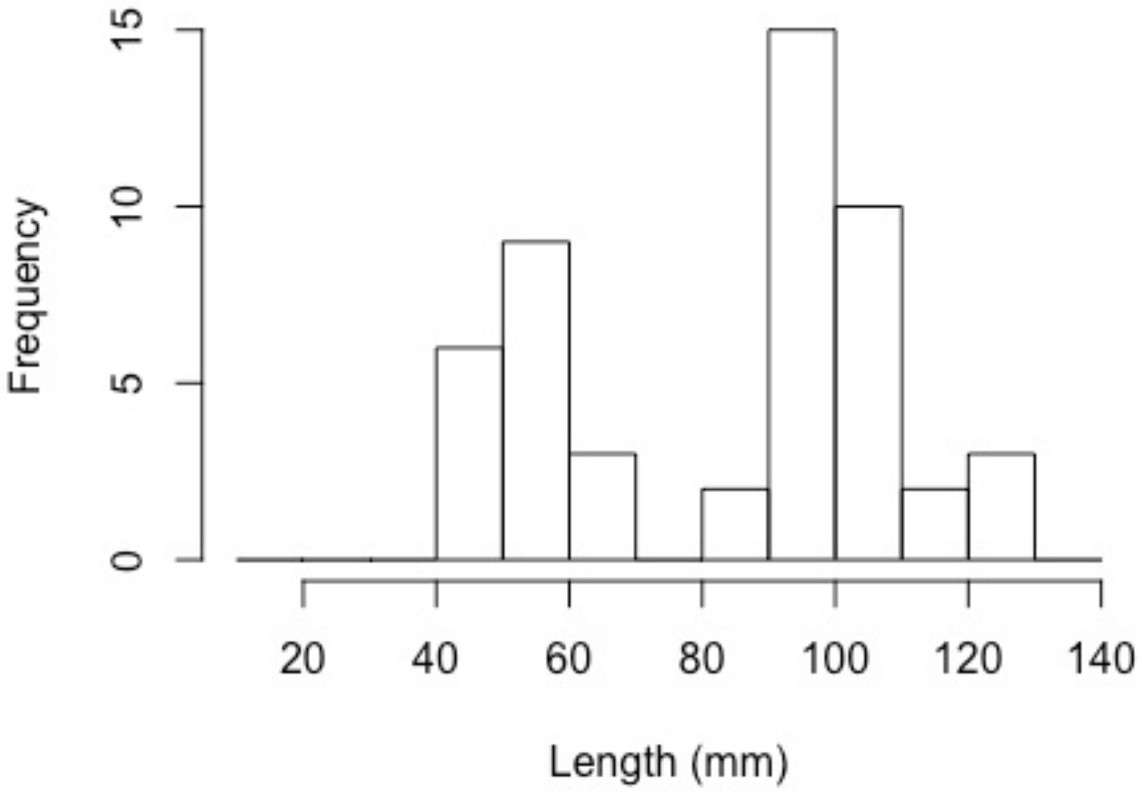

Figure 2. Histogram of logperch length (mm) distributions in the restoration reach of the Grand River. Collections were done via tote-barge electrofishing and two distinct year classes can be seen.

Chapter 5

Literature Review and Extended Methodology

Literature Review

River redhorse (*Moxostoma carinatum*) are one of the largest members of the Catostomidae family, growing as long as 75 centimeters and reaching weights of over 4.5 kilograms (Becker 1983). In Michigan, it is often confused with greater redhorse (*Moxostoma valenciennesi*) and shorthead redhorse (*Moxostoma macrolepidotum*), both of which also possess the red fins that are often first noted when attempting identification. While it is often considered difficult to differentiate between species in the *Moxostoma* genus, the river redhorse can be identified in the field by its large size, red asymmetrical caudal fin, lack of transverse grooves on its lips, large head, and distinct number of scales around its caudal peduncle (Jenkins 1970, O'Keefe 2002). In addition, river redhorse possess unique pharyngeal teeth which are only shared with its cousin the copper redhorse (*Moxostoma hubbsi*; Becker 1983a). While these teeth can be used to identify the species, they are difficult to see in a living specimen and so are not often used as a distinguishing characteristic in the field.

Original reports of the river redhorse's lifespan indicated longevity between 12 and 17 years, although those ages were considered an underestimate of their true life span (Beckman and Hutson 2012). More precise aging techniques using opercles have yielded more accurate estimates and have been used to document individuals living as long as 28 years (Reid et al. 2006). This longevity allows an individual multiple chances to reproduce but is accompanied by late sexual maturation. Late maturation can allow an individual to achieve greater reproductive output once mature (Roff 1992), but it can also prevent reproduction altogether should the individual die before it comes of age (Roff 1992, Fonseca and Cabral 2007). This has consequences for both the short-term population dynamics and the long-term evolution of the species. These effects are exacerbated in its northern range as colder temperatures slow its maturation (Kuparinen et al. 2011). For river redhorse, late maturation may have prevented it from adapting to the changing environment and contributed to its decline.

Related issues arise as anglers target older, larger fish, which are often the most fecund (Birkeland and Dayton 2005) reducing the size of the breeding population. Despite its threatened status in Michigan, this is likely occurring with the river redhorse. Examination of the Michigan Master Angler records shows a number of large redhorse, many approaching 30 inches in length, taken from around the state. Many of these large redhorse, while not documented to species in the records, were found to likely be river redhorse through later investigation (O'Keefe 2002) . Removal of these large adults can have long lasting consequences on the populations. It can reduce the abundance of future generations, as even a small female can produce 20,000 eggs in a single spawning season (Hackney et al. 1968), artificially eliminate the best adapted individuals, and can alter the reproductive timing and maturation of the population (Olsen et al. 2004).

The river redhorse is a late spawner when compared with other species in its genus. They have been reported to begin spawning from early April in southern waters to May and June in northern waters. Temperatures in northern waters at these times range from 17 to 20°C (Becker 1983a, Campbell 2002, Reid et al. 2006), with fish moving into the spawning area at temperatures as low as 15°C (Reid 2003). Spawning takes place in swift current overtop of gravel and other large substrate. Male river redhorse enter areas with suitable spawning habitat first, at lower temperatures than the females (Campbell 2002). Once on the spawning grounds Hackney et al. (1968) observed that males build redds up to eight feet wide and twelve inches deep. However, other studies have reported smaller redds and suggested that they are the result of other mating habits as opposed to nest constructing behavior (Jenkins 1970, Parker 1988). Whatever the causes of these sediment depressions, males are noted to hold position overtop of them while presenting mating displays to entice nearby females. This display involves two males darting back and forth across the spawning area until a female joins them. The males then flank the female releasing sperm as she releases eggs into the gravel below (Hackney et al. 1968). This has been observed by other studies and follows what is commonly known about sucker reproduction (Parker and McKee 1984, Reid 2003).

Following deposition, eggs remain in the gravel for 3 to 4 days until the young hatch and enter the water column (Hackney et al. 1968). While experiments and observations on river redhorse at this age are rare, lack of information can actually allow us to make important conclusions. For example, most individuals captured during studies are adults occupying swift flowing areas of rivers (Yoder and Beaumier 1986, Reid et al. 2006). The lack of juveniles captured in these studies suggests that they likely occupy different habitats than the adults. Experiments on similar species can give us insight into this potential hypothesis. One such experiment on the robust redhorse (*Moxostoma robustum*), a closely related cousin of the river redhorse, showed that juveniles of the species preferred slow moving areas of rivers and streams (Mosley and Jennings 2007). Eddies and backwaters provide refuge from high flow rates and predators, and they allow juveniles to feed on the aquatic organisms and organic matter deposited there. The use of eddies and backwaters has also been shown in a number of other sucker species including northern hogsuckers (*Hypentilium nigricans*), bluehead suckers (*Catostomus discobolus*), and flannelmouth suckers (*Catostomus latipinnis*; Matheney and

Rabeni 1995, Klein et al. 2017). It is therefore likely that juvenile river redhorse occupy similar slow flowing areas as they grow into adults.

An alternative hypothesis explaining the lack of juveniles captured during studies is their wariness of approaching people (Hackney et al. 1968). Hackney and colleagues noted that, even during feeding time, tank-reared juveniles would become skittish and nervous when researchers approached. This hypothesis could confound any researcher attempting to understand their physiology, but there has been no confirmation that juvenile river redhorse avoid humans any more than the adults.

As juveniles grow into adults they begin to inhabit large rivers with medium to fast flows and large particle substrates (Hackney et al. 1968, White and Trautman 1981, Becker 1983a, Yoder and Beaumier 1986). While Yoder and Beaumier (1986) showed that river redhorse prefer to avoid depositional zones, other studies have suggested that they may use these areas as refuges from excess flows and temperatures (Campbell 2002, Reid 2003). Campbell (2002) found river redhorse miles from the nearest riffle in up to 12 meters of water, while Reid (2003) found few specimens in their fast moving habitat during the fall, suggesting that they may be utilizing alternative areas of the river during different times of year. This phenomenon is well known in many fish species (Nickelson et al. 1992, Pander and Geist 2010) and has even been documented in catostomids. For example, northern hog suckers (*Hypentelium nigricans*) have been shown to occupy slower, deeper water with smaller substrates during the winter (Matheney and Rabeni 1995) and robust redhorse are documented as returning to the same overwintering and spawning grounds from year to year (Grabowski and Isely 2006). Diversity in habitat use seems likely for adult river redhorse but their dependence on fast moving water for spawning is well documented.

Fast water flushes fine sediment, preventing its accumulation on the riverbed and allowing the exposure of the river redhorse's main food source, mollusks (Becker 1983).

Mussels, perhaps more so than most organisms, exhibit patchy distributions (Ries et al. 2016). The locations of their populations within rivers can be influenced by a number of factors including fish distributions, water chemistry, and hydrologic conditions (Lefevre and Curtis 1912, Strayer et al. 2004, Allen and Vaughn 2010, Strayer 2014), and they are often greatly hindered by degraded water quality. Because of this and other anthropogenic threats, approximately 70 percent of known mussel species are extinct or in peril (Stein et al. 2000). This presents a dangerous issue for river redhorse, which have specifically evolved to feed on mussels. The decline of their main food source has likely influenced their own declines and is identified as a contributing factor for their potential extirpations (Reid et al. 2006). While the feeding specialization could prove disastrous for the river redhorse, it could also serve as an important management tool.

A number of non-native mollusks have invaded Michigan waterways including zebra and quagga mussels (*Dreissena polymorpha and D. bugensis*) and Asian clams (*Corbicula fluminea*). These species have few predators and often outcompete many native fauna leading to drastic changes in food webs (Kraemer 1979, Fahnenstiel et al. 1995, Gherardi and Acquistapace 2007). It is suggested that river redhorse, with their fairly unique feeding structures, could serve as effective biological controls for some of these aquatic invasive species (French 1993), and there is some evidence of this. Hackney et al. (1968) found that river redhorse in the Cahaba River, where their populations are fairly secure, fed largely on Asian clams. This could mean that a resurgence of river redhorse in Michigan could bring down Asian clam densities, though further research is needed to determine the potential effects on dreissenid mussels.

While river redhorse have the potential to be effective biologic controls for non-native mollusks, their current abundance is not sufficient to have a significant effect on invasive populations. Prior to European colonization, river redhorse occupied a large range that included the central Mississippi River basin, the Great Lakes, and the St. Lawrence River (Lee et al. 1980, White and Trautman 1981, Yoder and Beaumier 1986). This range covered much of the eastern United States and Canada but was in decline for many years (Scott and Crossman 1973). In the mid to late 1900's the species experienced serious population losses in Wisconsin, Illinois, Kansas, Iowa, Pennsylvania, Missouri, and Alabama, and was even thought to be extirpated from Michigan, Iowa, and Indiana (Becker 1983b). In Michigan, the river redhorse is currently listed as threatened with its populations restricted to the Muskegon, Grand, and St. Joseph rivers (Michigan Natural Features Inventory 2007). Beyond its Michigan range, the river redhorse is threatened in Wisconsin and Illinois, and noted as a species of concern in Ohio, Kansas, Ontario, and Quebec. With apparently secure populations in Alabama and Pennsylvania the species is in less danger than in the past but still faces a number of problems throughout its range.

Threats facing the river redhorse include flow control structures (Weyers et al. 2011), river fragmentation (Reid et al. 2008), loss of its main food source (Jenkins 1970), siltation, and degraded water quality (Becker 1983a). While physical threats have been credited with its drastic decline through the 20th century (Becker 1983b), river redhorse have not been well studied which has prevented the effective management of the species (Parker 1988).

Most fisheries management in the United States is geared toward "game species" that represent important sources of income for the country (Reynolds et al. 2008). This is no fault of the agencies that manage the fish communities but instead a result of simple economics where the primary focus is to acquire the most gain for our investment. As a result, non-game species are often left unmanaged with little attention or knowledge of their loss (Ricciardi and Rasmussen 1999). This is true for river redhorse as their range throughout the state of Michigan, and much of the rest of the country, are not wholly known. In addition, even known locations are called into question as sampling this species has proven difficult (White and Trautman 1981), and identification can be challenging to the untrained eye (Cooke et al. 2005).

While improper identification can be solved through training, sampling difficulties require more knowledge to fix. Sampling techniques for river redhorse have included fish traps and seine nets (White and Trautman 1981), boat mounted electrofishing units (Yoder and Beaumier 1986), hoop nets, and minnow nets (Campbell 2002). Varying degrees of success have been reported with these techniques and in some cases one or more have proven inadequate. White and Trautman (1981) noted the failure of experimental gill nets and seine nets in collecting river redhorse and the subsequent success of fish traps in the same sampling areas. Yoder and Beaumier (1986) further commented on the ineffectiveness of seining for adult river redhorse and encouraged the use of boat mounted electrofishing units when attempting to sample the species. Campbell (2002) attempted a number of sampling techniques for spawning and post-spawn adult river redhorse and found that electrofishing produced the greatest catch per unit effort followed by trap nets. While hoop nets and minnow nets were also employed, they represented just four percent of the total net catch. Trap nets and boat electrofishing have proven the most effective in these studies but boat electrofishing has been shown to be more effective when sampling catostomids in a lacustrine environment (Ruetz et al. 2007).

Most river redhorse sampling has occurred during spawning in their “preferred habitat”, as outlined by a number of fish identification and encyclopedic books. Few studies have attempted to sample during other times of the year or in alternative habitat types, and as a result little is known about river redhorse juveniles or adults outside of the spawning period. However, Campbell (2002) did make note of river redhorse captures in vegetated areas with slow flow and fine sediment. He suggested that this could indicate a broader range in habitat use by the species and should be considered when attempting to provide it with necessary habitat. This finding, accompanied by Reid's lack of river redhorse found in their traditional habitat during the fall, and studies on other suckers' use of slow flowing areas as wintering and nursery habitat suggest that more areas should be sampled if we are to better understand the full extent of the river redhorse's required habitats (Matheney and Rabeni 1995, Grabowski and Isely 2006, Mosley and Jennings 2007, Klein et al. 2017).

Extended Methodology

Tag implantation – Telemetry tags were inserted via the shielded needle technique (Ross and Kleiner 1982). A vertical incision approximately half an inch long was made in the abdominal wall of the fish above the pelvic girdle. A metal shield was inserted into the incision and beneath the pelvic girdle to allow a hollow needle to be inserted into the fish's body cavity without fear of damaging the internal organs. The needle was inserted into the body cavity below the pelvic girdle, into the metal shield, and out the incision site. The metal shield was removed. The trailing whip of the telemetry tag was then inserted into the hollow needle and out the pin hole created by the needle once removed. The telemetry tag was inserted into the body cavity and the incision site was sutured shut and sealed with surgical glue.

DNA Processing – DNA from tissue and water samples was extracted with a Qiagen DNeasy Blood and Tissue Kit. Approximately 25mg of fin tissue or one nitrocellulose filter paper was cut into small sections and placed in a 1.5ml microcentrifuge tube. 180µl of ATL buffer was added followed by 20µl of proteinase K and vortexed. Samples were incubated overnight at 56°C to ensure that all tissue had been lysed. The microcentrifuge tube was vortexed again upon completion and 200µl of AL buffer was added and vortexed. 200µl of 98% ethanol was added and vortexed. The mixture, minus the filter paper in water samples, was pipetted into a DNeasy mini spin column and the spin column was placed in a collection tube before being centrifuged at 8,000rpm for 1 minute. The flow through was discarded, 500 µl of AW1 buffer was added, and the spin column was centrifuged again at 8,000rpm for 1 minute. This step was repeated with AW2 buffer but was centrifuged at 14,000rpm for 3 minutes. The spin column was transferred to a 1.5ml microcentrifuge tube, and 200 µl of AE buffer was added and incubated on the membrane for one minute at room temperature before being centrifuged at 8,000rpm. This step was repeated for water samples to increase DNA yield.

Tissue and water samples were amplified in a Mastercycler nexus gradient (Eppendorf). Samples were denatured at 95°C for ten minutes and then cycled 30 times at 95°C for 1 minute, 60°C for 30 seconds, and 72°C for 30 seconds. Amplication ended with 72°C for 4 minutes and preserved at 4°C until collected. After amplification all samples were digested with the *Cac*8I restriction enzyme in a Mastercycler nexus gradient for one hour at 37°C. Digestion reactions included 1µl of *Cac*8I, 5µl of 1X CutSmart buffer, 2.5µl of amplified DNA, and 41.5µl of nuclease free water.

Water and tissue samples were visualized using gel electrophoresis with a 2% agarose gel. DNA fragments were sized using a 50bp to 10kb Fast DNA Ladder (New England Biolabs). Water samples were run alongside a positive control containing known river redhorse DNA, a negative control containing no DNA, an equipment blank, and a cooler blank.

Appendices

Table A1a. Ranked use data for river redhorse in the Grand River, Michigan. Only fish with developed home ranges were included in Kruskal-Wallis comparisons with available habitat. Habitat types used most often were ranked 1 and all subsequent use values were given higher ranks. Habitat categories are separated by vertical lines. Available habitat is denoted with the letter "A" before a given fish ID.

Fish ID	Sand	Gravel	Cobble	Mollusk	No Mollusk
271	2	1	3	1	2
393	3	1	2	1	2
171	3	1	2	1	2
453	3	1	2	1	2
201	3	1	2	1	2
152	3	1	2	1	2
231	1	2	3	1	2
372	1	2	3	1	2
349	2	1	3	1	2
A271	1	2	3	2	1
A393	3	1	2	2	1
A171	3	1	2	2	1
A453	3	1	2	2	1
A201	1	2	3	2	1
A152	3	1	2	2	1
A231	3	1	2	2	1
A372	1	2.5	2.5	2	1
A349	1	2.5	2.5	2	1

Table A1b. Ranked use data for river redhorse in the Grand River, Michigan. Only fish with over 10 tracked locations were included. Habitat types used most often were ranked 1 and all subsequent use values were given higher ranks. Habitat categories are separated by vertical lines. Available habitat is denoted with the letter "A" before a given fish ID.

	Velocity (m/s)					Depth (m)						
Fish ID	0-.25	.25_.5	.5_.75	.75_1	>1	0_.5	.5_1	1_1.5	1.5_2	2_2.5	2.5_3	>3
271	3	1	2	4.5	4.5	7	4.5	1	2	4.5	4.5	4.5
393	5	3	1.5	1.5	4	3.5	1.5	1.5	3.5	6	6	6
171	5	2.5	2.5	1	4	2.5	1	2.5	5.5	5.5	5.5	5.5
453	5	2.5	2.5	2.5	2.5	2	1	3	5.5	5.5	5.5	5.5
201	2	1	3	4.5	4.5	6	6	6	2	3.5	3.5	1
152	5	2.5	4	1	2.5	1.5	1.5	3	4	6	6	6
231	2.5	1	4	2.5	5	1	3	2	4	6	6	6
372	1	4	2	4	4	5	5	5	5	2	1	5
349	3	1	2	4.5	4.5	7	1.5	5	3	1.5	5	5
A271	3	1	2	4.5	4.5	6.5	3	1	2	5	4	6.5
A393	5	4	3	2	1	2	1	3	5.5	5.5	5.5	5.5
A171	5	4	3	2	1	2	1	3	5.5	5.5	5.5	5.5
A453	5	4	3	2	1	2	1	3	5.5	5.5	5.5	5.5
A201	2	1	3	4.5	4.5	6.5	6.5	5	4	2	1	3
A152	5	4	3	2	1	2	1	3	5.5	5.5	5.5	5.5
A231	5	4	3	2	1	2	1	3	5.5	5.5	5.5	5.5
A372	1	2	4	4	4	6	6	6	4	3	1	2
A349	2	1	3	4	5	5.5	5.5	5.5	3	1	2	5.5

Table A2. Fish species abundance found in four study reaches of the Grand River, Michigan. Fish were collected using tote-barge electrofishing in the restoration reach and boom shocking electrofishing in the other reaches. The sites at Plaster Creek, the wastewater treatment outflow, and Johnson Park are included separately to provide greater clarity as to where each species was located.

Species	Restoration	Adjacent	Adjacent Down	Plaster	Wastewater	Johnson	Total Abundance
Black buffalo	0	2	0	0	1	0	3
Black crappie	1	2	1	0	1	1	6
Black redhorse	0	1	0	0	0	0	1
Blackside darter	2	0	0	0	0	0	2
Bluegill	82	6	15	8	3	5	119
Bowfin	0	0	1	0	0	0	1
Channel catfish	0	11	9	0	5	0	25
Common carp	0	3	1	0	1	0	5

Common shiner	2	0	0	0	0	0	2
Emerald shiner	19	1	3	0	0	0	23
Flathead catfish	0	1	0	0	0	0	1
Gizzard shad	26	1	0	1	0	0	28
Golden redhorse	37	15	29	10	1	3	95
Greater redhorse	0	1	1	0	0	0	2
Green sunfish	0	0	2	0	0	0	2
Greenside darter	21	0	0	0	0	1	22
Hornyhead chub	1	0	0	0	0	0	1
Largemouth bass	5	1	6	4	1	1	18
Logperch	50	1	2	2		1	56
Longnose gar	N/A	Visual	Visual	N/A	N/A	N/A	N/A
Mimic shiner	61	0	0	0	0	0	61
Muskellunge	N/A	N/A	N/A	Visual	N/A	N/A	N/A
Northern hogsucker	5	0	0	0	0	0	5
Northern pike	0	1	0	1	0	0	2
Pumpkinseed	7	0	2	0	0	0	9
Quilback	0	0	4	0	0	0	4
Rainbow darter	2	0	0	0	0	0	2
River chub	2	0	0	0	0	0	2
River redhorse	1	0	3	2	1	0	7
Rock bass	52	1	1	0	0	1	55
Rosyface shiner	16	0	0	0	0	0	16
Round goby	80	0	1	0	0	0	81
Sand shiner	125	3	0	0	0	0	128
Shorthead redhorse	83	36	15	6	11	0	151
Silver lamprey	0	1	0	0	0	0	1
Silver redhorse	1	1	3	0	0	0	5
Smallmouth bass	27	7	10	2	1	1	48
Spotfin shiner	25	0	0	0	0	0	25
Spottail shiner	120	3	2	0	0	2	127
Striped shiner	17	0	0	0	0	0	17
Walleye	1	4	0	0	1	0	6
Western banded killifish	10	0	0	0	0	0	10
White bass	0	1	0	0	0	0	1
White drum	0	1		1	1	1	4
White sucker	1	0	0	0	0	0	1
Yellow perch	2	0	2	0	0	0	4
Total Abundance	884	105	113	37	28	17	1184
Total Species	30	25	21	10	12	10	45

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