BARCODING, METABARCODING, AND EXPERIMENTAL ANALYSES OF COMMUNITY DYNAMICS AND ENVIRONMENTAL CONDITIONS AFFECTING PREDATION OF LARVAL LAKE STURGEON IN THE BLACK RIVER, MICHIGAN

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Fisheries and Wildlife–Master of Science

2017

ABSTRACT

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The larval stage of most fishes is characterized by high levels of mortality and is likely a bottleneck to recruitment for many populations. Predation is an important source of mortality for the larval stage of many fish species, and is a possible factor driving high mortality in some populations. Lake sturgeon are a species of conservation concern in the Great Lakes region, with many populations experiencing little to no natural recruitment and high mortality rates during the vulnerable egg and larval early life stages. Predation of larval lake sturgeon, and larval fishes generally, has been difficult to quantify with morphological diet analyses due to rapid digestion times in the gastrointestinal (GI) tracts of predators. This study developed and utilized alternative molecular genetic methods to detect larval lake sturgeon in the diets of predator fishes, as well as conducting an experiment to further examine findings of the molecular diet analysis. Sturgeon-specific barcoding analysis of the COI mtDNA region quantified the predation frequency predation of larval lake sturgeon and revealed increased abundance of alternative prey and abiotic factors that lowered visibility could reduce predation of larval lake sturgeon. Metabarcoding analysis of predator diets using universal 18S rRNA primers revealed seasonal dietary shifts of predators when larval lake sturgeon were present in the prey community compared to after lake sturgeon larvae were no longer available, but that lake sturgeon larvae made up a small portion of the overall diets of predator fishes. Experimental manipulations of relative prey abundance quantified how the prey community could affect predator preferences. This study exemplifies the utility and improved accuracy of molecular tools in detecting predation of larval fish and other soft-bodied prey compared to morphological analyses, as well as the importance of the biotic community and environmental factors influencing predation mortality in larval fishes.

ACKNOWLEDGEMENTS

This project was funded by the Michigan Department of Natural Resources, the Great Lakes Fisheries Trust, and the US Fish and Wildlife Service Coastal Program. Special thanks to the 2015 and 2016 field crews at the Black River Sturgeon Rearing Facility, Dr. Kim Scribner, Dr. Edward Baker, Dr. Gary Mittelbach, Dr. James Bence, Dr. Brian Maurer, Dr. Terrence Marsh, Danielle Blumstein, Shaley Valentine, Jeanette Kanefsky, and Sturgeon for Tomorrow – Black Lake Chapter.

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THESIS INTRODUCTION

Lake sturgeon (*Acipenser fulvescens*) are a long-lived species of cartilaginous benthic fish that had historically large populations throughout the Great Lakes region. However, overfishing and habitat loss have led to the species becoming locally threatened, endangered, or extirpated throughout much of its range. Sturgeon fisheries reached their height in the late 1800s, providing caviar for lucrative markets in Europe before populations crashed throughout the lake sturgeon's range. Apart from a few limited fisheries, harvest of lake sturgeon in the US and Canada has been banned since 1928. However, restoration efforts have been hampered by a lack of suitable spawning habitat and low natural recruitment (Auer 1996; Peterson et al. 2007; Bruch et al. 2016). Environmental degradation and reduced access to spawning grounds blocked by dams has prevented most sturgeon populations from recovering (Auer 1996; Peterson et al. 2007; Bruch et al. 2016).

The unique reproductive ecology of sturgeon has been the focus of much of the research to improve restoration and conservation efforts. With a life span that can reach 80+ years in age, lake sturgeon have delayed maturity, typically first spawning around age 10-20yrs (Auer 1996). Lake sturgeon are iteroparous, with males spawning every 1-3yrs and females typically spawning every 3-5yrs (Forsythe et al. 2012). Female lake sturgeon can produce between 49,000 and 667,000 eggs per spawning event, with especially large females capable of producing upwards of one million eggs. Spawning occurs throughout the spring, with some fish spawning in mid April and others spawning events through early June (Peterson et al. 2007). Fish migrate in from lakes to rivers and streams, spawning on reefs in large rivers or in relatively deep areas over gravel or cobble substrate in smaller streams (Nichols et al. 2003; Peterson et al. 2007). Females may mate with multiple males at once, releasing thousands of eggs at a time to be fertilized by nearby males before adhering to the substrate of the spawning grounds (Peterson et al. 2007). Once the eggs have been deposited, sturgeon give no further parental care (Bruch and Brinkowski 2002). The eggs hatch 5 to 11 days after fertilization, however, at this point, larvae do not leave the spawning ground and continue to feed on their yolk sac for 3 to 5 more days (Auer and Baker

2002; Duong et al. 2011). Once the yolk sac has been almost completely absorbed and the larvae are approximately ready to begin feeding exogenously, they rise out of the substrate of the breeding grounds and semi-passively drift downstream (Auer and Baker 2002). This drifting behavior typically begins at dusk, and larval sturgeon tend to remain close to the benthos as they travel downstream (Kempinger 1988; Auer and Baker 2002). The drifting phase appears to be a critical period in the Black River, with many drifting larvae (hundreds to thousands) being observed but very few age-0 juveniles discovered during surveys (Smith and King 2005a; Smith and King 2005b).

High mortality of the early life stages of lake sturgeon is well documented, with mortality rates often exceeding 99% for both egg and larval stages (Nichols et al. 2003; Caroffino et al. 2010a). Moreover, the transition from a stationary yolk sac larva to a drifting exogenously feeding larva is a particularly vulnerable point in development, although the mechanism behind this high mortality rate is unclear (Duong et al. 2011). Starvation has been suggested to be an important source of mortality among other species of fish during this transition (Miller et al. 1988; Keckeis et al. 2000; McCasker et al. 2014). Starvation is a possible driver of mortality in larval lake sturgeon, though it seems unlikely to be the main cause, at least in the Black River. Whereas variable maternal provisioning would cause some families to have higher survival rates than others, evidence suggests that rates of mortality is relatively uniform throughout the population of drifting larvae (Duong et al. 2011). Lake sturgeon also typically initiate drifting behavior slightly before the transition to exogenous feeding, with mean length of drifting larvae usually falls close to 18-20mm TL (Kempinger 1988; LaHaye et al. 1992; Smith and King, 2005a), while the beginning of exogenous feeding often happens at approximately 22-25mm TL (Harkness and Dymond 1961; Smith 2003).

Predation appears to be the one of the most likely sources of mortality affecting drifting larval sturgeon in the Black River. Predation has been implicated as a driving factor associated with mortality in larvae of other species [e.g. alewife predation on larval yellow perch (Mason and Brandt 1996); walleye, white bass, and white perch predation on larval yellow perch (Carreon-Martinez et al. 2014); and herring, cod, and haddock predation of larval capelin (Gjøsæter et al. 2016)]. At the point of development where

larval sturgeon initiate drift, they have very few defenses against predators, relying on camouflage (Auer and Baker 2002) and drifting in low light conditions late at night (Auer and Baker 2002; Smith and King 2005a) and particularly during the new moon (Forsythe 2010). They have a limited swimming ability for escaping predators, and protective bony scutes begin to develop after the drift period and are not fully developed until the beginning of the juvenile stage when sturgeon reach 400mm TL (Auer and Baker 2002; Peterson et al. 2007). Experiments have shown that piscine and crustacean predators will target larval sturgeon. Rusty crayfish (*Orconectes rusticus*) and rock bass (*Amblopites rupestris*) prey on larval lake sturgeon (Crossman 2008). Visibility and cover affected larval white sturgeon (*Acipenser transmontanus*) predation by prickly sculpin (*Cottus asper;* Gadomski and Parsley 2005a). Larval white sturgeon were also vulnerable to a number of other piscine predators including walleye (*Sander vitraeus*), smallmouth bass (*Micropterus dolomieu*), northern pikeminnow (*Ptychocheilus oregonensis*), and channel catfish (*Ictalurus punctatus;* Gadomski and Parsley 2005b). However, without direct observation, predation of larval fish has been very difficult to quantify or even detect in field studies.

Larval fish are almost entirely composed of soft tissue, and as a result are easily damaged when ingested by predators and quickly digested (Schooley et al. 2008). Attempts to quantify predation mortality of larval sturgeon using traditional diet analysis have been mostly unsuccessful, usually detecting no predation (Nichols et al. 2003), or only one sturgeon in hundreds of samples (Parsley et al. 2002; Caroffino et al. 2010b). In typical morphological diet studies on fishes, 20-40% of intact prey items in predator stomachs are unidentifiable even to the family level (Carreon-Martinez et al. 2011; Leray et al. 2012), and over 95% of predator stomachs contain unidentifiable loose tissue that may or may not be from any intact prey items present (Baker et al. 2014). Laboratory studies show that the half-life of detectability of larval fish similar in size to larval sturgeon is only 2-4 hours after ingestion using morphological methods (Hallfredsson et al. 2007; Legler et al. 2010). Furthermore, identification of larval fish may not even be possible for predators that masticate their prey, as many common cyprinids do (Schooley et al. 2008). Molecular analysis of diet is capable of greater taxonomic resolution than traditional visual techniques, as molecular genetics assays are able to discriminate between different prey

items after degradation of physical characteristics would have made morphological identification extremely difficult (Braley et al. 2010). Meanwhile, genetic assays are usually capable of detecting prey DNA in the digestive tracts of fish predators 12 - 24 hours after ingestion (Carreon-Martinez et al. 2011; Hunter et al. 2012; Ley et al. 2014). Clearly, more accurate methods of detection such as molecular genetic assays should continue to be developed to examine the role predation plays in the mortality of larval fishes.

Two main methodologies exist for diet analysis using molecular genetics; taxon-specific assays meant to identify a target species or closely related clade, and universal metabarcoding assays capable of identifying a broader array of prey items across larger taxonomic groups. This study utilizes both approaches. Specific assays have the advantage of being faster, cheaper, and simpler, without the need to sequence sample DNA to determine the presence of the target organism in a predator's diet. These types of assays could be especially useful in examining the predation of species of management concern, like lake sturgeon (Rosel and Kocher 2002; Ley et al. 2014). The taxon-specific assay does require the identification of a unique target region in the genome of the target taxon, and the development of primers capable of amplifying that region. The other method utilizes universal primers and next-generation sequencing to amplify and identify sequences from barcoding regions across a broad taxonomic range of organisms. These barcoding regions are preserved enough between different groups that a single set of primers is able to amplify the target region, but different enough that the generated sequences are able to distinguish taxa by comparing known sequences in a reference database (Carreon-Martinez and Heath 2010; Pompanon et al. 2012). This approach enables identification of any prey items present in a predator diet sample that can be amplified by the selected primers and have reference sequences available. This makes it an attractive method to explore the trophic and taxonomic breadth of predators and has been successfully applied to marine fishes (Smith et al. 2005; Barnett et al. 2010), and invasive freshwater fishes (Jo et al. 2014; Taguchi et al. 2014).

DNA-based diet analyses can be used in conjunction with information on prey species relative abundance to quantify the rates of predation on larval sturgeon and other co-distributed prey species. In

the Black River system, lake sturgeon co-migrate with larvae of two catostomid species, white sucker (*Catostomus commersoni*) and silver redhorse (*Moxostoma nigrum;* Smith and King, 2005a). Additionally, there are numerous families of aquatic insects present in large numbers in the drift during the period of larval sturgeon drift (Scribner, unpublished data). The relative abundance of different prey items could affect the vulnerability of larval lake sturgeon to predation. Co-migration of multiple prey species has the potential of reducing overall prey mortality due to predation through predator swamping, and may be particularly beneficial in reducing predation pressure on relatively rare species (Frank and Legget 1983; Ims 1990). Furthermore, predator preferences for certain prey items are influenced by the relative abundance of prey (Murdoch et al. 1975; Kean-Howie 1988; Willette et al. 2001; Reiss et al. 2014; McPhee et al. 2015). Predators will often switch diets and foraging strategies to target more abundant prey items (Willette et al. 2001; Reiss et al. 2014; McPhee et al. 2015). The predator-prey dynamics outlined above could have important implications for larval lake sturgeon, supplying possible mechanisms with which high abundance of co-distributed alternative prey could "protect" larval lake sturgeon from predation. If these dynamics between larval sturgeon and other co-distributed prey in the UBR and other rivers, it could have important management implications. For example, managers could work to increase the abundance of alternative prey types as a way to improve survival of larval sturgeon and, ultimately, increase recruitment to the adult population.

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CHAPTER 1: BARCODING PCR PRIMERS DETECT LARVAL LAKE STURGEON (*ACIPENSER FULVESCENS*) IN DIETS OF PISCINE PREDATORS

ABSTRACT

Population levels of recruitment are often affected by high rates of mortality during early life stages. Lake sturgeon (*Acipenser fulvescens*), a regionally threatened species, experiences high rates of mortality during the larval stage, likely driven by predation. The objective of this study was to quantify and compare relative rates of larval sturgeon predation by piscine predators in the upper Black River (Cheboygan County, MI, USA). A molecular barcoding assay was developed using lake sturgeon-specific primers that amplify a region of mitochondrial DNA cytochrome oxidase I (COI) as an alternative to morphological analysis of gastrointestinal (GI) contents to quantify the presence of larval fish collected from potential fish predators (353 specimens, 17 potential predator species). The assay was verified to be sturgeon-specific and sufficiently sensitive to amplify the low quantities of degraded DNA in GI samples. Lake sturgeon DNA was identified in 26 of 353 predator diet samples (7.34%) in 9 of 17 potential predator fish species present. There was a significant positive correlation between the numbers of predators that had consumed larval lake sturgeon and the number of samples from a predator species analyzed. No relationship between predation level and habitat type (sand or gravel substrate) was observed, though predator community composition varied between habitats. Genetic assays as described here can be used to investigate predator-prey dynamics affecting species of conservation interest during important life stages that may otherwise be under-represented in diet studies that rely solely on morphological analysis.

INTRODUCTION

Many fish species experience high mortality rates during early life stages, most commonly attributed to starvation and predation (Hjort 1914; Houde 2008). In many systems, high predation rates in particular strongly influence larval fish mortality (Caroffino et al. 2010a; Duong et al. 2011; Carreon-Martinez et al. 2014) and affect recruitment of age-0 juveniles (Mason and Brandt 1996; Gjøsæter et al. 2016). In these situations when the larval stage is a bottleneck, even a slight reduction in the mortality rates of larval fish could significantly increase recruitment (Pine et al. 2001).

Lake sturgeon (*Acipenser fulvescens*) is a species of conservation concern that is characterized by low recruitment because of high mortality rates during early life stages (Caroffino et al. 2010a, Duong et al. 2011). Lake sturgeon were historically abundant throughout the Great Lakes region, but overfishing and degradation of spawning habitat have caused population declines throughout their native range (Peterson et al. 2007). Many lake sturgeon populations experience little or no natural recruitment. Like many other fishes, lake sturgeon exhibit a type III age-specific survivorship curve; female lake sturgeon typically produce between 49,000 and 667,000 eggs per spawning event (Peterson et al. 2007). However, mortality can often exceed 99% during egg and larval stages, indicating that survival during these early life stages is a likely bottleneck to recruitment (Nichols et al. 2003; Caroffino et al. 2010a; Forsythe et al. 2013). High mortality is associated with the period when larval lake sturgeon emerge from the stream substrate of spawning grounds, disperse downstream, and begin exogenously feeding (Caroffino et al. 2010a, Duong et al. 2011). During the drift period, migration downstream places larvae at greater risk of starvation, injury, and predation (Auer and Baker 2002).

The potential of predation as a major source of mortality for larval fish can be difficult to measure in the field. Incongruence between results from field and laboratory-based predation studies are due to the fact that field-based predation studies of larval fishes including lake sturgeon had difficulty detecting predation or quantifying predation rates by piscine predators. Laboratory studies suggest that many predatory fishes will prey upon larval sturgeon (Gadomski and Parsley 2005a, b; Crossman 2008).

However, studies of predator diets using wild-caught predators identified few or no sturgeon remains in the GI tracts (Parsley et al. 2002; Nichols et al. 2003; Caroffino et al. 2010b). Typically, predation studies have relied upon morphological analysis of predator gastrointestinal (GI) contents and identify digested prey items based on exoskeletons, bones, and other structures resistant to digestion (Hyslop 1980, Schooley et al. 2008). However, larval fish have few hard structures and are quickly digested beyond recognition, making predation difficult to detect using morphological analysis alone (Hallfredsson et al. 2007, Legler et al. 2010). DNA-based diet analysis overcomes some of the limitations of morphological identification of GI tract content. Larval fish can only be identified morphologically 2-4 hours post-ingestion (Hallfredsson et al. 2007, Legler et al. 2010), whereas genetic analysis of diet samples can identify prey DNA as long as 24 hours after ingestion (Carreon-Martinez et al. 2011, Hunter et al. 2011, Ley et al. 2014). Several methods of DNA-based diet analysis have been successfully applied to larval fish. Universal barcoding primers have been used to detect predation of several species of larval fish (Carreon-Martinez et al. 2011). Species-specific molecular assays have been used to detect larval yellow perch (*Perca flavescens)* using TaqMan© (Life Technologies) assays on single nucleotide polymorphisms (SNPs; Carreon-Martinez et al. 2014). Larval Atlantic cod (*Gaddus morhua*) have been detected using family-specific mitochondrial 16S barcoding primers and species-specific hybridization probes (Rosel and Kocher 2002). Also, the number of razorback sucker larvae (*Xyrauchen texanus*) in predator GI contents was quantified using quantitative polymerase chain reactions (qPCR; Ley et al. 2014).

In this study, lake sturgeon-specific PCR primers were designed and field-tested to detect lake sturgeon in dissected GI tracts of riverine fish predators. Similarly to previous DNA-based barcoding studies of larval fish predation, this assay targets the cytochrome oxidase subunit I (COI) region of mitochondrial DNA (mtDNA). However, the assay describe here is more efficient in that it only requires the species-specific PCR primers without additional probes or markers to detect target DNA. The objectives of this study were (1) to evaluate the species specificity and sensitivity of the sturgeon barcoding PCR primers and (2) to quantify incidences of lake sturgeon predation by piscine predators of

different species, and (3) to quantify predation associated with different riverine habitats defined by substrate type.

MATERIALS AND METHODS

Study area and sample collection

This study was conducted in the Upper Black River (UBR; Cheboygan County, MI), a fourth order stream and largest tributary of Black Lake, a 4100 ha inland lake in Michigan (Breck 2004). The UBR serves as the primary spawning area for a lake sturgeon population of moderate size (N ~1200; Pledger et al. 2013) in Black Lake. This population has been isolated from the Great Lakes since the construction of Alverno Dam in 1903. The spawning area in the UBR has been restricted to an 11 km stretch extending to Kleber Dam, which was constructed in 1949. Population abundance has declined because of over harvest and the lack of natural recruitment (Baker and Borgeson 1999).

The spawning area in the UBR includes a 1.5 km stretch of river that is approximately 9 km upstream of the river mouth (Figure 1). Sampling for larval lake sturgeon was conducted during 2015 within two 0.5 km transects directly downstream of each of the spawning sites (Figure 1, gravel transects A and B), and two other 0.5 km transects further downstream (Figure 1, sand transects C and D). Drift samples were collected for five days during the lake sturgeon drift period (May 25th, June 4th-7th); one transect was sampled per day. The presence and abundance of larval lake sturgeon dispersing each night was quantified using D-frame drift nets as described by Auer and Baker (2002). Five D-frame drift nets with 1600 μm mesh and detachable cod ends were set at each sampling site at 21:00. Net contents were collected and sorted every hour beginning at 22:00 and concluding at 02:00. Total biomass of larval lake sturgeon in the river was estimated for each night (Table 1).

Potential piscine predators were collected during electrofishing surveys the day following larval drift sampling (n=353 samples from 17 predator species). Electrofishing surveys were conducted using a barge electrofishing unit with a three-person crew. The 0.5 km stream segment sampled was between the locations where drift sampling was conducted the previous night. Electrofisher settings were set to 400 V at 4 A. Two crew members carried anodes and netted fish while a third crew member pulled the barge and

transferred fish to a live well. Relative abundance of fish was estimated by the catch per unit effort (CPUE), calculated as the number of fish caught per minute of active electroshocking. To collect diet samples, an overdose of MS222 (0.4 mg/ml) was administered to euthanize predators according to MSU animal use and care specifications. Total length of each predator was recorded and predators were individually stored in Whirl-Paks (Nasco, Fort Atkinson, WI, USA) and placed in a -20 °C freezer for preservation. Fin clips from each species were taken to evaluate species-specificity of the lake sturgeon PCR primers. Each predator was dissected, the entire GI tract was removed, and contents were carefully extracted to minimize the amount of predator tissue in the sample. As predator GI tracts were dissected, any morphologically identifiable lake sturgeon larvae remains were recorded. Diet samples were preserved in 95% ethanol and stored at -20 °C prior to DNA extraction.

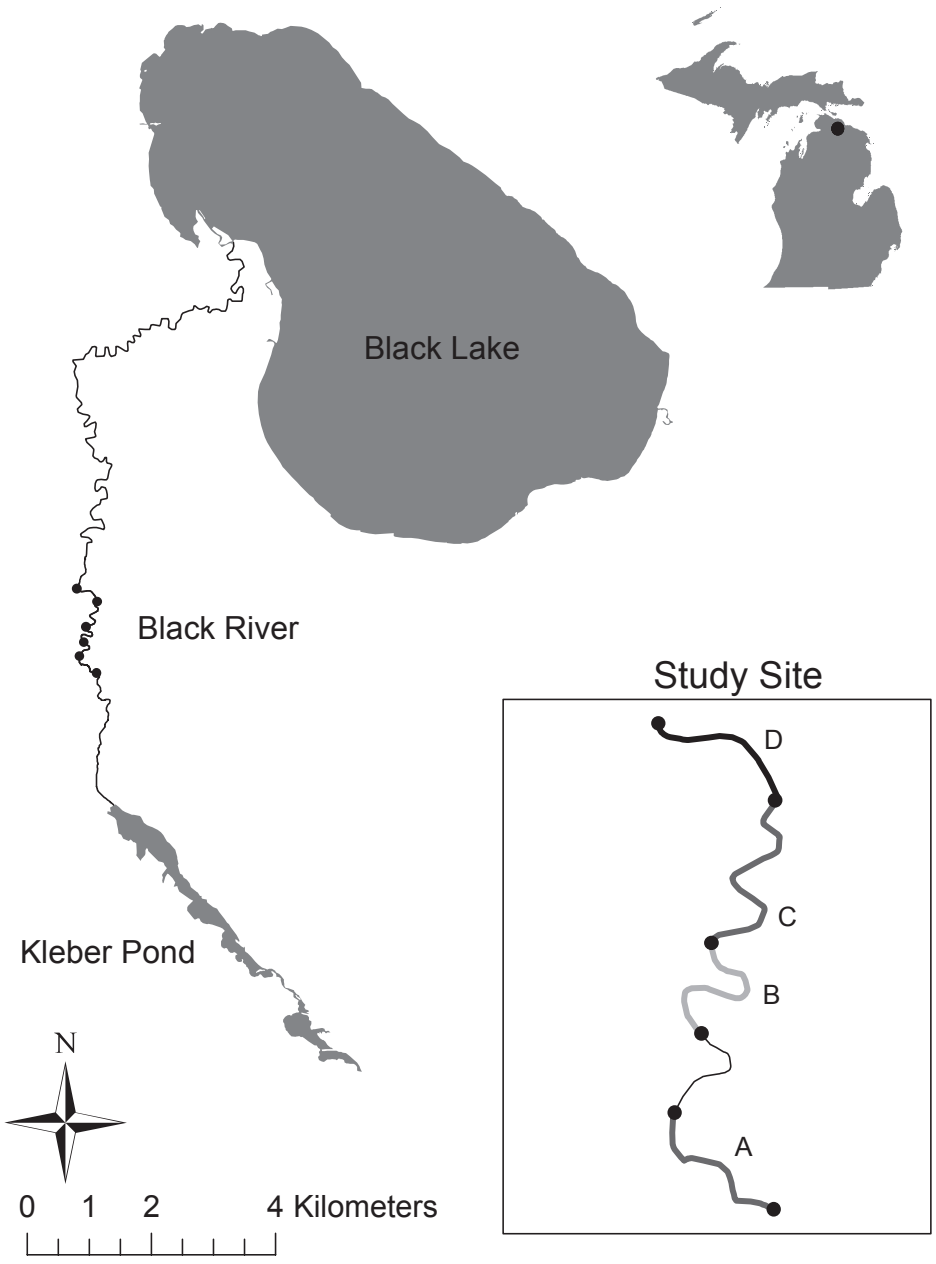

Figure 1. Overview of the study area showing the location in Michigan in the upper right, the location of the transects on the Upper Black River, and a pullout in the lower right showing each transect with labels. Larval drift sampling sites are marked by black circles and electroshocking transects are represented by thick lines next to the corresponding letter designation. Transects A and B were in the upstream gravel habitats directly below each of the main spawning sites. Transects C and D were in the downstream sand habitat.

Table 1. The number and total river dry weight biomass estimate of larval sturgeon caught at the drift sampling site at the upstream end of each transect for each sampling night.

Date	Catch of larval sturgeon (count)	Estimated total river dry weight biomass of larval lake sturgeon (g)
May 24th	131	8.99
June 4th	550	24.33
June 5th	291	13.42
June 6th	71	4.63
June 7th	6	0.60

DNA extraction

Ethanol was decanted from each sample and samples were washed twice with sterile water to remove excess ethanol that could interfere with DNA extraction and PCR amplification. Samples were then coarsely manually homogenized with forceps, sterile toothpicks, and vortexing. The extraction protocol used a modified version of the QIAamp Stool Mini Kit (QIAGEN, Hilden, Germany) protocol for human DNA analysis. For GI samples with less than 50 mg of material, the entire sample was used, otherwise a 50 mg subsample was taken for DNA extraction. Lysis in the InhibitEx Buffer from the QIAmp Stool Mini Kit was extended to 30 min. After proteinase and lysis buffer were added, an additional 10-min bead-beating step was added using 0.70 mm garnet beads (MOBIO, Carlsbad, CA, USA) to further homogenize samples and mechanically break apart cells difficult to lyse. DNA from lake sturgeon fin clips, the fin clips collected from all co-distributed fish, and aquatic macroinvertebrates from the UBR was extracted using the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). After elution, DNA concentration was quantified using a nanodrop spectrophotometer and samples were standardized to a concentration of 20 ng/μ1.

Primer design

The COI barcoding region of mtDNA was chosen to design lake sturgeon-specific primers, as many fish species and invertebrate families co-distributed with lake sturgeon in the UBR had COI sequences represented in the NCBI GenBank database. Primers were designed using the NCBI Primer-BLAST tool. Primer pairs were considered if they amplified regions <200 bp in size, contained at least 2

GC pairs in the 5' ends to promote specificity, and contained at least 5 mismatches from other fish and invertebrate sequences. Two primer pairs were selected for laboratory testing (Table 1). The only likely unintended targets with two or fewer mismatches for both primer pairs are other sturgeon species (Family: Acipenseridae) that are not present in the study area.

Table 2. Sequences and PCR amplicon lengths of the two lake sturgeon mtDNA COI primer pairs designed for molecular analysis to detect lake sturgeon in predator diets.

Primer Pair	Sequence	Amplicon Length
AfCOIF1	5'-CCATCATAATTGGCGGATTCGG-3'	138bp
AfCOIR1	5'-CCCCAGAGGAGGCTAAAAGG-3'	
AfCOIF2	5'-GCTCCTTTTAGCCTCCTCTGG-3'	151bp
AfCOIR2	5'-CCCCAAAATGGACGAAACCC-3'	

PCR optimization, primer specificity, and sensitivity

Optimal conditions for both primer pairs contained 20 ng of template DNA, 0.5 μM of each primer, 200 μM dNTPs, 1X reaction buffer, and 5U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and additional deionized water for a 25 μl total reaction volume. PCR conditions were set with an initial denaturation step of 94 ˚C for three minutes; followed by 35 cycles of 94 ˚C for 45 s, 56 ˚C annealing temperature for 30 s, and 72 ˚C for 30 s. Final extension was performed for 5 minutes at 72 ˚C. Primer pair AfCOIF1-AfCOIR1 was chosen as the primary primer pair for the rest of the experiment as it was less prone to primer-dimer formation.

To confirm specificity of the primer pairs, each primer pair was tested under optimal conditions as described above against genomic DNA from the 17 possible UBR predator fish species, three amphibian species, and 44 aquatic invertebrate families co-distributed with drifting larval lake sturgeon (Table 3). Each set of reactions included a positive control of lake sturgeon genomic DNA and a negative (no DNA) control to confirm reaction success. PCR products were visualized using 2% agarose gels stained with ethidium bromide under UV light.

Two dilution series experiments were performed to test the sensitivity of primer pair AfCOIF1-AfCOIR1. One dilution series started with an initial concentration of 4 ng/μl of lake sturgeon genomic

DNA and was diluted with sterile water by a ratio of 1:1 until the reaction failed to amplify. A second dilution series was performed using the same protocol on a diet sample that had tested positive for lake sturgeon DNA to examine how the diet extraction procedure, the presence of non-target DNA, and degradation of target DNA in predator GI tracts affect the minimum amplifiable DNA concentration.

A total of 353 diet samples were examined using primer pair AfCOIF1-AfCOIR1 under optimal PCR conditions and PCR products were visualized on 2% agarose gels as described previously. Successful amplification indicated the presence of lake sturgeon remains in diet samples. Two positive controls of lake sturgeon genomic DNA and two negative controls of PCR reaction mixtures without template DNA were included for each round of PCR and run on agarose gels. A 100 bp ladder was run next to the samples to approximate the size of the PCR product to ensure it was the expected size of the target region. Amplified DNA from diet samples was compared to the size of PCR products from lake sturgeon genomic DNA as evidence that the correct region was amplified in the diet samples. Agarose gels were visualized under UV light, an image was captured, and the electrophoresis gel image was used to score samples as positive or negative for the presence of lake sturgeon DNA. All positive samples were then subjected to additional PCR amplifications using both primer pairs. Only samples that were amplified by both primers were considered positive for lake sturgeon DNA. To check for possible error, a random 10% subset of all samples that were initially recorded as negative was subjected to an additional PCR amplification.

Table 3. List of all taxa present in the Upper Black River from which gDNA was extracted and tested with sturgeon-specific primers to ensure no amplification of non-target sequences.

Fishes (by species)			
Ambloplites rupestris	*Lepomis gibbosus*	*Notropis heterodon*	*Semotilus atromaculatus*
Ameiurus nebulosus	*Lota lota*	*Perca flavescens*	*Umbra limi*
Catostomus commersonii	*Luxilus cornutus*	*Percina caprodes*	
Etheostoma caeruleum	*Micropterus dolomeiu*	*Percina maculata*	
Etheostoma nigrum	*Nocomis biguttatus*	*Rhinichthys atratulus*	
Amphibians (by species)			
Necturus maculosus	*Rana catesbeiana*	*Rana clamitans*	
Aquatic invertebrates (by family)			
Aeshnidae	Corixidae	Helicopsychidae	Perlodidae
Athericidae	Corydalidae	Heptageniidae	Philopotamidae
Baetidae	Crambidae	Isonychiidae	Pleidae
Baetiscidae	Curcurlionidae	Lepidostomatidae	Polycentropodidae
Brachycentridae	Elmidae	Leptoceridae	Psephenidae
Caenidae	Ephemerellidae	Leptohyphidae	Sialidae
Calopterygidae	Ephemeridae	Leptophlebiidae	Simulidae
Chironomidae	Glossosomatide	Limnephilidae	Siphlonuridae
Coenagrionidae	Gomphidae	Macromiidae	Tabanidae
Cordulegastridae	Gyrinidae	Nocturidae	Veliidae
Cordulidae	Halipliidae	Perlidae	

Statistical analysis

Each diet sample was assigned a value of either 0 or 1 indicating the absence or presence of lake sturgeon DNA in the sample, respectively. Samples were categorized by predator species. The number of diet samples tested and the number of sturgeon-positive results were both ranked by species from highest (rank 1) to lowest (rank 17). The relationship between the number of diet samples and the number of lake sturgeon-positive diet samples from each predator species was analyzed using a Spearman's rank correlation test from the R package Hmisc v3.17-1 (Harrell Jr. 2015). The number of diet samples analyzed from each predator species closely corresponds with their relative abundance in the UBR (Pearson's correlation between number of samples per species and CPUE; $R^2 = 0.93, p < 0.0001$).

Diet samples were also categorized into groups by the dominant substrate type in the transect where the diet sample was collected (sand or gravel substrates). A Pearson's chi-squared test using R v3.2.2 (R Core Team 2015) was conducted to determine whether the frequency of lake sturgeon remains

identified in diet samples differed between samples collected from predators captured in sand or gravel substrates.

RESULTS

Primer performance

Both primer pairs successfully amplified the target region of lake sturgeon mtDNA and were specific for lake sturgeon. When tested against genomic DNA of co-distributed non-target UBR species, no amplification of non-target DNA was detected (data not shown).

All concentrations in the dilution series were successfully amplified using the AfCOIF1-AfCOIR1 primer set to a minimum concentration of 0.032 pg/μl of sturgeon genomic DNA. From the dilution series experiment using lake sturgeon positive diet samples, the target region of DNA was amplified to a minimum total DNA concentration of 0.12 pg/μl of DNA.

Larval lake sturgeon predation by species

Of the 353 diet samples tested, 26 samples (7.37%) tested positive for lake sturgeon remains using the genetic assay. Only one sample from a 152 mm smallmouth bass (*Micropterus dolomieu*) had lake sturgeon remains that were detected by morphological analysis. This sample also tested positive for lake sturgeon using the genetic assay. Lake sturgeon appeared in the diets of 9 of the 17 piscine predator species tested (Table 4). There was a positive correlation between number of diet samples and the number of lake sturgeon-positive diet samples from each predator species (Spearman's rank correlation test, $r_s = 0.604$, df1 = 1, df2 = 15, $p = 0.01$, Figure 2).

Table 4. The number of samples tested from each of the 17 predator species with the number and proportion that tested positive for lake sturgeon DNA, and the catch per unit effort.

Predator Species	Species Code	Total Samples	Lake Sturgeon-Positive Samples	Proportion Sturgeon Positive	CPUE (fish/min)
Blackchin shiner (*Notropis heterodon*)	BCS	3	1	0.33	0.013
Blacknose dace (*Rhinichthys atratulus*)	BND	4	1	0.25	0.017
Brown bullhead (*Ameiurus nebulosus*)	BRB	1	0	0	0.002
Blackside darter (*Percina maculata*)	BSD	8	0	0	0.025
Burbot (*Lota lota*)	BUR	6	0	0	0.025
Central mudminnow (*Umbra limi*)	CMM	12	0	0	0.080
Common shiner (*Luxilus cornutus*)	CMS	35	4	0.11	0.114
Creek chub (*Semotilus atromaculatus*)	CRC	33	2	0.06	0.209
Hornyhead chub (*Nocomis biguttatus*)	HHC	105	9	0.09	0.428
Johnny darter (*Etheostoma nigrum*)	JOD	2	1	0.50	0.019
Logperch (*Percina caprodes*)	LOP	19	3	0.16	0.051
Pumpkinseed (*Lepomis gibbosus*)	PUS	10	0	0	0.168
Rainbow darter (*Etheostoma caeruleum*)	RAD	43	3	0.07	0.142
Rock bass (*Amblopites rupestris*)	ROB	28	0	0	0.128
Smallmouth bass (*Micropterus dolomieu*)	SMB	17	1	0.06	0.058
White sucker (*Catostomus commersonii*)	WHS	4	0	0	0.024
Yellow perch (*Perca flavescens)*	YEP	23	1	0.04	0.101

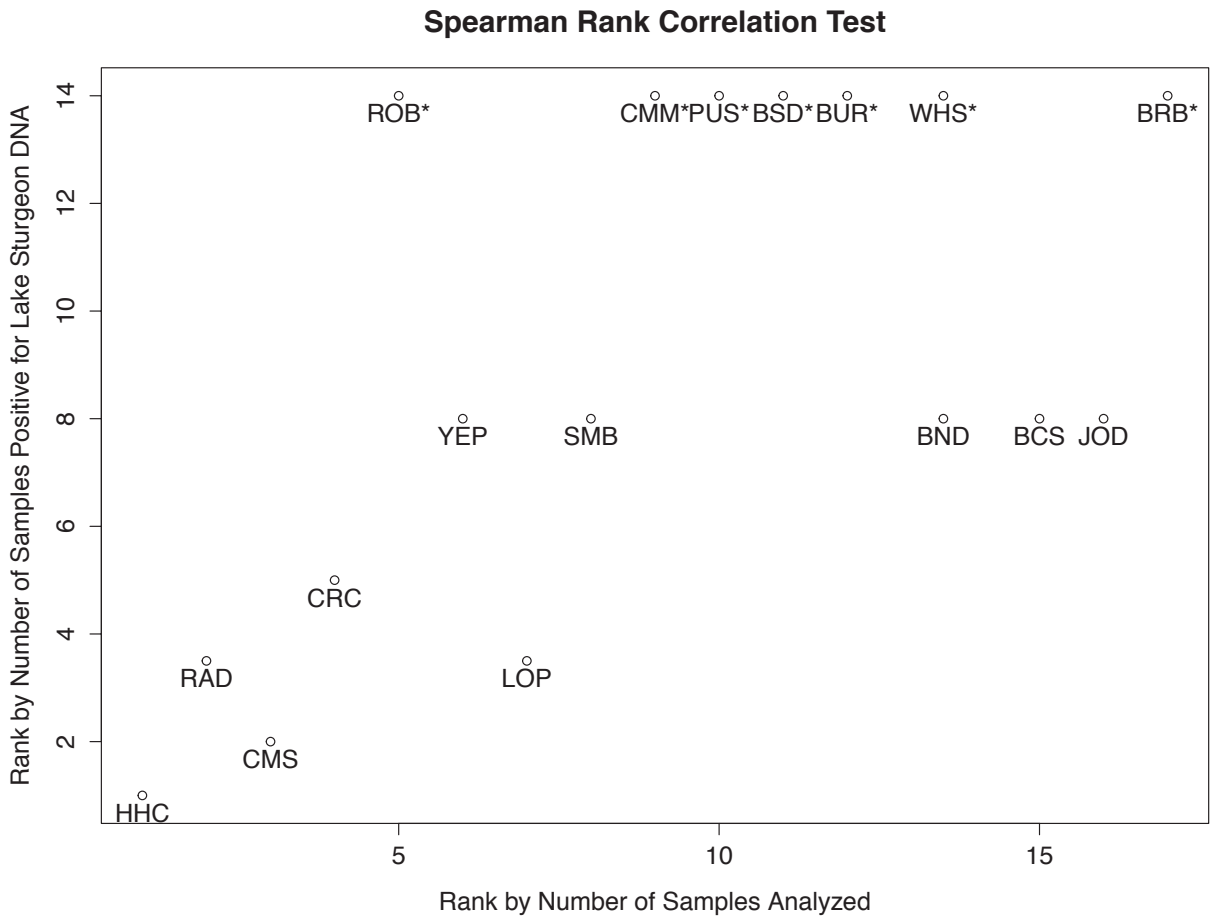

Figure 2. Results of the Spearman's rank correlation test showing each potential piscine predator species ranked by the number of samples analyzed and the number of samples that tested positive for lake sturgeon mtDNA. Ranks were assigned from highest to lowest values for each variable; e.g. the first ranked species for the number of positive samples was the species with the highest number of positive samples. Species with ties were given an average rank across the positions of the tied species. Seven species did not consume sturgeon and are tied for the last rank by number of samples positive for lake sturgeon DNA, indicated with a (*) next to the three-letter species code. The species names for each three-letter species code can be found in Table 4.

Larval lake sturgeon predation rates in different habitats defined by substrate

There was no significant interaction between substrate type and the presence of sturgeon DNA in predator diet samples (Chi-squared test, X^2 = 1.68e-30, df = 1, p = 1). Some predator species were associated with a certain substrate type; e.g. pumpkinseed sunfish (*Lepomis gibbosus*) and central mudminnow (*Umbra limi*) were found more frequently in gravel substrate while hornyhead chub

(*Nocomis biguttatus*) and logperch (*Percina caprodes*) were found more frequently in sand substrate. When the sand transects were sampled, density of larval lake sturgeon in the drift was much higher than when the gravel transects were sampled, despite sampling on consecutive days. The proportion of predators that had eaten lake sturgeon was not different between sand and gravel, but there were far fewer drifting larval lake sturgeon available in the gravel habitats. When GI samples were characterized by the substrate (gravel or sand) in which samples were collected, 16 of 224 samples (7.14%) collected in sand transects and 10 of 129 samples (7.75%) collected in gravel transects tested positive for sturgeon DNA.

DISCUSSION

The PCR assay designed for this study successfully detected lake sturgeon mtDNA in the GI tracts of piscine predators present during the larval drift period in the UBR. The assay was specific to lake sturgeon and did not amplify DNA of co-distributed predator and prey species. Fish species tested included seventeen species from seven families (Catostomidae, Centrarchidae, Cyprinidae, Gadidae, Ictaluridae, Percidae, Umbridae) that are common and widely distributed across North America and are widely co-distributed with lake sturgeon. Additionally, the primer pairs have very few mismatches with other species of Acipenseridae, and they may be capable of amplifying DNA from other species of sturgeon.

Sensitivity analysis revealed that the primers successfully amplified very low concentrations of DNA. Primer pairs were deliberately designed with short amplicons to maximize amplification success on degraded DNA that had been partially digested in the GI tracts of predator fish. However, it is unknown how long after ingestion sturgeon DNA is detectable by this assay. Similar assays targeting other fish species have been able to consistently amplify target DNA regions 12-24 hours after ingestion in some of the same predator species (Carreon-Martinez et al. 2011, Hunter et al. 2012, Ley et al. 2014). However, the post-ingestion detection success may also depend on the number and size of larval sturgeon consumed, as well as differences in the digestive systems of different predator species. Additionally, detection efficiency of ethidium bromide staining and UV light could be improved with quantitative (q)PCR. Further testing is necessary to address these issues and define the limitations of this assay.

The results of this study demonstrate the degree to which the use of morphological analysis alone can underestimate the presence of larval fish and other soft-bodied prey in diet studies and highlights the need for alternative methods to detect predation for easily digested prey. Morphological observations of lake sturgeon consumption in diet samples collected during this study (1 in 353 diet samples examined) were similar to other predation studies on larval sturgeon that relied on morphological analyses (Parsley et al. 2002; Nichols et al. 2003; Caroffino et al. 2010b). Evidence of predation on lake sturgeon was

corroborated in the one sample detected by morphological analysis using the molecular assay, and molecular analysis identified 25 additional samples as containing lake sturgeon. Morphological analysis has been shown to be an unreliable method of detecting larval fish in predator diets (Hallfredsson et al. 2007, Legler et al. 2010). Furthermore, this molecular assay revealed that a large percentage of lake sturgeon predators (61.54%) were cyprinids, which have pharyngeal teeth and masticate their prey. Detection of soft-bodied prey, even immediately after ingestion, would be especially difficult using a morphological diet analysis of predators with similar feeding behavior.

When quantified by predator species, analysis of predation revealed that the number of sturgeon-positive samples per predator species was correlated with the number of samples analyzed per predator species. Results suggest that predation rates of lake sturgeon are relatively similar for most of the potential piscine predator species present in the UBR. Total predation pressure exerted by a predator species appears to be roughly proportional to relative abundance. For example, hornyhead chub (*Nocomis biguttatus*) appear to be the most frequent predator of lake sturgeon larvae, accounting for 34.62% of all sturgeon-positive detections. Hornyhead chub were also the fish species most frequently caught during sampling, making up between 30-40% of the fish community in every transect. In other words, no fish predator species appeared to be targeting larval lake sturgeon more than any other species. More abundant species may have had more incidences of sturgeon predation, but these occurred in similar proportions to less common species.

There were several unexpected results from the analysis of predation by species. Rock bass (*Amblopites rupestris*) preyed upon larval sturgeon heavily in controlled experiments (Crossman 2008), but no predation of larval sturgeon was detected from samples collected from the river. Likewise, pumpkinseed (*Lepomis gibbosus*), smallmouth bass (*Micropterus dolomieu*), and yellow perch (*Perca flavescens*) will consume lake sturgeon in experimental settings (Waraniak, unpublished data), but few sturgeon were detected in diet samples collected from these species. Another unexpected result was finding sturgeon DNA in the gastrointestinal contents of several rainbow darter (*Etheostoma caeruleum*), Johnny darter (*Etheostoma nigrum*) and blackchin shiner (*Notropis heterodon*) that measured 55 mm TL

or smaller. Larval lake sturgeon typically drift at a size of 18 to 24mm (Auer and Baker 2005), and small darters or minnows would likely be gape-limited, and therefore not capable of consuming sturgeon larvae of that size. All of the small-bodied fish samples that contained lake sturgeon mtDNA were collected in the two gravel transects, which were directly downstream of the spawning areas where egg deposition occurs. Predation of lake sturgeon eggs, yolk-sac larvae, or scavenging pieces of eggs and larvae from predators like crayfish and stonefly (Family: Perlidae) that shred their prey could explain the sturgeon-positive results of the samples from these small-bodied fishes. Lake sturgeon spawning was ongoing at the time of the survey, and the molecular assay is not able to discriminate between different life stages of sturgeon. The data demonstrates that this assay could be applied beyond predation of drifting larvae for investigations of egg predation by fish, crayfish, and other invertebrate predators. Before applying this assay to other systems, however, further testing on a greater variety of fishes and invertebrates may be necessary to confirm the specificity of the primers.

There was no relationship between substrate and the proportion of predators that had consumed lake sturgeon, however, there were some confounding issues with this part of the analysis. When the sand transects were sampled, density of larval lake sturgeon in the drift was much higher than when the gravel transects were sampled, despite sampling on consecutive days. The proportion of predators that had eaten lake sturgeon was not different between sand and gravel, but there were far fewer drifting larval lake sturgeon available in the gravel habitats (Table 1). The predators in the gravel transects had access to the spawning sites where other early life history stages of lake sturgeon were available. Alternatively, Crossman (2008) found that juvenile sturgeon preferred sand substrate in the presence of piscine predators, so it is possible that young sturgeon might be less susceptible to predation on sandy substrate.

Contamination of predator diet samples could lead to false positives caused by environmental DNA (eDNA). Methods for the detection of eDNA and DNA from diet samples are similar and have to overcome many of the same obstacles (Taberlet et al. 2012). The main source of lake sturgeon eDNA in the UBR would be the spawning adult lake sturgeon. Detection of eDNA in rivers is dependent on abundance and biomass, distance from the source, and flow (Jerde et al. 2010; Jane et al. 2014). Jane et al.

(2014) found that eDNA was unlikely to be detected over 240 m from the sources in a stream with similar flow rates to the UBR, though the source in that study had a smaller biomass than a congregation of spawning sturgeon. If eDNA from adult sturgeon was prevalent in the GI tracts of predators, a higher proportion of samples collected near the spawning grounds would have been expected to test positive for lake sturgeon DNA. However the Chi-square test of sand and gravel transects shows that this is not the case. While false positives from eDNA cannot be completely ruled out, it seems to be unlikely that eDNA had an effect on results of this study.

Results of this study revealed predation of larval sturgeon is likely a more important source of mortality than previously observed in morphological diet analysis studies (Nichols et al. 2003; Caroffino et al. 2010a). Genetic tools could be used to investigate ecological factors such as predator and prey community composition, predator preferences, and habitat characteristics that affect sturgeon predation. Understanding the biotic and abiotic conditions that influence mortality due to predation can inform decisions on how lake sturgeon spawning streams and rivers are managed. Turbidity (Gadomski and Parsley 2005a; Carreon-Martinez et al. 2014), predator swamping through drifting in high densities (Furey et al. 2016) or with high densities of co-distributed larval fishes and aquatic insects (Kean-Howie et al. 1988), and indirect effects of large predators (Harvey 1991) have all been shown to affect the predation rate of young fish. The influence these factors have on predation of larval lake sturgeon and other species should be investigated to provide a better understanding of the ecological interactions within these systems and inform management decisions. Molecular assays like the one developed in this study can provide the necessary tools to do so.

This study revealed that the use of molecular genetics assays provide a more complete picture of predation of species that may be underrepresented using traditional morphological methods of diet analysis. Previous studies on larval sturgeon predation in the wild have been unable to draw meaningful conclusions because the likelihood of identifying sturgeon remains in the GI contents was extremely low. The molecular analysis used in this study suggests that predation on drifting larval sturgeon may be

prevalent in a variety of predatory fish species common throughout rivers in the Great Lakes region and could be hampering recovery of lake sturgeon populations.

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CHAPTER 2: MOLECULAR DIET ANALYSIS REVEALS PREDATOR-PREY COMMUNITY DYNAMICS AND ENVIRONMENTAL FACTORS AFFECT PREDATION OF LARVAL LAKE STURGEON (ACIPENSER FULVESCENS) IN A NATURAL SYSTEM

ABSTRACT

Predation is often implicated as a major cause of mortality during the early life stages of many fish species, and is often perceived as bottleneck to recruitment. Regionally threatened lake sturgeon (*Acipenser fulvescens*) experiences low natural recruitment throughout much of its native range, due in part to predation. This study utilizes emerging molecular tools to investigate the prevalence of predation during the vulnerable drifting larval life history stage of sturgeon. How predators, the co-distributed prey community, stream substrate, and abiotic environmental conditions affected levels of predation was quantified. D-frame drift net surveys were used to quantify the biomass of lake sturgeon and co-distributed prey. Gastrointestinal diet samples (n=1140) from potential fish predators were collected during electrofishing surveys. Sampling was conducted for 17 total days across two years, 2015 and 2016. Based on DNA barcoding analysis using sturgeon-specific mtDNA cytochrome oxidase I (COI) primers, lake sturgeon DNA was detected in 73 of 1140 diet samples (6.40%). A logistic regression model was used to analyze the effects of biotic and abiotic variables associated with the likelihood of larval lake sturgeon predation. Increasing lunar illumination and biomass of larval lake sturgeon increased predation rates on larval sturgeon. Higher discharge and greater biomass and proportions of alternative prey decreased predation rates of larval lake sturgeon. Predation rates were slightly higher in habitats with sand substrate. Most predator species preyed upon larval lake sturgeon at similar rates, with some exceptions. The study revealed much higher predation rates on larval sturgeon than previously thought, and how the predation rate was affected by co-distributed prey and abiotic environmental variables.

INTRODUCTION

The early life stages of many fishes are subject to high mortality rates, and impose a bottleneck to recruitment for many species (Hjort 1914; Houde 2008). Eggs and larval fishes are particularly vulnerable to predation (Pine et al. 2001; Mason and Brandt 1996; Gjøsæter et al. 2016). Predation on the early life stages of fishes can control recruitment of otherwise highly abundant populations (Silbernagel and Sorenson 2013), and inhibit recovery in populations already experiencing low natural recruitment (Dudley and Matter 2000; Köster and Möllmann 2000).

Predation of the early life stages of fishes has been difficult to quantify in natural systems. Previous studies focused on larval fish recruitment have relied upon inferences drawn from the relationship between predator abundance and the number of successful recruits (Mason and Brandt 1996; Gjøsæter et al. 2016) or extrapolated results from experimental studies showing predators readily consumed large numbers of fish larvae (Gadomski and Parsley 2005a; Silbernagel and Sorenson 2013). Attempts to estimate predation rates of larval fishes from predator diet analyses often underestimate the levels of predation on larval fish when using morphological methods to identify prey (Schooley et al. 2008). Larval fish have no long-lasting hard structures that can be identified when digested, and are often unidentifiable beyond 2 hours after consumption by piscivorous fishes (Hallfredsson et al. 2007; Schooley et al. 2008; Legler et al. 2010). An alternative is to use molecular methods to identify prey DNA in the gastrointestinal (GI) contents of predators. DNA of larval fishes has a much longer detection period in the GI tracts of piscivorous fishes, extending the detection period to 24-48 hours with genetic techniques (Carreon-Martinez et al. 2011; Hunter et al. 2011; Ley et al. 2014). With more accurate tools to identify larval fishes in the diets of predators with greater confidence, more rigorous field studies can be conducted to understand what factors impact predation on larval fishes.

The species and size-classes of predatory fish in a community can have important effects on the predation levels of larval prey (Chalcraft and Resetarits 2003; Parke et al. 2009). The prey preferences (Silbernagel and Sorenson 2013; Reiss et al. 2014), foraging efficiencies (Scharf et al. 2009), and

consumption rates (Gosch and Pope 2011) vary by predator. Recognition of certain species as important predators of larval fish can inform management decisions about harvest regulations, targeted removals, biological controls, or other strategies of predator control to improve recruitment of a target species (Carpenter and Mueller 2008). Additionally, identifying predators improves understanding about the ecological importance of larval fish and how these predator-prey relationships affect prey abundance and structure the overall community (Gjøsæter et al. 2016).

The prey community has the potential to affect predation rates of larval fish through indirect effects. Indirect effects can be mediated by predator switching, which occurs as prey items change in density and predators focus foraging efforts on more abundant prey (Murdoch 1969; Murdoch et al. 1975; Willette et al. 2001; Reiss et al. 2014; McPhee et al. 2015). Abundance and biomass of larval fishes is often highly variable through time and space (Reiss et al. 2002; Smith and King 2005; Kallasvuo et al. in press), making prey switching behaviors in predator species likely as the availability of prey fishes and encounter rates between predators and prey change. Overall abundance of prey species can also improve survival rates through predator swamping (Furey et al. 2016). Synchronized reproduction and migration, not just within a species, but also across species, has been hypothesized as an adaptive strategy to swamp predators and improve overall survival of early life stages (Frank and Leggett 1983; Ims 1990). Relatively rare larval fishes can be protected by more abundant co-distributed prey species (Frank and Leggett 1983; Kean-Howie et al. 1988).

Abiotic environmental conditions have important effects on predation rates of larval fishes, particularly environmental conditions that affect the foraging abilities of predators (Huusko et al. 1996; Camp et al. 2012; Carreon-Martinez et al. 2014). Turbidity can serve as cover from predators for larval fish, [e.g. larval yellow perch (*Perca flavescens*; Carreon-Martinez et al. 2014), juvenile Gila chub (*Gila cypha*; Dodrill et al. 2016), larval white sturgeon (*Acipenser transmontanus*; Gadomski & Parsley 2005a)]. Similarly, light levels affect the foraging success of visual predators, and adaptive prey behaviors coinciding with diel and lunar patterns in light levels reduce vulnerability to predation (Huusko et al. 1996; Beauchamp et al. 1999; Gadomski and Parsley 2005a; Prugh and Golden 2014). Substrate in

aquatic environments is crucial habitat that larval fish species use for cover. Insufficient habitat increases predation risk for larval fishes (e.g. substrate type; Gadomski and Parsley 2005a; McAdam 2011; Smith et al. 2012).

How biotic and abiotic factors are impacting predation rates during the early life stages is particularly important for species of conservation concern impacted by low recruitment. Lake sturgeon (*Acipenser fulvescens*) is one such species. Many populations have been reduced or extirpated by overfishing and loss of suitable spawning habitat in rivers (Auer 1996; Bruch et al. 2016). Stocking programs have been necessary to sustain populations with low natural recruitment in the Great Lakes region (Baker and Borgeson 1999; Peterson et al. 2007; Bruch et al. 2016). The high mortality rates experienced by egg and larval stages have been identified as likely bottlenecks to successful recruitment for lake sturgeon populations (Caroffino et al. 2010a; Forsythe 2010). The period when larval lake sturgeon begin feeding exogenously and drift downstream from spawning grounds leaves them particularly vulnerable to predation (Auer and Baker 2002; Duong et al. 2011). Predation of larval lake sturgeon and other sturgeon species has been difficult to reliably detect and quantify in field surveys using morphological diet analysis methods (Parsley et al. 2002; Caroffino et al. 2010b). The dynamics of predation on larval lake sturgeon are necessary to consider when attempting recovery of self-sustaining populations of lake sturgeon, as even small increases in survival could significantly improve recruitment (Pine et al. 2001).

This study used lake-sturgeon specific barcoding primers to detect the remains of larval lake sturgeon in the diets of piscivorous fish in the upper Black River (UBR) in northern lower peninsula of Michigan. Data collected during field surveys was used to build statistical models which included biotic and abiotic variables as parameters associated with the likelihood that a predator had consumed larval lake sturgeon. The objectives of this study were 1) to quantify frequency of lake sturgeon predation and 2) assess how the predator and prey communities, habitat, and environmental conditions affected the probability of lake sturgeon predation.

MATERIALS AND METHODS

Study area and sample collection

This study was conducted in the Upper Black River (UBR; Cheboygan County, MI), the largest tributary of Black Lake, a 4100 ha inland lake in the northern lower peninsula of Michigan with a population of ~1200 adult lake sturgeon (Pledger et al. 2013). The UBR serves as the sole spawning area for the lake sturgeon population, and is restricted to a 1.5 km stretch of river approximately 9 km upstream of the river mouth (Figure 3). Access further upstream is impeded by Kleber Dam, 11km from the river mouth. Sampling for larval lake sturgeon was conducted during 2015 and 2016 at sites directly downstream of each of the spawning sites (Figure 3; gravel sites PD1 and PD3), and two other sites further downstream (Figure 3; sand sites PD4 and PD5). Drift samples were collected for five days during the lake sturgeon drift period in 2015 (May 24th, June 4th-7th) and 12 days in 2016 (May 24th-27th, May 29th- June 1st, June 3rd – June 7th). High water conditions precluded sampling during parts of the larval sturgeon drift period in 2015. The abundance of larval lake sturgeon drifting downstream each night was quantified using D-frame drift nets (Auer and Baker 2002). Five D-frame drift nets with 1600 μm mesh and detachable cod ends were set at one sampling site per night beginning at 21:00. Total river discharge (m^3sec^{-1}) and the discharge entering nets were measured using a Marsh McBirney Flo-Mate 2000 (Hach Company, Loveland, CO, USA) to estimate the proportion of the river discharge being sampled. Net contents were collected and sorted hourly beginning at 22:00 and concluding at 02:00. Larval lake sturgeon were counted on site and returned to the river. A 5% subsample of the cod end contents was collected for each hour and preserved in 95% ethanol. Each subsample was examined under a dissecting microscope, and all larval suckers and aquatic macroinvertebrates were counted and identified to the family level to estimate the abundance of each family.

Electrofishing surveys were conducting the day following larval drift sampling to collect diet samples of potential predators (n=1140 samples from 27 predator species; Table 5). A barge electrofishing unit with a three-person crew sampled a 0.5 km stream segment immediately downstream

of sites where drift sampling was conducted the previous night. Electrofisher settings were set to 400 V at 4 A. Two crew members carried anodes and netted fish while a third crew member pulled the barge and transferred fish to a live well. Total length and species of all fish captured during the survey were recorded. A subset of 10 fish per species per day was sacrificed for gut content analysis with an overdose of MS222 (0.4 mg/ml). Sacrificed predators were individually stored in Whirl-Paks (Nasco, Fort Atkinson, WI, USA) and placed in a -20 °C freezer. Each predator was dissected, the entire GI tract was removed, and contents were carefully extracted to minimize the amount of predator tissue in the sample. During dissection, lake sturgeon larvae remains that could be morphologically identified were recorded. Diet samples were preserved in 95% ethanol and stored at -20 °C prior to DNA extraction.

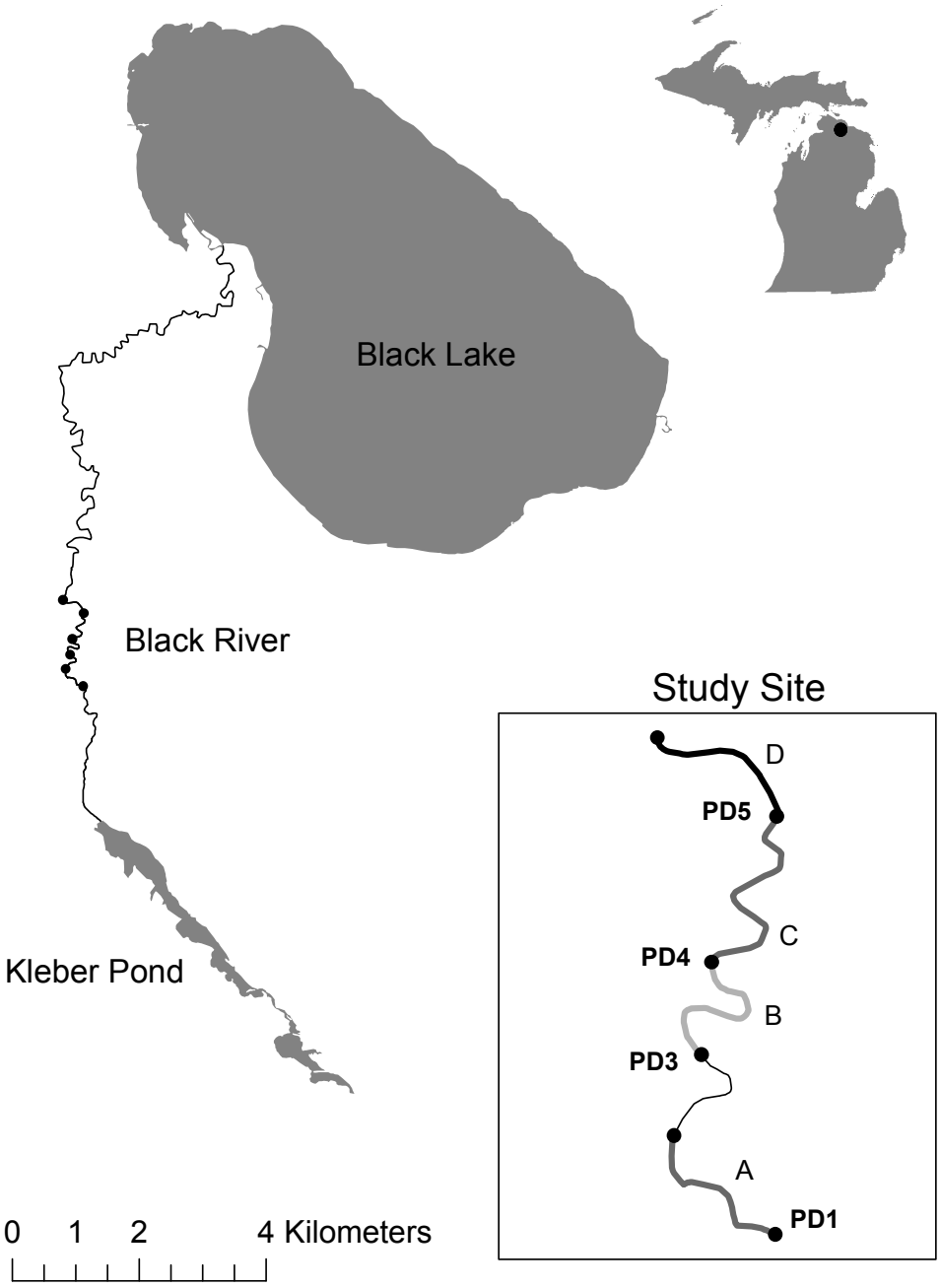

Figure 3. Map of the study site in the upper Black River, Cheboygan County, Michigan. Drift sampling was conducted at gravel sites PD1 and PD3, and sand sites PD4 and PD5 (in bold), with associated electrofishing transects A and B (gravel), and C and D (sand) respectively.

Table 5. Sample size for predators sampled during each year and total number of predator diet samples that tested positive for lake sturgeon DNA.

Predator Species	Sample Size 2015	Sample Size 2016	Total Sample Size	Total Positive for Sturgeon DNA
Amblopites rupestris	28	52	80	0
Ameiurus natalis	0	2	2	0
Ameiurus nebulosus	1	0	1	0
Catostomus commersonii	4	18	22	0
Chrosomus eos	0	3	3	0
Cottus bairdii	0	1	1	0
Culaea inconstans	0	4	4	1
*Etheostoma caeruleum***	43	129	172	7
Etheostoma exile	0	1	1	0
*Etheostoma nigrum***	2	20	22	1
Lepomis gibbosus	10	1	11	0
Lepomis macrochirus	0	1	1	0
*Lota lota***	6	16	22	1
*Luxilus cornutus***	35	81	116	8
*Micropterus dolomieu***	17	30	47	1
Moxostoma anisurum	0	1	1	0
*Nocomis biguttatus***	105	114	219	14
Notemigonus crysoleucas	0	1	1	1
Notropis heterodon	3	3	6	1
Notropis heterolepis	0	3	3	1
*Perca flavescens***	23	73	96	11
*Percina caprodes***	19	38	57	12
*Percina maculata***	8	16	24	1
Pomoxis annularis	0	1	1	0
Rhinichthys atratulus	4	0	4	1
*Semotilus atromaculatus***	33	74	107	7
*Umbra limi***	12	105	117	5
Total	353	788	1141	73

**- denotes predator species included in the predator species parameter used in the regression analysis.

DNA extraction and amplification

Diet samples were coarsely manually homogenized with forceps, sterile toothpicks, and vortexed to homogenize large pieces of tissue and ensure representative subsampling. Approximately 50-100mg of tissue dissected from predator GI tracts was used in each DNA extraction (this was often the entire sample, otherwise a subsample was taken) and washed with sterile water to remove excess ethanol. A modified version of the QIAamp Stool Mini Kit (QIAGEN, Hilden, Germany) protocol was used. The first modification extended lysis in InhibitEx Buffer from the QIAmp Stool Mini Kit to 30 min at 72ºC. Another modification to the QIAGEN protocol added a 10-min bead-beating step using 0.70 mm garnet

beads (MOBIO, Carlsbad, CA, USA) to further homogenize samples after lysis buffer and proteinase K were added to the sample. After elution, DNA concentration was quantified and presence of possible inhibitor proteins was examined with an ND-1000 nanodrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). If a high concentration of contaminants (260/280 < 1.7) remained in the sample, a salt precipitation using cold 100% ethanol and 0.15M sodium acetate was used to clean samples. All samples were diluted using sterile water to a standard concentration of 20ng/μl of DNA.

Two sturgeon-specific primer sets were used to test for the presence of lake sturgeon DNA in the diet samples, AfCOI1 and AfCOI2 (Waraniak et al. in review). PCR conditions for both primer pairs included 20 ng of template DNA, 0.5 μM for each forward and reverse primer, 200 μM dNTPs, 1X reaction buffer, and 5U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and deionized water for a reaction volume of 25μl. Amplification conditions included an initial denaturation step of 94 ˚C for three minutes; followed by 35 cycles of 94 ˚C (45 s), 56 ˚C (30 s), and 72 ˚C (30 s). Final extension lasted 5 minutes at 72 ˚C. 10μl of PCR products were visualized on 2% agarose gels stained with ethidium bromide. Successful amplification of a sequence of the appropriate size indicated the presence of lake sturgeon remains in diet samples. A positive control of lake sturgeon genomic DNA and a negative control of PCR reaction mixtures without template DNA were included for each round of PCR. 8μl of 100 bp ladders (Invitrogen, Carlsbad, CA, USA) were run on each gel to approximate the size of PCR products and ensure positive results were the expected size of the target region (138bp - AFCOI1, 151bp – AfCOI2). An image of each agarose gel was captured under UV light, and the image was used to score samples as positive or negative for the presence lake sturgeon DNA. Primer pair AfCOI1 was used as the primary primer pair tested on all samples, and is able to detect concentrations of lake sturgeon DNA as low as 0.032pg/μl (Waraniak et al. in review). Primer pair AfCOI2 was used to confirm positive results from primer pair AfCOI1. Only samples that were amplified by both primers were considered positive for lake sturgeon DNA.

Statistical analysis

Model description. Data of the presence or absence of lake sturgeon DNA in a predator diet sample was analyzed using binomial logistic regression. The regression models tested how the probability that a predator consumed a larval lake sturgeon was affected by biotic and abiotic variables. The full model included 15 explanatory variables that broadly encompassed three categories: availability of prey, predator type, and abiotic factors associated with visibility and cover (Model 1).

Model 1. (Full model)

P(Sturgeon consumed) = BLS + BC + BI + PLS + PC + Pred + TL + Lun + CC + Sub + Q + Year + E2 + E3 + E4

Variables corresponding to the availability of prey included nightly total biomass estimates for larval lake sturgeon, larval suckers, and aquatic macroinvertebrates (BLS, BC, and BI respectively). Dry mass estimates of individual larval fish and common families of macroinvertebrates (Walquist and Scribner, unpublished data) were applied to count data from the 5% drift subsamples to get an estimate for catch biomass. The catch biomass was extrapolated to the entire river by multiplying the catch biomass by the inverse of the proportion of discharge that was sampled by the drift nets (Equation 1).

Equation 1. (Estimate of total river biomass)

$$\text{Total River Biomass} = \text{Catch Biomass} * (\text{Net Discharge} / \text{River Discharge})^{-1}$$

The sum of biomasses from all macroinvertebrate families was used for a more general invertebrate biomass term. Estimated nightly proportions of the drift biomass made up by larval lake sturgeon and larval suckers (PLS and PC) were also included in the model. Only two proportions could be included so as not to violate independence assumptions.

Variables associated with the predator included the predator species (Pred) and total length (TL) of the fish from which a diet sample was collected. Samples from predator species in the dataset that had never consumed a sturgeon or had fewer than 10 total observations were removed from further analysis to improve the stability of the predator species term in regression models. This resulted in the predator species variable having 11 well-represented levels and the model being based on a sample size of n=999.

Abiotic variables included the predominant substrate type (sand or gravel) in the river transect from which a predator was collected (Sub), the percentage of the moon that was illuminated (Lun; US Naval Observatory, aa.usno.navy.mil/data/docs/RS_OneDay.php), the average nightly percentage of cloudy skies during drift surveys (CC; NOAA, www.ncdc.noaa.gov/cdo-web/datasets/GHCND/stations/GHCND:USC00201492/detail), the river discharge (Q), and the year in which the sample was collected (Year). Additionally, three temporal autocorrelation terms were included to account for similarities in prey biomass and environmental conditions across consecutive days, generated by an eigenfunction-based filtering method (Peres-Neto 2006). A temporal association matrix was constructed using the drift survey sampling dates. Because sampling occurred at regular intervals, the association between consecutive sampling days is equal to one and non-consecutive days are unrelated, having distances equal to 0. The temporal autocorrelation terms are the principal coordinates of the eigenanalysis of this temporal association matrix. The second (E2), third (E3), and fourth (E4) eigenvectors, associated with the sampling periods from 6/4/2015-6/8/2015, 5/24/2016-5/28/2016, and 5/30/2016-6/2/2016 respectively, were significantly correlated with the presence of lake sturgeon DNA in a diet sample, so those three variables were included in the full model.

Model selection. Models were fit using the glm function in R v 3.2.2 (R Core Team 2015). All possible combinations of variables included in the model were fit and AIC values and weights were calculated for each version of the model using functions from the MuMIn library (Barton 2016). The relative importance of each variable was calculated by dividing the sum of the weights from the 38

models with ΔAICc < 2, that included a variable by the weights of all 38 models with ΔAICc < 2 (Barton 2016).

Analysis of variable effects. Further exploration of important variables was carried out with the model averaged values across the 38 models with ΔAICc < 2. Differences between different levels in categorical variables and the effects of continuous variables were analyzed using the odds ratio values (OR). Wald's Chi-squared tests were used to further test for differences between levels of categorical variables.

The effects of selected continuous variables were visualized using the average model generated from the models with ΔAICc < 2. Predicted probabilities of larval lake sturgeon predation were calculated with the plogis function (R Core Team 2015). The values of one predictor variable would vary at a time across the range of values seen in the dataset for that variable, with all other variables being held constant at their mean value in the actual dataset. The 95% confidence intervals were generated for each variable by multiplying the standard error of the average odds ratio by the t-value for α=0.025 and df=18 (the degrees of freedom for the average model).

The effects of different predator species were tested against each other with the average odds ratios from the 38 best fit models that included predator species as an explanatory variable. Differences between species were then analyzed with Wald's tests (R library aod), contrasting one species against all others included in the model. For each species, a contrast was set up with the species of interest having a contrast coefficient of -1 while the other 10 species in the model were assigned contrast coefficients of 0.1. Significance was adjusted using a Holm-Bonferroni correction for multiple comparisons.

RESULTS

Field and molecular analysis data

A total of 74 predator diet samples tested positive for lake sturgeon DNA using both the AfCOI1 and AfCOI2 primer pairs out of 1140 total samples (6.49%). Only one larval lake sturgeon was morphologically identified in all of the diet samples (0.09%), identified from a smallmouth bass (*Micropterus dolomieu*) sampled in 2015.

Predator communities varied between the two years and between sand and gravel habitats. Table 5 shows the number of predators from each species and year from which diet samples were taken, as well as how many tested positive for lake sturgeon DNA. 16 predator species tested positive for lake sturgeon DNA, but only 11 of these species had sufficient sample sizes to be included in the final model parameter for predator species.

Estimated dry weight biomass and relative proportions of the three co-distributed prey types varied over the course of the study (Figure 4). Larval lake sturgeon nightly biomass varied from 2 g to 243 g with a mean of 65 g and median of 6 g. Larval sucker nightly biomass varied from 7 g to 2596 g with a mean of 477 g and median of 215 g. Aquatic macroinvertebrate nightly biomass varied from 47 g to 579 g with a mean of 314 g and median of 337 g. Larval lake sturgeon made up between 0.2-32.7% of the nightly biomass (mean = 9.1%), and larval suckers made up between 2.2-89.5% of the nightly biomass (mean = 40.9%).

Lunar illumination ranged from full moon (100%) to new moon (0%) conditions (mean = 42.6%). Likewise, cloud cover ranged from completely cloudy skies (100%) to clear skies (0%; mean =47.0%). River discharge varied from 4.93 m^3/s to 7.90 m^3/s (mean = 6.52 m^3/s).

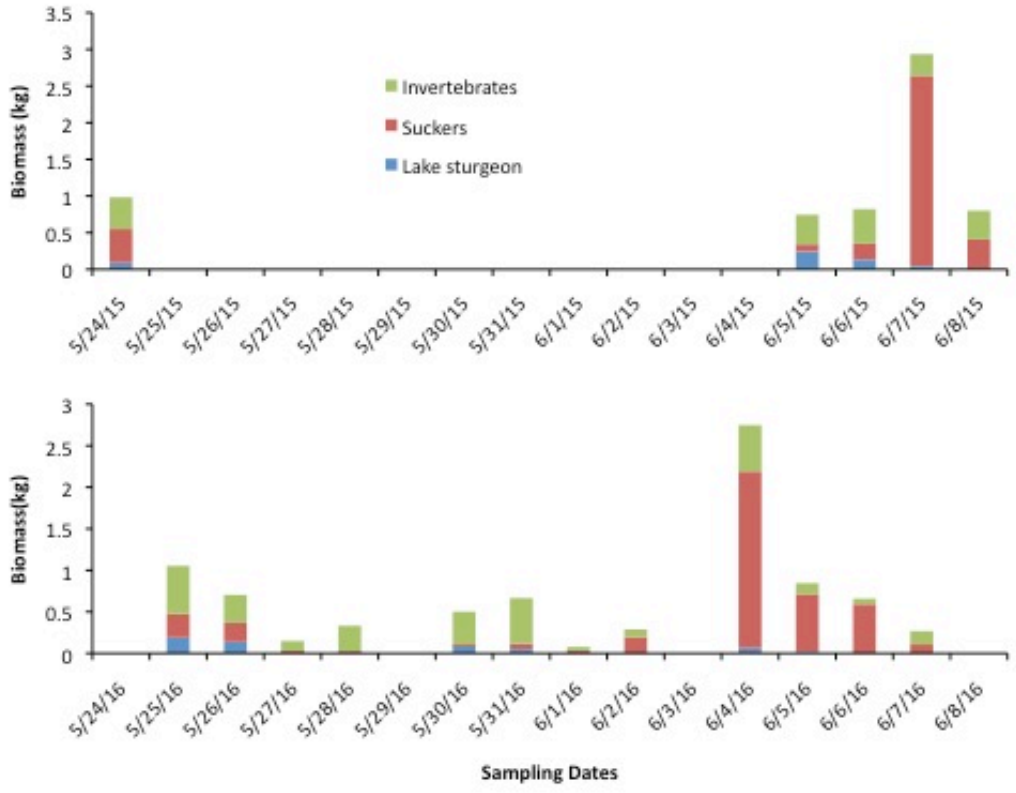

Figure 4. Estimated biomass from drift surveys for each of the three types of co-distributed prey for each night included in the study in 2015 (top) and 2016 (bottom).

Model selection and importance of variables

There were 38 well-performing "best-fit" models with $\Delta AIC < 2$ (Table 6). Across these models, the most important variables are temporal autocorrelation term E4, the proportions of drift biomass made up by larval lake sturgeon and larval catostomids, the biomasses of larval lake sturgeon and aquatic macroinvertebrates, and lunar illumination (Table 7; relative importance > 0.6). The other temporal autocorrelation terms, predator species, year, biomass of larval suckers, and cloud cover were relatively unimportant (relative importance < 0.4). The total length of predators was not included as a variable in any of the 38 best-fit models (relative importance = 0).

Table 6. Models with lowest AIC scores. BI – biomass of invertebrates, BLS – biomass of larval lake sturgeon, BC – biomass of larval suckers, PLS – proportion of biomass made up by larval lake sturgeon, PC – proportion of biomass made up by larval suckers, , Lun – percentage of moon illuminated, Sub – substrate type, Pred – predator species, Q – river discharge, CC – cloud cover. E2, E3, and E4 are temporal autocorrelation terms. The model with lowest AICc score is bolded.

Model	df	ΔAIC	wAIC
BI + BLS + BC + PLS + PC + CC + E4	8	1.65	0.022
BI + BLS + BC + PLS + PC + E2 + E4	9	1.22	0.027
BI + BLS + BC + PLS + PC + E4	7	1.44	0.024
BI + BLS + BC + PLS + PC + Lun + CC + E4	9	1.83	0.020
BI + BLS + BC + PLS + PC + Lun + E4	8	0.64	0.036
BI + BLS + BC + PLS + PC + Lun + Pred + E4	18	1.21	0.027
BI + BLS + BC + PLS + PC + Lun + Q + E4	9	1.82	0.020
BI + BLS + BC + PLS + PC + Lun + Sub + CC + E4	5	1.85	0.020
BI + BLS + BC + PLS + PC + Lun + Sub + E2 + E4	10	1.98	0.018
BI + BLS + BC + PLS + PC + Lun + Sub + E4	**9**	**0.00**	**0.050**
BI + BLS + BC + PLS + PC + Lun + Sub + Pred + E4	19	1.25	0.027
BI + BLS + BC + PLS + PC + Pred + E2 + E3 + E4	9	1.42	0.024
BI + BLS + BC + PLS + PC + Q + E2 + E3 + E4	10	1.49	0.024
BI + BLS + BC + PLS + PC + Sub + E2 + E3 + E4	10	1.59	0.022
BI + BLS + BC + PLS + PC + Sub + Pred + E2 + E3 + E4	18	1.01	0.030
BI + BLS + PLS + PC + CC + E2 + E3 + E4	9	1.69	0.021
BI + BLS + PLS + PC + E2 + E3 + E4	8	0.37	0.041
BI + BLS + PLS + PC + Lun + CC + E4	8	0.80	0.033
BI + BLS + PLS + PC + Lun + E4	7	0.23	0.044
BI + BLS + PLS + PC + Lun + Pred + E4	17	1.20	0.027
BI + BLS + PLS + PC + Lun + Q + E4	8	1.95	0.019
BI + BLS + PLS + PC + Lun + Sub + E4	8	1.17	0.028
BI + BLS + PLS + PC + Q + E2 + E3 + E4	19	1.42	0.024
BLS + Lun + Pred + E4	14	1.89	0.019
BLS + Lun + Sub + Pred + Q + E2 + E3 + E4	18	1.36	0.025
BLS + PC + Lun + Sub + Pred + Q + E2 +E3 + E4	19	1.92	0.019
BLS + PC + Lun + Sub + Q + E2 + E3 + E4	9	1.15	0.028
BLS + PLS + PC + E2 + E3 + E4	7	1.82	0.020
Lun + Sub + E2 + E3 + E4	8	1.28	0.026
Lun + Sub + Pred + E4	14	1.65	0.022
Lun + Sub + Q + E4	10	1.85	0.020
PLS + Lun + E4	4	1.59	0.023
PLS + Lun + Pred + E3 + E4	15	1.55	0.023
PLS + Lun + Pred + E4	14	1.11	0.029
PLS + Lun + Year + E4	5	1.08	0.029
PLS + PC + Lun + E4	5	1.18	0.028
PLS + PC + Lun + Pred + E4	15	1.29	0.026
PLS + PC + Lun + Pred + Year + E4	15	0.74	0.034

Table 7. Average log odds ratio across 38 models with ΔAICc < 2, standard error of model averaged odds ratio, and relative importance for each variable. Log odds ratios for categorical variables can only be interpreted compared to a standard category (standard category that other categories are compared to is included in parentheses after name of categorical factor). Relative importance is calculated by dividing AIC weights of all models that include the variable as a parameter by the AIC weights of all models in the 38 models with ΔAICc < 2. Importance values of at least 0.4 are required to be considered important.

Variable	Average Log Odds Ratio	± Standard Error of Odds Ratio	Relative Importance
E4 – temporal autocorrelation	23.34	11.87	1.00
Proportion of biomass made up by larval lake sturgeon	-0.190	0.113	0.84
Biomass of larval lake sturgeon	0.246	0.122	0.77
Proportion of biomass made up by larval suckers	-0.032	0.019	0.75
Lunar illumination	0.402	3.570	0.72
Biomass of invertebrates	-0.052	0.028	0.63
E3 – temporal autocorrelation	-26.36	26.01	0.36
Biomass of larval suckers	0.006	0.004	0.36
E2 – temporal autocorrelation	-29.73	32.59	0.35
Predator species (compared to BSD)	----	----	
BUR	-0.092	1.466	
CMM	-0.097	1.137	
CMS	0.345	1.101	
CRC	0.306	1.114	
HHC	0.277	1.071	0.33
JOD	0.420	1.476	
LOP	1.771	1.087	
RAD	-0.107	1.105	
SMB	-0.733	1.452	
YEP	0.875	1.088	
Substrate type (compared to gravel)	----	----	0.30
Sand	-0.679	2.351	
River discharge	-0.757	0.668	0.20
Cloud cover	-0.501	0.535	0.12
Year (compared to 2015)	----	----	0.06
2016	0.464	0.299	

Analysis of variable effects

The model averaging indicated a strong positive relationship between the likelihood that a predator would have consumed a larval lake sturgeon with the biomass of larval lake sturgeon present in the drift the night before (Figure 5a, OR =1.279). The odds ratio indicates that for each additional 24.3 g of lake sturgeon biomass present in the drift (10% of the observed range, ~2800 individual larvae), there

was an estimated 81% increase in lake sturgeon predation. There was a slightly less strong but still significant negative relationship between the probability of larval lake sturgeon predation and the biomass of invertebrates (Figure 5a, OR = 0.950), corresponding to a 29% decrease in sturgeon predation for each additional 52 g of aquatic invertebrates present in the drift (10% of the observed range; ~29,000 mayfly larvae, Family: Heptageniidae, the most abundant insect family). There was also a weak slightly positive relationship with the biomass of larval suckers (Fig. 5a, OR = 1.006), showing a 16% increase in the probability of larval lake sturgeon predation for each 258g of larval suckers present in the drift (10% of the observed range, ~216,000 individual larvae).

The proportional biomass of the drift made up by larval suckers had a moderately strong negative effect on the probability that a predator had consumed a lake sturgeon larva (Fig 5b, OR = 0.968). This corresponds to a 27% reduction in lake sturgeon predation for each 10% of the drift biomass made up by larval suckers. The proportion of the drift biomass made up by larval lake sturgeon had a large negative effect on the probability of lake sturgeon predation (OR=0.827), however, this effect was strongly dependent on whether or not the biomass of lake sturgeon was included in the model. The actual biomass (BLS) and proportional biomass (PLS) of larval lake sturgeon were correlated ($R^2 = 0.805$; $p<0.001$). Because of this, those two parameters explain much of the same variation, so the behavior of one parameter could be highly dependent on the presence of the other. The effect of BLS was relatively consistent across models. However, the behavior of PLS was dependent on the presence of BLS. In models without BLS, the proportional biomass of lake sturgeon was either weakly negative or weakly positive on the probability of lake sturgeon predation.

Lunar illumination had a moderately strong positive effect on the probability of lake sturgeon predation on average for all well-performing models (Fig 5c, OR = 1.495). For each 10% of the moon that was illuminated, there was an estimated 4% increase in the probability a predator had consumed a lake sturgeon larva.

Substrate type was a relatively unimportant variable, but did appear in several of the best-fit models (OR = 0.507). The models compared the probability of predation of larval lake sturgeon in sand-

dominated habitats to gravel dominated habitats, suggesting that larval lake sturgeon are less vulnerable in sand substrate habitats, but this difference was not significant between the two substrate types. Discharge and cloud cover were also relatively unimportant factors. In the best-performing models that the discharge parameter was included, increasing discharge has a moderate negative effect on the probability of sturgeon predation (OR = 0.469) and increasing cloud cover also decreased the probability of sturgeon predation (OR = 0.606).

Predator species relatively low variable importance in the analysis of all the "best-fit" models, however, some predators appeared to have more of an effect than others. In order to test the effects of different predator species, a model was fit with the predator species parameter as the only explanatory variable. In Wald's tests contrasts between one species and the average of the other 10 species included in the model, logperch (*Percina caprodes*) was the only species that was significantly more likely to consume larval lake sturgeon, and yellow perch (*Perca flavescens*) were nearly significantly more likely to consume larval lake sturgeon than the other species (Table 8).

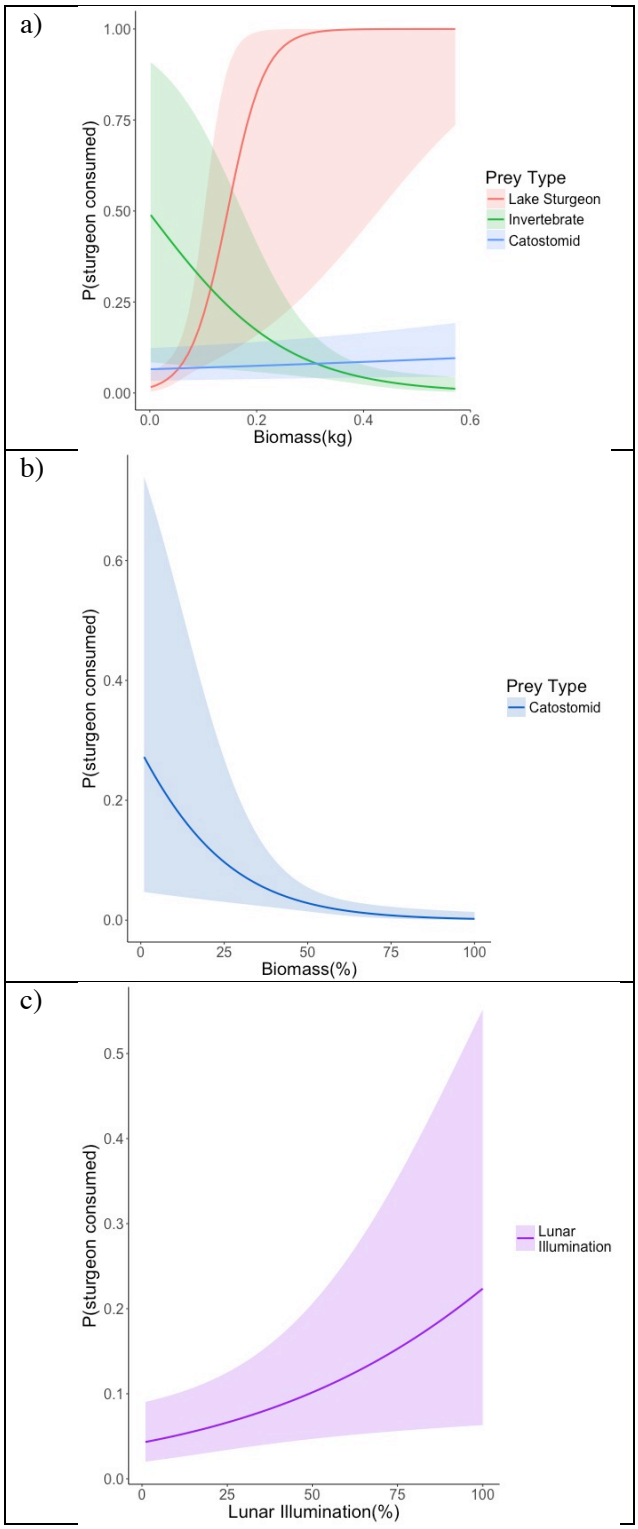

Figure 5. Simulated data based on the AIC selected model. Graphs show relationships between the probability that a predator diet sample contained lake sturgeon DNA and a) biomass of larval lake sturgeon (red), aquatic macroinvertebrates (green), and larval suckers (blue); b) the proportion of drift biomass made up by larval suckers, and c) the proportion of the moon illuminated. Shaded areas represent 95% confidence intervals.

Table 8. Results of Wald's Chi-squared test contrasts for each predator species compared to the other predator species that were included in the full model. Each Chi-squared test had df = 1.

Predator Species	Wald's χ^2	P-value
Blackside darter (*Percina maculata*)	2.9	0.086
Burbot (*Lota lota*)	0.0007	0.98
Central mudminnow (*Umbra limi*)	0.0064	0.94
Common shiner (*Luxilus cornutus*)	1.2	0.27
Creek chub (*Semotilus atromaculatus*)	0.87	0.35
Hornyhead chub (*Nocomis biguttatus*)	1.1	0.28
Johnny darter (*Etheostoma nigrum*)	0.0007	0.98
Logperch (*Percina caprodes*)	19.8	<0.001*
Rainbow darter (*Etheostoma caeruleum*)	0.043	0.84
Smallmouth bass (*Micropterus dolomieu*)	0.64	0.42
Yellow perch (*Perca flavescens*)	6.9	0.0085^

*- denotes statistical significance after Holm-Bonferroni adjustment. ^-denotes near statistical significance after Holm-Bonferroni adjustment.

DISCUSSION

The molecular methods used in this study were much more successful in identifying predators that had consumed larval lake sturgeon (73 out of 1140 predators tested) than a more traditional morphological diet analysis (1 out of 1140 predators tested), suggesting that predation is more common than previous studies have been able to detect (Parsley et al. 2002; Caroffino et al. 2010b). Furthermore, with intensive sampling over two years, this study was able to highlight possible biotic and abiotic factors that impact predation of larval lake sturgeon migrating out of their natal stream. Previous studies of predation examining this stage of sturgeon development have relied on artificial experimental setups or recorded too few observations of predation to effectively analyze. While the effects of co-distributed species (Frank and Leggett 1983; Pepin and Shears 1995) and abiotic factors (Gadomski and Parsley 2005a) on levels of predation of larval fishes have been hypothesized or examined in controlled experiments, there have been few studies evaluating these factors in natural systems. Using a large data set collected from drift and electrofishing field surveys; this study is one of the first using molecular methods to examine how multiple abiotic and biotic factors impact predation of a drifting larval fish.

Synchronized initiation of drifting behaviors is a common occurrence among many species with drifting larvae (Gale and Mohr 1978; Brown and Armstrong 1985; Carter et al. 1986). This behavior may be adaptively advantageous to the drifting larvae through predator swamping, overwhelming the predator community with large numbers of prey to reduce overall predation of prey items (Frank and Leggett 1983). The results of this study appear to support predator swamping as a mechanism reducing predation of larval lake sturgeon. Factors related to the biomass of alternative prey were present in 29 out of the 38 best-performing models, and all of the factors related to alternative prey abundance were included in the AIC selected model. The biomass of invertebrates and the proportion of larval sucker biomass in the drift both had consistent negative effects on the probability predators had consumed larval lake sturgeon and were both relatively important factors (Table 7). Synchronized emergence as a predator swamping strategy is most often considered in the context of one species (Ims 1990), however, predator swamping

with multiple co-distributed species may be more important for species that are relatively rare (Frank and Leggett 1983). In both years of this study, the drifting biomass of larval lake sturgeon peaked at approximately the same time as larval suckers, but the peak biomass for larval suckers was an order of magnitude larger than the peak biomass for larval sturgeon (Figure 4). Lake sturgeon larval production in the UBR is highly variable from year to year (Smith and King 2005), and there may be few years in which the sturgeon density alone is able to swamp predators. In most years, survival of larval lake sturgeon may more heavily depend on the abundance of co-distributed larval fishes and aquatic macroinvertebrates.

Some abiotic factors that affect the visibility of predators have also been shown to affect the predation of larval fishes. While turbidity was not directly measured, it is known to co-vary with discharge (Mather and Johnson 2014), which was a moderately important parameter in the logistic regression models. Turbidity has been shown to lower predation of larval perch in Lake Erie (Carreon-Martinez et al. 2014) and predation of white sturgeon larvae by sculpin in experimental settings (Gadomski and Parsley 2005a). Visual predators including darters (Becker et al. 2016), other percids (Chiu and Abrahams 2010; Carreon-Martinez et al. 2014), centrarchids (Johnson and Hines 1999; Ferrari et al. 2014), and piscivorous cyprinids (Bonner and Wilde 2002; Dodrill et al. 2016) all had reduced prey consumption in turbid conditions. Likewise, moonlight is known to have important effects on predator-prey dynamics in terrestrial systems (Prugh and Golden 2013). The results of this study suggest percentage of lunar illumination was one of the most important factors influencing predation of larval lake sturgeon in the UBR (Table 7). Lake sturgeon spawning, and concordantly larval drift, is known to coincide with lunar phases (Forsythe et al. 2012), so models that include lunar illumination but do not include the lake sturgeon biomass variable may overestimate the importance of lunar illumination. However, few of the best-performing models contain the lunar illumination term without the term for sturgeon biomass, so overestimation of the importance of lunar illumination is likely to be minor (Table 6). The effects of moonlight on predatory fish have not been rigorously tested, but many studies have demonstrated that predatory fish are highly sensitive to light levels, with many species exhibiting

crepuscular foraging behaviors (Peterson and Gadomski 1994, Huusko et al. 1996, Gadomski and Parsley 2005a) including some of the species in the UBR (Keast and Welch 1968). Low light levels, as would be expected during new moon phases, would reduce reaction distances of predatory fish and make foraging less efficient for nocturnal visual predators (Beauchamp et al. 1999). Additionally, there is circumstantial evidence that changing moon phases alter foraging behavior in predatory fishes (Horky et al. 2006, Whitty et al. 2009).

There was some evidence that predator species was an important predictor of larval lake sturgeon predation, indicating there were a few species that were consuming larval lake sturgeon at higher rates than others. Two species of percids included in this study were more likely to consume larval lake sturgeon than other species. 21.1% of logperch (*Percina caprodes*), and 11.5% of yellow perch (*Perca flavescens*) consumed larval lake sturgeon, significantly higher frequencies than other species (Table 8). Logperch are benthic predators (Leino and Mensinger 2017), and could have a higher encounter rate with benthic-drifting lake sturgeon larvae compared to other potential predators. The high predation rate of larval sturgeon by logperch is additionally concerning because the invasive round goby (*Neogobious melanostomus*) occupies a similar niche (Leino and Mensinger 2017), and while gobies have not been detected in the UBR, they have spread to other lake sturgeon breeding grounds in the Great Lakes region (Nichols et al. 2003). Most of the yellow perch sampled were age-1 juveniles with TLs approximately 80-100mm, a size at which they feed primarily on larval and age-0 juveniles of other fish species, making larval lake sturgeon likely to be targeted as prey items (Parke et al. 2009). While percids were the most likely to consume larval lake sturgeon, predatory cyprinids accounted for the most incidences of lake sturgeon predation (33 out of the 73 diet samples positive for lake sturgeon DNA), mainly due to the larger proportion of the fish community made up by cyprinids in the UBR. Apart from the two percid species highlighted above, most of the predators preyed on larval lake sturgeon at similar rates, and the species that most often consumed larval lake sturgeon were also the most numerically abundant fish species.

While most of the predators in this study that did have lake sturgeon DNA in their stomach could have reasonably consumed a larval lake sturgeon, there are a few small-bodied predators that would appear to be gape-limited (e.g. *Notropis* spp., stickleback, and some of the smaller rainbow darter). All of these small-bodied predators were captured in the gravel sections of the study area, which were located directly downstream of the main lake sturgeon spawning sites. The molecular assay is not able to discriminate between lake sturgeon DNA from larvae, eggs, or yolk-sac fry. Positive results of these small-bodied fishes could have resulted from direct predation of lake sturgeon life stages other than larvae, which were available throughout the study period, or from secondary predation of detritivorous invertebrates that were feeding on the remains of lake sturgeon eggs (Sheppard et al. 2005).

There is also the concern that environmental DNA (eDNA) from lake sturgeon could have gotten into the GI tracts of the predators and caused false positives, however, this is unlikely. The amount of eDNA depends on the abundance, biomass, and distance from the source of the genetic material (Jerde et al. 2010, Jane et al. 2014). The patterns of positive results from predator diet samples do not match what would be expected if eDNA accounted for most of the lake sturgeon DNA in predator GI tracts. The main source of lake sturgeon eDNA in the UBR would come from spawning adults, but periods of high abundance of spawning adults did not coincide with high numbers of predator diet samples that tested positive for lake sturgeon DNA, based on adult surveys conducted by the staff at the Black River Sturgeon Rearing Facility. Furthermore, the river distance of the study area is much farther than the distance eDNA can travel in a river the size of the UBR (Jane et al. 2014). With congregations of adult lake sturgeon in the main spawning areas, more predator diet samples collected from the nearby gravel sections would test positive with the genetic assay due to eDNA than predators further downstream in the sand sections. However, the diet samples of predators from the sand section were actually more likely to test positive for lake sturgeon DNA, and the probability of a positive result was positively correlated with the biomass of larval lake sturgeon present in the drift the night before, further evidence that predation was the most likely source of lake sturgeon DNA in predator GI tracts, not contamination due to eDNA.

This study took advantage of the regular, predictable periodicity of drifting behavior in larval sturgeon to semi-quantitatively assess levels of predation. Because larval drift occurred at approximately the same time each sampling day, and predators were always collected the same amount of time after larval lake sturgeon were available, day-to-day results should be comparable. Quantitatively estimating predation of larval fishes using molecular methods is difficult, but advances in the field are making it possible. Quantitative PCR (qPCR) can be used to estimate the number of copies of prey DNA in a predator GI tract, which is positively correlated with prey biomass and negatively correlated with the amount of time since consumption (Durbin et al. 2007). If the digestion rate of the predator and approximate time of consumption of the prey is known, the amount of prey ingested by the predator could be estimated (Durbin et al. 2007). Alternatively, genetic markers used for population genetics studies (e.g. microsatellites, SNPs - single nucleotide polymorphisms) could be used on diet samples to estimate the number of individual prey items of a certain species consumed by a predator (Carreon-Martinez et al. 2014).

The results of this study may be applicable to streams with similar fish and invertebrate communities and environmental conditions. This study was based on data collected from a single river system during two years that were qualitatively similar in terms of overall biomass of prey. Different river systems and even different years within the UBR may have environmental conditions and biotic communities outside the parameter space of the variables included in this model. For example, both years included in this study saw relatively similar peaks in magnitude for the biomass of drifting larval sturgeon (2015 - 243g, 2016 - 189g), there are records in the UBR historical dataset with peaks nearly an order of magnitude higher than the upper end of the range of biomasses of larval lake sturgeon represented in this study. Predators may switch to more actively targeting larval lake sturgeon if they are present at such high abundances (Sundell et al. 2003; Siddon and Whitman 2004). Likewise, sampling during high discharge events in 2015 was not possible (hence the lower sample size), and high flow rates could have reduced larval lake sturgeon predation if predators were more likely to seek out cover from the high flows than forage for prey (Kemp et al. 2006). How predators affect larval lake sturgeon mortality in larger river

systems, such as the St. Clair River, are likely to be quite different, as larger rivers have less variable flow rates and much different predator communities (Nichols et al. 2003). Additionally, using D-frame drift nets for sampling targeted benthic drifting taxa, potentially biasing biomass estimates by under-representing surface-drifting taxa, like catostomid larvae.

This study demonstrated that emerging molecular diet analysis techniques can semi-quantitatively evaluate predation and are likely to be more useful and accurate than morphological identification methods to evaluate how biotic communities and environmental conditions impact predation on vulnerable life history stages of a species of conservation concern. This study gives evidence that co-distributed larval fish and aquatic macroinvertebrate communities improve survival of larval lake sturgeon. In a system with many visual predators like the UBR, turbidity, moonlight, and other factors affecting visibility also appear to play important roles in the mortality of larval lake sturgeon. In order to ensure successful natural recruitment of lake sturgeon populations, factors such as these should be taken into consideration. How the predator community is managed (e.g. stocking large piscivorous species), habitat restoration that may not directly benefit lake sturgeon but increases abundance of co-distributed prey, and restoring connectivity to increase populations of the prey community could all be possible management actions with the potential to reduce predation pressure on larval lake sturgeon.

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CHAPTER 3: 18S METABARCODING DIET ANALYSIS OF THE PREDATORY FISH COMMUNITY IN THE BLACK RIVER, CHEBOYGAN COUNTY, MI, ACROSS SEASONAL CHANGES IN PREY AVAILABILITY

ABSTRACT

Predator-prey relationships are extremely important ecological interactions, affecting the composition of the biotic community and energy flow through a system, and are of great interest to ecologists and managers alike. Morphological diet analysis has been the primary method to quantify the diets of predators, but emerging molecular techniques using genetic markers have the potential to provide a more accurate method. This study used sequences from 18S V9 rRNA barcoding region to identify prey items in the gastrointestinal tracts (GI) of predators. GI samples were taken from predators in the Black River, Cheboygan Co., MI (n=367 samples from 12 predator species) during a period of high prey availability (including the larval stage of the regionally threatened lake sturgeon) in late May/early June of 2015 and a period of relatively lower prey availability in early July of 2015. DNA was extracted and sequenced from 355 samples (96.7%), and prey DNA was identified in 286 of the 355 samples (80.6%). Prey were grouped into 33 ecologically significant units based on the lowest taxonomic level sequences could be identified to on GenBank. Changes in the makeup of diets, diet overlap, and predator preference for certain prey items were analyzed comparing the two periods of prey abundance. Some predator species did undergo seasonal changes in diets, diet overlap was slightly but significantly higher during the period of high prey abundance, and there is little evidence for large changes in predator preference between the two periods. The metabarcoding technique used in this study was successful in quantifying and identifying differences between diets of different species in different time periods. Improvements could be made to this procedure by taking amplification biases of certain taxa into account and selecting more taxon-specific markers to yield higher taxonomic resolution. This study demonstrates the utility of molecular diet analysis in understanding predator-prey interactions in complex freshwater communities.

INTRODUCTION

Characterization of predator diets and food web interactions are generally greatly important in the understanding of community functioning and management of freshwater systems (Vaughn 2010; Thompson et al. 2012). DNA-based molecular methods offer a promising tool for analyzing the diets of fishes in freshwater food webs with greater accuracy and resolution than past methods (Carreon-Martinez and Heath 2010; Pompanon et al. 2012). Metabarcoding is one such molecular technique, utilizing conserved regions of DNA to amplify sequences in samples that can uniquely identify different taxa (King et al. 2008). Metabarcoding in ecological studies has primarily been used to assess biodiversity, detecting the presence of species in an area using environmental DNA (eDNA; Taberlet et al. 2012; Creer et al. 2016; Valentini et al. 2016). However, there is also a growing literature applying metabarcoding tools to estimate the diet composition of predators (Kartzinel and Pringle 2015; Albaina et al. 2016; Harms-Tuohy 2016). Molecular techniques have an advantage over morphological analyses of fish diets that require visual identification of partially digested prey items (Sheppard and Hardwood 2005; Schooley et al. 2008; Carreon-Martinez et al. 2011; Berry et al. 2015). Metabarcoding diet analysis has been used to characterize numerous predatory fish diets in marine food webs. Metabarcoding diet analysis had discriminated between the diets of clupeid zooplanktivorous fishes (Albaina et al. 2016), revealed niche differentiation between reef fishes (Leray et al. 2015), and provided a non-lethal way to sample the diets of endangered sharks (Barnett et al. 2010). In contrast, there are relatively few studies in freshwater systems that utilize this emerging molecular technique (Carreon-Martinez 2011; Jo et al. 2014; Shortridge 2016).

Metabarcoding methods can quantify diet compositions of predator fishes and compare them to the composition of the prey community in the environment to determine which prey taxa predators are selectively targeting (i.e. predator preference). Predator preference can indicate what members of the community act as important energetic links between trophic levels, or which prey taxa are required to maintain healthy fish populations (Ivlev 1961; Chesson 1978). Estimates of diet similarity between two

predator species can also be used as a measure of the degree of interspecific resource competition (Schoener 1970). Dietary composition data allow examination of how abundance of one species could slow recovery of another species (Jacobs et al. 2008), or what species may be at risk of competitive exclusion by invasive species (Pilger et al. 2010; Creque and Czesney 2012). The greater diet breadth and taxonomic resolution that can be achieved through metabarcoding diet analysis can better characterize the degree of niche partitioning among species, showing how finely species can partition resources to avoid high levels of interspecific competition (Katzinel et al. 2015; Leray et al. 2015) or how diet overlap was higher than non-molecular studies estimated due to high abundance of soft-bodied prey items in predator diets (Soininen et al. 2015; Gebremedhin et al. 2016), which are often difficult to detect in morphological diet studies due to rapid digestion times (Carreon-Martinez et al. 2011; Ley et al. 2014).

Predator preferences and diet overlap among predators is driven in part by the prey abundance. High abundance of prey leads to higher encounter rates, and in some cases can cause abundant prey taxa to be targeted by predators (Murdoch 1969; Ims 1990). Overabundance of prey can reduce pressure from inter-specific competition, allowing predators to coexist for periods of time despite having high overlap in utilization of prey resources (Gray et al. 1997; Michaeletz 1997; Kelling et al. 2016). Seasonal fluctuations in prey abundance and species composition are a common feature of many riverine communities (Brown and Armstrong 1985; Gray et al. 1997; Smith and King 2005). Synchronized emergence of larval fishes and aquatic macroinvertebrates may have evolved in some systems as an adaptive strategy to swamp predators, leading to periods where foraging predators are saturated by prey (Frank and Leggett 1983; Ims 1990). This seasonal influx of prey, comprised of the early life stages of river spawning fishes and emergence of certain aquatic macroinvertebrate taxa, can alter trophic interactions between predators and prey, and change the diet composition and diet overlap of the predatory fishes in rivers.

In temperate streams in northern Michigan where this study was conducted, the period of high prey dispersal in the drift is predominated by the emergence of larval suckers (Family: Catostomidae) and larval lake sturgeon (*Acipenser fulvescens*), a species of conservation concern, in mid-May to early June

(Auer and Baker 2002; Smith and King 2005). This period also coincides with the emergence of several aquatic insect taxa as well, including Heptageniidae, Isonychiidae and Perlidae (Scribner, unpublished data). This study examined how the abundance of prey in the drift affected the diets of predator community that potentially preys upon larval lake sturgeon. The goals of this research were to 1) characterize the diets of predatory fish during and after the larval lake sturgeon/catostomid drift period using metabarcoding molecular diet analysis, 2) measure diet overlap between predator species during and after the drift period, and 3) quantify predator diet preferences and changes in preference using metabarcoding diet data combined with surveys of the prey community.

MATERIALS AND METHODS

Study area and sample collection

Sampling was conducted in the Upper Black River (UBR; Cheboygan County, MI), the largest tributary of Black Lake, a 4100 ha inland lake in the northern lower peninsula of Michigan. Black Lake supports a population of ~1200 adult lake sturgeon (Pledger et al. 2013), which spawn solely in the UBR. Sampling of drifting larval stage lake sturgeon dispersing downstream from spawning areas has been conducted since 2003 (Smith and King, 2005). Abundance of co-distributed taxa in the drift has been collected since 2013. Larval lake sturgeon disperse from the UBR in late spring, often coinciding with the outmigration of larval suckers (white suckers, *Catostomus commersonii*; silver redhorse, *Moxostoma anisurum*; Family: Catostomidae), and the emergence of several species of aquatic insects (e.g. Heptageniidae, Isonychiidae, Perlidae), leading to a high abundance of available prey for the predatory fishes in the system. This high prey abundance contrasts with the relatively low abundance of available prey present in the drift by mid-summer in the UBR.

Sampling of drifting prey was conducted during 2015 at four sites downstream of the sturgeon spawning sites. Two sites consisted of predominately gravel substrate habitat (Figure 6; PD1 and PD3), and two sites further downstream were located in predominately sand substrate habitat (Figure 6; PD4 and PD5). The sampling dates were divided into two periods. The first period, "drift", occurred when larval lake sturgeon were observed in survey samples. "Drift" samples were collected for five days during the lake sturgeon drift period in 2015 (May 24th, June 4th-7th). The second period, "post-drift" occurred when larval lake sturgeon were no longer observed in the survey samples. The "post-drift" period began two days after no larval lake sturgeon were observed in the drift surveys and included drift sampling on two nights (July 3rd, July 5th). The abundance of drifting larval lake sturgeon and co-distributed prey taxa was quantified using D-frame drift nets (Auer and Baker 2002). Beginning at 21:00, five D-frame drift nets with 1600 μm mesh and detachable cod ends were set at one of the sampling sites each night. To estimate

the proportion of the river sampled by the drift nets, total river discharge (m^3sec^{-1}) and the discharge entering nets were measured using a Marsh McBurney Flow-Mate 2000 (Hach Company, Loveland, CO, USA). Contents of the cod ends were collected hourly between 22:00 and 02:00. Larval lake sturgeon were counted on site and returned to the river. 5% subsamples of the cod end contents were collected for each hour and preserved in 95% ethanol. Sucker larvae and invertebrates in the preserved samples were later counted and macroinvertebrate larvae were morphologically identified to the family level. Dry weight biomass estimates for individual fish and aquatic insect larvae were collected for most families observed during drift sampling (Table 9). These estimates (or, if a rare family had no estimate, the estimate from a closely related family) were used to estimate total catch biomass (Figure 7).

Electrofishing surveys were conducted the day following drift sampling to collect diet samples of predatory fishes (n=367 samples from 12 predator species). A barge electrofishing unit with a three-person crew sampled a 0.5km stream transect directly downstream of the site where drift sampling was conducted the previous night (Figure 6; Transects A, B, C, and D). Electrofishing voltage and amperage were set to 400 V at 4 A. Two crew members carried anodes and collected fish, and the third crew member moved the barge upstream and stored captured fish in a live well. Predator fish were sacrificed with an overdose of MS222 (0.4 mg/ml). Total length and species of all fish captured during the survey were recorded. Sacrificed fish were placed in Whirl-Paks (Nasco, Fort Atkinson, WI, USA) and stored in a -20 °C freezer within two hours. Predators were dissected, the entire GI tracts were removed, and contents were carefully extracted to minimize the amount of predator tissue in the sample. Diet samples were preserved in 95% ethanol and stored at -20 °C prior to DNA extraction.

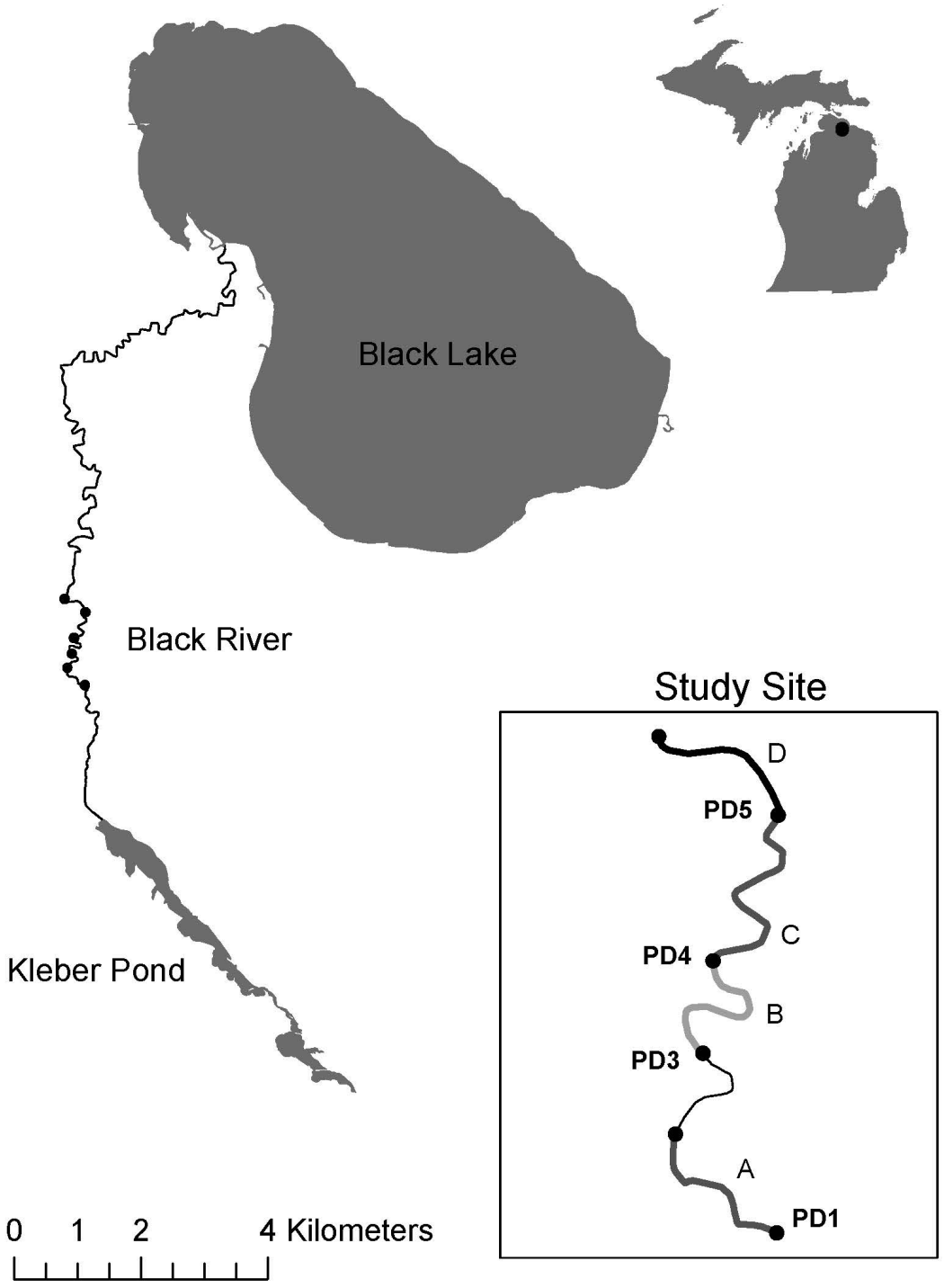


Figure 6. Map of the study area highlighting the D-frame drift net survey sites of the prey community (black points; PD1, PD3, PD4, and PD5) and the 0.5 km predator electrofishing transects (bold grey lines; A, B, C, and D) in the upper Black River, Cheboygan County, MI.

Table 9. Dry weight biomass estimates for individual prey for each family represented in the D-frame drift net surveys and the estimated catch biomass of each prey family for each night. Some prey families were grouped together under the same ecologically significant unit (ESU), indicated in parentheses after the family name.

Prey Family	Catostomidae	Acipenseridae	Heptageniidae	Baetidae	Ephemerellidae	Isonychiidae (ESU - Other Ephemeroptera)	Siphlonuridae (ESU - Other Epemeroptera)	Ephemeridae (ESU – Other Ephemeroptera)	Brachycentridae (ESU – Trichoptera)	Glossosomatidae (ESU – Trichoptera)	Helicopsychidae (ESU – Trichoptera)
Individual dry mass (mg)	1.193	8.5	1.768	0.366	1.255	4.145	6.829	5.578	1.458	6.948	0.914
5/23/15 estimated catch biomass (g)	8.42	1.65	1.03	0	0.15	2.98	0	0	0	0	0.02
6/4/15 estimated catch biomass (g)	1.89	4.71	0.74	0.02	0.18	2.98	0	0	0	0	0
6/5/15 estimated catch biomass (g)	3.53	2.21	0.57	0.02	0.08	0.50	0.14	0	0	0.14	0.53
6/6/15 estimated catch biomass (g)	36.70	0.66	0.46	0.05	0.18	0.08	0.14	0.11	0	0.14	0.02
6/7/15 estimated catch biomass (g)	7.90	0.12	0.57	0.01	0.13	0.33	0	0	0.03	0.14	0
7/3/15 estimated catch biomass (g)	0.67	0	0.18	0.14	0	0.33	0	0	0.26	0	0
7/5/15 estimated catch biomass (g)	0.84	0	0.53	0.07	0.03	0.58	0	0	0.12	0	0
Average catch biomass “drift” (g)	11.69	1.87	0.67	0.02	0.14	1.38	0.05	0.02	0.01	0.08	0.11
Average catch biomass “post-drift” (g)	0.75	0	0.35	0.10	0.01	0.46	0	0	0.19	0	0

Table 9 (cont'd)

Hydropsychidae (ESU – Trichoptera)	Lepidostomatidae (ESU – Trichoptera)	Leptoceridae (ESU – Trichoptera)	Limnephilidae (ESU – Trichoptera)	Perlidae (ESU – Plecoptera)	Perlodidae (ESU – Plecoptera)	Chironomidae	Simuliidae	Sialidae (ESU – Megaloptera)	Copepoda/Amphipoda	Elmidae (ESU – Coleoptera)	Psephenidae (ESU – Coleoptera)	Cordulegastridae (ESU – Odonata)	Gomphidae (ESU – Odonata)	Total Catch Biomass (g)
5.921	3.003	0.811	1.273	27.62	5.614	0.153	0.205	3.127	0.591	1.101	3.767	15.97	16.04	---
0.83	0.30	0.18	0	1.10	0.22	0.01	0	0	0.01	0.09	0.08	0	0.96	18.03
1.07	0.18	0.13	0	1.66	0	0.02	0	0	0	0.18	0	0	0.64	14.40
0.83	0.60	0.19	0.03	0.55	0.11	0.04	0	0	0	0.20	0.08	0.32	0.32	10.99
0.47	0.18	0.06	0	1.10	0	0.10	0	0.06	0.01	0.26	0.08	0	0.64	41.50
0.59	0	0.02	0	3.87	0.11	0	0	0	0.02	0.02	0	0	1.92	15.78
0.59	0	0.03	0	1.10	0	0.02	0.01	0	0	0.07	0	0	0.32	3.72
1.07	0	0.11	0	2.21	0	0.02	0.01	0	0.01	0.22	0	0	0	5.82
0.76	0.25	0.12	0.01	1.66	0.09	0.03	0	0.01	0.01	0.15	0.05	0.06	0.90	20.14
0.82	0	0.07	0	1.66	0	0.02	0.01	0	0.01	0.14	0	0	0.16	4.75

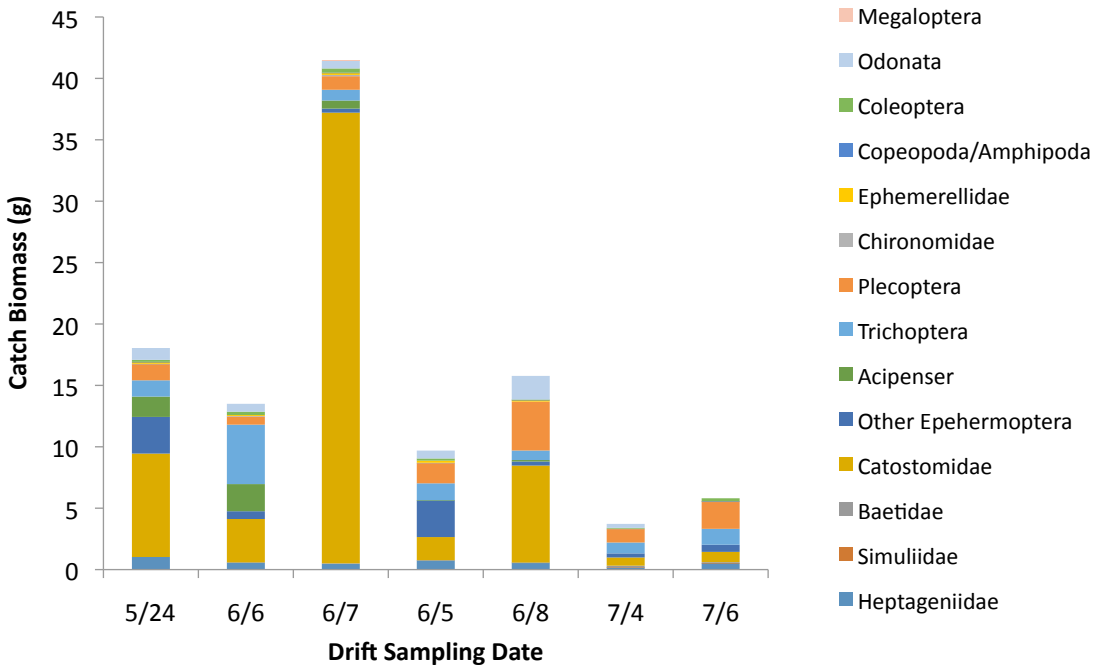


Figure 7. Estimated catch dry weight biomass for the aquatic macroinvertebrate and larval fish ecologically significant units (ESUs) observed during each night of the drift survey.

DNA extraction and sequencing

Diet samples were manually mixed and pieces of tissue were broken apart with forceps and sterile toothpicks, and thoroughly vortexed to homogenize the samples and ensure representative subsampling. 50-100mg of tissue from the GI tract diet samples was used in each DNA extraction and washed with sterile water to remove excess ethanol. DNA was extracted according to a modified version of the QIAamp Stool Mini Kit (QIAGEN, Hilden, Germany) protocol. Lysis in InhibitEx Buffer from the QIAmp Stool Mini Kit was extended to 30 min at 72ºC. Samples were also further homogenized with a 10-min bead-beating step using 0.70 mm garnet beads (MOBIO, Carlsbad, CA, USA) after lysis buffer and proteinase K were added to the sample. DNA was eluted, and concentration of DNA was quantified with an ND-1000 nanodrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). If the nanopdrop spectrophotometer revealed a high concentration of contaminants (260/280 < 1.7) in the sample, a salt precipitation using cold 100% ethanol and 0.15M sodium acetate was used to clean samples. All samples were diluted using sterile water to a standard concentration of 20ng/μl of DNA. An

empty microcentrifuge tube was used as a negative control for each extraction, and three negative controls were randomly selected for sequencing.

The coding region for 18S V9 rRNA (~200bp; Stoeck et al. 2010) was amplified with universal eukaryotic primers 1391F (5'-GTACACACCGCCCGTC-3'; Lane 1991) and EukB (5'-TGATCCTTCTGCAGGTTCACCTAC-3'; Medlin et al. 1988). These primers were chosen for their relatively short target sequence (~200bp), the large taxonomic breadth encompassed, and because preliminary screening indicated sequences from lake sturgeon and all of the major invertebrate families identified in UBR drift survey samples for the target region were available on GenBank (NCBI). Samples were sent to the Research Technology Support Facility (RTSF) at Michigan State University (East Lansing, MI, USA) for DNA sequencing. Creation of sequencing libraries, PCR amplification, and sequencing was performed according to standard Illumina (San Diego, CA, USA) protocols, with 100bp paired-end reads using an Illumina MiSeq sequencing platform (http://rtsf.natsci.msu.edu/genomics).

DNA sequence processing

Sequences were processed in *mothur* v 1.38 (Schloss et al. 2009). Similar paired-end reads (<2 bp difference) were merged to generate a list of unique sequences. Sequences were screened for quality by removing sequences that deviated from the target size, unique sequences that appeared only once, sequences with homopolymer regions ≥8 bp, and chimera checking. Operational taxonomic units (OTUs) were clustered and taxonomically assigned using a SILVA-based reference database provided with *mothur*, assigning OTU sequences to taxa with 80% identity. To standardize sequence sampling coverage between samples, all samples were rarefied to 1950 sequences. Rarefaction subsamples a consistent number of reads from each sample to standardize each sample to the same number of sequences while still accurately reflecting the relative abundance of each unique sequence present in the sample. Twelve samples with insufficient sequence numbers were discarded from further analysis.

The SILVA database only taxonomically identified OTU sequences to the subkingdom level. For greater taxonomic resolution of prey items, the metazoan OTUs appearing in the 1000 most common

unique OTU sequences were identified to order, class, or phylum (>90% sequence similarity or multiple lower taxonomical units with >95% sequence similarity) or family (>95% sequence similarity) using the *blastn* tool on GenBank (National Center for Bioinformatics). Prey taxa were divided into ecologically significant units (ESUs) based on the lowest taxonomic level that could be confidently identified. The number of OTU sequences from the same ESU was summed together within each sample. Most fish 18S sequences could not be identified to order from the sequences on GenBank. However, fish 18S sequences in the diet samples could be assigned to order or family based on some sequences occurring in high frequency in all samples from particular predator species or groups of species identified in the field. Assuming these sequences are from predator tissue, one fish order and four families could be identified (Order: Perciformes; Families: Catostomidae, Cyprinidae, Gadidae, Umbridae). The fish genus *Acipenser* and the fish order Petromyzontiformes could be identified by sequences available on GenBank. Sequences matching the identity of the predator the GI tract sample was taken from were removed from that sample, but retained in samples from predators of different taxa. GI tract diet samples with <20 sequences (1% of sequences) from likely diet items were removed from the dataset. These samples were likely taken from fish with empty or near empty stomachs, so most reads came from their own tissue, ingested environmental DNA, or resident parasites in their GI tract. All remaining samples were standardized so sequences of each diet item were represented as the proportion of all prey sequences in a sample.

Statistical analysis

Multivariate analyses were conducted in R Statistical Software v. 3.2.2 (R Core Team 2015) using the vegan library (Oksanen et al. 2016). A principal coordinates analysis (PCoA) was conducted on the proportions of diet items in each diet sample using Bray-Curtis distances. Correlations between the original matrix of diet item proportions and the eigenvectors of the first two principal coordinates were calculated to analyze which prey items were explaining most of the variation in the diet. The first two

principal coordinates were also plotted by predator species and by time period collected with 80% confidence intervals around each category.

To test the effects of predator species, sampling period, and substrate on the diet composition among members of the predator community, a PERMANOVA analysis was performed on the Bray-Curtis distance matrix of the diet proportions using the adonis function (Oksanen et al. 2016). Each PERMANOVA was run with 1000 permutations. Predator species, sampling period, and substrate were all treated as fixed effects and all interactions among the fixed effects were analyzed. If the three-way interaction was not significant, the interaction was removed and the model was fit again without it. The model was fit again if none of the two-way interactions between fixed effects was significant. If an interaction was significant, separate PERMANOVAs were performed on the data from each level of one the interacting factors, testing the effect of the other interacting factor.

Diet overlap between each species was calculated for both the drift and post-drift sampling periods. Schoener's index (α; Equation 1; Schoener 1970) was applied to the average diets of each pairwise comparison of predator species during each of the sampling periods.

$$\alpha = 1\text{-}0.5 * (\Sigma\, |p_{xi} - p_{yi}|) \qquad \text{Equation 1}$$

Where x and y represent different predator species and i represents the i^{th} prey taxa (in this case, the i^{th} ESU). A Schoener's α of 0 indicates no diet overlap, a Schoener's α of 1 indicates complete diet overlap, and a value of 0.6 is typically assumed to indicate substantial biologically relevant diet overlap (Schoener 1970). To compare diet overlap between the drift and post-drift periods, a permutation test was conducted. One of each pair of Schoener's index overlap value comparing the same two species was randomly assigned to either the drift or post-drift period and the mean difference between the two periods was calculated for each of 10000 iterations. The observed difference in mean Schoener's α was compared to the permutation results to estimate a p-value and determine significance.

Selectivity was analyzed using Chesson's selectivity index (ε; Equation 2; Chesson 1983).

$$\varepsilon = (m*\alpha_i - 1) * ((m-2)*\alpha_i + 1)^{-1} \quad \text{Equation 2}$$

Where m is the number of prey types in the environment and α_i is the Manley's selection index for the i^{th} prey type (Equation 3; Manley 1974).

$$\alpha = (r_i / n_i) * (\Sigma\, r_j / n_j)^{-1} \quad \text{Equation 3}$$

Where r is the proportion of the i^{th} prey taxa in the predator diet (by proportion of sequence reads in the diet sample), and n is the proportion of the i^{th} prey item in the environment (by biomass in the drift samples). Chesson's ε varies on a scale from -1 to 1, with negative values indicating negative selection, and positive values indicating positive selection for a given prey item. Community biomass data was only available for 14 of the 33 ESUs, so selectivity could only be calculated for these ESUs (Figure 7). Chesson's ε was calculated with the average diet of a predator species for each day a predator species was sampled during the electrofishing survey. A PERMANOVA was performed on Euclidean distance matrix of the daily Chesson's ε values, testing how predator species, sampling period, substrate, and interactions between factors affected selectivity values. If interactions were not significant, a new PERMANOVA was performed without the interactions to test for significance of factors. Principal componenets analysis (PCA) was performed on the Euclidean distance matrix and the first two principal coordinates were plotted.

RESULTS

DNA sequencing

Metabarcoding analysis of diet samples successfully amplified sequences of prey items from 286 of the predators collected during the electrofishing survey. Of the total fish collected, 355 GI tract samples (96.7%) successfully amplified the target 18S region. Of the samples that successfully amplified, 69 (19.4%) contained <1% of prey DNA and were removed from further analysis. Diet samples from 12 predator species (Table 10) were successfully amplified and contained prey DNA (n=286).

Samples rarified to 1950 sequences contained 10597 unique OTUs. Of the 1000 most abundant OTUs, 209 were identified as potential prey items based on alignment to reference sequences on GenBank. Suspected predator DNA accounted for 6.23% of the total sequence reads and were removed from further analyses. Of the rarified samples, sequences from the 33 ESUs accounted for 19.04% of all sequences from the GI tract samples. Prey sequences from these samples were grouped into 33 ESUs if they were identified as being from the same phylum, class, order, or family (Figure 8). The most abundant sequences from prey ESUs were Heptageniidae (16.42%), Simuliidae (8.89%), and other Ephemeroptera (8.11%). The 16 ESUs that were also represented in the drift biomass data and were used to examine selectivity account for 67.67% of the sequence reads from the 33 prey ESUs (12.88% of total sequences).

Table 10. Predator species caught during electrofishing surveys and sample sizes for diet samples collected during each sampling period ("drift" during larval lake sturgeon dispersal from spawning sites from late May to June, and "post-drift" after dispersal in early July). Sample sizes only include samples from fish that could be rarefied to 1950 sequences contained >1% of sequences from prey taxa.

Predator Species	**Three Letter Species Code**	**Sample Size Drift, Post-drift**	**Total**
Blackside darter (*Percina maculata*)	BSD	10, 6	16
Burbot (*Lota lota)*	BUR	6, 7	13
Central mudminnow (*Umbra limi*)	CMM	7, 6	13
Common shiner (*Luxilus cornutus*)	CMS	5, 3	8
Creek chub (*Semotilus atromaculatus*)	CRC	8, 9	17
Hornyhead chub (*Nocomis biguttatus*)	HHC	28, 13	41
Logperch (*Percina caprodes*)	LOP	11, 7	18
Pumpkinseed (*Lepomis gibbosus*)	PUS	12, 5	17
Rainbow darter (*Etheostoma caeruleum*)	RAD	22, 19	43
Rock bass (*Amblopites rupestris*)	ROB	24, 20	44
Smallmouth bass (*Micropterus dolomieu*)	SMB	19, 5	24
White sucker (*Catostomus commersonii*)	WHS	5, 5	10
Yellow perch (*Perca flavescens*)	YEP	18, 6	24
Total		175, 111	286

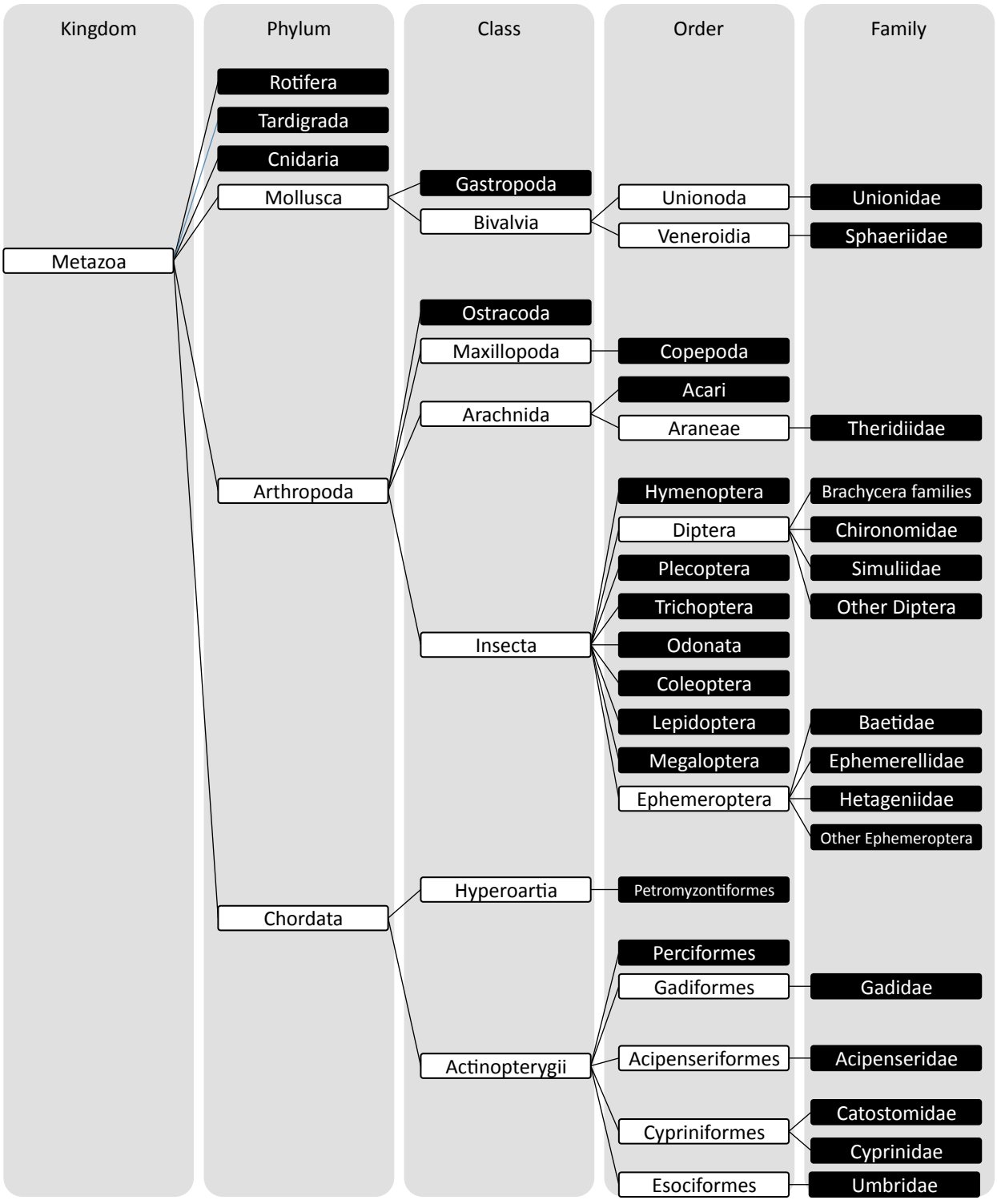


Figure 8. Taxonomic relationships of the prey & predator taxa identified by DNA sequencing of the 18S V9 rRNA gene in predator GI tracts. The 33 ESUs (in black) are the lowest taxonomical unit to which OTUs could be identified with >90% identity (phylum, class, order) or >95% identity (family).

Diet characterization

Predator diets contained between 2 to 12 diet items from different ESUs (mean = 4.5). The average proportion of reads from each ESU was calculated for each species of predator (Table 11). PCoA of the diet proportions revealed that diets segregated mainly by prevalence of a handful of prey items (Figure 9). High prevalence of heptageniid mayflies or rotifers tended to separate diets of percids and cyprinids respectively (PC1; Figure 9a), while diets of all fish collected during the high drift period typically contained more heptageniids compared to diets after the high drift period, which contained more simuliid sequences (PC2; Figure 9b).

PERMANOVA results indicated that there was no significant three-way interaction between substrate, sampling period, and predator species in influencing predator diets (pseudo-F=0.712, p=0.996). With the three-way interaction removed, there were significant interactions between the predator species and sampling period (pseudo-F=1.63, p=0.001) and between substrate and sampling period (pseudo-F=2.04, p=0.012; Table 12). PERMANOVA results for separate predator species testing the effect of sampling period on diet composition revealed that four predator species had significantly different diets in the two sampling periods after Holm-Bonferroni correction; blackside darter (*Percina maculata*; pseudo-F=5.70, p=0.003), logperch (*Percina caprodes*; pseuso-F=4.14, p=0.001), rainbow darter (*Etheostoma caeruleum*; pseudo-F=3.84, p=0.003), and rock bass (*Ambloplites rupestris*; pseudo-F=2.65, p=0.002; Table 13).

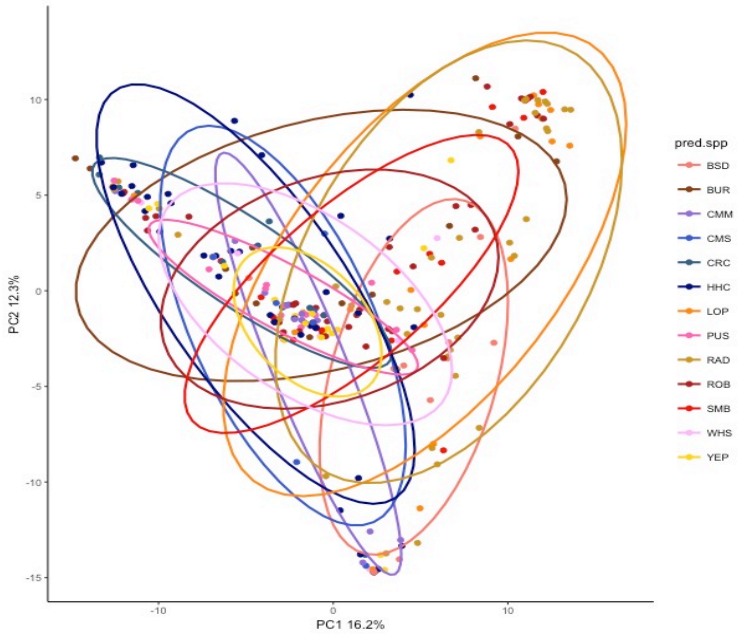
Table 11. Proportion of the DNA reads from all ecologically significant units (ESUs) observed in the diets of each predator species after suspected predator sequences had been removed.

Prey taxa	*P. maculata*	*P. caprodes*	*E. caeruleum*	*P. flavescens*	*L. gibbosus*	*M. dolomieu*	*A. rupestris*	*N. biguttatus*	*S. atromaculatus*	*L. cornutus*	*U. limi*	*L. lota*	*C. commersonii*
Perciformes	---	---	---	---	---	---	---	0.13	0.34	0.22	0.09	0.17	0.27
Cyprinidae	0.08	0.16	0.03	0.14	0.14	0.22	0.09	---	---	---	0.02	0.06	---
Umbridae	---	---	0.03	0.02	0.04	0.04	0.10	0.08	0.09	---	---	0.11	---
Catostomidae	---	0.01	---	0.09	0.02	0.14	0.09	0.02	---	---	---	0.06	----
Gadiformes	---	---	---	0.04	---	---	0.03	0.02	0.08	---	---	---	0.06
Acipenseridae	---	0.05	0.01	0.08	---	0.01	0.02	0.06	---	---	0.03	0.02	---
Petromyzontidae	---	---	---	0.12	---	---	0.04	---	---	---	---	---	---
Heptageniidae	0.06	0.20	0.24	0.02	---	0.19	0.11	0.03	0.01	---	---	0.15	0.03
Baetidae	0.44	0.03	0.13	0.01	---	0.02	0.05	---	0.02	0.01	---	0.04	---
Ephemerellidae	0.03	0.07	0.03	---	---	0.01	---	0.01	---	0.04	---	---	---
Other Mayfly	0.05	0.12	0.09	0.04	0.04	0.08	0.07	0.01	---	---	---	0.03	0.10
Simuliidae	0.21	0.12	0.10	0.08	0.01	0.02	---	0.17	0.05	0.20	0.27	0.01	---
Chironomidae	0.05	0.09	0.12	---	0.10	0.03	0.06	0.03	0.07	---	0.02	0.02	0.10
Brachycera	---	0.03	0.01	0.01	---	0.04	---	---	0.08	---	---	---	---
Other Diptera	---	---	---	---	---	---	---	---	---	---	---	---	---
Odonata	---	---	---	---	0.03	---	0.02	---	---	---	---	---	---
Trichoptera	0.02	0.05	0.07	0.04	0.06	0.04	0.02	0.06	---	---	0.01	0.08	0.01
Plecoptera	---	---	---	0.01	---	---	0.03	---	---	0.04	---	0.08	---
Megaloptera	---	---	---	0.02	---	---	---	0.01	---	---	---	---	---
Coleoptera	---	---	---	---	---	---	---	0.02	---	---	0.05	---	---
Lepidoptera	---	---	---	---	---	---	---	---	---	0.06	---	---	---

Table 11 (cont'd)

Prey taxa	*P. maculata*	*P. caprodes*	*E. caeruleum*	*P. flavescens*	*L. gibbosus*	*M. dolomieu*	*A. rupestris*	*N. biguttatus*	*S. atromaculatus*	*L. cornutus*	*U. limi*	*L. lota*	*C. commersonii*
Acari (Mites)	---	---	0.03	0.02	0.03	---	0.01	0.04	0.12	0.18	0.02	0.02	---
Theridiidae (Water spiders)	---	---	---	---	---	---	---	0.02	---	---	---	---	---
Hymenotpera	---	---	---	0.01	---	---	---	0.01	0.02	---	---	---	---
Gastropoda	---	---	---	---	0.18	---	0.01	---	---	---	0.08	---	---
Unionidae	0.05	---	---	0.02	---	---	---	---	---	---	---	---	---
Sphaeriidae	---	---	---	---	0.11	---	---	---	---	---	0.07	---	0.10
Annelidae	---	---	0.02	0.04	0.03	0.04	0.04	0.01	---	---	0.08	---	---
Ostracoda	0.01	---	---	0.06	0.04	0.04	0.04	0.01	---	---	0.02	---	0.25
Amphipoda/ Copepoda	---	0.02	---	0.08	0.02	---	0.03	---	---	0.13	0.21	0.06	0.05
Tardigrada	---	---	---	0.04	0.01	---	0.03	0.04	---	---	---	---	---
Hydra	---	---	---	---	---	0.01	---	---	---	---	---	---	---
Rotifera	0.01	0.05	0.08	0.05	0.15	0.05	0.09	0.19	0.11	0.13	0.04	0.10	0.02

a)



b)

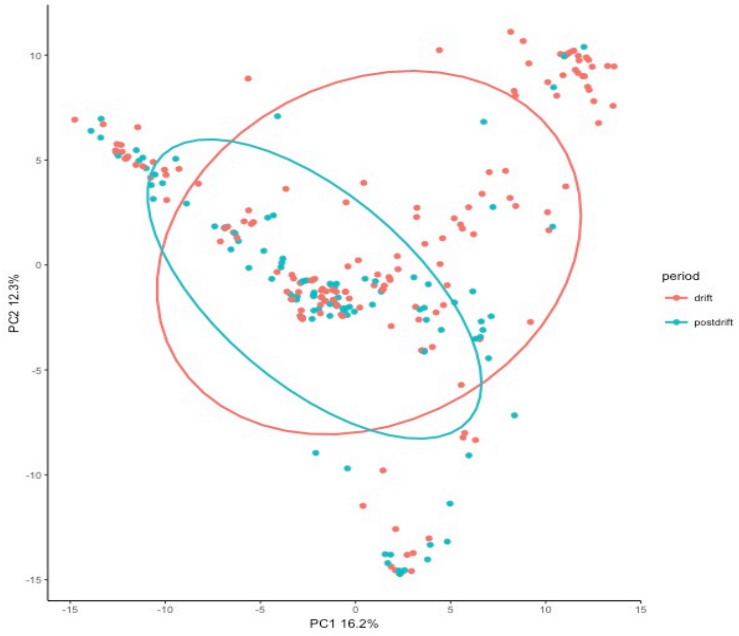


Figure 9. Principal coordinates analysis (PCoA) of diet taxonomic composition from all diet samples. PC1 is mainly associated with the prevalence of Heptageniidae (right) and Rotifera (left) sequences in diet samples. PC2 is mainly associated with the prevalence of Heptageniidae (top) and Simuliidae (bottom) sequences in diet samples. Each diet sample is represented by a point and color identifying predator species (a) or sampling period (b). See Table 10 for three-letter predator fish species codes.

Table 12. Results of PERMANOVA analysis testing effects of predator species (n=13), substrate (sand or gravel), and time period (during or after drift) on the prey composition of diet samples. The test shows that the responses of all predator species and predators in areas of the river with different substrates were not the same across different time periods.

Factor	DF	F	R^2	p-value
Predator Species	12	3.172	0.117	0.001*
Substrate	1	1.966	0.006	0.020*
Time Period	1	4.489	0.014	0.001*
Predator*Substrate	10	1.063	0.033	0.275
Substrate*Time	1	2.037	0.006	0.012*
Predator*Time	12	1.634	0.060	0.001*
Residual	248		0.764	

Table 13. PERMANOVA analysis results testing the effect of time period during or after the larval lake sturgeon drift on the diet composition of each predator species individually.

Predator Species	p-value
Blackside darter (*Percina maculata*)	0.003*
Burbot (*Lota lota)*	0.012
Central mudminnow (*Umbra limi*)	0.439
Common shiner (*Luxilus cornutus*)	0.909
Creek chub (*Semotilus atromaculatus*)	0.024
Hornyhead chub (*Nocomis biguttatus*)	0.632
Logperch (*Percina caprodes*)	0.001*
Pumpkinseed (*Lepomis gibbosus*)	0.206
Rainbow darter (*Etheostoma caeruleum*)	0.003*
Rock bass (*Amblopites rupestris*)	0.002*
Smallmouth bass (*Micropterus dolomieu*)	0.878
White sucker (*Catostomus commersonii*)	0.314
Yellow perch (*Perca flavescens*)	0.009

*- indicates diet compositions of predator species that were significantly different between the two time periods after Bonferroni correction for multiple comparisons ($\alpha = 0.004$)

Diet overlap

Schoener's index of diet overlap was calculated for each pair of predator species during each time period (Table 14). Five species pairs had significant diet overlap during the drift period; burbot and rock bass ($\alpha = 0.63$), rainbow darter and logperch ($\alpha = 0.69$), rainbow darter and rock bass ($\alpha = 0.63$), rainbow darter and smallmouth bass ($\alpha = 0.60$), and rock bass and smallmouth bass ($\alpha = 0.63$). There were no species pairs with substantial diet overlap during the post-drift period. A permutation test of the Schoener Index values for each species pair shows that overall, diet overlap slightly but significantly decreased from the drift period in late May/ early June to the post-drift period in early July (mean $\Delta\alpha = 0.05$, $p = 0.003$).

Table 14. Schoener's Index of diet overlap between each pair of predator fish species represented in the data set. Top values represent diet overlap during larval lake sturgeon drift, and the bottom values in parentheses indicate diet overlap following drift. Schoener's index values >0.6 are considered to be biologically relevant and indicate the possibility of competition. See Table 1 for three-letter predator fish codes.

	BSD	BUR	CMM	CMS	CRC	HHC	LOP	PUS	RAD	ROB	SMB	WHS
BUR	0.22 (0.13)											
CMM	0.08 (0.22)	0.26 (0.28)										
CMS	0.12 (0.26)	0.32 (0.27)	0.38 (0.38)									
CRC	0.10 (0.15)	0.22 (0.37)	0.09 (0.31)	0.36 (0.39)								
HHC	0.14 (0.36)	0.38 (0.36)	0.30 (0.41)	0.34 (0.47)	0.39 (0.40)							
LOP	0.25 (0.36)	0.52 (0.23)	0.18 (0.32)	0.13 (0.30)	0.17 (0.22)	0.28 (0.38)						
PUS	0.16 (0.11)	0.25 (0.08)	0.25 (0.23)	0.12 (0.06)	0.14 (0.10)	0.44 (0.10)	0.14 (0.24)					
RAD	0.34 (0.39)	0.19 (0.32)	0.19 (0.25)	0.14 (0.32)	0.18 (0.20)	0.32 (0.33)	0.69* (0.43)	0.26 (0.29)				
ROB	0.26 (0.14)	0.63* (0.53)	0.19 (0.23)	0.14 (0.18)	0.18 (0.19)	0.37 (0.32)	0.47 (0.32)	0.39 (0.18)	0.63* (0.29)			
SMB	0.32 (0.15)	0.45 (0.35)	0.18 (0.04)	0.11 (0.00)	0.19 (0.02)	0.29 (0.16)	0.51 (0.37)	0.44 (0.07)	0.60* (0.16)	0.63* (0.52)		
WHS	0.11 (0.08)	0.30 (0.34)	0.42 (0.35)	0.46 (0.17)	0.23 (0.43)	0.39 (0.25)	0.18 (0.22)	0.13 (0.44)	0.19 (0.22)	0.15 (0.38)	0.18 (0.23)	
YEP	0.23 (0.17)	0.32 (0.15)	0.37 (0.08)	0.29 (0.09)	0.11 (0.11)	0.40 (0.22)	0.25 (0.12)	0.50 (0.12)	0.31 (0.18)	0.40 (0.23)	0.57 (0.11)	0.33 (0.07)

Prey availability and diet selectivity

The proportions of total drift biomass and the proportions of total reads in the predator GI tract samples for 14 prey ESUs was estimated for each night (Table 15). On average, prey biomass was higher during the drift period than during the post-drift period (Figure 7; Table 9). Catostomid larvae and mayflies in the "Other Ephemeroptera" ESU (primarily the family Isonychiidae) were the most abundant prey by biomass during the drift period (mean nightly catch dry weight biomasses of 11.69g and 1.45g respectively). During the post-drift period, Trichoptera and Plecoptera became the most abundant prey taxa (mean nightly catch dry weight biomasses of 1.09g and 1.66g respectively). Mean biomass of Trichoptera and Plecoptera did not change much from drift to post-drift periods, but biomass of other prey taxa declined.

Chesson's selectivity index value (ε) was calculated for each prey ESU by combining all samples from the same predator species for each day. PERMANOVA of the ε values indicate that there were no significant two-way or three-way interactions between substrate, sampling period, and predator species in affecting selectivity of prey items. Only selectivity between the drift and post-drift periods showed were nearly significantly different (pseudo-F = 4.75, p=0.058; Table 16). PCA of the Chesson's ε distance matrix reveals differences in diet preferences between certain predator fish species (Figure 10a). Overall, predators were slightly less selective during the post-drift compared to the drift period (fewer extreme values for PC1 during post-drift period; Figure 10b).

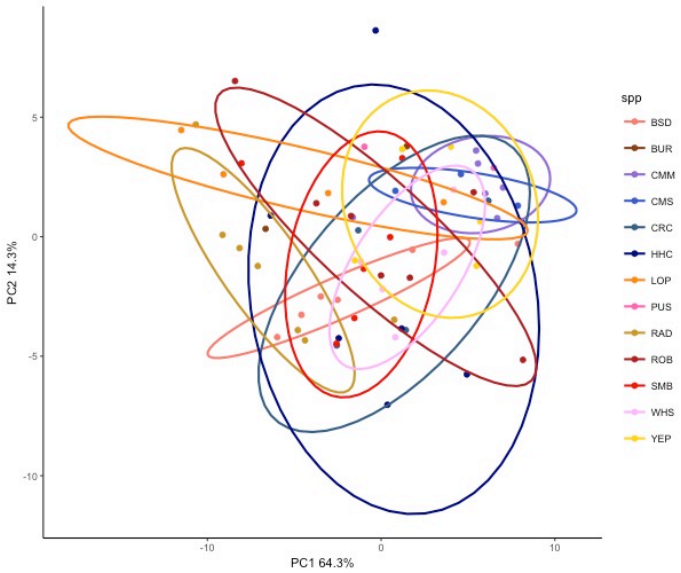
Table 15. Percentages of total estimated drift biomass (top) and percentages of reads in predator diet samples (bottom) for the 14 prey ecologically significant units (ESUs) morphologically identified from drift survey samples for each night of the drift survey.

Drift Sampling Date	**Catostomidae**	**Acipenseridae**	**Heptageniidae**	**Baetidae**	**Ephemerellidae**	**Other Ephemeroptera**	**Trichoptera**	**Plecoptera**	**Chironomidae**	**Simuliidae**	**Megaloptera**	**Copepoda/ Amphipoda**	**Coleoptera**	**Odonata**
05/23	46.68	9.17	5.68	---	0.83	16.54	7.34	7.37	0.07	---	---	0.13	1.81	---
	(0.36)	(2.26)	(20.95)	(10.56)	(3.04)	(17.95)	(3.81)	(1.38)	(4.62)	(6.79)	(0.13)	(0.31)	(---)	(1.42)
06/04	19.45	0.18	7.66	0.23	1.81	30.79	14.19	17.10	0.16	---	---	---	1.22	4.43
	(0.19)	(3.62)	(10.84)	(4.32)	(2.28)	(6.92)	(3.49)	(1.24)	(2.13)	(16.70)	(---)	(5.24)	(0.05)	(---)
06/05	26.16	16.37	4.19	0.16	0.56	4.70	35.85	4.92	0.32	---	---	0.07	0.34	1.85
	(0.74)	(4.25)	(18.68)	(7.70)	(1.78)	(1.52)	(2.40)	(2.06)	(5.17)	(4.17)	(0.03)	(3.81)	(---)	(---)
06/06	88.40	1.58	1.11	0.12	0.42	0.80	2.11	2.66	0.25	---	0.15	---	0.60	8.79
	(10.76)	(---)	(14.74)	(6.72)	(0.08)	(1.96)	(4.93)	(---)	(4.92)	(3.35)	(0.02)	(4.29)	(---)	(0.49)
06/07	50.07	0.76	3.59	0.05	0.80	2.10	4.92	25.22	---	---	---	---	1.72	3.93
	(8.76)	(2.05)	(2.83)	(6.71)	(0.47)	(3.10)	(4.28)	(0.31)	(2.17)	(2.96)	(---)	(4.61)	(0.05)	(1.97)
07/03	17.95	---	4.75	3.74	---	8.91	23.83	29.68	0.65	0.11	---	---	1.62	7.84
	(1.62)	(5.38)	(4.40)	(1.42)	(2.27)	(3.31)	(2.32)	(2.44)	(9.73)	(9.75)	(---)	(4.18)	(5.07)	(---)
07/05	14.38	---	9.13	1.13	0.43	9.99	22.31	38.04	0.37	0.21	---	0.20	3.71	---
	(2.65)	(3.16)	(2.81)	(3.46)	(---)	(1.12)	(4.67)	(---)	(9.91)	(8.93)	(0.21)	(2.56)	(---)	(---)

Table 16. Results of PERMANOVA analysis testing effects of predator species (n=13), substrate (sand or gravel), and time period (during or after drift) on the predator preferences for prey observed in the drift.

Factor	DF	F	R^2	p-value
Predator Species	12	1.588	0.318	0.225
Time Period	1	4.749	0.079	0.061
Substrate	1	-3.949	-0.066	0.994
Residual	40		0.764	

a)



b)

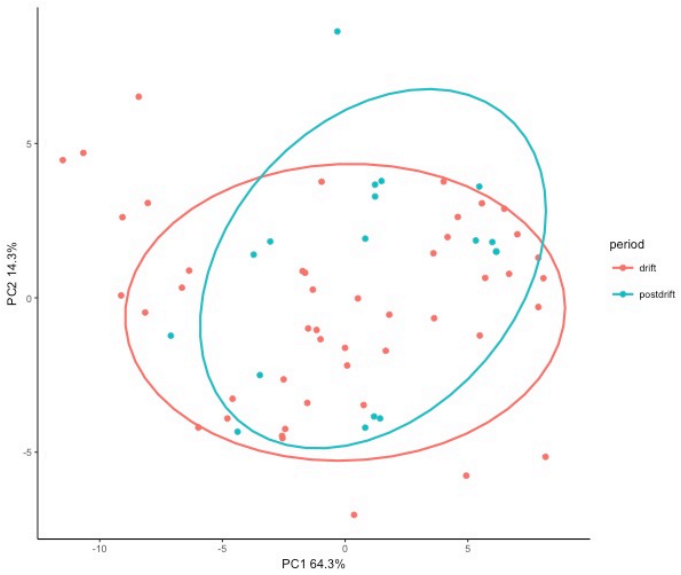


Figure 10. Principal components analysis (PCA) of predator preferences from daily averages of each predator species. PC1 is mainly associated with the prevalence of Simuliidae (right) and Lepidoptera/Plecoptera/Megaloptera/Acipenser (left) sequences in diet samples. PC2 is mainly associated with the preference for Baetidae (top) and Simuliidae (bottom) sequences in diet samples. Each diet sample is represented by a point and color identifying predator species (a) or sampling period (b). See Table 10 for three-letter predator fish species codes.

DISCUSSION

Metabarcoding of the 18S V9 region of rRNA combined with surveys of the prey community was able to quantify how predator diets changed as the availability and taxonomic composition of prey changed. Sequencing analysis revealed that many of the riverine fish predators analyzed in this study had diverse diets, with 9 or more prey taxa contributing at least 1% of the prey sequences within the diet samples of each predator species. This high diversity may be because of the ability of metabarcoding to detect quickly digested soft-bodied prey that are often difficult to identify in morphological analyses of diet samples (Alonso et al. 2014; Albaina et al. 2016; Moran et al. 2016; Sakaguchi et al. 2017). In fact, no prey taxa constituted an outright majority of diet composition from any predator species, in contrast with other studies on these species in other systems using different methodologies to quantify diets (Philips and Kilambi 1996; Gray et al. 1997; Roell and DiStefano 2010; Johnson 2015). Metabarcoding analysis observed consistently higher taxonomic richness in predator diets compared to morphological diet analyses of the same species. Diet breadth of rock bass (dietary richness – 10; Roell and DiStefano 2010), logperch (dietary richness – 10; Burkett and Jude 2015), and white sucker (dietary richness – 6; Ahlgren 1990) was wider in the metabarcoding analysis in this study (dietary richness – 20, 13, and 10, respectively). As displayed in this study, molecular tools offer more powerful methods for diet analyses to detect a greater diversity of prey, more accurately characterize predator diets, and better quantify the trophic links among species in a community.

Diet characterization

Diets of riverine fish predators were more diverse using metabarcoding analysis than morphological diet analysis of these species has recorded, but the identities of the most prevalent prey items were largely consistent. The three darter species (*Percina maculata, Percina caprodes*, *Etheostoma caeruleum*) preyed primarily on mayfly larvae and midge larvae (i.e. Chironomidae and Simuliidae), similar to what was found in previous studies (Philips and Kimbali 1996; Alford and Beckett 2007).

Pumpkinseed sunfish (*Lepomis gibbosus*) appeared to specialize on mollusks, snails (Gastropoda) and pea clams (Sphaeridae), along with smaller contributions to the diet from other benthic invertebrates and cyprinids, all of which have been observed in other systems (Mittelbach 1984; Garcia-Berthou and Marino-Amich 2000; Locke et al. 2013). Most of the yellow perch (*Perca flavescens*) collected during the electrofishing survey were age-1 fish, and diet analysis of these individuals supports previous findings that larval fishes are an important prey for juvenile yellow perch (Parke et al. 2009). Diets of cyprinids were quite diverse, but largely characterized by small dipteran larvae, consistent with past studies (Quist et al. 2006; Johnson 2015), however, metabarcoding analysis also revealed high proportions of rotifers and a high degree of piscivory, likely due to predation on larval and juvenile fishes abundant in the UBR during the study period. Central mudminnow (*Umbra limi*) exhibited a diet similar to other analyses that focused primarily on midge larvae and crustacean zooplankton (Copepoda, Amphipoda, Ostracoda; Chilton et al. 1984; Martin-Bergmann and Gee, 1985). White sucker (*Catostomus commersonii*) were shown to prey largely on ostracod crustaceans, a common prey item observed in other studies (Ahlgren 1990), but the molecular diet analysis also suggests that the white sucker in the UBR may also be engaging in piscivory, or consuming the eggs and larvae of spawning bass, perch, and darters, which has also been observed in other studies (Baldridge and Lodge 2013).

A range of size classes of burbot (*Lota lota*), rock bass (*Ambloplites rupestris*), and smallmouth bass (*Micropterus dolomieu*) were present in the UBR. The effect of size class on diet composition was not analyzed in this study. This does not take into account ontogenetic diet shifts known to occur in these species (Amundsen et al. 2003; Paterson et al. 2006; Dauwalter and Fisher 2008). As a result, the overall diet diversity of these species may be wider than expected for an individual fish, and the diets of some size classes may overlap more or less strongly with other species. It is likely that the smaller size classes of these species prey heavily on aquatic macroinvertebrates while larger fish account for most of the piscivory seen in the data from this study (Amundsen et al. 2003; Paterson et al. 2006; Dauwalter and Fisher 2008).

Diets of the predators largely segregated into the darter and bass predators that mainly consumed mayfly larvae and midge larvae and the piscivorous and rotifer-consuming cyprinid predators, respectively (Figure 9a). Darters and bass were also the most likely to exhibit a shift in diet from the drift period to the post-drift period (Table 13). In blackside and rainbow darters, this was a shift in diet composition from baetid and heptageniid mayfly prey during the drift period to simuliid midge prey during the post-drift period, despite only slight declines in the abundance of those families of mayfly (Figure 7; Table 8). In logperch and rock bass, diets also shifted away from mayfly prey during the drift period to a more piscivorous diet in early July when abundance of Ephemeroptera declined.

Diet overlap

All prey taxa were consumed by multiple predators, however, there was relatively little substantial diet overlap among predators. Rock bass diets significantly overlapped with burbot, rainbow darter, and smallmouth bass. Diets of logperch and rainbow darter also substantially overlapped during the drift period (Schoener $\alpha > 0.6$, Table 14), mainly due to all five species relying heavily upon heptageniid and "other" mayfly larvae as a primary prey source. Overall, diet overlap among all species was typically higher during the drift period when prey was more abundant. High diet overlap during the drift period does not necessarily indicate that predators are intensely competing with each other (Cardona 2001; Raborn et al. 2004; Jacobs et al. 2010). Niche theory actually predicts that the high abundance of prey reduces interspecific competition pressure, allowing predators to utilize the same resources (Pianka 1974; Schoener 1974; Pianka 1976). This pattern has also been observed in other fish communities (Michaletz 1996; Gray et al. 1997; Dantas et al. 2013; Correa and Winemiller 2014; Sánchez-Hernández et al. 2017). No predator species exhibited a high degree of diet overlap during the post-drift period, possibly indicating niche partitioning among the predator species when prey became relatively scarce (Gray et al. 1997; Raborn et al. 2004).

Biases and limitations

The analyses in this study used the assumption that the number of sequence reads in a diet sample was directly proportional to the biomass of a prey OTU. There is good evidence that the number of sequencing reads is generally a good approximation of the biomass of organisms in a sample (Elbrecht and Leese 2015; Evens et al. 2016; Hänfling et al. 2016; Clarke et al. 2017), including with the same set of universal primers used in this study (Albaina et al. 2016). However, the relationship between biomass and number of sequence reads can be highly variable among taxa due to amplification bias of the primers (Elbrecht and Leese 2015; Albaina et al. 2016). Amplification biases are heavily dependent on the primers and prey taxa in a study, and can be factored into analyses if control mixtures with known amounts of prey tissue are sequenced and regressed against taxon biomass to determine the relationship between sequence read count and biomass for each prey taxa of interest (Elbrecht and Leese 2015; Thomas et al. 2016). It is also important to note that rusty crayfish (*Orconectes rusticus*) were prevalent throughout the UBR, but no crayfish appeared in the diet sequences of fish predators, possibly because the crayfish sequence failed to amplify using the 18S V9 primers applied to diet samples in this study. Additionally, taxonomic resolution of the prey items could be further improved through the use of different sets of barcoding primers targeting different regions (Albaina et al. 2016; Hänfling et al. 2016).

Although metabarcoding can detect a wide array of prey items there are some drawbacks to that sensitivity. Incidental consumption of environmental DNA in the water and secondary predation (detection of prey of prey) can give false instances of predation on some prey taxa (King et al. 2008; Pompanon 2012). It can be difficult to determine whether fish are actually targeting some prey items (e.g. Rotifera) or whether the sequences from those taxa are showing up in fish diets because aquatic insects or other prey items were consuming certain prey taxa as well. Furthermore, because metabarcoding relies on unique sequences to detect prey items, prey with the same DNA sequence as the predator cannot be distinguished from predator sequences (King et al. 2008). This makes cannibalism and predation of related species underrepresented in predator diets, which could particularly affect the rock bass and smallmouth bass diets in the study. Bass were observed to prey upon darters, and likely prey on other

centrarchids (Dauwalter and Fisher 2008), but all of those fishes have indistinguishable sequences at the 18S V9 region used in this study. Likewise, cannibalism has been shown to be an important component of burbot diets (Jacobs et al. 2010), but would be extremely difficult to detect using standard metabarcoding techniques.

The relative abundance of the prey community estimated from the drift survey could have been biased and may not have represented the true availability of prey in the UBR. D-frame drift nets were deployed to maximize the catch of larval lake sturgeon (Auer and Baker 2002; Smith and King 2005), so drift surveys might have overestimated the abundance of taxa with similar benthic drifting behaviors. Prey that drifted near the surface (e.g. catostomid larvae; Corbett and Powles 1986) are likely underrepresented in prey community relative abundance data, and prey taxa that do not drift (e.g. Gastropoda), were too small to be sampled by the 1600μm mesh of the D-frame drift nets (e.g. Rotifera), or could escape from the drift nets (e.g. Cyprinidae) were not represented in the prey community relative abundance estimates at all. Only 14 of the 33 ESUs identified in this study were represented in the drift survey. As a result, the selectivity values based on the prey community composition should only be interpreted in the context of the 14 ESUs identified in the drift surveys.

Conclusions

The 18S V9 rRNA metabarcoding approach implemented in this study shows promise as a powerful tool to investigate the diets of freshwater predatory fishes. Diet items could be identified to similar taxonomic levels as morphological diet analyses, with the potential for metabarcoding to have even higher taxonomic resolution as more sequences become available. Metabarcoding also revealed that predator diets were more diverse than previously thought, detecting predation on taxa such as larval fishes and rotifers that are unlikely to be accounted for using morphological diet analysis (Carreon-Martinez et al. 2011; Hunter et al. 2011; Ley et al. 2014).

This study also demonstrated the importance of how fluctuating seasonal abundance of drifting aquatic insect and larval fishes can impact predator diets (Michaletz 1997; Raborn et al. 2004; Correa and

Winemiller 2014; Sánchez-Hernández et al. 2017). High resource abundance appeared to break down niche partitioning due to interspecific competition (Pianka 1974). Seasonal drift may serve as an important influx of energy and nutrients into riverine systems and as a competitive release for certain species, allowing them to utilize preferred prey resources without having intense resource competition from other predator species. The combination of more representative diet analysis using metabarcoding and the sampling of diets at very different periods of prey availability allow for a more complete understanding of the trophic links within complex riverine ecosystems

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CHAPTER 4: EFFECTS OF CHANGES IN ATERNATIVE PREY DENSITIES ON PREDATION OF DRIFTING LARVAL LAKE STURGEON (*ACIPENSER FULVESCENS*)

ABSTRACT

Predator-prey interactions are critical in determining the co-existence of species in dynamic systems. Interactions including predator switching, predator swamping, and size selectivity can all play important roles in maintaining multi-species systems. In fishes, early life stages are often cited as a recruitment bottleneck due to high mortality rates caused, in part, by predation. High mortality rates during the early life stages are particularly important for species of conservation concern, such as lake sturgeon (*Acipenser fulvescens)*. In this study, two predatory species, rock bass (*Ambloplites rupestris)* and hornyhead chub (*Nocomis biguttatus)* and three larval prey species groups, lake sturgeon, mayflies (Family: Heptageniidae), and suckers (Family: Catostomidae) were subjected to experimental raceway trials. Two pulses of prey were introduced over two treatments types, mirroring how nocturnally migrating prey densities change over short time periods. In the initial low density treatment, predators were offered prey at a 13:13:4 ratio of mayfly, suckers, and lake sturgeon respectively during the first pulse, and a 1:1:1 prey ratio during the second pulse 30 min into the trial. In the equal density treatment, all prey numbers were equivalent during both pulses. Trials were concluded after one hour and predation rates, predation preference, and size selection were calculated from the remaining prey items. For both predators, lake sturgeon was the least preferred prey species while mayfly were positively selected for. Between the two treatments, hornyhead chub had higher preference for sturgeon in the equal density treatment than in the low density, indicating initial availability had an effect on predator foraging behavior. Rock bass preference did not change between the treatments. The proportion of surviving lake sturgeon was similar between both treatments for each predator, indicating little evidence for predator swamping at the prey densities tested. Hornyhead chub did not exhibit size selectivity while rock bass selected smaller catostomid larvae. This study revealed strong preferences for mayfly prey and some effects of short time-scale changes in prey density on consumption by predators. Mayflies and other

aquatic macroinvertebrates in particular are likely an important food source in the wild, protecting threatened lake sturgeon from predation by these two predators.

INTRODUCTION

Predator-prey interactions play a crucial role in structuring communities, through both direct and indirect effects. Direct effects of predation include higher mortality rates and altered densities of prey populations. Indirect effects can manifest as changes in prey behavior while in the presence of predators (e.g. shifts in foraging behavior; Semlitsch 1987; Schmitz 1998; Creel and Christianson 2008), but can also include reduced predation pressure on one prey species caused by high abundance of another prey species (Pepin and Shears 1995). Reduction of predation pressure can be due to density-dependent predator preference (predator switching; Murdoch 1969; Ims 1990; Sundell et al. 2003) for certain prey items, as well as reduced predator effectiveness for capturing preferred prey when there is high relative abundance of other prey (multi-species predator swamping; Ims 1990; Aukema and Raffa 2004). Predator preference, predator switching, and predator swamping help maintain the coexistence of multiple prey species in an environment, and could be important factors affecting successful recruitment in some fisheries (Fryxell and Lundberg 1994; Godiksen et al. 2006).

Abundance of alternative prey can reduce predation pressure on a target prey species through predator switching or predator swamping (Ims 1990). Switching occurs when one prey item becomes more abundant, and the feeding habits of the predator change so that they consistently preferentially consume more abundant prey (Kean-Howie 1988; Sundell et al. 2003). Predator switching is particularly pronounced when alternative prey items require different feeding strategies (Murdoch et al. 1975; Humphries et al. 2016). How this behavior is mediated over short time scales is particularly important for predator-prey dynamics of larval fishes and their predators, as some larval fishes undergo diel vertical migrations or lotic drifting behaviors, causing their availability to predators to temporally fluctuate in many systems (Johnson and McKenna 2007; Humphries et al. 2016).

Predator swamping dilutes direct predation effects on a prey species through a high abundance of prey items. Typically, predator swamping is associated with synchronous reproductive events (Ims 1990) or schooling prey (Major 1978; Stier et al. 2013). The high abundance of prey items as a short-term pulse

reduces the chance that any one prey item will be eaten. In addition, the high overall prey abundance may reduce the predation rate on relatively rare prey taxa, making preferred but rare prey more difficult or relatively less advantageous to selectively forage for (prey shielding; Kean-Howie et al. 1988; Aukema and Raffa 2004; Koss et al. 2004). The potential for high numbers of prey items to reduce overall predation on a single prey species or multiple prey species is crucial to understand in dynamic environments and across species.

The larval stage of fishes is a critical period in determining population levels of recruitment, and predation is the cause of a large portion of larval mortality for many fish species (Bailey and Houde 1989). In some systems, predation is the driving factor affecting larval recruitment [e.g. larval yellow perch (*Perca flavescens)* and alewife (*Alosa pseudoharengus)* predation (Mason and Brandt 1996); haddock (*Melanogrammus aeglefinus)* predation of larval capelin (*Mallotus villosus;* Gjøsæter et al. 2016)]. It can be difficult to investigate these dynamics in natural systems, as fish larvae often require specialized techniques to be detected in predator diets (e.g. molecular genetic assays, stable isotope analysis, or inferences from population models; Schooley et al. 2008). Experimental studies can simplify complex systems and offer direct evidence of how specified ecological factors can influence the predator-prey dynamics of larval fishes (Stier et al. 2013).

The importance of quantifying predator-prey relationships in ecological systems that control of population dynamics and potential recruitment is especially critical for species of conservation concern. Sturgeons (Family: Acipenseridae) are a taxon of global conservation concern as the majority of species are threatened (Duncan and Lockwood 2001). Predation may be an important factor in the recruitment of multiple sturgeon species (Gadomski and Parsley 2005a; Gadomski and Parsley 2005b; Flowers et al. 2011). At the larval stage, lack of protective scutes and drifting behavior leaves larval sturgeons susceptible to predation (Peterson et al. 2007). Predator preference for juvenile sturgeon has been studied in pallid sturgeon (*Scaphirhynchus albus*; French et al. 2014) and white sturgeon (*Acipenser trasmontanus*; Gadomski and Parsley 2005a; Gadomski and Parsley 2005b). However, comparatively

little information is available on the predator-prey interactions for other sturgeon species (Parsley et al. 2002), including the lake sturgeon (*Acipenser fulvescens)*.

Lake sturgeon is a species of conservation concern in the Laurentian Great Lakes region (Peterson et al. 2007). Adults are highly fecund, but early-life stage mortality can be high, leading to variable recruitment (Smith and King 2005; Caroffino et al. 2010a). Alterations to river systems, including impoundments, pollution, and reduced spawning habitat have affected lake sturgeon recruitment (Peterson et al. 2007). In addition, reductions in populations of co-distributed prey species may expose larval lake sturgeon to high levels of predation. Lake sturgeon larvae are susceptible to predation by a variety of predator taxa, including fishes (Waraniak et al., in review) and crayfish (Crossman, 2008). Research has quantified egg predation, but information on losses due to predation during the larval stage is lacking (Crossman 2008; Caroffino et al. 2010b). Predation has been a major factor affecting recruitment of other sturgeon species (Parsley et al. 2002; Steffenson et al. 2015), and may be an important factor contributing to the lack of natural lake sturgeon recruitment. Understanding the predator-prey relationships between lake sturgeon, other prey species, and their potential predators will be useful in understanding why natural recruitment is extremely low and suggest possible management actions to alter the ecology of sturgeon spawning rivers to improve larval sturgeon survival (Parsley et al. 2002).

In this study, experiments were conducted with two common predators from the Upper Black River (UBR), rock bass (*Ambloplites rupestris)* and hornyhead chub (*Nocomis biguttatus),* and lake sturgeon larvae, and two common co-distributed prey taxa, larval suckers (Family: Catostomidae), and mayfly larvae (Family: Heptageniidae). Densities of prey taxa were manipulated to address two primary objectives. First, the study assessed whether short-term predator switching behavior influenced the predation rate on larval lake sturgeon. Second, the study tested whether high alternative prey abundances provided a shielding effect for larval lake sturgeon. Additionally, analysis of predator size-selectivity was conducted.

MATERIALS AND METHODS

Study site and experimental enclosures

The UBR in Cheboygan County, Michigan, USA is the largest tributary of Black Lake and is used as spawning grounds for a well-studied population of lake sturgeon (~1200 adults; Pledger et al. 2013). This study was conducted in spring 2016 at the Black River Streamside Rearing Facility (BR-SRF) near Kleber Dam. Water supplied to the BR-SRF was taken directly from the UBR (Kleber Reservoir). Experiments were conducted in two 12.2m x 0.5m flow-through fiberglass raceways (Figure 11). No substrate was added to raceways. Raceways were filled with UBR water to a depth of 0.27m. Recirculating pumps were used to generate a laminar flow rate of 0.10-0.14m/s, a relatively slow but realistic flow in the UBR (Smith and King 2005). Predator exclusion areas were created for the introduction and collection of prey items at both ends of the raceways with 1.5cm x 1.5cm steel mesh. Aquarium dip nets were placed over the outflows during each trial to catch prey that had traveled the length of the raceway and were not consumed by predators. Trials were conducted in dark conditions to mimic night light levels when prey drift (Smith and King 2005). A vinyl tarp was erected around the raceways to reduce the risk of disturbances that might affect prey and predator behaviors during the experimental trial.

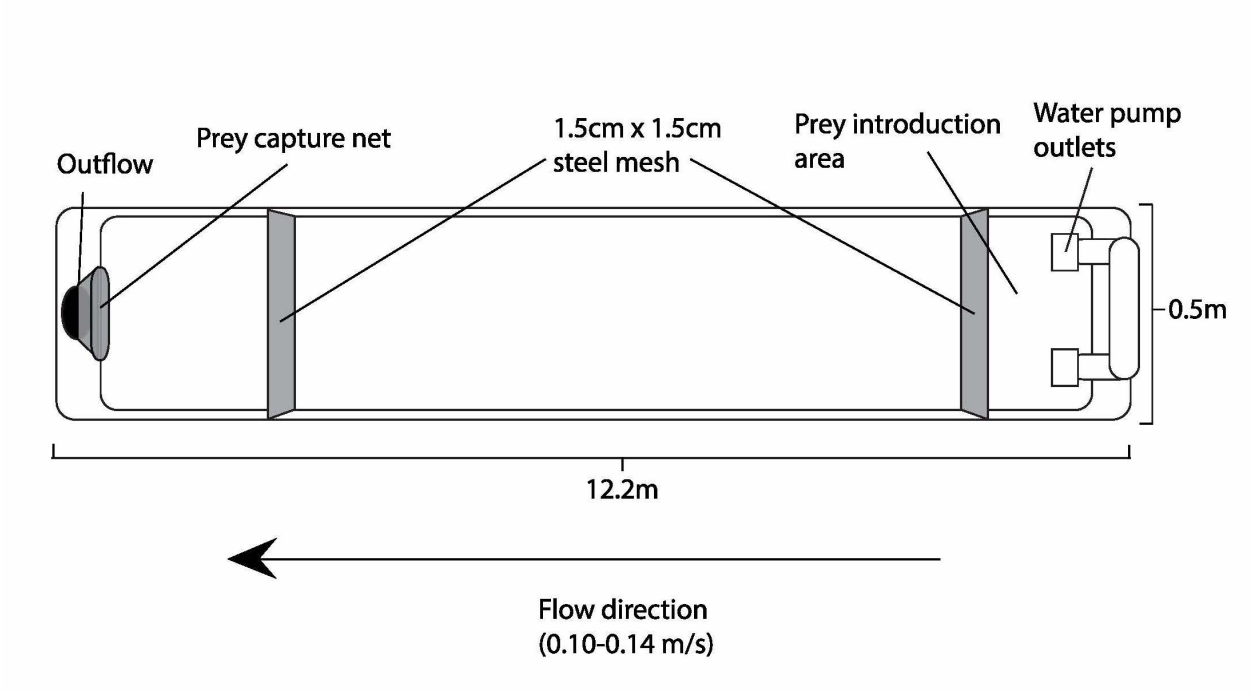

Figure 11. Diagram of experimental raceways used in predation trials.

Predator and prey collection and holding

Rock bass and hornyhead chub predators were collected near the lake sturgeon spawning grounds in the UBR with a barge electroshocker with a three person crew (settings – 18V, 4A) and transported back to the BR-SRF. To maintain similar ratios of predator and prey biomass between trials, only predators of a certain size class were used for trials. For rock bass, individuals were between 123-209 mm total length (TL; mean ± SD, 154 mm ± 21.7 mm), and for hornyhead chub, 80-157 mm TL (mean ± SD, 111 mm ± 19.3 mm). All predators were naive to trials. Food was withheld from predators for 24-26 before being used in a trial. Predators were maintained in covered, outdoor flow-through fiberglass raceways before trials were conducted. Four hours before trials began, predators were moved to the indoor trial raceways for acclimation. Recirculating pumps were turned on only one hour before the start of trials so predators could be acclimated to the higher flow conditions but not become fatigued from prolonged swimming in laminar flow without cover. After trials were completed, predators were released and new naive predators were collected for the next trials.

Larval white sucker (*Catostomus commersoni*), silver redhorse (*Moxostoma nigrum*) and mayflies (Family: Heptageniidae) were selected as alternative prey because of their high abundance throughout the majority of the lake sturgeon drift period (mid-May to early July; Scribner, unpublished data). Lake sturgeon, sucker, and mayfly larvae were collected during nightly surveys using D-frame drift nets as described by Auer and Baker (2002) and Smith and King (2005). As needed, mayfly larvae were also collected in the UBR during the day using kick-nets. Wild lake sturgeon larvae were used, rather than hatchery produced, to increasing applicability of results to the UBR system and because hatchery-reared larvae may be more susceptible than wild larvae to predation (Fritts et al. 2007). Sucker and mayfly larvae were retained in a covered flow-through outdoor raceway and lake sturgeon larvae were kept in 3.0-L polycarbonate flow-through aquaria. All prey were naïve to trials.

Density treatment design

Two treatments were included in experimental trials: (i) initial low number of sturgeon (low) and (ii) even numbers of all three prey species (even). Six trials per treatment (n = 6 experimental units) were conducted for each predator species in a randomized complete block design. Trials were blocked by raceways, with treatment type alternating between the two raceways each time a new trial was conducted. Each trial lasted a total of 60min and consisted of two pulses of prey introduced to raceways 30min apart. For the low treatment, the species composition of the first pulse was 43.3% sucker larvae, 43.3% mayfly larvae, and 13.3% lake sturgeon larvae. The second pulse for the low treatment and both pulses for the even treatment had equal densities of the three prey species. For rock bass trials, 60 prey items per pulse were used (120 prey items total), and in hornyhead chub trials, 30 prey items per pulse were used (60 prey items total). The number of prey items for rock bass was doubled to ensure some prey was recovered, as previous studies suggested rock bass were capable of consuming much more prey than hornyhead chub (Gezon, unpublished data; Waraniak, unpublished data). One rock bass or two to three hornyhead chubs were used per trial. Multiple hornyhead chubs were used because some cyprinids will not feed unless in the presence of conspecifics (Persson 1982).

At the end of each trial, predators were immediately removed from the raceways, total length (TL) was measured for each predator, and predators were released back into the UBR. Prey items were collected from raceways using dip nets after predators were removed. The number of prey recovered was subtracted from the number of introduced during the trial to estimate the number of prey consumed by the predator. A control trial was run without a predator, and >97% of prey items were recovered, suggesting loss due to causes other than predation was minimal. Photographs of prey items were taken before and after trials with a reference ruler. For each prey item, TL was measured using ImageJ 1.49 (NIH Image). The average TL for each species group was calculated based on measurements using ImageJ.

Statistical analyses

To analyze the prevalence of predator switching behavior, predator preference for each trial was calculated using Chesson's selectivity index (Equation 1; Chesson, 1978).

$$\alpha = (r_i/n_i) * (\Sigma\, r_j/n_j)^{-1} \qquad \text{Equation 1.}$$

Where r_i is the proportion of the i^{th} prey item in a predator diet, and n_i is the proportion of the i^{th} prey item in the environment. Chesson's selectivity index is given on a scale from 0 to 1 with values under 0.33 indicating negative selection for a prey item, values over 0.33 indicate positive selection, and values near 0.33 indicate no preference in a system with 3 types of prey. The selectivity index compared the predator species' preference for each prey between the two treatments. Each predator species was analyzed separately in a one-factor analysis. Chesson's selectivity index values were fit to a linear mixed effects model with the prey density treatment as a fixed factor, the raceway identity as a block, and the interaction between the fixed factor and block as a random factor. The statistical significance of factors and interactions were calculated by approximating likelihood ratios of models with and without variables and interactions to a χ^2 distribution.

To test the effects of predator switching, one-way ANOVAs compared Chesson's selectivity index values between the two treatments for each predator species. To test the effects of predator swamping, one-way ANOVAs compared the proportions of larval sturgeon that survived trials between the two treatments for each predator species.

To test predator size selectivity, the size distributions of each prey taxa before and after trials were compared using a Kolmogorov-Smirnoff (KS) test. Holm-Bonferroni corrections adjusted for type-I error with multiple comparisons. All statistical analyses were conducted using R statistical software (v. 3.2.2, R Core Team). Mixed models were fit and analyzed with the lme4 package in R (Bates et al. 2015).

RESULTS

Chesson's selectivity index and predator switching

Lake sturgeon were the least preferred prey item (mean Chesson's alpha = 0.14 for rock bass, 0.11 for hornyhead chub; Table 17). Overall, preference for larval catostomids was slightly higher than preference for lake sturgeon larvae but still negative (mean Chesson's alpha = 0.16 for rock bass, 0.15 for hornyhead chub). Heptageniid mayfly larvae were the most preferred prey for both species, with both predator species exhibiting a positive preference (mean Chesson's alpha = 0.70 for rock bass, 0.79 for hornyhead chub).

Hornyhead chub exhibited a significantly higher preference for sturgeon in the equal treatment than the low treatment ($\chi^2 = 5.642$, $P = 0.018$), even though hornyhead chub still negatively selected lake sturgeon in both treatments (Figure 12). Hornyhead chub only consumed lake sturgeon larvae once in the low treatment and still showed strong negative selection against lake sturgeon in the equal treatment (mean Chesson's alpha = 0.11). Rock bass did not exhibit a difference in preference for lake sturgeon between the two treatments ($\chi^2 = 0.224$, $P = 0.636$; mean Chesson's alpha = 0.14; Figure 12).

Table 17. Mean (± SE) of Chesson's α and proportional survival for larval lake sturgeon in each density treatment and for each predator species.

Predator Species	Density Treatment	Mean Chesson's α	Mean proportion survival
Rock bass	Even	0.164 (± 0.097)	0.842 (± 0.159)
	Low sturgeon	0.121 (± 0.141)	0.923 (± 0.094)
Hornyhead chub	Even	0.110 (± 0.091)	0.950 (± 0.032)
	Low sturgeon	0.009 (± 0.021)	0.988 (± 0.029)

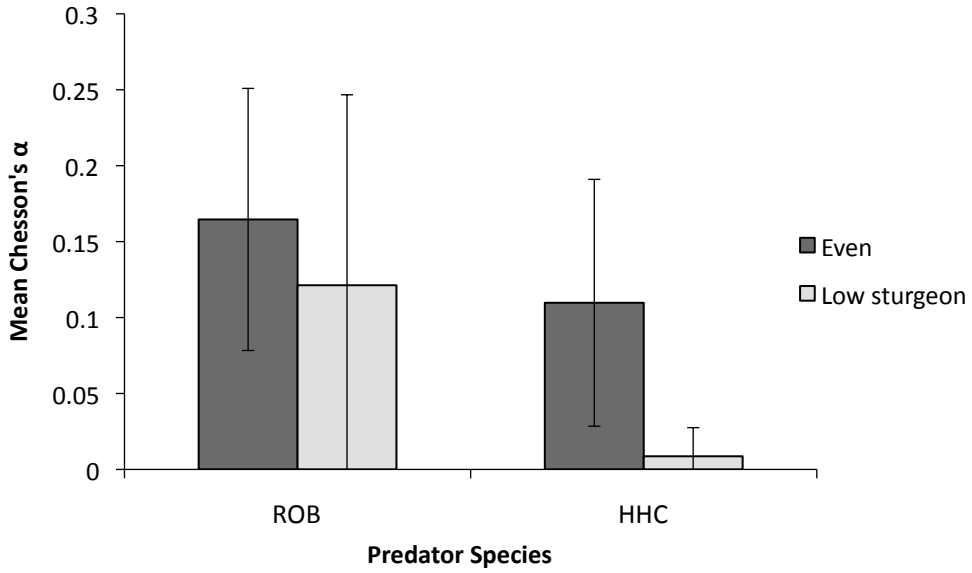

Figure 12. Mean Chesson's α showing preference for larval lake sturgeon for each predator species and each prey density treatment (color). Error bars represent ± 95% CIs.

Proportional survival and predator swamping

In predator trials, there was no significant difference in the proportion of surviving lake sturgeon between the two treatments for either rock bass ($\chi^2 = 1.237$, $P = 0.266$) or hornyhead chub ($\chi^2 = 3.629$, $P = 0.057$; Figure 13).

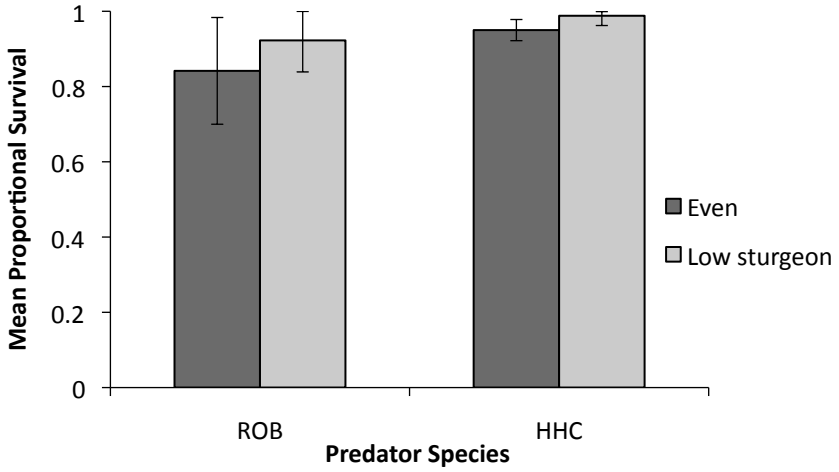

Figure 13. Mean proportional survival of larval lake sturgeon in trials with each predator species and prey density treatment (color). Error bars represent 95% CIs.

Size selectivity

Lake sturgeon were the largest larvae (mean TL ± SD, 22.7 ± 2.7mm) followed by catostomids (mean TL ± SD, 15.2 ± 1.0mm) and heptageniid mayflies (mean TL ± SD, 7.9 ± 1.4mm). Hornyhead chub did not exhibit size selectivity for prey. However, rock bass selected for small and mid-size catostomid larvae in both treatments (KS test; D_{low}= 0.208, $P < 0.001$, corrected alpha = 0.008; D_{even} = 0.184, $P < 0.01$, corrected alpha = 0.01). Rock bass did not select for mayfly larvae on the basis of size, and small lake sturgeon larvae were nearly significantly selected for (D = 0.144, $P = 0.039$, corrected alpha = 0.013; Figure 14).

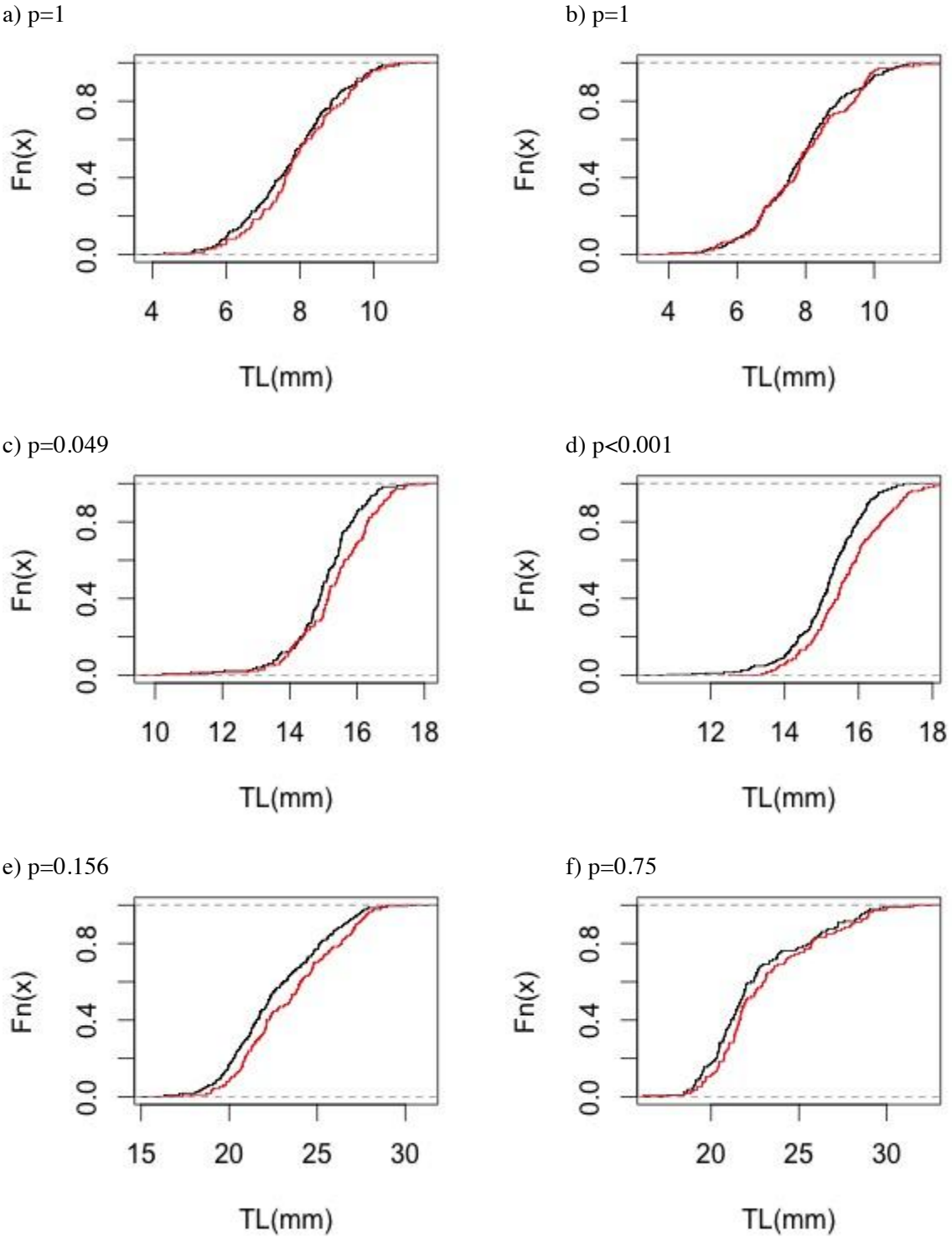

Figure 13. Cumulative distribution functions showing the distribution in size of prey items before (black) and after (red) predation trials with rock bass predators (p = Holm-Bonferroni corrected significance of Kolmogorov-Smirnov test comparing prey size distributions before and after predator trials). Prey size data were pooled for each treatment. Any trials in which the predator did not consume a certain prey item were excluded from the analysis of that prey item. There was no significant change in the size

Figure 13 (cont'd)

distributions for heptageniid (a,b) in either the equal density treatment (a) or the initial low sturgeon density treatment (b). Both treatments showed significant size-selective predation for catostomid larvae (c,d). Finally, there was near significant size-selective predation for larval lake sturgeon in the equal density treatment (e), but not in the initial low sturgeon density treatment (f).

DISCUSSION

Experimental evidence was found for changes in predator preference due to exposure to different prey densities and size selectivity among predators and prey types, but there was no evidence of predator swamping on the larval lake sturgeon predation rate at the prey densities tested. Studies including these predator-prey interaction behaviors are lacking for lake sturgeon, but provide useful insights into predator-prey dynamics, showing how alternative prey can mediate predation pressure on lake sturgeon larvae. Recovery of many lake sturgeon populations is limited because of little to no natural recruitment due to a variety of environmental and biological factors (Baker and Borgeson 1999; Caroffino et al. 2010a). Predator-prey interactions among lake sturgeon, other prey species, and predators may inform management decisions regarding lake sturgeon recruitment problems. Additionally, this study shows how short-term changes in prey communities can have demonstrable effects on predator foraging behavior.

The consistent negative selection of lake sturgeon as a food source and high preference for heptageniid mayfly larvae indicate little evidence of strong predator switching. Mayfly larvae were preferred by both predator species while lake sturgeon and catostomid larvae were selected against. Both rock bass (Elrod et al. 1981) and hornyhead chub (Poff and Allan 1995) display generalist feeding habits. Hornyhead chub diets consist primarily of benthic macroinvertebrates, with some piscivory and herbivory (Scott and Crossman 1973). Rock bass diets typically consist of benthic macroinvertebrates and crayfish, with piscivory common in larger adults (Paterson et al. 2006). Molecular diet analysis of these species also show that benthic macroinvertebrates are more commonly exploited prey than larval fishes in the UBR system (Waraniak et al., unpublished data). These results are similar to French et al. (2010) and French et al. (2014), where channel catfish (*Ictalurus punctatus)*, smallmouth bass (*Micropterus dolomieu)*, walleye (*Sander vitreus)*, and flathead catfish (*Pylodictis olivaris)* exhibited negative or neutral preference for pallid sturgeon (*Scaphirhynchus albus)* juveniles in the presence of fathead minnow (*Pimephales promelas)* alternative prey.

Some evidence suggested that varying prey densities had an effect on predator preference, at least for one of the predator species in this study. Rock bass did not exhibit a difference in preference for lake sturgeon in the two treatments. Instead, they matched consumption with the abundance of prey within the environment, so some predator switching may have occurred. However, when hornyhead chub were initially offered low numbers of lake sturgeon with high numbers of alternative prey, they continued to consume the alternative prey at higher rates even after more lake sturgeon larvae were available. They did not exhibit predator switching. Only one lake sturgeon larvae was consumed in all low treatment trials combined for hornyhead chub, whereas at least one lake sturgeon larvae was consumed in all but one of the even treatments. This modest disparity appears to support the hypothesis that the prey selection of some predators is affected by the prey items they are initially exposed to. These predators appear to continue to target prey items that were previously more available even if other prey items become abundant over short time scales. Experiments using fifteen-spined stickleback (*Spinachia spinachia)* showed switching behavior could change diet preferences on a similar short-term time scale (30 min; Hughes and Croy 1993). In this study, this pattern may have been created by satiation of the predators. However, it seems unlikely that predators were satiated by consuming prey in the first pulse, as both predator species routinely consumed more mayfly larvae than were initially offered.

From the perspective of lake sturgeon, predator swamping did not appear to occur. The proportions of surviving lake sturgeon were not statistically significantly different between the two treatments for each predator species, though the sturgeon survival was slightly higher in the low sturgeon density treatment. There might have been a small improvement in lake sturgeon survival due in the low-density treatment to predator swamping, but the small sample size may have precluded observation of a statistically significant difference. In theory, predator swamping should occur as larvae of many different species initiate drift under similar conditions and at similar times (Brown and Armstrong 1985; Carter et al. 1986; Lechner et al. 2013). In addition, the reduction of predation through the dilution effect has been hypothesized as a possible evolutionary driver of that synchronicity (Frank and Leggett 1983; Ims 1990). Experimentally, it has been shown that high densities of alternative prey can reduce predation on a

preferred target species (Kean-Howie et al. 1988). For example, for three larval marine fishes, the presence of *Artemia* nauplii (i.e. the alternative prey species) reduced the number of larval fish eaten (Pepin and Shears 1995). Contrarily, other experiments have shown that the presence of alternative prey items did not change the consumption rate of the species of interest. Alternative prey species did not reduce the number of ringed salamanders (*Ambystoma annulatum)* consumed by mosquitofish (*Gambusia holbrooki)* and fathead minnows (Drake et al. 2014) or the number of Pacific treefrogs (*Pseudacris regilla)* consumed by mosquitofish (Goodsell and Kats 1999). In addition, gudgeon (*Gobio gobio)* and stone loach (*Barbatula barbatula)* preyed on the most consistently abundant prey items, regardless of alternative prey species (Worischka et al. 2015). However, whether a predator switches prey may depend on the identity of the predator species and its behavior and the identity and behavior of the prey items. For example, perlid stoneflies (Family: Perlidae) did not exhibit predator switching, while perlode stoneflies (Family : Perlodidae) did (Elliott 2004). Pepin and Shears (1995) suggest that this effect may occur most often when prey are of similar sizes. In this experiment, there was little overlap in prey sizes, with larval lake sturgeon being larger than larval catostomids and heptageniid mayfly larvae.

Some size selection for prey occurred. No size selection occurred for hornyhead chub, but rock bass selected smaller catostomid larvae. In addition, some evidence, although not significant, showed rock bass also selected for small lake sturgeon larvae. The distribution of surviving larval catostomids corroborates the observation made by Paradis et al. (1996) that posits vulnerability to predation is maximized when larval size is 1/10th that of the predator size. The greatest difference in the size distributions (the K-S test D statistic) is located around 15-16 mm, close to 1/10th the mean TL of rock bass used in the experimental trials (154 mm). Small and mid-size predator species, including rock bass and hornyhead chub, exhibit size selectivity toward small prey due to gape limitation (Specziár 2011). Predators select smaller prey items to save energy while foraging as larger prey items require more energy to hunt and process (Floeter and Temming 2005). Preferred prey taxa, mayflies, had little size variation, and relatively few larval fishes were consumed. These preferences are heavily affected by species-specific characteristics of prey and predators (Worischka et al. 2015).

The three prey species are behaviorally and morphometrically dissimilar to one another, which may affect the ability of predators to detect and capture them. Heptageniid larvae are grazers that cling to rocks and other hard surfaces to feed and are relatively poor swimmers compared to larval fishes (Wellnitz and Poff 2006). The two larval fishes also have different drifting behaviors. Catostomid larvae typically drift near the surface or the middle of the water column (Clifford 1972; Gale and Mohr 1978; Corbett and Powles 1986), whereas lake sturgeon larvae are more benthic drifters, especially at relatively low flow rates, similar to conditions used in this study (Kempinger 1988; D'Amours et al. 2000; Smith and King 2005). Additionally, there are apparent qualitative differences between the swimming locomotion of larval catostomids and larval lake sturgeon. Catostomid larvae exhibit a sub-carangiform swimming movement, enabling short, fast bursts of speed, while larval lake sturgeon swim with a slower anguillid motion. These differences in movement may affect the ability of predators to capture either type of larvae. However, Hall-Scharf and Stallings (2014) used morphologically dissimilar prey species as well, but results were not affected by this difference in prey type. In the case of this experiment, the differences in morphology and behavior of the prey types likely had a stronger effect on predator preference than the different density treatments. Specziár (2011) found predators had an affinity toward slower-moving, benthic prey species, which would be mayflies in this study. Prey densities may have a stronger effect on predator preference if prey items are more functionally similar, as there would be fewer differences in handling time, energy density, or other factors influencing predator preference.

Only two predator species and two alternate prey species were used, which greatly simplifies the UBR ecosystem. Logperch (*Percina caprodes)*, another common predator in the UBR, may exhibit a higher preference for lake sturgeon than hornyhead chub or rock bass (Waraniak, unpublished data), so different alternative prey combinations and predator species may alter results. In addition, predator species were used in separate trials, not together, so the effect of interspecific competition at the predator-guild was not tested. Exploitative competition does affect predator-prey systems, yielding different numbers of prey items ingested per capita (Raborn et al. 2004). Finally, this experiment could only evaluate predator preference over the course of an entire trial, not how predator preference might have

changed during each pulse. Predation rates were not directly measured to prevent researchers from disturbing foraging predators which could alter predator behavior. Instead, recovered numbers of larvae were used as a proxy of the number of predation events per prey species. Video or another method that would avoid disturbing predators could be used in future studies to obtain direct observation data and estimate more instantaneous changes in predator diet preference as prey availability changes.

In conclusion, this study revealed potentially important implications for predator-prey dynamics between larval fishes, macroinvertebrates, and adult fishes in riverine systems. Some species appear to maintain diet preferences over the short term even as relative availability of different prey changes. Additionally, this study highlighted the importance of aquatic macroinvertebrates in the diets of two common native stream predators, indicating that high abundance of macroinvertebrate taxa could be important in reducing predation pressure on the larval stage of threatened fishes. Especially at the pre-larval and larval stages, predation is often a major factor affecting recruitment (Leggett and Deblois 1994), so investigating predator-prey dynamics at these life stages is critical. How foraging strategies and decisions of predators change in dynamic environments are important topics to relate to natural systems, and have significant implications for taxa, including larval fish, that undergo periods of increased exposure to predators over short time scales.

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