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METHODS AND RESOURCES ARTICLE

Barcoding PCR primers detect larval lake sturgeon (*Acipenser fulvescens*) in diets of piscine predators

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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, partially due to 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 as an alternative to morphological analysis of gastrointestinal (GI) contents to quantify the presence or absence 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.

Keywords Lake sturgeon · Predation · Larval fish · Molecular diet analysis · Cytochrome c oxidase · mtDNA barcoding

Introduction

Many fish species experience high mortality rates during early life stages (Hjort 1914; Houde 2008), most commonly attributed to starvation and predation. 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; Pritt et al. 2014; 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. Female

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lake sturgeon typically produce between 49,000 and 667,000 eggs per spawning event (Peterson et al. 2007). However, mortality can exceed 99% during egg and larval stages, indicating that survival during these early life stages is a 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 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).

Field-based predation studies of larval fishes, including lake sturgeon, have 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 that are 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 h post-ingestion (Hallfredsson et al. 2007; Legler et al. 2010), whereas genetic analysis of diet samples can identify prey DNA as long as 24 h after ingestion (Carreon-Martinez et al. 2011; Hunter et al. 2012; 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 numerous 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 described here is 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 potential 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 (Fig. 1). Sampling for larval lake sturgeon was conducted during 2015 within two 0.5 km transects directly downstream of each of the spawning sites (Fig. 1, gravel transects A and B), and two 0.5 km transects further downstream (Fig. 1, sand transects C and D). Drift samples were collected for 5 days during the lake sturgeon drift period (May 25th, June 4th–7th); one transect was sampled per day. The presence and relative 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 larval lake sturgeon were counted every hour beginning at 22:00 and concluding at 02:00 (nightly sturgeon captures; Online Resource 1, Table S1).

Potential piscine predators were collected during electrofishing surveys the day following larval drift sampling (approximately 12 h after conclusion of drift survey). 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

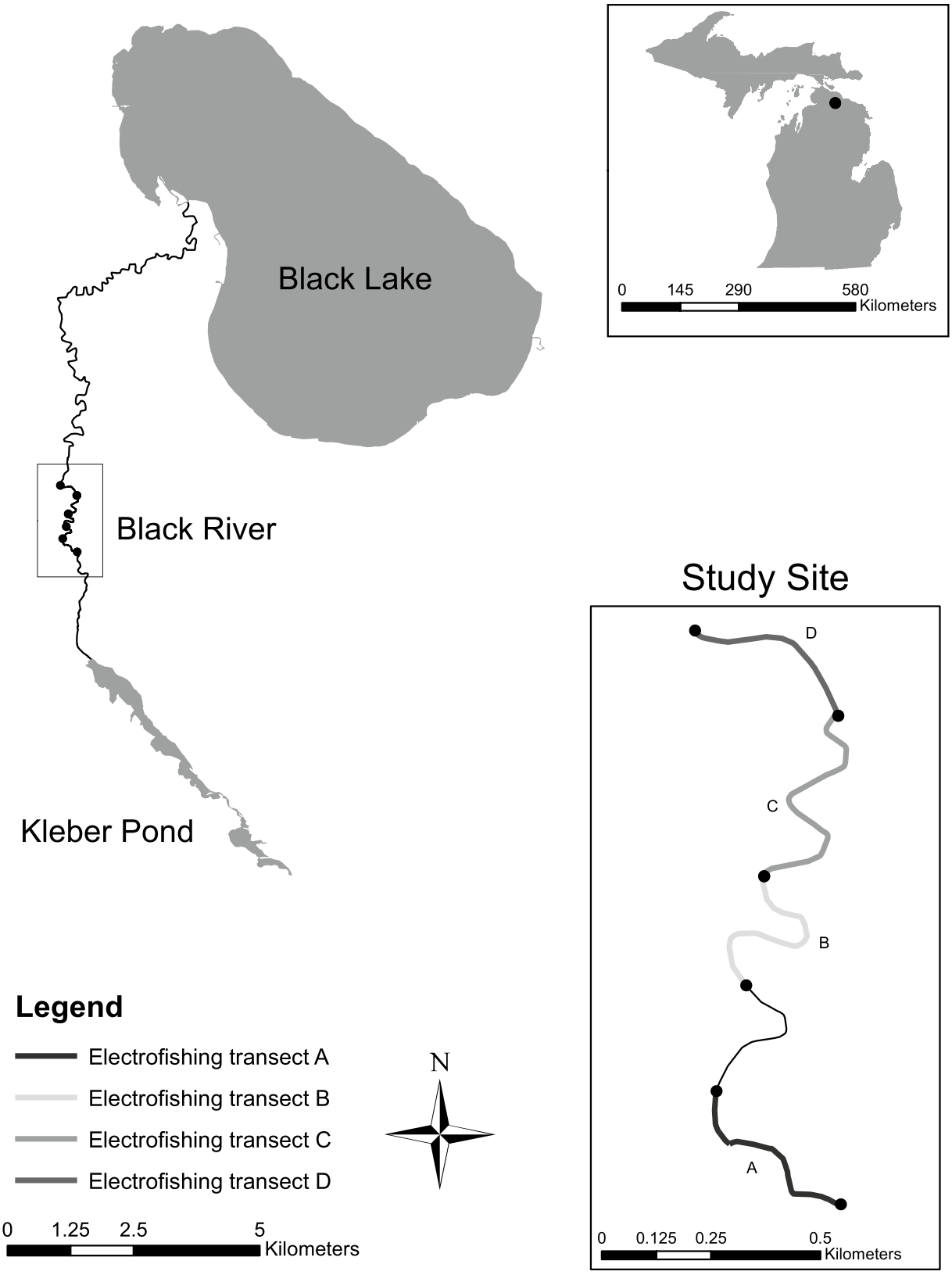

Fig. 1 Overview of the study area showing the location in Michigan in the *upper right*, the location of the transects on the Upper Black River, and a pullout in the *lower right* showing each transect with labels. Larval drift sampling sites are marked by *black circles* and electrofishing transects 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

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 potential predator was recorded and were individually stored in Whirl-Paks (Nasco) and placed in a −20 °C freezer for preservation (n = 353 samples from 17 predator species). Fin clips from each species were taken to evaluate species-specificity of the lake sturgeon PCR primers. Each potential 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 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.

DNA extraction

Ethanol was decanted from each sample and samples were rinsed 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 and sterile toothpicks and thoroughly vortexed. The extraction protocol used a modified version of the QIAamp Stool Mini Kit (QIAGEN) 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 of the thoroughly manually homogenized full sample 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) 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). After elution, DNA concentration was quantified using a nanodrop spectrophotometer and samples were standardized to a concentration of 20 ng/µl.

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 sequences of other fish and invertebrates known to occur in the UBR (Online Resource 1, Table S2). Two primer pairs were selected for laboratory testing (Table 1). The only likely unintended targets found on GenBank with two or fewer mismatches for both primer pairs are other sturgeon species (Family: Acipenseridae) not present in the study area.

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, 1× reaction buffer, and 5U of Taq DNA polymerase (Invitrogen), 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 min at 72 °C. Primer pair AfCOI1 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 potential predator fish species, three amphibian species, and 44 aquatic invertebrate families (Online Resource 1, Table S2) co-distributed with drifting larval lake sturgeon. 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.

Table 1 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
AfCOI1	F: 5′-CCATCATAATTGGCGGATTCGG-3′	138 bp
	R: 5′-CCCCAGAGGAGGCTAAAAGG-3′	
AfCOI2	F: 5′-GCTCCTTTTAGCCTCCTCTGG-3′	151 bp
	R: 5′-CCCCAAAATGGACGAAACCC-3′	

Two dilution series experiments were performed to test the sensitivity of primer pair AfCOI1. 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 AfCOI1 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 of the presence 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.

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 potential 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 potential predator species was analyzed using a Spearman's rank correlation test from the R package Hmisc v3.17-1 (Harrell 2015). The number of diet samples analyzed from each potential predator species closely corresponds with their relative abundance in the UBR (Pearson's correlation between number of samples per species and CPUE from electrofishing survey; $R^2 = 0.93$, $p < 0.0001$).

A Monte Carlo simulation test was conducted to determine the likelihood of the observed results for the Spearman's rank correlation given the sample sizes of potential predator species. Simulated data sets (n = 999) were created maintaining the same sample size for each fish species observed in the original data, but replacing the lake sturgeon DNA presence-absence data with randomly generated binomial data with a 0.0737 probability (the proportion of diet samples that tested positive for lake sturgeon DNA) of a "success" (i.e. containing lake sturgeon DNA) for each observation. Spearman rank correlations were carried out on each of the simulated data sets. The Spearman's rho values calculated from these simulated data sets were then compared to the Spearman's rho of the observed data to estimate the probability of the observed Spearman's rho versus what would be expected if all predator species were preying on larval lake sturgeon at the same frequency.

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 fish 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.

All concentrations in the dilution series were successfully amplified using the AfCOI1 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 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 potential predator species tested (Table 2). There was a positive correlation between number of diet samples and the number of lake sturgeon-positive diet samples from each species (Spearman's rank correlation test, r_s= 0.604, df1 = 1, df2 = 15, p = 0.01, Fig. 2).

Results of the Monte Carlo simulation test showed that the observed rho value of the Spearman's rank correlation was slightly lower, but not significantly different from what would be expected given the observed sample size of each potential predator species and assuming each fish had the same probability of consuming a lake sturgeon regardless of predator species (observed r_s = 0.604; simulated mean r_s = 0.754; p = 0.161).

Larval sturgeon predation rates in different habitats defined by substrate

There was no significant interaction between substrate type and the presence of sturgeon DNA in diet samples (Chi-squared test, X^2 = 1.68e-30, df = 1, p = 1). Some potential predator species were associated with a certain substrate type (Online Resource 1, Table S3). For example, 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, the abundance 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 potential predators that had consumed lake sturgeon was

Table 2 The number of samples tested from each of the 17 potential 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

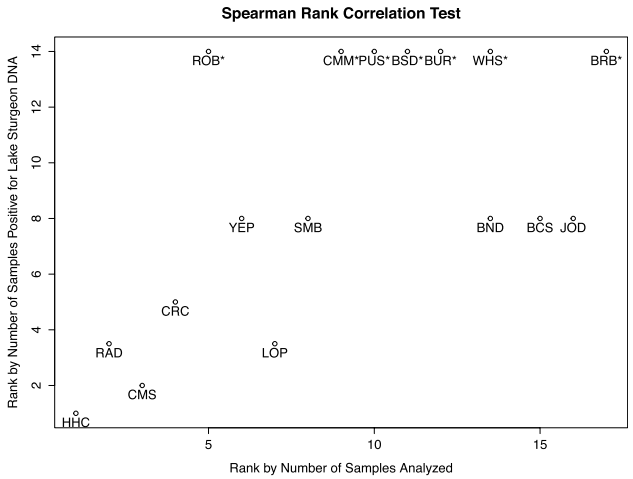

Fig. 2 Results of the Spearman's rank correlation test showing each piscine potential predator species ranked by the number of samples analyzed and the number of samples that tested positive for lake sturgeon mtDNA. Ranks were assigned from highest to lowest values for each variable (e.g. the first ranked 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 were tied for the lowest rank by number of samples positive for lake sturgeon DNA, indicated with a (*asterisk*) next to the three-letter species code. The species names for each three-letter species code can be found in Table 2

not different between sand and gravel, however 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 potential predators present during the larval drift period in the UBR, which is believed to be the life stage where mortality is highest. The assay was specific to lake sturgeon and did not amplify DNA of co-distributed predator and prey species. Fish species tested included 17 species from 5 families (Catostomidae, Centrarchidae, Cyprinidae, Gadidae, Ictaluridae, Percidae, Umbridae) that are common and widely distributed across North America and 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 potential 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 h after ingestion in some of the same species in this study (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 predator species. Additionally, sensitivity ethidium bromide staining and UV light is relatively low compared to other methods, and detection efficiency of DNA could be improved by adapting these primers for qPCR. 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 analysis of diet samples collected during this study (1 in 353 diet samples examined) was similar to other predation studies on larval sturgeon (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 masticate their prey. Detection of soft-bodied prey, even immediately after ingestion, would be especially difficult using morphological diet analysis of predators with similar feeding behavior.

When quantified by potential predator species, analysis of consumption of lake sturgeon revealed that the number of sturgeon-positive samples per species was correlated with the number of samples analyzed per predator species, and this correlation was not significantly different to what would be expected if predation rates of lake sturgeon were the same among the piscine potential predator species present in the UBR. Many of the species with diet samples that tested positive for lake sturgeon DNA are known to have crepuscular or nocturnal feeding habits (Keast and Welsh 1968; Magnan and Fitzgerald 1984; Jansen and Mackay 1992; Johnson 2015), and it is likely these species were foraging during the larval drift. Total lake sturgeon

consumption by a predator species appears to be roughly proportional to relative abundance. For example, hornyhead chub (*Nocomis biguttatus*) appeared to have the highest number of incidences of larval lake sturgeon consumption, 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 and 40% of the fish community in every transect. No potential predator species appeared to be targeting larval lake sturgeon more frequently than any other species, however, low sample sizes may be inaccurately estimating the frequency of larval lake sturgeon predation for some species. The observed Spearman's rho was in the bottom 10th percentile of expected rho values from the simulated data sets, suggesting the assumption that all potential predator species are equally likely to consume larval lake sturgeon explains somewhat less of the variation in the observed data than would be expected if the assumption was true. There may be some minor differences in predation frequencies between potential predator species that could be discerned with larger sample sizes.

There were several unexpected results from the analysis of predation by species. Rock bass (*Amblopites rupestris*) preyed upon larval sturgeon in controlled experiments (Crossman 2008), but no predation of larval sturgeon was detected from samples collected from the river. Another unexpected result was the finding that sturgeon DNA in the gastrointestinal contents of several species including 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 18–24 mm (Smith and King 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 gravel transects, which were directly downstream of the spawning areas where egg deposition occurs. Predation of lake sturgeon eggs or yolk-sac larvae, or scavenged 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 lake sturgeon. 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 potential predators that had consumed lake sturgeon, however, there were some confounding issues with this part of the analysis. Abundance of larval lake sturgeon in the drift was much higher in sand transects than the gravel transects, despite sampling on consecutive days. The proportion of fish 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 (Online Resource 1, Table S1). The fish in the gravel transects had access to the spawning sites where lake sturgeon eggs and yolk-sac larvae 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 diet samples caused by environmental DNA (eDNA) could lead to false positives, but does not appear to have a large effect on the results of this study. Methods for the detection of eDNA and DNA from diet samples 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. 2011; Jane et al. 2015). eDNA was unlikely to be detected over 240 m from the sources in a stream with similar flow rates to the UBR (Jane et al. 2015). 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.

This study provides the first diet-based analysis in a field setting that evaluated the prevalence of predation at a vulnerable early life stage during sturgeon development (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) all can 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 genetic 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.

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