FIELD AND EXPERIMENTAL STUDIES FIND VARIATION IN LEVELS OF LARVAL LAKE STURGEON PREDATION IS ASSOCIATED WITH BEHAVIOR AND ENVIRONMENTAL CONDITIONS

By

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ABSTRACT

FIELD AND EXPERIMENTAL STUDIES FIND VARIATION IN LEVELS OF LARVAL LAKE STURGEON PREDATION IS ASSOCIATED WITH BEHAVIOR AND ENVIRONMENTAL CONDITIONS

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Species are vulnerable to predation during early in life stages, especially those who provide little or no parental care. Predation risk is further elevated during periods of migration when individuals are exposed to a greater abundance and diversity of predators. Levels of predation during migration are often variable and can be heavily influenced by environmental conditions and changes in behavior. It is important to understand how these factors influence predation rates of threatened and endangered species. Lake sturgeon are an imperiled species native to the Great Lakes, Mississippi River, and Hudson Bay drainages that undergo a downstream from spawning grounds to nursery habitat at the beginning of the larval stage. This migration begins at night and larvae are exposed to several known predator species as they migrate the length of the spawning river in event. In this dissertation, I examine influences of ecological factors and behavior on levels of predation during the downstream migration. In Chapters 1 and 2, I used mesocosm experiments to determine the effects of night light level, rearing temperature, and prey abundance on survival during migration. Survival is highest during the environmental conditions present early in the season: new moon light levels, colder rearing conditions, and when prey abundance is high. In Chapters 3 and 4, I returned to the mesocosms to examine the survival implications of the behavioral responses to alarm cues during the larval stage. Lake sturgeon larvae exposed to a predator species odor and lake sturgeon alarm cue have higher survival rates when later exposed to the predator species. Additionally, lake sturgeon can use alarm cues from unrelated allopatric and sympatric species as

kairomones similarly to conspecific alarm cues. In Chapter 5, I collected lake sturgeon larvae and predator diets from a natural lake sturgeon spawning stream to estimate nightly mortality rates, identify the predatory species that consumed, and determine the environmental factors that influence lake sturgeon mortality in a wild population. Survival rates varied greatly, but on average, approximately one third of lake sturgeon larvae survived the section of the river sampled. Additionally, roughly one third of all potential predator diets sampled contained lake sturgeon. Similar to previous chapters, light level and the abundance of prey had the greatest influence on the survival rates of lake sturgeon and the probability of detecting lake sturgeon in the diets of potential predators. Results indicate the timing of lake sturgeon spawning influences larval survival by determining the ecological conditions present during offspring development and downstream migration. Work presented here identifies the factors with the strongest influence on larval lake sturgeon survival. Though variation in larval lake sturgeon mortality is high, managers may begin to use these factors to forecast larval lake sturgeon survival rates in populations. It will be important to monitor changes to these ecological variables, such as fluctuations in co-distributed fish and invertebrate populations, and variation in water temperatures during development, because these factors have strong and predictable influences lake sturgeon recruitment rates.

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KEY TO ABBREVIATIONS

AES	Aquatic eco-systems
Ag/AgCl	Silver/silver chloride
AICc	Corrected Akaike information criterion
AMB	Ambient temperature
CA	California
CAT	Catostomidae
Cat.	Catostomidae
CI	Confidence intervals
cm	Centimeters
COI	Cytochrome c oxidase subunit I gene
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
CT	Connecticut
D^{-1}	Inverse Simpson's diversity index
df	Degrees of freedom
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotide triphosphates
DPEF	Days post exogenous feeding
EOG	Electro-olfactogram
Eq.	Equation
FL	Florida
g	Grams

GA	Georgia
glmmTMB	Generalized linear mixed models using template model builder
H	Hypothesis
H’	Shannon diversity index
HEPT	Heptageniidae mayfly
Hept.	Heptageniidae
HHC	Hornyhead chub
HP	Horsepower
KCl	Potassium chloride
kg	Kilograms
km	Kilometers
L	Liter
L.S.	Lake Sturgeon
lme4	Linear mixed-effects models using Eigen and S4
LS	Lake sturgeon
m	Meters
M	Molarity
m/s	Meters per second
m^3/s	Meters cubed per second
MA	Massachusetts
MD	Maryland
mg	Milligrams
MI	Michigan

min	Minutes
mL	Milliliters
mm	Millimeters
MS-222	Tricaine methanesulfonate
mtDNA	Mitochondrial deoxyribonucleic acid
multcomp	Tukey’s general linear hypothesis test for multiple comparisons
MuMIn	Multi-model inference
mV	Millivolts
n	Sample size
ng	Nanograms
NH	New Hampshire
NJ	New Jersey
NTU	Nephelometric turbidity units
^{o}C	Degrees Celsius
OH	Ohio
PCR	Polymerase chain reaction
PPM	Parts per million
pred.	Predator
Pred_cm	Predator size in centimeters
Ra	Response magnitude to 10^{-5} Molar $_{\text{L}}$-alanine
Rb	Response magnitude to the blank
RB	Rock bass
rRNA	Ribosomal ribonucleic acid

Rt	Response magnitude to the alarm cue test stimulus
s	Seconds
SE	Standard error
Std. Error	Standard error
temp.	Temperature
UBR	Upper Black River
USA	United States of America
UV	Ultraviolet
v	Version
VAR	Variable temperature
vs.	Versus
W	Watt
WI	Wisconsin
ΔAICc	Change in corrected Akaike information criterion
μL	Microliters
μM	Micromolar

INTRODUCTION

Early life stages of many organisms are highly susceptible to predation, particularly in species that do not provide post-ovulatory parental care (Klug & Bonsall, 2010). Predation rates during early life stages are especially high for species that undergo migrations early in life (Furey et al., 2016; Jonsson et al., 2017), where they are exposed to taxonomically diverse groups of predators and different habitats. Phenotypic, physiological, and behavioral traits that allow early life stages to escape predation should be selected for, especially for populations at relatively low abundances where recruitment rates are more dependent on predation rates than resource availability. However, trait expression may be environmentally dependent. It is important to understand the factors driving population dynamics of species of conservation concern, however for species that experience high levels of predation during early life stages, it is often difficult to link predation rates to specific ecological variables including temperature, weather conditions, community diversity, and population density (Waraniak et al., 2018) a several factors likely contribute simultaneously.

In my dissertation, I used field and mesocosm studies to test hypotheses that link levels of predation of larval lake sturgeon (*Acipenser fulvescens*) to behavioral traits and ecological factors. Lake sturgeon are a primitive teleost fish species that are native to the Great Lakes and portions of the Mississippi River and Hudson Bay drainages. Lake sturgeon are a species of conservation concern across much of their range due to over harvest and habitat loss in the late 1800s and early 1900s (Bruch et al., 2016). Recruitment rates in many lake sturgeon populations are generally low, and highly variable among years (Caroffino et al., 2010; COSEWIC 2006). Adult lake sturgeon are protandrous and swim up rivers to spawn over gravel and cobble substrates (Bruch & Binkowski, 2002). After hatch and development to the larval stage, young lake sturgeon migrate downstream beginning at night, from spawning to rearing habitats. During migration, larvae are highly susceptible to predation by numerous predatory species (Waraniak et al., 2018). Mortality rates during early life history have been estimated to be high (Caroffino et al., 2010), and likely strongly influence population levels of recruitment. My dissertation examines the influence of several ecological factors and predator evasion behaviors on larval lake sturgeon predation during downstream migration.

In chapter 1, Experimental evidence for the effects of nocturnal light conditions on predation levels by riverine predators on larval prey, I investigated the effect of nighttime light level on predation rates of drifting lake sturgeon and co-distributed larvae by two common predatory fish. Larvae are believed to be at greater predation risk by visual riverine predators during periods of higher lunar illumination. Predation rates may also be affected by the taxonomic composition and relative abundance of alternative prey. To quantify associations between larval mortality levels and light levels, I experimentally tested whether differences in larval lake sturgeon predation levels were attributed to a change in prey selectivity, or to an overall decrease in consumption of co-distributed and dispersing prey consumed by predatory riverine species under different light conditions. Larvae from three common riverine prey taxa observed nocturnally dispersing together, lake sturgeon, white suckers (*Catostomus commersonii*), and Hetptageniid mayflies, were released into raceway mesocosms. Raceways contained one of two abundant predacious riverine fishes [either rock bass (*Ambloplites rupestris*) or hornyhead chub (*Nocomis biguttatus*)]. Trials were conducted in light conditions simulating a full or new moon. Both predators consumed fewer prey under new moon relative to full moon light conditions. In full moon conditions, rock bass selected for mayflies and against white suckers, but in new moon conditions rock bass selectivity was similar for all prey. Hornyhead chub selected for mayflies under both light conditions. Results indicate rock bass and hornyhead chub rely on visual cues to detect and consume prey. Rock bass also appear to visually differentiate between prey taxa, whereas hornyhead chub may use alternative senses to identify prey taxa. Nocturnal light levels likely influence predation rates in natural populations of nocturnally dispersing larvae. Variation in larval lake sturgeon mortality during the downstream drifting period is a function of the taxonomic composition and size distribution of predators, and composition and relative abundance of alternative co-distributed prey. Light levels during larval dispersal should be considered when interpreting intra- and inter-annual variation in recruitment.

In Chapter 2, Experimental evidence indicates lake sturgeon are vulnerable to an evolutionary trap, I examine how environmental conditions during larval lake sturgeon development and drift periods influenced larval predation rates. Many species depend on environmental cues to begin mating. These cues are predictive of ecological conditions that are favorable for the survival of offspring. Climate change can cause temperature to become an unreliable cue, creating an evolutionary trap (Schlaepfer et al., 2002). In this case, individuals may begin mating at temperatures typically associated with maximal offspring survival, however realized survival is diminished due to a mismatch with other ecological factors. Our goal in this study was to determine whether lake sturgeon may be susceptible to an anthropogenically-mediated evolutionary trap. Lake sturgeon adults use environmental cues, including temperature, to time migration into spawning streams (Forsythe et al., 2012). Sturgeon spawn in distinct temporally separated groups (runs), each present at spawning sites at a different stream temperature. Larvae produced during each run develop and migrate downstream associated with different environmental conditions. Larvae produced by early spawning adults are larger in size, disperse with a higher density of co-dispersing larvae, and disperse under lower lunar illumination than larvae produced by late spawning adults. If temperature becomes an unreliable cue to initiate reproduction, larvae may disperse under a mismatched combination of ecological conditions. If lake sturgeon are susceptible to an evolutionary trap caused by climate change, this mismatch in ecological conditions during dispersal would be expected to lower larval survival. I used an artificial stream mesocosm to measure the influences that larval size, disperser density, and lunar illumination, and the interaction between factors, had on larval sturgeon survival when exposed to a common predator. Results indicated overall survival decreased when the combination of ecological factors larvae experience is mismatched and therefore, lake sturgeon are likely vulnerable to a climatically induced evolutionary traps. Larval survival in threatened populations should be monitored to determine whether mismatched ecological conditions influence recruitment.

In Chapter 3, Stressful rearing environment diminishes the ability of larval lake sturgeon to use alarm cues to avoid predation, I tested whether larval lake sturgeon can use associative learning to avoid predators after exposure to lake sturgeon alarm cue, and whether behavioral change leads to changes in predation and feeding rates. During early life stages, aquatic species are vulnerable to high levels of predation. Several traits have evolved to allow individuals to detect predators and reduce predation risk. Levels of stress during early life stages also influences the ability of individuals to evade predators. Rearing environments in aquatic habitats are predicted to become more stressful due to high levels of variation in temperature associated with climate change. Many aquatic taxa are capable of associative learning, where a novel stimulus is detected coincident with a threatening stimulus (e.g., alarm cue), and in the future the novel stimulus induces the same behavior as the threatening stimulus. Lake sturgeon have been shown to behaviorally respond to alarm cue (Wishingrad et al., 2014), although the fitness implications of the changes in behavior have not been well-characterized. In this study, I sought to determine whether lake sturgeon are capable of associative learning to associate predatory odor with sturgeon alarm cue, and subsequently avoid predation by two common riverine predators (rock bass and rusty crayfish), and whether this trait was influenced by stress level during early ontogeny. Additionally, I tested whether a trade-off exists between evading predators and feeding rates. Lake sturgeon eggs and larvae were reared in ambient or artificially variable temperatures. Treatment groups were exposed to either a predator odor and lake sturgeon alarm cue, or control. Concentrations of odors used were shown to be detected by lake sturgeon via electrophysiology experiments. Larvae were exposed to rock bass and crayfish predators in mesocosms. Groups exposed to alarm cue and predatory odor had higher survival rates indicating larval lake sturgeon are able to use associative learning to associate alarm cue with predatory odor and increase survival when subsequently exposed to the predator. However, rearing in stressful conditions led to a decline in survival rates. Additionally, treatment groups were fed brine shrimp with and without predatory odor. Larvae that had been exposed to alarm cue and predator odor consumed less food when predator odor was added to food. Results indicate larval lake sturgeon use associative learning to recognize and reduce predation risk, but this trait is influenced by stress during rearing. Results also indicate a trade-off between predator evasion and feeding rate responses.

In Chapter 4, Lake sturgeon larvae use kairomone cues of sympatric and allopatric species to evade predation, I determined larval lake sturgeon can use alarm cues produced by other species as kairomones to recognize a threat and reduce predation risk. Fish rely on multiple cues to warn of the presence of predators. Alarm cues, the odors released from the damaged tissue of conspecifics, alert individuals of nearby predation and induce defensive behavioral responses in several fish species. Kairomones, odors that are released from heterospecifics, may be similarly used to alert individuals of predation of other species. It is common for fish to use alarm cues of closely related or sympatric species as kairomones to warn of predation. Here, I assessed whether lake sturgeon larvae can use alarm cues released by sympatric species and allopatric species to recognize predators via associative learning and reduced predation risk when exposed to predators. Replicate treatment groups of larval lake sturgeon were exposed to predatory rock bass odor along with either alarm cue of lake sturgeon, white sucker (sympatric species), round goby (allopatric species), or water (control). Larvae from each treatment group were released into the upstream end of a stream-mimicking mesocosm and allowed to passively drift by two predatory rock bass, simulating natural larval dispersal. Survival rates were highest for larvae exposed to lake sturgeon alarm cue and rock bass odor. Survival rates in white sucker and round goby alarm cue treatment groups were higher than the control group, indicating lake sturgeon can use these odors as kairomones to detect predators and increase survival. The ability for sturgeon to use kairomones to increase survival is an adaptive trait in heterogeneous tributary-Great Lakes environments. Additionally, it is rare for a species to use the alarm cue of an unrelated, allopatric species as a kairomone.

In Chapter 5, Mortality and predation on dispersing larval lake sturgeon are influenced by ecological factors, I examined the influence of ecological factors on mortality rates of larval lake sturgeon drifting downstream in a wild population. Additionally, using molecular diet analysis, I documented which ecological factors (nightly light level, river discharge, and larval lake sturgeon size) influenced the probability that different predatory fish species consumed migrating larval lake sturgeon. Nets were used to capture drifting lake sturgeon larvae and co-dispersing taxa at three sites, each 2 km apart on a lake sturgeon spawning stream. Nets were deployed on 13 nights over two years. I estimated the total number and biomass of larval lake sturgeon, suckers, and invertebrates each night. From these data I estimated the mortality level between sites. Mortality rates over the 4 km stretch sampled were estimated to range 10-96%, with a mean of 66% per night. Analyses indicated sturgeon mortality between drift sites was highest on nights with higher light levels, and when the abundance of larval suckers was higher. I also conducted a diet study, where predatory fish were captured via electroshocking and DNA was extracted from digestive tracts of a subset of fish. PCR and gel electrophoresis were used to determine whether lake sturgeon DNA was present in the digestive tracts. The probability of finding lake sturgeon DNA in the diet of a predator was strongly associated with the light level during the night prior to collection for most species. The relative effects of other ecological variables, including mean sturgeon size, and abundance of co-distributed prey taxa, differed depending on the predator species analyzed. Results indicate predation rates on drifting larval lake sturgeon were high yet variable. Variation in predation rates was strongly influenced by light conditions and the abundances of co-distributed taxa during downstream migration. Although night light level will vary with moon phase and cloud cover, managers may expect to find lowest mortality rates during the larval drift period in populations that co-disperse with a large abundance of other fish larvae, highlighting the need for a taxonomically diverse and abundant riverine prey community.

My dissertation research focused on understanding the survival of early life stage lake sturgeon, a long-lived, imperiled fish species in the Great Lakes region, and how this survival is influenced by behavior and ecological factors. In my first two chapters, I used mesocosm experiments to test hypotheses about how combinations of ecological factors influenced drifting lake sturgeon predation. In my third and fourth chapters, I investigate how the use of lake sturgeon and heterospecific alarm cues can lead to decreased predation rates. My fifth and capstone chapter encompassed a large field study where I examined the relative influences of the factors considered in my previous chapters on the predation of migrating lake sturgeon in a wild population.

The findings presented in this dissertation provide a greater understanding of how behavior and ecological factors affect the survival of larval lake sturgeon, a species of conservation concern. Though managers can expect to find a high degree of variation in lake sturgeon survival rates during the critical larval migration period, the data presented here suggest a great deal of this variation can be explained by ecological variables including light level, and the abundance of other prey species. These ecological variables are much easier to quantify than lake sturgeon survival and may serve to help forecast lake sturgeon recruitment rates. Additionally, changes in these ecological variables due to climate change or other anthropogenic influences may substantially impact lake sturgeon population recruitment and should be closely monitored.

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CHAPTER 1:
Experimental evidence for the effects of nocturnal light conditions on predation levels by riverine predators on larval prey

Introduction

Most fish species experience high mortality during early life stages (Demetrius 1978). Larval fish are susceptible to both predation and starvation because small size, and restricted mobility limits their ability to evade predators and forage (Almany and Webster 2006; Durant et al., 2007). Annual variation in early life mortality in larval fish can influence population levels of recruitment (Doropoulos et al., 2016; Gagliano et al., 2007). Consequently, early life mortality remains a heavily investigated topic (Li and Mathias 1982; Houde 1989; Berkeley et al., 2004; Johnson et al., 2014). Early life stages of many fish species disperse from spawning areas to rearing areas following hatch (Almany et al., 2017; Radinger & Wolter 2014). Understanding how environmental factors influence mortality during periods of early life dispersal is critical to understand and predict mortality levels during the vulnerable larval stage (Houde 1989).

Many studies focusing on impacts of environmental factors on larval fish mortality have examined commercially harvested species (Houde 1989, Garrido et al., 2014, Johnson et al., 2014). Studies are of particular importance for species of conservation concern. In this study, we seek to examine how environmental factors influence the mortality rates of larval lake sturgeon (*Acipenser fulvescens*), a regionally threatened species (Léonard et al., 2004). Adult lake sturgeon generally inhabit the benthic zone of lakes and large rivers, and forage over sand and silt substrate, but migrate up rivers to spawn on rocky substrate (Harkness and Dymond 1961; Threader et al., 1988; Haxton et al., 2008). Like many adfluvial, river-spawning species, lake sturgeon larvae passively disperse downstream following hatch (Auer and Baker 2002), along with larvae from other taxa, including other co-distributed fish and macroinvertebrates, and are consumed by predators (Waraniak et al., 2019).

Previous research on lake sturgeon dispersal has shown that as nightly lunar illumination increases, the probability of detecting lake sturgeon in the diets of predatory fish increases (Waraniak et al., 2018). These findings imply that lake sturgeon are more easily detected by predators during periods of higher lunar illumination. Other studies have shown high turbidity during larval dispersal is associated with higher larval fish survival rates (Fisken et al., 1998, Carreon-Martinez et al., 2014), also suggesting that impediments to predator visual acuity is associated with higher larval survival.

Our objective in this study was to quantify the impact of night light level on larval lake sturgeon mortality rates. Because of the high level of complexity in natural stream environments, we used replicated experimental raceways to simulate flowing stream habitats to evaluate three hypotheses: (H1) larval lake sturgeon mortality rates increase with higher lunar illumination, (H2) low light levels will impair the ability of predators to differentiate between different types of prey, and (H3) larval lake sturgeon predation rates are affected by lower levels of detection in low light and alteration of predation risk due to predator selectivity for alternate co-distributed prey.

Methods

Study site and subject

All experiments were conducted at the Black River Streamside Rearing Facility, a lake sturgeon hatchery and research facility on the Upper Black River (UBR) in Cheboygan County, MI (USA). A well-characterized population of lake sturgeon inhabiting Black Lake spawn annually in the UBR during late April through early June (Forsythe et al., 2012a). Lake sturgeon from other populations have been prevented from entering Black Lake and part of the Lower Black River since 1903 due to the construction of a hydroelectric dam on the Lower Black River, located downstream of Black Lake (Smith & Baker, 2005). Adult lake sturgeon spawn over rock and gravel substrate, where eggs and yolk sac larvae remain until yolk sacs have been absorbed, at which point the larvae enter the water column at night and disperse downstream to foraging areas in the river (Auer & Baker, 2002). During this dispersal stage, lake sturgeon larvae are highly susceptible to predation by a taxonomically diverse group of predators (Waraniak et al., 2018; Waraniak et al., 2019)

Lake sturgeon larvae in the UBR have been observed drifting concurrently with larval Catostomids including white sucker (*Catostomus commersonii*) and river redhorse (*Moxostoma carinatum*), as well as a diverse community of macroinvertebrates (Waraniak et al., 2019). To simulate natural stream conditions, prey taxa used in this experiment included naturally occurring lake sturgeon (LS) larvae, Catostomid (CAT) larvae, and Heptageniid mayfly (HEPT) larvae. Catostomid larvae used in these experiments were silver redhorse. Prey species were captured from the UBR using D-frame drift nets deployed nightly downstream of sturgeon spawning grounds. Prey items were housed in 3 L or 5 L tanks with filtered UBR river water for no more than 24 hours before being used in experimental raceway trials.

Two abundant predatory species inhabiting the UBR are rock bass (*Ambloplites rupestris*, RB) and hornyhead chub (*Nocomis biguttatus*, HHC). These species were selected as predatory species because they have been shown to consume a diversity of prey in previous experiments (Waraniak et al., 2017). Individuals of both species used in experiments were collected by barge electroshocking in the UBR and were housed in covered tanks with filtered UBR water. RB total length ranged from 9.3 to 13.1 cm. HHC total length ranged from 8.8 to 11.5 cm. Individuals of both species were held for 24-32 hours before being used in a trial. Collection, handling, and experimental protocols were all performed under conditions approved by the Michigan State University Animal Use and Care Committee.

Experimental Design

All experimental trials were conducted in one of two flow-through raceway mesocosms with dimensions 7.15 m x 0.5 m, housed in a darkened Quonset hut. Six hours prior to the start of a trial, two predators (either RB or HHC) were placed in 5 m x 0.5 m gated areas within the raceway. For the first five hours of acclimation, sand filtered water from the UBR was pumped at a flow of approximately 0.085 m/s. One hour prior to the start of a trial, recirculating water pumps were activated in order to increase the water flow to 0.134 ± 0.003 m/s. Light conditions were adjusted in the building housing the raceways so that the light at the surface of the water in the center of the raceways measured 0.20 lux simulating full moon conditions or 0.00 lux simulating new moon conditions. 0.20 lux and 0.00 lux were chosen for light and dark trials, respectively, because 0.20 lux is a plausible brightness of a relatively bright full moon and 0.00 lux is characteristic of a new moon (Kyba et al., 2017). Flow rates were measured with a Marsh-McBirny Flo-Mate 2000 flow meter (Frederick, MD) and lux was measured with an Extech LT300 light meter (Waltham, MA). Downstream of the gated area, fine-mesh aquarium nets were placed over the raceway outflow to capture drifting prey items that were not consumed by the predator.

At the start of the trial, 10 individuals of each prey taxa (LS, CAT, HEPT) were released simultaneously at the area of the raceway upstream from the gated section. Prey releases were repeated at 15 minutes, 30 minutes, and 45 minutes, for a total of 40 individuals of each prey species in each raceway and experimental trial. After 60 minutes, the trial ended, and the predatory fish were removed from the raceway. The entire raceway was searched for surviving prey. All surviving prey items were collected to determine counts of each prey taxa consumed during the trial. After each trial, predators were fin-clipped and released into Upper Black River to avoid using individuals in more than one trial. Eight replicate trials of each predator and light level combination were conducted.

Statistical Analysis

Binomial generalized linear models were used to quantify sources of variation in the number of each prey taxa and the total number of prey consumed during a trial as a function of light level (lux), predator species, predator body size (total length), and water temperature during the trial. The full model included interactions between all variables. Temperature and predator total length variables were nested within each predatory species. Temperature was included in the models because it influences the predator's metabolism, and potentially feeding rate (Volkoff & Rønnestad, 2020). Predator body size likewise could affect feeding rate (Ball et al., 2012). Competing models were ranked via AICc.

Chesson's alpha values (Chesson 1983) were calculated to determine the degree to which each predator selected for or against each prey species. If Chesson's alpha exceeded or was less than one third, the predator selected for or against the prey item, respectively, because equal numbers of three taxa were introduced to the predator. Predators select neutrally for a prey item if Chesson's alpha is approximately one third. Generalized linear models were used to explain prey selectivity as a function of light level (lux), predator species, predator total length, and water temperature. Similar models describing the number of prey consumed as a function of temperature, and predator size as nested variables within predator species were created and competing models were ranked by AICc.

Results

Total prey consumed

The top ranked model explaining total prey consumed included light level, predator species, predator size, the interaction between light level and predator species, the interaction between light level and predator size, and the three-way interaction between light level, predator species, and predator size (Table 1.1). Across all trials, the average total number of individual prey consumed was higher in full moon (0.20 lux) than new moon (0.00 lux) trials (mean ± SE full moon: 36.69 ± 2.33; mean ± SE new moon: 19.6 ± 2.32; Figure 1.1). The top ranked indicated that the probability an individual prey would be consumed in a full moon trial was 0.16 (95% CI: 0.10 – 0.21) higher than that in new moon trials (p-value = 2.53e-08).

Table 1.1. The top five AICc-ranked binomial generalized linear models describing the total number prey taxa consumed during raceway trials.

Total prey consumed	**Independent variables included**	**AICc**	**ΔAICc**
	light * predator species* predator size	236.86	0.00
	light + predator species* predator size	239.39	2.53
	light + predator species* predator size* temperature	246.73	9.87
	light * predator species	260.60	23.20
	light + predator species	261.63	24.77

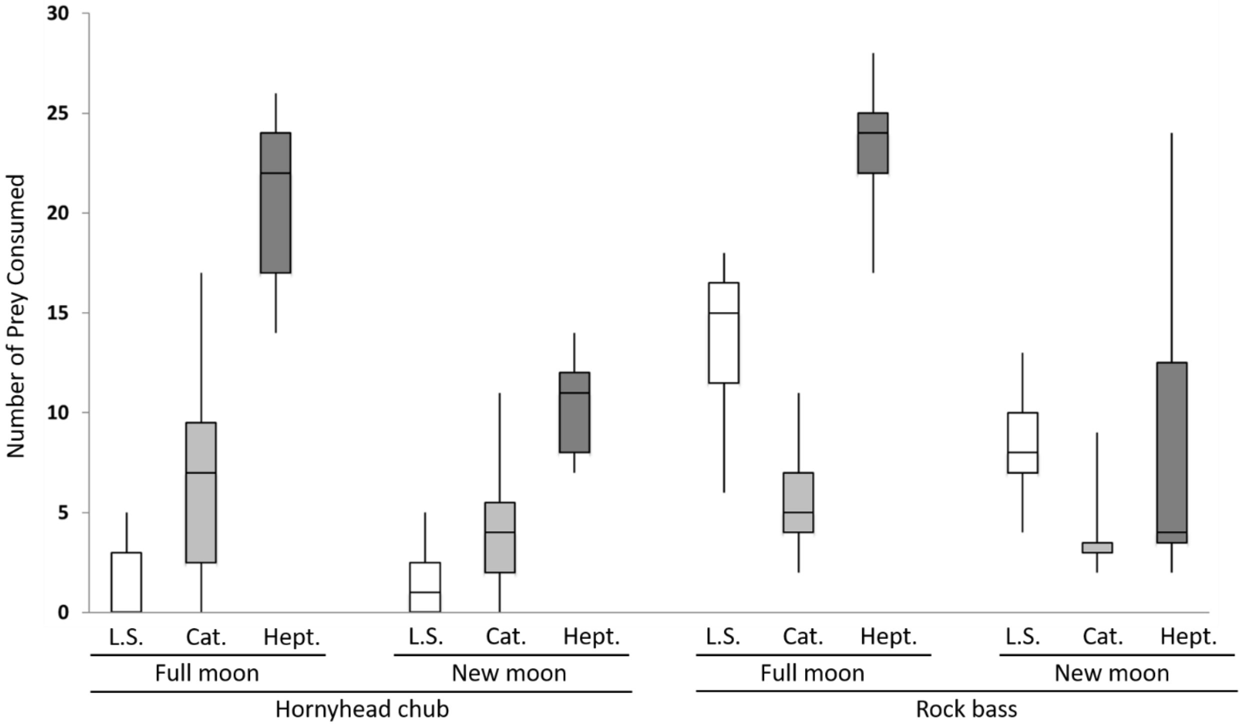

Figure 1.1. Boxplot showing the number of lake sturgeon (L.S.), Catostomid (Cat.), and Heptageniid (Hept.) larvae in trials with either full moon or new moon light conditions, and with either hornyhead chub or rock bass as the predatory species.

Lake sturgeon consumed

The top ranked model explaining the number of LS consumed based on AICc included light level, predator species, predator size, and the interaction between predator species and predator size (Table 1.2). The average number of LS consumed was higher in full moon lit trials than new moon lit trials (mean ± SE full moon: 7.31 ± 2.33; mean ± SE new moon: 4.93 ± 1.00; Figure 1.1). The top ranked model based on AICc indicated the probability an LS individual would be consumed in a full moon trial was 0.11 (95% CI: 0.02 – 0.18) higher than that in new moon trials (p-value = 0.01; Table 1.3). The average number of LS consumed in RB trials was higher than in HHC trials (mean ± SE RB: 10.44 ± 1.11; mean ± SE HHC: 1.81 ± 0.48). The top model also indicated that the probability an LS individual would be consumed in an RB trial was 0.39 (95% CI: 0.35 – 0.43) higher than in a HHC trials. The number of LS consumed in a trial was positively correlated with the size of the predator (Table 1.3). The top model indicated for an increase in mean predator total length by 1 cm, the probability of an LS individual getting consumed increased by 0.16 (95% CI: 0.16 – 0.26) in HHC trials and 0.06 (95% CI: -0.06 – 0.15) in RB trials.

Table 1.2. Parameters included in the top five ranked models by AICc explaining the total number of each prey taxa consumed during raceway trials.

Dependent variable	**Independent variables included**	**AICc**	**ΔAICc**
Lake sturgeon consumed	light + predator species* predator size	147.03	0.00
	predator species* predator size	150.98	3.95
	light * predator species* predator size	155.76	8.73
	light + predator species* predator size* temperature	160.18	13.16
	predator species* predator size* temperature	161.85	162.78
Catostomids consumed	light * predator species* predator size	203.47	0.00
	light + predator species* predator size	206.92	3.44
	predator species* predator size	212.01	8.54
	light + predator species* predator size* temperature	212.40	8.93
	predator species* predator size* temperature	216.02	12.55
Heptageniids consumed	light * predator species* predator size	188.03	0.00
	light + predator species* predator size* temperature	198.38	10.36
	light + predator species* predator size	201.31	13.29
	light * predator species* predator size* temperature	221.78	33.76
	light * predator species	222.06	34.03

Table 1.3. Parameter estimates included in the top AICc-ranked model describing the number of lake sturgeon, Catostomids, and Heptageniids consumed during raceway predation trials.

Independent variable	**Parameter**	**Estimate**	**Std. Error**	**P-value**
Lake sturgeon consumed	Intercept	-3.448	0.2438	< 0.001
	Lux-0.2	0.4383	0.1693	0.009
	Predator species-RB	2.1423	0.2384	< 0.001
	Predator size	0.6807	0.2309	0.003
	Predator species-RB: Predator size	-0.4222	0.2442	0.084
Catostomids consumed	Intercept	-1.8392	0.1669	< 0.001
	Lux-0.2	0.2833	0.2328	0.2236
	Predator species-RB	-0.1767	0.2427	0.4666
	Predator size	0.309	0.152	0.0421
	Lux-0.2: Predator species-RB	0.1334	0.3319	0.6878
	Lux-0.2: Predator size	0.8239	0.2773	0.0029
	Predator species-RB:Pred_cm	-0.2359	0.1905	0.2156
	Lux-0.2: Predator species-RB: Predator size	-1.2786	0.3667	0.0004
Heptageniids consumed	(Intercept)	-1.0308	0.1272	< 0.001
	Lux-0.2	1.075	0.1703	< 0.001
	Predator-RB	-0.3073	0.1976	0.1199
	Predator size	-0.0945	0.1181	0.4235
	Lux-0.2: Predator-RB	0.7006	0.2586	0.0067
	Lux-0.2: Predator size	-0.3103	0.1928	0.1074
	Predator species-RB: Predator size	0.6731	0.154	< 0.001
	Lux-0.2: Predator species-RB: Predator size	-0.367	0.2542	0.1489

Catostomids consumed

The top ranked model based on AICc indicated that the best supported model included effects of light level, predator species, predator size, the interaction between lux and predator species, the interaction between predator species and predator size, and the three-way interaction between lux, predator species, and predator size (Table 1.2). The effect of predator size on the number of CAT consumed was greater during full moon trials than new moon trials (Table 1.3). The probability an individual CAT would be consumed increased by 0.24 (95% CI: 0.15 – 0.34) for every 1 cm increase in mean predator total length in full moon trials, but 0.07 (95% CI: 0.00 – 0.15) for new moon trials (p-value = 0.003; Table 1.3).

Heptageniids consumed

The top ranked model based on AICc indicated, accounting for estimated effects of predator species, predator size, the interaction between predator species and predator size, the interaction between light level and predator species, the interaction between light level and predator size, and the three-way interaction between light level, predator species, and predator size (Table 1.2). The number of HEPT consumed was higher in full moon trials than new moon trials (mean ± SE full moon: 22.31 ± 1.06 vs. mean ± SE new moon: 9.50 ± 1.43; Figure 1.1). The difference in magnitude of light level effects was greater for RB trials (mean±SE RB full moon: 24.00 ± 1.35 vs. mean ± SE RB new moon: 8.5 ± 2.78 relative to the HHC trials; mean ± SE HHC full moon: 20.63 ± 1.49, vs. mean ± SE HHC new moon: 10.5 ± 0.91). The probability an HEPT individual would be consumed in a full moon trial was 0.25 (95% CI: 0.18 – 0.30) was higher than that in new moon trials (p-value = 2.76e-10; Table 1.3). The effect of light level was larger in RB trials than HHC trials. During RB trials, the probability an LS individual would be consumed in a full moon lit trial was 0.36 (95% CI: 0.28 – 0.41) higher than new moon lit trials, and in HHC trials, the probability an HEPT individual would be consumed in a full moon trail was 0.25 (95% CI: 0.17 – 0.30) higher than new moon trials (p = 0.006; Table 1.3). The relationship between predator size and number of HEPT consumed is more strongly negative in HHC trials than in RB trials (Table 1.3). Across HHC trials, as mean predator total length increased by 1 cm, the probability of a HEPT being consumed decreases by 0.02 (95% CI: -0.08 – 0.03) whereas across RB trials, as mean predator total length increased by 1 cm, the probability of a HEPT being consumed increased by 0.14 (95% CI: 0.07 – 0.21; p-value = 1.25e-5; Table

1.3). Although other covariates were included in the top model based by AICc, no other co-variate was found to have a significant influence on the probability of HEPT consumption (Table 1.2).

Lake sturgeon selectivity

Lake sturgeon larvae were selected against by HHC and were neutrally selected for by RB. The average of top ranked models (ΔAICc < 2) indicated, accounting for estimated effects of light level, predator size, and the interaction between predator species and predator size (Table 1.4). LS were selected for by RB more than by HHC (mean ± SE Chesson's alpha RB: 0.35 ± 0.03, mean ± SE Chesson's alpha HHC: 0.08 ± 0.02; Figure 1.2). The probability LS would be selected for in a RB trial was 0.87 (95% CI: 0.90 – 0.92) higher than that in HHC trials (p-value < 2e-16). LS were more likely to be selected for in new moon trials than in full moon trials (mean ± SE new moon Chesson's selectivity: 0.26 ± 0.04; mean ± SE full moon Chesson's selectivity: 0.17 ± 0.04). The average of top ranked models indicated the probability LS would be selected for in new moon trials was 0.07 (95% CI: 0.01 – 0.18) higher than in full moon trials (p-value = 0.03). As predator size increased, selection for LS also increased in HHC trials, but not RB trials. For every 1 cm increase in predator total length, the probability LS would be selected for increases by 0.14 (95% CI: 0.02 – 0.24) in HHC trials and 0.01 (95% CI: -0.12 – 0.13) in RB trials (interaction p-value = 0.364).

Table 1.4. The top five ranked models by AICc explaining the selectivity for each prey taxa consumed during raceway trials.

Dependent variable	**Independent variables included**	**AICc**	**ΔAICc**
Lake sturgeon selection	light + predator species* predator size	144.70	0.00
	light + predator species	145.71	1.00
	predator species* predator size	146.60	1.90
	light + predator species* temperature	147.21	2.51
	light * predator species	148.79	3.24
Catostomids selection	light + predator species* predator size	165.97	0.00
	predator species* predator size	166.43	0.46
	light * predator species* predator size	168.14	2.16
	light + predator species* predator size* temperature	171.52	5.55
	predator species* predator size* temperature	171.63	5.65
Heptageniids selection	light * predator species* predator size	177.65	0.00
	predator species* predator size	178.08	0.43
	light * predator species* predator size	181.37	3.72
	light + predator species* predator size* temperature	186.91	9.26
	predator species* predator size* temperature	195.90	18.26

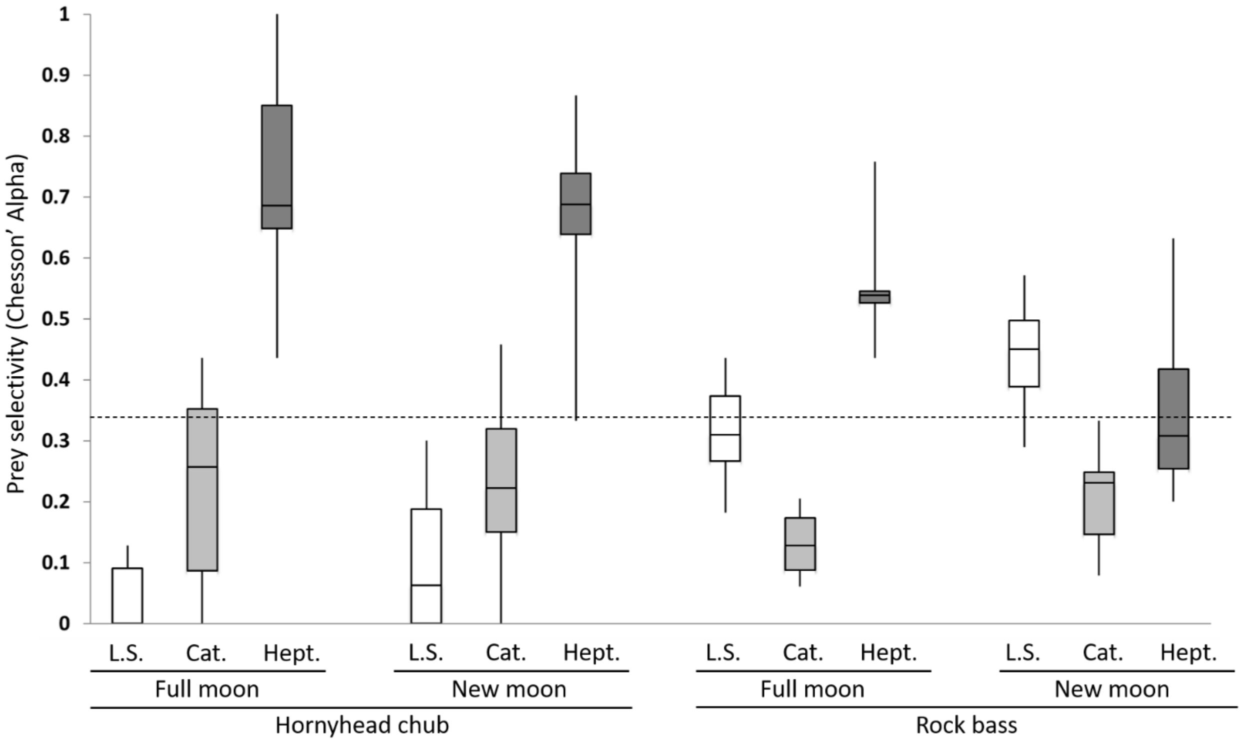

Figure 1.2. Boxplot showing the prey selectivity of hornyhead chub and rock bass for lake

Figure 1.2 (cont'd)
sturgeon (L.S.), Catostomid (Cat.), and Heptageniid (Hept.) larvae in trials with either full moon or new moon light conditions. Dotted line indicates Chesson's alpha value of 0.333, indicating neutral selectivity.

Catostomid selectivity

Catostomids were neutrally selected for by HHC and selected against by RB. CAT were selected for by HHC more than by RB (mean ± SE Chesson's alpha HHC: 0.25 ± 0.04, mean ± SE Chesson's alpha HHC: 0.19 ± 0.03; Figure 1.2). The average of top ranked models (ΔAICc < 2) indicated, accounting for estimated effects of light level, predator size, and the interaction between predator species and predator size (Table 1.4), the probability CAT would be selected for in a HHC trial was 0.12 (95% CI: 0.04 – 0.20) higher than that in a RB trial (p-value = 0.004). As predator size increased, selection for CAT also increased in HHC trials, but not RB trials. For every 1 cm increase in predator total length, the probability CAT would be selected for increases by 0.13 (95% CI: 0.07 – 0.19) in HHC trials and decreases 0.07 (95% CI: -0.15 – 0.02) in RB trials (interaction p-value = 4.24e-06). The effect of light level was also included in the averaged top models but did not significantly impact selectivity for CAT.

Heptageniid selectivity

Heptageniids were selected for by HHC and RB, though levels of selection were dependent on light level, predator species, and predator size. HEPT were selected for by HHC more than by RB (mean ± SE Chesson's alpha RB: 0.45 ± 0.04, mean ± SE Chesson's alpha HHC: 0.67 ± 0.05). The average of top ranked models (ΔAICc < 2) indicated that when accounting for estimated effects of light level, predator size, and the interaction between predator species and predator size, light level and predator species, light level and prey size, and the three way interaction between light level, prey size and prey species (Table 1.4), the probability HEPT would be selected for in a RB trial was 0.19 (95% CI: 0.09 – 0.28) higher than that in HHC trials (p-value = 3.13e-04). HEPT were more likely to be selected for in full moon trials than new moon trials (mean ± SE new moon Chesson's selectivity: 0.48 ± 0.05; full moon Chesson's selectivity: 0.63 ± 0.04). The average of top ranked models indicated the probability HEPT would be selected for in new moon trials was 0.11 (95% CI: 0.02 – 0.21) higher than in full moon trials (p-value = 0.017). As predator size increased, selection for HEPT also increased in HHC trials, but not RB trials. For every 1 cm increase in predator total length, the probability LS would be selected for increases by 0.14 (95% CI: 0.02 – 0.24) in HHC trials and 0.01 (95% CI: -0.12 – 0.13) in RB trials (interaction p-value = 0.364).

Discussion

Results indicate both common stream predatory fishes rely on light to locate prey. Total prey consumed was significantly less in trials reflecting new moon conditions than full moon conditions, for both predators. Data further revealed that RB selected prey more efficiently under full moon conditions, whereas HHC prey selection was consistently focused on HEPT regardless light level. Results explain the observed correlation between levels of lunar illumination and the probability of finding dispersing sturgeon in the diets of riverine predators (Waraniak et al., 2018). Findings suggest any decrease in larval LS predation during nights of lower lunar illumination can be attributed to the inability of riverine predators to efficiently detect and consume prey in low light conditions, as Chesson's alpha was slightly higher in new moon trials for both RB and HHC (indicating higher selection for LS in new moon conditions), but both predator species consumed fewer LS in new moon trials.

Other species of juvenile fish that are vulnerable to predation rely on the cue of the new moon to signal the beginning of their out migration from spawning grounds. For example, Coho salmon (*Oncorhynchus kisutch*) smolts were more likely to migrate downstream during the new moon, though the strength of this relationship varied by population (Spence & Dick 2014). The relationship between Coho smolt outmigration and new moon is thought to increase smolt survival due to the inability of visual predators to locate smolts (Grau et al., 1981). There is evidence that Coho smolts released during a new moon have higher recruitment rates than those released at a random date (Nishioka et al., 1989). However, larval LS migrate downstream passively when their yolk-sac energy reserve is exhausted, therefore outmigration timing strongly dependent on the timing of spawning, and water temperature (Duong et al., 2011), which influences their metabolic rate.

It is common for fish populations to rely on environmental cues to initiate spawning. Lunar phase and temperature have been shown to be predictive of the onset of spawning several species including European eel (*Anguilla anguilla*; Miyai et al 2004), Japanese eels (*Anguilla japonica*; Sudo et al., 2014), multi-species coral reef aggregations (Fisher et al., 2018), and honeycomb grouper (*Epinephelus merra*; Lee et al., 2002). The onset of spawning in LS can be predicted based upon lunar phase, temperature, and river discharge. Spawning generally begins during a new moon and when temperatures are near 10 °C (Forsythe et al., 2012a). Temperatures early in the spawning season result in larval sturgeon consuming the last of their yolk-sac and beginning to drift approximately 25-30 days (total observed range 21-36 days) after fertilization (Duong et al., 2011). If an egg is fertilized during the new moon and the larva disperses 25-30 days afterwards, the larva will be drifting near the next new moon, in the darkest night conditions. Results in this study indicate that sturgeon drifting during the lowest light levels have the highest survival rates. This means adult LS spawning during a new moon at the beginning of the spawning season may have an optimal probability of their offspring surviving during the drifting stage, and therefore optimal fitness.

Previous research has shown that there is a high repeatability of the timing of the onset of LS spawning for individuals, suggesting it is a heritable trait (Forsythe et al., 2012b). Taken together, results from this study along with known characteristics of LS spawning ecology indicate that the timing of lake sturgeon spawning could be a heritable trait that is selected for through higher survival rates of larval lake sturgeon drifting in lower light levels. More research into the heritability of spawning time, the relative survival of larvae under different environmental conditions, and the replication of studies across numerous lake sturgeon populations is needed to make definitive conclusions as to whether selection has acted on the timing of the first LS spawning period.

Examination of the morphology of RB and HHC may explain why RB appeared to depend on sight to select prey, whereas HHC were able to strongly select for HEPT in both light conditions. Centrarchids in general have eyes with a higher cone density than other groups, and RB in particular have larger eyes and larger cone surface area than other Centrarchids, allowing RB to see in low levels of light (Williamson & Keast, 1988). This highly adapted trait likely allows Centrarchids and especially RB to rely on using sight to detect and select prey, even in low-light conditions. Likewise, we found that RB were able to capture more prey and select for prey in full moon conditions. In new moon conditions, where no light was detected by lux meter, RB consumed less total prey and did not exhibit the same selectivity displayed in full moon conditions, suggesting low levels of light are required for RB prey detection and identification. Previous studies have also found Centrarchid’s are capable of prey detection and identification in low visibility conditions. Andree & Wahl (2019) found both black crappie

(*Pomoxis nigromaculatus*) and white crappie (*Pomoxis annularis*), other Centrarchid visual predators, were able to select for prey species similarly in water 0-50 nephelometric turbidity units (NTU), though selectivity for size of prey diminished as turbidity increased.

Conversely, HHC, which do not possess eyes highly-adapted for low-light conditions, exhibited positive selection for HEPT and negative selectivity for LS under low light conditions. HHC belong to the supergroup Ostariophysi, which also includes Cypriniformes (minnows, suckers, and carp), Characiformes (characins), Gymnotiformes (electric eels), and Siluriformes (catfish). A major synapomorphy of this group is the Weberian Apparatus, a bony structure connecting the swim bladder to the inner ear, which effectively uses the swim bladder to amplify sounds to improve hearing ability. The Weberian Apparatus has divergently evolved in families of fish, allowing each optimal hearing in their environment (Bird & Hernandez, 2007). HHC likely use their Weberian Apparatus to select for prey when they cannot see the prey. Cyprinids have been documented using hearing alone to find prey. Holt and Johnston (2011) found that when sounds mimicking prey were played on underwater speakers, several Ostariophysi species, mainly Cyprinids, approached the speakers, but no species without a Weberian Apparatus approached speakers, displaying the efficacy of Cyprinids relying on hearing ability to hunt prey.

Predator size was included in all top ranked models for number of each prey taxa consumed and prey selection. Across all trials, as predator size increased, the number of LS consumed increased, and the number of HEPT consumed decreased, with the exception of HEPT consumed by RB in new moon trials. These trends are expected, as generalist diets tend to shift through ontogeny from zooplankton, to invertebrates, and to small fish, and often continues to larger fish as the generalists grow (Kitagawa & Fujioka 2017; Jacobson et al., 2019). Ontogenetic diet shifts to piscivory has been documented in Centrarchids (Dauwalter & Fisher

2008; Probst et al., 1984). Larger RB may consume more HEPT in darker conditions because larger predators consume more prey (Table 1.1), but RB cannot efficiently differentiate between prey types in new moon conditions. It is relatively uncommon for Cyprinids to become piscivorous (Özdilek 2017; Vejřík et al., 2016). However, LS in the drifting period are relatively small (16.0-24.4 mm; Auer & Baker 2002), similar to the size of aquatic macroinvertebrates, so HHC are not gape-limited. One study detected LS in diets of 5 Cyprinid species during the larval LS drifting period, including 9% of HHC sampled (Waraniak et al., 2019). Results in this study indicate RB and HHC may be important predators of LS, particularly when predators are relatively large.

Mortality rates of larval aquatic macroinvertebrate species and lake sturgeon have been thought to have high inter-annual variation (McElravy et al., 1989; Caroffino et al., 2010a). Numerous biotic and abiotic variables, including river discharge, temperature, competition, and predation have been shown to affect macroinvertebrate mortality (Gasith & Resh 1999). Results of this study suggest that macroinvertebrate mortality rates may also be influenced by lunar illumination, as both RB and HHC consumed more HEPT, and both predators selected for HEPT in full moon light conditions. Predation rates of larval Heptageniid populations are likely more influenced by lunar illumination than predation on larval lake sturgeon populations.

Survival of LS during early life stages and subsequent recruitment has been studied across several populations (Barth et al., 2020; Caroffino et al., 2010a; Caroffino et al., 2010b; McDougall et al., 2014; Pratt et al., 2014, Waraniak et al., 2018; Waraniak et al., 2019). LS population survival rates across ontogeny are an important metric for managers to model to allocate resources to conserve threatened populations. Evidence of the impact of nightly lunar illumination on predation rates of LS exemplify the need for lunar illumination during drift period to be included in future models describing larval LS survival. However, lunar phase is not the only factor influencing available light at night for visual predators; the timing of moonrise, local weather conditions, depth, and turbidity (Andree & Wahl 2019; Carreon-Martinez et al., 2014, Fisken et al., 2002) will all influence the amount of light and therefore prey detectability.

Population sizes of invertebrates likely influence LS predation rates. In this study, as well as others in the Black River system by Waraniak et al., (2017) and Waraniak et al., (2019), found that the majority studied fish species do not select for LS larvae and instead exhibit positive selectivity for drifting macroinvertebrates, particularly HEPT. This suggests that if there were an abundance of more desirable prey drifting with LS larvae, predation rates on LS are likely to decrease. Managers interested in increasing lake sturgeon survival rates during critical early life stages may benefit from managing river ecosystems to promote high population abundance of taxa such as mayflies that are selected by riverine predators but are sensitive to poor water quality.

The impact of night-time light level on natural larval mortality is of interest to ecologists and managers with the goals of modelling larval fish and aquatic macroinvertebrate recruitment. Variation in a predators' ability to see prey is influenced by lunar phase but is also likely dependent on the height of the moon, local cloud cover, forest canopy cover of the river, and turbidity of the water. These are likely relevant parameters for all larval fish and invertebrate mortality estimations, as light level was shown not only to affect survival of lake sturgeon, our species of interest, but also overall predation rates.

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CHAPTER 2:
Experimental evidence indicates lake sturgeon are susceptible to an evolutionary trap

Introduction

Organisms use environmental cues, including changes in temperature, precipitation, and lunar phase to guide the timing of life history events including migration, maturation, and mating (Iwasa & Levin, 1995). For species that congregate to mate, environmental cues are used to coordinate migrations to suitable habitat for mating (Post et al., 2001), and synchronize mating behaviors (Takemura et al., 2004). For environmental cues to be adaptive, they should be predictive of the ecological conditions that will be present during offspring development, such as the availability of resources or changes in temperatures (Durant et al., 2007). Adults that begin mating in response to historically reliable environmental cues are more likely to be reproductively successful (Levitan et al., 2011). Offspring have higher survival rates when they develop under the ecological conditions associated with historically adaptive environmental cues that guided parental mating (Durant et al., 2007). As a result, there has been strong selection for adults to begin mating under specific environmental conditions.

Climate change has caused environmental cues that have historically been indicative of optimal conditions for offspring development to become increasingly unreliable (Robertson et al., 2013). For example, species that have historically relied on temperature to cue the onset of spring mating have begun reproducing earlier in the year, as average temperatures increase with climate change (Mayor et al., 2017). Earlier periods are also frequently characterized by temperature variability, even on a diel temporal scale. If current climatic warming trends continue, mismatches between resource availability and resource needs during life cycle stages are predicted to arise for many species (Carey, 2009). This is an example of an evolutionary trap, where an adapted response to a cue has become detrimental to fitness due to changes in the environment (Schlaepfer et al, 2002). It is important to understand whether species of conservation concern that rely on environmental cues to initiate reproductive activities may be susceptible to an evolutionary trap due to climate change.

Lake sturgeon (*Acipenser fulvescens*) is a long-lived ancestral teleost fish species that is threatened or endangered throughout much of its range (Bruch et al., 2016). Lake sturgeon rely on environmental cues including temperature, river discharge, and lunar phase to time spawning migrations up rivers to begin reproduction (Forsythe et al., 2012). Within each year, there are distinct lake sturgeon spawning periods, each occurring at different temperature ranges as spawning rivers warm in the spring (Duong et al., 2011). Females generally spawn in one spawning period within a year, whereas males have been observed making up to three intra-annual migrations to spawning grounds (Larson et al., 2020). After developing to the larval stage, lake sturgeon emerge from the river substrate at night to feed and drift downstream with the flow of the river to depositional nursery habitat (Auer & Baker, 2002). During downstream drift, larvae are exposed to diverse groups of predators and mortality is high (Waraniak et al., 2018).

Lake sturgeon larvae produced during each spawning period develop at different temperatures and at when exogenous feeding begins, drift alongside other larval vertebrate and invertebrate members of the stream community. Larvae produced early in the spawning season develop in colder water conditions, resulting in larger body sizes because more energy from yolk sac reserves can contribute to somatic growth instead of respiration (Jonsson et al., 2022). Black River larvae produced early in the spawning period drift downstream with a co-distributed prey community with approximately four times the abundance of the community present later in the drifting period (Waraniak et al., 2019). Additionally, larvae produced during the early spawning period are more likely to drift during a new moon, because early spawning adults use the new moon as a cue to begin spawning (Forsythe et al. 2012), and it takes larvae produced by early spawning approximately 30 days, or one lunar phase cycle, to reach the larval stage and begin drifting (Duong et al., 2011). To summarize, lake sturgeon larvae produced early in the spawning season on average have larger body size, drift with a larger abundance of co-distributed prey, and are more likely to drift during low light levels than larvae produced later in the spawning season.

We hypothesized that since lake sturgeon have evolved to use temperature, discharge, and lunar phase as cues to guide spawning behaviors, the combination of ecological variables (i.e., body size, prey abundance, and light level) present during early and late drift periods likely result in high survival rates. Subsequently, if environmental cues become unreliable and do not predict the conditions present during larval development, survival during the larval drift period should be reduced. In this study, our objective was to determine whether lake sturgeon are susceptible to an environmental trap by comparing larval survival rates in mesocosm experiments under combinations of ecological factors that are set to match or mismatch the ecological conditions present during the early and late drift periods. Results will demonstrate the fitness consequences of adult lake sturgeon spawning in response to unreliable environmental cues and will indicate whether sturgeon are susceptible to an evolutionary trap.

Methods

Lake sturgeon rearing

Eggs and sperm were collected from lake sturgeon spawning in the Upper Black River, Cheboygan County, MI, USA. Eggs from a single female were fertilized with sperm from a single male, resulting in full sibling families. Four families were reared in 2018 and four additional families were reared in 2019. Fertilized eggs were disinfected with povidone-iodine (Betadine, Stamford, CT) and were treated with Fuller's earth for de-adhesion, following established lake sturgeon hatchery protocols (Crossman et al., 2011; Bauman et al., 2016). After fertilization, eggs from each female were evenly divided into two temperature exposure groups. One group was reared beginning at the incubation stage at 10 °C, simulating temperatures present early in the spawning season, while the other group was reared at 18 °C, representative of temperatures late in the spawning season. Each group was reared in a partially recirculating system supplied with water pumped from the Upper Black River and equipped with a heater and chiller to allow for temperature control (1800W Single Tube Bottom Heater, Pentair AES, Apopka, FL; Arctica ¼ HP 1000W Aquarium Chiller, Transworld Aquatic Enterprises, Inglewood, CA). At hatch, yolk sac larvae were transferred to 3 L tanks (Aquatic Habitats; Pentair, Apopka, FL) containing 32 bio balls (CBB1-S; Pentair, Apopka, FL) to simulate spawning stream substrate. Tanks held up to 100 lake sturgeon yolk-sac larvae. Within 24 hours of transitioning into the larval stage, individuals were placed in 3 L tanks containing no bio balls and were used in predation trials.

Predation experiment

Flow through mesocosm raceways were used to simulate conditions experienced by drifting lake sturgeon larvae in the stream. Rock bass (*Ambloplites rupestris*) were captured via electrofishing from upstream of the Kleber Dam on the Upper Black River. Rock bass were held in 3.58 x 0.5 m tanks for 24-36 hours prior to predation trials. Two hours before a trial began, two rock bass were placed in separate gated sections of a 7.15 x 0.5 m raceway tank, to allow predators to acclimate to the mesocosms while preventing aggressive interactions between individuals. One hour before trials, flow rates were increased to 0.134 ± 0.003 m/s in the raceways to simulate flow rates lake sturgeon could experience during the larval drift period. At this time, the light level in the building housing the raceways was set to either 0.00 lux, simulating new moon conditions that are often present during the drift period of lake sturgeon larvae produced early in the spawning season, or 0.20 lux, simulating a moon lit night that is more likely to be experienced by larvae produced later in the spawning season.

At the beginning of each trial, nets were placed over raceway outflows and co-dispersing larvae were released into the upstream end of the raceways. Equal numbers of larval lake sturgeon, white suckers (*Catostomus commersonii*), and Heptageniid mayflies were released simultaneously at the beginning of each trial to simulate the co-distributed prey community present during the larval drift period. The lake sturgeon used in a single trial were from the same family and temperature group. The white sucker larvae and Heptageniid mayfly larvae were captured in the Upper Black River with D-frame nets deployed at night, and via kick-netting. Additional pulses of the three prey taxa were released 15, 30, and 45 minutes after the beginning of the trial. Each pulse contained eight individuals of each taxon in trials simulating prey density early in the lake sturgeon larval drift period, or two individuals of each taxa for trials simulating later period prey densities, for a total of either 32 or 8 individuals of each taxa released per trial. After one hour, predators were removed from the mesocosms, and surviving larvae were enumerated. Within each family, a trial was conducted for every unique rearing temperature group by light level by drifting density combination, resulting in 8 trials per family, and 56 trials total.

Statistical analysis

We fit a series of binomial family generalized linear mixed effect models to describe the proportion of lake sturgeon surviving predation trials as a function of rearing temperature, light level, prey density, as well as the interactions between these effects. Additionally, the random effect of family was included in each model. Models were ranked via AICc. Models were averaged if ΔAICc < 2 for two or more models. Models were produced in R v 4.2.0 using packages lme4 and MuMIn.

Results

The AICc-selected model describing the proportion of larval lake sturgeon consumed in predation trials included light level, density, rearing temperature, and the interactions between light level and density, light level and rearing temperature, as well as the inteaction between density and rearing temperature (Table 2.1). The variance in larval lake sturgeon survival among families was esitmated to be 0.053 by the model of best fit. Top ranking models were not averaged because no alternate model had an ΔAICc < 2 (Table 2.1).

Table 2.1. Competing models describing the proportion of larval lake sturgoen consumed in predation trials as a function of independent variabels including prey density, light level (lux), lake sturgeon rearing temperature, and the interactions between these terms. ":" indicates the interaction between the terms was also included in the model. The random effect of family was included in every model.

Parameters included	**ΔAICc**	**df**
Density + Lux + Rearing temp. + Density * Lux + Density * Rearing temp. + Lux * Rearing temp.	0.0	8
Density + Lux + Rearing temp. + Density * Lux + Density * Rearing temp. + Lux * Rearing temp. + Density * Lux * Rearing temp.	2.8	9
Density + Lux + Rearing temp. + Density * Rearing temp. + Lux * Rearing temp.	3.6	7
Density + Lux + Rearing temp. + Lux * Rearing temp.	4.9	6
Density + Lux + Rearing temp. + Density * Lux + Lux * Rearing temp.	6.9	7
Density + Lux + Rearing temp. + Lux * Rearing temp.	8	6
Density + Lux + Rearing temp. + Density * Rearing temp	12.1	6
Density + Lux + Rearing temp. + Density * Lux	15.1	5
Density + Lux + Rearing temp.	18.5	5
Density + Lux + Density * Lux	37.2	4
Density + Lux	40.4	4
Density + Rearing temp. + Density * Rearing temp.	140	3
Density + Rearing temp.	145.2	4
Density	165.9	3
Lux + Rearing temp. + Lux * Rearing temp.	367.4	5
Lux + Rearing temp.	379	4
Lux	398	3
Rearing temp.	485.8	3
Intercept only	504	2

Table 2.2. Parameter estimates and standard error for the terms included in the AICc selected model identified in Table 2.1 describing the proportion of lake sturgeon larvae conusmed during predation trials.

Parameter	**Estimate**	**Std. Error**
(Intercept)	0.7991	0.1431
Rearing temp. 18	-0.4751	0.1696
Density low	-2.4121	0.1776
Lux 0.2	-1.0928	0.1563
Rearing temp. 18: Density low	0.6166	0.2318
Rearing temp. 18: Lux 0.2	-0.7027	0.2247
Density low: Lux 0.2	0.5566	0.2316

Larval lake sturgeon reared at 18 °C had lower survival rates than those reared at 10 °C (Table 2.2; Figure 2.1). As documented in previous studies, larvae reared at 18 °C were smaller on average than those reared at 10 °C (19.1 vs 21.9 mm). Larval lake sturgeon drifting with low densities of co-distributed prey taxa also had significantly lower survival rates (Table 2.2; Figure 2.1). Also, larval lake sturgeon in trials with the light level imitating a full moon had lower survival rates than in with lower levels of light (Table 2.2; Figure 2.1).

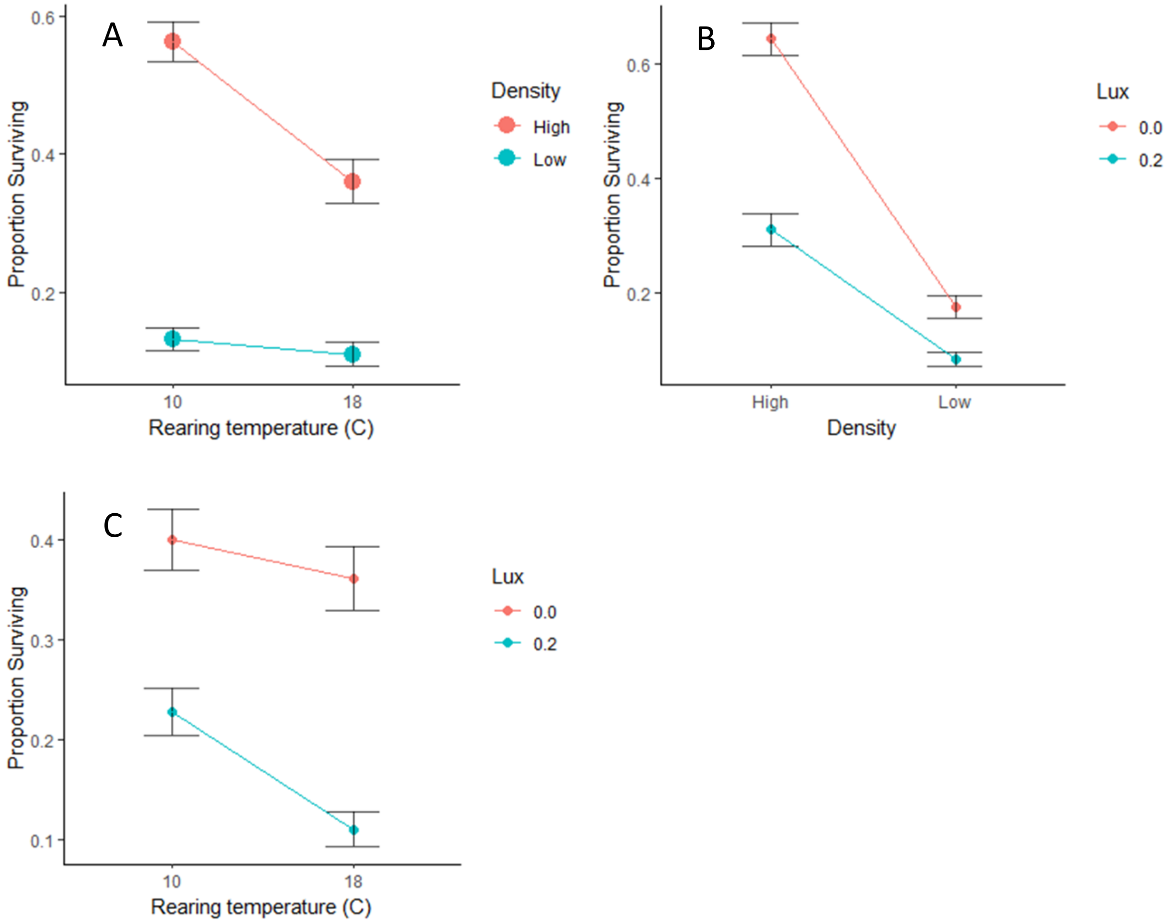

Figure 2.1. The proportion of larval lake sturgeon surviving in predation trials when exposed to rock bass. Points and error bars reflect predictions and 95% confidence intervals produced by the AICc-selected model indicated in Table 2.1. A) displays the interaction between the effects of taxa density included in trials and lake sturgeon rearing temperature. B) displays the interaction between light level (lux) during trials and taxa density. C) displays the interaction between light level and rearing temperatures.

The interaction between density of co-distributed prey and rearing temperature was included in the top model (Table 2.1). Rearing temperature did not have a strong influence on survival rates of lake sturgeon larvae drifting with high densities of co-distributed prey, however at high densities, larvae reared at 10 °C experienced higher survival than those reared at 18 °C (Table 2.2; Figure 2.1A). The interaction between light level and co-distributed prey density was also included in the top model (Table 2.1). The effect of density was greater at 0.0 lux than at 0.2 lux (Table 2.2; Figure 2.1B). Specifically, drifting with a low density of co-distributed prey was more detrimental to lake sturgeon survival rates during new moon conditions than during full moon conditions. Lastly, the interaction between light level and rearing temperature was also included in the top model (Table 2.1). During new moon conditions, rearing temperature did not have a strong influence on the survival rates of larval lake sturgeon in predation trials, however in full moon conditions, larvae reared at 10 °C had higher survival rates than the smaller lake sturgeon reared at 18 °C (Table 2.2; Figure 2.1C).

Discussion

Results indicated the effect each ecological factor tested had on lake sturgeon survival during a simulated larval drift period. Each two-way interaction was included in the AICc-selected model (Table 2.1), and all interaction terms were significant (Table 2.2) demonstrating the complex interactions among ecologically relevant physical and biotic stream conditions, even under a reduced set of experimental conditions. It is important to understand how these interactions could influence lake sturgeon populations in natural and increasingly highly variable stream environmental conditions if environmental cues used to time spawning become unreliable, and therefore larval lake sturgeon encounter abnormal combinations of ecological factors during critical early developmental periods, including the drift stage.

Larval lake sturgeon produced early in the spawning season are typically reared in colder temperatures and drift downstream with higher densities of co-dispersing larvae than larvae produced later in the spawning season (Waraniak et al., 2019). Climate change may cause temperatures during the springtime to increase more quickly (Hoegh-Guldberg & Bruno, 2010), resulting in larvae produced early in the spawning season developing under warmer conditions. Subsequently, lake sturgeon dispersing downstream early in the drift period would have smaller body size and drift with high densities of co-distributed prey taxa. Lake sturgeon larvae reared at 10 °C were approximately 14% larger than those reared at 18 °C for in our experiments. The interaction between prey drifting density and rearing temperature (Figure 2.1A) indicates the potential for a decrease in larval sturgeon survival rates relative to sturgeon reared at 10 °C drifting at high densities, because small body size negatively impacted survival rates of lake sturgeon larvae drifting with high densities of co-distributed larvae. However, if the reverse were to take place, and lake sturgeon produced later in the season and drift with low densities of co-dispersing prey taxa were larger due to rearing at lower temperatures, results suggest there would be little change in survival rates because rearing temperature does not strongly influence survival rates when lake sturgeon larvae are drifting with low densities of co-distributed taxa.

Adult lake sturgeon use the new moon phase as a cue to initiate spawning at the beginning of the reproductive season (Forsythe et al., 2012). Larvae produced during this time take roughly 30 days to reach the larval stage and initiate drift (Duong et al., 2011). Therefore, larvae produced early in the spawning season are often drifting during the next period of low lunar illumination. If water temperatures increase after the onset of spawning, the time it takes larvae to develop to the larval stage will be decreased (Duong et al., 2011), and therefore the lake sturgeon larvae could be drifting under full moon light conditions. The interaction between light level and co-distributed prey density suggests that if lake sturgeon larvae drifting early in the season high densities drift under full moon conditions instead of new moon conditions, survival rates would be decrease (Figure 2.1B). This result provides additional evidence that lake sturgeon could be susceptible to an evolutionary trap.

Larger lake sturgeon larvae, reared at colder temperatures, are more likely to drift near a new moon. The interaction between light level and rearing temperature (Figure 2.1C) indicates that if the larvae drifting during a new moon were reared in warmer water due to spawning cues becoming unreliable, there would be relatively little change in the survival rates of these larvae. Additionally, if unreliable cues result in larvae produced later in the season drifting during the new moon, or being reared at colder temperatures, survival rates of larvae in the population could increase. This is the only important interaction that does not support the hypothesis that lake sturgeon are vulnerable to an evolutionary trap.

The fixed effects of rearing temperature, co-distributed prey density, and light level are also important to understand because all three had a significant influence on the lake sturgeon larval survival rates (Table 2.2), and these effects are likely to have similar influences on contemporary populations of drifting larval lake sturgeon. The effect of co-distributed prey density had the largest influences on survival rate (Table 2.2). Results are likely due to predator swamping, where prey availability exceeds the maximum consumptive rate of predators, decreasing the probability that an individual will be consumed (Fury et al., 2016). Predator swamping is an evolutionary strategy to escape predation that has been documented in insects, birds, reptiles, and fish (Corkum et al., 2006; Tucker et al., 2008; Descamps, 2019; Fury et al., 2016). If survival rates of drifting lake sturgeon larvae are influenced by predator swamping, the timing of spawning is important because lake sturgeon larvae must be drifting at the same time other fish and invertebrate larvae.

Light level influenced larval lake sturgeon survival, likely due to predatory rock bass, a visual predator, having difficulty in detecting prey taxa in darker conditions (Chapter 2). Night light level also has been associated with fish predation rates in other riverine species (Fury et al., 2016). Rearing temperature also influenced lake sturgeon survival, likely because of the influence rearing temperature has on the body size of larvae. Lake sturgeon reared at 10 °C were larger and had higher survival rates (Table 2.2). Larger prey often experience lower rates of predation due to gape limitations of predators, requiring longer handling time by predators (Nilsson & Brönmark, 2003), or being more adept at evading predation as swimming velocity and larval size are correlated (Fisher et al., 2000). In these experiments, no rock bass predators were gape limited. Therefore, predation rates may be lower on larger lake sturgeon larvae due to increased handling times by predators, or due to a relatively fast swimming to evade rock bass predators.

In conclusion, larval lake sturgeon survival in mesocosm experiments simulating the larval drift period was dependent on light level, the density of co-distributed larvae present during an experiment, and rearing temperature. The interactions between these effects indicate larval survival rates may decrease if the environmental cues are no longer predictive of optimal rearing and subsequent drifting conditions. As climate change influences the mean and variation of temperatures and precipitation levels, the changes in temperature and river discharge that lake sturgeon adults use as a cue to signal the beginning of the mating season may become detrimental to fitness.

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CHAPTER 3:
Stressful rearing environment diminishes the ability of larval lake sturgeon to use alarm cues to avoid predation

Introduction

Early life stages of most aquatic organisms are highly susceptible to predation, particularly in species with little or no post-ovulatory parental care (Allen 2008; Miller et al., 1988). During early life stages, individuals must successfully detect and avoid predators to survive. Consequently, two of the first sensory abilities to develop are vision and olfaction, facilitating the detection of predators via visual and chemical cues (Kelley & Magurran, 2003). Alarm cues are a type of chemical signal used by organisms to warn of proximity to predators. When attacked by a predator, prey passively release alarm cues that warn conspecifics of nearby predation and initiates a change in behavior to evade predators. Alarm cues have been documented in a wide diversity of aquatic taxa, including but not limited to insects, mollusks, gastropods, crustaceans, fish, and amphibians, indicating this trait has independently evolved multiple times and confers fitness benefits (Reviewed in Chivers and Smith 1998). Olfactory systems are often highly sensitive to these odors and can often be detected at low concentrations (Mirza et al., 2009; Ou et al., 2015).

Innate behavioral responses to alarm cues, referred to as alarm responses, are context dependent. Prey organisms often employ one of two adaptive alarm responses to reduce predation risk: 1) increase activity rates and speed to escape predators as documented in sea lamprey (*Petromyzon marinus)* and zebrafish (*Danio rerio*) (Hume et al.,2015; Speedie & Gerlai 2008, respectively) or 2) reduce activity rates and remain immobile to hide from predators as has been described in rainbow trout (*Oncorhynchus mykiss*) and darter species (*Etheostoma spp.*)

(Mirza & Chivers 2003; Commens-Carson & Mathis 2007, respectively). Additionally, responses to alarm cue can be life stage dependent as has been observed in early life stages of lake sturgeon (*Acipenser fulvescens*) (Wishingrad et al., 2014, Wassink et al.,2019). The magnitude of the alarm response is often concentration dependent, whereby higher concentrations of alarm cues elicit stronger responses (Mirza & Chivers 2003; Speedie & Gerlai 2008). Behaviors similar to the innate alarm responses are also triggered when prey detects the odor of a predator. Behavioral responses to predator odors are usually learned and therefore occur only after conspecific alarm cue is detected at the same time as a predator's odor (Kelley & Magurran, 2003). For example, an Iberian frog (*Rana iberica*) simultaneously exposed to predator odors and conspecific alarm cue displays alarm response behaviors following a subsequent exposure to only predator odor (Gonzalo et al., 2007). Additionally, European minnow (*Phoxinus phoxinus*) display a fright response when exposed to the odor of a predacious northern pike (*Esox lucius*) only after a previous exposure to European minnow alarm cue and northern pike odor together (Magurran, 1989). These are both examples of associative learning, where a known conspecific cue that elicits an innate response is associated with a novel cue such as a predator odor, and a response is elicited when that novel cue is detected in the future (Morand-Ferron 2017).

Despite substantial evidence for associative learning associated with predator odors and alarm cues in taxonomically diverse taxa (Reviewed in Kelley & Magurran, 2003), the survival implications of alarm responses are less commonly investigated, with a few notable exceptions (e.g., Chivers et al., 2006; Vilhunen, 2006). Alarm responses are often presumed to be associated with substantial decreases in predation rates because of the inherent trade-off between spending energy to alter behavior in response to a predation threat and choosing other essential behaviors such as feeding (Houston et al., 1993). However, the trade-offs associated with behaviors induced by alarm cues are not often investigated. If trade-offs do exist between feeding and predator evasion behaviors, it would likely be most apparent earlier in life, when individuals are most vulnerable to predation (Werner et al., 1983).

Deviations from historical environmental conditions have also been shown to induce chronic stress and influence behavioral responses (Alfonso et al., 2021). Climate change models predict the environment will become more stressful to aquatic communities due to increases in mean and variation in temperature (Hoegh-Guldberg & Bruno, 2010). For example, juvenile Chinook salmon (*Oncorhynchus tshawytscha*) reared in temperatures consistent with climate change projections experienced reduced survival rates when exposed to predators compared to juveniles reared in water temperatures of their native stream (Marine & Cech, 2004). The influence of variable temperature-induced stress on behavior and survival is important to consider, particularly in species of low abundance and of conservation concern (Lande, 1988).

Recent evidence demonstrated the behavior and survival of lake sturgeon, a long-lived fish species of conservation concern in the Laurentian Great Lakes region (Bruch et al., 2016), are negatively influenced by temperature-induced chronic stress during rearing (Wassink et al., 2019). Lake sturgeon spend most of their time as juveniles and adults in large lakes and migrate up rivers to spawn (Peterson et al., 2007). Individuals demonstrate context-dependent alarm responses when exposed to alarm cue. During the early larval life stage, activity level and swimming speed increase after exposure to alarm cue, as a likely mechanism to escape predation (Wassink et al., 2019). In contrast, juveniles decrease activity when exposed to alarm cue, likely as a likely tactic to use their cryptic coloration to hide from predators (Wishingrad et al., 2014). Additionally, sturgeon behavior has been shown to be influenced by stress levels (Lankford et al., 2005, Wassink et al., 2019, Wassink et al., 2021). Larval lake sturgeon are consumed by numerous predator species (Waraniak et al., 2018), and accordingly, sturgeon use different behavioral tactics to evade different forms of predation. Larval lake sturgeon are susceptible to fish predators their downstream dispersal from spawning grounds to lentic nursery habitats (Waraniak et al., 2018), and accordingly, use different behavioral tactics to evade different forms of predation (Crossman et al., 2018). Individuals are susceptible to crayfish predators in nursery habitats characterized by comparatively low stream flow regimes (Crossman et al., 2018). It is important to understand how an increasingly stressful rearing environment due to climate change may influence the ability of larval lake sturgeon to effectively escape predation during the vulnerable larval stage.

Our objectives were to 1) assess lake sturgeon olfactory sensitivity to conspecific alarm cue and predator odors, 2) determine the impact of associative learning of conspecific alarm cue on survival when exposed to two distinct predators, 3) characterize potential trade-offs between feeding and predator evasion, and 4) determine the effect of variable rearing environment on predator evasion and feeding efficiency. We hypothesized that lake sturgeon can associate predator odors with alarm cue and change their behavior accordingly to avoid predation. Results will demonstrate the survival implications of alarm cue behaviors in larval lake sturgeon as well as the impact a changing climate may have on fitness and population levels of recruitment.

Methods

Rearing lake sturgeon

Gametes were collected from spawning lake sturgeon in the Upper Black River, Cheboygan County, MI, USA. On the day of collection, eggs from each female were fertilized with sperm of a unique male so that offspring families consisted of full siblings. Four families were raised in 2020, and eight families were raised in 2021. Following Fuller's earth de-adhesion and povidone-iodine disinfection (Betadine, Stamford, CT), fertilized eggs and larvae were reared in a stream-side rearing facility under standard lake sturgeon hatchery conditions (Crossman et al., 2011; Bauman et al., 2016). Eggs were reared in tumbling jars (Pentair J32, Apopka, FL). After hatch, yolk-sac larvae were transferred to 3 L tanks each containing 32 bio balls (CBB1-S; Pentair, Apopka, FL), mimicking natural substrate. Bio balls were removed from tanks once lake sturgeon consumed their yolk-sacs, indicating the beginning of the larval stage. Each sturgeon family was split into two groups of equal size and reared in ambient or variable temperatures. The ambient group was supplied with water pumped directly from the Upper Black River, ensuring the temperature of the tanks closely matched the temperature of their natal stream environment. The variable group was supplied with water from a partially recirculating system equipped with a heater and chiller (1800W Single Tube Bottom Heater, Pentair AES, Apopka, FL; Arctica ¼ HP 1000W Aquarium Chiller, Transworld Aquatic Enterprises, Inglewood, CA), allowing the temperature to be manipulated. Increased variation in water temperature is predicted with climate change and increases stress levels in fish (Hoegh-Guldberg & Bruno, 2010; Alfonso & Sadoul, 2021). To simulate the predicted increase variation in temperature, water temperatures for the variable group were set 2 °C warmer than ambient temperature during the day and 2 °C cooler than ambient during the night, resulting in a 4 °C larger diel temperature range.

Collecting odors

Lake sturgeon alarm cue was prepared following previously established methods (Wishingrad et al., 2014). One hundred larval lake sturgeon were euthanized by cervical dislocation according to approved Michigan State University animal use and care protocols and were homogenized with mortar and pestle. The whole-body homogenate was enclosed in 20-micron filter paper and immersed in 1 L of ground water for one hour. The filter paper containing the sturgeon was removed and the remaining water constituted lake sturgeon alarm cue. Five rusty crayfish (*Faxonius rusticus*), weighing a combined 37.8 g, were placed in an aerated tank containing 15L of water from the Upper Black River. After one hour, crayfish were removed from the tank, 2 L of the water was collected and used as crayfish odor. Two rock bass (*Ambloplites rupestris*), weighing a combined 38.6 g, were placed in an aerated tank containing 15 L of water from the Upper Black River. After one hour, rock bass were removed from the tank, 2 L of the water was collected and used as rock bass odor. All odor samples were stored at -20 °C.

Electro-olfactogram recordings

Juvenile lake sturgeon used for electro-olfactogram (EOG) recordings were transported to the University Research Containment Facility at Michigan State University, East Lansing, MI, USA and held in continuous flow-through tanks supplied with aerated well water. The EOG setup and recordings were conducted following previously established procedures (Scott et al., 2019) with modification to determine whether the lake sturgeon olfactory organ was sensitive to lake sturgeon alarm cue, crayfish odor, or rock bass odor. Briefly, lake sturgeon were anesthetized with tricaine methanesulfonate (50 mg L^{-1}, MS-222, Sigma-Aldrich, St. Louis, MO) and immobilized with an injection of gallamine triethiodide (30 mg kg^{-1} of body weight, Sigma-Aldrich). Gills were continuously irrigated with aerated water containing 25 mg L^{-1} MS222 throughout the experiment. The olfactory lamellae were surgically exposed, and the differential EOG response magnitude was recorded using glass capillary borosilicate electrodes filled with

0.4% agar in 0.9% saline and connected to solid state electrodes with Ag/AgCl pellets (Warner Instruments LLC, model ESP-M15N, Hamden, CT) in 3 M KCl. EOG signals were amplified (NeuroLog system, Digitimer, model NL102, Hertfordshire, UK), filtered (low-pass 60 Hz, Digitimer, model NL125), digitized (Molecular Devices LLC, Digidata 1440A, San Jose, CA), and recorded on a computer running AxoScope 10.4 software (Molecular Devices LLC).

Prepared lake sturgeon alarm cue stored at -20 °C was serially diluted with filtered water to yield 0.027 to 27000 PPM dilutions for the lake sturgeon alarm cue dilution-response recordings. We chose these dilutions because approximately 270 PPM lake sturgeon alarm cue dilution was shown to influence larval lake sturgeon behavior in Wishingrad et al.,2014. A 10^{-3} M stock solution of L-alanine in deionized water was prepared, stored at 4 °C, and diluted with filtered water to yield a 10^{-5} M solution. A 10^{-5} M L-alanine solution was introduced to the olfactory epithelium for 4 seconds, and the response was recorded to correct for variations in olfactory sensitivity among fish. The olfactory epithelium was flushed with filtered water for 2 minutes, and then the blank control was introduced and recorded. Next, the alarm cue (starting at 0.027 to 27000 PPM) was applied, recorded, and flushed. Blank control and 10^{-5} M L-alanine standard were measured repeatedly (approximately after every 3 dilutions of stimuli) throughout each recording session. The EOG response magnitudes were measured in mV. The normalized EOG response was calculated as normalized EOG amplitude = (Rt − Rb) ÷ (Ra − Rb), where Rt is the response magnitude to the alarm cue test stimulus, Rb is the response magnitude to the blank, and Ra is the response magnitude to 10^{-5} M L-alanine (Scott et al., 2018). The threshold of detection was defined as the lowest dilution in which the alarm cue elicited a larger response than the blank (paired t-test, n = 5, one tailed). The same EOG methods as the lake sturgeon alarm cue dilution-response recordings were used to record the olfactory response to crayfish and rock bass odors. Prepared crayfish and rock bass odors stored at -20 °C were serially diluted with filtered water to yield 2.7, 270, and 27000 PPM dilutions.

Crayfish predation trials

Rusty crayfish were captured via minnow traps in the shallow backwater habitats of the Upper Black River at the base of Kleber dam, an area upstream of where larval sturgeon can be found. Crayfish were held communally in circular tank (1 m diameter) supplied with water from the Upper Black River for 24-36 hours with no food. Replicate 3 L tanks in ambient and variable temperature groups were randomly selected to be exposed to either crayfish odor and alarm cue, or ground water. Twelve hours prior to predation trials, 0.8 mL of alarm cue and 0.8 mL of predator odor were added to treatment tanks, resulting in an alarm cue dilution of 270 PPM, while 0.8 mL of ground water and 0.8 mL of river water was added to control tanks. This dilution of alarm cue was shown to induce behavioral responses in larval lake sturgeon in Wishingrad et al. (2014).

One hour prior to predation trials, a rusty crayfish (carapace length range: 20-26 mm) was placed in an aerated 15 L tank to acclimate and ensure the water in the test arena tank contained crayfish odor. Each individual was used in one trial. At the beginning of predation trials, 10 larval lake sturgeon were added to the tank. The sturgeon included in each trial were from one of four families, from either ambient or variable temperature groups, and had been previously exposed to the mixture of sturgeon alarm cue and crayfish odor, or ground water control. After a three-hour exposure, predators were removed, measured, and surviving lake sturgeon were quantified. Within each family, five predation trials were conducted with each combination of rearing temperature group and exposure odor group (ambient and alarm cue & rusty crayfish odor, ambient and water, variable and alarm cue & rusty crayfish odor, variable and water) for a total of 80 trials. We fit generalized linear models using a binomial distribution to model the probability of sturgeon larvae surviving as a function of exposure odor, rearing temperature group, and predator size in R v 4.2.0. Competing models were ranked via AICc.

Rock bass predation trials

Rock bass were captured via barge electrofishing, fyke net, or hook-and-line angling and held communally in 3.58 m x 0.5 m tank supplied with water from the Upper Black River for 24-36 hours with no food. All rock bass were collected between Kleber and Tower dams on the Upper Black River, a section not inhabited by lake sturgeon. Replicate 3 L tanks in ambient and variable temperature groups were randomly selected to be exposed to either rock bass odor and alarm cue, or ground water. Trials were conducted with the four families of lake sturgeon larvae reared in 2020. Within each family, two predation trials were conducted with each combination of rearing temperature group and exposure odor group (ambient and alarm cue & rock bass odor; ambient and water; variable and alarm cue & rock bass odor; variable and water) for a total of 32 trials. Twelve hours prior to predation trials, 0.8 mL of alarm cue and 0.8 mL of predator odor were added to treatment tanks, while 0.8 mL of ground water and 0.8 mL of river water was added to control tanks. Two hours prior to predation trials, two rock bass (length range: 10.8-24.8 cm) were placed into one of two flow-through raceways with dimensions 7.15 m x 0.5 m with a flow rate of approximately 0.085 m/s. Each individual was used in one trial.

One hour prior to the trial, flow rates were increased to 0.134 ± 0.003 m/s with recirculating pumps in order to reflect flow rates possible during larval drift period and to ensure that rock bass odor was present throughout the raceway. Additionally, one hour before the trial, the light levels in the raceway were set to 0.20 lux to simulate the light levels of a moonlit night. At the beginning of predation trials, 8 lake sturgeon larvae, 8 white sucker larvae (*Catostomus* *commersonii*), and 8 mayfly larvae (Heptageniidae) were added to the upstream end of the raceway simultaneously. White sucker and Heptageniid mayfly larvae were included in these trials to more accurately portray the community of prey available to potential lake sturgeon larvae predators during the larval drift period (Waraniak et al., 2017). White sucker larvae were captured in drift nets placed in the Upper Black River at night. Heptageniid mayflies were found on cobble in the Upper Black River. Eight individuals of each taxon were added to the upstream end of the raceway every 15 min (0, 15, 30, 45 min) for a total of 32 individuals of each taxa introduced during a one hour trial. Surviving prey were captured in nets placed over the outflows of the raceway. The sturgeon included in each trial were from one of five families, from either ambient or variable temperature groups, and had been previously exposed to the mixture of sturgeon alarm cue and rock bass odor, or ground water control.

Each trial was complete after one hour. Predators were removed from raceways and measured while surviving individuals were quantified. We fit generalized linear models using a binomial distribution to model the probability of sturgeon larvae surviving as a function of exposure odor, rearing temperature group, and predator size in R v 4.2.0. Competing models were ranked via AICc.

Feeding trials

After lake sturgeon from ambient or variable groups reached the larval stage, they were fed brine shrimp in accordance with common lake sturgeon hatchery practices (Crossman et al., 2011). Feeding trials began 1 or 3 days after exogenous feeding began. Trials were conducted with the eight families of lake sturgeon larvae reared in 2021. Fourteen hours prior to feeding trials, 10 larval lake sturgeon from the same family and temperature group (ambient or variable) were placed in a 3 L tank. Twelve hours prior to trials, 0.8 mL of alarm cue and 0.8 mL of rock bass odor was added to treatment tanks, and 0.8 mL of ground water and 0.8 mL of river water was added to control tanks. At the beginning of the trial, a meal of brine shrimp (8.6% of body weight; Bauman et al., 2016) was added to the 3 L tank along with either 0.8 mL of rock bass odor or 0.8 mL of river water. Brine shrimp not consumed by sturgeon was collected in a filter placed on outflow of the tank. Fifteen minutes after feeding, larval sturgeon were removed from the tanks and the remaining water was poured through the filter to capture any remaining brine shrimp. The filters containing excess brine shrimp were then dried and weighed to the nearest 0.1 mg with an analytical balance. The dry weight of the filter before the trial was subtracted from the dry weight of the filter containing excess brine shrimp to determine the amount of uneaten food. The proportion of food consumed was calculated by subtracting the weight of excess food from the initial amount added to the tank, then dividing by the initial amount added. Within each family, up to four feeding trials were conducted with each combination of rearing temperature group, exposure odor group, and food odor for a total of 244 feeding trials. We fit generalized linear mixed models using a beta distribution (R package glmmTMB; Brooks et al., 2017) to model the proportion of brine shrimp consumed as a function of exposure odor, odor added to food, days post exogenous feeding (DPEF), and rearing temperature group, and included the random effect of family. Competing models were ranked via AICc.

Results

Electro-olfactogram recordings

Electro-olfactogram recordings demonstrated that lake sturgeon were capable of detecting odors at dilutions used in exposures prior to predation and feeding experiments. Lake sturgeon alarm cue elicited dilution-dependent olfactory responses in six-month-old juvenile lake sturgeon (Figure 3.1). Lake sturgeon alarm cue was a potent odor with a threshold of detection less than 0.027 PPM, the lowest concentration tested (paired t-test, one tailed, t= 6.08, p-value = 0.0018). The EOG recordings showed the crayfish and rock bass odors at 270 PPM, the dilution used in predation trials, were detected by lake sturgeon olfactory epithelia (Figure 3.2). Crayfish and rock bass odors (270 PPM) induced larger olfactory responses than background water (paired t test, one tailed, t = 5.91, p-value = 0.0020 and t = 7.26, p-value = 0.0009, respectively).

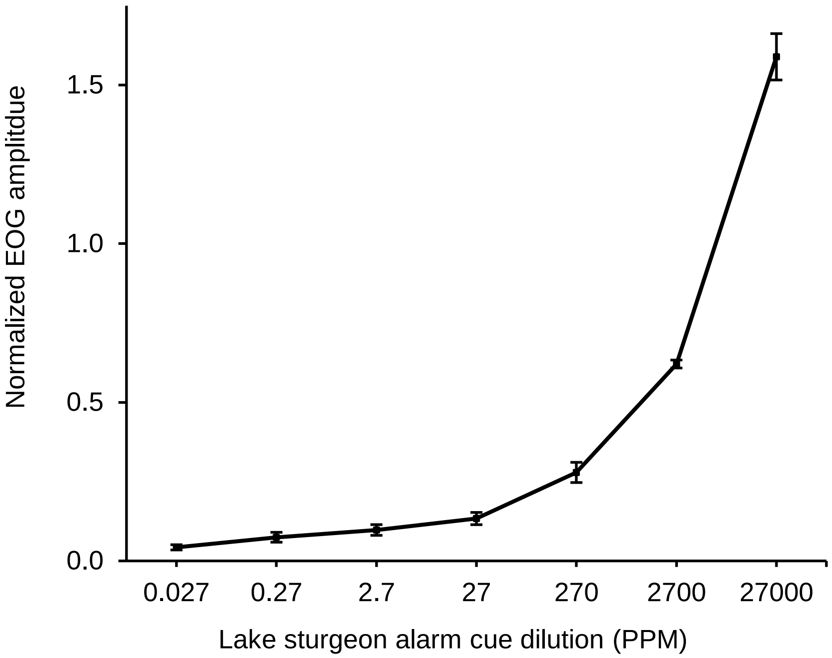

Figure 3.1. Lake sturgeon alarm cue elicited dilution-dependent responses measured with electro-olfactogram (EOG) recordings in six-month-old juvenile lake sturgeon (mean ± SE, n= 5). The alarm cue dilutions are displayed as parts per million (PPM). The response amplitude was normalized to the response amplitude of 10^{-5} M L-alanine.

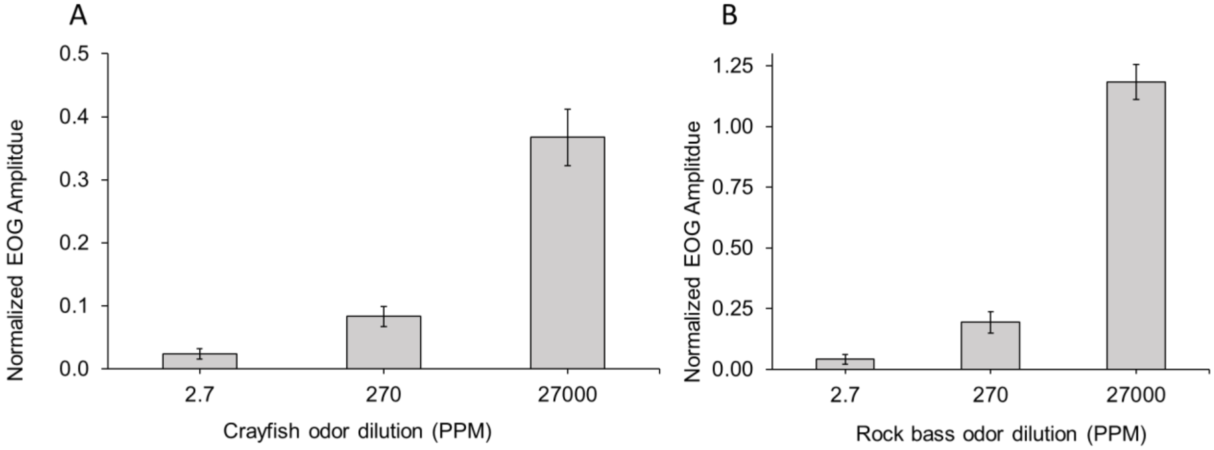

Figure 3.2. Six-month-old juvenile lake sturgeon detect (A) crayfish and (B) rock bass odor in the olfactory epithelium as measured with electro-olfactogram (EOG) recordings (mean ± SE, n= 5). The odor dilutions are displayed as parts per million (PPM). The response amplitude was normalized to the response amplitude of 10^{-5} M L-alanine.

Crayfish predation trials

The top model for sturgeon predation during rusty crayfish trials included predator size, prior odor exposure, rearing temperature group, and the interaction between exposure odor and rearing temperature (Table 3.1). Predator size was positively associated with the probability an individual would be consumed by a crayfish (Table 3.2). The effect of rearing temperature and the interaction between rearing temperature group and odor exposure were also significant (Table 3.2). Focusing on lake sturgeon larvae reared in ambient temperatures, survival was higher when the larvae were previously exposed to alarm cue and rusty crayfish odor (Figure 3.3). Results indicate that in ambient rearing conditions, larval lake sturgeon are capable of associative learning and associate the odor of a predator with alarm cue to escape predation, thereby increasing lake sturgeon survival rates. Conversely, lake sturgeon larvae reared in the variable, more stressful temperature regime, had reduced survival when pre-exposed to alarm cue and rusty crayfish odor compared individuals pre-exposed to ground water (Figure 3.3). Results indicate lake sturgeon associative learning did not reduce risk of rusty crayfish predation when reared in variable temperature (more stressful) environments. Instead, survival decreased after pre-exposure to alarm cue and rusty crayfish odor.

Table 3.1. Competing models describing the proportion of lake sturgeon larvae surviving experimental rusty crayfish and rock bass predation trials as a function of independent variables including exposure odor, rearing temperature, and predator size, as well as the interactions between these terms. "*" indicates the interaction between the terms was also included in the model. Models are ranked via AICc.

Independent variable	**Parameters included**	**ΔAICc**	**df**
Rusty crayfish predation	Exposure odor * Rearing temperature + Predator size	0	5
	Exposure odor * Rearing temperature * Predator size	3.6	8
	Rearing temperature * Exposure odor	4.0	4
	Exposure odor + Rearing temperature * Predator size	6.9	5
	Exposure odor + Rearing temperature + Predator size	7.3	4
	Predator size	8.5	2
	Exposure odor + Predator size	9.1	3
	Rearing temperature	11.8	2
	Exposure odor + Rearing temperature	12.4	3
	Exposure odor	14.0	2
Rock bass predation	Exposure odor + Rearing temperature + Predator size	0	4
	Rearing temperature + Exposure odor * Predator size	2.2	3
	Exposure odor + Rearing temperature * Predator size	2.6	5
	Exposure odor * Rearing temperature * Predator size	2.8	8
	Exposure odor * Rearing temperature + Predator size	2.8	5
	Exposure odor	8.2	2
	Exposure odor + Rearing temperature	8.9	3
	Predator size	10.5	2
	Rearing temperature * Exposure odor	11.5	4
	Rearing temperature	15.1	2

Table 3.2. Parameters included in AICc-selected models describing the proportion of larval lake sturgeon surviving rusty crayfish and rock bass predation trials.

Independent variable	**Parameter**	**Estimate**	**Std. Error**
Rusty crayfish predation	Intercept	-0.69572	1.1242
	Exposure odor	-0.42828	0.2918
	Rearing temperature	-0.9545	0.2757
	Predator size	0.12127	0.0491
	Exposure odor * Rearing temperature	1.21776	0.3981
Rock bass predation	Intercept	-1.82199	0.60677
	Exposure odor	0.45571	0.13067
	Rearing temperature	-0.29313	0.13347
	Predator size	0.11646	0.03448

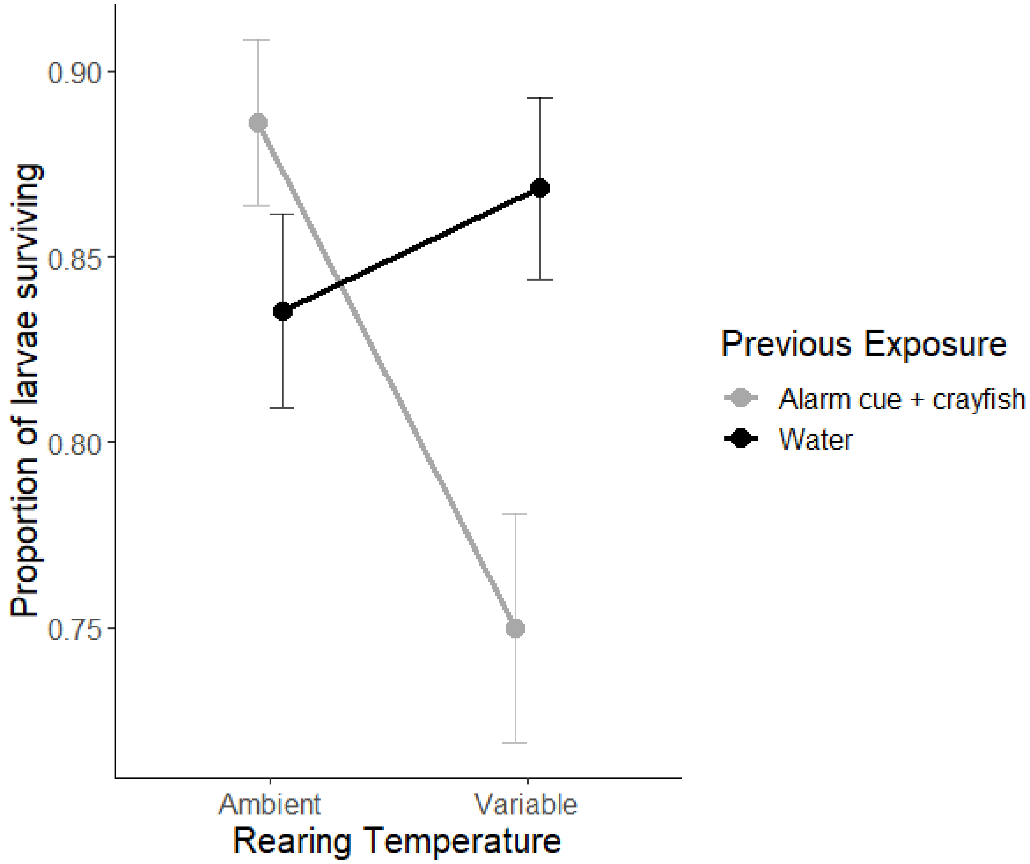

Figure 3.3. The proportion of lake sturgeon larvae surviving after a 3-hour exposure to a rusty crayfish in a 15 L tank. Points and error bars reflect predictions and 95% confidence intervals from the AICc-selected model described in Table 3.1.

Rock bass predation trials

The top model for larval lake sturgeon predation during rock bass trials included predator size, exposure odor, and rearing temperature group (Table 3.1). Predator size was positively associated with the probability an individual would be consumed by a rock bass (Table 3.2). Sturgeon reared in ambient temperatures had 7.3% higher survival than those reared in variable temperatures (Table 3.2; Figure 3.4), indicating larvae reared in stressful, variable temperature environments were more susceptible to rock bass predation. Additionally, sturgeon previously exposed to alarm cue and rock bass odor had 10.8% higher survival (Table 3.2; Figure 3.4). Results indicate lake sturgeon larvae can associate sturgeon alarm cue with predator odor and subsequently changed behavior to reduce risk rock bass predation. Unlike rusty crayfish predation, sturgeon reared in variable and ambient temperature regimes used associative learning to escape predation.

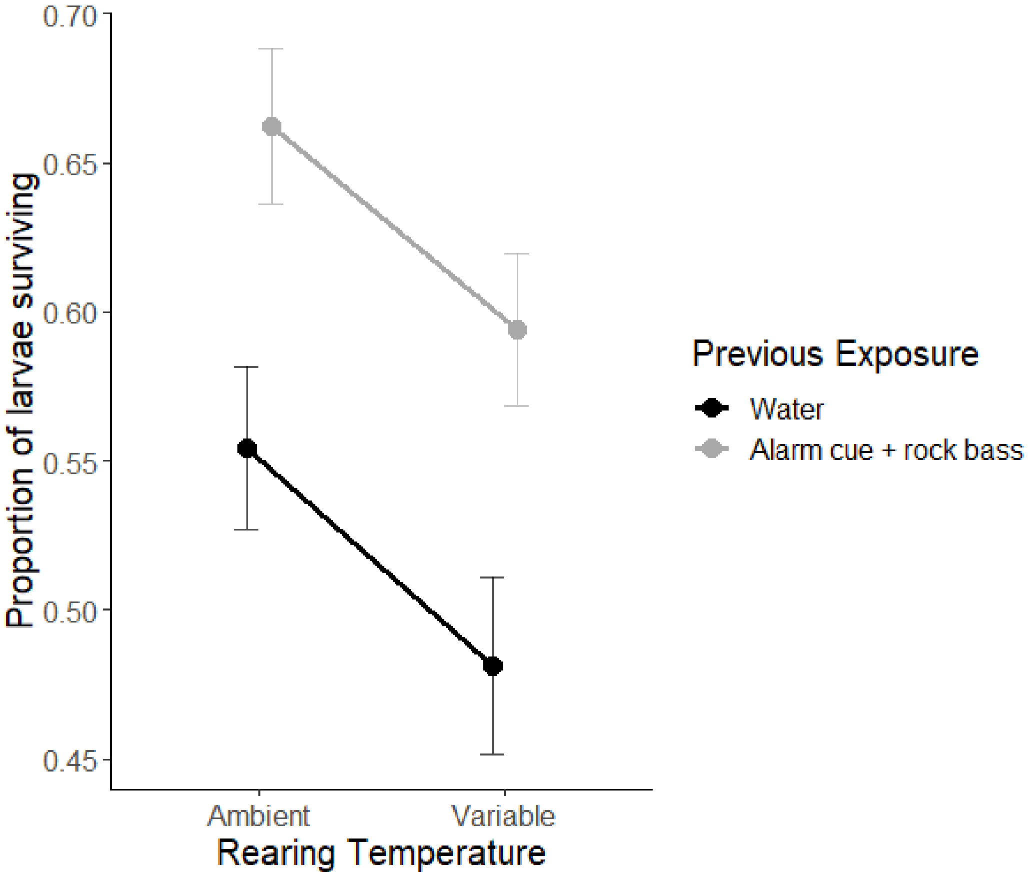

Figure 3.4. The proportion of lake sturgeon larvae surviving after being exposed to rock bass predators in a 1-hour flow through raceway assay. Points and error bars reflect predictions and 95% confidence intervals from the AICc-selected model described in Table 3.2.

Feeding experiments

The top model describing the proportion of food a tank of 10 larval lake sturgeon consumed included the odor added to the food, exposure odor, larval age (days post exogenous feeding, DPEF), rearing temperature group, as well as the interaction between exposure odor and food odor, interaction between exposure odor and days post exogenous feeding, interaction between food odor and larval age, and finally, the three-way interaction between exposure odor, food odor, and larval age (Table 3.3). The top model also indicated the level of variance in the proportion of food consumed was 0.097. Our AICc-selected model indicates lake sturgeon larvae reared in variable temperatures consumed more food than larvae reared in ambient temperatures (Table 3.4; Figure 5). Sturgeon feeding 3 days post-exogenous feeding (DPEF) consumed a higher proportion of food than those 1 DPEF (Table 3.4; Figure 3.5). Additionally, the top model included interactions between exposure odor and DPEF, between food odor and DPEF, and the three-way interaction between exposure odor, food odor, and days post exogenous feeding (Table 3.3). Within trials where sturgeon were 1 DPEF, the proportion of food consumed by fish previously exposed to alarm cue and predator odor and those exposed to only ground water was similar when ground water was added to the food. However, 1 DPEF fish exposed to alarm cue and predator odor consumed less food than fish exposed to ground water when predator odor was added to the food (Figure 3.4). Results demonstrate that larval lake sturgeon exposed to a predator odor with alarm cue change behavior in a subsequent exposure to the predator odor. Additionally, larval lake sturgeon that detected a predatory threat changed behavior and reduced feeding rates, indicating a trade-off between feeding rate and predator avoidance. The proportion of food consumed by sturgeon 3 DPEF was less influenced by exposure to alarm cue and rock bass odor and rock bass odor in food than sturgeon 1 DPEF (Table 3.3). In these trials, similar proportions of food were consumed when predator odor or water had been added to the food, regardless of the exposure odor (Figure 3.4). Results indicate that larvae 3 DPEF do not change feeding behaviors in response to detecting predators in the same manner as larvae 1 DPEF.

Table 3.3. Competing models describing the proportion of food consumed by lake sturgeon larvae during feeding trials as a function of independent variables including exposure odor, odor added to food, days post exogenous feeding (DPEF), and rearing temperature group, as well as the interactions between these terms. "*" indicates the interaction between the terms was also included in the model. Models are ranked via AICc.

Parameters	**ΔAICc**	**df**
Exposure odor * Food odor * DPEF + Rearing temperature	0	11
Exposure odor + Food odor * DPEF + Rearing temperature	7.9	8
Exposure odor * Food odor * DPEF * Rearing temperature	8.4	18
Exposure odor * Food odor * DPEF	12.0	10
Exposure odor + Food odor + DPEF + Rearing temperature	19.7	7
Exposure odor + DPEF + Rearing temperature	22.1	6
Exposure odor * Food odor + Rearing temperature	24.6	7
Food odor + DPEF + Rearing temperature	25.7	6
Exposure odor * Food odor + DPEF	28.5	7
Exposure odor * Food odor	36.0	6

Table 3.4. Summary of the AICc-selected model describing the proportion of food consumed by lake sturgeon larvae during feeding trials. DPEF is an abbreviation for days post exogenous feeding.

Parameter	**Estimate**	**Std. Error**
Intercept	0.9014	0.0323
Exposure odor	0.02597	0.0299
Food odor	-0.0315	0.0299
DPEF	-0.27048	0.0466
Rearing temperature	0.06044	0.0158
Exposure odor * Food odor	0.02051	0.0426
Exposure odor * DPEF	0.11891	0.0437
Food odor * DPEF	0.21551	0.0437
Exposure odor * Food odor * DPEF	-0.19076	0.0624

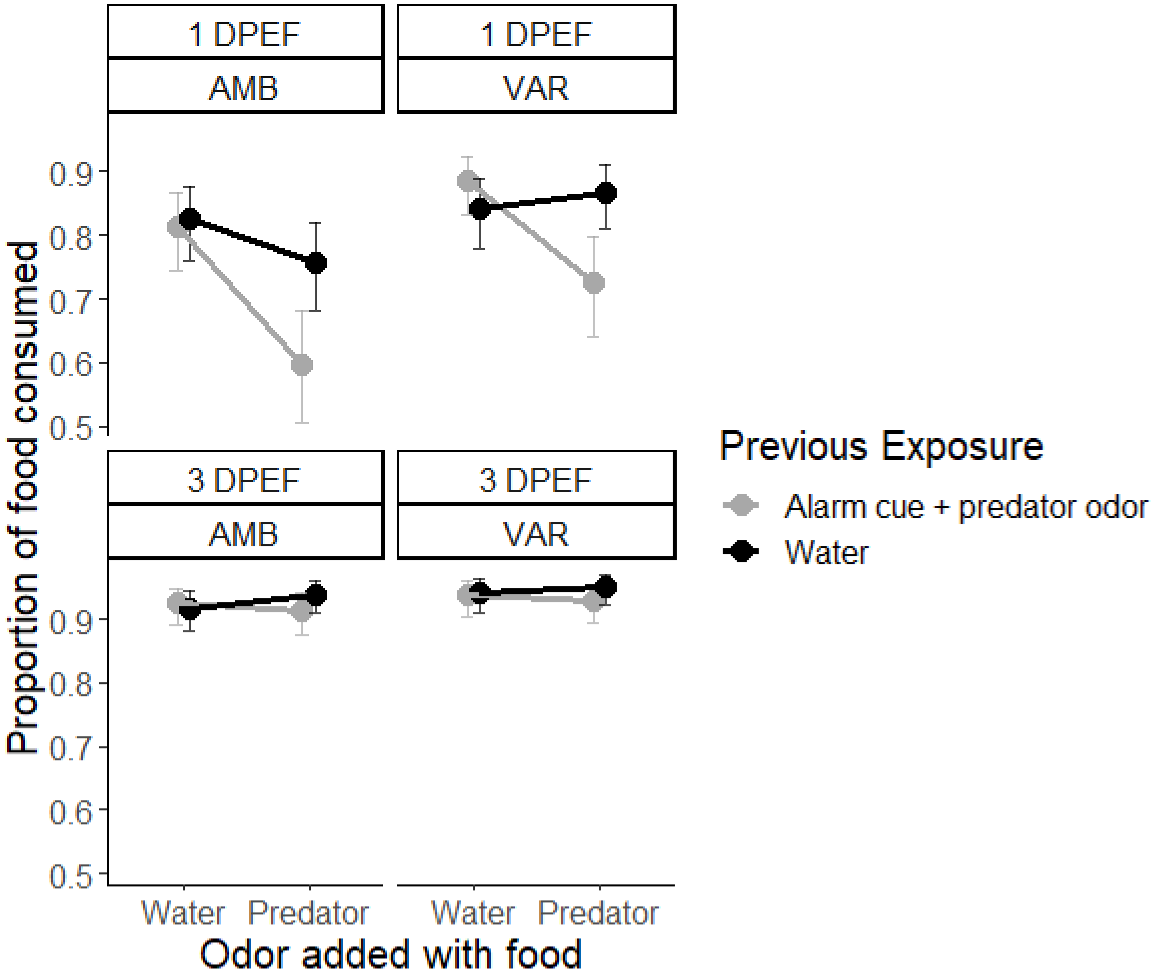

Figure 3.5. The proportion of food consumed by lake sturgeon larvae in feeding trials. Points and error bars reflect predictions and standard error from the AICc selected model identified in Table 3.3.

Discussion

Results support our hypothesis that larval lake sturgeon are capable of using associative learning to associate predator odors with alarm cues, and subsequently change behavior to reduce predation risk when predator odors are detected. Previous research has documented lake sturgeon behaviorally respond to alarm cue (Wishingrad et al., 2014; Wassink et al., 2021). Here, we demonstrate these changes in larval lake sturgeon behavior result in higher survival when exposed to rock bass and rusty crayfish, two known lake sturgeon predators. Additionally, we found variable rearing temperatures led to reduced survival rates in predation trials for groups exposed to alarm cue and predator odor, relative to those reared in ambient temperatures. Variable temperatures associated with climate change may result in lower recruitment rates of lake sturgeon due to the diminished ability of larvae to use chemical cues to escape predation. Further, we show a trade-off exists between predator avoidance and feeding behaviors, where treatment groups with the lowest feeding rate also had highest survival rates when exposed to predators. It is necessary for some larvae to be consumed by predators for other individuals to detect alarm cue and realize the survival benefits of associative learning. Therefore, this effect is most likely to be observed in larval populations with higher densities and may be one mechanism for depensatory effects which have prevented populations from recovering (Pollock et al., 2015).

Response to alarm cue

Behaviors in response to alarm cues have evolved in diverse groups of aquatic organisms with presumed fitness benefits (i.e., decreased predation rates; Chivers & Smith, 1998), though the survival implications and potential trade-offs of these behaviors are seldom assessed. Species associate odors of predators with conspecific alarm cues when detected simultaneously, and express evasion behaviors when the predator odor is subsequently detected (Kelley & Magurran, 2003). Our study indicates lake sturgeon during early life stages have the ability to associate odors of predators with conspecific alarm cues and increase rates of survival when exposed to taxonomically and ecologically diverse predators. Electrophysiology experiments show the lake sturgeon olfactory epithelia detect lake sturgeon alarm cue and predator odors at low dilutions (Figure 3.1 and Figure 3.2, respectively). When exposed to rock bass, lake sturgeon larvae previously exposed to alarm cue and rock bass odors had higher rates of survival than those exposed to water (Figure 3.4), indicating the behavior expressed by larval sturgeon increased fitness. Trials were designed to simulate the conditions present during larval lake sturgeon dispersal, when larval lake sturgeon are susceptible to high levels of predation as they drift from spawning grounds downstream to nursery habitat (Smith & King, 2006). One field study from the Upper Black River population found lake sturgeon present in the diets of 16 of the 29 fish species sampled (Waraniak et al., 2018). Our results indicate a high degree of selection may be occurring for any advantageous traits, such as efficacious use of chemical cues, to escape predation.

Impact of variable temperature rearing environment

Rearing in a stressful environment due to increased variation in water temperature influenced survival of larval lake sturgeon in both rock bass and rusty crayfish predation trials. In rock bass trials, larval lake sturgeon reared in variable temperatures experienced lower survival rates, regardless of whether they were pre-exposed to alarm cue and predator odor, or water (Figure 3.4). As environments become more stressful with climate change (Alfonso et al., 2021), data suggests that larval lake sturgeon survival rates during the critical drifting period may decrease. High stress levels have been associated with poor evasion from predators in other fish species including Chinook salmon, coho salmon, bonefish (*Albula vulpes*), and great barracuda (*Sphyraena barracuda*; Marine & Cech, 2004; Olla & Davis, 1992; Danylchuk et al., 2007; Brownscombe et al., 2014).

In contrast, when considering rusty crayfish predation of larval lake sturgeon, variable rearing temperatures and higher stress levels were not always detrimental. When lake sturgeon larvae were exposed to only ground water, survival was higher among fish that were reared in the stressful, variable temperature regime (Figure 3.3). Similar results were reported by Wassink et al. (2019), where rusty crayfish predation rates were lower on sturgeon reared in stressful conditions. This counterintuitive effect of temperature-induced chronic stress on predation by rusty crayfish may be due to higher sturgeon activity rates. Green sturgeon (*Acipenser medirostris*) and lake sturgeon with elevated cortisol levels have been shown to display higher baseline levels of activity (Lankford et al., 2005; Wassink et al., 2019, respectively), which likely allows the sturgeon to escape rusty crayfish predation (Crossman et al., 2012). However, rearing in a stressful environment has a negative impact on larval lake sturgeon ability to use chemical cues to escape predation. Although ambient-reared sturgeon previously exposed to alarm cue and rusty crayfish odor had higher survival rates than those exposed to only ground water (88.6% vs. 83.5%), variable-reared sturgeon exposed to alarm cue and rusty crayfish odor had lower survival rates than those exposed to ground water (74.9% vs. 86.8%). Results indicate that the chronic stress imparted by the variable temperature rearing environment caused larval lake sturgeon behavioral response after detecting a known predator to become maladaptive. Lake sturgeon larvae have increased activity when reared in stressful environments (Lankford et al., 2005; Wassink et al., 2019), and when the threat of predation is detected (Wassink et al., 2020; Wassink et al., 2021). We reasoned an increase in activity levels due to stressful rearing environment, paired with an increase in activity level due to larval lake sturgeon recognizing the odor of the rusty crayfish as a potential predator, resulted in maladaptive behaviors. If lake sturgeon larvae became fatigued during the trial and activity levels dropped, they would be more easily captured by the rusty crayfish. As a result, the behaviors associated with predator recognition via associative learning, which had a positive influence on survival rates during rock bass predation trials and rusty crayfish predation trials when fish were reared in ambient temperatures, had a negative influence on survival in fish reared at variable temperatures. To summarize, a stressful rearing environment negatively influenced larval sturgeon survival when exposed to a predator. Fish reared in variable temperatures had lower survival rates when exposed fish predators regardless of odor treatment, whereas the stress influenced larval survival rates differently depending on the odor treatment when exposed to crayfish predators. Although many studies assessing the effects climate change may have on ectothermic species focus on physiological responses to changes in temperature (meta-analysis by Bennett et al., 2018), it is also important to understand the effects a changing climate has on chemical cue-mediated predator evasion behaviors, as these exist in a wide diversity of aquatic taxa (Chivers & Smith 1998).

Larvae reared in ambient temperature conditions and exposed to alarm cue and rusty crayfish odor have higher rates of survival during rusty crayfish predation trials than those exposed to ground water alone (Figure 3.3). A previous experiment showed young lake sturgeon were consumed by rusty crayfish at higher rates than by fish predators (Crossman et al., 2018), indicating rusty crayfish are an important predator of lake sturgeon. This, paired with the fact that rusty crayfish have invaded much of the range of lake sturgeon (Olden et al., 2006), highlight the importance of the ability of larval lake sturgeon to recognize crayfish as predators and choose behaviors to escape predation. Lake sturgeon larvae display different behaviors to escape predation by fish versus crayfish (Crossman et al., 2018). Our results indicate lake sturgeon larvae are able to change behavior contextually, based on olfactory cues, to evade both types of predators, thus increasing the probability of survival.

Trade-offs

We have demonstrated sturgeon larvae recognize predator odors as threats and change their behavior accordingly, resulting in increased survival when exposed to fish and crayfish predators. Results also demonstrate that larval lake sturgeon do not always display antipredator behavior; if this behavior was always exhibited, survival would be equal across treatment groups. This is intuitive; evading predators is energetically costly and necessitates that the potential prey must cease other behaviors such as feeding. In our feeding experiments, we found larval lake sturgeon 1 DPEF that were previously exposed to predator odor and alarm cue consumed less food if predator odor was administered with the food (Figure 3.5). The larvae that had been pre-exposed to predator odor and alarm cue had the highest survival rates during rusty crayfish and rock bass predation trials but consumed the smallest proportion of food when predator odor was present during feeding trials. Results provide evidence for a trade-off, where the larval lake sturgeon that recognize the predator odor as a risk of predation display antipredator behavior, and as a result, consume less food than groups that were not exposed to alarm cue and predator odor, or groups that did not have predator odor added to the food. Interestingly, this trade-off was not apparent in lake sturgeon 3 DPEF; instead, all treatment groups consumed similar proportions of food. This phenomenon may be explained by lake sturgeon early life history. Following the absorption of the yolk-sac, lake sturgeon larvae exit the cover of the stream substrate in riverine spawning areas and enter the water column to disperse and feed. As a result, the larvae drift downstream with the flow of the river, travelling several kilometers until they reach lentic nursery habitat (Auer & Baker, 2002). Assuming population densities of sturgeon predators are relatively similar in the river and nursery habitats, we reason the threat of predation on larvae is higher during the dispersal because drifting individuals will encounter a greater number and taxonomic diversity of predators as they traverse a large stretch of the river. Therefore, antipredator behaviors would be most advantageous immediately following the transition to exogenous feeding, whereas the need to feed may outweigh the risk of predation in nursery habitats. This may help explain the differences in proportion of food consumed by fish 1 and 3 DPEF. Alternatively, it is possible that the 3 DPEF lake sturgeon larvae habituated to feeding in a hatchery setting so that they did not change their feeding rate in response to recognizing a risk of predation. However, other species reared and fed in aquaria for much longer periods of time have been documented changing feeding rates in response to predator odor (Holmes & McCormick, 2010; Mitchell et al., 2012).

Summary

In conclusion, our study shows that larval lake sturgeon can associate predator odor with conspecific alarm cue, resulting in higher survival rates when encountering two abundant and ecologically different aquatic predators. Lake sturgeon detected alarm cue and predator odors at low concentration, indicating an olfactory system highly specialized to recognize these cues. Additionally, sturgeon reared in a variable temperature, stressful environment had lower survival rates when exposed to a fish predator and a maladaptive behavioral response when detecting a rusty crayfish predator. Finally, lake sturgeon larvae decrease feeding rates when a predator odor is detected immediately following the onset of exogenous feeding, but not days later when the overall risk of predation was lower. Moving forward, additional studies could be conducted to determine the influence of other variables associated with climate change (e.g., elevated temperature, variable discharge) may have on the use of chemical cues to modulate antipredator behaviors of other aquatic species of conservation concern.

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CHAPTER 4:
Lake sturgeon larvae use kairomone cues of sympatric and allopatric species to evade predation

Introduction

Alarm cues are composed of compounds released when an individual is attacked by a predator. Conspecifics detect alarm cues and recognize that the presence of the cue indicates predation risk. Many aquatic organisms use alarm cues including crustaceans, bivalves, jawless fish, ray-finned fish, and amphibians (Reviewed in Chivers and Smith 1998). Some species can additionally recognize or ‘eavesdrop’ on the alarm cues of other species. Heterospecific alarm cues can act as kairomones, chemical cues given off by one species and are detected by and benefit another species, and serve a similar role as conspecific alarm cues by warning the receiver of proximity to predators (Ferrari et al., 2010).

The degree to which a species responds to a heterospecific alarm cue is often based on the taxonomic relatedness between species, or whether the two species are sympatric and have evolved the ability to detect and recognize the heterospecific alarm cue from a co-distributed predator. Species often use heterospecific alarm cues to warn of predation threats in communities where multiple prey species share predators (Ferrari et al., 2010). For example, rainbow darters (*Etheostoma caeruleum*) respond behaviorally to alarm cues given off by sympatric Ozark minnows (*Notropis nubilus*) but not allopatric bumblebee gobies (*Brachygobius doriae*; Anderson and Mathis 2016). Rainbow darters from populations living in sympatry with Oklahoma salamander (*Eurycea tynerensis*) respond to the salamander alarm cue, while individuals from populations allopatric to Oklahoma salamanders do not, demonstrating the potential for geographical specificity in alarm cue recognition (Anderson & Mathis 2016).

Closely related species have been shown to respond to one another's alarm cues, regardless of whether the species live in sympatry. This reciprocity has been well-characterized in the Pomacentridae (clownfishes and damselfishes; Mitchell et al., 2012) and Petromyzontidae (northern lampreys; Hume & Wagner, 2018). In both cases, individuals respond to heterospecific alarm cues, and the magnitude of the response is dependent on the relationship between the species. More phylogenetically closely related species generally display a greater response to kairomones (Mitchell et al, 2012; Hume & Wagner 2018).

It is particularly advantageous for species with low population abundance, such as threatened and endangered species, to behaviorally respond to heterospecific alarm cues because there are relatively few nearby conspecifics that could emit alarm cues to warn of predation. One threatened species that is known to respond to alarm cues is the lake sturgeon (*Acipenser fulvescens*). Lake sturgeon populations are threatened or endangered throughout most of their native range (Bruch et al., 2016). Adults live primarily in large lakes and are exposed to a different suite of species than early life stages, which are produced and remain in spawning streams before dispersing downstream to nursery habitats (Peterson et al., 2007). Larvae experience high levels of predation during the dispersal period and are vulnerable to predation by numerous taxa (Caroffino et al., 2010a; Waraniak et al., 2019). Lake sturgeon demonstrate innate behavioral responses to conspecific alarm cue at the larval and juvenile stages (Wassink et al., 2019; Wishingrad et al., 2014). In this study, we sought to determine whether lake sturgeon can also use heterospecific alarm cues as kairomones to recognize and evade predators.

In small remnant populations, where spawning lake sturgeon abundance is low and larvae are relatively uncommon, the ability to perceive and respond (i.e., eavesdrop; Schoeppner & Relyea, 2009) to alarm cues emitted by heterospecific species would be advantageous. Lake sturgeon disperse at the same time as other, more abundant fish larvae such as white suckers (*Catostomus commersonii*; Smith & King, 2005), so it would therefore be advantageous for lake sturgeon larvae to respond to alarm cues released by white sucker larvae. To determine whether lake sturgeon larvae use other species' alarm cues as kairomones to avoid predation, we evaluated three competing hypotheses:

1) Lake sturgeon are unable to detect and respond to other species alarm cues as kairomones
2) Lake sturgeon are able to detect and respond to only alarm cues of sympatric species as kairomones
3) Lake sturgeon are able to detect and respond to alarm cues of taxonomically diverse species as kairomones

To test these hypotheses, we exposed larval lake sturgeon to one of four odor treatments: (1) predator odor with lake sturgeon alarm cue, (2) predator odor with white sucker (sympatric species) alarm cue, (3) predator odor with round goby (*Neogobius melanostomus*; allopatric species) alarm cue, or (4) predator odor with ground water (control). Next, we conducted predation trials where individuals from a treatment group were exposed to predators in a mesocosm simulating flowing stream conditions during the larval dispersal period. Surviving larvae were captured to quantify and compare survival rates of each treatment group and determine the survival implications of exposure to alarm cues produced by each species.

Methods

Lake sturgeon rearing

Experiments were populated using lake sturgeon gametes collected from five spawning females in the Upper Black River, Cheboygan County, MI, USA between May 2 and May 18,

2021. Multiple families of larvae were used because there can be important differences in phenotypes, stress responses, and behavioral responses between families (Crossman et al., 2011; Dammerman et al., 2015; Wassink et al., 2021). Eggs were fertilized within five hours with sperm collected on the same day, following standard lake sturgeon hatchery procedures described in Crossman et al. (2011) and Bauman et al. (2016) at the Black River Sturgeon Rearing Facility, Cheboygan County, MI, USA. After fertilization, povidone-iodine (Betadine, Stamford, CT) disinfection, and clay de-adhesion, eggs were reared in tumbling jars (Pentair, Apopka, FL). Upon hatch, yolk-sac larvae were raised in 3 L tanks (Aquatic Habitats; Pentair, Apopka, FL) containing 32 bio balls (CBB1-S; Pentair, Apopka, FL) to simulate the natural substrate in spawning streams, allowing larvae to burrow until emergence and initiation of exogenous feeding. Individuals ($n \approx 400$) from each family were split evenly among 8 tanks. Water flow into these tanks was set to 0.3 L per minute. Eggs and yolk-sac larvae were reared in a partial recirculating system supplied with water pumped from the Upper Black River and filtered with a sand filter. A chiller in the partial recirculating system (Arctica ¼ HP 1000W Aquarium Chiller; Transworld Aquatic Enterprises, Inglewood, CA) maintained water temperature during rearing (10-12 °C). When individuals emerged from artificial substrate indicated by the absence of a yolk sac, they were transferred to new 3 L tanks with no bio balls at densities of 35-40 individuals per tank.

Alarm cue preparation

Lake sturgeon alarm cue collection followed protocols described by Wishingrad et al. (2014). Whole body homogenate was created by euthanizing one hundred larval lake sturgeon (mean length 21mm) by cervical dislocation, according to approved Michigan State University animal use and care protocols, and homogenizing samples with a mortar and pestle. Similarly, whole body homogenate of larval white suckers was created using 712 individuals following the same protocols as above, such that the same dry weight of fish tissue were used for each lake sturgeon and white sucker alarm cue preparation (0.85 g according to average dry weight values reported in Waraniak et al., 2019). White sucker larvae were captured in D-frame drift nets deployed in the Upper Black River on May 24, 2021. White sucker was chosen because they live in sympatry with lake sturgeon throughout most of their range, including the Black River, and the white sucker larval dispersal period overlaps with the lake sturgeon larvae dispersal period (Smith & King, 2005). Whole body homogenate was also created from a euthanized round goby, collected from Lake Michigan on June 1, 2021. A 3.54 g subsample of the round goby homogenate was taken so that the dry weight matched the dry weight of the larval lake sturgeon and white suckers used to create alarm cue, based on the round goby dry to wet weight conversion by Tarsa (2021). Round goby was chosen because this species historically lived in allopatry with lake sturgeon until introduction of round goby into the Great Lakes in the 1990s (Brown & Stepien, 2008). However, round goby have not invaded Black Lake and remain allopatric with the population of lake sturgeon used in this study (personal communication, Tim Cwalinski, Fisheries Biologist, Michigan Department of Natural Resources, May 2022). Homogenate from each species were enclosed in 20-micron filter papers and placed in Nalgene bottles (ThermoFisher Scientific, Waltham, MA) containing 1 L of ground water to minimize the probability of odors of other species being present. After one hour, the homogenate was removed, creating 1 L each of lake sturgeon, white sucker, and round goby alarm cue. Lake sturgeon alarm cue was prepared in May 2020 and was stored at -20 °C until trials began in May 2021. Round goby and white sucker alarm cue odors were prepared in May 2021 and stored at -20 °C.

Predator odor preparation

Two rock bass (*Ambloplites rupestris*) weighing a combined 38.6 g were collected from upstream of Kleber Dam in the Upper Black River and held in a 15 L tank (diameter of 30.5 cm) containing aerated Black River water. Rock bass occur sympatrically with lake sturgeon in high abundance and are a known larval lake sturgeon predator (Waraniak et al., 2019). After one hour, the fish were removed and 1 L of the water was collected, and stored at -20 °C.

Predation trials

Within 24 hours after lake sturgeon emerged from the artificial substrate and entered the larval stage, individuals were transferred to new 3 L tanks at a density of 35-40 individuals per tank. Larval lake sturgeon were exposed to predatory rock bass odor along with either a ground water control, lake sturgeon, white sucker, or round goby alarm cue; 0.8 mL of each odor was added to 3 L tanks to create concentrations consistent with previous behavioral studies (Wishingrad et al. 2014; Wassink et al. 2021).

Rock bass were captured via angling or electrofishing from upstream of Kleber Dam on the Upper Black River. Predators were held in 3.58 m x 0.5 m tanks supplied with water from the Black River for 24-36 hours with no food prior to predation trials. Ten hours after lake sturgeon larvae were exposed to odors, two rock bass were placed in one of two flow-through raceways measuring 7.15 m x 0.5 m, at a flow rate of approximately 0.085 m/s. Each rock bass was placed in a separate gated section within a raceway to minimize interactions between individuals. Eleven hours after larvae were exposed to odors, flow rates were increased to 0.134 ± 0.003 m/s with recirculating pumps to simulate flow rates of the Black River during larval dispersal, and to ensure that the rock bass odor was present throughout the raceway. During all trials, light levels in the raceways were also set to 0.20 lux to reflect a moonlit night. Twelve hours after lake sturgeon larvae were exposed to odors, eight pre-exposed larval lake sturgeon, larval white suckers, and larval mayflies (family Heptageniidae) were released into the upstream end of the raceway to mimic the taxonomically heterogeneous prey community present in the Upper Black River (Waraniak et al., 2017). Sturgeon larvae used in each trial were from the same family and odor treatment group. Eight more individuals of each taxon were also released into the raceway after 15, 30, and 45 minutes (total n = 32 individuals of each taxa per trial). Surviving larvae were captured in nets placed over the outflows of the raceway. Two trials were conducted for each family and odor treatment group combination for a total of 40 trials. Across trials, water temperatures ranged from 23-26 °C.

Statistical analyses

We fit a series of binomial generalized linear mixed models in R v 4.2.0 describing the proportion of larval lake sturgeon surviving as a function of odor treatment, mean predator length, water temperature during the trial, as well as the interactions between these effects. Temperature was included because feeding rates of fish may be influenced by temperature (Volkoff & Rønnestad, 2020). Additionally, each model included family as a random effect. Competing models were ranked via AICc. A Tukey's general linear hypothesis test for multiple comparisons (R package multcomp) was conducted using the top ranked model to determine whether there were significant differences in the proportion of larval lake sturgeon surviving from different odor treatment groups ($\alpha = 0.05$).

We also fit series of binomial generalized linear models in R v 4.2.0 describing the proportion of larval mayflies and larval suckers surviving predation trials as a function of mean predator length, water temperature during the trial, the number of lake sturgeon consumed during the trial, as well as the interactions between these effects. The number of sturgeon larvae was included to determine whether predators consumed more of other prey taxa when sturgeon survival rates were higher. Competing models were ranked via AICc and models with ΔAICc < 2 were averaged (R package MuMIn).

Results

The AICc-selected model describing larval lake sturgeon survival rates in predation trials included the fixed effects of treatment odor, water temperature during the trial, and mean predator length, as well as the random effect of family (Table 4.1). Model averaging was not neccessary because no other model had a ΔAICc < 2. As temperature increased, the proportion of lake sturgeon surviving decreased (Table 4.2). Additionally, as mean predator length increased, the proportion of sturgeon surviving increased (Table 4.2). Variance attributed to family group by the model was 1.53e-10, indicating survival rates were consistent across lake sturgeon families.

Table 4.1. Competing models describing the proportion of larval lake sturgeon surviving in experimental rock bass predation trials as a function of independent variables including treatment odor, water temperature during trial, mean predator length, and the interactions between these terms. "*" indicates the interaction between the terms was also included in the model.

Parameters included	**ΔAICc**	**df**
Treatment odor + Temp. + Mean pred. length	0.0	7
Treatment odor	2.3	5
Treatment odor + Temp. * Mean pred. length	3.1	8
Treatment odor + Temp.	3.6	6
Treatment odor * Temp. + Mean pred. length	4.4	10
Treatment odor * Mean pred. length + Temp.	6.3	10
Treatment odor + Pred. length	8.1	6
Treatment odor * Temp. * Mean pred. length	23.0	17
Temp + Mean pred. length	38.2	4
Mean pred. length	40.9	3
Null	41.5	2
Temp.	42.2	3

Table 4.2. Parameter estimates of AICc-selected model describing the proportion of lake sturgeon larvae surviving predation trials.

Parameter	**Estimate**	**Std. Error**
Intercept	4.337	1.677
Round Goby Alarm Cue	0.663	0.161
White sucker Alarm Cue	0.645	0.162
Lake sturgeon Alarm Cue	1.099	0.167
Temp.	-0.241	0.079
Pred. length	0.075	0.029

Lake sturgeon survival rates were highest for the treatment group previously exposed to lake sturgeon alarm cue (Figure 4.1). Survival rates of lake sturgeon previously exposed to round goby alarm cue and white sucker alarm cue were nearly identical (Table 4.2; Figure 4.1). Lake sturgeon larvae in the round goby and white sucker treatment groups experienced signficiantly lower survival than individuals in the lake sturgeon alarm cue treatment group (Figure 4.1, p-value = 0.0448 and p-value = 0.0336, respectively), and significantly higher survival rates than the control group (Figure 4.1, p-value = 3.85e-5, p-value < 0.001, respectively). Survival of larval lake sturgeon in the control group was also significantly lower than larvae from the lake sturgeon alarm cue group (Figure 4.1, p-value < 0.001).

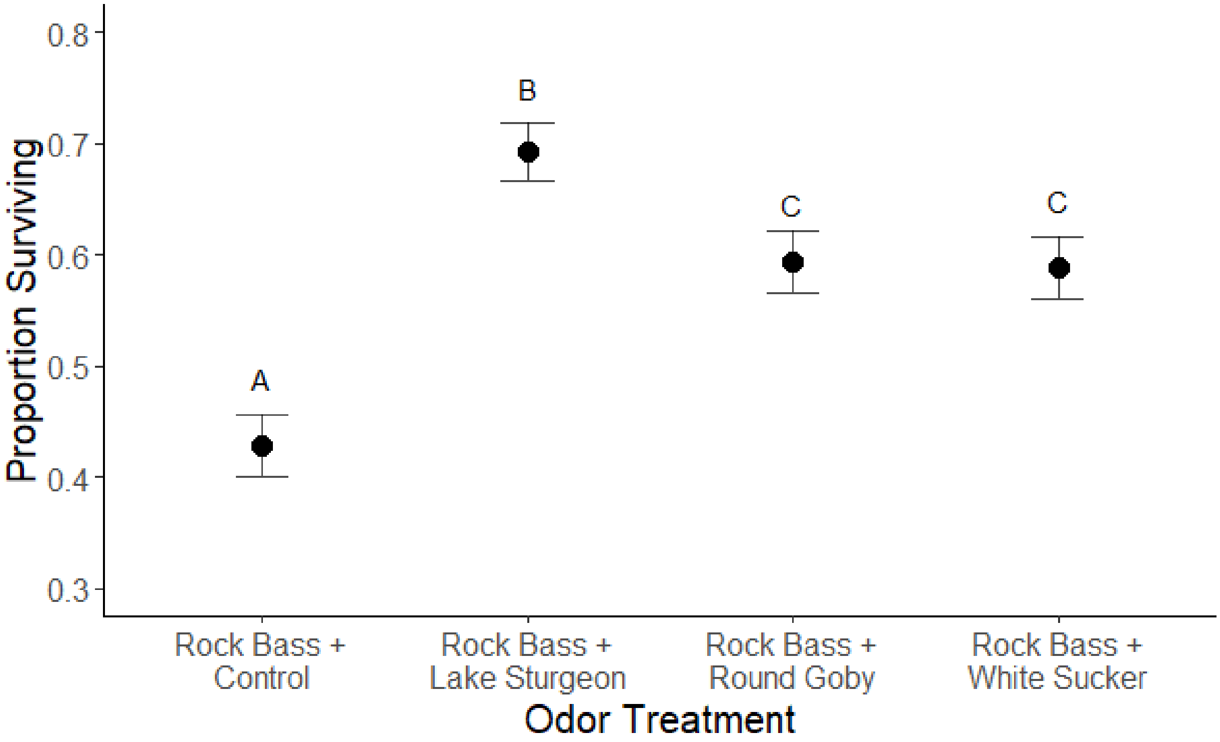

Figure 4.1. The proportion of lake sturgeon larvae surviving predation trials as a function of odor treatment group. Each treatment group was exposed to predatory rock bass odor and the alarm cue of one species or ground water (control). Points and error bars represent the predictions and standard error from the AICc-selected model identified in Table 4.1. Different letters signify significant differences ($\alpha = 0.05$) according to a Tukey's general linear hypothesis test for multiple comparisons.

Table 4.3. Competing models describing the proportion of mayfly (Heptageniidae) and white sucker larvae surviving in experimental rock bass predation trials as a function of independent variables including treatment odor, water temperature during trial, mean predator length, the number of lake sturgeon consumed, and the interactions between these terms. "*" indicates the interaction between the terms was also included in the model. Weight indicates the weight models were given when averaged.

Independent variable	**Parameters included**	**ΔAICc**	**df**	**Weight**
Mayflies consumed	Intercept only	0.0	1	0.54
	Sturgeon consumed	1.5	2	0.26
	Temp.	1.9	2	0.20
	Pred. length	2.2	2	
	Temp. + Sturgeon consumed	3.7	3	
	Temp. + Pred. length	4.1	3	
	Temp. * Sturgeon consumed	5.9	4	
	Temp. + Sturgeon consumed + Pred. length	6.1	4	
	Sturgeon consumed * Pred. length	6.3	4	
	Temp. * Sturgeon consumed + Pred. length	8.5	5	
	Temp. + Sturgeon consumed * Pred. length	8.6	5	
	Temp. + Pred. length * Sturgeon consumed	8.6	5	
	Temp. * Sturgeon consumed * Pred. length	17.1	8	
White sucker consumed	Temp.	0.0	2	0.55
	Temp. + Sturgeon consumed	1.7	3	0.23
	Temp. + Pred. length	1.9	3	0.21
	Pred. length	2.7	2	
	Temp. + Sturgeon consumed + Pred. length	3.9	4	
	Temp. * Sturgeon consumed	4.2	4	
	Intercept only	4.7	1	
	Temp. + Pred. length * Sturgeon consumed	6.2	5	
	Temp. + Sturgeon consumed * Pred. length	6.5	5	
	Temp. * Sturgeon consumed + Pred. length	6.5	5	
	Sturgeon consumed	6.8	2	
	Sturgeon consumed * Pred. length	7.5	4	
	Temp. * Sturgeon consumed * Pred. length	12.2	8	

Table 4.4. Parameter estimates included in models describing the proportion of Heptageniid mayflies and white sucker larvae surviving in predation trials. Models shown are the result of averaging competing models with ΔAICc < 2 indicated in Table 3.

Independent variable	**Parameter**	**Estimate**	**Std. Error**
Mayflies consumed	Intercept	-0.9801	0.8771
	Sturgeon consumed	-0.0098	0.0115
	Temp.	-0.0370	0.0728
White sucker consumed	Intercept	5.40735	1.7332
	Temp	-0.1773	0.0744
	Sturgeon consumed	0.00873	0.0112
	Mean pred. length	-0.0212	0.0315

Three models describing Heptageniidae survival rates had ΔAICc < 2 (Table 4.3) and were therefore averaged to produce the model described in Table 4. This model indicates Heptageniidae survival rates are lower when more lake sturgeon are consumed during a trial, and when water temperature was higher (Table 4.4). Three models describing white sucker survival rates had ΔAICc < 2 (Table 4.3) and were also averaged to produce the model described in Table 4.4. This model indicates white sucker surival rates decreased as the water temperature was increased (Table 4.4). Additionally, white sucker survival was higher when the number of sturgeon consumed increased and when the mean predator length decreased (Table 4.4).

Discussion

Data demonstrate that lake sturgeon larvae were able to use alarm cues of both sympatric white suckers and allopatric round goby as kairomones to identify a novel predator as a threat and subsequently elicit an alarm response, which was reflected by increased survival rates when exposed to a common stream predator. Results support our third alternative hypothesis: larval lake sturgeon use alarm cues emitted by a diverse group of species, regardless of overlapping population ranges or phylogenetic relationship, to warn of predation risk. However, the survival benefits imparted by these kairomones are not as strong as those imparted by the conspecific alarm cue (Figure 4.1).

The fact that kairomones do not impart the same benefit as the conspecific alarm cue has been documented in diverse groups of fish including lamprey, reef fish, and darters (Hume & Wagner, 2018; Anderson & Mathis, 2016; Mitchell et al., 2012). However, results for larval lake sturgeon were unexpected in that exposure to a sympatric and allopatric species alarm cues (white sucker and round goby, respectively), resulted in equivalent lake sturgeon larval survival rates during predation trials. Although it is common for closely related species or sympatric species to respond to one another's alarm cue (Chivers & Smith, 1998), it appears that this is the first described example of one species behavior being influenced similarly by alarm cues of distantly related sympatric and allopatric species.

Behavioral responses to heterospecific alarm cues are thought to have evolved in two main ways. If the two species are closely related, behavioral responses are most likely triggered by compounds that are present in the skin of both species (Chivers & Smith, 1998; Pfeiffer et al., 1985). Alternatively, if two species live sympatrically and share common predators, there is likely selection occurring for individuals of both species to detect and respond to the heterospecific alarm cue (Chivers et al., 1995; Ferrari et al., 2010). This may explain the selection that occurred which resulted in larval lake sturgeon being influenced by the sympatric white sucker alarm cue; however, it does not explain the response to the allopatric round goby alarm cue.

Most previous studies on fish species using heterospecific alarm cues as kairomones focus on teleosts. Hume and Wagner (2018) reported sea lamprey (*Petromyzon marinus*), an ancestral, non-teleost fish respond to other lamprey alarm cues but not sympatric white sucker (teleost) alarm cue. Our results indicate lake sturgeon, another non-teleost fish, are able to respond to alarm cues from taxonomically differentiated fish species and therefore most likely rely on detecting different compounds than lamprey or teleost fishes. Future work is warranted to determine whether other species of sturgeon also respond to alarm cues from diverse groups of fish, and to determine the chemical identity of the compounds in alarm cue odors that are responsible for eliciting the responses observed in sturgeon.

Mortality rates of larval lake sturgeon are high and variable (Caroffino et al. 2010). Subsequently, year class strength is dependent on survival during the larval stage (Pollock et al. 2015). As lake sturgeon larvae drift downstream from spawning grounds to nursery habitat during this stage, they may travel dozens of kilometers without stopping (Auer & Baker, 2020) while being exposed to abundant and diverse predators (Waraniak et al., 2019). In our experiment, we found that sturgeon larvae that had been exposed to rock bass odor along with either round goby alarm cue or white sucker alarm cue had approximately 15% higher survival rates than larvae exposed to rock bass odor alone. Though this increase in survival due to kairomone recognition may appear modest, it may have important population-level implications when extrapolated to the numerous opportunities of predation that wild larvae encounter in spawning streams.

Survival rates of larval lake sturgeon, Heptageniid mayflies, and white suckers were lower during trials with higher water temperatures (Table 4.2; Table 4.4). As water temperature increases, the metabolic rate and feeding rate of fish also increase, assuming the temperature does not approach the critical thermal maxima of the predator (Volkoff & Ronnestad, 2020). The critical thermal maxima for Centrarchids are generally 35-40 °C (Dent & Lutterschmidt, 2003; Brewer et al., 2022), and the maximum temperature during a predation trial was 26 °C. Therefore, the relationship between larval survival and temperature is likely due to increased feeding rates of the predatory rock bass.

Lake sturgeon conservation efforts often include constructing passage systems to allow mature adults access to historic spawning grounds (Theim et al., 2011; Pratt et al., 2021) and creating ideal spawning habitat for lake sturgeon by placing large cobble in historic spawning rivers (Chiotti et al., 2008; Roseman et al., 2011). Our study shows that lake sturgeon larvae benefit from the alarm cues emitted from other fish species. Therefore, lake sturgeon conservation efforts should also include improving the ability of other anadromous fish to migrate upstream concurrently with lake sturgeon and restoring adequate habitat for aquatic taxa to successfully reproduce. Fortunately, recent spawning habitat restoration efforts have resulted in several species of fish spawning in the same location as lake sturgeon (Fischer et al., 2018) and fish passage systems that allow lake sturgeon to circumvent dams en route to spawning grounds often allow other species of fish to access spawning grounds upstream as well (Schulze 2017, Cooke et al., 2020). As new fish passages are constructed and active fish passage systems are operated (i.e., fish elevator; Schulze 2017), there should be efforts to maximize the fish migrating to spawning grounds concurrently with lake sturgeon because sturgeon larvae will likely have higher survival rates when exposed to alarm cues emitted by these co-occurring species.

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CHAPTER 5:
Mortality and predation on dispersing larval lake sturgeon are influenced by ecological factors

Introduction

Organisms are generally at increased risk of predation during periods of migration (Milner-Gulland et al., 2011). However, migration is often necessary to access optimal habitats for feeding, mating, and rearing offspring (Alerstam et al., 2003). Levels of predation risk during migration can be affected by multiple and concurrently acting ecological factors, such as the number and size of individuals undergoing migration, weather conditions and other physical environmental factors during migration, and the number and diversity of predators inhabiting the migration route (Furey et al., 2016a, Furey et al., 2016b, Jonsson et al., 2017). As a result, species have evolved to begin migrating under conditions that will increase the probability of survival (Schmaljohann & Dierschke, 2005; Pavlov & Mikheev, 2017).

The risk of predation during migration is particularly high for species during early life stages (Osterback et al., 2013; Pavlov & Mikheev, 2017). For example, threatened and endangered sea turtles provide a well-documented example of this phenomenon. Just after hatching, young sea turtles must exit nests, traverse a sandy beach, enter the ocean, and complete their migration by reaching deep water (Wyneken, 2000). Predation rates during migration are dependent on the number and species of predators present, the number of and size young turtles undergoing migrations, and local environmental conditions (Janzen et al., 2001; Martins et al., 2021; Peterson et al., 2013). Species of potamodromous and anadromous fish larvae undergo similar migrations during early life stages, where young fish disperse from spawning habitats downstream to nursery habitats (Pavlov & Mikheev, 2017). Estimates of predation levels on fish migrating at early life stages are high (Caroffino et al., 2010); however, the ecological conditions influencing predation rates on larval fish are less extensively investigated. The importance of understanding the relative effects of ecological factors that influence the levels of predation during early life migrations are pronounced in fish populations of conservation concern.

Throughout their native range, lake sturgeon (*Acipenser fulvescens*) are considered to be threatened or endangered (Bruch et al., 2016). Lake sturgeon is a potamodromous species that migrate upstream in rivers to spawn over rocky substrate in the spring (Bruch & Binkowski, 2002). As young individuals reach the larval stage, they leave the spawning grounds and undergo downstream migration until they reach depositional nursery habitats (Auer & Baker, 2002). During this larval migration, also referred to as the drift period, lake sturgeon larvae are susceptible to predation by numerous species of riverine fish (Waraniak et al., 2018). Though few studies have quantified mortality rates during the early life stages of lake sturgeon, mortality estimates at the larval stage have been estimated to be greater than 90%, though there is evidence of high variability in survival during ontogeny (Caroffino et al., 2010). As a result, recruitment rates in lake sturgeon populations are highly variable; observations of 5-100-fold differences in cohort abundances in different years have been reported (COSEWIC, 2006). Harvest has been banned or heavily regulated in most lake sturgeon populations for over fifty years, yet most populations have been slow to recover demographically (Bruch et al., 2016). Slow population recovery has been attributed to low rates of recruitment, as mature adult mortality is low (Vaugeois et al., 2022).

Ecological factors have been posited to influence predation rates on larval lake sturgeon during the drift period. Experimental evidence has shown light level during dispersal influences the ability for predators to locate and consume larval lake sturgeon (Chapter 1). Likewise, a field study showed the probability lake sturgeon were found in the diet of a predator increased as the lunar illumination increased during lake sturgeon dispersal (Waraniak et al., 2018). Experiments have also shown size of larvae, which is a product of temperatures during early development, influences predation rates on drifting lake sturgeon (Chapter 2). Additionally, the presence and abundance of other taxa migrating concurrently with larval lake sturgeon may influence predation rates, as some predatory species have selected against larval lake sturgeon when invertebrates were available (Waraniak et al., 2017, Chapter 1).

In this study, our general objective was to estimate mortality rates of larval lake sturgeon in a natural field setting during downstream migration, and to assess whether rates were associated with ecological factors including light level, larval size, river discharge, and the biomass of co-distributed fish and invertebrate larvae. Additionally, we evaluated whether ecological variables influenced the probability predatory stream fishes consumed lake sturgeon, and whether explanatory ecological variables were constant across predator species.

Methods

All field work was conducted in the in the Upper Black River (UBR), Cheboygan County, Michigan, USA. Lake sturgeon spawning and larval drift periods have been well described in this population (Smith & King, 2006; Larson et al., 2021). Our work consisted of two main approaches. First, we collected drifting larval sturgeon to estimate abundance and mortality within nights. Second, we collected potential predators to estimate the proportion of different taxa that preyed upon lake sturgeon on the previous night. Relevant ecological data were also collected daily to produce models that may explain variation in mortality and variation in the proportions of diets containing lake sturgeon.

Collecting drifting lake sturgeon larvae

Three larval lake sturgeon drift sites were selected on the UBR (Figure 5.1). Site 1, the most upstream site, was immediately downstream of the known spawning grounds (Smith & King, 2005; Forsythe et al., 2012). Site 2 was located 2 km downstream of the spawning grounds, where drifting larval lake sturgeon have been collected during previous years (Smith & King, 2005, Duong et al., 2011). Site 3 was 2 km downstream of Site 2.

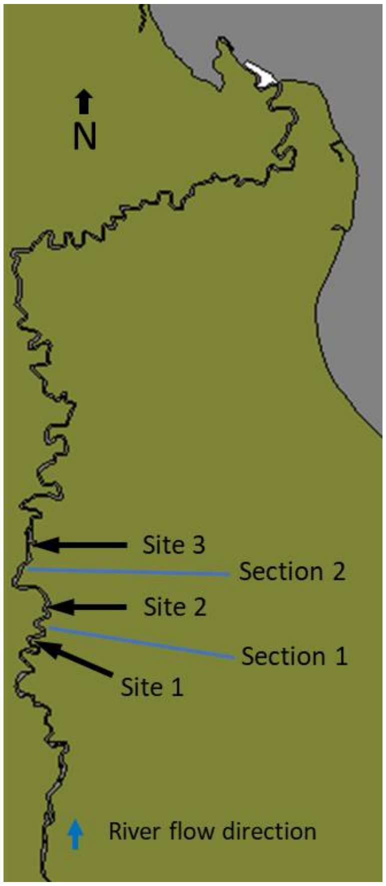

Figure 5.1. Map of the Upper Black River, Cheboygan County, MI, USA. Drifting larval lake sturgeon and co-distributed organisms were captures at Sites 1, 2, and 3. Site 1 was immediately downstream of spawning habitat. Sites 2 and 3 were 2 km and 4 km downstream of the spawning grounds, respectively. Predatory fish included in the diet study were collected in river Section 1 and Section 2, located between drift collection sites.

At each drift site, seven D-frame drift nets were deployed to capture and quantify nocturnally drifting larvae (Auer & Baker, 2002). Nets were deployed from 20:00-1:00 at Site 1, 21:00-2:00

at Site 2, and 22:00-3:00 at Site 3. Collection periods were delayed at Site 2 and 3 because we estimated passively drifting larvae would travel 2 km in approximately one hour given flow rates in the thalweg of the UBR. River depth and flow were recorded using a flow meter (OTT MF pro Flow Meter, Fondriest Environmental Inc, Fairborn, OH and Flo-Mate 2000, Marsh-McBirney Inc, Frederick, MD) at four locations on each net, as well as at 1 m intervals across the width of the river at each site. Each hour, cod ends of D-frame nets were collected and larval lake sturgeon were enumerated. Additionally, each hour light level (lux), was recorded with a light meter (Extech, Nashua, NH, USA). At each site, 10 lake sturgeon larvae were photographed each hour, and later measured via ImageJ (National Institute of Health, Bethesda, MD). Average larval lake sturgeon biomass was estimated by multiplying 0.0051 by the average length to the 2.2639 power, based on the line of best fit produced when comparing the known lengths and weights (Larson, unpublished data). Estimates of the total number lake sturgeon drifting by each site were produced by dividing the number of lake sturgeon larvae enumerated by the proportion of stream estimated discharge sampled by the D-frame nets. The estimated biomass of lake sturgeon drifting on any night was the estimated average biomass multiplied by the lake sturgeon abundance estimate. A 5% subsample of larval suckers and invertebrates captured in D-frame nets were preserved in ethanol. The estimated biomass of preserved taxa was calculated by multiplying the average biomass of that taxa (Waraniak et al., 2019) was by the count of preserved individuals divided by 0.05 (to account for taking a 5% subsample), and then divided by the proportion of the discharge sampled. This provides point estimates of the numbers and biomass of taxa drifting downstream at each collection site, but do not account for measurement error. Additionally, estimates assume that the proportion of discharge sampled is directly proportional to the proportion of hourly drifting taxa collected, catchability of drifting taxa was constant across sites and across sampling nights, the sturgeon larvae measured were representative of the population, the proportion of discharge sampled remained constant throughout each night, and differences is estimates between sites were due to predation and not larvae settling between sites.

Collecting predatory fish diets

Predators were captured between larval drift sites using a barge electroshocker (ETS Electrofishing Systems LLC, Madison, WI, USA) beginning the morning after drifting larvae were collected at the three drift sites. In one day, we sampled either Section 1, located between Sites 1 & 2, or Section 2, located between Sites 2 & 3. Each section was sampled twice in 2019 and 2021. Habitats in both sections were similar and were characterized by sand substrate with large woody debris providing cover for fish (Waraniak et al., 2018). Sections were divided into three approximately 670 m transects. After completing shocking each transect, all collected fish were identified to species, weighed, and total length was measured. A subset of these fish were euthanized by overdosing with tricaine methanesulfonate (MS-222) using approved Michigan State University Animal Use and Care protocols and stored on ice before freezing at -20 °C. No more than four fish of the same species were euthanized from the same transect on a single day, and no more than ten fish of the same species were euthanized from the same river section in a single day. The individuals of each species chosen for preservation were selected at random. We used t-tests to compare measures of fish community taxonomic diversity including Shannon diversity (Shannon, 1948) and inverse Simpson index (Hill, 1978) between river sections and between years. Additionally, t-tests were used to compare the number of fish of each species between river sections, nights and years as a measure of species relative abundance.

DNA extraction

Preserved predators were thawed and dissected. Up to 50 mg of contents of gastrointestinal tracts were removed for DNA extraction. For diet samples greater than 50 mg, the entire contents were homogenized with a sterile pipette tip, and a 50 mg subsample was used for DNA extraction. The protocol for DNA extraction was a modified version of the QIAamp Stool Mini Kit (QIAGEN, Hilden, Germany) described by Waraniak et al. (2018). Lysis while submerged in InhibitEx Buffer was lengthened to 30 min. Also, 0.5 g of 0.7 mm garnet beads (OMNI, Kennesaw, GA, USA) were added to samples for a 90 second bead beating (Mini-Beadbeater-16, Glenn Mills, Clifton, NJ) after proteinase and lysis buffer were added to lyse cells. After extraction, DNA concentrations were quantified with a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA).

DNA amplification

PCR conditions for amplification of lake sturgeon DNA from fish gastrointestinal tracts were based on the protocol provided in Waraniak et al. (2018). Each reaction contained 0.5 µM of forward and reverse AfCOI1 primers, 200 dNTPs, 5U of Taq DNA polymerase, 1x reaction buffer, 100 ng of DNA, and sterile deionized water to bring the volume to 25 µL. AfCOI1 primers were designed to bind the COI region of mtDNA lake sturgeon DNA and do not amplify the DNA of other fish, amphibians, or invertebrates present in the UBR (Waraniak et al., 2018). PCR conditions included initial denaturation at 94 °C for three min, 35 cycles of denaturation at 94 °C for 45s, annealing at 56 °C for 30s, and extension at 72 °C for 30s, followed by a final elongation at 72 °C for five min. Each series of PCR reactions included a positive control, containing 20 ng of lake sturgeon genomic DNA, and a negative control, containing no DNA.

PCR products from all gastrointestinal tracts were run on an ethidium bromide stained 2% agarose gel and visualized under UV light to assess presence or absence of lake sturgeon DNA.

Statistical analyses

Larval lake sturgeon mortality rates between drift sites were calculated by subtracting the downstream abundance from the upstream abundance, and then dividing by the upstream abundance. Competing beta family generalized linear models were created describing the mortality rate between two sites as a function of average larval sturgeon size (mm), river discharge (m^3/s), river section, average lux level across the night, percent lunar illumination, and the estimated biomasses (mg) of drifting larval lake sturgeon, suckers, and invertebrates (R packages glmmTMB and MuMIn) and ranked via AICc. Wald confidence intervals for the parameters included in the model of best fit were calculated (R package effects).

Competing binomial family generalized linear models were created describing the probability of predator diets containing lake sturgeon DNA as a function of average larval lake sturgeon body size (mm), river discharge (m^3/s), river section, average hourly lux level across the 5 hour sampling period, and the estimated biomasses (mg) of drifting larval lake sturgeon, suckers, and invertebrates at the drift site upstream of the section where the predator was capture on the night prior to the day it was captured. Additionally, a series of competing binomial family generalized linear modes were created describing the probability of predator diets of different taxa groups containing lake sturgeon DNA as a function of the same ecological variables listed above, as well as the size (cm) of the individual because the predation of lake sturgeon larvae by different predatory taxa are likely influenced by different ecological factors. Predator species were placed into taxa groups based on phylogenetic relatedness. Table 5.1 shows the predator taxa groups evaluated and the species included in each group. Burbot (*Lota lota*) and central mudminnow (*Umbra limi*) were not placed in a taxa group and were evaluated separately because no closely related species were collected. Yellow perch (*Perca flavescens*) was not placed in a taxa group because the most closely related groups, *Etheostoma* and *Percina* genera, include benthic darter species whereas yellow perch are found in a variety of habitats and depths (Mrnak et al., 2021). Diets of northern pike (*Esox lucius*) were not evaluated by these models because no closely related species were collected and too few diets (n = 8) were analyzed to produce a reliable model. Models were ranked via AICc and models with Δ AICc < 2 were averaged.

Table 5.1. The predatory species captured in the Upper Black River were assigned to each taxonomic groups for diet analysis modelling.

Taxa group	Common name	Scientific name
Bullhead	Black bullhead	*Ameiurus melas*
	Yellow bullhead	*Ameiurus natalis*
Burbot	Burbot	*Lota lota*
Central mudminnow	Central mudminnow	*Umbra limi*
Centrarchid	Black crappie	*Pomoxis nigromaculatus*
	Pumpkinseed	*Lepomis gibbosus*
	Bluegill	*Lepomis macrochirus*
	Rock bass	*Ambloplites rupestris*
	Smallmouth bass	*Micropterus dolomieu*
Chubs	Creek chub	*Semotilus atromaculatus*
	Hornyhead chub	*Nocomis biguttatus*
Etheostoma	Iowa darter	*Etheostoma exile*
	Johnny darter	*Etheostoma nigrum*
	Rainbow darter	*Etheostoma caeruleum*
Percina	Blackside darter	*Percina maculata*
	Logperch	*Percina caprodes*
Minnows	Blacknose shiner	*Notropis heterolepis*
	Common shiner	*Luxilus cornutus*
	Emerald shiner	*Notropis atherinoides*
	Northern redbelly dace	*Chrosomus eos*
Yellow perch	Yellow perch	*Perca flavescens*

Results

Larval drift collection

Drifting lake sturgeon larvae were collected at drift Sites 1-3 during six nights (June 4-8 and June 24) in 2019, and during seven nights (May 24-30) in 2021. Heavy rainfall caused a drastic increase in river discharge and prevented sampling from June 9-23, 2019. The number of lake sturgeon larvae drifting varied greatly between years; the nightly estimated number of lake sturgeon at drift Site 1 ranged from 764-2,174 in 2019 and 4,525-37,934 in 2021 (Figure 5.2). Discharge also varied between years, ranging from 7.09-7.98 m^3/s in 2019 and 4.62-5.90 m^3/s in 2021 (Table 5.2). Recorded light levels, average sturgeon length, and estimated biomass of invertebrate and sucker larvae overlapped between 2019 and 2021 (Table 5.2).

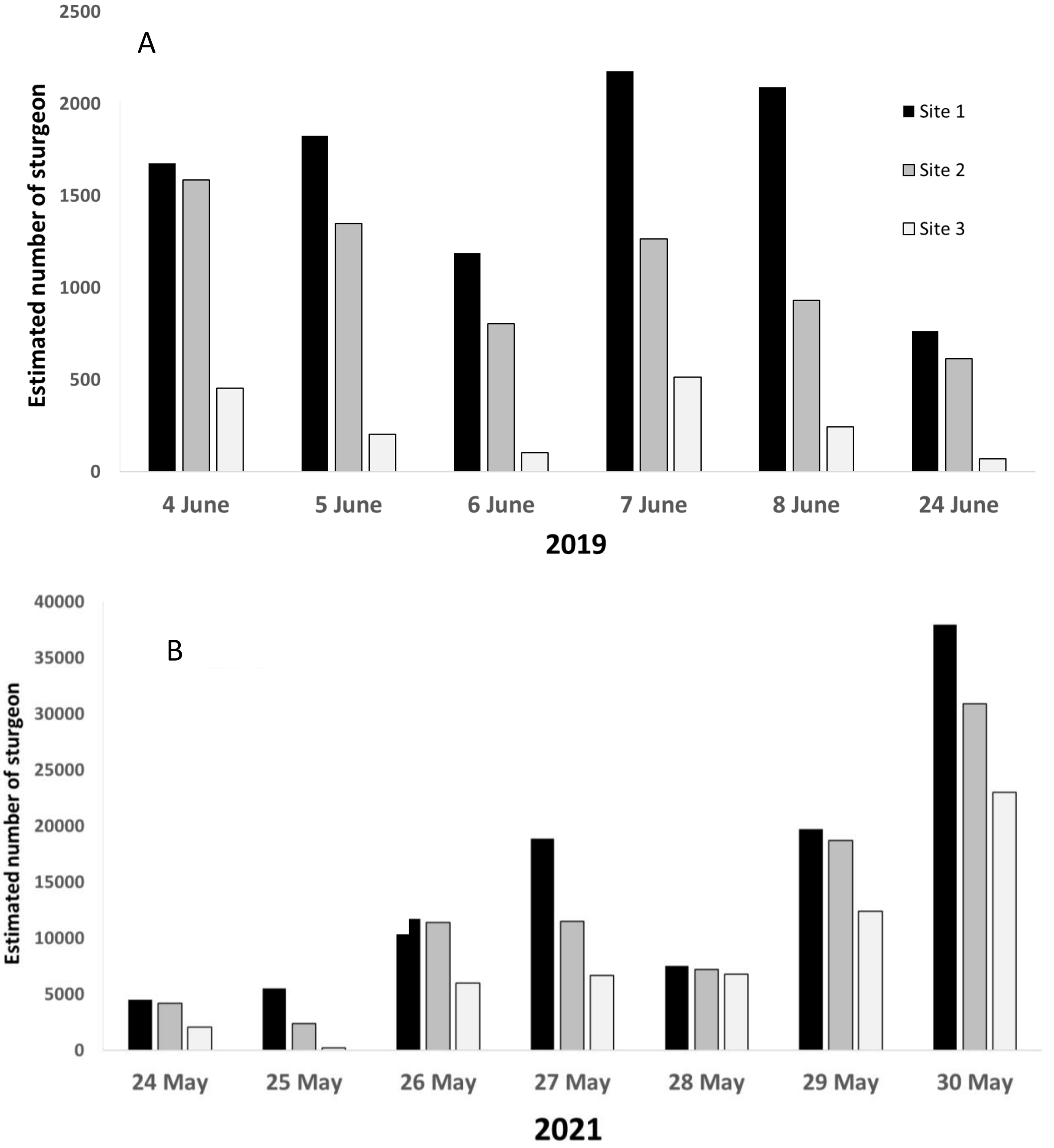

Figure 5.2. Estimates of the total number of larval lake sturgeon drifting at each drift site (A) in 2019 or (B) in 2021. Note the ~10-fold difference in estimated abundance on the y-axis between A and B. Site 1 was located just downstream of the known lake sturgeon spawning grounds. Sites 2 and 3 were located approximately 2 and 4 km downstream of Site 1, respectively.

Table 5.2. The light level, discharge (m^3/s), mean larval lake sturgeon body size (mm), and estimated biomasses of invertebrates, larval suckers, and larval lake sturgeon present during each night drift was collected.

Year	Date	Lux	Discharge (m^3/s)	Lake sturgeon size (mm)	Invertebrates (mg)	Lake sturgeon (mg)
2019	4 June	0.094	7.35	21.0	3200.4	7955.5
2019	5 June	0.186	7.72	20.6	1481.9	6484.1
2019	6 June	0.122	7.98	20.8	2376.2	3953.2
2019	7 June	0.116	7.94	20.4	1726.9	5943.2
2019	8 June	0.163	7.72	20.4	1507.0	4383.4
2019	24 June	0.124	7.09	18.4	1032.5	2287.2
2021	24 May	0.083	5.90	19.0	158.9	16717.7
2021	25 May	0.141	5.19	19.8	166.0	10471.6
2021	26 May	0.034	4.67	20.9	384.9	52159.2
2021	27 May	0.061	6.25	20.9	1313.1	57021.2
2021	28 May	0.078	4.62	19.8	3485.3	31747.7
2021	29 May	0.09	5.31	19.8	1558.9	82255.8
2021	30 May	0.104	5.35	20.1	2879.5	140620.2

Predator communities

A total of 630 fish were captured during 2019, and 724 fish were captured during 2021. Of the fish captured, 260 were preserved in 2019 and 349 were preserved in 2021. Across both years, 23 species of fish were captured and preserved. Shannon diversity did not differ significantly between years (H’ = 2.16 in 2019, H’ = 2.29 in 2021; t = 0.96, p-value = 0.37) nor between section (H’ = 2.22 in Section 1, H’ = 2.23 in Section 2; t = 0.10, p-value = 0.92). Likewise, inverse Simpson’s diversity did not differ significantly between years (D^{-1} = 6.71 in 2019, D^{-1} = 7.53 in 2021; p-value = 0.56) or between sections (D^{-1} = 7.29 in Section 1, D^{-1} = 6.94 in Section 2; p-value = 0.81). The number of fish captured on each sampling day while sampling across approximately equal distances of the Upper Black River was also similar between years (2019 mean = 157.5, 2021 mean = 181.0; t = 0.75, p-value = 0.50) and sections (Section 1 mean = 156.8, Section 2 mean = 181.8; t = 0.80, p-value = 0.45). The only predator species with unequal number of captures between river sections was rock bass (Section 1 mean = 16.5, Section 2 mean = 47.3; t = 3.24, P = 0.025).

Estimated larval lake sturgeon mortality rates

Nightly mortality rate estimates ranged from 3-58% with a mean of 24% between drift sites 1 and 2 and ranged from 6-91% with a mean of 58% between sites 2 and 3. Mortality between sites 1 and 3, the entire 4 km section of the river sampled, ranged from 10-96% with a mean of 66% (Figure 5.2). The AICc-selected model describing the estimated mortality rates between drift sites included lux, section, and estimated grams of larval suckers drifting concurrently with the larval lake sturgeon (Table 5.3). This model indicated mortality rate was positively associated with light level, negatively associated with the amount of larval suckers drifting, and mortality rates were higher between drift sites 2 and 3 than between sites 1 and 2 (Figure 5.3).

Table 5.3. Parameter estimates of the AICc selected model describing the estimated mortality rate between drift sites. No other model had Δ AICc <2.

Parameter	Estimate	Std. Error
Intercept	-2.422	0.5024
Lux	13.61	3.789
River section 2	1.645	0.2954
Sucker abundance (mg)	-2.812E-04	9.782E-05

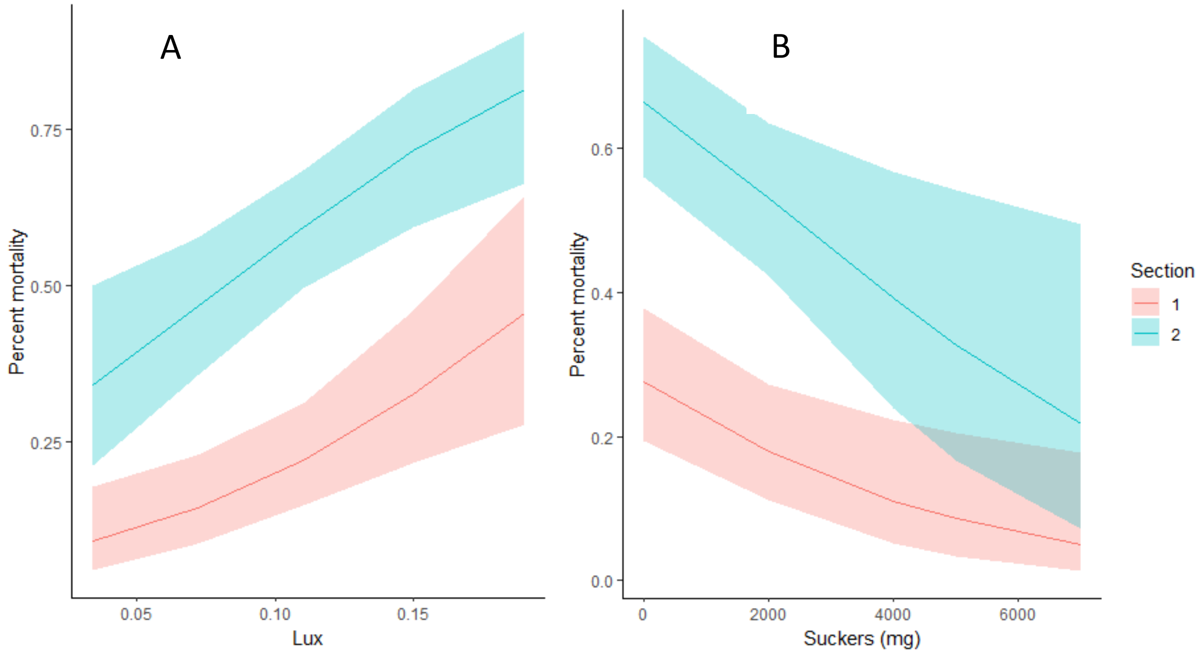

Figure 5.3. Predicted larval lake sturgeon mortality rates with 95% CI during larval drift based on the AICc-selected model described in Table 5.3. Plots show the relationships between mortality rate, river section, and (A) night light level (lux), or (B) estimated biomass (mg) of sucker larvae drifting concurrently with lake sturgeon larvae. Section 1 is the 2 km stretch of the Black River immediately downstream of the spawning grounds and section 2 is the 2 km stretch immediately downstream of section 1.

Estimated predator diets

Lake sturgeon DNA was detected in 30.5% of total preserved predator diets and was detected at least once in 19 of 23 species sampled. The proportion of preserved diets that contained lake sturgeon DNA varied between species (Figure 5.4). The probability larval sturgeon was present in the diet of any predator was best described by a model including nightly light level (lux), estimated sucker biomass (mg) and estimated invertebrate biomass (mg) drifting in the river (Table 5.4). However, models assessing the probability of sturgeon DNA being present in different fish taxa revealed important species differences between the ecological factors that influence the probability that different taxa consume larval lake sturgeon (Table 5.5).

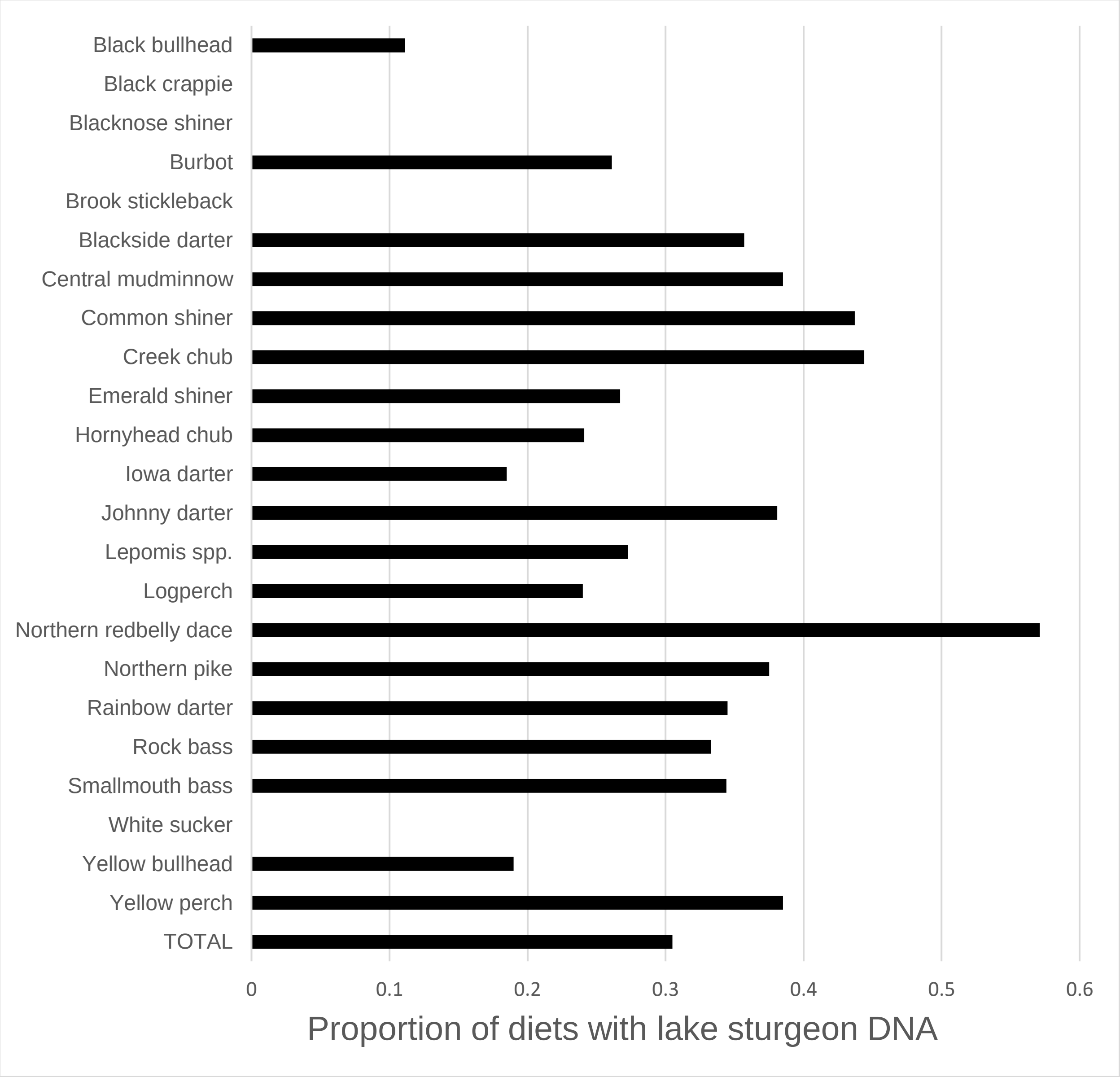

Figure 5.4. The proportion of predator fish diets collected that contained lake sturgeon DNA.

Table 5.4. A) The parameters included in the models describing the probability lake sturgeon DNA was detected in the diet of predatory fish taxa with AICc < 2. These models were averaged to produce the parameter estimates in B).

A) Models averaged	Parameters	Δ AICc	Weight
	Lux	0	0.4
	Lux + Sturgeon.mm	1.9	0.15
	River discharge (m3/s) + Lux	1.93	0.15
	Lux + Sucker abundance (mg)	1.93	0.15
	Invertebrate abundance (mg) + Lux	1.99	0.15

B) Parameters included	Parameter	Estimate	Std. Error
	Intercept	-2.242	0.9305
	Lux	11.71	2.2440
	Sturgeon size (mm)	0.03628	0.1067
	River discharge (m^3/s)	0.03695	0.1216
	Sucker abundance (mg)	-1.4E-05	4.59E-05
	Invertebrate abundance (mg)	3.11E-05	0.00017

Lux was strongly positively associated with the probability of diets containing lake sturgeon in the model which included all species (Table 5.4; Figure 5.3), but there was no relationship between lux and sturgeon consumption in burbot and *Percina* (Table 5.5), and there was a negative association between lux and lake sturgeon consumption by minnows (Table 5.6). The biomass of drifting invertebrates was positively associated with sturgeon predation for bullhead, central mudminnow, Centrarchids, *Etheostoma*, yellow perch, and negatively associated with sturgeon predation for burbot, chubs, and minnows. Also, the biomass of drifting larval suckers was positively associated with sturgeon predation for chubs, *Etheostoma*, and *Percina*, but negatively associated with sturgeon predation for bullhead, Centrarchids, and yellow perch. Predator size also had different effects on lake sturgeon consumption across taxa. The probability of detecting lake sturgeon in diets was positively associated with predator length for burbot, *Etheostoma*, and minnows and negatively associated with length for chubs and *Percina*.

Table 5.5. The parameters included in models describing the probability lake sturgeon DNA was detected in a predator diet that were averaged to produce the models displayed in and Table 5.6.

Bullhead	Parameters included	Δ AICc	Weight
	Lux	0	0.3
	Null	0.2	0.28
	Invertebrate abundance (mg)	1.23	0.16
	Sucker abundance (mg)	1.58	0.14
	Invertebrate abundance (mg) + Lux	1.9	0.12

Burbot	Parameters included	Δ AICc	Weight
	River section	0	0.14
	Length	0.03	0.14
	Null	0.82	0.09
	Length + River section	0.99	0.08
	River discharge (m^3/s) + River section	1.37	0.07
	Invertebrate abundance (mg) + Length	1.54	0.06
	River discharge (m^3/s)	1.58	0.06
	River discharge (m^3/s) + Length	1.59	0.06
	Sucker abundance (mg)	1.64	0.06
	Length + Sucker abundance (mg)	1.64	0.06
	Length + Sturgeon abundance (mg)	1.73	0.06
	River section + Sturgeon abundance (mg)	1.76	0.06
	River section + Sucker abundance (mg)	1.84	0.05

Central mudminnow	Parameters included	Δ AICc	Weight
	Lux	0	0.62
	Sturgeon.mm	0.97	0.38

Centrarchid	Parameters included	Δ AICc	Weight
	Lux	0	0.56
	Lux + Sucker abundance (mg)	1.85	0.22
	Lux + Sturgeon.mm	1.9	0.22

Table 5.5 (cont'd)

Chubs	Parameters included	Δ AICc	Weight
	Length + Lux	0	0.22
	Length + Lux + Sturgeon abundance (mg)	0.47	0.18
	Invertebrate abundance (mg) + Length + Lux + Sturgeon abundance (mg)	1.28	0.12
	Lux	1.61	0.1
	Invertebrate abundance (mg) + Length + Lux	1.61	0.1
	Length + Lux + Sucker abundance (mg)	1.67	0.1
	Length + Sucker abundance (mg)	1.7	0.1
	Length + River section + Sucker abundance (mg)	1.91	0.09

Etheostoma	Parameters included	Δ AICc	Weight
	Lux	0	0.47
	Lux + River section	1.86	0.18
	Length + Lux	1.92	0.18
	Invertebrate abundance (mg) + Lux	1.98	0.17

Percina	Parameters included	Δ AICc	Weight
	Sucker abundance (mg)	0	0.27
	Length + Sucker abundance (mg)	0.25	0.24
	Null	0.42	0.22
	Length	0.96	0.17
	Invertebrate abundance (mg)	1.79	0.11

Minnows	Parameters included	Δ AICc	Weight
	Null	0	0.35
	Length	1.28	0.19
	Invertebrate abundance (mg)	1.29	0.18
	River section	1.84	0.14
	Lux	1.9	0.14

Yellow perch	Parameters included	Δ AICc	Weight
	Lux	0	0.34
	Invertebrate abundance (mg)	1.07	0.2
	Lux + Sucker abundance (mg)	1.2	0.18
	River discharge (m^3/s) + River section	1.7	0.14
	Lux + River section	1.78	0.14

Table 5.6. Parameters included in AICc-selected models describing the probability lake sturgeon DNA was detected the diets of predators in each taxa group. Top ranking models were averaged if one or more model had AICc < 2. Component models that were averaged to produce these estimates are listed in Table 5.5.

Bullhead	Parameter	Estimate	Std. Error
	Intercept	-2.6627	1.4422
	Lux	22.8709	15.483
	Invertebrate abundance (mg)	0.0009	0.0009
	Sucker abundance (mg)	0.0002	0.0002

Burbot	Parameter	Estimate	Std. Error
	Intercept	-1.925	2.243
	River section 2	1.203	0.8947
	Length	0.1147	0.0820
	River discharge (m^3/s)	-0.3402	0.3499
	Invertebrate abundance (mg)	-0.0005	0.0006
	Sucker abundance (mg)	-0.0001	0.0002
	Sturgeon abundance (mg)	1.26E-06	9.81E-06

Central mudminnow	Parameter	Estimate	Std. Error
	Intercept	-23.19	22.881
	Lux	31.952	13.782
	Sturgeon size (mm)	1.562	1.013

Centrarchid	Parameter	Estimate	Std. Error
	Intercept	-2.279	2.439
	Lux	17.85	5.09
	Sucker abundance (mg)	-6.2E-05	0.0001
	Sturgeon size (mm)	-0.112	0.2407

Chubs	Parameter	Estimate	Std. Error
	Intercept	-0.4581	0.9953
	Length	-0.1603	0.08371
	Lux	11.14	5.562
	Sturgeon abundance (mg)	6.01E-06	4.31E-06
	Invertebrate abundance (mg)	-0.0004	0.0004
	Sucker abundance (mg)	0.0001	0.0001
	River section 2	0.5576	0.4050

Table 5.6 (cont'd)

Etheostoma	Parameter	Estimate	Std. Error
	Intercept	-2.5136	1.4330
	Lux	12.6895	5.6814
	River section 2	0.3570	0.6479
	Length	0.2243	0.4529
	Invertebrate abundance (mg)	-0.0003	0.0006

Percina	Parameter	Estimate	Std. Error
	Intercept	3.2757	8.6381
	Sucker abundance (mg)	0.0007	0.0005
	Length	-1.9634	1.7509
	Invertebrate abundance (mg)	0.0012	0.0011

Minnows	Parameter	Estimate	Std. Error
	Intercept	-0.5902	0.8459
	Length	0.1183	0.1268
	Invertebrate abundance (mg)	-0.0005	0.0006
	River section 2	-0.3185	0.5719
	Lux	-3.2637	6.5019

Yellow perch	Parameter	Estimate	Std. Error
	Intercept	-3.1576	1.3064
	Lux	18.0410	7.0024
	Invertebrate abundance (mg)	-0.0006	0.0006
	Sucker abundance (mg)	-0.0002	0.0002
	River discharge (m^3/s)	0.5617	0.2366
	River section 2	0.7195	0.6813

Discussion

We demonstrated that variation in nightly mortality rate of drifting lake sturgeon larvae could be explained as a function of ecological factors in the UBR over a two-year period. Specifically, the light level and estimated biomass of suckers, fish taxa known to disperse at the same time as lake sturgeon, influenced the mortality rate of lake sturgeon larvae (Figure 5.3, Table 5.3). Additionally, we showed that the probability a predatory fish consumed drifting lake sturgeon larvae was also influenced by light level and larval sucker biomass, as well as average sturgeon size, river discharge, and invertebrate biomass (Table 5.4). However, ecological factors had disparate effects on the probability different predatory taxa consumed lake sturgeon (Table 5.5). Taken together, results elucidate the large variation in lake sturgeon survival rates during the downstream migration from spawning grounds to nursery habitat.

Effect of light level

Mortality rate increased with nightly light level and decreased with estimated sucker biomass (Figure 5.3). These results are consistent with experiments in Chapter 1, where sturgeon predation in a mesocosm was lower when the lighting level was set to simulate new moon conditions, and a diet analysis that found the probability of detecting lake sturgeon in the stomach of a predator was positively associated with percent lunar illumination (Waraniak et al., 2018). However, our results indicated the measured lux, and not percent lunar illumination, influenced lake sturgeon predation. The light level measured at the river in lux is dependent on not only percent lunar illumination, but also the timing of moonrise and moonset, and cloud cover.

Light level during the night was also an important predictor in models assessing the probability lake sturgeon DNA would be detected in the diet of a predator (Table 5.4; Table 5.5). However, light level did not influence the probability burbot and *Percina* consumed lake sturgeon (Table 5.5), likely due to feeding behaviors of these species. *Percina* species primarily feed during the day (Greenberg, 1991), so it is unlikely that night light level would influence their diet. Interestingly, 36% of *Percina* diets sampled had sturgeon DNA, even though lake sturgeon do not begin drifting in high numbers until after sunset (Smith & King, 2006). It is possible *Percina* fish consumed the first lake sturgeon larvae drifting. Conversely, burbot are a nocturnal species that often lives in deep water habitats where light levels are low (McPhail & Paragamian, 2000; Boyer et al., 1989). It is likely that burbot are better adapted to feeding in lower light levels than other predators sampled. In the minnow predator group, the probability of an individual consuming lake sturgeon increased as light level decreased (Table 5.6). Fish in this taxa group have a Weberian apparatus, a specialized swim bladder that amplifies sound and allows predators to detect prey (Holt & Johnston, 2011). The Weberian apparatus likely allows fish in this group to detect larval sturgeon, regardless of light level. On nights with low light, it is possible that predation rates of minnows on lake sturgeon larvae increase due to lower competition from visual predators.

Effect of sucker biomass

Larval suckers are known to co-disperse with lake sturgeon larvae (Smith & King, 2005). We demonstrated the biomass of larval suckers present was inversely related to larval lake sturgeon mortality and the probability a predator's diet contained lake sturgeon DNA (Figure 5.3, Table 5.4). There are multiple mechanisms that could explain this effect. Increased biomass of sucker larvae could result in predator switching (Waraniak et al., 2017), where predators select for the most abundant source of food. Experimental evidence has shown that lake sturgeon predators that were exposed to relatively high ratios of invertebrate and sucker prey subsequently consumed fewer lake sturgeon (Waraniak et al., 2017). However, sucker biomass in the UBR was generally two orders of magnitude less than lake sturgeon biomass across all drift sampling days (Table 5.2). Therefore, it is unlikely that predators would select for larval suckers due to predator switching. Alternatively, higher abundances of larval suckers could cause predator swamping, where predators feeding rate is maximized, so that additional prey experience high survival rates (Furey et al., 2016a). Data indicate lake sturgeon biomass was consistently higher and varied more greatly than sucker biomass (Table 5.2), and the biomass of lake sturgeon drifting did not influence the mortality rate of drifting lake sturgeon (Table 5.3), it is unlikely that the addition of a relatively small biomass of larval suckers maximized the feeding rates of riverine predators. Lastly, larval lake sturgeon could be using chemical cues emitted by predated sucker larvae to alter behavior and avoid predation. Experimental evidence shows larval lake sturgeon can use white sucker alarm cue to identify predator presence and elevated predation risk, which increased survival in conditions simulating the larval drift period (Chapter 4). When drifting sucker larvae biomass was greater, it is likely that lake sturgeon larvae were exposed to the sucker alarm cue as suckers are predated upon, which may cause more sturgeon to display alarm behavioral responses to evade predation. More research is warranted to determine if release of heterospecific alarm cues is the mechanism that explains why sturgeon survival increases when sucker larvae are present in high biomass.

Effect of river section

Although both river sections were approximately 2 km long, are characterized by similar habitat (Waraniak et al., 2018), and had similar diversity and densities of predators, sturgeon mortality was consistently higher in Section 2, the downstream section (Figure 5.2; Figure 5.3). There are two explanations for this effect. The first potential explanation is the abundance of rock bass. On average, approximately three times more rock bass were captured in Section 2 than in Section 1. Additionally, 33% of rock bass diets contained lake sturgeon. Rock bass were among the largest predators collected (weighing on average 31.4 ± 5.1 g, vs. 18.7 ± 1.4 g for all other predators), suggesting a rock bass with sturgeon DNA present in its diet possibly consumed more individuals than an average predator. The second possible explanation is that predators are more efficient in capturing lake sturgeon in section 2 than in section 1. The probability of detecting sturgeon DNA in the diets of predator taxa including chubs, *Etheostoma*, and yellow perch was higher in Section 2 than in Section 1 (Table 5.6), indicating there may be additional important differences between these two similar sections. These results demonstrate that drifting sturgeon mortality rates may be variable across similar habitats.

It is important to note this study focused on predation of drifting lake sturgeon over 4 km, a relatively short section of river. Mortality rates of drifting lake sturgeon over the entire distance of the UBR are assumed to be much greater than the rates reported here. The UBR is a relatively short spawning stream; it is not uncommon for lake sturgeon to migrate hundreds of km upstream to spawn (Auer, 1996). Lake sturgeon larvae do not stop drifting until reaching suitable nursery habitat, which may take days in larger river systems (Auer & Baker, 2020). Predation rates of drifting larval lake sturgeon in these longer rivers are likely to far exceed the rates we estimated in the UBR, but the same ecological factors likely have similar influences on predation rates.

Sturgeon predators

Larval lake sturgeon are susceptible to predation from diverse groups of predators. Sturgeon were found in the diets of 19/23 species sampled. The species with no sturgeon recorded in diet contents were either sampled at low frequencies (black crappie, blacknose shiner, and brook stickleback; Table 5.4) or have a mouth specialized for consuming invertebrates (white sucker; Saint-Jacques et al., 2000). Lake sturgeon were detected in 30.5% of diets. A similar diet study detected lake sturgeon in 6% of diets sampled (Wararniak et al., 2018), though the biomass of drifting lake sturgeon present in that study were often orders of magnitude lower than the estimated biomass of lake sturgeon in this study. Another diet study found one out of 862 sampled diets collected in the Peshtigo River, WI during 2006 and 2007 contained lake sturgeon larvae during the larval drift period (Caroffino et al., 2010b). Sampling over the course of the entire larval drift periods in the Peshtigo River in 2006 and 2007 gave larval lake sturgeon abundance estimates of 6,208 and 13,207, respectively (Caroffino et al., 2010a), whereas drifting larval lake sturgeon sampling on the UBR across only six nights in 2019 and seven nights in 2021 gave estimates of 9,720 and 105,818 larvae, respectively. The difference in larval lake sturgeon abundance may explain the differences the results of the diet study in the Peshtigo River (Caroffino et al., 2010b), and the study presented here.

Conclusion

Results demonstrate larval lake sturgeon mortality during the vulnerable larval dispersal period are high yet variable between nights and is influenced by ecological factors. Diet analyses show lake sturgeon during this period are vulnerable to predation by the majority of piscivorous fish in the river. Night light levels and estimated biomass of co-distributed sucker larvae had the greatest influence on both drifting lake sturgeon mortality rates, and the probability of a predator consuming lake sturgeon. Results indicate in order to increase the number of sturgeon surviving this critical dispersal period, it is important to not only to have a large sturgeon spawning population producing many eggs, but also to have abundant and taxonomically diverse populations of co-occurring larvae.

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