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Rainbow Darter (*Etheostoma caeruleum*, Storer, 1845) predation on early ontogenetic stages of Lake Sturgeon (*Acipenser fulvescens*, Rafinesque, 1817)

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Summary

Previous molecular diet analysis identified lake sturgeon (*Acipenser fulvescens*, Rafinesque, 1817) DNA in the gastrointestinal tracts of stream-resident rainbow darters (*Etheostoma caeruleum*, Storer, 1845) during the egg incubation, free embryo, and larval drift stages. The objectives of this experimental study were to: (a) quantify levels of predation by rainbow darters on lake sturgeon at the egg and free-embryo stages; and (b) evaluate whether predation varied as a function of substrate size and rainbow darter body size. We conducted experimental trials in 23-L polycarbonate tanks 0.41 m (L) × 0.33 m (W) × 0.30 m (D) with a standardized benthic area of 0.14 m^2. The tanks were randomly assigned one of two different substrate size classes: large rock (51.35 mm ± 0.91 mm) or small rock (27.68 mm ± 0.57 mm). We stocked individual rainbow darter, which were deprived of feed for 48 hr, with lake sturgeon (133 individuals/m^2) in each of 12 replicates per ontogenetic stage and substrate type. The number of surviving lake sturgeon was quantified following a 24-hr predation exposure period. We used a generalized linear model with a binomial distribution to assess the influence of ontogenetic stage, substrate size, and rainbow darter body size on proportional lake sturgeon survival. Predation on lake sturgeon occurred at both egg (6.25 ± 1.16 individuals, mean ± 2*SE*) and free embryo (3.08 ± 1.08 individuals, mean ± 2*SE*) stages. Egg proportional survival was generally lower than at the free embryo stage in both substrate sizes; however, free embryo proportional survival was greater in small substrate trials. Rainbow darter total length did not affect the probability of lake sturgeon survival at either developmental stage. Results demonstrate that rainbow darters prey on early ontogenetic stages of lake sturgeon, corroborating previous results based on genetic diet analysis. Results fill a major knowledge gap concerning the vulnerability of pre-drift sturgeon to predation by an abundant river resident species that was previously discounted as a predator for early ontogenetic stages of lake sturgeon due to its small body size.

1 | INTRODUCTION

Predation is a fundamental population regulatory mechanism, and is widely demonstrated to have indirect and direct effects on prey communities. Indirect (i.e., non-lethal) effects have been found to cause behavioral (Crossman, Scribner, Forsythe, & Baker, 2017) and life history changes among prey species (Peckarsky, McIntosh, Taylor, & Dahl, 2002). Direct (i.e., lethal) effects of predation can influence prey species population demographics by increasing mortality rates and/or decreasing reproductive success (Bailey & Houde, 1989; Crossman et al., 2017; Leggett & DeBlois, 1994; Waraniak, Blumstein, & Scribner, 2017). Predation efficiency is influenced by factors including habitat (Carreon-Martinez, Wellband, Johnson, Ludsin, & Heath, 2014; Denno, Finke, & Langellotto, 2005; Gadomski & Parsley, 2005) and predator physiological or phenotypic limitations, such as gape-limitation (Mittelbach, 1981; Rakocinski, 1991; Truemper & Lauer, 2005).

Predation is a leading determinant of annual recruitment of fishes in many ecosystems (e.g., Bailey & Houde, 1989; Leggett & DeBlois, 1994), including lake sturgeon (*Acipenser fulvescens*, Rafinesque, 1817; Peterson, Vecsei, & Jennings, 2007; Waraniak, Valentine, & Scribner, 2017). As with many fishes, lake sturgeon exhibit a type III survivorship curve (Deevey Jr., 1947) characterized by high mortality in the early life stages (Caroffino, Sutton, Elliott, & Donofrio, 2011; Forsythe, Scribner, Crossman, Ragavendran, & Baker, 2013; Nichols et al., 2003). Lake sturgeon are prey for many river-resident and adfluvial fish species before and during the larval drift life stage (Caroffino et al., 2011; Duong et al., 2011; Forsythe et al., 2013; Smith & King, 2005; Waraniak, Blumstein, et al., 2017; Waraniak, Marsh, & Scribner, 2019). Lake sturgeon eggs adhere to substrate for approximately 8–14 days before hatching into free embryos, which rely on endogenous yolk reserves (Peterson et al., 2007). During the larval drift stage, lake sturgeon emerge from the substrate, disperse downstream, and begin to feed exogenously. The magnitude of predation risk facing lake sturgeon varies according to the timing of spawning and larval drift, availability of co-distributed prey, and taxonomic composition of piscine predators (Waraniak, Valentine, et al., 2017). Quantifying these relationships in complex riverine systems is critical to the management of threatened and endangered species (e.g., Crossman et al., 2017; Waraniak, Blumstein, et al., 2017; Waraniak et al., 2019).

Laboratory studies (e.g., Crossman et al., 2017) found smaller size classes of lake sturgeon larvae tended to have greater mortality when exposed to native predators such as smallmouth bass (*Micropterus dolomieu*, Lacépède, 1802) and rock bass (*Ambloplites rupestris*, Rafinesque, 1817), while larger size classes suffered greater mortality from non-native rusty crayfish (*Orconectes rusticus*, Girard, 1852). In field studies (Waraniak, Blumstein, et al., 2017; Waraniak, Marsh, & Scribner, 2019), lake sturgeon DNA was found in the gastrointestinal tracts in nine of 17 piscine predators during the larval drift period, including numerically abundant members of the Cyprinid, Centrachid, and Percid families in the Upper Black River, MI. An especially interesting result in Waraniak, Blumstein, et al. (2017) was the discovery of lake sturgeon DNA in the gastrointestinal tracts of presumably gape-limited (<55 mm TL) species such as blackchin shiner (*Notropis heterodon*, Cope, 1865), Johnny darter (*Etheostoma nigrum*, Rafinesque, 1820), and rainbow darter (*Etheostoma caeruleum*, Storer, 1845). As these species were likely unable to consume exogenously feeding larval lake sturgeon (18–24 mm TL; Smith & King, 2005), the findings in Waraniak, Blumstein, et al. (2017), Waraniak et al. (2019) suggest smaller, riverine species feed on eggs and/or free embryo stages of lake sturgeon in the Upper Black River, MI.

Rainbow darters are small, benthic fish that occur in riffle habitats throughout small-order midwestern rivers in the USA (Harding, Burkey, & Way, 1998). Rainbow darters spawn in spring and early summer when water temperatures are between 15 and 18°C (Heins, Baker, & Tylicki, 1996; Winn, 1958). Foraging rainbow darters are often found in areas dominated by gravel substrate during their spawning period (Vogt, Jr., & Coon, 1990; Winn, 1958), which is concurrent with areas where lake sturgeon eggs are deposited and free embryos prefer to reside prior to dispersal (Hastings, Bauman, Baker, & Scribner, 2013). Rainbow darters are typically considered insectivores (Hlohowskyj & White, 1983; Wynes & Wissing, 1982). However, darters have also been observed to engage in filial cannibalism on eggs (i.e., predation on an individual's own viable offspring; Lindstrom & Sargent, 1997), and opportunistically feeding on the eggs of other fish species (Adamson & Wissing, 1977; Kuehne & Barbour, 1983; Wynes & Wissing, 1982). Given the species' propensity to consume eggs and findings of lake sturgeon DNA in the gastrointestinal tracts of rainbow darters (Waraniak, Blumstein, et al., 2017; Waraniak, Marsh, & Scribner, 2019), it is likely that rainbow darters are preying on early ontogenetic stages of lake sturgeon. The extent of rainbow darter predation on lake sturgeon eggs and free embryos has not been established, however given the species' abundance and distribution, further knowledge of the species impact to lake sturgeon early life mortality is warranted.

Little is known about predation on early life stages of lake sturgeon prior to larval drift. This study will help fill this knowledge gap by evaluating the effect of rainbow darter predation on two ontogenetic stages of lake sturgeon before initiation of exogenous feeding and drift begins. The objectives of this study were to: (a) quantify levels of predation by rainbow darters on lake sturgeon at the egg and free-embryo stages; and (b) to evaluate whether predation varied as a function of substrate size and rainbow darter body size.

2 | MATERIALS AND METHODS

2.1 | Study site

This project was conducted during May 2017 at the Black River Streamside Rearing Facility (BR-SRF) on the Upper Black River, Cheboygan County, MI, USA. The BR-SRF is a flow-through hatchery (~700 L/min) supplied with ambient river water from the Kleber Dam Reservoir. Kleber Dam is the upstream barrier on the Upper Black

River. (Baker, 1980). Lake sturgeon migrate upstream from Blake Lake and spawn downstream of Kleber Dam in several discrete areas across a 1.5 km stretch of the Upper Black River from late April to early June (Baker, 1980; Forsythe et al., 2012).

2.2 | Gamete collection and handling for free embryo trials

We collected gametes from single male and female lake sturgeon spawning in the Upper Black River, MI on 4 May 2017 for the free embryo portion of the experimental trials. Gametes were collected following methods described in Crossman, Forsythe, Scribner, and Baker (2011). Gametes were returned to the hatchery and eggs were fertilized within 4 hr of collection using methods described in Bauman, Baker, Marsh, and Scribner (2015). On the day of hatch (14 May 2017), free embryos were randomly selected in quantities of 18 per trial replicate consistent with densities described in Dammerman (2015). Free embryos were released into tank replicates immediately after hatch, allowed to disperse randomly, and remained unmolested during the 48-hr predator starvation period.

2.3 | Gamete collection and handling for egg trials

We collected gametes from a single male and female lake sturgeon spawning in the Upper Black River, MI on 16 May 2017 for the egg portion of the experimental trials. The male and female from which we collected gametes for the egg trials were different from the male and female used in the free embryo trials. We collected gametes using methods described in Crossman et al. (2011). Gametes were returned to the hatchery and the eggs were fertilized within 4 hr of collection using methods described in Bauman et al. (2015). However, eggs were not de-adhered with Fuller's earth solution so that they would adhere to the substrate in tanks. Immediately following fertilization, eggs were randomly dispersed through the experimental arena at a density of 18 eggs per tank consistent with known densities in the Upper Black River, MI (Dammerman, 2015; Finley, Forsythe, Crossman, Baker, & Scribner, 2018). Eggs were left to adhere to the respective substrates for 48 hr following introduction to accommodate the predator starvation period. Eggs were visually inspected for clumping following dispersal, and there was no visual evidence that eggs clumped together, and no eggs had to be artificially moved to avoid aggregation.

2.4 | Predator collection

We collected rainbow darters on 12 May 2017, in areas adjacent to known lake sturgeon spawning sites using a 5 mm mesh seine. We brought the rainbow darters back to the BR-SRF and placed them in a holding tank (100-L). We used the same group of darters for the free-embryo (14 May 2017) and egg trials (18 May 2017). We conducted the egg trials with the rainbow darters used in the free embryo trials because river discharge increased, which prevented additional capture of wild rainbow darters. Moreover, the availability of live lake sturgeon eggs is time sensitive, so we conducted the trials as soon as the eggs were available. The free embryo trial ended on 15 May 2017. To reduce the likelihood of learning behavior, individual darter were pooled in a 1 m diameter fiberglass tank and fed a diet of frozen bloodworms (Brine Shrimp Direct) three times per day between experiments. Additionally, the BR-SRF is a surface water facility, in which small prey items (<100 µm) are available from a single pass flow-through system (Valentine, Bauman, & Scribner, 2017). Predators were again excluded from feed for 48-hr beginning on 16 May 2019 prior to the lake sturgeon egg consumption trials.

2.5 | Experimental design

We conducted experimental trials with 12 replicates of each substrate size for both egg and free embryo stages (48 total trials) in 23-L polycarbonate tanks 0.41 m (L) × 0.33 m (W) × 0.30 m (D) with a standardized benthic area of 0.14 m^2. We standardized substrate depth to 10 cm per trial representing at least two layers of substrate for each substrate size class to allow adequate interstitial space. We stocked one rainbow darter per tank, which was deprived of food for 48 hr, with 18 lake sturgeon eggs or free embryos in each of 12 replicates per ontogenetic stage and substrate type. Prior to introduction into each tank, we measured rainbow darter weight and total length.

Tanks were randomly assigned to one of two different substrate size classes ($\mu \pm 2SE$): large rock (51.35 mm ± 0.91 mm) or small rock (27.68 mm ± 1.14 mm). We collected substrate samples from the known Upper Black River spawning grounds and returned those pieces to the experimental arena for use. These substrate sizes were selected because they provided a reasonable representation of the substrate where overlap can be expected in rainbow darter distributions and egg and free embryo lake sturgeon, not as a measure of the optimal substrate for each ontogenetic stage (Dammerman, Steibel, & Scribner, in review). A sub-sample of 15 rocks from each rock size replicate was digitally photographed to measure the largest linear distance (mm) using IMAGE-J 1.5 software. We standardized water depth in all tanks to 0.15 m above the substrate. To ensure eggs, free embryos, and predators did not escape from the trials we fitted outflow standpipes with a mesh screen. The tank inflows were standardized to a water velocity of 0.22 m/s for the egg trial, and 0.24 m/s for the free embryo trial consistent with flows in the riffle areas of the Upper Black River during the lake sturgeon spawning period (Finley et al., 2018). To achieve these velocities on the substrate, a 0.33 m long × 13 mm diameter PVC spray bar was placed on the substrate. Each spray bar featured six 2.8 mm holes spaced 6 cm apart along the long axis of the spray bar and oriented to direct water flow horizontally over the top of the substrate. Surface velocity was not measured owing to the benthic nature of the predators, and the position of the eggs/ free embryos. To limit the distraction of outside noise and movement, we surrounded the tanks with an exclusion curtain of 24″ plastic.

We deprived rainbow darters of food for 48 hr prior to the start of each trial. An individual darter was randomly selected, then measured and weighed before being added to each tank. Rainbow darter length ranged between 36–66 mm (55.79 ± 1.78 mm, $\mu \pm 2SE$). We recorded the time at which fish were added to the tank and after 24 hr, we removed the rainbow darters and placed them into a holding tank. We then terminated the flow, removed each rock and inspected them individually for adhered eggs or free embryos. We also inspected the tank once all rocks were removed for any remaining eggs or free embryos.

2.6 | Statistical analysis

We conducted all analyses in the statistical program, R (v. 3.1.5; R Core Team, 2018). We first calculated summary statistics (e.g., mean ± two standard errors) for the number of eggs and free embryos consumed per trial type. We used a generalized linear model with a binomial distribution (i.e., logistic regression; Hosmer & Lemeshow, 2000) to assess the influence of rainbow darter total length (cm), lake sturgeon ontogenetic stage (egg/ free embryo), substrate size (small/large) and an interaction term between ontogenetic stage and substrate size on the probability of lake sturgeon survival. We chose to use logistic regression to estimate the probability of lake sturgeon survival from proportional lake sturgeon survival, because Zhao, Chen, and Schaffner (2001) found that logistic regression performed better than linear regression at analyzing percentage data; therefore we followed this principle in our analysis. Specifically, we used the generalized linear model (i.e., glm) function from the base R 'stats' package (R Core Team, 2018). We also generated 95% confidence intervals (CI) based on standard errors for each coefficient included in the model. Rainbow darter weight was strongly correlated with rainbow darter total length (Pearson's $R = .93$); therefore we only included total length as a measure of rainbow darter size in the model.

3 | RESULTS

Rainbow darters consumed lake sturgeon at both the egg and free embryo ontogenetic stages and on large and small substrate sizes (Table 1). Rainbow darters consumed more eggs (6.25 ± 1.16 individuals, mean ± 2*SE*) than free embryos (3.08 ± 1.08 individuals, mean ± 2*SE*; 17.6% more individuals consumed). The mean number of eggs consumed by rainbow darters varied little between the small (6.08 ± 1.51 individuals, mean ± 2*SE*) and large (6.42 ± 1.83 individuals, mean ± 2*SE*) substrate size classes (Table 1, Figure 1). In contrast, 11.1% fewer free embryos were consumed in the tanks with small substrate (2.08 ± 1.19 individuals, mean ± 2*SE*) than those in large substrate (4.08 ± 1.66 individuals, mean ± 2*SE*).

The results from the logistic regression model (Table 2) were consistent with the patterns observed in Figure 1. Ontogenetic stage had a significant effect on the probability of lake sturgeon survival, where sturgeon in the free embryo stage had a greater chance of survival than eggs. Substrate size did not have a significant effect on the probability of survival, but had a significant, negative effect when included as an interaction term with ontogenetic stage (Figure 1). The probability of survival was lower when lake sturgeon at the free embryo stage were associated in large substrate. Rainbow darters ranged in body size (TL) from 36 to 66 mm (55.8 ± 1.8 mm; mean ± 2*SE*). The probability of lake sturgeon survival decreased

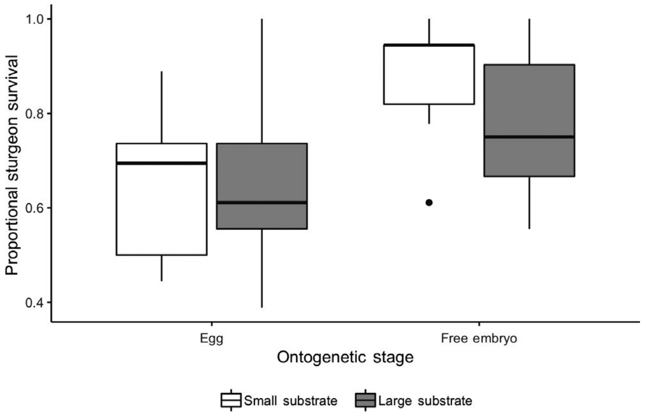

FIGURE 1 Proportion of lake sturgeon that survived rainbow darter predation at each ontogenetic stage by substrate size

TABLE 1 Mean (±2*SE*) number and percent of lake sturgeon (*Acipenser fulvescens*) individuals consumed by rainbow darter (*Etheostoma caeruleum*) per ontogenetic stage (egg/free embryo) and substrate size (small/large)

		Substrate size		Ontogenetic stage		All			
		Small	Large	Egg	Free Embryo	Small/Egg	Large/Egg	Small/FE	Large/FE
#	Mean	4.08	5.25	6.25	3.08	6.08	6.42	2.08	4.08
	2*SE*	1.26	1.30	1.16	1.08	1.51	1.83	1.19	1.66
%	Mean	22.69	29.17	34.72	17.13	33.80	35.65	11.57	22.69
	2*SE*	6.98	7.24	6.46	6.02	8.37	10.19	6.62	9.22

Abbreviations: #, number of consumed lake sturgeon; %, percent of consumed lake sturgeon; FE, free embryo; *SE*, standard error.

TABLE 2 Model coefficients with two standard errors associated with variables from our generalized linear model predicting proportional lake sturgeon (*Acipenser fulvescens*) survival

Coefficients	Mean	2*SE	95% CI	p-Value
Intercept	2.15	1.93	(0.26, 4.03)	.026*
Stage	0.97	0.41	(0.57, 1.38)	<.001**
Substrate	−0.02	0.35	(−0.37, 0.33)	.905
Darter TL	−0.03	0.03	(−0.06, 0.01)	.112
Stage*Substrate	−0.58	0.59	(−1.16, −0.01)	.048*

Note: 95% confidence intervals (CI) and *p*-values are provided for each coefficient.
**p* < .05 and ***p* < .01: Levels of significance.

slightly as rainbow darter TL increased, however this effect was non-significant.

4 | DISCUSSION

Results from this experiment corroborated genetic diet analyses by Waraniak, Blumstein, et al. (2017), Waraniak, Marsh, and Scribner (2019) in the Upper Black River by demonstrating that rainbow darters prey on early lake sturgeon ontogenetic stages. Lake sturgeon eggs were equally vulnerable to rainbow darter predation on small and large substrates. Free embryo survival was higher than egg survival, and was particularly associated with small substrate (Figure 1). The higher free embryo survival in smaller substrate may be due to differential rainbow darter foraging efficiency between relatively larger versus smaller substrate sizes.

Lake sturgeon eggs were likely more vulnerable to predation because they adhere to substrate and remain relatively stationary and visible until hatching (Scott & Crossman, 1973; Thiem, Hatin, Dumont, Kraak, & Cooke, 2013). Moreover, the ability of small-bodied predators to prey on lake sturgeon eggs within smaller substrates increases their predation risk in comparison with lake sturgeon free embryos. For example, Nichols et al. (2003) found non-native round gobies (*Neogobius melanostomus*, Pallas, 1814) present an additional threat for lake sturgeon eggs in rubble substrates, which previously provided protection from predation, but found no evidence of predation on lake sturgeon larvae within their study. Our egg predation rates are likely higher than might be seen in the wild, because the eggs only adhered to the surface of the substrate and not within the interstitial spaces provided by the layers of substrate.

In contrast, lake sturgeon free embryos are mobile and likely have greater opportunity to evade predation or escape detection by burrowing in substrate. Kempinger (1988) suggested that free embryo predator avoidance mechanisms, such as hiding in substrate, may be one of the most important adaptations for newly hatched free embryos. In our study, the smaller substrate with subsequently smaller interstitial space may have either afforded the free embryos more opportunities to hide or hindered the darter's ability to maneuver and search for prey. Given our two-layer substrate design, we believe the free embryos had adequate opportunities to hide and predation rates were not greatly exaggerated. We also recognize that lake sturgeon eggs and free embryos face predation threats from species with and without gape-limitations other than rainbow darters (Waraniak, Blumstein, et al., 2017) where predatory mechanisms are not yet understood. A more thorough understanding of predator-prey interactions with other stream predators will be necessary to gauge the vulnerabilities of early ontogenetic stages of lake sturgeon.

Our results indicate that rainbow darter total length did not affect the probability of lake sturgeon survival. Previous work has documented gape-limitations for species closely related to rainbow darters (Rakocinski, 1991; Truemper & Lauer, 2005). Thus, we expected a similar gape-limitation to influence rainbow darter consumption of lake sturgeon, where larger fish should consume greater proportions of lake sturgeon. Several factors may explain why we did not observe variability in lake sturgeon predation as a function of rainbow darter body size. Rainbow darter total lengths ranged from 36 to 66 mm, which does not encompass the upper bounds of known length distribution (maximum recorded rainbow darter length = 77 mm; Page & Burr, 1991). Additionally, the size of the eggs and free embryos may be small enough that gape-limitation does not affect adult (i.e., reproductively active) rainbow darter consumption of early ontogenetic stages of lake sturgeon. Another possibility is that the interstitial space afforded by the substrate may have restricted the searching patterns of larger rainbow darters, thus preventing them from eating larger numbers of lake sturgeon. We used rainbow darter total length as a correlate of gape-length, which represents a weakness in our study design. To better understand the mechanisms underlying these patterns, future experiments should consider a wider range of rainbow darter total lengths and incorporate measurements of predator gape-size and lake sturgeon egg and free embryo sizes.

We acknowledge that prior exposure of darters to lake sturgeon in the free embryo trials may have been a confounding factor in the egg survival portion of the experiment. Heavy precipitation resulted in elevated river flow during the late spawning run in 2017 and prevented us from being able to capture a novel set of rainbow darters for the lake sturgeon egg trials. Therefore, we used predators exposed to lake sturgeon free embryos in the first set of trials as predators for the lake sturgeon egg trials. This prior exposure to lake sturgeon free embryos could have allowed the rainbow darters to develop a “search image” for early ontogenetic stages of lake sturgeon, thus exaggerating the egg predation rates. Further experimentation including use of larger numbers of replicates would be useful.

Interpreting the results of the current experiment in light of the possible confounding effects of rainbow darter reuse should include consideration of the species' ecology and experimental conditions. Darters are heavily reliant on visual cues for feeding (Daugherty, Daugherty, & Blair, 1976; Kellogg & Leipzig-Scott, 2017; Roberts & Winn, 1962), and are susceptible to minor extrinsic variability (2017).

Lake sturgeon free embryos burrow into substrate following hatch, and their distribution is both intrinsically and extrinsically variable (Hastings et al., 2013). In contrast, lake sturgeon egg distribution is largely dependent on adult site selection (Finley et al., 2018; Forsythe et al., 2013) and consumption can be highly variable as a function of substrate size (Forsythe, Crossman, Firkus, Scribner, & Baker, 2018). In other studies, where both egg and larval sturgeon consumption was quantified, the difference between each stage was substantial (Caroffino et al., 2011). This suggests that visual cues may be sufficiently different between ontogenetic stages to overcome the lack of naivety of the predators used in both trials. Similarly, in studies of foraging in other fish species, Croy and Hughes (1991) found fifteen-spined stickleback (*Spinachia spinachea*, Linneaus, 1758) foraging efficiency was reduced when exposed to variable prey types even after exhibiting learning behavior for both prey items individually. Warburton (2003) suggested predacious fish go through a 'reorientation period' when different prey types are variable between trials (i.e., one prey type per trial). As the rainbow darters were regularly fed bloodworms between trials, it is likely that when they were introduced to the egg experimental tanks, their foraging tactics would have been initially focused towards bloodworms, rather than lake sturgeon eggs. Social learning behavior (Brown & Laland, 2003) was not possible as each experimental tank housed a single rainbow darter.

It is possible that the depth and size of substrate in this experiment resulted in a higher consumption rate of free embryos than would be observed naturally. These substrate sizes were selected because they provide a reasonable representation of the substrate in which overlap can be expected in rainbow darters and lake sturgeon eggs and free embryos, and not as a measure of the best substrate for each ontogenetic stage (Dammerman et al., in review). While substrate depths used in this study may not be comparable to those in the Upper Black River, they are consistent with similarly designed studies to evaluate predator risk (e.g., Forsythe et al., 2018).

In conclusion, our study increased understanding of predation risks to pre-drift lake sturgeon. We confirmed rainbow darter predation on early ontogenetic stages of lake sturgeon, as was suggested by the genetic data presented in Waraniak, Blumstein, et al. (2017), Waraniak, Marsh, and Scribner (2019). These findings are important, because they emphasize that even small-bodied and gape-limited species benefit from ephemeral resources such as lake sturgeon eggs deposited during the spawning season. Eggs have been widely shown to be a seasonally important nutrient import, as commonly noted for Pacific (*Oncorhynchus* spp.) and Atlantic (*Salmo salar*, Linnaeus, 1758) salmon spawning runs (e.g., Jonsson & Jonsson, 2003; Moore & Schindler, 2004). Information is less commonly reported for other adfluvial species like sturgeons. Our results demonstrate the need for additional research on the vulnerabilities of lake sturgeon to predation during early life stages in experimental and field settings. Within these studies, predator densities should be estimated to assess the effects of predator interactions and whether predation risk facing early life stages of lake sturgeon increases linearly with predator abundance. Additionally, predation on lake sturgeon free embryos varied across substrate types, coincident with differences in amounts of interstitial space. Previous studies have found that increased habitat complexity can increase prey survival by providing refuge and hindering the maneuverability of predators (e.g., Humphries, Peyre, & Decossas, 2011; Persson & Eklov, 1995; Warfe & Barmuta, 2004). Researchers would benefit from future studies pertaining to how habitat complexity affects mortality risk for lake sturgeon eggs and free embryos in the wild, where they are both exposed to a host of predators and surrounded by other potential prey species (e.g., macroinvertebrates). Additionally, as regulated rivers (e.g., Upper Black River) tend to have less geomorphic complexity than unregulated rivers (Graf, 2006), future studies should also consider how reduced habitat complexity affects predation risk for lake sturgeon. This information would help us understand what habitat types in wild systems help provide lake sturgeon refuge from a large suite of predators during vulnerable early stages of development.

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CONFLICT OF INTEREST

The authors listed above certify that they have no affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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