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Lethal and non-lethal effects of predation by native fish and an invasive crayfish on hatchery-reared age-0 lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817)

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Summary

The objective was to investigate the effects of two native fish predators (adult rock bass *Ambloplites rupestris*, and smallmouth bass *Micropterus dolomieue*) and an invasive benthic crayfish (*Orconectes rusticus*) on habitat choice and survival of hatchery-reared age-0 lake sturgeon (*Acipenser fulvescens*). Three length-classes (mean total length ± 1 *SD*) of 68.4 ± 12.7 cm, 85.9 ± 15.0, and 111.6 ± 15.4 cm corresponding to 8–9, 11–13, and 15–16 weeks of age, respectively, were examined. Experiments were conducted in a circular (2.44 m diameter, 0.6 m deep) fiberglass tank divided into two equal sections (volume of each section: 2.81 m^3). Each section included three contiguous and equal sized substrate types [sand, small gravel (4–10 mm diameter), and cobbles (30–50 mm diameter)] along with the water column providing a choice of habitats. Survival and substrate choice were quantified with and without predators over 24 hr with 30 sturgeon per trial. Multiple trials were repeated for each length-class and predator type. Survival was also evaluated when alternate prey (emerald shiners *Notropis atherinoides*) were present in equal numbers (*n* = 15 per trial). Sturgeon used sand in the absence of a predator or in the presence of fish predators, but in the presence of crayfish the water column (58 ± 5%) was used in increasingly higher proportions by the end of the trial. Survival was significantly lower for sturgeon exposed to crayfish (54%) compared to all other fish predators (97%). Fish predators consumed significantly more alternate prey (32%) than sturgeon (0%) while crayfish consumed significantly more sturgeon (35%) compared to alternate prey (0%). Results demonstrate that non-lethal effects may contribute to higher rates of mortality between alternate predator types through predator-induced occupancy of non-preferred habitats.

1 | INTRODUCTION

Predator-induced effects on prey communities, whether lethal or non-lethal, represent a significant component of ecosystem level processes (Peacor & Werner, 2004; Walsh & Reznick, 2008; Werner & Anholt, 1996). Predators can influence the behavior of prey species, which in turn modifies the effects incurred from other predators (Miller & Kerfoot, 1987). Non-lethal effects of predators may be as important as the direct mortality of prey in understanding the outcome of species interactions (Mittelbach, 1986; Werner & Anholt, 1996; Werner & Peacor, 2003). For example, predation risk and effects on foraging have been shown to influence habitat choice across a number of species (Belk, Whitney, & Schaalje, 2001; Byström, Persson, Wahlström, & Westman, 2003; Kneib, 1987; Lima, 1998; Winkelman & Aho, 1993). Furthermore, altered prey behavior can affect probabilities of detection or capture (Lima, 1998). Estimating behavioral responses related to ontogenetic changes in body size and morphology during early life history stages is particularly relevant to understanding the ecology of predator-prey interactions (Werner & Anholt, 1993; Werner & Gilliam, 1984).

Invasive species have changed aquatic community compositions in many regions (Gurevitch & Padilla, 2004). Although the direct impacts of invasive species on commercially or recreationally important species have been acknowledged for some time (Mills, Leach, Carlton, & Secor, 1994; Ogutu-Ohwayo, 1990), their effects on native fish communities are still being quantified. Invasive species can have both lethal (direct predation) and non-lethal (e.g. competition for resources) effects on prey species, especially those occupying similar habitats. Invasive predators have the potential to indirectly affect prey (Kuehne & Olden, 2012), including responses that increase exposure to other predators (Klose & Cooper, 2012). One example, the invasive rusty crayfish (*Orconectes rusticus*), has rapidly displaced native crayfish in the Great Lakes basin (Hill, Sinars, & Lodge, 1993; Olden, McCarthy, Maxted, Fetzer, & Vander Zanden, 2006), resulting in reductions in abundance (Kuhlmann & Hazelton, 2007) and reduced growth rates of native fish species (Matsuzaki, Sakamoto, Kawabek, & Takamura, 2012). The relative influences of lethal and non-lethal effects of the invasive rusty crayfish on native fish species as a function of habitat use and age has yet to be fully evaluated.

Understanding how native and invasive predators affect prey habitat use, predation risk, and anti-predator behavior during different ontogenetic life stages is important for predicting community composition and rates of prey recruitment. This is particularity important for understudied and imperiled species, such as the lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817), a species that is threatened throughout much of its range. Little is known about the interactions of young lake sturgeon with predators and the effect of those interactions on survival or behavior. Estimating the direct (e.g., lethal) effects of different predators, including invasive species, on juvenile lake sturgeon survival and the non-lethal effects on behavior would help direct restoration efforts throughout the Great Lakes (Holey, Baker, Thuemler, & Elliott, 2000) as age-0 juveniles are released annually to mitigate low natural recruitment. Further, sources of mortality in a stream setting, particularly mortality attributed to predation, for age-0 lake sturgeon are largely unknown despite studies assessing the vulnerability of sturgeon eggs and larvae (Caroffino, Sutton, Elliott, & Donofrio, 2010; Gadomski & Parsley, 2005a; Miller & Beckman, 1996), and age-0 juveniles (Caroffino et al., 2010; Gadomski & Parsley, 2005b, 2005c) to predation. Identification of predator types, habitats, and the interaction between these factors and age is important when designing restoration programs. This is especially important for sturgeon programs that focus on supplementation with hatchery-produced progeny, as these fish likely lack a strong anti-predator response immediately following release.

Documenting sources and levels of mortality and non-lethal (behavioral) responses during early ontogenetic life stages is difficult in natural settings (Houde, 1987; Schlosser, 1987). By reducing the complexity of natural environments, laboratory mesocosms provide an efficient means to study lethal and non-lethal effects on prey as a function of habitat types and predator species (Bernot & Turner, 2001; Stunz & Minello, 2001; Werner, Gilliam, Hall, & Mittelbach, 1983; Werner & Hall, 1988). In this study, laboratory mesocosms were used to quantify rates of predation on hatchery-reared age-0 lake sturgeon (*A. fulvescens*), to determine if rates of mortality varied as a function of predator species, presence of alternative prey, and size. Specific objectives were to: (i) determine whether habitat used by age-0 lake sturgeon of different sizes varied with and without different predator types, (ii) quantify the effects of sturgeon size on levels of predation by native fish and an invasive crayfish, and (iii) quantify rates of predation of age-0 lake sturgeon exposed to different predators in the presence of alternate prey.

2 | MATERIALS AND METHODS

Age-0 *A. fulvescens* used for the study were from paternal half-sib family groups produced from a population that spawns in the Upper Black River, a tributary of Black Lake, Michigan. Individuals were reared separately in two hatchery environments that are used to support the conservation aquaculture program for this population. Half of the sturgeon used in predation trials were reared in a streamside hatchery located on the natal Upper Black River (near Onaway, Michigan) with a natural temperature regime of 19.7 ± 1.9°C (*M* ± *SD*) during the course of the experiment. The remaining fish were reared in heated ground water (approximately 20°C) at a state hatchery in southwestern Michigan (Wolf Lake State Hatchery), representing a traditional hatchery environment. Both hatcheries used a flow-through design. At the streamside hatchery water was pumped directly from the Upper Black River, mechanically filtered through a sand filter to remove coarse sediments and gravity-fed through the rearing tanks before returning to the river. At the traditional hatchery, water was pumped from the ground and allowed to gravity feed through the rearing tanks without any filtration. Flow rates (18L/min) at both hatcheries were kept constant and at a level equivalent to the volume of the tank every 30 min for all rearing tanks (1.22 m diameter, 0.5 m deep). Details regarding rearing tanks, cleaning, feeding, and lighting regimes were consistent between the two hatchery environments (described in Crossman, Scribner, Davis, Forsythe, & Baker, 2014).

Predators chosen for the experiment consisted of two native fish predators, rock bass (*Ambloplites rupestris* Rafinesque, 1817) and smallmouth bass (*Micropterus dolomieu* Lacépède, 1802), which have been demonstrated to be abundant in areas occupied by larval and age-0 lake sturgeon (Caroffino et al., 2010). We also tested the invasive rusty crayfish (*O. rusticus*, Girard, 1852), a benthic predator that has spread through the Great Lakes over the past 50 years (Olden et al., 2006) and is linked to increasing ecosystem vulnerability (Olden, Vanderzanden, & Johnson, 2011). These predators were chosen due to their presence in most natural river systems that age-0 lake sturgeon occupy. Although crayfish prefer to remain in the vicinity of habitats with cobble substrate (Wilson et al., 2004), they have also been observed in the lower reaches of the Upper Black River on sand during visual night-time surveys for age-0 lake sturgeon (J. Crossman, Michigan State University, personal observation). Fish predators were collected from the Upper Black River using fyke nets, backpack electro-fishing, and hook and line fishing. Crayfish were collected using dip nets. Predator species were held separately at the streamside hatchery in indoor, circular fiberglass tanks (0.61 m diameter, 0.7 m deep). All indoor tanks were supplied with flow-through river water and overhead lighting that mimicked natural photoperiod. Predators were collected ≤4 days prior to the start of each experimental trial and were not fed during captivity. We measured (cm) total length, gape width, and gape height for all native fish predators used in the experiment. Measurements (mm) of total carapace length and pincer width were collected for the invasive crayfish.

2.1 | Experimental design

All experiments were conducted at the streamside hatchery. Experiments were conducted in a circular (2.44 m diameter, 0.6 m deep) fiberglass tank that was encircled with a black tarp to minimize human-induced behavioral effects. The tank was divided into two equal sections (volume of each section: 2.81 m^3) thus two trials could be run simultaneously. Each section included three contiguous and equal-sized substrate types: sand, small gravel (4–10 mm diameter), and cobbles (30–50 mm diameter). These substrate types were chosen due to their availability in most natural river systems that age-0 lake sturgeon occupy. The water column was also included as a habitat type. Substrate types were removed and randomly redistributed prior to the beginning of each trial. The incoming flow of water was positioned so that excessive currents were not generated in the tank. Trials were conducted for 24-hr duration beginning at 07.30 h and conducted with white sturgeon at 8–9 (mean total length ± 1 *SD*, 68.4 ± 12.7 cm; *n* = 120), 11–13 (85.9 ± 15.0; *n* = 120), and 15–16 (111.6 ± 15.4, *n* = 120) weeks of age. These specific length classes were chosen due to differences in survival observed when released into the Black River (Crossman, Scribner, Forsythe, & Baker, 2011). Further, these groupings were used because only two trials could be conducted simultaneously and several weeks were needed to complete trials with all combinations of lake sturgeon and predators. Fish reared at the traditional hatchery environment were transported to the streamside rearing facility a few days prior to the start of the trials for the different age classes tested. Acclimation of fish from the traditional hatchery environment to the streamside hatchery was conducted when water temperatures differed. Although not a specific objective of the study, we followed fish from different hatchery environments and different family groups, as both could theoretically influence variation in the results (Dammerman, Steibel, & Scribner, 2015). Fish were marked individually with a visible implant elastomer dye (Northwest Marine Technology, WA, USA) to distinguish fish reared in different hatchery environments and family groups. Elastomer was injected on the ventral side of the rostrum where the colors were most easily distinguished and not visible to predators. All fish were measured for total length before introduction into the tank and surviving fish were re-measured at the termination of the trial to determine which fish within a specific family or hatchery group were consumed by predators.

2.1.1 | Experiment 1: Tests of indirect effects of predation risk on substrate use

This experiment was conducted to evaluate whether the presence of predators, either native or invasive, influenced the choice of habitats (substrates) used by age-0 lake sturgeon. A secondary objective was to determine if the hatchery-rearing environment (streamside and traditional) influenced the distribution of sturgeon among habitats with and without predators. Fish from traditional and streamside hatcheries were used in separate trials because we were unable to follow individual fish during the trial. We conducted a total of 12 trials with four trials per length grouping. Within each length group class we conducted two trials with fish from each of the streamside and traditional hatchery. Within each trial we used five juveniles from each of six half-sib families (total *N* = 30) marked correspondingly. This density of sturgeon (10.7 sturgeon/m^3) is considered high compared to what might occur in nature for this age of sturgeon based on catch results from studies conducted on this age class (Benson, Sutton, Elliott, & Meronek, 2005; Caroffino et al., 2010). However, releases of hatchery fish occur in large numbers and sturgeon tend to remain in larger groups immediately following release (Crossman, Scribner, Duong, et al., 2011), when the majority of predation likely occurs. Juvenile lake sturgeon were introduced into tanks without a predator. Observations of fish locations, either on one of three substrate types or in the water column, were taken hourly until nightfall and then again in the morning. Sturgeon were then removed and the predator was introduced. The predator was allowed to acclimate to the tank for a 24-hr period and a new set of naive sturgeon were introduced into the tank with the predator. Individual lake sturgeon and predators were never used twice to avoid any learned behaviors; however, numerous observations of habitat selection were recorded for an individual lake sturgeon over time when the predator was present, as well as repeated observations of the predator's location. Observations were recorded hourly during daylight hours and then again at dawn the following morning. The predator was removed immediately following the termination of the trial.

All statistical analyses were performed using R (R Development Core Team, 2016). We used a multifactor analysis of variance (ANOVA) to analyze the data. The response variable was the proportion of juvenile sturgeon observed using each substrate type. The response variable was transformed using a square root transformation to achieve normality. Factors in this analysis included the presence or absence of a predator, the length class, rearing environment, and the trial number. The interaction between trial and fish size (length class) was examined to determine if this factor was confounded by the fact that it took several weeks to complete trials for each length grouping. The trial was not a significant factor within either the presence or absence of a predator, therefore all trial data were pooled. Data were reanalyzed using repeated measures ANOVA to incorporate hour of observation as an additional factor. Separate analyses were conducted for the pelagic predators (fish) and the benthic predator (crayfish).

2.1.2 | Experiment 2: Direct effects of predation on size-specific survival

This experiment was conducted to determine if different sizes of age-0 lake sturgeon were differentially vulnerable to predation. A secondary objective was to test if a hatchery rearing environment influenced the rate of predation observed in the experiment. Experimental design was consistent with experiment 1 with the exception that we could follow individual survival associated with two factors, hatchery environments and families. Family was followed as a group due to maternal differences noted during early life stages in the hatchery environment (Crossman et al., 2014). Ten trials were conducted per each fish predator (n = 1 per trial), with 4, 4, and 2 trials conducted at the 8–9, 11–13, and 15–16 week length-classes, respectively. Eight trials were conducted with rusty crayfish (n = 4 per trial), with 4 trials in each of the 11–13 and 15–16 week length-classes. Within each trial we used 5 juveniles from each of 6 uniquely marked families (N = 30 total per trial). All fish were measured for total length prior to the trial. Surviving fish were re-measured at the termination of each trial.

A general linear model was used to quantify associations between percent survival and independent variables that included: size, predator species, rearing environment, and family as fixed effects. Survival was square root transformed prior to analysis to achieve normality. To determine if there was size selective predation within each length grouping we used a paired t test to test for differences in the mean total lengths of fish that were killed and those that survived.

2.1.3 | Experiment 3: Alternate species predation trials

This experiment was conducted to evaluate whether predation rates on juvenile lake sturgeon were significantly different with and without the presence of an alternate prey species. An equal mixture of juvenile lake sturgeon (n = 15) and emerald shiners (*Notropis atherinoides* Rafinesque, 1818, n = 15) was introduced into a tank containing an acclimated predator: rock bass (n = 1 per trial), smallmouth bass (n = 1 per trial), or crayfish (n = 4 per trial). Two trials were conducted per predator species, using age-0 lake sturgeon from the larger two length-classes. Emerald shiners were chosen because they are a native species and readily available; the shiners were not reused. Family and rearing environment treatments were not included in alternate species trials due to the reduced number of sturgeon used in each replicate. Total lengths of sturgeon and shiners were measured prior to the trial. A t test was conducted to test for differences in the size distributions between sturgeon and shiners for each age-class. Substrate use and survival were recorded hourly until nightfall and then again the following morning. A three-factor ANOVA was used to analyze variation in survival as a function of length class, alternate prey species presence/absence, and predator species.

3 | RESULTS

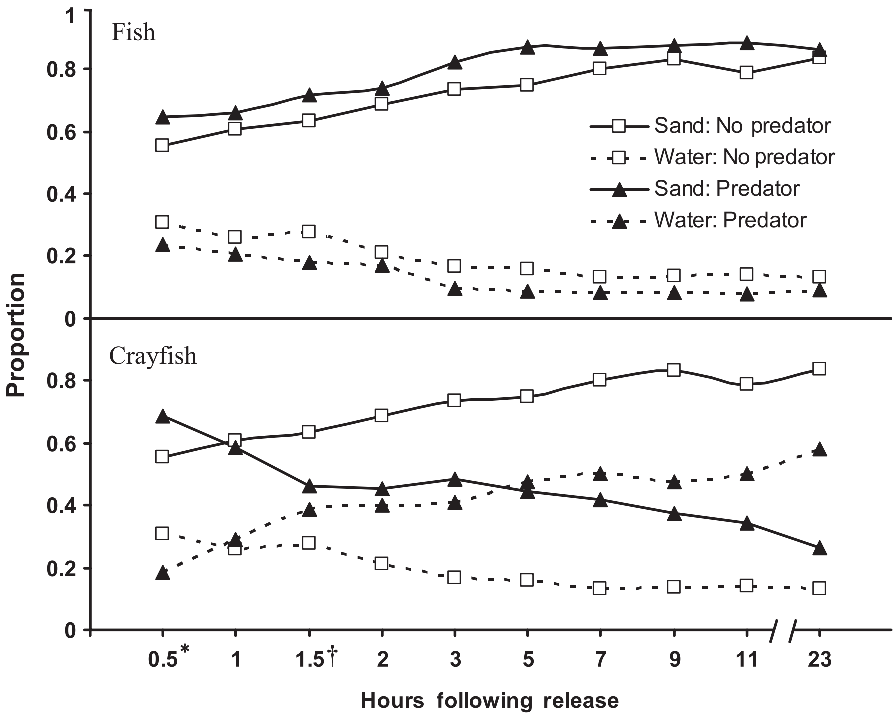
3.1 | Experiment 1: Tests of indirect effects of predation risk on substrate use

In the absence of a predator, the sand occupied by lake sturgeon was for significantly greater periods of time than rocks, gravel, and the water column ($F_{3,1276}$ = 4,670, p < .01; Figure 1). Substrate use by juvenile lake sturgeon changed in the presence of predators. There were no significant differences among the predatory fish species ($F_{2,793}$ = 0.01, p = .99) or between the main effect of hatchery rearing environment ($F_{1,793}$ = 0.02, p = .87) in our analyses, and as such, results are presented across all fish predators used. Juvenile lake sturgeon exposed to fish predators used sand substrate at a significantly higher proportion ($F_{59,1220}$ = 172.6, p = .005) when compared to small gravel, cobble, and the water column (Figure 1). Furthermore, juvenile lake sturgeon preference for sand remained significant ($F_{59,1220}$, p < .001) throughout the trial while preference for the water column significantly decreased over the same 24-hr period ($F_{59,1220}$ = 172.6, p = .016; Figure 1). Age-0 lake sturgeon at the smallest size (8–9 week length class) spent significantly less time ($F_{59,1220}$ = 172.6, p = .006) in the water column compared to older fish at both 11–13, and 15–16 weeks of age. In general, habitat occupancy was divided between sand substrate and the water column (Figure 1), with small gravel and cobbles combining for <15% total occupancy across all trials containing the fish predators.

Proportional substrate use by sturgeon was significantly different when crayfish were used as the predator compared to the two fish predators ($F_{11,755}$ = 373.6, p < .01). Use of the water column increased significantly ($F_{55,584}$ = 65.39, p < .001; Figure 1) over time following crayfish introduction, while use of sand decreased ($F_{55,584}$ = 65.39, p < .001; Figure 1). In the absence of crayfish, sturgeon preferred sand, with their preference for sand increasing significantly over time ($F_{55,584}$ = 65.39, p = .004; Figure 1). There was no effect of hatchery environment or age on fish distributions with or without crayfish predators.

3.2 | Experiment 2: Size-dependent predation rates

Size measurements recorded from all predators used in the study are provided in Table 1. Survival rates across all age classes were high when lake sturgeon were exposed to fish predators (Table 2). Survival rates ranged from 93.3%–100%, 96.7%–100%, and 100% for 8–9, 11–13, and 15–16 week length-classes. Survival was significantly less in trials conducted with the youngest length class compared to the other two ($F_{15,224}$ = 3.48, p < .001). While low, predation rates between the two predatory fish species tested were similar (Table 2). There were no significant effects of predator size, rearing environment, or time on the survival of juvenile lake sturgeon exposed to the fish predators. Family was not significantly related to mortality, likely because of low overall predation rates. However, in three trials where only one juvenile lake sturgeon was consumed, all mortalities were from the same family. The probability of this occurring by chance is



* First observation of predation by crayfish predators.
† First observation of predation by fish predators.

FIGURE 1 The effect of two predator types (fish, crayfish) on the proportion of juvenile lake sturgeon observed on sandy substrate or that were swimming in the water column. Predators included rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieue*), and the rusty crayfish (*Orconectes rusticus*). Predator and no predator refer to trials conducted with and without the presence of predators over a 24 h period

.005 (16.7% per trial multiplied by 3 trials). Total lengths of individuals that died were not significantly different from the surviving fish across all ages (*df* = 162, *t* = 0.468, *p* = .64).

Survival rates were significantly lower for juvenile lake sturgeon exposed to crayfish ($F_{1,286}$ = 1,393.6, $p < .001$) compared to the fish predators (Table 2). There was a significant effect of time on juvenile sturgeon survival, with a higher proportion of mortality occurring at hours immediately following release into the tank ($F_{11,36}$ = 3.94, $p < .001$). There was no effect of length class on survival, with rates being 0.66 ± 0.01 and 0.65 ± 0.09 for age 11–13 and 15–16 weeks, respectively. There was no effect of hatchery rearing environment on survival. A test was not done to determine if there was a relationship with crayfish morphometric data as multiple crayfish were used in each trial simultaneously. No differences were attributed to family or size across different ages or trials. The total lengths of individuals that were killed in the crayfish trials were not significantly different from the surviving fish across all ages (*df* = 100, *t* = 1.187, *p* = .238).

3.3 | Experiment 3: Alternate species predation trials

Prey choice differed significantly between predatory fish species and crayfish ($F_{1,140}$ = 313.94, $p < .01$). Length class was not a significant factor ($F_{1,138}$ = 1.41, p = .238) in the analysis so the data were pooled. A significant difference ($F_{1,92}$ = 94.52, $p < .01$) was found between predation rates on sturgeon and shiners with larger proportions of shiners being consumed by fish relative to crayfish predators over the 24-hr period (Table 3). All lake sturgeon survived in all trials with fish predators while average survival of shiners was 0.77 ± 0.09 and 0.69 ± 0.01 in rock bass and smallmouth bass trials, respectively. The opposite result was found for crayfish, which consumed significantly more lake sturgeon ($F_{1,44}$ = 457.8, p = <.01) during the 24-hr period compared to shiners, with overall survival rates of 0.62 ± 0.06 and 1.0 for sturgeon and shiners, respectively (Table 3). There was no significant difference in the total length of sturgeon (mean ± 1 *SD*, 89.4 ± 11.1) compared to shiners (89.3 ± 10.9) at the 11–13 week length-class (*df* = 118, *t* = 0.092, *p* = .927). However, sturgeon (120.0 ± 9.1) were significantly larger than the shiners (85.5 ± 8.7) during the 15–16 week length-class trials (*df* = 118, *t* = 34.04, $p < .001$).

4 | DISCUSSION

A shift in habitat use by hatchery-reared age-0 juvenile lake sturgeon was documented in the presence of both native fish predators and an invasive benthic predator. Predation risk has been shown to influence habitat shifts in numerous studies (Lima, 1998). The shift in habitat use was most pronounced in the presence of the invasive crayfish (Figure 1). Studies have indicated that fish rapidly develop antipredator behaviors after witnessing only a few attacks on conspecifics (Berejikian, Smith, Tezak, Schroder, & Knudsen, 1999). Predation rates for naïve juvenile lake sturgeon that had never been conditioned to predators was highest with crayfish relative to the fish predators evaluated, which supports the dramatic shift in habitat occupancy in the crayfish trials. While tests were completed with groups of hatchery-reared fish, these non-lethal effects may play a large role in survival in the wild. When crayfish were present, sturgeon shifted from a preferred (Peake, 1999) benthic habitat (sand) to a pelagic habitat, which may increase their vulnerability due to an increased risk of exposure to

TABLE 1 Size measurements recorded from predators used in this study, including total length (TL; cm; *M* ± *SD*), gape width (GW), and gape height (GH) for fish predators. Carapace length (CL) and pincher width (PW) were measured for crayfish

Predator	N	TL	GW	GH	CL	PW
Rock bass	10	18.0 ± 1.9	2.8 ± 0.2	2.6 ± 0.2		
Smallmouth bass	10	28.0 ± 1.0	3.4 ± 0.1	3.5 ± 0.1		
Crayfish	32				28.8 ± 2.1	13.4 ± 1.0

TABLE 2 Mean survival (*SD*) of juvenile lake sturgeon, *Acipenser fluvescens*, from three different length groupings and ages classes exposed to three different predators for a 24-hr period

		Predator		
Length group	Age group	Rock bass	Smallmouth bass	Rusty crayfish
68.4 (12.7)	8–9	0.95 (0.03)	0.94 (0.02)	
85.9 (15.0)	11–13	0.98 (0.02)	0.98 (0.02)	0.66 (0.01)
111.6 (15.4)	15–16	1	1	0.65 (0.09)

TABLE 3 Mean survival (*SD*) of juvenile lake sturgeon (LS) (*Acipenser fulvescens*) and an alternate prey species, emerald shiners (ES), exposed to three different predators at two different length groupings and corresponding ages for a 24-hr period

			Predator					
LS		ES	Rock bass		Smallmouth		Rusty crayfish	
Length group	Age group	Length group	LS	ES	LS	ES	LS	ES
89.4 (11.1)	11–13	89.3 (10.9)	1	0.70 (0.05)	1	0.68 (0.22)	0.60 (0.09)	1
120.0 (9.1)	15–16	85.5 (8.7)	1	0.83 (0.05)	1	0.70 (0.16)	0.63 (0.05)	1

predation by other predator types. Rahel and Stein (1988) showed that a prey darter species (*Etheostoma nigrum* Rafinesque, 1820) avoided predatory bass by hiding under provided cover. However, individuals became vulnerable when flushed from the cover by a second species, a benthic crayfish. In such instances, selection might be expected to favor alternative avoidance responses such as shifts to other refuge, which would be an important extension of this work. Previously, it has been hypothesized that changing habitat use to avoid predation risk is unlikely once juvenile shortnose sturgeon (*Acipenser brevirostrum* Lesueur, 1818) obtain a size too large for most predators (Kynard, Horgan, Kieffer, & Seibel, 2000). We observed habitat shifts in each juvenile lake sturgeon length-class evaluated.

Documenting the rates of predation by fish predators is important for understanding lake sturgeon ecology and for defining barriers to recruitment. Our results represent some of the first data on predation as it relates to the size and relative age of for juvenile lake sturgeon provided with habitat choices. Efforts have been made to document rates of predation on sturgeon embryos (Forsythe, 2010; Kynard & Horgan, 2002; Miller & Beckman, 1996; Nichols et al., 2003) and larvae (Gadomski & Parsley, 2005a; Kynard & Horgan, 2002), but limited work has been conducted on juveniles using size as an experimental factor. Gadomski and Parsley (2005b) found that predation was a likely cause of mortality in age-0 white sturgeon (*Acipenser transmontanus*) and that predation rates decreased with age when exposed to four predator species. They documented higher rates of predation for smaller sized fish. However, direct comparison with our results is difficult due to differences in the experimental design. Gadomski and Parsley (2005b) used smaller sized (larval) individuals initially, large prey size ranges within each trial, and predators that were predominantly benthic-feeding fish. Results from our work help to extend their study by providing a heterogeneous tank (substrate) environment and by documenting non-lethal effects (anti-predator behaviors), which are important ecological factors.

It is generally accepted that mortality rates of young-of the-year sturgeon are high (Nilo, Dumont, & Fortin, 1997), although there is a lack of knowledge regarding predation as a source of mortality for different ages of juvenile lake sturgeon. Our results show that rates of predation on the juveniles by fish predators were low and similar to results documented by both Caroffino et al. (2010) and French et al. (2010). In all of our trials, none of the predators used were gape-limited for the size of juvenile lake sturgeon used. Rates of predation on the smallest lake sturgeon (8–9 week age class) were significantly higher than larger size groups, but were still low (Table 2). Juvenile lake sturgeon responded behaviorally to the presence of fish predators by reducing activity levels (remained motionless on the sand), a behavior that has been documented in other predator-prey studies (Anholt, Werner, & Skelly, 2000; Skelly & Werner, 1990). Observations taken during trials with the fish predators revealed unsuccessful attacks on juvenile lake sturgeon at all age-classes by all predator types (data not shown). Alternate species trials were included in part to help determine if the streamside hatchery environment or tank had any effect on the feeding behavior of the predator species. Significantly higher predation rates on the alternate prey species confirmed that fish predators were actively feeding. In future sturgeon predation trials, a more benthic prey species (e.g. sculpin, Cottidae spp.) would be logical to test as an alternate prey choice for predators.

The types or complexity of habitats simulated in mesocosm studies can affect the outcomes of predator-prey interactions. Trials in this experiment were conducted within a large tank space with only one predator, and predators were never reused, thus eliminating learned behavior as a confounding variable. This created conditions where the rates of interaction between predators and prey were not excessively elevated by confinement, a common problem in small-scale predation trials. This is particularly relevant for hatchery-raised fish, which are commonly used as prey in experimental predation studies. Furthermore, crayfish densities were low compared to the natural environment, thereby minimizing aggressive or agonistic interaction (Bruski & Dunham, 1987) among individuals over prey resources.

Different results were found between predator types when an alternate species was present (Table 3). Significantly more shiners were consumed by visual fish predators compared to the same initial amount of juvenile lake sturgeon. The opposite trend was documented in the crayfish trials. Crayfish were very efficient at capturing juvenile lake sturgeon at both 11–13 and 15–16 week length-classes, despite their larger body sizes. Gadomski and Parsley (2005c) found a similar difference in prey choice between two different predators (prickly sculpins, *Cottus asper* and northern pikeminnow, *Ptychocheilus oregonensis*), one preferring juvenile white sturgeon over the alternate prey choice (goldfish, *Carassius auratus*) and the opposite with the other predator. French et al. (2010) found that fish predators presented with both age-0 pallid sturgeon (*Scaphirhynchus albus* (Forbes and Richardson, 1905) and fathead minnows (*Pimephales promelas* Rafinesque, 1820) consumed more of the alternate prey. Predation rates in our study would have been much higher if more than four crayfish had been used. Predation by crayfish was immediate (Figure 1), and observations were that each crayfish immediately captured a sturgeon. However, the entire trial period was needed for a single crayfish to consume a single juvenile lake sturgeon. Rates of predation were likely constrained by handling time and degree of satiation. Although not quantitatively measured, the high levels of vigilance, schooling, and avoidance behaviors observed visually with the shiners helped to preclude their capture by crayfish. Results showing high levels of predation by crayfish regardless of juvenile age suggest that lake sturgeon remain vulnerable to crayfish predation over prolonged periods. The rusty crayfish used in our trials is an invasive species (Olden et al., 2006) abundant in the range of this population. Crayfish are active nocturnal predators and this could be detrimental for juvenile lake sturgeon due to their known nocturnal behavior at young ages (Crossman, Scribner, Forsythe, et al., 2011; Holtgren & Auer, 2004). Direct crayfish predation on fishes, including sturgeon, has not been well documented, despite many studies that have focused on fish predation on crayfish (Roth & Kitchell, 2005). Crayfish predation on juvenile sturgeon in a natural setting has been documented for sturgeon up to 13 weeks of age (Crossman, Scribner, Forsythe, et al., 2011), but crayfish were previously more associated with mortality at the egg stage (Forsythe, 2010). Furthermore, because many large fish predators (e.g. smallmouth bass) prey upon crayfish, examining rates of juvenile lake sturgeon predation with two predator types simultaneously would be an important extension. Although predation rates on rusty crayfish by fish are lower than on native crayfish species (Kuhlmann, Badylak, & Carvin, 2008), the presence of smallmouth bass may alter crayfish behavior (Stein & Magnuson, 1976) and indirectly affect crayfish predation on sturgeon.

Streamside hatcheries placed on natal rivers have recently been implemented for lake sturgeon restoration throughout the Great Lakes (Crossman, 2008; Holtgren, Ogren, Paquet, & Fajfer, 2007). These environments have also been demonstrated as increasing phenotypic variability and genetic diversity at critical early life history stages (e.g., eggs, larvae, and juveniles) relative to more traditional hatchery environments, an effect attributed to rearing conditions (temperature, nutrients, odorants, etc.) that are more reflective of the natural environment (Crossman, Scribner, Duong, et al., 2011; Crossman, Scribner, Forsythe, et al., 2011). Despite these differences, in the experiments reported here we observed no significant results attributed to the different hatchery rearing environments (streamside vs. traditional). This is likely due to the fact that the experiments were conducted at the streamside hatchery. Fish from the traditional hatchery were transported to the streamside facility a few days prior to the start of the predation trials to allow for acclimation. If incoming water contained chemical odorants or cues from predator species (as suggested by Berejikian et al., 1999) or injured conspecifics, then the fish could have already been conditioned prior to introduction into the trial. This would be another benefit of streamside rearing for lake sturgeon restoration. Further research that examines juvenile lake sturgeon mortality within each of the different hatchery environments would be desirable. The significant shift in habitat use with and without the presence of a predator suggests that even after lengthy periods of captivity, predator avoidance by juvenile lake sturgeon still occurred which is important for lake sturgeon restoration.

5 | CONCLUSIONS

Our results revealed a strong anti-predator response in juvenile lake sturgeon when exposed to an invasive crayfish species in a heterogeneous environment. A similar response was observed with fish predators although the result was not as strong. Although overall survival was high for the fish predators, this source of mortality combined with high mortality due to crayfish cannot be discounted as a barrier to recruitment. Importantly, our data were restricted to juvenile sturgeon with fully developed armoring (scutes) (Jollie, 1980) that may have reduced predation by the fish predators. Younger ages than those tested in this study could inform the susceptibility across a similar suite of predator types. Low survival of sturgeon in crayfish trials suggests that release locations for lake sturgeon restoration programs could consider crayfish densities prior to stocking to reduce post-release mortality. Finally, non-lethal effects as reflected in shifts in habitat occupancy identified in this study are likely to predispose juvenile lake sturgeon to higher rates of predation by alternate predator species.

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REFERENCES

Anholt, B. R., Werner, E. E., & Skelly, D. K. (2000). Effect of food and predators on the activity of four larval ranid frogs. *Ecology*, *81*, 3509–3521.

Belk, M. C., Whitney, M. J., & Schaalje, G. B. (2001). Complex effects of predators: Determining vulnerability of the endangered June sucker to an introduced predator. *Animal Conservation*, *4*, 251–256.

Benson, A. C., Sutton, T. M., Elliott, R. F., & Meronek, T. G. (2005). Evaluation of sampling techniques for age-0 lake sturgeon in a Lake Michigan tributary. *North American Journal of Fisheries Management*, *25*, 1378–1385.

Berejikian, B. A., Smith, R. J. F., Tezak, E. P., Schroder, S. L., & Knudsen, C. M. (1999). Chemical alarm signals and complex hatchery rearing habitats affect antipredator behavior and survival of chinook salmon (*Oncorhynchus tshawytscha*) juveniles. *Canadian Journal of Fisheries and Aquatic Sciences*, *56*, 830–838.

Bernot, R. J., & Turner, A. M. (2001). Predator identity and trait-mediated indirect effects in a littoral food web. *Oecologia*, *129*, 139–146.

Bruski, C. A., & Dunham, D. W. (1987). The importance of vision in agonistic communication of the crayfish *Orconectes rusticus*. I: An analysis of bout dynamics. *Behaviour*, *103*, 83–107.

Byström, P., Persson, L., Wahlström, E., & Westman, E. (2003). Size- and density-dependent habitat use in predators: Consequences for habitat shifts in young fish. *The Journal of Animal Ecology*, *72*, 156–168.

Caroffino, D. C., Sutton, T. M., Elliott, R. F., & Donofrio, M. C. (2010). Predation on early life stages of lake sturgeon in the Peshtigo River, Wisconsin. *Transactions of the American Fisheries Society*, *139*, 1846–1856.

Crossman, J. A. (2008). *Evaluating lake sturgeon (Acipenser fulvescens) restoration programs in the Great Lakes: Effects of collection method, hatchery rearing environment, and age of stocking on genetic diversity, growth, survival, and predation*. PhD. dissertation, Michigan State University, East Lansing MI, 192 pp.

Crossman, J. A., Scribner, K. T., Davis, C. A., Forsythe, P. S., & Baker, E. A. (2014). Survival and growth of Lake Sturgeon during early life stages as a function of rearing environment. *Transactions of the American Fisheries Society*, *143*, 104–116.

Crossman, J. A., Scribner, K. T., Duong, Y. T., Davis, C. A., Forsythe, P. S., & Baker, E. A. (2011). Gamete and larval collection methods and hatchery rearing environments affect levels of genetic diversity in early life stages of lake sturgeon (*Acipenser fulvescens*). *Aquaculture*, *310*, 312–324.

Crossman, J. A., Scribner, K. T., Forsythe, P. S., & Baker, E. A. (2011). Hatchery rearing environment and age affect survival and movements of stocked juvenile lake sturgeon. *Fisheries Management and Ecology*, *18*, 132–144.

Dammerman, K. J., Steibel, J. P., & Scribner, K. T. (2015). Genetic and environmental components of phenotypic and behavioral trait variation during lake sturgeon (*Acipenser fulvescens*) early ontogeny. *Environmental Biology of Fishes*, *98*, 1659–1670.

Forsythe, P. S. (2010). *xogenous correlates of migration, spawning, egg deposition and egg mortality in the lake sturgeon (Acipenser fulvescens)*. PhD. dissertation, Michigan State University, East Lansing, MI. 190 pp.

French, W. E., Graeb, B. D. S., Chipps, S. R., Bertrand, K. N., Selch, T. M., & Klumb, R. A. (2010). Vulnerability of age-0 pallid sturgeon *Scaphirhynchus albus* to fish predation. *Journal of Applied Ichthyology*, *26*, 6–10.

Gadomski, D. M., & Parsley, M. J. (2005a). Effects of turbidity, light level, and cover on predation of white sturgeon larvae by prickly sculpins. *Transactions of the American Fisheries Society*, *134*, 369–374.

Gadomski, D. M., & Parsley, M. J. (2005b). Laboratory studies on the vulnerability of young white sturgeon to predation. *North American Journal of Fisheries Management*, *25*, 667–674.

Gadomski, D. M., & Parsley, M. J. (2005c). Vulnerability of young white sturgeon, *Acipenser transmontanus*, to predation in the presence of alternative prey. *Environmental Biology of Fishes*, *74*, 389–396.

Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution*, *19*, 470–474.

Hill, A. M., Sinars, D. M., & Lodge, D. M. (1993). Invasion of an occupied niche by the crayfish *Orconectes rusticus*: Potential importance of growth and mortality. *Oecologia*, *94*, 303–306.

Holey, M. E., Baker, E. A., Thuemler, T. F., & Elliott, R. F. (2000). Research assessment needs to restore lake sturgeon in the Great Lakes. Great Lakes Fishery Trust, Workshop Results, Muskegon, Michigan.

Holtgren, J. M., & Auer, N. A. (2004). Movement and habitat of juvenile lake sturgeon (*Acipenser fulvescens*) in the Sturgeon River/Portage Lake system, Michigan. *Journal of Freshwater Ecology*, *19*, 419–432.

Holtgren, J. M., Ogren, S. A., Paquet, A. J., & Fajfer, S. (2007). Design of a portable streamside rearing facility for lake sturgeon. *North American Journal of Aquaculture*, *69*, 317–323.

Houde, E. D. (1987). Fish early life dynamics and recruitment variability. *American Fisheries Society Symposium*, *2*, 17–29.

Jollie, M. (1980). Development of head and pectoral girdle skeleton and scales in Acipenser. *Copeia*, *2*, 226–249.

Klose, K., & Cooper, S. D. (2012). Contrasting effects of an invasive crayfish (*Procambarus clarkii*) on two temperate stream communities. *Freshwater Biology*, *57*, 526–540.

Kneib, R. T. (1987). Predation risk and use of intertidal habitats by young fishes and shrimp. *Ecology*, *68*, 379–386.

Kuehne, L. M., & Olden, J. D. (2012). Prey naivety in the behavioural responses of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) to an invasive predator. *Freshwater Biology*, *57*, 1126–1137.

Kuhlmann, M. L., Badylak, S. M., & Carvin, E. L. (2008). Testing the differential predation hypothesis for the invasion of rusty crayfish in a stream community: Laboratory and field experiments. *Freshwater Biology*, *53*, 113–128.

Kuhlmann, M. L., & Hazelton, P. D. (2007). Invasion of the Upper Susquehanna River watershed by rusty crayfish (*Orconectes rusticus*). *Northeastern Naturalist*, *14*, 507–518.

Kynard, B., & Horgan, M. (2002). Ontogenetic behavior and migration of Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*, and shortnose sturgeon, *A. brevirostrum*, with notes on social behavior. *Environmental Biology of Fishes*, *63*, 137–150.

Kynard, B., Horgan, M., Kieffer, M., & Seibel, D. (2000). Habitats used by shortnose sturgeon in two Massachusetts rivers, with notes on estuarine Atlantic sturgeon: A hierarchical approach. *Transactions of the American Fisheries Society*, *129*, 487–503.

Lima, S. L. (1998). Nonlethal effects in the ecology of predator-prey interactions. *BioScience*, *48*, 25–34.

Matsuzaki, S. S., Sakamoto, S., Kawabek, K., & Takamura, N. (2012). A laboratory study of the effects of shelter availability and invasive crayfish on the growth of native stream fish. *Freshwater Biology*, *57*, 874–882.

Miller, A. I., & Beckman, L. G. (1996). First record of predation on white sturgeon eggs by sympatric fishes. *Transactions of the American Fisheries Society*, *125*, 338–340.

Miller, T. E., & Kerfoot, W. C. (1987). Redefining indirect effects. In W. C. Kerfoot, & A. Sih (Eds.), *Predation: Direct and indirect impacts on aquatic communities* (pp. 33–37). Hanover, New Hamsphire: University Press of New England.

Mills, E. L., Leach, J. H., Carlton, J. T., & Secor, C. L. (1994). Exotic species and the integrity of the Great Lakes. *BioScience*, *44*, 666–676.

Mittelbach, G. (1986). Predator-mediated habitat use: Some consequences for species interactions. *Environmental Biology of Fishes*, *16*, 159–169.

Nichols, S. J., Kennedy, G., Crawford, E., Allen, J., French, J. I. I. I., Black, G., … Thomas, M. (2003). Assessment of lake sturgeon (*Acipenser fulvescens*) spawning efforts in the Lower St. Clair River, Michigan. *Journal of Great Lakes Research*, *29*, 383–391.

Nilo, P., Dumont, P., & Fortin, R. (1997). Climatic and hydrological determinants of year-class strength of St. Lawrence River lake sturgeon. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*, 774–780.

Ogutu-Ohwayo, R. (1990). The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, *Lates niloticus*, and the Nile tilapia, *Oreochromis niloticus*. *Environmental Biology of Fishes*, *27*, 81–96.

Olden, J. D., McCarthy, J. M., Maxted, J. T., Fetzer, W. W., & Vander Zanden, M. J. (2006). The rapid spread of rusty crayfish (*Orconectes rusticus*) with observations on native crayfish declines in Wisconsin (U.S.A.) over the past 130 years. *Biological Invasions*, *8*, 1621–1628.

Olden, J., Vanderzanden, J. M., & Johnson, P. (2011). Assessing ecosystem vulnerability to invasive rusty crayfish (*Orconectes rusticus*). *Ecological Applications*, *21*, 2587–2599.

Peacor, S. D., & Werner, E. E. (2004). How dependent are species-pair interaction strengths on other species in the food web? *Ecology*, *85*, 2754–2763.

Peake, S. (1999). Substrate preferences of juvenile hatchery-reared lake sturgeon, *Acipenser fulvescens*. *Environmental Biology of Fishes*, *56*, 1573–5133.

R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. URL https://www.R-project.org/.

Rahel, F. J., & Stein, R. A. (1988). Complex predator-prey interactions and predator intimidation among crayfish, piscivorous fish, and small benthic fish. *Oecologia*, *75*, 94–98.

Roth, B. M., & Kitchell, J. F. (2005). The role of size-selective predation in the displacement of Orconectes crayfishes following rusty crayfish invasion. *Crustaceana*, *78*, 297–310.

Schlosser, I. J. (1987). The role of predation in age- and size-related habitat use by stream fishes. *Ecology*, *68*, 651–659.

Skelly, D. K., & Werner, E. E. (1990). Behavioral and life-historical responses of larval American toads to an odonate predator. *Ecology*, *7 l*, 2313–2322.

Stein, R. A., & Magnuson, J. J. (1976). Behavioral response of crayfish to a fish predator. *Ecology*, *57*, 751–761.

Stunz, G. W., & Minello, T. J. (2001). Habitat-related predation on juvenile wild-caught and hatchery-reared red drum *Sciaenops ocellatus* (Linnaeus). *Journal of Experimental Marine Biology and Ecology*, *260*, 13–25.

Walsh, M. R., & Reznick, D. N. (2008). Interactions between the direct and indirect effects of predators determine life history evolution in a killifish. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 594–599.

Werner, E. E., & Anholt, B. R. (1993). Ecological consequences of the tradeoff between growth and mortality rates mediated by foraging activity. *American Naturalist*, *142*, 242–272.

Werner, E. E., & Anholt, B. R. (1996). Predator-induced behavioral indirect effects: Consequences to competitive interactions in Anuran larvae. *Ecology*, *77*, 157–169.

Werner, E. E., & Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecological Systems*, *15*, 393–125.

Werner, E. E., Gilliam, J. F., Hall, D. J., & Mittelbach, G. G. (1983). An experimental test of the effects of predation risk on habitat use in fish. *Ecology*, *64*, 1540–1548.

Werner, E. E., & Hall, D. J. (1988). Ontogenetic habitat shifts in bluegill: The foraging rate-predation risk trade-off. *Ecology*, *69*, 1352–1366.

Werner, E. E., & Peacor, S. D. (2003). A review of trait-mediated indirect interactions in ecological communities. *Ecology*, *84*, 1083–1100.

Wilson, K. A., Magnuson, J. J., Lodge, D. M., Hill, A. M., Kratz, T. K., Perry, W. L., & Willis, T. V. (2004). A long-term rusty crayfish (*Orconectes rusticus*) invasion: Dispersal patterns and community change in a north temperate lake. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*, 2255–2266.

Winkelman, D. L., & Aho, J. M. (1993). Direct and indirect effects of predation on mosquitofish behavior and survival. *Oecologia*, *96*, 300–303.

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