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ARTICLE

Assessing Antipredator Behavior and the Potential to Enhance It in a Species of Conservation Concern

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Abstract

Animal behavior has the potential to guide the management of populations at risk of extirpation. This includes insights into how animals respond to novel predators and whether those responses can be enhanced to improve survival. Training hatchery-reared fish to recognize predators has been proposed as a way to reduce postrelease mortalities in the wild. Identifying the presence of innate predator recognition and the capacity for learning to recognize predators in specific species or populations can inform conservation management practices. Here, antipredator behavior (time spent moving and distance from a predator) and the efficacy of predator training were assessed for three populations of a species of conservation concern, the Arkansas Darter *Etheostoma cragini*, which is vulnerable to predation by esocid predators like the introduced Northern Pike *Esox lucius*. Arkansas Darters demonstrated some innate ability to recognize and respond to a novel esocid predator and also responded to predator cues (training), although the direction of the response to cues was opposite our prediction. Populations differed in their response to the predator treatment, highlighting the value of managing populations separately. Although the fitness consequences of exposure to predator cues remain to be tested in Arkansas Darters, our results suggest that antipredator behavior is innate and that exposure to predator cues does affect behavior; however, whether or how those behavioral changes affect survival is unclear. This study demonstrates the importance of testing enrichment practices and incorporating behavioral observations into conservation programs to guide population-specific management decisions.

The study of animal behavior can be an effective tool that conservation practitioners use to successfully manage species of conservation concern (Blumstein and Fernández-Juricic 2010). For example, the study of antipredator behavior can elucidate how animals respond to human-caused changes in the predator community,

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including the loss and introduction of predators, and whether those changes may pose problems for at-risk populations (Carthey and Blumstein 2018). Furthermore, behavioral studies can aid in the development of tools to enhance the antipredator behavior of animals in conservation breeding programs in order to increase their survival after release (McLean et al. 1996).

Behavioral tools are being explored across multiple taxa to reduce the postrelease predation of animals reared in captivity (Griffin et al. 2000). For example, Shier and Owings (2007) found that training black-tailed prairie dogs *Cynomys ludovicianus* to recognize novel predators prior to release decreased their susceptibility to predation. Similar findings have been documented in other taxa, including birds (e.g., White et al. 2005; Guadioso et al. 2011; Cortez et al. 2015), reptiles (Burunat-Pérez et al. 2018), amphibians (Teixeira and Young 2014), and fishes (Mirza and Chivers 2000; Vilhunen 2006; D'Anna et al. 2012). However, the potential advantages of predator training vary by species and perhaps even population (e.g., Smith and Blumstein 2012; Belgrad and Griffen 2016; Toscano 2017; Jolly et al. 2018; Blumstein et al. 2019; Greggor et al. 2019; Muralidhar et al. 2019; Tetzlaff et al. 2019).

Conservation breeding programs that rear fish for population augmentation may benefit from assessing and enhancing behavioral responses to predators. In many cases, hatchery-reared fish demonstrate high rates of mortality immediately after release (Olla et al. 1998; Fraser 2008), which are thought to be caused in part by high predation rates and a lack of experience in interacting with predators (Olla et al. 1998; Jackson and Brown 2011). Because hatchery-reared fish often lack experience with predators, both over their lifetime and across generations, they demonstrate reduced predator recognition, reduced antipredator behavior, and reduced survival compared to their wild counterparts (Fraser 2008; Jackson and Brown 2011; Crane et al. 2015). Experience with predators or predator cues in captivity may improve the development and expression of antipredator behavior (Brown and Laland 2001). Furthermore, introduced and invasive novel predators can be particularly devastating to native populations, as the effect of predation can be very strong due to a lack of co-evolutionary history between predator and prey (Sih et al. 2010; Smith and Blumstein 2012; Belgrad and Griffen 2016).

Predator training has been suggested as a way to increase antipredator behavior and decrease the postrelease mortality of fish (Brown and Laland 2001; Jackson and Brown 2011; Crane et al. 2015). One method of training fish to recognize and respond to novel predators is to associate predators with a conspecific alarm cue—a chemical that is released from the skin during predation (Smith 1979; Brown and Laland 2001). Fish can learn to recognize a novel predator as a threat through associative learning when alarm cues are detected in combination with an identifying cue of a novel predator, such as the predator's odor (kairomone) and/or a visual representation of the predator (Griffin et al. 2000; Brown and Laland 2001). This approach could be initiated in conservation hatcheries, potentially increasing the survival of trained fish upon release. However, it is necessary to identify the presence of innate predator recognition and the capacity for learning to recognize novel predators (e.g., Kopack et al. 2015, 2016).

The Arkansas Darter *Etheostoma cragini* is a species of conservation concern in Colorado that inhabits the Arkansas River drainage. It is currently threatened by loss of habitat and by predation from introduced species, including the Northern Pike *Esox lucius*, a generalist apex predator that has been shown to prey on Arkansas Darters and to directly reduce darter distribution and abundance in Colorado (Labbe and Fausch 2000; Groce et al. 2012; Fitzpatrick et al. 2014). Captive breeding of Arkansas Darters for supplementation into wild populations has been initiated by the state of Colorado (Groce et al. 2012), but its efficacy is still unclear. Fitzpatrick et al. (2014) found that hatchery-reared Arkansas Darters contributed very little genetically to wild populations in areas where they had been released. Arkansas Darters are able to reproduce in the hatchery, suggesting that their lack of genetic contribution is unlikely to be attributable to reproductive barriers. Instead, it is suspected that hatchery-reared darters cannot reach reproductive age in the wild due to high mortalities from predation (Olla et al. 1998), prompting the state of Colorado to consider the use of predator training as a way to decrease the number of darters that are lost to predation after release. Little is known about the antipredator behavior of Arkansas Darters. To our knowledge, no studies have investigated the response of Arkansas Darters to predation cues or the potential to enhance that response in hatchery-reared individuals intended for release.

The goal of this study was to determine whether hatchery-reared Arkansas Darters have an innate capability to detect and behaviorally respond to the threat of predation from a novel esocid predator (tiger muskellunge [Northern Pike × Muskellunge *Esox masquinongy*]) and whether predator training could be used to enhance the darters' recognition of and antipredator responses to the novel predator. Objectives of the study included determining (1) whether Arkansas Darters are capable of responding to a nonnative predator by reducing movement and maintaining their distance from the threat, (2) whether exposing darters to chemical and visual predator cues prior to an encounter with a tiger muskellunge increases their antipredator responses, and (3) whether responses to the tiger muskellunge or predator recognition training differ

TABLE 1. Predator species that are known to coexist with each population of Arkansas Darters (Colorado Parks and Wildlife, unpublished data). In each column (darter populations 1, 2, and 3), an asterisk (*) indicates that the predator species has been detected in association with that population of darters.

Predator species	Population 1 (Big Sandy Creek)	2 (Black Squirrel Creek)	3 (Horse Creek)
Yellow Perch *Perca flavescens*[a]	*		
Northern Pike *Esox lucius*[a]	*		
Black Bullhead *Ameiurus melas*	*	*	*
Green Sunfish *Lepomis cyanellus*	*	*	*
Largemouth Bass *Micropterus salmoides*[a]	*		
Channel Catfish *Ictalurus punctatus*	*		*
Orangespotted Sunfish *L. humilis*	*		

[a] Nonnative predator.

among three darter populations that are known to exist with and without esocid predators. Because Arkansas Darters have not coevolved with tiger muskellunge, we predicted that darters would not have an innate predator response to them. We predicted that exposure to alarm cues coupled with tiger muskellunge kairomones and a visual model would increase antipredator behavior through associative learning. Finally, because Arkansas Darter populations are known to differ genetically and in the predator community they experience, we expected to find differences among populations in their response to predators and perhaps in their response to predator training.

METHODS

Animal husbandry.— We conducted this experiment using three populations of Arkansas Darters: (1) Big Sandy Creek (UTM coordinates 717,413, 4,269,881), (2) Black Squirrel Creek (524,117, 4,317,294), and (3) Horse Creek (613,691, 4,260,607; hereafter referred to as populations 1, 2, and 3). These three populations serve as source populations for conservation hatchery propagation and stocking in Colorado, and we suspected that behavioral responses could differ by population due to genetic differences (Fitzpatrick et al. 2014) and due to the differences in native and introduced predators that are known to coexist with these populations. Specifically, population 1 experiences a broader community of predators, including an introduced esocid (Northern Pike), compared to populations 2 and 3 (Table 1).

Both darters and predators for this experiment were sourced from hatcheries in Colorado. One-hundred young-of-year Arkansas Darters from each population (size ranging from 37 to 58 mm TL, with no size differences among populations) were obtained from the Colorado Parks and Wildlife (CPW) John W. Mumma Native Aquatic Species Restoration Facility (Alamosa, Colorado). The genetic integrity of these populations is maintained in the hatchery through careful breeding practices, including frequent supplementation using wild individuals, meaning that the hatchery-reared Arkansas Darters are only a few generations removed from wild individuals (Fitzpatrick et al. 2014). Seventeen young-of-year tiger muskellunge (233–295 mm TL) were obtained from the CPW Wray Fish Hatchery (Wray, Colorado). Tiger muskellunge were selected as predators because they were readily available, free of disease, and easy to maintain and are a hybrid cross of Northern Pike and Muskellunge.

Arkansas Darters and tiger muskellunge were transported to the CPW Salmonid Disease and Sport Fish Research Lab (Fort Collins, Colorado), where experiments took place. For 4 weeks prior to the start of the experiment, each species was held in 76-L flow-through tanks (31.75 × 61.0 × 42.0 cm), with darters separated by population and predators divided into groups of 5–6 individuals/tank. Water was supplied from a 4.88-m well, filtered through a coke ring headbox for degassing and aeration, and then held in a head tank before being gravity fed to the tanks at a flow rate of 7.5 L/min. Water temperature was maintained at 13.5 ± 2.0°C for the duration of the experiment. The laboratory was illuminated by 32-W fluorescent lights (General Electric Electrolux) with a light cycle of 14 h light : 10 h dark, and all experiments and observations took place when lights were on. Darters were fed thawed bloodworms (San Francisco Bay Brand, Newark, California), while tiger muskellunge were fed trout feed (Rangen, Buhl, Idaho) ad libitum once daily. One week before we conducted any behavioral observations, darters were moved to individual 10-L flow-through tanks (23 × 31 × 19 cm; water depth = 17.75 cm) for the remainder of the experiment and continued to receive thawed bloodworms ad libitum once daily.

Chemical cue extraction.— As part of the predator training treatment, we exposed darters to conspecific alarm cues and kairomones from tiger muskellunge. Conspecific alarm cues were collected from the epidermal

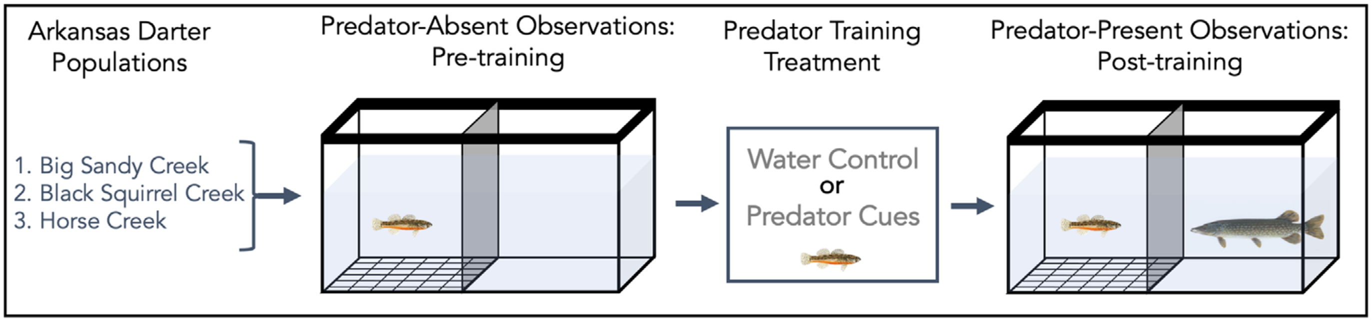

FIGURE 1. Flow diagram of experimental procedures. We evaluated the antipredator behavior of three populations of Arkansas Darters (n = 180). First, we measured behavior without a predator (predator-absent observation; 20 min). Next, darters were randomly assigned to either a predator recognition training (predator cues) or a control (water control) treatment. Finally, we measured the same behaviors in response to a live predator (tiger muskellunge; predator-present observation; 20 min). Food was introduced after 10 min during both the predator-absent and predator-present observations. Pre- and posttraining observations were conducted in a 76-L tank, and training was conducted in individual 10-L tanks.

tissue of 51 recently decapitated Arkansas Darters (mixed tissue from 17 fish/population). Immediately after decapitation, fish were scored 50 times on either side in a crosshatch pattern using a razor blade. Bodies were then rinsed with 10 mL of distilled water filtered through cheesecloth, and the water with the cues was immediately stored in a freezer at −20°C until the day of its use (adapted from Nordell 1998 and following Kopack et al. 2015). Predator kairomones were acquired by collecting water from a closed circulatory 19-L tank that housed two tiger muskellunge predators for 48 h. During this time, the predators were fed six darters (two from each population), so the water likely contained conspecific alarm cues in addition to the predator kairomones. The collected water was stored at −20°C until the day it was used (following Kopack et al. 2015).

Experimental design.— This experiment had three parts: (1) we observed darter behavior in the absence of a predator (hereafter, “predator-absent” observation) to establish a pretraining baseline, (2) we randomly assigned darters to a treatment (either predator training or a control) and subjected them to the treatment, and (3) we then observed darter behavior in the presence of a predator (hereafter, “predator-present” observation; Figure 1). Each individual darter participated in all three parts. In the predator-present observation, the behavior of the control darters represents innate antipredator behavior, while the behavior of the darters from the treatment group reflects the added effect of the training.

Both the predator-absent and predator-present observations were conducted in the same 76-L tank (31.75 × 61.0 × 42.0 cm; water depth = 30.5 cm), and the tank was emptied and sterilized with iodophor between each trial. The tank was fitted with (1) a permanent, semi-permeable, transparent plastic divider in the center to separate darters from the tiger muskellunge, which allowed water and thus odor to move between the two sides; and (2) a removable opaque divider that was used to control visual access to the tiger muskellunge. On the bottom of the tank on the side occupied by the darter, there was a 30- × 30-cm grid (consisting of thirty-six 5-cm^2 blocks), which was used to reference the distance between the darter and the divider (Figure 1). A camera (Model CHDHS-101; GoPro Hero 4 Session) was mounted above each tank to record behavior, and a blind was placed in front of each tank to hide researcher movements during the experiment.

Predator-absent and predator-present observations of darter behavior were conducted sequentially and identically except that a live predator was present during only the predator-present observation. Bloodworms were withheld from darters for 24 h prior to the start of the observations. At the beginning of each pretraining, predator-absent observation, nothing (i.e., no tiger muskellunge predator) was placed on one side of the permanent transparent divider. With the opaque divider in place, we introduced a darter into the opposite side of the tank and allowed 30 min for acclimation. After 30 min, the opaque divider was removed, allowing the darter to view the opposite side of the tank through the transparent divider. The video camera then recorded behavior for 20 min. During the observations, we introduced food, consisting of five thawed bloodworms placed along the darter side of the transparent divider using a 1-mL pipet, midway through the 20-min experiment (i.e., after 10 min). With no threat of predation on the other side of the divider, we expected darters to move about the tank normally and to approach the divider in an attempt to feed following the introduction of food.

After all observations were complete, a trained observer that was not aware of the treatments scored each video to extract two dependent variables: (1) time spent moving and (2) distance from the divider. For movement, the observer recorded the amount of time (s) that the darter spent moving during the first 10 min of the

20-min observation period (before food introduction) and during the last 10 min of the observation period (after food introduction). For distance, we used the grid on the bottom of the tank to measure the darter's distance from the divider before and after food was added. As a measure of distance before the introduction of food, we recorded distance at 0 and 10 min and averaged the two values; similarly, as a measure of distance after food introduction, we recorded distance at 11 and 20 min and averaged the values.

Upon completing the initial pretraining behavioral observations in the absence of a tiger muskellunge, darters were placed back into their individual 10-L tanks for treatment. One week later, half of the individuals from each of the three populations were assigned to either a water control treatment or a predator cues treatment. The water control treatment received 10 mL of distilled water, while the predator cues treatment received 5 mL of alarm cue mixed with 5 mL of predator kairomone (previously thawed at room temperature) as well as exposure to a visual predator model. Blinds were placed over all individual tanks to prevent researcher movements from influencing the treatment, and darters were allowed 30 min to acclimate after the placement of the blind before the treatment was initiated. To add the liquid to each individual tank, air tubing was taped to the front right corner of the tank, extended outward past the viewing blind where the researcher was positioned, and attached to a 10-mL syringe that was loaded with water or cue. We turned off the water in the flow-through system to prevent dilution of the cues during the treatment period, and the treatment lasted 5 min. For the visual predator model used in the predator cues treatment, a 102-mm plastic lure that resembled a tiger muskellunge (Model SGK022; Savage Gear USA) was attached to a wand with fishing line. A researcher standing behind the blind used the wand to introduce the model into the individual tank when the liquid was added (water or cue). After 5 min, the model was removed and the water was turned back on to flush the tank.

Twenty-four hours after darters received the treatment, they participated in the predator-present observation. We used the same methods described above to record darter behavior, but with 1 of 17 randomly chosen tiger muskellunge predators present on the other side of the transparent divider. If darters perceived the predator as a threat, we expected them to reduce movement and maintain or increase their distance from the divider rather than approaching the divider in an attempt to feed.

Statistical analysis.— To evaluate whether Arkansas Darters respond to tiger muskellunge predators and to assess whether predator training enhanced those responses, darter behavior was measured before (predator-absent observation) and after (predator-present observation) predator training as well as before and after the introduction of food using two metrics: (1) time spent moving and (2) distance from the divider. These behaviors were used as measures of antipredator response, as we expected darters to spend less time moving and to maintain a greater distance from the divider in the presence of a tiger muskellunge predator if they exhibit the normal antipredator behavior typically seen in prey fish (Januchowski-Hartley et al. 2011; Crane et al. 2015). Time spent moving had a skewed distribution and unequal variance around the mean (Levene's test of equal variance: $F_{23,\ 334} = 4.40$, $P < 0.01$; leveneTest in the R package car; Fox and Weisberg 2019) and was therefore standardized using a natural log transformation prior to analysis. During posttraining, predator-present observations, some tiger muskellunge breached the transparent divider, consuming darters when they did. In total, 14 darters were consumed during predator-present observations. These trials were excluded from the analyses, resulting in fewer predator-present observations ($N = 106$) than predator-absent observations ($N = 120$).

We compared antipredator behavior between treatments (water control and predator cues), among populations (1, 2, and 3), before and after food introduction, and in the absence or presence of a tiger muskellunge in a mixed model. Because each individual was tested with a predator absent and then with a predator present and before and after the introduction of food, we used a repeated-measures ANOVA for each behavior: movement and distance (lme4 package: Bates et al. 2015; lmerTest package: Kuznetsova et al. 2017). Presence of a tiger muskellunge (present or absent), treatment (water control or predator cues), the introduction of food (before or after), and population (1, 2, or 3) were treated as fixed effects, while individual identity was treated as a random effect for both analyses (movement and distance). All two-way, tertiary, and quaternary interactions between the fixed effects of behavior were also investigated. We retained and report significant and nonsignificant effects from the full model because (1) each of these fixed effects and interactions were part of our experimental design, (2) we had a priori predictions for all factors in our model, and (3) others have argued against removing nonsignificant effects from models, as it can bias the results (e.g., Whittingham et al. 2006; Forstmeier and Schielzeth 2011). Values were reported from the type III sum of squares. If fixed effects were found to be significant in a model ($P \leq 0.05$), the least-squares means method (lsmeans package; Lenth 2016) was used to determine differences in behavior between treatments, among populations, before and after food introduction, and in the absence and presence of a tiger muskellunge. All statistical analyses were performed using R statistical software (R Core Team 2017).

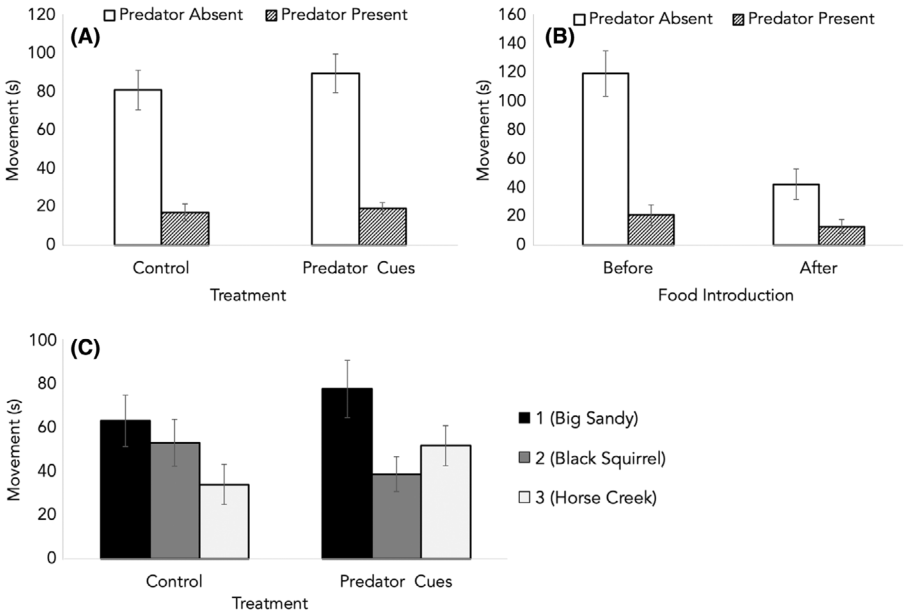

FIGURE 2. **(A)** Time spent moving (s; mean ± SE) by Arkansas Darters during predator-absent (open bars) and predator-present (hatched bars) observations, before (left) and after (right) darters received the water control (Control) or predator cues treatment. Both main effects were significant, but their interaction was not (see Supplementary Table 1.1). **(B)** Time spent moving (mean ± SE) by darters during predator-absent (open bars) and predator-present (hatched bars) observations, before (left) and after (right) the introduction of food halfway through the observation, is shown (see Supplementary Table 1.2). There was a significant interaction between treatment and food introduction. In panels A and B, all three darter populations are pooled. **(C)** The time spent moving (mean ± SE) in the presence of a predator between water control and predator cues treatments differed among darter populations (see Supplementary Tables 1.3 and 1.4). In panels A, B, and C, error bars show SE (N = 180).

RESULTS

The amount of time (s) that darters spent moving varied in response to the presence of a predator, the introduction of food, and the treatment (exposure to the water control or to predator cues). First, darters spent less time moving when there was a tiger muskellunge predator present compared to when the predator was absent ($F_{1,\ 258} = 61.84$, $P < 0.001$; Figure 2A). This was true for both treatment groups, but treatment also had an effect; on average, the group that was exposed to the water control moved less (mean ± SE = 49.89 ± 6.14 s) than the treatment group that was exposed to predator cues (56.51 ± 6.11 s; $F_{1,\ 89} = 4.26$, $P = 0.04$; Figure 2A). There was no interaction between treatment and the presence of a predator ($F_{1,\ 271} = 1.89$, $P = 0.17$). The introduction of food caused a decrease in movement in the absence of a predator ($t_{246} = 6.12$, $P < 0.001$) but did not affect the already low levels of movement when a predator was present ($t_{251} = 0.86$, $P = 0.39$; interaction term: $F_{1,\ 249} = 12.97$, $P = 0.0004$; Figure 2B). When a predator was absent, 8.5% of all darters attempted to feed, whereas 2.1% fed when a predator was present. The three darter populations also responded differently to the treatment (interaction term: $F_{2,\ 88} = 3.48$, $P = 0.04$; Figure 2C), and the main effect of population was marginally significant ($F_{2,\ 88} = 3.46$, $P = 0.06$). When a predator was present, individuals from population 3 that experienced the predator cues treatment spent more time moving than those from the water control treatment ($t_{88} = -2.95$, $P = 0.004$), whereas there was no significant difference in time spent moving between treatments for populations 1 and 2 (population 1: $t_{90} = -1.36$, $P = 0.18$; population 2: $t_{86} = 0.78$, $P = 0.44$; Figure 2C). Within the water control treatment, darters from population 3 spent significantly less time moving in the presence of a tiger muskellunge than darters from populations 1 ($t_{89} = 2.38$, $P = 0.05$) and 2 ($t_{86} = 3.15$, $P = 0.006$). There were no significant differences in time spent moving among populations in the predator cues treatment ($-0.59 \leq t \leq 1.42$, $P \geq 0.05$), as fish from all three populations converged on an intermediate amount of movement when a tiger muskellunge was present (Figure 2C; Supplementary Tables 1.3 and 1.4 available in the online version of this article).

Darters changed their distance from the divider in response to predator presence and treatment (Figure 3), but population ($F_{2,\ 89} = 0.13$, $P = 0.88$) and food introduction ($F_{1,\ 248} = 0.05$, $P = 0.83$) had no effect. When there was a tiger muskellunge predator present, darters increased their distance from the divider ($F_{1,\ 269} = 27.90$, $P < 0.001$; Figure 3). Darters in the predator cues treatment decreased their distance to the divider compared to darters in the water control treatment ($F_{1,\ 89} = 4.53$,

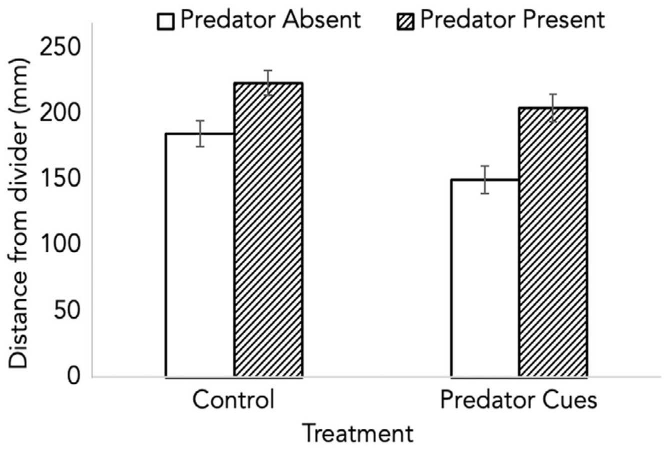

FIGURE 3. Distance of Arkansas Darters from the divider (mm; mean ± SE) when the predator was absent (open bars) and present (hatched bars) for fish from the water control and predator cues treatments. All three darter populations are pooled. Both main effects were significant, but the interaction was not (see Supplementary Table 2).

$P = 0.04$; Figure 3). There was no interaction between these two main effects ($F_{1,\ 269} = 0.77$, $P = 0.38$).

DISCUSSION

Our results suggest that Arkansas Darters possess some innate ability to detect, recognize, and respond to the threat of predation from a novel esocid predator. Opposite our prediction, we observed antipredator behavior in untrained, control fish when in the presence of a predator. Regardless of the treatment they received, Arkansas Darters strongly decreased their time spent moving and increased their distance from the divider when a tiger muskellunge was present, and both responses are in the direction indicative of antipredator behavior. This suggests an innate ability of the darters to recognize and respond to a novel esocid predator, despite not having coevolved with this species (Ferrari et al. 2008; Sih et al. 2010). Innate responses may not be uncommon; Kopack et al. (2015) found that hatchery-reared Rainbow Trout *Oncorhynchus mykiss* exhibited innate antipredator responses to predator cues. This innate response may reflect the ability of Arkansas Darters to generalize predatory threats across suites of species (Ferrari et al. 2008) and may stem from the high predation pressure they experience given their small size at maturity (Jørgensen and Fiksen 2010). Generalizing predatory threats may offer Arkansas Darters a way to minimize costs of maintaining species-specific responses given the uncertainty of which predators will be encountered throughout their lives (Ferrari et al. 2008). Future experiments should further explore whether Arkansas Darters generalize predatory threats or whether other predatory species elicit greater responses; this can be accomplished by observing antipredator behavior in response to training with different native and nonnative predators.

Arkansas Darters altered their behavior in response to predator training, but it was not in the direction predicted. In the presence of a predator, darters that were treated with predator cues spent more time moving compared to control fish. A decrease in movement, rather than an increase, is thought to increase the probability of surviving initial encounters with predators (in Rainbow Darters *Etheostoma caeruleum*; Crane et al. 2015); however, whether a reduction of movement in the presence of an esocid predator is adaptive in Arkansas Darters is unknown. Future studies should directly measure survival, especially since exposure to predator cues does not always translate to increased survival after release (e.g., Kopack et al. 2016) and because the factors correlated with the success of reintroduction programs are diverse (e.g., water quality, prey availability, and the duration of the stocking event; Cochran-Biederman et al. 2015). Furthermore, the magnitude of the treatment effects was small; treatment fish moved for 6.6 s more than control fish. It is unclear whether these differences would translate to differences in survival upon initial encounters with a novel predator.

We also found an effect of the predator training on distance from the divider; fish that were exposed to the predator cues were 28.1 mm closer to the tiger muskellunge than fish that were treated with the water control. This effect is small, and we are cautious in our interpretation of this result. There was a slight difference in the predator-absent responses between treatment groups (predator cues versus water control) before we assigned and administered these treatments, potentially due to sampling error and/or variability in individual responses measured during the predator-absent observation period. This could have affected differences between treatments in our distance measurements when the predator was present after treatment occurred. Additionally, our measure of distance from the divider was an average of only four time points rather than continuous measurements across the entire trial. Despite these limitations, the patterns we detected for distance from the divider mirrored our results for movement, providing support for the general result that predator presence and training affected darter behavior.

A similar study in Rainbow Darters found effects of predator cues on behavior for wild populations but not for hatchery populations (Crane et al. 2015). It is possible that wild Arkansas Darters would also be more responsive to a predator cues treatment than the hatchery-reared fish used in our study, and this should be explored in future work, as relaxed and/or reversed selection in captivity could impact the response to predator cues (Brown and Laland 2001; Fraser 2008; Crane et al. 2015). This information could inform the decisions of aquatic resource managers. For instance, if wild Arkansas Darters are more responsive than hatchery populations, then continuous and opportunistic supplementation of hatchery populations and their broodstock using wild darters would be necessary to maintain appropriate antipredator behavior in hatchery-reared fish over time.

The three Arkansas Darter populations differed in their response to the treatment for movement. Exposure to predator cues affected movement for population 3 but did not have a significant effect on the other two populations, thus highlighting the importance of considering discrete populations independently rather than viewing the species as a single homogeneous unit. The cause of this difference among populations is unclear. This pattern does not match population differences in exposure to esocid predators, as only population 1 coexists with Northern Pike. Although we know that Northern Pike do forage on and directly affect the abundance and distribution of Arkansas Darters (Labbe and Fausch 2000), there are certainly other abiotic and biotic sources of mortality (e.g., pools freezing; Labbe and Fausch 2000) that could affect population-specific treatment efficacy and poststocking survival among these populations.

To test the trade-off between safety and foraging, we presented darters with food during observations with and without a predator present. If darters perceived the predator as a threat, it should have affected foraging behavior. However, very few animals fed during our experiment (8.5% fed when a predator was absent; 2.1% fed when a predator was present), thereby limiting our ability to make inference from this study. We did find that in the absence of a predator, there was a reduction in movement after food was added, closer to the low levels of movement observed in the presence of a predator. This could indicate that the addition of food had a startling effect that induced antipredator behavior, or it could reflect a reduction in exploratory behavior over time in the tank. Unfortunately, we cannot tease apart these two alternatives or draw conclusions about potential trade-offs between foraging and antipredator behavior from our study, but future work should incorporate foraging/antipredator trade-offs to more closely mimic the decisions facing wild individuals.

Our study demonstrates how behavioral observations can be applied in conservation programs to guide the decisions of managers. Our experimental design allowed us to mimic what Arkansas Darters experience during initial encounters with nonnative esocid predators after release and to gain insight into how they respond. Arkansas Darters may have an innate potential to respond to novel esocid predators, which managers could leverage, but ideally these responses should be compared to the antipredator responses of wild darters in future studies. Predator recognition training has been suggested as a way to increase the postrelease survival of captive-reared, predator-naïve species of conservation concern (Brown and Laland 2001; Crane et al. 2015). To our knowledge, our study is the first to investigate the potential to enhance antipredator behavior in Arkansas Darters. We did find effects of predator training, but the behavioral responses were not in the direction predicted and the responses also varied across populations; further, it is unclear whether or how these responses might affect survival and fitness. This highlights the importance of testing enrichment strategies before implementing them widely in hatcheries. Several other methods that could potentially increase antipredator behavior and survival in this species have yet to be explored, such as multiple training events, training in groups rather than individually (which would be more logistically feasible in a hatchery), and different forms of predator training (e.g., multiple predator species, exposure to live predators, etc.). Furthermore, because hatcheries rarely mimic natural environments, abiotic enrichment is a promising future research avenue (Lamothe and Drake 2019). Fish must not only recognize predators, but also be able to escape them, and both abiotic (e.g., refugia, water flow, and temperature) and biotic (e.g., competition for resources, predatory threats) environmental factors can shape behavioral, morphological, and physiological traits associated with surviving predation (Sgrò and Hoffmann 2004; Conover and Baumann 2009; D'Anna et al. 2012). The use of multiple populations in this experiment allowed us to detect population-level variation in behavior, validating the importance of continuing to manage Arkansas Darters at the population level. This study lays important groundwork for assessing the efficacy of predator training that future research can build upon to inform the management of behavior in hatcheries.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.