Received: 12 September 2022 | Revised: 28 May 2023 | Accepted: 3 July 2023

DOI: 10.1111/csp2.12999

Conservation Science and Practice
A Journal of the Society for Conservation Biology
Open Access
WILEY

CONTRIBUTED PAPER

The effects of abiotic enrichment and predator training on the behavior, morphology, and survival of a species of conservation concern

Christopher J. Kopack[1] | **Eric R. Fetherman**[2] | **E. Dale Broder**[3] | **Ryan M. Fitzpatrick**[2] | **Lisa M. Angeloni**[1]

[1]Department of Biology, Colorado State University, Fort Collins, Colorado, USA

[2]Colorado Parks and Wildlife, Aquatic Research Section, Fort Collins, Colorado, USA

[3]Department of Biological Sciences, University of Denver, Denver, Colorado, USA

Correspondence
Christopher J. Kopack, Department of Biology, Colorado State University, Fort Collins, CO 80523-1878, USA.
Email: cjkopack@rams.colostate.edu

Funding information
Colorado Parks and Wildlife Species Conservation Trust Fund, Grant/Award Number: Grant no. SCTF911C; National Science Foundation Graduate Research Fellowship Program; Colorado Parks and Wildlife's Species Conservation Trust Fund; National Science Foundation

Abstract

Conservation practitioners often rely on captive breeding programs to supplement wild populations at risk of extinction. While supplementation has been successful for some taxa, the success of using hatchery fish to supplement wild populations is severely impacted by predation. Elevated predation on hatchery fish may arise because hatchery environments often differ from wild environments and constrain the ability of hatchery fish to be adapted to the environments in which they are released. We assessed the effects of abiotic enrichment on the expression of behavioral and morphological phenotypes across three populations of a species of conservation concern, the Arkansas darter (*Etheostoma cragini*). We also used a factorial approach to assess whether abiotic enrichment and predator training increases survival during encounters with a novel predator. We found that abiotic enrichment affected ecomorphological attributes associated with fins; generally, measures of the caudal and pectoral fin decreased in the treatment group compared to the control treatment. Behaviorally, darters reared with abiotic enrichment increased feeding and decreased movement compared to the control group. Importantly, we found that in combination with predator training, abiotic enrichment increased the probability of surviving first encounters with a predator. We therefore recommend conservation practitioners incorporate abiotic enrichment and predator training in hatchery programs. Captive breeding programs are used to supplement wild populations at risk of extinction, but hatchery-reared fish often do not survive after release. Using the threatened Arkansas darter, we show that abiotic enrichment and predator training of hatchery populations impact behavior and morphology and increase the probability of surviving first encounters with a non-native predator.

KEYWORDS

antipredator behavior, Arkansas darter, *Etheostoma cragini*, fitness, hatchery, phenotypic plasticity

---

1 | INTRODUCTION

As biodiversity declines globally, conservation programs that supplement wild populations offer one possible solution for maintaining or increasing the abundance of threatened and endangered species (Mallinson, 1995). For such conservation efforts to be successful, captive breeding programs must produce animals capable of surviving and reproducing in the wild. While there are several examples of captive breeding and reintroduction efforts supplementing terrestrial populations with individuals capable of surviving and reproducing in the wild (e.g., black-footed ferret: Biggins et al., 1999; California condor: Utt et al., 2008; Golden lion tamarins: Stonski et al., 2003; Caribbean rock iguanas: Alberts, 2007), aquatic conservation efforts have struggled to enhance self-sustaining, wild populations of fish (Cochran-Biederman et al., 2014; Fraser, 2008; Griffin et al., 2000; Lamothe & Drake, 2019; Shumway, 1999). Though conservation hatcheries can produce large quantities of fish for supplementation in their native ranges, survival after release is often very low (Brown & Laland, 2001; Crane et al., 2015; Fitzpatrick et al., 2014; Fraser, 2008; Griffin et al., 2000; Hawkins et al., 2008; Jackson & Brown, 2011).

One reason why hatchery-raised fish do poorly after release is that the hatchery environment favors traits better suited for life in captivity than in the wild. For example, fish reared in captivity often demonstrate riskier behaviors like increased time spent exploring and foraging when a predator is present (Crane et al., 2015). Additionally, hatchery fish tend to exhibit greater morphological abnormalities (Belk et al., 2008; Hutchison et al., 2012; Saraiva & Pompeu, 2016) and physiological limitations (e.g., swim performance) that further constrain the probability of surviving after release (Chittenden et al., 2010; Fuss & Byrne, 2002; Hutchison et al., 2012). As such, predation is suspected to be the leading cause of mortality immediately following release (Hutchison et al., 2012; Mesquita & Young, 2007; Olla et al., 1998).

Predator training and abiotic enrichment have been suggested as ways to promote antipredator behavior and increase post-release survival by incorporating more natural conditions into the hatchery rearing environment that closely mimic those experienced following release (Crane et al., 2015; Hawkins et al., 2008; Saraiva & Pompeu, 2016; Vilhunen, 2006). Behavioral, morphological, and physiological traits associated with detecting, recognizing, avoiding, and escaping predators can be highly correlated and often work in tandem to increase the breadth of an animal's response to the threat of predation, as well as its probability of surviving encounters with predators (Chittenden et al., 2010). For example, training fish to recognize predators by exposing them to predator cues can increase antipredator behavior (Becker & Gabor, 2012; Crane et al., 2015; Keefe, 1992; but see Kopack et al., 2023), and fish can learn to associate a visual cue, like a model of a predator, with danger when paired with conspecific alarm cue or an identifying predator odor (Becker & Gabor, 2012; Brown & Laland, 2001; Olla et al., 1998; Vilhunen, 2006). Similarly, abiotic enrichment to make the captive environment more natural shows promise for increasing expression of phenotypes associated with surviving predator encounters after release (Belk et al., 2008; Chittenden et al., 2010; Fuss & Byrne, 2002; Hutchison et al., 2012; Saraiva & Pompeu, 2016; Ullah et al., 2017). Subjecting hatchery-reared fish to variable temperatures, variable flow, and/or structure (e.g., artificial plants and refugia) can shift traits like thermal tolerance (Blair & Glover, 2019), metabolic rate (Cook et al., 2018), muscle development (Evans et al., 2015), swimming performance (Bergendahl et al., 2017; Chittenden et al., 2010; Higham et al., 2015), personality (Brignon et al., 2018; Lee & Berejikian, 2008), neural plasticity (Salvanes et al., 2013), and stress levels (Zhang et al., 2020) to more closely match those observed in wild fish. Shifts in one or more of these traits may improve the ability of fish to survive initial predator encounters (D'Anna et al., 2012; Higham et al., 2015).

A more complete understanding of the effects of predator training and abiotic enrichment on behavior and survival is required before recommending the adoption of these tools by conservation practitioners. While studies have traditionally focused on the effects of either abiotic enrichment (e.g., Belk et al., 2008; Chittenden et al., 2010; Fuss & Byrne, 2002; Hutchison et al., 2012; Hyvärinen & Rodewald, 2013; Saraiva & Pompeu, 2016; Ullah et al., 2017) or predator training (e.g., Brown, 2003; Kopack et al., 2016, 2015) individually, few have evaluated both simultaneously to determine their relative contributions and potential interactions in shaping antipredator behavior and survival (but see D'Anna et al., 2012, *Diplodus sargus*; Tave et al., 2019, *Hybognathus amarus*). Furthermore, populations within a species may differ in their responses to training due to underlying genetic variation and/or differences in predation pressure experienced among them (Mery & Burns, 2010). As such, conservation efforts may benefit from considering population-level effects of training during initial assessments of its efficacy.

Here, we assess the effect of abiotic enrichment on morphology and behavior as well as the relative contributions of abiotic enrichment and predator training on the survival of Arkansas darters (*Etheostoma cragini*; Figure 1) when encountering a predator for the first time. The Arkansas darter is a threatened species in the state of Colorado due to population declines observed since the

FIGURE 1 Photo of a male (left) and female (right) Arkansas darter. Photo by Andrew Treble, Colorado Parks and Wildlife.

1980s (Fitzpatrick et al., 2014; Groce et al., 2012; Labbe & Fausch, 2000). Abiotic factors like thermal regime and water flows have been linked to population declines (Fitzpatrick et al., 2014; Labbe & Fausch, 2000). One particular pressure on Arkansas darters in Colorado are non-native Northern Pike, which predate on darters and directly affect their abundance and distribution across their native range (Labbe & Fausch, 2000). In an effort to conserve the species, Colorado Parks and Wildlife (CPW) began an artificial propagation program for the Arkansas darter in 1999 (Groce et al., 2012). Despite intense propagation and supplementation efforts, hatchery-reared Arkansas darters have contributed little genetic material to wild populations where released, suggesting they experience high mortality following release (Fitzpatrick et al., 2014), perhaps facilitated by a lack of experience with predators or an inability to survive in wild stream environments due to being reared in the hatchery environment.

Hatchery-reared Arkansas darters inhabit an environment that differs drastically from the wild, which may affect their behavioral and morphological development and contribute to their low post-release survival. For example, wild environments offer refugia, low base water flow with occasional high flow events, and temperatures that shift daily, while hatchery environments typically lack structural complexity, provide minimal consistent water flow, and maintain constant water temperatures over time (Piper et al., 1986). Hatcheries also lack predators, which can inhibit the proper development and expression of antipredator behaviors and predator recognition in prey (Crane et al., 2015). As such, hatchery-reared Arkansas darters may benefit from both abiotic enrichment and predator training prior to release if such strategies increase their chances of surviving predation. The objectives of this study were to determine: (1) if abiotic enrichment altered feeding behavior and/or morphology of hatchery-reared darters; (2) if populations differed in their responses to abiotic enrichment; and (3) the relative contributions, if any, abiotic enrichment and predator training had on surviving an initial encounter with a novel predator.

2 | MATERIALS AND METHODS

2.1 | Animal husbandry and abiotic enrichment

We obtained 240 fully grown and developmentally mature Arkansas darters (ranging from 37 to 58 mm in total length, aproximately 10 months old) from three source populations (80 darters from each) from the CPW John W. Mumma Native Aquatic Species Restoration Facility (Alamosa, CO). The three populations (Big Sandy Creek, Black Squirrel Creek, and Horse Creek) are maintained as separate brood stocks in the hatchery breeding program. Genetic differences among these populations is high due to a lack of gene flow in the wild (Fitzpatrick et al., 2014). To maintain the genetic integrity of the individual source populations and introduce wild genetic variation to prevent domestication, hatchery brood stocks were previously supplemented with wild individuals (Fitzpatrick et al., 2014), although it had been several generations prior to this study since the populations were last supplemented with wild fish. The three source population creeks are all physically similar, in that they are spring fed systems with cool, clear water, low velocity flows, and lots of vegetation (especially watercress).

We held darters in 76-L flow-through tanks in the CPW Salmonid Disease and Sport Fish Research Lab (Fort Collins, CO), maintained water temperatures of

13.5 ± 2°C (comparable to spring-fed stream temperatures), and fed them thawed bloodworms (San Francisco Bay Brand Inc., Newark, CA) ad libitum, once daily. Populations were kept separate, with two tanks for each population (40 darters in each tank; thus replication occurred at the level of the individual). Within each population, one tank was not enriched ("abiotic control"), while the other was enriched by incorporating structural cover, increased flow, and diurnal temperature cycles ("abiotic enrichment"). For structural cover, we added three, three-inch long, ¾-inch diameter PVC tubes (as cave structures) and nine artificial plants (multi-pack B1; Marineland®, Blacksburg, VA) dispersed randomly throughout the tanks. Plants were secured to the bottom of tanks using suction cups attached to plant bases via zip ties. Enriched tanks also included two powerheads, each producing turbulent flows of 155 L/min (Hydor USA Inc., Sacramento, CA) mounted side by side at one end of the tank, as well as two in-tank 100-W water heaters (Eheim™, Buffalo, NY) mounted in the middle of the long side of the tanks and connected to timers. We set the heater timers to turn on after sunrise (8 a.m.) following the natural light cycle and shut off 10 h after turning on. On average, tank temperatures fluctuated by 3 ± 0.5°C daily. Fish were exposed to these conditions for 90 days before commencement of morphological evaluations and predator training.

Seventeen tiger muskellunge (*Esox masquinongy* × *E. lucius*; hereafter "tiger muskie") predators were obtained from the CPW Wray Fish Hatchery (Wray, CO) for use in survival trials. We selected tiger muskie because they were readily available, free of disease, easy to maintain, and are the hybrid cross of muskellunge (*E. masquinongy*) and northern pike (*E. lucius*), a non-native predator in some Arkansas darter natural habitats. Predators ranged from 233 to 295 mm total length. We housed tiger muskies in five 76-L flow-through tanks maintained at 13.5 ± 2°C and fed them trout feed (Rangen Inc.™, Buhl, ID) ad libitum, once daily.

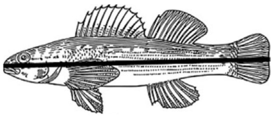
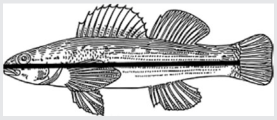
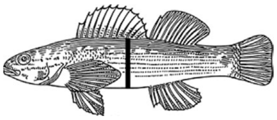
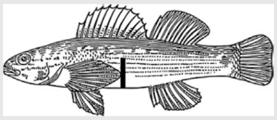
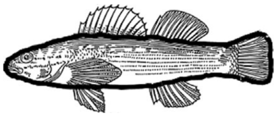
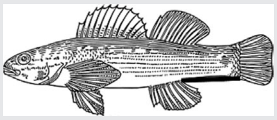
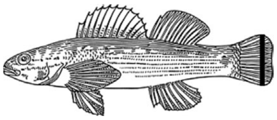
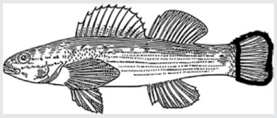
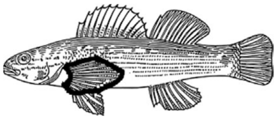
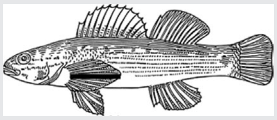
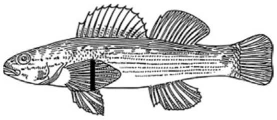
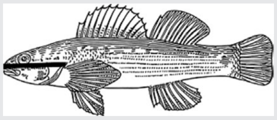
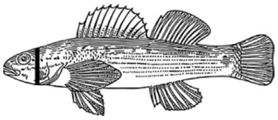
2.2 | Behavior

To determine if abiotic enrichment influenced Arkansas darter feeding behavior (i.e., feeding and/or moving), feeding trials were conducted 7 weeks into the experiment. Feeding behavior could be affected by abiotic enrichment as increased temperatures and flows may increase activity (Maynard et al., 2004; Stoner, 2004), metabolism (Cook et al., 2018; Stoner, 2004), and calorie intake needs (Jørgensen & Jobling, 1993; Stoner, 2004), while the presence of structure might provide a greater sense of safety by allowing for greater concealment (D'Anna et al., 2012; Moberg et al., 2011; Ullah et al., 2017). We withheld food from darters in both the abiotic enrichment and control tanks for 24 h prior to the start of the feeding trials to encourage them to feed during the experiment. On the day of a feeding trial, we added one thawed bloodworm cube into the top of each tank such that the bloodworms were distributed throughout and sank to the bottom of the tank as the cube disintegrated. An observer recorded the number of fish feeding and/or moving during instantaneous scans conducted every 2 min over a 30-min observation period which began the moment that the cube was added to the tank (15 scans in each tank). We repeated this process two more times, every other week, for a total of three feeding trials per tank. We expected darters in abiotic enrichment tanks to move and feed more than darters in abiotic control tanks because enrichment typically increases movement and feeding due to the high energetic demand of the enriched environment (Braithwaite & Salvanes, 2005; Higham et al., 2015).

2.3 | Morphology

Body shape in fish has been shown to influence swimming behavior and escape performance (Saraiva & Pompeu, 2016). To test if the abiotic enrichment altered body shape, we photographed fish (treatment group and control group) at the end of the 90-day treatment period with a high-definition camera (Coolpix AW100, Nikon Inc, Melville, NY) mounted to a tripod and facing down on a white board illuminated by two 30-cm, 415-lumen LED light bars mounted on either side. For each photograph, a researcher (CJK) haphazardly netted each fish out of its abiotic treatment tank, positioned it horizontally on the board above a 16-cm ruler for scale, and fanned caudal and first-dorsal fins with a wet paintbrush. We photographed 20 fish from each population (10 from each tank), but all photographs of the Big Sandy Creek population were lost, as well as two individuals from the abiotic enrichment group in the Black Squirrel Creek population due to an error in camera operation. From each remaining photograph, 13 linear morphological measurements were recorded (Table 1) using Image J software (Abramoff et al., 2004), from which nine ecomorphological attributes were calculated and assessed (following Pessanha et al., 2015; Saraiva & Pompeu, 2016; Table 2). We used proportional ratios of the morphological measurements (Table 1) to generate nine ecomorphological attributes that control for differences in body size and reflect an individual's ability to perform under various ecological contexts, including predator–prey interactions (Saraiva & Pompeu, 2016; Table 2). We chose

TABLE 1 Morphological measurements, their description, and illustration (following Saraiva & Pompeu, 2016).

Morphological measurements		
Measurement	**Description**	**Illustration**
Total Length (TL)	The distance from the tip of the snout to the tip of the caudal fin.	
Standard Length (SL)	The distance from the tip of the snout to the tip of the caudal peduncle.	
Body Height (BH)	The greatest distance between the ventral and dorsal sides of the body, perpendicular to its length.	
Average Body Height (ABH)	The greatest vertical distance from the abdomen to the lateral line.	
Body Area (BA)	The area of the body plus the area of the caudal fin.	
Caudal Peduncle Length (CPL)	The distance from the posterior end of the anal fin to the tip of the caudal peduncle.	
Caudal Fin Width (CFW)	The greatest distance between the anterior and posterior ends of the fully extended caudal fin.	
Caudal Fin Area (CFA)	The area of the fully extended caudal fin.	
Pectoral Fin Area (PFA)	The area of the fully extended pectoral fin.	
Pectoral Fin Length (PFL)	The greatest distance between the base and tip of the pectoral fin.	
Pectoral Fin Width (PFW)	The greatest distance from the dorsal end to the ventral end of the fully extended pectoral fin, perpendicular to its length.	
Head Length (HL)	The distance from the tip of the snout to the posterior end of the operculum.	
Head Height (HH)	The distance from the ventral side of the mandible to the dorsal side of the cranium.	

attributes considered ecologically relevant in high flow environments and potentially important for escaping predators after release (Table 2). If these traits exhibit plasticity in response to the abiotic environment, we expected darters exposed to abiotic enrichment to decrease the Ventral Flattening Index (VFI), Relative Height of the Body (RH), and Relative Height of the Head (RHH), and increase all other attributes, based on patterns associated with high flow environments and/or swimming ability (Pessanha et al., 2015; Saraiva & Pompeu, 2016; Table 2).

2.4 | Predator training

At the end of the 90-day abiotic treatment period, darters were randomly selected and moved to 10-L flow-through group-tanks for the remainder of the experiment. After being moved, darters were allowed to acclimate for 1 week prior to starting the predator treatments described below. For each population, 16 group-tanks housed five darters each. Eight tanks held darters from the abiotic enrichment treatment and eight held darters from the abiotic control treatment that continued to be fed thawed bloodworms ad libitum, once daily. Next, for each population we randomly assigned four of its eight abiotic treatment tanks (and all individuals contained therein) to a predator control treatment (exposed to distilled water only), and we assigned the other four tanks to a predator training treatment (exposed to conspecific alarm cue, predator kairomone, and a predator model) following Kopack et al. (2023), so that groups of individuals in each abiotic and predator treatment were maintained separately, without mixing. We tagged darters with a color-coded mark on either side of their dorsal fins using Visible Implant Elastomer (VIE; Northwest Marine Technology Inc., Shaw Island, WA) to identify each individual's treatment combination ("abiotic control" vs. "abiotic enrichment") and predator treatment ("predator control" vs. "predator training").

Conspecific alarm cue was collected by scoring the epidermis of 51 recently decapitated Arkansas darters (17 additional darters obtained from each population, mixed together) with a razor blade. Bodies were then rinsed with 10 mL of distilled water, which was filtered through cheesecloth and immediately stored in a freezer

TABLE 2 Ecomorphological attributes, their calculation, ecological significance, and the direction of change expected and observed for each population (Black Squirrel Creek: "BSQ" and Horse Creek: "HRS") in response to abiotic enrichment.

Ecomorphological attributes			Direction of change		
Attribute	**Calculation**	**Ecological significance**	**Expected**	**BSQ**	**HRS**
Ventral Flattening Index (VFI)	VFI = ABH/BH	Lower values reflect fish living in high flows with a greater ability to maintain position without swimming.	−	O	O
Relative Height of the Body (RH)	RH = BH/SL	Lower values reflect fish living in high flows.	−	O	O
Relative Length of the Caudal Peduncle (RLCP)	RLCP = CPL/SL	Higher values reflect fish living in high flows with better swimming ability.	+	O	O
Relative Area of the Caudal Fin (RACF)	RACF = CFA/BA	Higher values reflect fish with a benthic lifestyle and greater burst swimming capabilities.	+	−	+
Aspect Ratio of the Caudal Fin (ARCF)	ARCF = CFW/CFA	Higher values reflect fish that swim more.	+	−	−
Relative Area of the Pectoral Fin (RAPTF)	RAPTF = PFA/BA	Higher values reflect fish living in high flows with greater maneuverability.	+	−	−
Aspect Ratio of the Pectoral Fin (ARPTF)	ARPTF = PFL/PFW	Higher values reflect fish living in high flows with longer fins and greater swimming ability.	+	−	−
Relative Length of the Head (RLH)	RLH = HL/SL	Higher values reflect fish living in high flows.	+	O	O
Relative Height of the Head (RHH)	RHH = HH/SL	Lower values reflect fish living in high flows	−	O	O

Note: Direction of change is indicated by an increase ("+") or decrease ("−"), with "O" representing no change in attribute expression, in response to abiotic enrichment (obtained from Pessanha et al., 2015; Saraiva & Pompeu, 2016).

at −20°C until the day of its use (following Kopack et al., 2015 and adapted from Nordell, 1998). We obtained predator kairomone by collecting water from a 19-L non-circulating tank that housed two tiger muskies for 48 h. During this time, tiger muskies were fed and consumed six darters (two from each population), so the water likely contained conspecific alarm cues in addition to the predator kairomone. The collected water was stored at −20°C until the day it was used (following Kopack et al., 2015; Kopack et al., 2023).

Twenty-four hours prior to the start of survival trials, we treated darters with a 5-min exposure to predator control or training conditions in their group tanks following Kopack et al. (2023). To administer the treatments, 10 mL syringes, loaded with either distilled water ("predator control") or 5 mL of alarm cue mixed with 5 mL of predator kairomone thawed at room temperature ("predator training"), were connected to air tubing taped to the front right corner of each group-tank. We placed a blind over all tanks to prevent darters from being visually disturbed and turned off the water flow to prevent dilution of cues during the treatment period. Following placement of the blind, we allowed darters 30 min to acclimate before adding the 10 mL of liquid through the air tubing. For fish in the predator training groups, we additionally exposed each tank to a predator model, a 102 mm plastic lure that resembled a tiger muskie predator (Savage Gear USA®, Ontario, CA), for 5 min immediately following the introduction of chemical cues. The model was attached to a wand with fishing line so that it could be introduced and removed from the tank by a researcher standing behind the blind. After the 5-min exposure period, we removed the model and water flow was resumed (following Kopack et al., 2023).

2.5 | Survival trials

Prior to the start of survival trials, five tiger muskie predators were randomly assigned to one of five 151-L flow-through (7.5 L per minute) predator encounter tanks, where they were housed for the remainder of the experiment. Similar to the abiotic enrichment tanks, each encounter tank included 12 artificial plants and three PVC caves for structure, two powerheads for generating flow, and two in-tank heaters for fluctuating temperatures, simulating conditions darters may experience during release. Survival trials were conducted for each population separately, one population at a time, using a 2 × 2 factorial design consisting of 20 individuals per abiotic and predator treatment combination ("control" = abiotic control + predator control; "enrichment" = abiotic enrichment + predator control; "training" = abiotic control + predator training; and "enrichment + training" = abiotic enrichment + predator training). Twenty-four hours after receiving predator treatments, darters from a given population were assigned to one of the five encounter tanks, so that each encounter tank included four darters, one from each treatment combination. On the day of survival trials, the four darters were collected into a 13 cm long × 7 cm wide × 12 cm tall plastic specimen container (Lee's Aquarium and Pet Products; San Marcos, TX) and released into their respective predator encounter tanks on the side opposite the predator, marking the start of a trial. A trial ended once the tiger muskie consumed a darter, at which point the tiger muskie was removed from the tank, the three surviving darters were retrieved, and then the tiger muskie was returned to the tank. We did not use a blind for this experiment so that the observer could rapidly remove the tiger muskie from the tank before it was able to consume additional darters. Next, using the VIE tags to identify the three remaining survivors, we identified the treatment combination of the consumed individual. We then placed each of the three survivors into one of four 10-L holding tanks, separated by treatment combination. We repeated this process daily until every darter had been through one encounter trial with a predator, conducting one trial per predator, per day, to prevent predator satiation.

Once every surviving fish within a given population had completed an initial survival trial, one fish from each of the four holding tanks (representing each treatment combination) was randomly selected to participate in a second predator encounter trial. This process was repeated for each population until one of the four treatment combinations was eliminated. Up to three rounds of predator encounter trials were conducted per individual over a 1-week period before this occurred.

2.6 | Statistical analyses

All statistical analyses were performed using R statistical software version 4.0.2 (R Core Team, 2020), except cumulative survival, which was assessed using Program MARK (White & Burnham, 1999). To compare Arkansas darter feeding behavior between abiotic treatments (tanks with or without enrichment) and among populations, we conducted a linear mixed model (lme4 package: Bates et al., 2014; lmerTest package: Kuznetsova et al., 2017) for each of the two behaviors recorded: (1) the number of individuals feeding in any given scan and (2) the number of individuals moving in any given scan. Because these were count data, we treated them as Poisson distributions. We included abiotic treatment, population, and their interaction as fixed effects and feeding trial as a random effect in both analyses. We reported Wald Chi-Squared values, identified significant fixed effects, and used the estimated marginal means method (emmeans package: Lenth, 2020) to determine significant differences for interactions between treatments and populations.

To assess morphological differences across abiotic treatments and populations, we performed a redundancy analysis (RDA; adapted from Borcard et al., 2011; following Marques et al., 2019) using the vegan package (Oksanen et al., 2020), followed by a nonparametric, permutational multivariate analysis of variance (PERMANOVA; Legendre & Legendre, 2012), as it is particularly robust when sample sizes are limiting. Briefly, this approach allows one to reduce complex multivariate variation into a few axis, analyze these axis of variation for differences among treatments and populations, and then can reveal direction and magnitude of any changes to individual ecomorphological attributes by using scores obtained from the RDA in MANOVAs (adapted from Borcard et al., 2011; following Marques et al., 2019). Before conducting the analysis, we partitioned the data into two distinct data sets, one representing individual response variables (ecomorphological attributes; Table 2) and the other, a data frame of their corresponding predictor variables (abiotic treatment and population). Next, the data were submitted to the RDA, from which permutation tests (999 permutations) were conducted to determine the relative contribution of each main effect and their interactions to the variance partitioning of each constrained canonical axis (Legendre et al., 2010; McArdle & Anderson, 2001). While all nine unconstrained canonical axes were retained from the redundancy analysis (Table S1), of the three constrained canonical axes produced, the first axis (RDA1) explained 51% of the variation observed and the second axis (RDA2) explained 34%, cumulatively representing 85% of the total variation observed. Permutation tests performed on the eigenvalues of the constrained canonical axes found that a significant proportion of observed variation across all attributes was explained by RDA1 (variance $= 0.96$, $F_{3,34} = 4.60$, $p = .004$) and RDA2 (variance $= 0.65$, $F_{3,34} = 3.12$, $p = .008$) but not RDA3 (variance $= 0.29$, $F_{3,34} = 1.39$, $p = .20$). To determine the effects of treatment, population, and their interaction on the variance partitioning for each constrained canonical axis found to be significant in the RDA, we performed an ANOVA using the ecomorphological attribute scores obtained from the analysis. Last, to assess changes to individual attributes in response to abiotic treatment, we conducted a nonparametric, permutational MANOVA (999 permutations; "adonis2" in R, Oksanen et al., 2020) using a Euclidean distance matrix of the ecomorphological attributes (Legendre & Anderson, 1999; McArdle & Anderson, 2001). We included treatment, population, and their interaction as fixed effects in all models, reporting statistics from the type III sum of squares, followed by pairwise comparisons of means for interactions found to be statistically significant (McArdle & Anderson, 2001; RVAideMemoire package, Hervé, 2021).

Lastly, to determine survival differences among abiotic and predator treatments, survival rates were calculated and compared across their combinations, both within and among trials. Using a binomial distribution, with one indicating survival and zero indicating mortality, we calculated instantaneous survival rates for each trial using a generalized linear model and compared survival differences among the trials using an RM ANOVA, with trial, treatment, and population included as fixed effects and predator identification as a random effect. However, because no differences were detected among populations ($X^2 = 2.25$, $p = .33$), instantaneous survival rates were pooled for comparison. Additionally, because the model's random effect variance was estimated to be at or near zero, predator identification was dropped as a random effect to prevent overfitting the model. Because we had a 100% recapture probability and we could identify the treatment combination of the consumed individual immediately after a trial was completed, cumulative

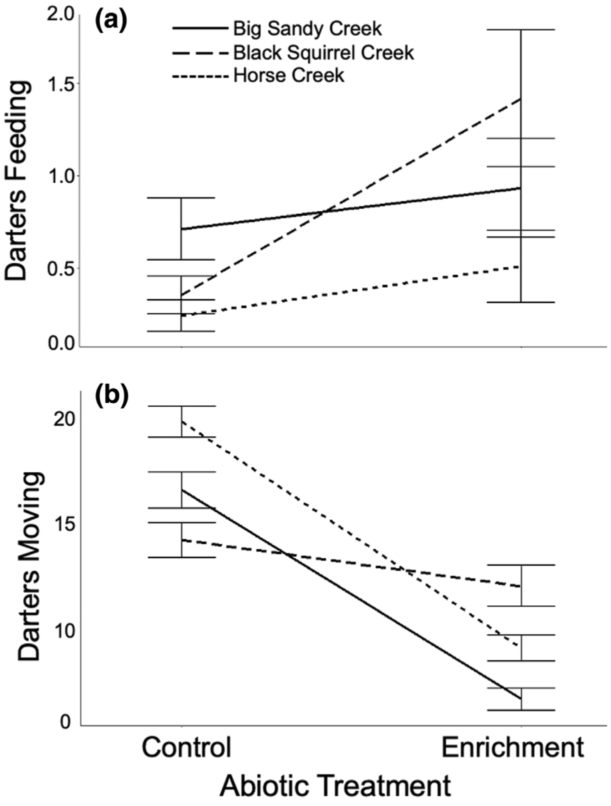


FIGURE 2 The mean (± SE) number of darters feeding (panel a) and moving (panel b) during instantaneous scans made every 2 min during a 30-min observation period for fish treated with ("Enrichment") and without ("Control") abiotic enrichment in each population (Big Sandy Creek, Black Squirrel Creek, and Horse Creek). There was a significant interaction between treatment and population in both a and b.

TABLE 3 Models used to analyze data from each experiment. Random effects are not included.

Experiment	Model Used	Response Variable(s)	Predictor Variable(s)	Test Statistic(s) Reported
Behavior	RM ANOVA	Number of Darters Feeding or Moving in a given scan	Abiotic treatment, population, and interaction	X^2, z-test statistics, and P-values
Morphology	PERMANOVA	Differences in Ecomorphological Attributes*	Abiotic treatment, population, and interaction	t-test statistics, F-test statistics, and P-values
Survival	GLMM; AIC	Number of Surviving darters during encounters with a live predator	Abiotic treatment, predator training treatment, population, and trial number	z-test statistics and P-values; confidence intervals

*Ecomorphological attribute scores used in PERMANOVA were obtained from a Redundancy Analysis (RDA).

survival rates were estimated across all trials using a known fate capture-recapture estimator in Program MARK (White & Burnham, 1999). Cumulative survival rates were estimated for the four treatment combinations only, with data from all three populations combined into the same analysis, as there were not enough encounters overall to parse out population effects. The model set included an intercept model, models with treatment and trial effects, and models with the additive and interactive combinations of those effects. Cumulative survival (± unconditional standard error) is reported as a model-averaged derived parameter from the known fate model set. See Table 3 for a summary of the statistical models used.

3 | RESULTS

3.1 | Feeding behavior

In our assessment of feeding behavior, abiotic enrichment had no effect on the number of darters feeding overall ($X^2 = 1.38$, $p = .24$), but the number of individuals feeding differed by population ($X^2 = 13.03$, $p = .002$), as the Big Sandy Creek ($z = 3.89$, $p < .001$) and Horse Creek ($z = -2.80$, $p < .05$) populations fed more than the Black Squirrel Creek population but did not differ from each other ($z = 1.01$, $p > .1$). There was a significant interaction between abiotic treatment and population ($X^2 = 7.49$, $p = .02$), as abiotic enrichment caused an increase in feeding for the Horse Creek population ($z = 4.44$, $p < .001$) but not the Big Sandy Creek ($z = 1.18$, $p > .1$) and Black Squirrel Creek populations ($z = 2.04$, $p > .1$; Figure 2a; Table S2).

Fewer individuals were observed moving in abiotic enrichment tanks than the control tanks ($X^2 = 174$, $p < .001$). Movement also differed by population ($X^2 = 61.60$, $p < .001$), as the Black Squirrel Creek ($z = -5.32$, $p < .001$) and Horse Creek ($z = -4.81$, $p < .001$) populations moved more than the Big Sandy Creek population but did not differ from one another ($z = 0.32$, $p > .1$). There was a significant abiotic treatment and population interaction ($X^2 = 77.45$, $p < .001$); abiotic enrichment caused a decrease in movement in the Big Sandy Creek ($z = -13.19$, $p < .001$) and Black Squirrel Creek ($z = -12.95$, $p < .001$) populations but not the Horse Creek population ($z = -2.10$, $p > .1$; Figure 2b; Table S3).

3.2 | Morphology

In our assessment of morphology, we found abiotic treatment ($F_{3,34} = 2.62$, $p = .01$), population ($F_{3,34} = 3.43$, $p = .001$), and their interaction ($F_{3,34} = 3.06$, $p = .01$) were significant predictors, explaining 55%, 72%, and 64% of the variation observed across all ecomorphological attributes, respectively. Our redundancy analysis produced three constrained canonical axes, two of which (RDA1 and RDA2) explained a significant proportion of variation across ecomorphological attributes. The first constrained canonical axis (RDA1) was attributed to differences in Aspect Ratio of the Caudal Fin (ARCF), Relative Length of the Head (RLH), RHH, Relative Area of the Pectoral Fin (RAPTF), Relative Length of the Caudal Peduncle (RLCP), and Aspect Ratio of the Pectoral Fin (ARPTF), while the second axis (RDA2) was attributed to differences in ARPTF, and ARCF. An ANOVA attributed variation in RDA1 to the main effects of abiotic treatment ($F_{3,34} = 3.98$, $p = .05$) and population ($F_{3,34} = 6.52$, $p = .02$), while the variation in RDA2 was associated with the effects of abiotic treatment ($F_{3,34} = 40.02$, $p < .001$), population ($F_{3,34} = 25.14$, $p < .001$), and their interaction ($F_{3,34} = 52.60$, $p < .001$; Figure S1). An analysis of the significant interaction for RDA2 revealed that populations responded differently to abiotic treatment, with Black Squirrel Creek increasing ($t = -6.33$, $p < .001$) and Horse Creek decreasing ($t = 3.86$, $p = .002$) overall expression of attributes when exposed to abiotic enrichment.

To understand the multivariate RDA, we analyzed individual ecomorphological attributes using scores obtained from the RDA in MANOVAs. This revealed an

TABLE 4 PERMANOVA results showing the effect of abiotic treatment, population, and their interaction for each ecomorphological attribute (adapted from Saraiva & Pompeu, 2016).

				Effect					
	Treatment			Population			Interaction		
Attribute	R^2	$F_{(1,34)}$	***p* value**	R^2	$F_{(1,34)}$	***p* value**	R^2	$F_{(1,34)}$	***p* value**
VFI	0.012	0.428	.541	<0.001	0.019	.911	0.006	0.222	.660
RH	0.016	0.611	.422	0.006	0.205	.665	0.066	2.461	.115
RLCP	0.012	0.586	.461	0.265	12.511	**.003**	0.004	0.196	.650
RACF	0.017	1.207	.266	0.027	1.862	.169	0.464	32.130	**.001**
ARCF	0.082	3.319	*.062*	0.082	3.324	*.067*	<0.001	0.005	.950
RAPTF	0.090	3.485	*.070*	0.033	1.287	.273	<0.001	0.084	.796
ARPTF	0.252	12.875	**.002**	<0.001	0.016	.919	0.084	4.304	**.038**
RLH	0.065	2.976	.104	0.196	9.060	**.005**	0.003	0.119	.744
RHH	0.001	0.029	.887	0.108	4.155	**.049**	0.009	0.341	.552

Note: *p* values ≤.05 were considered significant (bold). *p* values >.05 but ≤.10 were considered trends (italic). Attribute acronyms are defined in Table 2.
Abbreviations: ARCF, Aspect Ratio of the Caudal Fin; ARPTF, Aspect Ratio of the Pectoral Fin; RACF, Relative Area of the Caudal Fin; RAPTF, Relative Area of the Pectoral Fin; RH, Relative Height of the Body; RHH, Relative Height of the Head; RLCP, Relative Length of the Caudal Peduncle; RLH, Relative Length of the Head; VFI, Ventral Flattening Index.

effect of abiotic treatment, population, and/or their interaction for several of the attributes examined (Tables 2 and 4). Counter to our predictions, abiotic enrichment caused a decrease in ARPTF for all darters (Table 4), though the decrease was greater in the Black Squirrel Creek population ($F_{3,34} = 10.71$, $p < .05$; Figure 3a). Also counter to predictions, there was a nonsignificant trend where RAPTF and ARCF decreased when exposed to abiotic enrichment (Table 2; Figure 3c,d). The two populations were found to differ morphologically from one another regardless of the abiotic treatment they received, with Black Squirrel Creek having a lower RLCP ($F_{3,34} = 12.45$, $p = .001$), but higher RLH ($F_{3,34} = 8.09$, $p = .008$) and RHH ($F_{3,34} = 4.29$, $p = .04$) compared to Horse Creek (Table 4). Populations also responded differently to abiotic treatment with respect to RACF, as abiotic enrichment caused an increase in RACF for Horse Creek ($F_{3,34} = 51.04$, $p = .006$), which matched expectations, but a trend for a reduced RACF for Black Squirrel Creek ($F_{3,34} = 6.76$, $p < .1$; Table 4; Figure 3b).

3.3 | Survival

The combination of abiotic enrichment and predator training significantly increased survival of darters in the presence of a predator ($z = 8.27$, $p = .04$; Figure 4a). During the first survival trial (trial 1), those that received abiotic enrichment and predator training ("enrichment + training") had significantly higher instantaneous survival than the other three abiotic enrichment and predator training combinations ($z = 2.04$, $p = .04$; Figure 4a), which did not differ from each other ($0.00 \leq z \leq 1.67$, $p > .05$). Overall, cumulative survival, calculated across trials, was also highest in fish receiving both abiotic enrichment and predator training, but it did not differ among combinations (Figure 4b). Darters that received only abiotic enrichment ("enrichment") were the first treatment to be eliminated during survival trials for the Big Sandy Creek and Black Squirrel Creek populations, whereas those that received only predator training ("training") were the first to be eliminated in the Horse Creek population.

4 | DISCUSSION

Preparing captive-reared animals for the environments they will encounter after release has been suggested to increase survival and improve the success of conservation breeding programs (D'Anna et al., 2012; Tave et al., 2019). The results of this study suggest that abiotic enrichment, including physical structure, increased water flow, and varied temperatures, can alter the behavior and morphology of Arkansas darters, and the combination of abiotic enrichment and predator training can increase the probability of surviving initial encounters with novel predators. Additionally, as predicted, populations differed in their behavioral and morphological responses to abiotic enrichment; however, populations did not differ in survival.

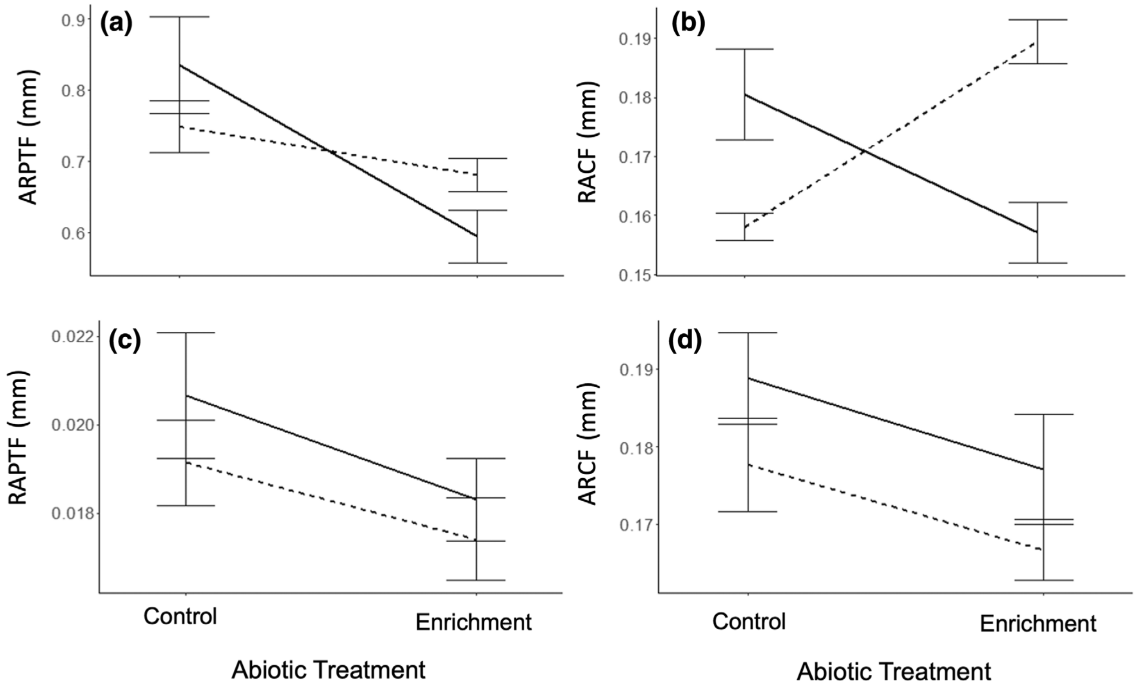


FIGURE 3 The mean (± SE) Aspect Ratio of the Pectoral Fin (ARPTF, panel a), Relative Area of the Caudal Fin (RACF, panel b), Relative Area of the Pectoral Fin (RAPTF, panel c), and Aspect Ratio of the Caudal Fin (ARCF, panel d) for Arkansas darters from two populations (Horse Creek: dotted line; Black Squirrel Creek: solid line) reared with ("Enrichment") and without ("Control") abiotic enrichment. There was a significant interaction between abiotic treatment and population for a and b and trends for treatment effects for c and d (Table S3).

4.1 | Feeding behavior

We assessed the effect of abiotic enrichment on rates of feeding and movement to determine whether changes in temperature, flow, and structure might alter Arkansas darter behavior within hatcheries. We expected abiotic enrichment to increase both movement and feeding if darters increased their caloric intake in response to the high energetic demands associated with greater physical activity in the enriched environment (Braithwaite & Salvanes, 2005; Brown et al., 2003). However, Arkansas darters exposed to abiotic enrichment moved less and, in some populations, fed more compared to those in abiotic control tanks. The decrease in movement, as well as the increase in feeding in abiotic enrichment tanks may have been the result of food dispersion caused by increased flow produced by powerheads (Gu et al., 2018). Flows affected dispersion of bloodworms throughout the enriched tanks, which could have allowed darters more opportunities to encounter prey and feed without needing to move. Similarly, Gu et al. (2018) found benthic juvenile Chinese sturgeon (*Acipenser sinensis*) experienced greater foraging efficiency on drifting prey in a lotic system with high flows, because of an increase in prey encounter rates and a decrease in prey escape ability.

In contrast, the lack of flow in abiotic control tanks caused food to remain concentrated in the location it was introduced, requiring darters to move in order to feed. This may also have caused individuals to cluster around the food, increasing competition and decreasing foraging efficiency. Increased intraspecific competition caused by high rearing densities, as well as selection in hatcheries, is thought to contribute to the bolder and riskier behaviors hatchery-reared fish often express compared to their wild counterparts, potentially increasing their susceptibility to predation (Lima & Dill, 1990; Tyler, 1993) and reducing survival immediately following their release, especially if they fail to discriminate novel predatory threats (Braithwaite & Salvanes, 2005; D'Anna et al., 2012; Moberg et al., 2011; Roberts et al., 2011). Thus, abiotic enrichment may offer conservation practitioners a way to reduce the maladaptive effects of traditional hatchery environments and impart a survival advantage if the behaviors observed here persist after release into the wild (Braithwaite & Salvanes, 2005; D'Anna et al., 2012; Moberg et al., 2011; Roberts et al., 2011).

Feeding and movement behavior differed among populations and abiotic treatments. For the number of fish feeding, only Horse Creek was significantly impacted by the abiotic treatment with more fish feeding in

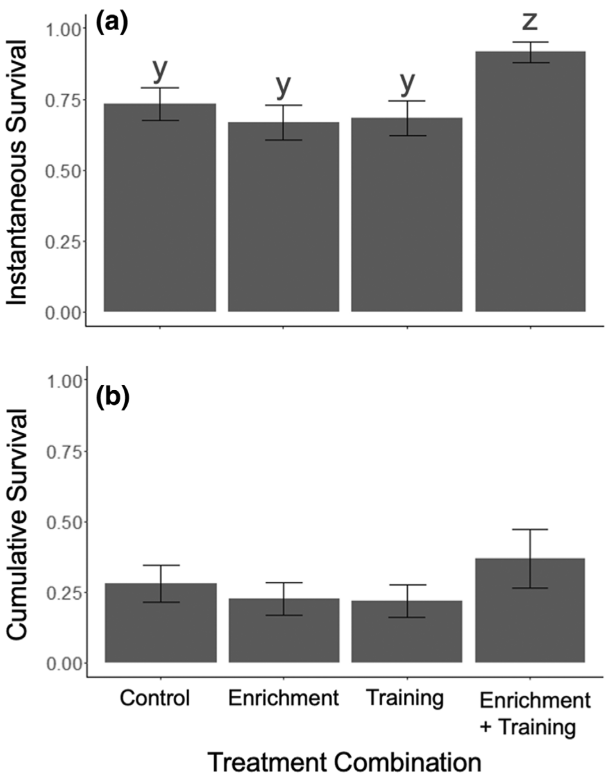


FIGURE 4 The mean (± SE) instantaneous (panel a) and cumulative (± unconditional SE; panel b) survival of Arkansas darters that received no abiotic enrichment and no predator training ("Control"), only abiotic enrichment ("Enrichment"), only predator training ("Training"), or received both abiotic enrichment and predator training ("Enrichment + Training"). Panel a shows instantaneous survival for only the first encounter trial with a tiger muskie predator, and different letters above the bars indicate statistical significance ($p < .05$). There were no significant differences in panel b.

enriched tanks compared to the control. However, this increase (approximately a difference of one fish) was so slight, it may not be biologically meaningful. For movement, the Horse Creek population did not differ between treatments while the other two populations moved less in the abiotic enrichment compared to the control (approximately a difference of 10 individuals). The higher number of Horse Creek individuals moving in the abiotic enrichment treatment relative to the Big Sandy Creek or Black Squirrel Creek populations could increase their conspicuousness and translate to higher susceptibility to predation after release (Lima & Dill, 1990) if behavioral differences persist in the wild. As such, we suggest conservation managers continue to explore the effects of enrichment on behavior of Arkansas darters at the population level, while continuing efforts to prevent esocid predators from establishing themselves where darters are particularly vulnerable to predation.

4.2 | Morphology

In our assessment of Arkansas darter morphology, we found that abiotic enrichment affected four of the nine ecomorphological attributes, indicating some degree of morphological plasticity, particularly for traits associated with the caudal and pectoral fins. Darters exposed to abiotic enrichment had a lower ARPTF, lower ARCF, and lower RAPTF compared to those in abiotic control tanks (Table 2). However, these reductions contradicted our prediction based on what we expected would increase swim performance (Saraiva & Pompeu, 2016). It is important to note that the original study that examined the ecological relevance of these attributes (i.e., whether they were adaptive and increased swim performance) did so in 33 species, of which only four were darters (Gatz Jr., 1979), and therefore the ecological relevance of these attributes may have been different for all species combined versus the species-specific relevance to darters. The difference between our predictions and our findings may reflect the benthic lifestyle of darters and their lack of a swim bladder. Movement is more energetically costly for darters, which do not actively swim as much as other species and/or those with swim bladders (Gatz Jr., 1979). Because darters rely on their pectoral and caudal fins to maintain their position in space (Carlson & Lauder, 2010), the reductions to ARCF, RAPTF, and ARPTF we observed in abiotic enrichment tanks may allow darters to combat the effects of increased drag resulting from having large fins in high flows, thereby reducing the amount of energy and activity required to maintain their position in space (Carlson & Lauder, 2010; Franssen, Harris, et al., 2013). Indeed, the fact that darters in the abiotic enrichment with predator treatment had the highest survival suggests that the morphological differences we observed may be adaptive. Furthermore, the lack of change observed in other attributes may be partially due to the inclusion of structure in abiotic enrichment tanks, as darters could have used these structures as refuge to shelter themselves from the physical stressors that accompany high flows, providing potential opportunities to overcome the high energetic cost of shifting morphological traits in response to the environment (Carlson & Lauder, 2010; Liao, 2007).

Some ecomorphological attributes differed between populations regardless of which abiotic treatment they received and are likely the result of the genetic differences that exist between them. Because various biotic and abiotic factors contribute to selection pressures that shape morphology, differences between the environments of the source populations (e.g., differences in available prey), may have led to morphological divergence and the significant effect of population we detected in this study (Franssen, Harris, et al., 2013; Franssen, Stewart, & Schaefer, 2013; Pessanha et al., 2015). Furthermore, we anticipated the possibility that populations would morphologically differ from one another due to the high genetic variation and lack of gene flow known to exist between them (Fitzpatrick et al., 2014). As with the feeding experiment, we also detected differences in how the populations responded to the abiotic treatment (gene-by-environment interaction). Specifically, for RACF Horse Creek differed in the expected direction as it was larger in the enrichment treatment while it was in the opposite direction in the Black Squirrel Creek population. Differences in plasticity among populations should be explored in future work by investigating differences in gene expression among populations in response to various treatments like abiotic enrichment and predator exposure. Because phenotypic and genetic differences exist, conservation efforts would benefit from knowing the degree to which the abiotic and biotic environments of the source populations work independently and/or in tandem to shape the morphological differences among them, as understanding the environmental forces that shape these differences may allow practitioners to design captive environments that promote the morphological variation observed in the wild.

4.3 | Survival

In our assessments of survival, the abiotic enrichment and predator training together increased the probability of surviving first encounters with a predator. Additionally, although not statistically significant, the trend towards higher cumulative survival observed for darters exposed to both abiotic enrichment and predator training suggests this combination may provide lasting effects that span beyond first encounters with a novel predator. Recall that the cumulative survival experiment used the same surviving fish in repeated trials, which would have provided opportunities for the fish to learn through encounters with live predators, potentially explaining the lack of significance between treatments.

Prey must be able to both recognize and escape a predator in order to survive. Our results suggest that predator recognition can be enhanced via predator training and escape ability enhanced via abiotic enrichment, and this aligns with other work. The post-release survival of hatchery-reared White seabream (*Diplodus sargus*) doubled when they were conditioned to recognize predators and reared with refugia (D'Anna et al., 2012). Methods to rear endangered Rio Grande Silvery Minnows (*Hybognathus amarus*) in semi-natural environments with both abiotic and biotic stressors (including predation) also showed promise for increasing survival after release (Tave et al., 2019). To our knowledge, our study is the first to measure survival in Arkansas darters exposed to abiotic enrichment and predator training. We found that these treatments together increased survival despite using a single predator training event and making relatively simple changes to the abiotic environment compared to the wild. Considering that thousands of hatchery-reared Arkansas darters are released into the wild annually, even a small survival difference could result in more cost-effective efforts to conserve this species.

We were not able to detect differences in survival among populations, likely due to small sample sizes. Because Arkansas darters are a threatened species, it is difficult to acquire animals for research purposes, which was a challenge for this research. Among populations, the darters that experienced only abiotic enrichment were the first to be eliminated for the Big Sandy Creek and Black Squirrel Creek populations, while darters that experienced only predator training were the first to be eliminated for Horse Creek, suggesting that there may be some differences in survival among populations and treatments. We suggest conservation managers, therefore, continue to explore population differences to determine if population-specific management is necessary for the persistence of darters in the wild. We tested survival for each population independently; future research could test all populations simultaneously during survival trials to explore whether particular Arkansas darter populations are more likely to survive predation. Knowing if certain populations are better at surviving predation could help managers determine which populations are the best sources for translocations and population augmentation.

4.4 | Limitations and future work

This study was the first to explore the efficacy of enrichment for the Arkansas darter, and it lays the foundation for future work. We tested a non-native introduced predator in this study because we know that they heavily predate on darters in at least one of the populations we studied; however, it would be valuable to also test the efficacy of predator training with native predators such as black bullhead catfish (*Ameiurus melas*) and green sunfish (*Lepomis cyanellus*), especially since predators vary in foraging strategy (e.g., ambush or active). While our survival experiment yielded promising results, our inference is limited because the experiment was conducted in a laboratory which cannot capture the full complexity of a wild environment. Finally, our abiotic enrichment treatment was relatively simple; future efforts may be even more effective if the abiotic enrichment (e.g., regarding water quality, benthic structure, vegetation) matches wild environments from which populations are sourced, especially considering that we found differences among populations in our study.

Future work should test survival in the wild by releasing darters reared with abiotic enrichment and predator training and using mark-recapture methods to see if they survive longer than darters reared under standard hatchery conditions. It would also be beneficial to understand how darters and predators interact in the wild, which could be assessed using a telemetry study with predation tags that indicate when a fish is consumed. Because the goal of these conservation efforts is to produce fish that not only survive but also reproduce in the wild, future studies would ideally use genomics (e.g., whole genome sequencing) to determine whether fish reared in various enrichment treatments have higher fitness in the wild, contributing genetically to wild populations over generations. Studies are also needed to determine if increasing post-stocking survival will help increase population growth rates over time, or if other limiting factors need to be addressed to adequately conserve the species, such as ongoing efforts to secure water availability and habitat quality for existing populations, identify new reintroduction sites within the species' native range, and fence over-grazed riparian reaches (Colorado Parks and Wildlife, 2015).

5 | CONCLUSIONS

By mimicking a predatory encounter that darters might experience after release, we gained valuable insight into how the biotic and abiotic environments can interact to influence survival. We found that abiotic enrichment altered both behavior and morphology of Arkansas darters. We also found that, in combination with predator training, abiotic enrichment increased survival during an initial encounter with a novel predator. Thus, these types of hatchery practices show promise for increasing Arkansas darter survival after release. We recommend that conservation practitioners employ predator training and abiotic enrichment at the hatchery level by incorporating powerheads, in-tank heaters, artificial structure, and other forms of enrichment. Because we detected differences among populations, we suggest that practitioners continue to manage Arkansas darters at the population level. While it may seem challenging to implement abiotic enrichment and predator training at the hatchery level, simple methods like those we tested have the potential to increase survival and bolster efforts to conserve this species. Finally, stocking programs are a commonly employed conservation strategy for many threatened and endangered fish species, yet many hatchery-reared fish have low survival post-release in the wild. Our work adds to the growing body of literature suggesting that abiotic enrichment and predator training may be valuable tools for the conservation of fishes.

ACKNOWLEDGMENTS

We would like to thank H. Crockett, P. Foutz, G. Schisler, A. Treble, T. Smith, and the staff of the Colorado Parks and Wildlife John W. Mumma Native Aquatic Species Restoration Facility and Wray Fish Hatchery for providing fish, equipment and supplies, logistic support, and expertise. The authors would also like to thank B. Avila, E. Vigil, R. Osmundson, T. Hubbard, K. Dick, F. Horne, and T. Klee for help with setup, animal husbandry, and data collection. Fish were cared for under the guidelines and requirements of the Colorado State University Animal Care and Use Committee (Protocol no. 16-6518A). Funding for this work was provided by Colorado Parks and Wildlife's Species Conservation Trust Fund (Grant no. SCTF911C) and a Graduate Research Fellowship awarded to C. Kopack by the National Science Foundation.

ORCID

Christopher J. Kopack https://orcid.org/0000-0002-7689-4683

REFERENCES

Abramoff, M. D., Magalhaes, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*, 36–42.

Alberts, A. C. (2007). Behavioral considerations of headstarting as a conservation strategy for endangered Caribbean rock iguanas. *Applied Animal Behavior Science*, *102*, 380–391.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Becker, L. J. S., & Gabor, C. R. (2012). Effects of turbidity and visual vs. chemical cues on anti-predator response in the endangered fountain darter (*Etheostoma fonticola*). *Ethology*, *118*, 994–1000.

Belk, C. M., Benson, L. J., Rasmussen, J., & Peck, S. L. (2008). Hatchery-induced morphological variation in an endangered fish: A challenge for hatchery-based recovery efforts. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*, 401–408.

Bergendahl, I. A., Miller, S., Depasquale, C., Giralico, L., & Braithwaite, V. A. (2017). Becoming a better swimmer: Structural complexity enhances agility in a captive-reared fish. *Journal of Fish Biology*, *90*, 1112–1117.

Biggins, D. E., Vargas, A., Godbey, J., & Anderson, S. H. (1999). Influence of pre-release experience on reintroduced black-footed ferrets (*Mustela nigripes*). *Biological Conservation*, *89*, 121–129.

Blair, S. D., & Glover, C. N. (2019). Acute exposure of larval rainbow trout (*Oncorhynchus mykiss*) to elevated temperature limits *hsp70b* expression and influences future thermal tolerance. *Hydrobiologia*, *836*, 155–167.

Borcard, D., Gillet, F., & Legendre, P. (2011). *Numerical ecology with R* (2nd ed.). Springer.

Braithwaite, V. A., & Salvanes, A. G. V. (2005). Environmental variability in the early rearing environment generates behaviourally flexible cod: Implications for rehabilitating wild populations. *Proceedings of the Royal Society Series B: Biological Sciences*, *272*, 1107–1113.

Brignon, W. R., Pike, M. M., Ebbesson, L. O. E., Schaller, H., Peterson, J. T., & Shreck, C. B. (2018). Rearing environment influences boldness and prey acquisition behavior, and brain and lens development of bull trout. *Environmental Biology of Fishes*, *101*, 383–401.

Brown, C., Davidson, T., & Laland, K. (2003). Environmental enrichment and prior experience of live prey improve foraging behaviour in hatchery-reared Atlantic salmon. *Journal of Fish Biology*, *63*, 187–196.

Brown, C., & Laland, K. (2001). Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology*, *59*, 471–493.

Brown, G. E. (2003). Learning about danger: Chemical alarm cues and local risk assessment in prey fishes. *Fish and Fisheries*, *4*, 227–234.

Carlson, R. L., & Lauder, G. V. (2010). Living on the bottom: Kinematics of benthic station-holding in darter fishes (Percidae: Etheostomatinae). *Journal of Morphology*, *271*, 25–35.

Chittenden, C. M., Biagi, C. A., Davidsen, J. G., Davidsen, A. G., Kondo, H., McKnight, A., Pedersen, O., Raven, P. A., Rikardsen, A. H., Shrimpton, J. M., Zuehlke, B., McKinley, R. S., & Devlin, R. H. (2010). Genetic versus rearing-environment effects on phenotype: Hatchery and natural rearing effects on hatchery- and wild-born Coho salmon. *PLoS One*, *5*, e12261.

Cochran-Biederman, J. L., Wyman, K. E., French, W. E., & Loppnow, G. L. (2014). Identifying correlates of success and failure of native freshwater fish reintroductions. *Conservation Biology*, *29*, 175–186.

Colorado Parks and Wildlife. (2015). *State wildlife action plan: A strategy for conserving wildlife in Colorado*. Colorado Parks and Wildlife.

Cook, C. J., Wilson, C. C., & Burness, G. (2018). Impacts of environmental matching on the routine metabolic rate and mass of native and mixed-ancestry brook trout (*Salvelinus fontinalis*) fry. *Conservation Physiology*, *6*, 1–12.

Crane, A. L., Lampe, M. J., & Mathis, A. (2015). Maladaptive behavioural phenotypes in captive-reared darters (*Etheostoma caeruleum*, Storer 1845). *Journal of Applied Ichthyology*, *31*, 787–792.

D'Anna, G., Giacalone, V. M., Fernández, T. V., Vaccaro, A. M., Pipitone, C., Mirto, S., Mazzola, S., & Badalamenti, F. (2012). Effects of predator and shelter conditioning on hatchery-reared white seabream *Diplodus sargus* (L., 1758) released at sea. *Aquaculture*, *356-357*, 91–97.

Evans, M. L., Hori, T. S., Rise, M. L., & Fleming, I. A. (2015). Transcriptomic responses of Atlantic salmon (*Salmo salar*) to environmental enrichment during juvenile rearing. *PLoS One*, *10*, e0118378.

Fitzpatrick, S. W., Crockett, H., & Funk, W. C. (2014). Water availability strongly impacts genetic patterns of an imperiled Great Plains stream fish. *Conservation Genetics*, *15*, 771–788.

Franssen, N. R., Harris, J., Clark, S. R., Schaefer, J. F., & Stewart, L. K. (2013). Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proceedings of the Royal Society Series B: Biological Sciences*, *280*, 20122715.

Franssen, N. R., Stewart, L. K., & Schaefer, J. F. (2013). Morphological divergence and flow-induced phenotypic plasticity in a native fish from anthropogenically altered stream habitats. *Ecology and Evolution*, *3*, 4648–4657.

Fraser, D. J. (2008). How well can captive breeding programs conserve biodiversity? A review of salmonids. *Evolutionary Applications*, *1*, 535–586.

Fuss, H., & Byrne, J. (2002). Differences in survival and physiology between coho salmon reared in semi-natural and conventional ponds. *North American Journal of Aquaculture*, *64*, 267–277.

Gatz, A. J., Jr. (1979). Ecological morphology of freshwater stream fishes. *Tulanne Studies in Zoology Botany*, *21*, 91–124.

Griffin, A. S., Blumstein, D. T., & Evens, C. S. (2000). Training captive-bred or translocated animals to avoid predators. *Conservation Biology*, *14*, 1317–1326.

Groce, M. C., Bailey, L. L., & Fausch, K. D. (2012). Evaluating the success of Arkansas darter translocations in Colorado: An occupancy sampling approach. *Transactions of the American Fisheries Society*, *141*, 825–840.

Gu, X. L., Zhao, F., Zhuang, P., Shi, X. T., Feng, G. P., Zhang, T., Liu, J. Y., & Kynard, B. (2018). The effect of water velocity on feeding efficiency of juvenile Chinese sturgeon, *Acipenser sinensis*. *Journal of Applied Ichthyology*, *34*, 29–32.

Hawkins, L. A., Magurran, A. E., & Armstrong, J. D. (2008). Ontogenetic learning of predator recognition in hatchery-reared Atlantic salmon, *Salmo salar*. *Animal Behaviour*, *75*, 1663–1671.

Hervé, M. (2021). *RVAideMemoire: Testing and plotting procedures for biostatistics. R Package 0.9-79*. https://CRAN.R-project.org/package=RVAideMemoire.

Higham, T. E., Stewart, W. J., & Wainwright, P. C. (2015). Turbulence, temperature, and turbidity: The echomechanics of predator-prey interactions in fishes. *Integrative and Comparative Biology*, *55*, 6–20.

Hutchison, M., Butcher, A., Norris, A., Kirkwood, J., & Chilcott, K. (2012). A review of domestication effects on stocked fishes, strategies to improve post stocking survival of fishes and their potential application to threatened fish species recovery programs in the Murray-Darling basin. *Murray-Darling Basin Authority*, *48*, 1–34.

Hyvärinen, P., & Rodewald, P. (2013). Enriched rearing improves survival of hatchery-reared Atlantic salmon smolts during migration in the river Tornionjoki. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*, 1386–1395.

Jackson, C. E., & Brown, G. E. (2011). Differences in antipredator behaviour between wild and hatchery-reared juvenile Atlantic salmon (*Salmo salar*) under seminatural conditions. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*, 2157–2165.

Jørgensen, E. H., & Jobling, M. (1993). The effects of exercise on growth, food utilization and osmoregulatory capacity of juvenile Atlantic salmon, *Salmo salar*. *Aquaculture*, *116*, 233–246.

Keefe, M. (1992). Chemically mediated avoidance behavior in wild brook trout, *Salvelinus fontinalis*: The response to familiar and unfamiliar predaceous fishes and the influence of fish diet. *Canadian Journal of Zoology*, *70*(2), 288–292.

Kopack, C. J., Broder, E. D., Fetherman, E. R., Fitzpatrick, R. M., & Angeloni, L. M. (2023). Assessing antipredator behavior and the potential to enhance it in a species of conservation concern. *North American Journal of Aquaculture*, *85*, 136–145.

Kopack, C. J., Broder, E. D., Fetherman, E. R., Lepak, J. M., & Angeloni, L. M. (2016). The effect of a single prerelease exposure to conspecific alarm cue on poststocking survival in three strains of rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Zoology*, *94*, 661–664.

Kopack, C. J., Broder, E. D., Lepak, J. M., Fetherman, E. R., & Angeloni, L. M. (2015). Behavioral responses of a highly domesticated, predator naïve rainbow trout to chemical cues of predation. *Fisheries Research*, *169*, 1–7.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26.

Labbe, T. R., & Fausch, K. D. (2000). Dynamics of intermittent stream habitat regulate persistence of a threatened fish at multiple scales. *Ecological Applications*, *10*, 1774–1791.

Lamothe, K. A., & Drake, D. A. R. (2019). Moving repatriation efforts forward for imperilled Canadian freshwater fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*, 1914–1921.

Lee, J. S. F., & Berejikian, B. A. (2008). Effects of the rearing environment on average behaviour and behavioural variation in steelhead. *Journal of Fish Biology*, *72*, 1736–1749.

Legendre, P., & Anderson, M. J. (1999). Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs*, *69*, 1–24.

Legendre, P., & Legendre, L. (2012). *Numerical ecology* (3rd ed.). Elsevier.

Legendre, P., Oksanen, J., & ter Braak, C. J. F. (2010). Testing the significance of canonical axes in redundancy analysis. *Methods in Ecology and Evolution*, *2*, 269–277.

Lenth, R. (2020). *emmeans: Estimated marginal means, AKA Least-squares means. R Package Version 1.4.8*. https://CRAN.R-project.org/package=emmeans.

Liao, J. C. (2007). A review of fish swimming mechanics and behavior in altered flows. *Philosophical Transactions of the Royal Society Series B: Biological Sciences*, *362*, 1973–1993.

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, *68*, 619–640.

Mallinson, J. J. (1995). Conservation breeding programmes: An important ingredient for species survival. *Biodiversity and Conservation*, *4*, 617–635.

Marques, N. C. S., Fava, F. G., & Nomura, F. (2019). Morphology-environment interaction in ecomorphological guilds of tadpoles. *South American Journal of Herpetology*, *14*(2), 116–122.

Maynard, D. J., Flagg, T. A., Iwamoto, R. N., & Mahnken, C. V. W. (2004). A review of recent studies investigating seminatural rearing strategies as a tool for increasing Pacific salmon postrelease survival. *American Fisheries Society Symposium*, *44*, 573–584.

McArdle, B. H., & Anderson, M. J. (2001). Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, *82*, 290–297.

Mery, F., & Burns, J. G. (2010). Behavioural plasticity: An interaction between evolution and experience. *Evolutionary Ecology*, *24*, 571–583.

Mesquita, F. O., & Young, R. J. (2007). The behavioural responses of Nile tilapia (*Oreochromis niloticus*) to anti-predator training. *Applied Animal Behaviour Science*, *106*, 144–154.

Moberg, O., Braithwaite, V. A., Jensen, K. H., & Salvanes, A. G. V. (2011). Effects of habitat enrichment on the foraging behaviour of juvenile Atlantic cod (*Gadus morhua* L). *Environmental Biology of Fishes*, *91*, 449–457.

Nordell, S. E. (1998). The response of female guppies, *Poecilia reticulata*, to chemical stimuli from injured conspecifics. *Environmental Biology of Fishes*, *51*, 331–338.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H., Szoecs, E., and Wagner, H. (2020). *vegan: Community Ecology Package. R Package Version 2.5-7*.

Olla, B. L., Davis, M. W., & Ryer, C. H. (1998). Understanding how the hatchery environment represses or promotes the development of behavioral survival skills. *Bulletin of Marine Science*, *62*, 531–550.

Pessanha, A. L. M., Araújo, F. G., Oliveira, R. E. M. C. C., da Silva, A. F., & Sales, N. S. (2015). Ecomorphology and resource use by dominant species of tropical estuarine juvenile fish. *Neotropical Ichthyology*, *13*, 401–412.

Piper, R. G., McElwain, I. B., Orme, L. E., McCraren, J. P., Fowler, L. G., & Leonard, J. R. (1986). *Fish hatchery management* (3rd ed.). United States Fish and Wildlife Service.

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Roberts, L. J., Taylor, J., & de Leaniz, C. G. (2011). Environmental enrichment reduces maladaptive risk-taking behavior in salmon reared for conservation. *Biological Conservation*, *144*, 1972–1979.

Salvanes, A. G. V., Moberg, O., Ebbesson, L. O. E., Nilsen, T. O., Jensen, K. H., & Braithwaite, V. A. (2013). Environmental enrichment promotes neural plasticity and cognitive ability in fish. *Proceedings of the Royal Society Series B: Biological Sciences*, *280*, 20131331.

Saraiva, S. O., & Pompeu, P. S. (2016). Fish hatchering and its effects on the morphology of *Prochilodus lineatus* (Actinopterygii: Prochilodontidae). *Brazilian Journal of Biology*, *76*, 209–217.

Shumway, C. A. (1999). A neglected science: Applying behavior to aquatic conservation. *Environmental Biology of Fishes*, *55*, 183–201.

Stoner, A. W. (2004). Effects of environmental variables on fish feeding ecology: Implications for the performance of baited fishing gear and stock assessment. *Journal of Fish Biology*, *65*, 1445–1471.

Stonski, T. S., Beck, B. B., Bloomsmith, M. A., & Maple, T. L. (2003). A behavioral comparison of captive-born, reintroduced golden lion tamarins and their wild-born offspring. *Behavior*, *140*, 137–160.

Tave, D., Toya, L. A., & Hutson, A. M. (2019). Raising fish in a purpose-built conservation aquaculture facility using conservation aquaculture management. *North American Journal of Aquaculture, Special Selection: Captive Propagation of Imperiled Species*, *84*(4), 326–332.

Tyler, J. A. (1993). Effects of water velocity, group size, and prey availability on the stream-drift capture efficiency of blacknose dace, *Rhinicthys atratulus*. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*, 1055–1061.

Ullah, I., Zuberi, A., Khan, K. U., Ahmad, S., Thörnqvist, P., & Winberg, S. (2017). Effects of enrichment on the development of behavior in an endangered fish mahseer (*Tor putitora*). *Applied Animal Behavior Science*, *186*, 93–100.

Utt, A. C., Harvey, N. C., Hayes, W. K., & Carter, R. L. (2008). The effects of rearing method on social behaviors of mentored, captive-reared juvenile California condors. *Zoo Biology*, *27*, 1–18.

Vilhunen, S. (2006). Repeated antipredator conditioning: A pathway to habituation or to better avoidance? *Journal of Fish Biology*, *68*, 25–43.

White, G. C., & Burnham, K. P. (1999). Program MARK: Survival estimation from populations of marked animals. *Bird Study*, *46*-(Supplement), 120–138.

Zhang, Z., Bai, Q., Xu, X., Guo, H., & Zhang, X. (2020). Effects of environmental enrichment on the welfare of juvenile black rockfish *Sebastes schlegelii*: Growth, behavior and physiology. *Aquaculture*, *518*, 734782.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kopack, C. J., Fetherman, E. R., Broder, E. D., Fitzpatrick, R. M., & Angeloni, L. M. (2023). The effects of abiotic enrichment and predator training on the behavior, morphology, and survival of a species of conservation concern. *Conservation Science and Practice*, e12999. https://doi.org/10.1111/csp2.12999