Effects of Heavy Metal Pollution on the Antipredator Behavior of Orangethroat Darters (Etheostoma spectabile)

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EFFECTS OF HEAVY METAL POLLUTION ON THE ANTIPREDATOR BEHAVIOR OF ORANGETHROAT DARTERS (*ETHEOSTOMA SPECTABILE*)

A Master's Thesis

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In Partial Fulfillment

Of the Requirements for the Degree

Master of Natural and Applied Science, Biology

By

Caleb Stephen O'Neal

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ABSTRACT

Heavy metal pollution can have numerous negative impacts on stream fishes, including both lethal and sublethal effects. Because of the sensitivity of fishes to toxins, they are excellent environmental indicators of stream and watershed health. The Tri-State Mining District is a Superfund site located in parts of Missouri, Kansas and Oklahoma that offers a good opportunity to study sublethal effects of heavy metal pollutants on fish behavior. I observed the antipredator behavior of Orangethroat Darters (*Etheostoma spectabile*) from 3 streams that varied in the abundance of heavy metal pollutants. In the lab, darters from the most polluted site were less active overall, but darters from both polluted sites produced alarm cues and responded to the cues with an appropriate fright response. In field trials, I observed darters by snorkeling and recording how darters from each stream responded to the threat of an approaching predator. Darters from heavily and moderately polluted streams showed a reduced tendency to flee, relative to uncontaminated stream darters when approached by a predator. Therefore, long-term exposure to heavy metals from mining pollution is associated with changes in behavior of stream fishes.

KEYWORDS: stream pollution, antipredator behavior, flight initiation distance, alarm cue

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INTRODUCTION

Approximately 10% of all known species currently recorded and 1/3 of all vertebrate species are supported by freshwater stream ecosystems (Strayer & Dudgeon, 2010). Consequently, the relative health of streams and their contributing watersheds is of great interest to conservationists and environmental scientists across the globe. Human activities contribute negatively to the health of aquatic ecosystems in a variety of ways, many of which are associated with terrestrial runoff. These pollutants include sewage and agricultural runoff, plastics, and heavy metals (reviews: Bukola et al., 2015; Häder et al., 2020).

Contamination of streams and watersheds by heavy metals is particularly problematic because these metals are stable, tend to bioaccumulate, and in many cases are toxic even at low concentrations (Yousafzai & Shakoori, 2008; Fatima et al., 2014). Human activities such as industrial production, agricultural chemical runoffs, and mining can result in heavy metals leeching into streams (Eisler, 1993; Tchounwou et al., 2012). Mining wastes can result in substantial quantities of heavy metal wastes, with lead (Pb), zinc (Zn), copper (CU), arsenic (AS) and cadmium (CD) causing the most environmental concern (Dudka & Adriano, 1997). In Missouri/Kansas/Oklahoma, the Tri-state Mining District had active lead and zinc mines for over a century, ending in 1970 (U.S. Department of Interior 2008). Although some remediation activities have occurred, elevated levels of contaminants continue to be an environmental hazard at many sites (Gutiérrez et al., 2020).

In affected areas, the relative health of potentially impacted ecosystems should be monitored to determine the need for conservation and remediation efforts. These indicators include potential effects on the biota that live in impacted areas (Cunto, 2012). For fresh-water streams, both invertebrate and vertebrate animal species can be particularly sensitive to environmental pollutants because their entire bodies are in direct contact with the water for long periods of time, and their gills are highly permeable (Birge et al., 2009). Among vertebrates, fishes have proven to be effective health indicators for streams and their contributing watersheds (Fausch et al., 1990; Lazorchak et al., 2003).

For lead, zinc, and cadmium, which are among the most common heavy metal pollutants, high levels of contamination can have lethal effects on many fish and other stream-dwelling species (Gerhardt, 1993), whereas low to moderate levels more typically lead to sub-lethal effects on morphology, physiology, and behavior of the affected species (Martinez et al., 2004). In my study, I will examine whether heavy metal pollution is associated with changes in antipredator responses of fish in both laboratory settings and in natural stream habitats. Study sites affected by heavy metal pollution are in Jasper County, MO, which is part of the Tri-State Mining District (Geel et al., 2009).

Orangethroat Darters (*Etheostoma spectabile*), the focal species in this study, is widespread and occurs throughout the Ozark region of the USA in small streams (Pflieger, 1975). Because they are small (32—63 mm), these darters are subject to predation by a wide range of predators, including piscivorus fishes. Orangethroat Darters commonly occupy small and shallow creeks with cobble and gravel streambed material under sluggish riffles. Darters do not possess a swim bladder to keep them suspended in the water column, rather they rest on the stream bottoms and anchor themselves using their pectoral fins. This close association of darters to the streambed potentially makes them even more vulnerable to heavy metal pollutants accumulated in the sediment bedload. I hypothesize that darters collected from streams polluted with heavy metals will exhibit a lower antipredator response than darters from uncontaminated streams.

Antipredator behavior of darters has been well studied, indicating that decreased activity is a typical fright response (e.g., Crane et al., 2011; Gibson & Mathis, 2006). A field study of Iowa Darters, *E. exile*, in Canada indicated that darters from an uncontaminated stream avoided areas marked with conspecific chemical alarm cues, but darters from a metal-contaminated stream did not (McPherson et al., 2004). Although no published studies have examined whether heavy metals influence antipredator behavior of Orangethroat Darters, unpublished data from a Missouri State University lab studies indicate that antipredator behavior of focal darters collected from polluted streams in the Tri-State area showed comparatively decreased antipredator responses to alarm cues from conspecifics (unpublished data, Blecha & Mathis, 2014; Figure 1). In the Mcpherson et al., (2004) study, the authors did not provide the population source for the donors of the alarm cue. However, in the Blecha & Mathis (unpublished) study, darters that provided the alarm cue were from the same populations as the focal darters. Therefore, there are several possible hypotheses that could explain the lack of response by the focal darters from impacted sites: (1) heavy metal pollution is associated with lower responses to alarm cues by focal darters, (2) heavy metal pollution is associated with lower production of alarm cues by donor darters, or (3) factors independent of pollution that vary between sites may have resulted in local adpatations in polluted stream populations resulting in darters having an altered alarm cue response. These hypotheses are not mutually exclusive.

Although most studies of darter antipredator behavior have been conducted under controlled conditions in the laboratory, some field studies have also been reported (Wisenden et al., 2004; McCormick et al., 2007). McPherson et al., (2004) marked traps with alarm or control cues and recorded the number of darters that were taken in the traps to determine whether avoidance occurred.

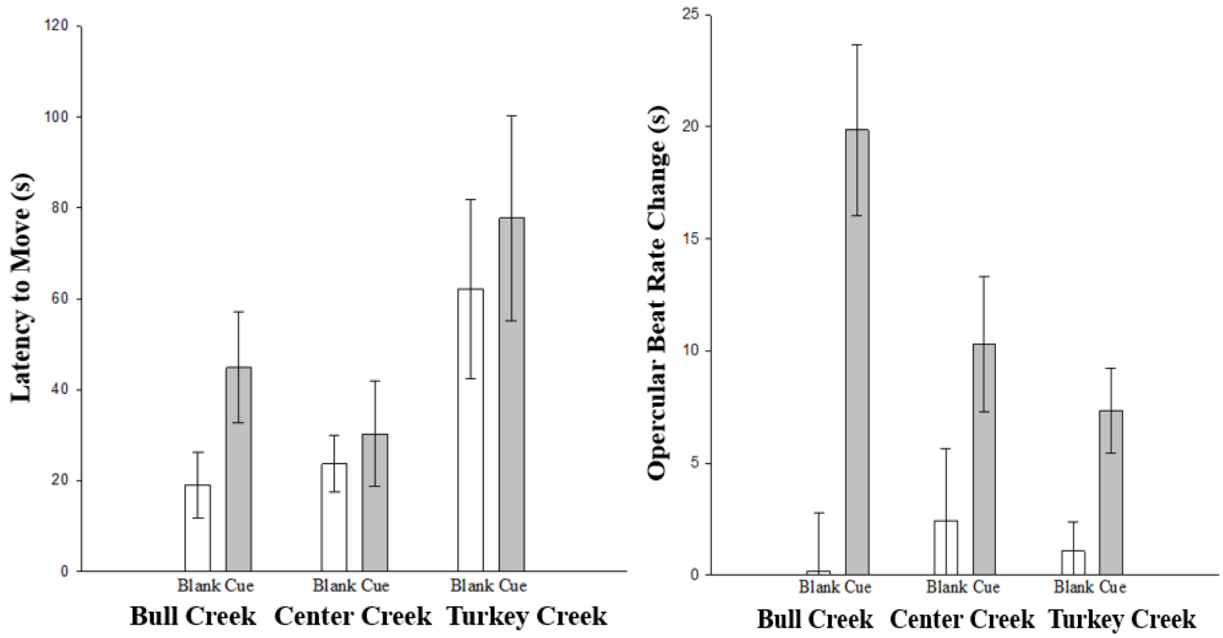

Figure 1. Mean (± SE) latency to move (left) and change in opercular beat rate (right) of darters from a non-impacted control stream (Bull Creek), a moderately impacted stream (Center Creek) and a heavily impacted stream (Turkey Creek) following exposure to a blank control or chemical alarm cue. Donor fish that provided the alarm cue were from the same population as the focal darters. Graphs are from unpublished data of Blecha & Mathis, 2014.

Snorkeling also has been successfully used to document freezing responses of Rainbow Darters in response to alarm cues in naturally occurring streams (Crane et al., 2009). In addition, snorkeling has been used to quantify "wariness" of darters in streams with and without introduced trout (Johnson & Mathis, 2021). The wariness assay used in Johnson & Mathis' study was Flight Initiation Distance (FID)—the distance at which individuals flee from an approaching threat (Ydenberg & Dill, 1986). More wary individuals respond by having longer FID scores; that is, they flee when predators are at further distances than less wary individuals. FID scores have been successfully used to quantify this measure of antipredator behavior in a wide range of vertebrate species (e.g., mammals: Bonenfant & Kramer, 1996; birds: Geist et al., 2005; fish: Gotanda et al., 2009; amphibians: Cloyed & Eason, 2014)

My goal in this study is to examine two questions about whether antipredator behavior of Orangethroat Darters from streams polluted with heavy metals is depressed in comparison to darters from a control stream: (1) Do Orangethroat Darters from polluted streams produce and respond to chemical alarm cues in the laboratory, and (2) Are Orangethroat Darters in uncontaminated streams more wary than darters in contaminated streams as indicated by FID scores in the field?

METHODS

Study Sites

The Institutional Animal Care and Use Committee approved protocol 19-007.0 for this study on 4/30/2019 (Appendix) and specimens were collected using permits granted by the Missouri Department of Conservation. In both lab and field portions of my study, I sampled darters from three streams in the Missouri Ozarks that were chosen to represent a gradient in heavy metal pollutants (Figure 2). Two streams polluted with run-off from mining tailings are part of the Spring River basin, with Turkey Creek (Jasper County, MO) designated as heavily polluted, and Center Creek (Jasper County, MO) designated as moderately polluted (Gutiérrez et al., 2020). The control stream, Bull Creek (Christian County, MO), which has no mining effluence, is part of the White River basin.

To determine whether habitat variables other than concentration of heavy metals might vary among the streams, I conducted a habitat analysis at each site during the same season and locations where I conducted the field study. As I moved down the stream to collect data on darter responses (see below), I stopped at 100 random points in the stream approximately 1 m apart and used a handheld Geographic Resource Solutions (GRS) densitometer to record the presence or absence of canopy cover (England et al., 2004). I also conducted Wolman (1954) pebble counts using a gravelometer to gauge the size of 100 randomly selected pieces of coarse stream substrate. I started selecting substrate samples (pebbles, cobble, boulders) at the tail of each riffle and worked my way upstream in a zigzag pattern to the head of the riffle. Stream temperature data were only collected once at each site at the time of the field tests.

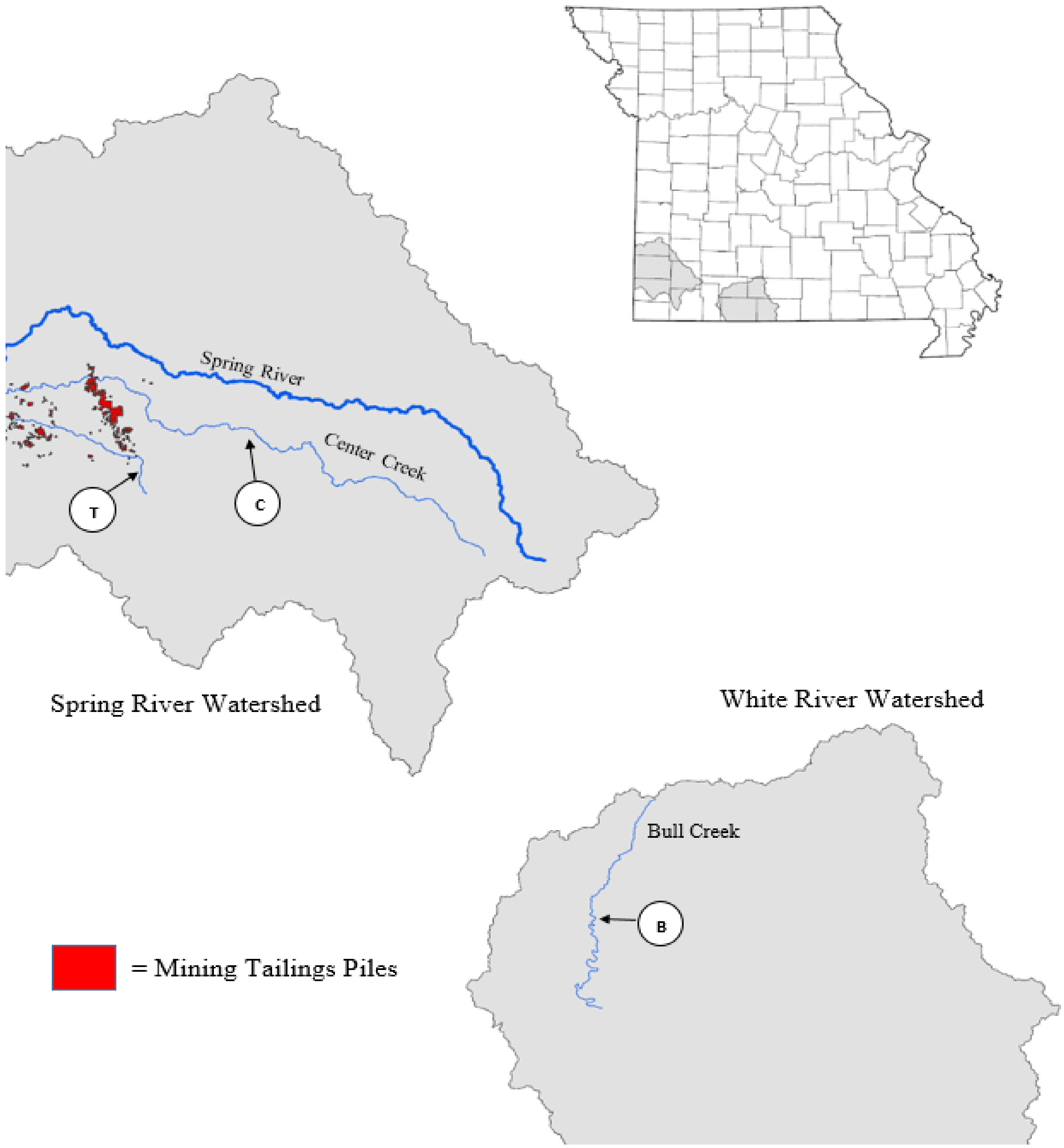

Figure 2. Map of study sites. “T” indicates Turkey Creek, “C” Center Creek, and “B” Bull creek. Red markings indicate known locations of chat piles from mining waste.

Sediment Analyses

To verify that the heavy metal concentrations at my study sights were similar to those reported by Gutiérrez (2020), I conducted sediment analyses for lead, zinc, and cadmium from each site. At each of the three sites, I collected sediment samples from the streambeds during the spring of 2021. I used a small shovel to dig sediment samples at a depth of 5 cm at the head and tail of each riffle and placed the sediment samples in plastic bags. I dried samples by placing the open bags in ovens set at 55°C for 72 h. Once samples were thoroughly dried, I then disaggregated and sieved samples through a stack of 8 mm, 6 mm, 4 mm, 2 mm, and finally 250 µm sieves. I then sent the < 2 mm and < 250 µm fractions to the Ozarks Environmental and Water Resources Institute, where the heavy metal quantities in each sample were measured using an X-MET3000TXS+ handheld x-ray fluorescence (XRF) analyzer in accordance with established lab procedures for this device (OEWRI, 2007).

Do Darters from Polluted Streams Both Produce and Recognize Alarm Cues?

In this laboratory experiment, I compared the response of darters from contaminated sites to alarm cues produced by darters from contaminated and uncontaminated sites. If darters from contaminated sites have decreased production of chemical alarm cues, I predict that focal darters will show decreased responses to cues from these sites in comparison to cues from darters from the uncontaminated site.

I collected adult darters from the contaminated and uncontaminated sites in the months of October and November of 2019 using a kick-seine or hand-held D-net. I then transferred darters to 18.9 L containers fitted with aerators for transportation to the laboratory on the Missouri State University campus. Three 75.7-L holding tanks housed the darters, which were separated based on the stream from which they were collected. The floor of each holding tank contained natural river rock, and pot shards as cover. A mixture of thawed bloodworms (*Tubifex* spp.) and mysis shrimp (*Mysis* spp.) was fed to darters every other day. The lab was kept at 18-20°C, with a 12:12 light:dark cycle, except during testing.

Testing Procedure

Chemical alarm cues were collected from adult donor darters (standard length (SL) = 49 – 56 mm) from each of the contaminated streams and from the uncontaminated control stream. To prepare the alarm cues, I arbitrarily selected donor darters from the pool of darters from each testing site, with n = 6 darters from each stream. Stream identity was coded so that I was blind to the treatment. Because anesthesia would have contaminated the chemical collected, I sacrificed donor darters by stunning them with a sharp blow to the head followed by pithing as described by Anderson and Mathis (2016) and approved by the Missouri State University IACUC. I then laid the darter carcasses on a sterile block and, following standard procedures for collection of darter alarm cues (Smith, 1981), I made shallow vertical cuts (25) with a scalpel on each side of the darter. Once cuts were made, I placed the fish carcass in a beaker with 60 mL of distilled water and a magnetic stirrer and agitated the solution for 300 s. I then removed the fish carcass from the beaker, and placed the resulting solution, containing the alarm cue, into 10-mL aliquots in syringes and then rested them on ice. Thus, each donor fish provided alarm cue for 6 trials. I prepared a blank control cue in the same way as the alarm cue except that no fish carcass was present.

Behavioral tests followed a 2-factor design, with source population of focal darters (Turkey Creek and Center Creek – both polluted streams) and source of alarm cues as the two factors. Alarm cue sources were Turkey Creek (heavy pollution), Center Creek (moderate pollution), Bull Creek (no pollution) and the blank control. There were 15 replicates from both populations for each of the four cue treatments.

I randomly assigned darters to individual tanks in an aquatic habitat system (AHAB) 48 h prior to behavioral response trials. The AHAB is a circulating system composed of 48 individual 1.5-L tanks holding one fish per tank. Tanks were left bare of any substrate, and each was covered with automotive window tinting (visible light transmission 15%) to reduce the visibility of the researcher to the fish. Laboratory lights were turned off 1 h before testing, and AHAB tanks were illuminated by overhead fluorescent fixtures placed directly over each tank. Water circulation continued until 1 h prior to testing for the row of tanks that were to be tested that day so that the cue treatments were not dispersed throughout the entire AHAB system.

During the first 20 s, I measured the pre-stimulus opercular beat rate for each fish by counting the number of times the operculum opened and then multiplied that number by three to extrapolate to opercular beat rate per min. I then injected the 10 mL of one of the four randomly determined treatment solutions (three population sources of alarm cues or a blank) into the corner of the tank and waited 30 s to allow the solution to disperse. I then recorded the post-stimulus opercular beat rate for another 20 s.

Solitary darters in undisturbed tanks can exhibit low activity. Therefore, before collecting activity data, I injected 1 mL of thawed mysis shrimp into the corner of the focal darter's tank to incentivize movement. I then immediately recorded the darter's latency to move (LTM) and the number of moves the darter made for the next 300 seconds. Darters typically move in short bursts ("darts"), so counting the number of moves is effective for quantifying activity (e.g. Commens & Mathis, 1999).

Do Darters from Polluted Sites Differ in Their Response to Risk in Comparison to Darters from Uncontaminated Sites?

In this field study, I compared the response of Orangethroat Darters from the three populations in their natural stream habitats to increased risk. I simulated increased risk by approaching focal darters while snorkeling in the stream. The fright response of each individual is measured by flight initiation distance (FID), which is the distance between the approaching threat and the location of the darter when it first begins to move away (Ydenberg & Dill, 1986). If antipredator behavior such as fright response of darters is affected by the heavy metal pollution, darters from impacted streams should have different FID scores than darters in the control stream.

I measured FID scores of free-living darters from Turkey Creek (heavily polluted), Center Creek (moderately polluted), and Bull Creek (control), following the methods of Johnson & Mathis (2021). As I snorkeled upstream, I selected focal darters that were solitary adults located away from cobble or boulders. I then slowly moved upstream toward the focal darter. As soon as the darter moved away, I dropped a weighted marker to the stream bed directly below my mask and then set another weighted marker at the darter's original position before it fled. I then measured the distance between the two markers in the stream as the FID score for that individual. I collected the weights and continued to move upstream looking for another suitable focal darter, at least 3 m from the last focal darter to ensure that no darters were tested twice.

Data Analysis

All data were analyzed using Minitab, v. 20. I used a Kruskal-Wallis test to detect differences in stream bed substrate size between the three populations and Chi-Square goodness of fit test for differences in canopy cover at each site.

I transformed data from the lab experiment using an aligned rank transformation (ART; Higgins & Tashtoush, 1994). I then analyzed each factor using an ANOVA. The factors were (1) population source (Turkey Creek, heavy; Center Creek, Moderate; and Bull Creek, uncontaminated) and (2) treatment (alarm cue and blank). Separate analyses were made for each response variable: number of moves, latency to move, and opercular beat rate. Because I have data pre- and post-stimulus introduction for opercular beat rates, I converted these data to a change score: pre-stimulus beats – post-stimulus beats.

Since the data did not differ from a normal distribution according to Kolmogorov-Smirnov test, I ran a one-way ANOVA with a post-hoc Tukey test to detect differences in FID scores between the three populations.

RESULTS

Habitat and Sediment Analysis

Stream habitat assessment revealed no significant differences among substrate sizes for the three study streams (H = 3.34, DF = 2, $P = 0.188$). Canopy cover was dense at all three sites, and there was no significant difference among sites ($\chi^2 = 2.47$, $P = 0.291$). Water temperature data were only taken once (during the field experiment) and so was not statistically analyzed; temperatures were similar at all three sites (Table 1).

Sediments from Bull Creek, the uncontaminated control site, contained no detectable levels of lead or cadmium and had barely detectable amounts of zinc (Table 2). Center Creek, the moderately impacted site, also contained no cadmium, but contained lead, and almost 4× the amount of zinc as the control creek. In comparison to the other two sites, sediments from Turkey Creek, the heavily impacted site, contained the highest levels of heavy metals, with cadmium present and 32× the amount of zinc and 24× the amount of lead as Center Creek (Table 2).

Do Darters from Polluted Streams Both Produce and Recognize Alarm Cues?

Focal darters from the heavily polluted stream (Turkey Creek) were significantly less active overall than darters from the moderately polluted stream ($F_{3,\,112} = 9.04$, DF = 1, $P = 0.003$; Figure 3). Predation risk significantly affected activity of darters ($F_{3,\,112} = 2.34$, DF = 3, $P <$ 0.001), with darters being less active in response to the conspecific alarm cues than to the blank control. There was no interaction between population source of focal darters and cue type with respect to number of moves ($F_{3,\,112} = 1.41$, DF = 3, $P = 0.243$). Similar results were seen for latency to move with focal darters from the most polluted stream (Turkey Creek) having significantly longer freezing response ($F_{3, 112}$ = 14.58, DF = 1, $P < 0.001$; Figure 4). Freezing response from both focal darter populations was significantly longer in response to the alarm cue than to the blank control ($F_{3, 112}$ = 12.18, $P < 0.001$). There was no interaction between focal darter population and cue with respect to latency to move ($F_{3, 112}$ = 1.36, $P = 0.260$). Opercular beat rate was not significantly affected by focal darter population ($F_{3, 112}$ = 0.14, P = 0.712), cue type ($F_{3, 112}$ = 0.08, P = 0.973) or an interaction between the two variables ($F_{3, 112}$ = 1.94, P = 0.127; Figure 5).

Do Darters from Polluted Sites Differ in Their Response to Risk in Comparison to Darters from Uncontaminated Sites?

Darters from the three streams differed significantly in their flight initiation distance (FID) scores ($F_{2, 177}$ = 242.98, $P < 0.001$). Tukey post-hoc tests indicated that darters from the uncontaminated stream exhibited longer FID's than darters from either of the contaminated sites and that the two contaminated sites did not differ from each other (Figure 6).

Table 1. Mean ± SE of sediment size and canopy cover estimates at the three sample sites. Water temperature was taken once at the beginning of the FID testing day at each location.

Location	Substrate Size (mm)	Canopy Cover %	Temp (°C)
Bull Creek	43.38 ± 2.47	79	11.67
Center Creek	41.46 ± 3.37	78	12.78
Turkey Creek	46.96 ± 3.46	86	10.00

Table 2. Concentrations of lead, zinc, and cadmium in sediment samples (n = 2) from streambeds of each sampling site.

Location	Pb (ppm)	Zn (ppm)	Cd (ppm)
Bull Creek	Not detectable	37, 30	Not detectable
Center Creek	39, 48	120, 124	Not detectable
Turkey Creek	1995, 728	2242, 3679	33.9, 25.0

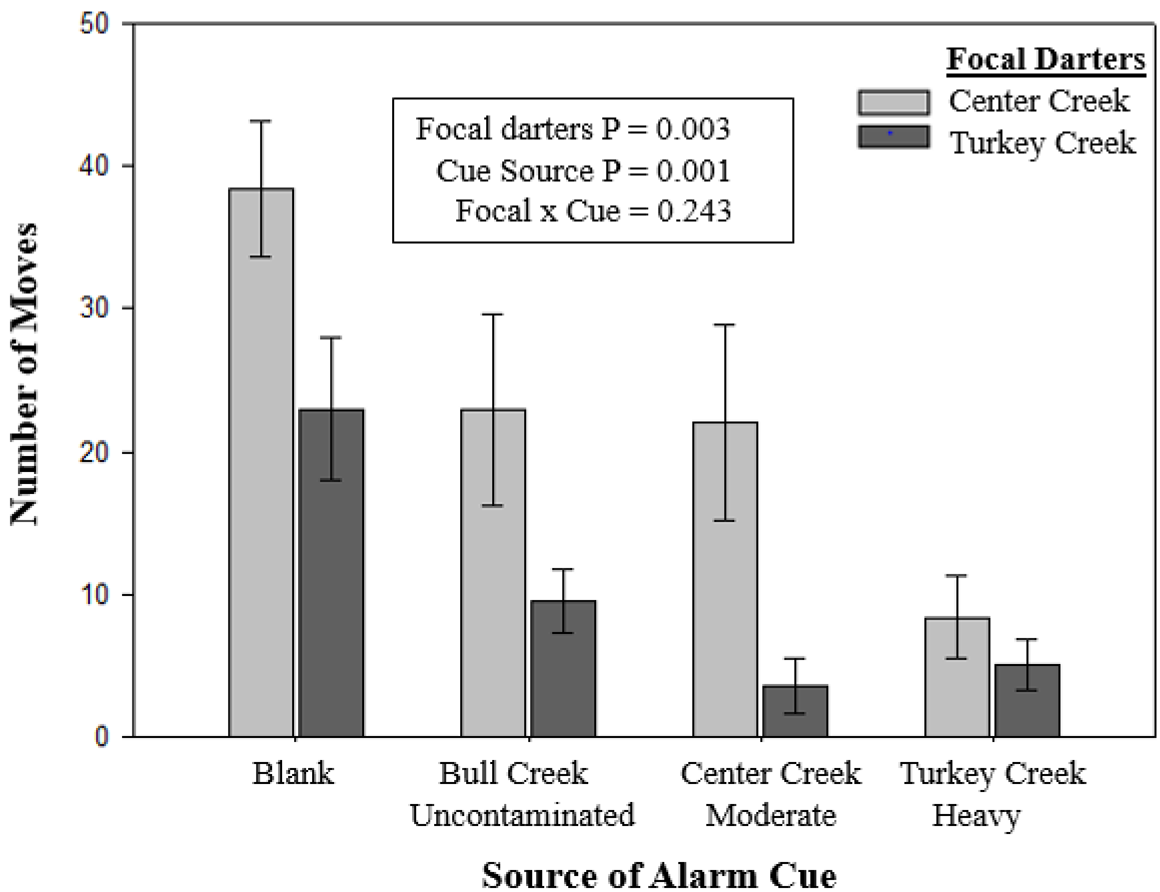

Figure 3. Mean latency to move (± SE) by Orangethroat Darters from Center Creek and Turkey Creek when presented with alarm cues of darters from each location or a blank. N = 15 per treatment per population.

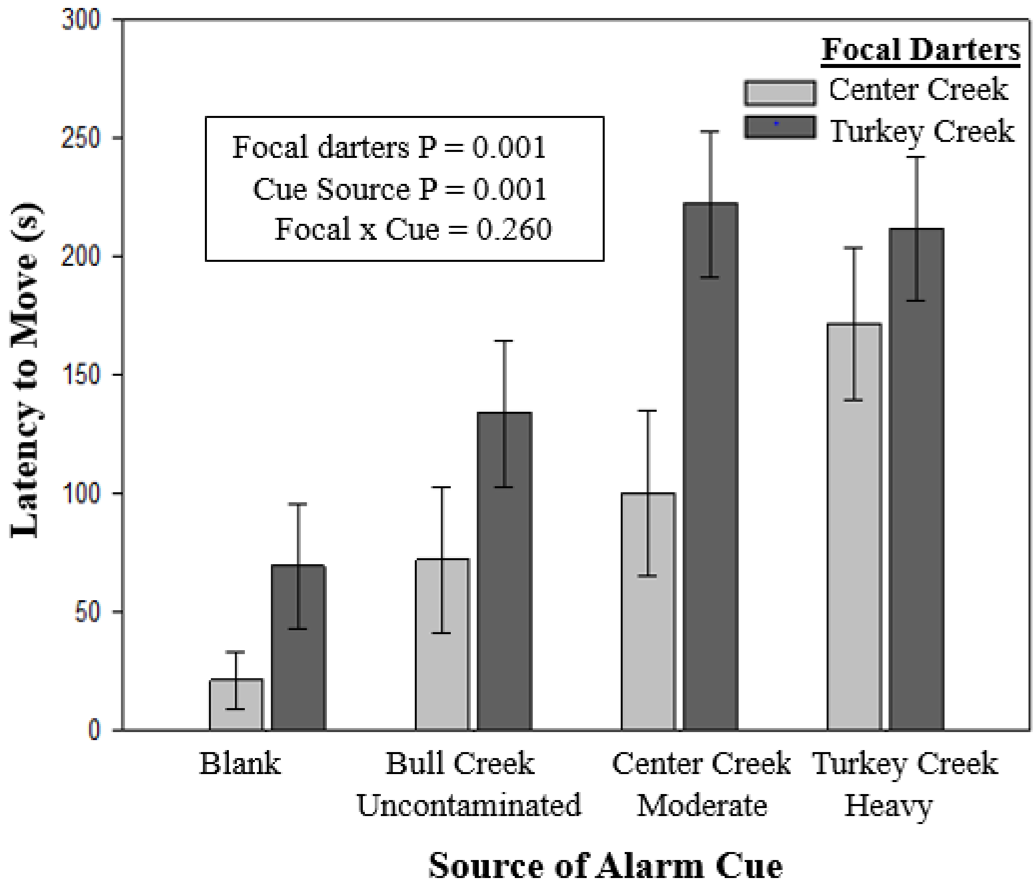

Figure 4. Mean number of moves (± SE) made by Orangethroat Darters from Center Creek and Turkey Creek when presented with alarm cues of darters from each location or a blank. N = 15 per treatment per population.

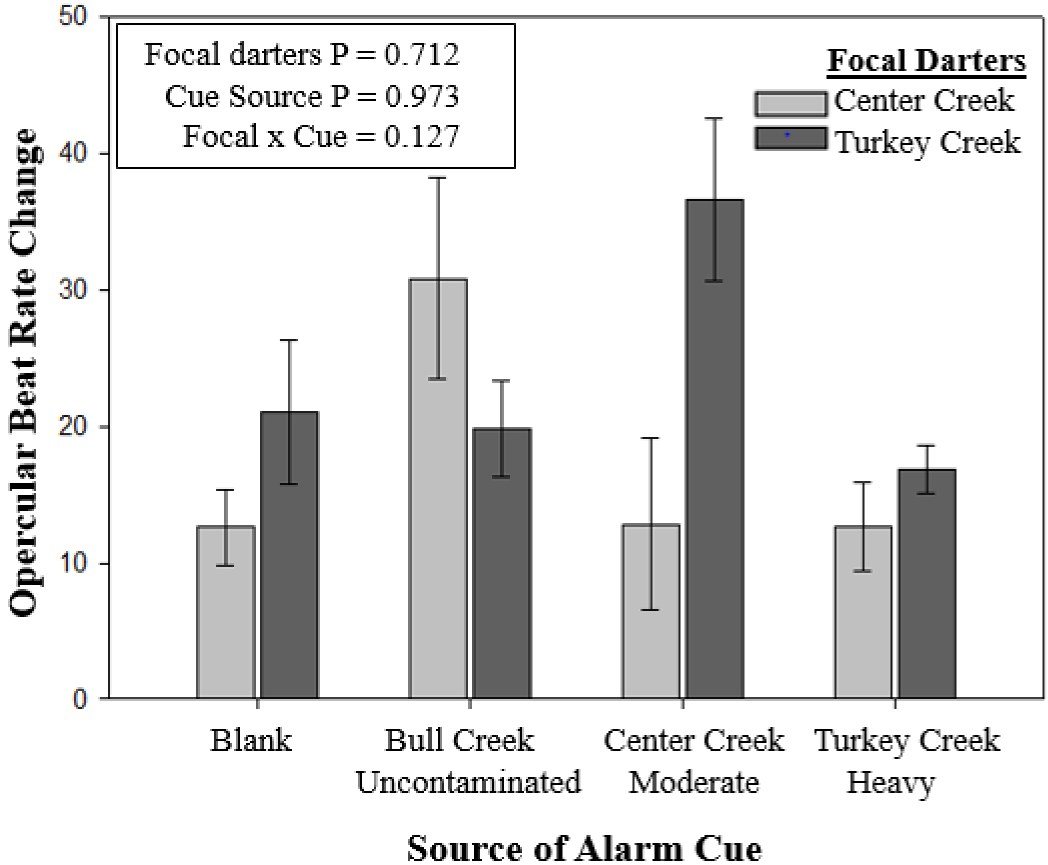

Figure 5. Mean change in opercular beat rate (± SE) by Orangethroat Darters from Center Creek and Turkey Creek when presented with alarm cues of darters from each location or a blank. N = 15 per treatment per population.

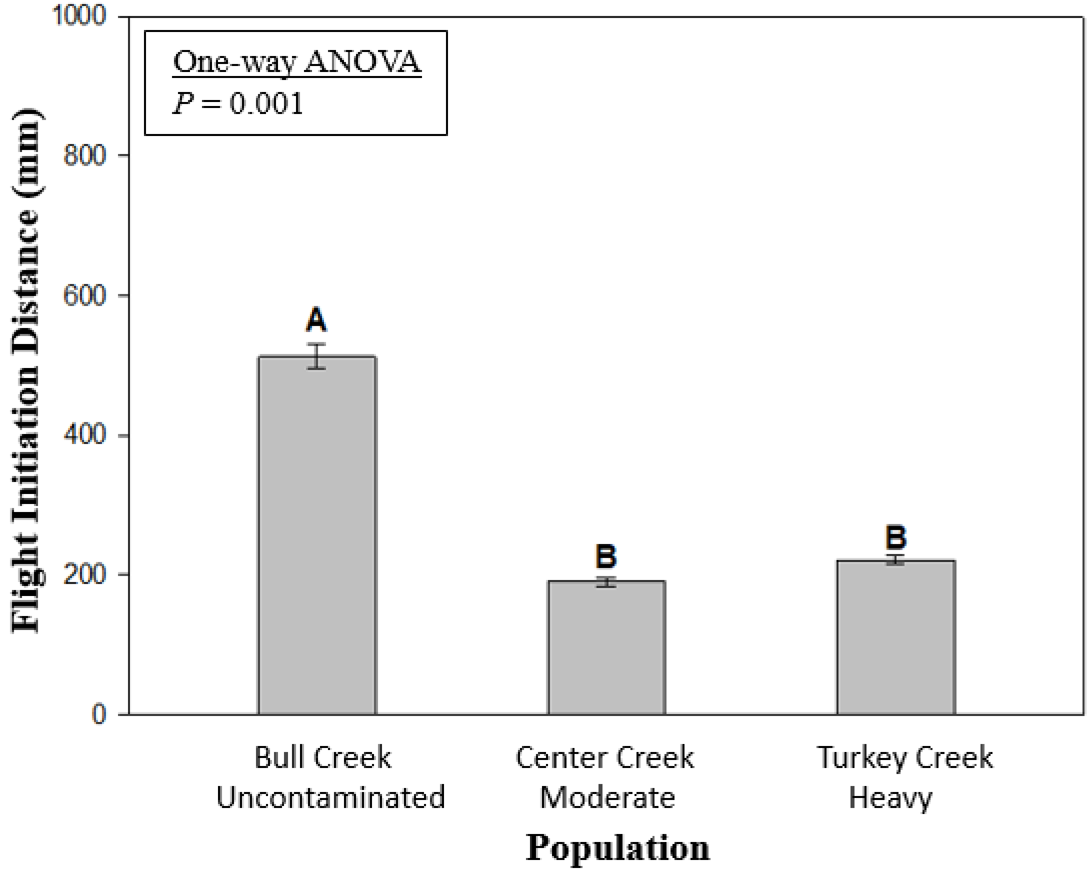

Figure 6. Mean flight initiation distances (± SE) of Orangethroat Darters in natural stream habitats at each location. N = 60 per population.

DISCUSSION

A previous unpublished study from the Mathis lab indicated that Orangethroat Darters from polluted streams had a lessened antipredator response when exposed to alarm cues from conspecific individuals than did darters from an uncontaminated stream (Figure 1; opercular beat data). The explanation of the reduced antipredator response seen in this study could be either that polluted-stream darters were less able to detect the alarm cues or that darters from polluted areas had decreased production of alarm cues. Results from my lab experiment indicated that darters from streams polluted with heavy metals responded similarly with respect to activity measures to conspecific alarm cues regardless of the source population. Therefore, darters from polluted streams are producing alarm cues at detectable levels. To my knowledge, no other studies have examined effects of heavy metal pollution on the ability of fish to produce chemical alarm cues.

As in other studies of darters (Blecha & Mathis, unpublished data), darters from both contaminated populations in my study did demonstrate the ability to detect the alarm cues, at least with respect to the freezing response. In contrast, Scott et al., (2003) reported short (1-wk) exposures to water-borne heavy metal pollution in the lab resulted in Rainbow Trout (*Oncorhynchus mykiss*) eliminating their normal antipredator response to conspecific alarm cues. They found that heavy metals accumulated in the olfactory organs and hypothesized that this accumulation interfered with the reception of the alarm cue. Similar findings of fish exposed to heavy metals having a decreased antipredator response when presented with alarm cues in the lab setting were reported by Woods (2008), in a closely related species of darter (*E. caeruleum*). Rapid detoxification of a bioaccumulated heavy metals in a marine fish has been reported (Zhang et al., 2012). Although the darters I tested had long-term exposure to heavy metals, they had time to acclimate in the uncontaminated water in the laboratory, which could have given their bodies time to detoxify from the heavy metal pollution that they experienced in their natal streams.

In natural stream habitats, darters from polluted streams showed significantly reduced wariness by allowing the snorkeler to approach significantly closer than darters from the uncontaminated stream before initiating fleeing. A decreased antipredator response was also reported for Iowa Darters in streams contaminated with heavy metals (copper, nickel, zinc) (McPherson et al., 2004). In that study, darters in uncontaminated streams avoided traps marked with alarm cue, but this avoidance response disappeared for darters in contaminated streams. Another interpretation of my field data is that the shorter FID scores indicate a freezing response by darters from contaminated sites instead of flight antipredator response. This interpretation might be expected if darters were in poorer condition, and thus might be choosing the lower-cost response in terms of energetics. However, anecdotally, darters from contaminated streams appeared to be in excellent body condition.

Because this study is correlational rather than experimental, uncontrolled variables in addition to heavy metal concentration could contribute to differences in antipredator behavior at the three sites. Temperature frequently influences the behavior of stream fishes (Bartolini et al., 2014), but there was little variation between the three populations in temperature at the time of testing in the field experiment. Whether fleeing versus freezing is the most appropriate response for fishes could depend on other habitat features. Cover availability can influence FID (Dill, 1990), but stream substrate characteristics were similar between study streams with respect to % canopy cover and substrate size. Predator type and abundance can also influence the FID of stream fish (Csányi & Dóka, 1993), and although I did not conduct a predator abundance survey, anecdotally I found many of the same predators at each site. Ambush predators like Banded

Sculpin (*Cottus carolinae*) were in abundance and active predator fish in the genus *Lepomis* were frequently seen at all three locations. In Pearson's (2010) more extensive survey of fishes in the Tri-state Mining Area, she found that while some sensitive species were affected by concentration of pollutants, overall fish diversity, as indicated by an index of biotic integrity, was not.

Heavy metals such as Pb, Zn, and Cd have all been reported to accumulate in fish tissues, whether they are taken in through the gills, the skin, or through ingestion (Afshan et al., 2013; Vinodhini et al., 2008). Increased stress can also influence behavior of freshwater fish (Israeli & Kimmel, 1996), which is likely a contributing factor to the differences seen in antipredator behavior between the three study sites. Bioaccumulation of heavy metals in fish tissues can lead to increased levels of blood plasma cortisol in freshwater fish (Pratap & Bonga, 1990; Mishra & Mohanty, 2009). The decreased antipredator responses seen in the field study of darters from polluted streams could be the result of conditioning to high levels of stress due to the pollution in their stream habitat. If levels of plasma cortisol in a prey fish are already elevated due to bioaccumulation of heavy metals in the fish tissues, an additional stressor like an approaching predator may not elicit as severe an antipredator response from the stressed fish as it would from a fish with lower circulating plasma cortisol levels.

The reduced antipredator response seen in Orangethroat Darters from polluted streams in the field could have the direct effect of leaving them more vulnerable to predation. Heavy metal pollution in streams can also have various indirect negative impacts on stream fish, such as reduction in time spent foraging (Kasumyan, 2001; Woods, 2008), and reduced fecundity (Sindhe & Kulkarni, 2005), all of which can lead to population declines of prey fish such as darters. Elucidating the effects of heavy metal pollution on stream inhabitants is a daunting task, but it is one of importance, especially in areas such as the Tri-State Mining District where the improper disposal of industrial byproducts is prevalent. My study adds to our understanding of potential mechanisms for population declines in polluted areas, which can help us better protect and conserve native freshwater species.

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APPENDIX

From: LabTracks <REDIPComputing@missouristate.edu>
Sent: Friday, December 20, 2019 12:14 PM
To: Mathis, S Alicia <AliciaMathis@MissouriState.edu>
Subject: LabTracks: Protocol Approved

Protocol 19-007.0 has been approved.