Friends in Low Places: Responses of a Benthic Stream Fish to Intra-Guild Alarm Cues

Kelsey Ann Anderson

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FRIENDS IN LOW PLACES: RESPONSES OF A BENTHIC STREAM FISH TO INTRA-GUILD ALARM CUES

A Master’s Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Kelsey Anderson

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FRIENDS IN LOW PLACES: RESPONSES OF A BENTHIC STREAM FISH TO INTRA-GUILD ALARM CUES

Biology

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Master of Science

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ABSTRACT

Many aquatic species produce chemical alarm cues that serve as a warning to nearby conspecifics. In mixed-species aggregations, individuals may also benefit by “eavesdropping” on the chemical alarm cues of other species that are in the same prey-guild. Rainbow Darters (*Etheostoma caeruleum*) are benthic fish that co-occur with native Ozark Minnows (*Notropis nubilus*), recently-introduced Western Mosquitofish (*Gambusia affinis*), and native Oklahoma Salamanders (*Eurycea tyrnerensis*), all of whom are vulnerable to the same predators. We tested the responses of darters to the damage-released alarm cues of conspecifics (positive control), minnows, and mosquitofish; alarm cues from Bumblebee Gobies (*Brachygobius doriae*) served as a negative (allopatric) control. We also tested the response of sympatric and allopatric darters to the damage-released alarm cues of Oklahoma Salamander. Darters exhibited a fright response to conspecific and minnow alarm cues, but not to cues from mosquitofish or gobies. Lack of response to mosquitofish cues could be because they are introduced or because they typically occur higher in the water column than darters. Darters that were sympatric with the salamander exhibited a fright response to the alarm cues of the salamander, while allopatric darters did not. Rainbow Darters can develop responses to the alarm cues of syntopic species (minnows and Oklahoma Salamander) within their prey guild.

KEYWORDS: group living, prey-guild, communication, alarm cues, antipredation, evolution,

This abstract is approved as to form and content

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INTRODUCTION

One of the advantages to living in groups is that individuals can gain information from group members about issues such as the location of potential food and the presence of danger (Krause & Ruxton 2002; see reviews in Zuluaga 2013). The efficacy of information transfer is typically enhanced by larger group size, with each new individual not only adding to the detection abilities of the group (e.g., “many eyes”: see reviews in Zuluaga 2013), but also bringing potentially different experiences to the group.

“Warnings” about the presence of predators are often transmitted to conspecifics via visual (e.g., Caro 1986a, b; Keenleyside 1955; Magurran & Higham 1988), auditory (e.g., Klump & Shalter 1984; Myrberg 1981; Seyfarth et al. 1980 a,b) or chemical (e.g., Blum 1985; Bowers et al. 1972; von Frisch 1938, 1941) alarm cues.

Many of the benefits of group size are augmented by the incorporation of individuals from other species in the group (Stensland et al. 2003). Mixed species groupings of animals are common in nature, and occur for insects (Menzel et al. 2008), arachnids (Hodge & Uetz 1992), mammals (see review in Stensland et al. 2003), birds (reviewed in Zuluaga 2013), and fishes (Morse 1977; Welty 1934). Alarm cues of closely related species are often similar (e.g., McCracken & Sheldon 1997; Miller 1996), and members of one species can benefit by eavesdropping on this “public information” (Wisenden & Chivers 2006). For example, white-browed scrubwrens, *Sericornis frontalis*, and superb fairy-wrens, *Malurus cyaneus*, have overlapping territories in the breeding season, can occur in mixed-species flocks in the nonbreeding season, and respond to each other's alarm calls (Fallow & Magrath 2010). In contrast, signals of
phylogenetically distant species tend to be increasingly dissimilar (e.g., Kort & Cate 2001). Despite the dissimilarity that results from increasing taxonomic distance, natural selection can still favor eavesdropping in mixed-species groups under certain conditions, as when prey are sympatric and benefit from avoidance of the same predators (Hauser 1988; Shriner 1998; Pays et al. 2014). Most mixed-species groups are composed of species that are at least somewhat closely related: flocks of birds, schools of fish, and herds of ungulates. Consequently, most studies of eavesdropping of alarm signals have been on mixed groups of similar species (Moore 1977; Windfelder 2001). Studies of eavesdropping of alarm signals between very dissimilar species are rarer, such as terrestrial salamanders (Chordata, Amphibia) detecting the alarm cues of earthworms (Annelida, Oligochaeta, Lumbricus sp.) that share their microhabitat and common predators (Crane et al. 2013).

In aquatic habitats, chemical alarm cues typically are superior to visual displays because chemicals can go around visual barriers, such as vegetation (Stauffer & Semlitsch 1993; Takahara et al. 2012), and are available when vision is limited by turbidity or darkness (at depths or at night) (Chivers et al. 1996; Mathis & Smith 1993; Wisenden et al., 1995). Unlike most auditory alarm cues, chemical cues do not require a sophisticated vocal apparatus. Additionally, water provides an excellent medium in which chemical substances can disperse (Hara 1992).

Chemical alarm cues that warn conspecifics of danger are particularly common in aquatic species, including invertebrates (e.g., amphipods: Williams & Moore 1985; crayfish: Hazlett 1994) and vertebrates (e.g., frogs and toads: see review in Chivers & Smith 1998; Hews & Blaustein 1985; Wilson & Lefcort 1993, and salamanders: Mathis
The best studied chemical alarm cues are produced by fishes of the Superorder Ostariophysi, which comprises more than 70% of all freshwater fish species, including minnows, suckers, catfishes and carps (see review in Smith 1992). In Ostariophysans, the presence of epidermal club cells that contain alarm substance, “Schreckstoff”, is considered ubiquitous (Mathis et al. 1995; Pfeiffer 1966, 1977; Smith 1979). The chemicals within these cells are released into the water after the skin is abraded, typically during a predatory attack (Smith 1979, 1992; Wisenden 2003). Similar but non-homologous alarm cues are found in other taxa of nonostariophysan fishes including live-bearers, sticklebacks, darters, gobies, sunfishes, and cichlids (Mathis 2009). The release of alarm cues by fishes has been hypothesized to be analogous to the distress calls of birds in that they are produced after capture and may attract other predators in addition to alerting conspecifics (Mathis et al. 1995).

When individuals of different prey are vulnerable to the same predators and co-occur both spatially and temporally, the species are be members of a prey-guild (Chivers et al. 1997). Individuals that can associate the alarm cues of heterospecifics within their prey-guild with threat gain an additional opportunity to perform antipredator tactics that ultimately increase the probability of survival (Chivers et al. 1997; Mathis & Smith 1993). In aquatic habitats, responses to alarm cues within prey-guilds have been studied in a number of species, including salamanders (Chivers et al. 1997; Crane et al. 2013; Sullivan et al. 2003), insects (Wisenden et al. 1997), gastropods (Dalesman et al. 2007; Snyder 1976; Stenzler & Atema 1977), and some fish (Mirza 2003; Wisenden et al. 1997; Wisenden 2008).
Darters (Osteichthyes: Perciformes: Percidae: Etheostominae) are benthic stream fishes commonly found co-habiting with other species. Some species of darters have epidermal sacciform cells, rather than club cells, that are thought to function in the production and release of the alarm chemical (Commens & Mathis 1999; Haney et al. 2001; Smith 1979). One species of darter whose alarm cues have been relatively well-studied is the Rainbow Darter (*Etheostoma caeruleum*) (see review in Mathis 2009). This species is found in rocky stream habitats and riffles with relatively high flow rates and is found in almost all streams of the Ozark Region (Pflieger 1997). Fright responses of Rainbow Darters include a suite of antipredator behaviors, including freezing, decreased activity, and increased opercular beat rates in response to the alarm cues of conspecifics and at least some other species of fish (reviewed in Mathis, 2009).

This study examined the responses of Rainbow Darters presented with alarm cues of three of their prey-guild members to test for antipredator responses. The Rainbow Darter frequently occurs with other fishes and amphibians, including the native Ozark Minnows (Osteichthyes: Cypriniformes: Cyprinidae: *Notropis nubilus*), introduced Western Mosquitofish (Osteichthyes: Cyprinodontiformes: Poecilidae: *Gambusia affinis*), and native Oklahoma Salamanders (Amphibia: Caudata: Plethodontidae; *Eurycea tynerensis*).

Minnows and darters frequently co-occur, and minnows are the dominant fish groups present in the Ozarks (Pflieger 1997). Ozark Minnows school near the bottom of the water column in creeks and small rivers with rocky bottoms and strong flow, in microhabitats similar to those occupied by darters (Becker 1983; Pflieger 1997). Western Mosquitofish occur in the same areas but typically are found near the top of the water
column and prefer slower backwaters and sloughs of darter-containing rivers and streams (Krumholz 1948; Pflieger 1997); Western Mosquitofish have been widely introduced for mosquito control, and likely reached the Ozarks Region between the 1940’s and 1960’s (Pflieger 1997). A more phylogenetically distant prey species, the Oklahoma Salamander, is benthic, stream-dwelling, and endemic to the Ozark Plateau (Bonett & Chippindale 2004; Tumlison & Cline 1990). Some populations of the Oklahoma Salamander are paedomorphic, with individuals retaining juvenile aquatic morphology and lifestyle into adulthood. The life-long exposure of paedormorphic individuals to aquatic predators, many of whom are cryptic, should create strong selection pressure for recognition of potential aquatic predators via non-visual cues (Epp & Gabor 2008; Mathis & Unger 2012; Whitham & Mathis 2000).

Where the Ozark Minnow, Western Mosquitofish, Oklahoma Salamander, and Rainbow Darter co-occur, they form a prey-guild with a variety of likely shared predators, including predatory fishes such as Banded Sculpin (Cottus carolinae) (Miller & Robison 1980; Phillips & Kilambi 1996; Robison & Buchanan 1988; Tumlison & Cline 2002), Smallmouth Bass (Micropterus dolomieu) (Page 1983; Robertson & Winemiller 2001), and several species of crappie (Pomoxis sp. (Pflieger 1997). Because predators that prey upon one of the prey species are also dangerous to the other species, selection should favor cross-species reactions to alarm cues of members within the prey-guild.

In the first experiment, I tested the response of Rainbow Darters to damaged-released alarm cues from: (1) conspecifics (positive control), (2) Ozark Minnows (native prey-guild member), (3) Western Mosquitofish (introduced prey-guild member), and (4)
Bumblebee Gobies (*Brachygobius* sp., negative control). Bumblebee Gobies are not a member of the darter prey-guild, as they are allopatric to Rainbow Darters, and they have successfully been used as a negative control for darter alarm cue studies (Commens & Mathis 1999; Commens-Carson & Mathis 2007; Gibson & Mathis 2006). I hypothesized that darters would show a fright response to alarm cues of conspecifics, somewhat benthic and native species (minnow), and to the introduced, higher-water column species (mosquitofish), but would not show a fright response to the allopatric species (goby).

A second experiment tested for the presence of a fright response by Rainbow Darters exposed to damaged-released alarm cues from a more distantly-related species, the Oklahoma Salamander. In this experiment I tested Rainbow Darters that were either sympatric or allopatric with Oklahoma Salamanders. I hypothesized that Rainbow Darters that were sympatric with Oklahoma Salamanders would exhibit a fright response to the salamander alarm cue, and that this fright response would be absent in allopatric darters.
METHODS

Experiment 1: Response to Alarm Cues from Heterospecific Fishes

**Collection and Maintenance.** Rainbow Darters (n = 200) and Ozark Minnows (n = 50) were collected in October 2015 and January 2016 by seine net from James River (Greene CO., MO). Western Mosquitofish (n = 50) were collected by electroshock from the same location. Bumblebee Gobies (n = 24) were purchased and shipped from an online supplier (LiveAquaria).

All fishes were housed in 37.9 or 75.7-L tanks filled with dechlorinated tap water for at least one week before testing. After acclimation, darters were randomly placed into individual 1.5-L containers in an Aquatic Habitat System (AHAB; Aquatic Habitats, Inc., Apopka, FL USA), which provides a continuous flow of filtered, dechlorinated tap water, and sex and standard length of each darter were recorded. Fishes acclimated to testing aquaria for one week on a 12:12 light:dark cycle at 20 ± 2º C, and darters, minnows, and mosquitofish were fed 1 mL of frozen brine shrimp (*Artemia* sp.) three times each week. Gobies were fed 1 mL frozen bloodworms twice weekly (*Chironomidae tetans*).

**Alarm Cue Collection.** Alarm cues were collected using the methods of Smith (1982). Donor fish (x ± SE standard length (STDL) = darter: 43.55 ± 1.08 mm; minnow: 47.76 ± 1.29 mm; mosquitofish: 33.85 ± 1.70 mm; goby: 24.05 ± 0.58 mm) were sacrificed as approved by the MSU IACUC (Protocol #13.010) via a blow to the head followed by the severing of the spinal cord and pithing. A razor blade was used to make 25 shallow epithelial cuts on each side of the body to release the alarm cue from the epithelial cells. The donor fish was then placed in a beaker in 40 mL of dechlorinated
water and stirred on a stir plate for 5 min before being removed from the solution. The solution was drained through glass wool to filter out particulates and drawn into two 20-mL syringes and placed on ice until testing. This procedure was repeated for each donor animal, and all equipment was cleaned with bleach and rinsed between preparations. All alarm cues were used within 2 h of preparation, with syringes being removed from ice 20 min prior to testing and warmed to room temperature.

**Experimental Protocol.** Approximately 24 h prior to testing, cardboard dividers were inserted between AHAB tanks to visually isolate adjacent fish. Window tinting was placed over the front of the tanks to inhibit the fish’s ability to detect the observer and to reduce visual distraction. Fluorescent lights were turned on above tanks at least 30 min prior to testing, and water flow was turned off at least 60 min before testing. To standardize hunger levels, fish were fed one day prior to testing.

Trials were conducted from 15 November–20 February by a single observer. Test darters were randomly assigned treatments, which included exposures to conspecific (positive control), minnow, mosquitofish, or goby (negative control) alarm cues. Each fish was observed for 5 min, and the number of moves made during this time was recorded as “pre-stimulus”, with a “move” being defined as a whole-body hop or dart on a horizontal surface, and each second of vertical swimming equating to one move. If the individual did not move during the pre-stimulus period, it was not included in the experiment. A 28-cm length of aquarium tubing was connected to each syringe so that the tubing could be inserted through a hole in the lid of the tank for introduction of the stimulus, and the randomly-selected stimulus was injected at a rate of 1 mL/second and allowed to disperse through the tank for 30 s. The fish was observed for an additional 5
min, and the number of moves made during this time was recorded as “post-stimulus”.

Time until the first movement made after stimulus had been injected was recorded as latency to move.

The change in moves was calculated by subtracting pre-stimulus from post-stimulus number of moves such that negative numbers indicated decreased activity and positive numbers indicated increased activity. Both response variables are indicators of a ‘fright’ response, with decreased movement and long latencies indicating a strong fright response. Neither response variable (change in moves and latency to move) was normally distributed, and all data were transformed using ARTool 1.5.1 (Wobbrock et al. 2011) and analyzed with one-way ANOVAs followed by post-hoc Tukey’s analyses.

Two individuals were excluded due to accidental disturbance during presentation of stimulus that resulted in an immediate, extreme flight reaction in the fish. This experiment was approved by the MSU IACUC (13-010.0 and 16-019.0).

**Experiment 2: Response to Alarm Cues from a Salamander**

**Collection and Maintenance.** Rainbow Darters (n = 165) were collected in June 2014 by seine net from the James River (Greene CO., MO) (allopatric population) and from Bull Creek (Christian CO., MO) (sympatric population). Fish were separated by population and housed as in Experiment 1. After one week of acclimation, 33 Rainbow Darters from the James River and 33 darters from Bull Creek were then randomly placed into individual tanks in the AHAB system (see Experiment 1) such that population of origin was unknown to the observer. Darters were allowed to acclimate to the testing aquaria for one week before testing. Darters from the allopatric (\(\bar{x} \pm SE\) total length (TL))
populations did not differ in size ($F_{1,66} = 0.15, p = 0.70$).

Oklahoma Salamanders ($n = 120$) were collected from Christian CO., MO in November 2013, where salamanders have been previously collected (Moore & Hughes 1939; Daniel et al. 2014). Oklahoma Salamanders were housed in a separate AHAB unit under the same conditions as the darters and received 1 mL of live black worms ($Lumbriculus variegatus$) twice weekly.

**Alarm Cue Collection.** Oklahoma Salamanders ($\bar{x} \pm SE$ snout-vent length (SVL) = 29.40 \pm 1.20 mm; $\bar{x} \pm SE$ mass = 0.48 \pm 0.04 g) that were selected for collection of alarm cue were arbitrarily chosen from their holding tank. For *Eurycea*, the alarm cue is located in the skin and not in other tissues (Marvin et al. 2004). Before collecting skin extracts, salamanders were placed in a petri dish with approximately 10 mL of water and placed into a freezer (4 °C) for 15 min to slow the salamander’s brain activity (Shine et al. 2015) before being decapitated and pithed, as approved by the MSU IACUC (Protocol #14-011). Salamander skin was removed using a razor blade and measured for length and width. The salamander’s skin plus 20 mL of dechlorinated water per cm² of skin was added to a mortar and pestle. The skin was ground by hand for 5 min, and the mixture was filtered through glass wool into a graduated cylinder. Dechlorinated water was added to bring the total volume of the stimulus mix to 100 mL, which was separated into five 20-mL aliquots. I used the same procedure to prepare a non-alarm control using the remaining cartilaginous spine and muscle tissue. A blank control was prepared by swirling a clean razor blade in 20 mL of dechlorinated tap water for 5 min. All cues and blank preparations were frozen at -20 °, and thawed to room temperature prior to each
trial; Rainbow Darters do not respond to frozen conspecific alarm cues. (R.J. F. Smith, personal observation, as cited in Chivers et al. 1995).

**Experimental Protocol.** Darters were tested from 5 - 15 July 2014. Darters were randomly assigned one of three treatments: salamander alarm cue, salamander non-alarm cue, or dechlorinated water blank. Set up of testing aquaria and experimental procedures were as in Experiment 1.

Latency to move was not measured in this experiment. The change in moves was not normally distributed; data were transformed using ARTool 1.5.1 (Wobbrock et al. 2011) and analyzed using generalized linear modeling (GLM) followed by post-hoc Tukey’s tests. This experiment was approved by the MSU IACUC (13-010.0 and 16-019.0).
RESULTS

Experiment 1: Response to Alarm Cues from Heterospecific Fishes

Treatment significantly influenced both latency to move ($F_{3,134} = 8.53$, $df = 3$, $p < 0.01$; Figure 1) and change in number of moves ($F_{3,135} = 6.33$, $p < 0.01$; Figure 2). Darters that received conspecific alarm cue exhibited significantly longer latencies to move than did darters exposed to mosquitofish or goby alarm cues ($p < 0.05$; Figure 1). Latency to move was not significantly different between darters that received conspecific or minnow alarm cues ($p > 0.05$; Figure 1), but these responses were significantly different from the increase in movement exhibited to mosquitofish or goby alarm cue ($p < 0.05$; Figure 2).

Experiment 2: Response to Alarm Cues from a Salamander

There was a significant main effect of population ($F_{1,64} = 7.46$, $p = 0.01$; Figure 3), with darters from the sympatric population showing an overall lower level of activity. Although treatment did not have a significant main effect ($F_{2,64} = 1.30$, $p = 0.28$; Figure 3) there was an interaction between population and treatment ($F_{2,64} = 4.93$, $p = 0.01$; Figure 3). Allopatric darters responded similarly to all three treatments ($p$’s > 0.05; Figure 3). Sympatric darters showed an average decrease in activity in response to the skin extracts, and this decrease was significantly different from the average increase in activity by allopatric darters to skin extracts ($p < 0.05$; Figure 3). Sympatric and allopatric darters did not differ in their response to the blank or non-skin controls ($p$’s > 0.05; Figure 3).
Figure 1: Latency to move (mean ± SE) by darters exposed to alarm cues from Bumblebee Gobies (Goby), Western Mosquitofish (Mosquitofish), Ozark Minnows (Minnow), or conspecific Rainbow Darters (Darter). Means that do not share a letter are significantly different.
Figure 2: Change in number of moves (mean ± SE) by darters exposed to alarm cues from Bumblebee Gobies (Goby), Western Mosquitofish (Mosquitofish), Ozark Minnows (Minnow), or conspecific Rainbow Darters (Darter). Means that do not share a letter are significantly different.
Figure 3: Change in number of moves (mean ± SE) by darters that were allopatric or sympatric with Oklahoma Salamanders to salamander alarm cue (skin), salamander muscle/bone/cartilage (non-skin control) and water (blank control). GLM statistics were calculated using align rank transformed data.
DISCUSSION

Experiment 1: Response to Alarm Cues from Heterospecific Fishes

Individuals should experience fitness benefits if they respond appropriately to alarm cues from conspecifs or from heterospecifics that are members of the same prey-guild. The results of this study confirmed the response to conspecific chemical alarm cues that has been reported in other studies (Commens & Mathis 1999; Crane et al. 2009; Gibson & Mathis 2006). Darters showed a stronger fright response (longer latency to move and fewer moves) to alarm cues from conspecifs than to alarm cues from other fish species (minnow, mosquitofish, goby). Reduced activity, which is a common fright response in darters (Commens & Mathis 1999) and other fishes (see review in Mathis 2009), effectively reduces the probability of detection by predators (Azevedo-Ramos et al. 1992; Skelly 1994). Although decreased activity can lead to increased survivorship when predation risk is high, it can result in lost feeding or reproductive opportunities (Lima & Dill 1990; Mathis 2009).

Darters frequently co-occur in mixed species aggregations with minnows in the Ozarks, where they are the two dominant fish types (Pflieger 1997). Although darters in this study responded the most strongly to conspecific alarm cues, they also significantly decreased activity in response to alarm cues from Ozark Minnows in comparison to cues from the other species. Rainbow Darters and Ozark Minnows clearly are members of the same prey-guild occurring in close proximity (benthic or near-benthic microhabitats: Becker 1983, Pflieger 1997), and sharing predators such as piscivorous fishes and wading birds. Therefore, darters should benefit by exhibiting antipredator behavior in response to
alarm cues from Ozark Minnows. Rainbow Darters also have been reported to respond to alarm cues from Fathead Minnows, *Pimephales promelas*, in a way that is consistent with a fright response (decreased activity) (Gibson & Mathis 2006). Chemical alarm cues are likely to be homologous across the Superorder Ostariophysi (Brown et al. 2003; Smith 1977, 1986), so if Rainbow Darters respond to alarm cues from one species it is not surprising that they would also respond to cues from related species. Darters have shared a long evolutionary history with Ozark Minnows (since the Illinoian glacial cycle, about 191,000—130,000 mya: Berendzen et al. 2010; Page 1983), allowing sufficient time for selection to have led to the acquisition of innate responses of darters to the minnow alarm cue. However, it is also possible that darters learn to respond to the minnow alarm cue. For example, Fathead Minnows that are allopatric with Brook Stickleback, *Culae inconstans*, can learn to respond to their alarm cues after associating with them (Pollock et al. 2003).

When exposed to invasive mosquitofish alarm cue, Rainbow Darters increased movement and exhibited a latency to move that was significantly shorter than latency to conspecific alarm cues. Increased activity is typically interpreted as an exploratory response rather than a fright response (Godin 1997; Wisenden et al. 1995). This lack of fright response to mosquitofish alarm cue by darters has several possible explanations. First, these species may not have co-existed in sympatry long enough for a fright response to have evolved. Western Mosquitofish have been introduced world-wide since the beginning of the twentieth century in efforts to control mosquitos and mosquito-borne illnesses (Pyke 2005). Originally, Western Mosquitofish were discovered in the US residing in drainages associated with the Mississippi river, as far north as Illinois (Jordan
et al. 1930). Pflieger (1997) noted that Western Mosquitofish may have been introduced to the Central Missouri region in 1944 from Michigan and that the species is increasingly more widespread since its introduction. Second, mosquitofish tend to occur in areas with little to no current in comparison to the darters’ preferred riffle habitats; Rainbow Darters may have little or infrequent contact with the alarm cues of Western Mosquitofish. Third, because mosquitofish tend to occupy the water column near the surface (Pflieger 1977), predation on mosquitofish is not a reliable indicator of risk for the benthic darters.

The failure of darters to respond with antipredator behavior to alarm cues from Bumblebee Gobies has been reported in previous studies (Commens & Mathis 1999; Commens-Carson & Mathis 2007, Gibson & Mathis 2006). No members of the Family Gobiidae co-occur with Rainbow Darters, and so there has been no opportunity for either evolved or learned fright responses to develop. As with mosquitofish alarm cues, Rainbow Darters increased movement when exposed to goby alarm cue, which is consistent with a foraging or exploratory response rather than fright (Godin 1997; Smith 1997; Wisenden et al. 1995). The result confirms that gobies are useful as a negative control in studies of darter alarm cues.

**Experiment 2: Response to Alarm Cues from Salamander**

In some areas, darters occur in the same prey-guild as Oklahoma Salamanders, and so may benefit by responding to the salamander alarm cues. Rainbow Darters from populations that are sympatric with the salamanders responded to salamander skin extract (containing alarm cue) with decreased movement in comparison to the controls of salamander muscle-tissue extract (no alarm cue) and a water blank. The decrease in
movement by sympatric darters is qualitatively the same as their response to conspecific alarm cue (Commens & Mathis 1999, this study). In contrast, darters allopatric with the salamander failed to distinguish between the salamander alarm cue and the control stimuli. Qualitatively, allopatric darters tended to increase movement following exposure to all three treatments.

Eavesdropping on the alarm cues of others has most often been documented between closely related species, such as fish responding to alarm cues of other fish (Brown et al. 2001; Brown & Godin 1997; Chivers & Smith 1994; Wisenden 2008), salamanders responding to alarm cues of other salamanders (Chivers et al. 1997; Sullivan et al. 2003), birds responding to alarm calls of other birds (e.g., Leger & Nelson 1982; Ridley et al. 2014), and mammals responding to alarm calls of other mammals (e.g., Fichtel 2004; Kirchhof & Hammerschmidt 2006). However, some species have also been shown to eavesdrop on the alarm cues of more distantly-related taxa. Magrath et al. (2015) examined responses to heterospecific calls by terrestrial vertebrates. Of the 37 responses to heterospecific alarm calls by birds, all but 2 were to calls of other birds. Similarly, of 26 responses to heterospecific alarm calls by mammals, all but 9 were to calls from other mammals. For amphibians, one species of terrestrial salamander, (Ozark Zigzag Salamander, *Plethodon angusticlavius*), has been reported to respond to alarm secretions of non-amphibians (earthworms, *Lumbricus* sp.: Crane et al. 2013). Currently, I am only aware of one other study in which fish (small sculpin, *Cottus carolinae*) show a cross-taxonomic response to a non-fish (amphibian alarm cue from hellbenders, *Cryptobranchus alleganiensis*) (Gall & Mathis 2011). My study adds a second example of a fish responding to a non-fish alarm cue.
Concluding

The response by Rainbow Darters to alarm cues of Ozark Minnows and Oklahoma Salamanders indicates the presence of intra-prey-guild eavesdropping for this species. Eavesdropping on the signals of other species likely provides a greater amount and broader range of information than can be obtained from conspecific signals alone (Seppänen et al. 2007). In addition to immediate benefits in terms of increased probability of survival, detecting alarm calls from heterospecifics can have longer-term benefits, potentially including gaining spatial information about danger, allowing for decreased per capita vigilance, and providing opportunities for learning about unfamiliar predators (Magrath et al. 2015). Overall, relatively few studies have documented responses to heterospecific alarm cues, particularly between species that are phylogenetically distant. However, given the potential fitness benefits to individuals that make maximal use of public information, this paucity of examples may be due to a lack of attention from researchers.
REFERENCES


Krumholz, L. A. 1948: Reproduction in the Western Mosquitofish, Gambusia affinis affinis (Baird and Girard), and its use in mosquito control. Ecol. Monogr. 18, 1—43.


Smith, R. J. F. 1979: Alarm reaction of Iowa and Johnny Darters (Etheostoma, Percidae, Pisces) to chemicals from injured conspecifics. Canadian Journal of Zoology 57, 1278—1282.


Wisenden, B. D., Chivers, D. P., Brown, G. E., & Smith, R. J. F. 1995: The role of
everience in risk assessment: Avoidance of areas chemically labelled with
Fathead Minnow alarm pheromone by conspecifics and heterospecifics.
Écoscience 2, 116—122.

Transform for nonparametric factorial analyses using only ANOVA
procedures. Proc ACM Conf Hum Comp Sys (CHI ’11). New York: AMP

Woody, D. R., & Mathis, A. 1997: Avoidance of areas labeled with chemical stimuli
from damaged conspecifics by adult newts, Notophthalmus viridescens, in a
natural habitat. J. Herpetol. 31, 316—318.

Zuluaga, G. J. C. 2013: Why animals come together, with the special case of mixed-