



Olfactory cues of risk and visual cues of safety interact with sympatry and phylogeny in shaping behavioral responses by littoral fishes

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Abstract

Prey incorporate information about risk, safety, and the reliability of both of these cues when assessing risk of predation. Here, we report results of an experiment testing avoidance of chemical alarm cues derived from skin extract of blacknose shiners, *Notropis heterolepis* (BNS), attraction toward a visible shoal of five BNS, and the combination of both, on fishes in Deming Lake where BNS occur. We then repeated the experiment in nearby Budd Lake where BNS do not occur. BNS avoided traps with conspecific alarm cues but did not respond to the presence of BNS shoals. We recorded responses by similar-sized heterospecifics that share predators with BNS to see if they respond to BNS as indicators of risk and safety. Fathead minnows, *Pimephales promelas* (FHM) avoided traps chemically labeled with BNS alarm cue when a shoal was absent and avoided BNS shoals when traps were labeled with water (control). When both BNS alarm cue and BNS shoal were combined, FHM antipredator response to BNS alarm cue invoked a shoaling response with the BNS shoal in the trap. The more distantly related redbelly dace, *Chrosomus eos* (RBD), responded weakly to BNS cues in Deming Lake and ignored them in Budd Lake. Non-cyprinid species (brook stickleback *Culaea inconstans* and pumpkinseed sunfish, *Lepomis gibbosus*) did not respond to either olfactory or visual cues of BNS in Deming Lake. In Budd Lake, neither avoidance of BNS alarm cue or attraction to BNS shoals surpassed the threshold of statistical significance, indicating a role of ecological familiarity and learned recognition of risk and safety in cross-species reactions to olfactory and visual cues of heterospecifics.

Significance statement

Small fish, such as minnows, avoid areas marked by injury-released chemical cues because these cues are released only in the context of predation and are attracted to groups of fish because shoals offer safety from predation. In this study fathead minnows *Pimephales promelas* (FHM) avoided alarm cue of blacknose shiners *Notropis heterolepis* (BNS) in Deming Lake where both species occur but not in Budd Lake where BNS do not occur. FHM were not attracted to BNS shoals in either lake. However, when a shoal of BNS was combined with BNS alarm cue a synergism occurred where antipredator responses to BNS alarm cue induced FHM to seek safety by shoaling with BNS shoals. The synergism occurred in Deming Lake where BNS alarm cue and shoals are ecologically familiar but not in Budd Lake where BNS do not occur. These data show that behavioral responses by prey to olfactory and visual cues are tempered by sympatry (ecological familiarity) and confidence in the reliability of those cues.

Keywords Chemical alarm cues · Shoaling · Risk assessment · Information reliability · Predator–prey · Fathead minnows

Introduction

Navigating the predation landscape while simultaneously solving ecological imperatives such as foraging, reproduction, and other activities linked to fitness presents many tradeoffs (e.g., Lima and Dill 1990; Gaynor et al. 2019). This is made

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especially complex because risk of predation varies spatially across a species' geographic range, and varies temporally over daily, seasonal, and evolutionary time scales. Further, predation risk shifts dynamically with ontogenetic development of prey and predators (Dall et al. 2005; Dall 2010) as reflected in intra-guild predation (Henkanaththegedara and Stockwell 2014).

In aquatic habitats, risk of predation is often detected by chemical cues that reliably indicate the presence of a predator (Ferrari et al. 2010; Wisenden 2015). Aquatic animals rely on semiochemicals because water is an excellent solvent, chemical cues are detectable in highly structured or turbid habitats and chemical information is retained in the water for ecologically relevant durations of time. There is an extensive literature detailing ways in which small fishes detect and respond to chemically-mediated indicators of predation risk (Ferrari et al. 2010). Most of this work has been done with behavioral responses to chemical alarm cues, i.e., chemical compounds released from epidermal tissue damaged by predators during an attack. These alarm chemicals are released in no other context making them a reliable indicator of imminent risk. In the field, fishes avoid the area where cue is detected (Mathis and Smith 1992; Friesen and Chivers 2006; Wisenden and Barbour 2005), the area avoided is between 2 and 8 m (Wisenden 2008) and avoidance of chemically-labeled areas lasts for 2–4 h (Wisenden et al. 1994, 1995, 2009).

Fish use multiple sensory modalities for risk assessment. For example, visual and chemical indicators of risk produce an additive assessment of risk for free-swimming natural populations of blacknose shiners *Notropis heterolepis*. Area avoidance of equal intensity occurred after either release of conspecific alarm cue or the visual presentation of a model predator (Wisenden et al. 2004). When both the model and alarm cues were presented simultaneously, the response was greater than when either visual or olfactory cues were presented alone (Wisenden et al. 2004).

There is a large literature devoted to ways in which prey detect alarm cues that indicate when and where risk of predation is high (e.g., Ferrari et al. 2010). Equally, or more important, detecting low predation risk allow prey to safely engage in other life tasks that contribute to fitness (Luttbeg et al. 2020). Because small fishes occupy structureless habitats such as the open water column, they seek shelter in each other and form shoals (Krause and Ruxton 2002). When risk of predation is detected, fish shoal more cohesively (Keenleyside 1955; Hamilton 1971; Krause and Ruxton 2002; Schaerf et al. 2017) and shoal with conspecifics or heterospecifics (Pollock et al. 2006). When a small shoal of conspecifics or heterospecifics is experimentally added to a minnow trap, they indicate safety and attract other fish to join the shoal in the trap. Wisenden et al. (2003) conducted a field experiment with minnow traps to examine the interactive effects of chemical alarm cues that indicate risk with the visual presence of a shoal that indicates safety. For traps

that did not contain a shoal (an empty jar), traps labeled with water caught more fathead minnows *Pimephales promelas* and more northern redbelly dace *Chrosomus eos* than traps labeled with alarm cue derived from the skin of fathead minnows. For traps chemically labeled with water, traps containing captive shoals of fathead minnows caught more fish than traps seeded with an empty jar. When both chemical alarm cue and the captive shoal were presented, a synergism occurred. Fish were induced by alarm cue to engage in antipredator behavior, and rather than flee the area (avoid the trap) they instead engaged in a shoaling response and entered the trap to join the captive shoal even though the trap was itself the source of alarm cue (Wisenden et al. 2003).

Behavioral responses could, however, be contingent on whether the cues are novel. Information uncertainty mediates intensity of interspecific interactions and behavioral responses to indicators of risk (Dall et al. 2005; Dall 2010; Johnson et al. 2013). The risk of failing to respond to an unknown novel stimulus increases with uncertain information about that stimulus. For example, tadpoles had reduced neophobic responses after being exposed to consistent kairomones cues from a familiar predator (Ferrari et al. 2018). Although fishes generally prefer to shoal with conspecifics than with heterospecifics, this can be overcome if the heterospecifics are familiar individuals (Ward et al. 2003). Responses to heterospecific chemical alarm cues is socially-mediated by the presence of conspecific and heterospecific shoals. For example, anemone fishes *Amphiprion percula* adjust the response to alarm cues of conspecifics and alarm cues of its congener *A. melanopus* depending on whether or not they are in the presence of a shoal of conspecifics or a mix-species shoal that includes both conspecifics and *A. melanopus* individuals (Manassa et al. 2013). Behavioral reactions to alarm cue from either *A. percula* or *A. melanopus* were muted when accompanied by a shoal of *A. percula*. However, responses to heterospecific alarm cue derived from *A. melanopus* were reduced in the company of a shoal of *A. percula* but not when in the company of a shoal of *A. melanopus* (Manassa et al. 2013). Guppies *Poecilia reticulata* increase latency to approach a novel predator if the visual presentation of the predator is paired with a novel chemical cue, increasing information uncertainty (Feyten et al. 2019).

Here, we report responses by wild free-swimming littoral community of prey fishes to chemical and visual stimuli of blacknose shiners *Notropis heterolepis* (BNS). We predicted that blacknose shiners should avoid conspecific alarm cue, be attracted to shoals of blacknose shiners, and demonstrate a synergistic attraction to the combination of alarm cue and conspecific shoal. Other taxonomically-related and ecologically-similar species such as fathead minnows (FHM) and redbelly dace (RBD) are similar in size, occupy similar habitat and presumably are vulnerable to the same suite of predators as blacknose shiners. Therefore, we predicted that FHM and RBD should avoid BNS-derived chemical indicators of risk,

be attracted to a heterospecific shoal of BNS, and may also exhibit a synergistic response to the combination of BNS alarm cue and a BNS shoal. We predicted that that responses by other fishes such as brook sticklebacks and pumpkinseed sunfish would be muted due to their antipredator morphology (spines).

To test for the effects of sympatry (= ecological familiarity) and information uncertainty on response intensity to chemical cues of risk and visual cues of safety from heterospecifics, we repeated our experiment using BNS cues in a nearby lake, which contains FHM and RBD but lacks BNS. We asked if FHM and RBD in the second lake, that have never been exposed to BNS alarm cue or the visual presence of BNS shoals for at least 20 years (BDW, pers. obs.), would recognize and avoid BNS alarm cue, be attracted to a shoal of allopatric BNS heterospecifics, or exhibit a synergistic response when these stimuli were combined.

Materials and methods

Two study sites were selected for this experiment, Deming Lake (47.170183, -95.168064, surface area approximately 5.5 ha) and Budd Lake (47.166725, -95.174828, surface area approximately 3 ha), both within the boundaries of Itasca State Park, Minnesota, USA. Both lakes are meromictic and devoid of large piscivorous fish species because conditions of low dissolved oxygen during prolonged periods of ice cover are lethal to large-bodied fishes (Tonn and Magnusson 1982). Fish species composition of Deming Lake varies over time due to stochastic colonization events followed by extirpation by winter kill. At the time of this study (May–June 2022), Deming Lake contained northern redbelly dace (RBD, *Chrosomus eos*), fathead minnows (FHM, *Pimephales promelas*), blacknose shiners (BNS, *Notropis heterolepis*), pumpkinseed sunfish (PKS, *Lepomis gibbosus*), brook stickleback (BSB, *Culaea inconstans*), golden shiners (*Notemigonus crysoleucas*), Iowa darters (*Etheostoma exile*) and black bullhead catfish (*Ameiurus melas*). BNS were observed in Deming Lake in 2001 and 2002, were absent from catch records from 2006–2014, but have been present in the lake since 2015. Catch data from 2003–2022 in Budd Lake have consistently contained only two fish species, fathead minnows and northern redbelly dace.

Preparation of alarm cue

Alarm cue was prepared in two batches. The batch used in Deming Lake was prepared from BNS collected by seine net from Deming Lake and transported to the University of Minnesota Itasca Biological Field Station. Fish were euthanized by an overdose of methane tricaine sulfonate (University of Minnesota IACUC protocol 2103-38900A).

Epidermal tissue was dissected from each flank of 30 blacknose shiners (mean \pm SE TL = 56.8 ± 0.6 mm, $n = 30$),

length and width of each section of skin measured and then placed in a beaker containing reverse-osmosis de-ionized (RODI) water on a bed of ice to minimize biochemical degradation during cue preparation. A total of 89.5 cm² of skin was collected. Epidermal tissue was homogenized using a hand blender for 60 s, filtered through gauze to remove sheets of connective tissue and diluted with RODI water to a final volume of 640 mL. Alarm cue was infused into 32 blocks of cellulose sponge (approximate dimensions of 47.5 \times 36.3 \times 50 mm). Each sponge received 20 mL, equivalent to 2.80 cm² of skin. Previous field studies in Deming Lake have demonstrated that this method of presenting skin extract creates an active space with a radius greater than 2 m but less 8 m (Wisenden 2008) and persists for more than 3 h but less than 6 h (Wisenden et al. 2009). We prepared 32 additional blocks of sponge and infused them with 20 mL of RODI water as a control. All sponge blocks were frozen at -20 °C until needed. The sponges used for Budd Lake were prepared a week later, using BNS seined from Deming Lake. A total of 24 BNS (TL = 56.8 ± 0.6 mm, $n = 24$) were euthanized and skin harvested as described above. A total of 92.1 cm² of skin was used to prepare BNS alarm cue, which was aliquoted into 20 mL infusions into 32 sponges, equivalent to 2.87 cm² of skin per sponge. We made 32 sponges with RODI water to be used as controls. Sponges were frozen at -20 °C until needed.

Testing protocol

Gee's minnow traps (43 cm long, 22 cm in diameter, Fig. 1) were set from shore at a depth of approximately 1 m spaced apart by approximately 10 m. There were four treatments in a 2 \times 2 factorial design; (1) BNS alarm cue sponge affixed to the inside of each trap by a short piece of stainless-steel wire + 5 BNS in a 973 mL jar with the lid replaced with mosquito window screen, (2) Water sponge + 5 BNS in a 973 mL jar, (3) BNS alarm cue sponge + a water-filled jar without fish, (4) water sponge + empty jar. BNS used for the shoals were the same stock collected by seine from Deming Lake to make alarm cue. We set traps in blocks of four traps representing one of each treatment type, waited 5 min, then set the next block of 4 traps, and so on until 16 blocks of four had been set for a total of 64 traps (Deming Lake) or 15 blocks of traps for a total of 60 traps (Budd Lake). Each block of traps was pulled after 120 min and the fish captured were sorted by species, counted, and released back into the lake. Processing a trap required about 5 min, so that each successive block of traps was pulled after approximately 120 min of fishing time. When large numbers of fish were captured that required more than 5 min to process, we delayed pulling the next block of traps so that any delays in processing affected all treatments equally. It was not possible to record data blind because our study required careful labeling of each trap location in the field.



Fig. 1 One of the minnow traps used in this study containing a block of sponge attached with a short piece of wire and a jar with window-screen lid used for holding a shoal of five blacknose shiners

Data analysis

Number of fish caught were not normally distributed (Kilgore-Smirnov tests $P < 0.05$) therefore catch data were ranked within species within each lake. That is, within each lake and for each species, the trap that caught the fewest number of fish received a rank of 1, the trap that caught the second-most fish received a rank of 2, and so on, up to the total number of traps in the lake (64 in Deming and 60 in Budd). Treatments were compared by factorial ANOVA using SPSS v26 (Brunner et al. 2017). We used the phyloT tool based on NCBI Taxonomy (Madeira et al. 2019) to examine the phylogenetic relationships of blacknose shiner to the five other species sampled in this study.

Results

In Deming Lake, mean \pm SE fishing time was 123 ± 0.45 min, in which time 64 traps caught 965 FHM, 2549 RBD, 185 BNS, 273 PKS, 68 BSB and one Iowa darter

(total = 4041 fish). In Budd Lake, mean \pm SE fishing time was 141 ± 2.93 min, in which time 60 traps caught 7591 FHM and 3379 RBD (total = 10,970 fish).

Blacknose shiners

In Deming Lake, there was a significant effect of BNS alarm cue on the number of BNS caught per trap ($F_{1,60} = 4.185$, $P = 0.045$) but there was no effect of the presence of a shoal of BNS in a jar ($F_{1,60} = 2.552$, $P = 0.115$) nor was there an interaction between sponge cue and jar treatment ($F_{1,60} = 0.393$, $P = 0.533$; Fig. 2).

Fathead minnows

The number of FHM caught per trap showed a 3-way interaction among sponge x jar x lake ($F_{1,116} = 5.753$, $P = 0.018$; Fig. 3). Within Deming Lake, there was a significant interaction between sponge and jar treatments in the number of FHM caught per trap ($F_{1,60} = 10.664$, $P = 0.002$). For traps

containing an empty jar, traps labeled with BNS alarm cue caught significantly fewer FHM than traps labeled with water ($t = 2.754$, $df = 30$, $P = 0.010$). When a shoal of BNS was present, there was a weak but statistically non-significant tendency to catch more FHM in traps labeled with BNS alarm cue than in traps labeled with water ($t = 1.863$, $df = 30$, $P = 0.072$). For traps chemically labeled with water sponges, traps containing a shoal of BNS caught significantly fewer FHM than traps containing an empty jar ($t = 2.36$, $df = 30$, $P = 0.025$). For traps chemically labeled with BNS alarm cue, traps containing a jar shoal caught significantly more FHM than traps

containing an empty jar ($t = 2.258$, $df = 30$, $P = 0.031$). In Budd Lake, the number of FHM caught per trap showed a weak but statistically non-significant avoidance response to BNS alarm cue ($F_{1,56} = 3.068$, $P = 0.085$), no response to BNS shoals in jars ($F_{1,56} = 0.192$, $P = 0.663$) and there was no interaction between the two treatments ($F_{1,56} = 0.093$, $P = 0.762$).

Redbelly dace

Overall, there was no effect of lake ($F_{1,120} = 2.409$, $P = 0.123$), BNS alarm cue ($F_{1,120} = 1.470$, $P = 0.228$) or BNS shoal ($F_{1,120} = 0.981$, $P = 0.324$) on the number of RBD caught per trap (Fig. 4). Within Deming Lake, there was a weak but statistically non-significant interaction between sponge and jar treatments ($F_{1,60} = 2.908$, $P = 0.093$), consistent with a synergism between treatment cues as seen in FHM. There was no effect of chemical cue ($F_{1,60} = 0.014$, $P = 0.840$) or shoal ($F_{1,60} = 0.445$, $P = 0.507$). Within Budd Lake, the effect of BNS alarm cue ($F_{1,56} = 0.041$, $P = 0.840$), BNS shoal ($F_{1,56} = 0.044$, $P = 0.834$) and their interaction ($F_{1,56} = 0.361$, $P = 0.550$) were all non-significant.

Pumpkinseed sunfish and brook stickleback

There was no effect of BNS alarm cue or BNS shoal on catch numbers for the two non-cyprinid fish species in Deming Lake (PKS Alarm Cue $F_{1,60} = 0.028$, $P = 0.868$; Shoal $F_{1,60} = 0.156$, $P = 0.695$; Cue*Shoal $F_{1,60} = 1.448$, $P = 0.234$; and BSB Alarm Cue $F_{1,60} = 1.209$, $P = 0.276$; Shoal $F_{1,60} = 2.438$, $P = 0.124$; Cue*Shoal $F_{1,60} = 1.859$, $P = 0.178$; Fig. 5).

Heterospecific responses to BNS alarm cue and BNS shoals was correlated with phylogenetic distance (Fig. 6). BNS, FHM and RBD are all in the family Cyprinidae (the minnows), superorder Ostariophysi, and form a cluster that is distinct from PKS and BSB, which are in the superorder Acanthopterygii. Within the Cyprinidae, FHM and BNS are more closely related to each other compared to their relationships to RBD (Fig. 6).

Discussion

BNS avoided alarm cue from their own species consistent with observations from an earlier study with this species (Wisenden et al. 2004). We did not observe a statistically detectable attraction to a shoal of conspecifics in either the presence or absence of conspecific alarm cue even though the treatment means trended in this direction. The shoaling

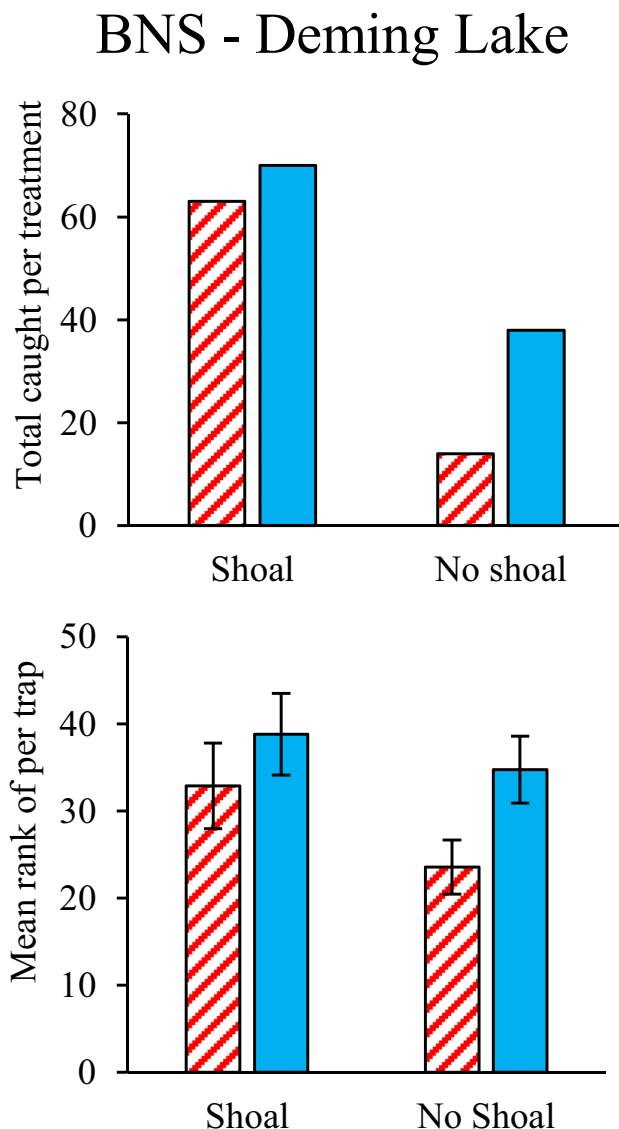


Fig. 2 Total number caught (upper) and mean \pm SE rank (lower) of blacknose shiners in Deming Lake. Traps were labeled chemically with either water (blue fill) or alarm cue (red hatched fill) from blacknose shiners, and visually with either a shoal of five blacknose shiners or an empty jar

Fig. 3 Total number caught (upper) and mean \pm SE rank (lower) of fathead minnows in Deming Lake (left panel) and Budd Lake (right panel) for traps labeled chemically with either water (blue fill) or alarm cue (red hatched fill) from blacknose shiners, and visually with either a shoal of five blacknose shiners or an empty jar

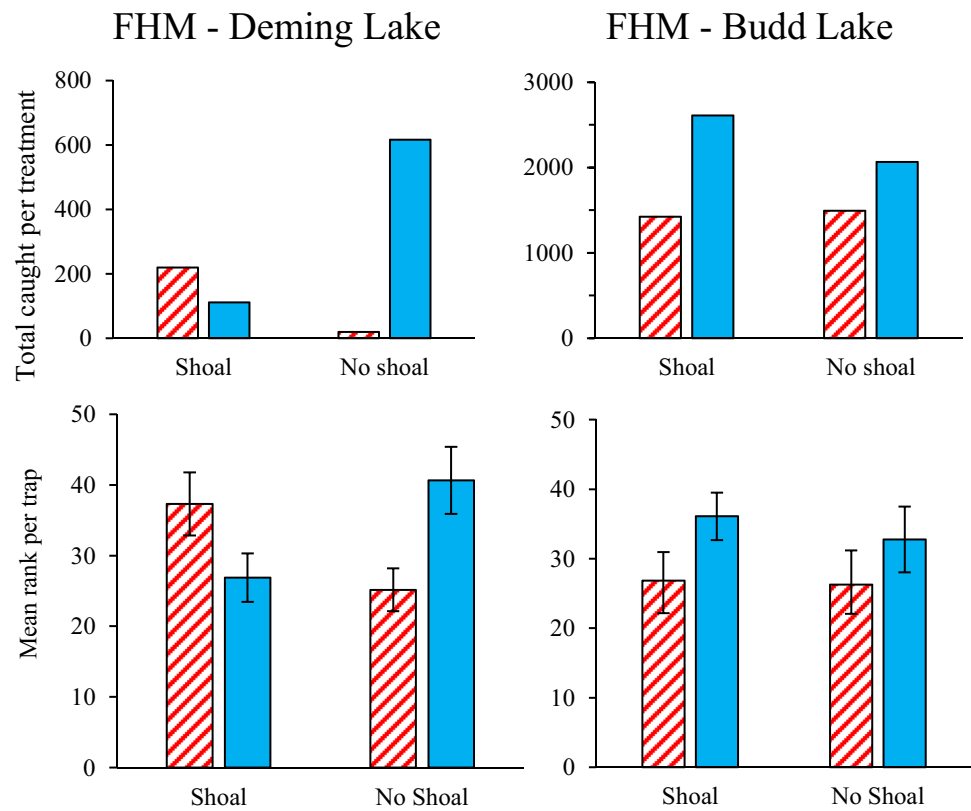


Fig. 4 Total number caught (upper) and mean \pm SE rank (lower) of redbelly dace in Deming Lake (left panel) and Budd Lake (right panel) for traps labeled chemically with either water (blue fill) or alarm cue (red hatched fill) from blacknose shiners, and visually with either a shoal of five blacknose shiners or an empty jar

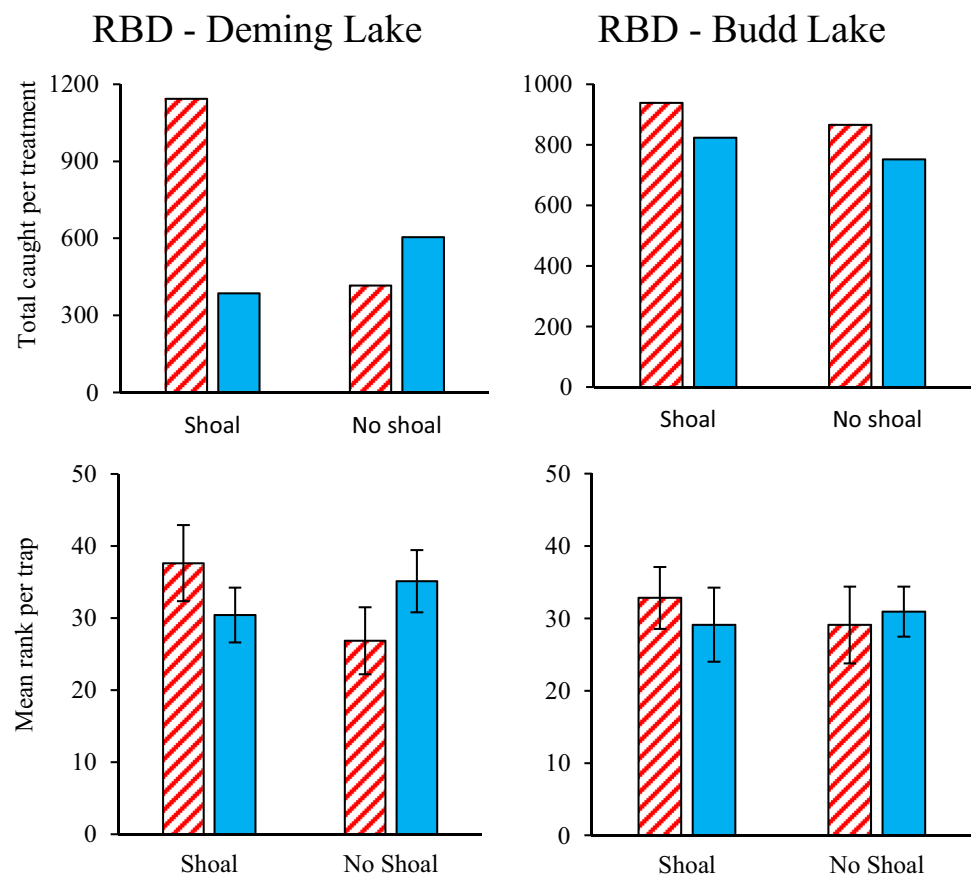


Fig. 5 Total number caught (upper) and mean \pm SE (lower) for pumpkinseed sunfish and brook stickleback in Deming Lake for traps labeled chemically with either water (blue fill) or alarm cue (red hatched fill) from BNS, and visually with either a shoal of five BNS or an empty jar

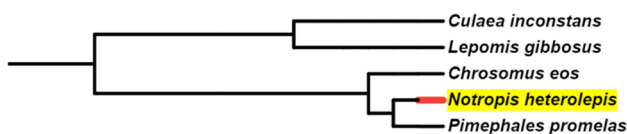
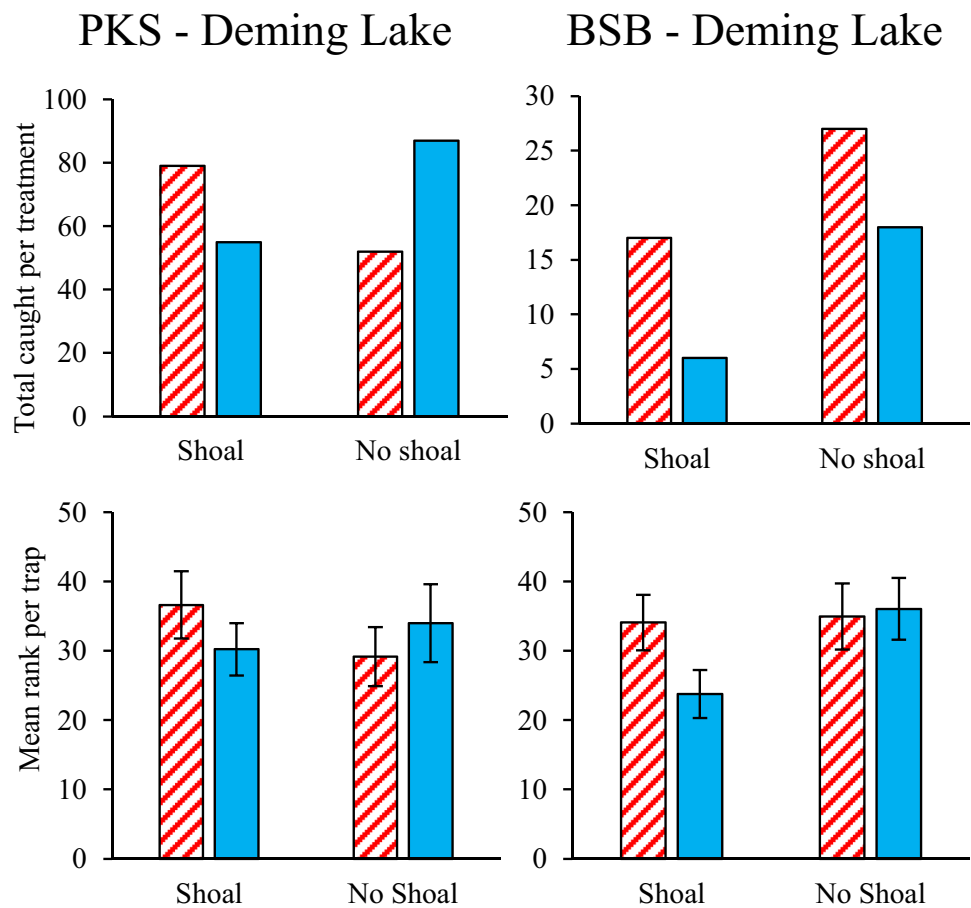


Fig. 6 A phylogenetic tree of fish species captured in Deming Lake. Brook stickleback *Culaea inconstans*, pumpkinseed sunfish *Lepomis gibbosus*, northern redbelly dace *Chrosomus eos*, blacknose shiner *Notropis heterolepis* (highlighted species), fathead minnows *Pimephales promelas*

response in BNS may not be as strong as it is in other species such as FHM.

Fathead minnows avoided BNS alarm cue in Deming Lake, where BNS occur, but only weakly avoided BNS alarm cue in Budd Lake where BNS are absent. In Deming Lake, FHM avoided BNS shoals when alarm cues were absent but FHM were attracted to BNS shoals when BNS alarm cue was present. Therefore, FHM were able to detect and recognize the significance of BNS alarm cue in Deming Lake because BNS were sympatric and therefore an ecologically familiar heterospecific, but FHM did not respond to BNS cues in Budd Lake where alarm cue from BNS is novel. The responses by FHM in Deming Lake

corroborate the synergistic interaction between chemical indicators of risk and visual indicators of safety first observed in Deming Lake by Wisenden et al. (2003). When FHM perceived risk of predation via a heterospecific alarm cue, they sought safety by joining the shoal of BNS inside the trap even though the trap was itself the source of alarm cue. In Budd Lake, FHM gave no evidence of attraction to, or avoidance of, BNS shoals under any context.

The absence of an attractive effect of BNS shoals in the absence of alarm cues in Deming Lake was contrary to our expectation based on earlier work that showed that FHM and RBD were attracted to shoals of FHM (Wisenden et al. 2003). BNS have been in Deming Lake in catchable numbers for at least seven years before this study was conducted, therefore all FHM and RBD in Deming Lake were likely familiar with BNS. Further, BNS and FHM are similar enough in size and external appearance that students need to be trained to distinguish them. Ecological familiarity, and similarity of size and morphology can favor heterospecific shoaling even if there are small differences in behavior and ecology. For example, when Chinese bream *Parabramis pekinensis* and qingbo *Spinibarbus sinensis* form heterospecific shoals, each species shifts their preferred swimming speed and inter-individual distances to create a homogenous shoal (Tang et al.

2017). On the other hand, antipredator benefits of homogeneity in size, appearance and behavior are known to favor shoals assorted by species, and body size within species (Krause et al. 1996; Pajmians et al. 2019). It is only during times of heightened risk (as indicated by the presence of alarm cue) that antipredator benefits of shoaling, even with heterospecifics, leads to the formation of heterospecific shoals (Krause and Godin 1994; Wisenden et al. 2003).

There are two factors that likely contribute to cross-species reactions to heterospecific alarm cue: shared and conserved biochemistry of cue due to close phylogenetic relatedness and learned responses of heterospecific members of the same prey guild. Both of these mechanisms rely upon the evaluation of external cues as indirect assessment of risk (Dall et al. 2005).

Experimental tests of naïve test subjects indicate that the strength of reactivity is inversely proportional to phylogenetic distance between two species (e.g., Schutz 1956) suggesting that alarm cue comprises multiple biochemical components, some shared among sister taxa and others unique to each species (e.g., Mirza and Chivers 2001a; Ferrari et al. 2007; Hume and Wagner 2018). The biochemistry of fish alarm cues remains woefully understudied. There is evidence that components of cyprinid alarm cue may include chondroitin sulfate (Mathuru et al. 2012; Farnsley et al. 2016; Faulkner et al. 2017), hypoxanthine-3N-oxide (Pfeiffer et al. 1985; Brown et al. 2000, 2001; Parra et al. 2009), polypeptides (Kasumyan and Lebedeva 1979), and may or may not include components within epidermal club cells (Pfeiffer 1977; Chivers et al. 2007; Carreau-Green et al. 2008; Pandey et al. 2021) or the microbiome associated with the skin surface (Chia et al. 2019). Whatever these components may be, it is plausible that the biochemistry of BNS alarm cue would share some critical properties or perhaps in similar ratios, with alarm cue of other minnow species such as FHM and RBD.

Indeed, avoidance of heterospecific alarm cue in this study was correlated with phylogenetic relatedness (Fig. 6). The Ostariophysan cyprinid lineages for FHM, BNS and RBD share a common ancestor approximately 34 mya (Stout 2017) whereas the Acanthopterygii that include sunfish and stickleback diverged from the Ostariophysi approximately 290 mya (Steinke et al. 2006). In Deming Lake, avoidance of BNS alarm cues was observed for both conspecifics (BNS) and FHM, weakly for RBD, but absent in the non-cyprinid species in this study. The weak response by RBD is correlated with its more distant phylogenetic relationship to BNS relative to the relationship between FHM and BNS. However, avoidance of BNS alarm cue was not observed for FHM in Budd Lake, suggesting that ecological familiarity is important for recognizing and responding to heterospecific alarm cues. This finding suggests that phylogenetic proximity is necessary but not sufficient for alarm cue responses.

Thus, the findings may reflect phylogenetic inertia in the evolution of the production and/or cross-species detection of chemical alarm cues. This hypothesis would suggest graded responses and would require more fine scale sampling of other minnow species such as congeners of BNS.

Recognition of BNS alarm cue by FHM in Deming Lake is an example of a cross-species response to alarm cue of a heterospecific within the same prey guild, i.e., another species of similar size, habitat and presumably vulnerable to similar predators. Thus, alarm cue of any species within a prey guild indicates the presence of risk to any and all members of the prey guild (e.g., Pollock et al. 2003; Wisenden et al. 2003; Friesen and Chivers 2006; Wisenden and Barbour 2005; Wisenden 2008; Anderson and Mathis 2016). These cross-species reactions are acquired through associative learning by pairing conspecific alarm cue with heterospecific alarm cue in a predator with a mixed diet (Mirza and Chivers 2001b) or by associating novel heterospecific alarm cue with a known predator (Chivers and Mirza 2003).

The weak avoidance of novel BNS alarm cue by FHM in Budd Lake suggests that BNS alarm cue may have been recognized by FHM as a weak or uncertain indicator risk (Dall 2010; Ferrari et al. 2016; Feyten et al. 2021; Crane et al. 2022; Johnson et al. 2023). Because RBD did not respond to visual or chemical cues from BNS in either lake, it may be that the threshold for behavioral responses to indicators of predation risk are lower in FHM than they are in RBD, i.e., there are species differences in responses to information uncertainty about risk.

Measuring dynamic social interactions among sympatric species in a natural field setting is complex because most traps with empty jars contained a multi-species “shoal” by the end of the two-hour fishing time. Individual decision-making is based upon information gathered directly from sensory receptors and information gathered indirectly cued by the behavior of others (Dall et al. 2005). From our data, it is not possible to determine the effect, if any, that one species (e.g., FHM) may have had on responses by other species (e.g., RBD). Differences among treatment groups detected and reported here likely reflect effects that were most pronounced soon after each trap was set, and dissipated over time. The muted response of FHM and RBD to BNS-based cues in Budd Lake may, in part, reflect that the high density of fish in Budd Lake partially swamped the effects of chemical and visual indicators of risk and safety. Be that as it may, these data were collected from free-swimming wild fish under natural field conditions. The temporal dynamics of navigating a landscape of risk may be shorter in the context of dense prey populations, and vary in response to abiotic conditions of such as light, temperature, turbidity, and photoperiod, which may all differ from species to species. Future work on the role of information uncertainty on behavioral decision making could try to isolate and control the influences of some of these other factors.

This study provides corroborating evidence for a behavioral synergism by fathead minnows between the aversive effect of alarm cue, and the attractive effect of the safety represented by a shoal, when the alarm cue and shoal are derived from a sympatric heterospecific (Deming Lake). When those olfactory and visual cues are from an allopatric heterospecific, as was the case in Budd Lake, neither cue had much effect, suggesting a role of experience and acquired recognition of these cues of both threat and safety in the cognitive ecology of fathead minnows. Other small littoral fishes at the study site did not show these responses suggesting that fathead minnows may process chemical and visual information about predation risk with a greater degree of sensitivity to information uncertainty than other species do.

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Data availability https://datadryad.org/stash/share/9gUG3yDqxV0Cd46kMmi5TqwF_zeN7JQGDk9T0PzLL6k.

Key to variables in dataset: Deming1Budd2=source lake of catch data, SpongeAlarm1Water2=chemical content of sponge, JarShoalYes1No2=whether the jar contained a shoal or not, Set=replicate set within each lake, FHM=number of fathead minnows per trap, FHM-rank=rank of fathead minnow catch per trap within lake, RBD=number of northern redbelly dace per trap, rbdrank=rank of redbelly dace catch per trap within lake, BNS=number of blacknose shiners per trap, bnsrank=rank of blacknose shiner catch per trap in Deming Lake, PKS=number of pumpkinseed sunfish per trap, pksrank=rank of pumpkinseed sunfish per trap in Deming Lake, Stickleback=number of brook stickleback per trap, sbrank=rank of stickleback per trap in Deming Lake.

Declarations

Ethics approval All applicable international, national, and/or institutional guidelines for the use of animals were followed. All protocols used in this study were reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee, protocol 2103-38900A.

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