



Original Article

Niche separation, ontogeny, and heterospecific alarm responses in centrarchid sunfish

Jigang Xia,^{a,•} Chris K. Elvidge,^{b,•} and Steven J. Cooke^b

^aLaboratory of Evolutionary Physiology and Behavior, Chongqing Key Laboratory of Animal Biology, College of Life Sciences, Chongqing Normal University, Chongqing 401331, China, and ^bFish Ecology and Conservation Physiology Laboratory, Department of Biology and Institute of Environmental Science, Carleton University, 1125 Colonel By Drive, Ottawa, ON K1S 5B6, Canada

Received 14 December 2017; revised 20 March 2018; editorial decision 22 March 2018; accepted 4 April 2018; Advance Access publication 19 April 2018.

Behavioral responses to alarm cues in aquatic species are typically examined with emphasis on the potential survival benefits accrued by conspecific receivers. By contrast, heterospecific responses to alarm cues and changes in responses with ontogeny in fishes are relatively unexplored. Taking an ecological niche perspective, we hypothesized that the response patterns of fish to risky chemical cues should be closely related to their degree of niche differentiation, which increases with ontogeny. We tested this hypothesis using the responses of adults from sympatric bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) populations to the alarm cues of conspecific and heterospecific adults and juveniles, including water as a control treatment. Responses measured consisted of changes in body posture (time spent with the dorsal fin <30°, between 30° and 60°, or >60°) and behavior (times spent still, frozen, at the surface, or on the bottom of the tank). Both adult bluegill and pumpkinseed spent significantly more time with their fins held >60° in response to adult versus juvenile alarm cues, with these responses mediated by donor species such that adult conspecific cues resulted in greater responses than heterospecific cues. The same general pattern was observed in the behavioral measures. These results demonstrate that behavioral response patterns to chemical alarm cues in sunfishes are highly plastic and are likely related to niche separation in adults. Our findings open new lines of research into the role of ecological niches in shaping behavioral responses of fish to risky information.

Key words: antipredator behavior, alarm cues, chemical information use, niche shifts.

INTRODUCTION

Chemical information is pervasive and a dominant form of communication among animals in aquatic systems (Hara 1992; Ferrari et al. 2010; Elvidge and Brown 2015). Chemical cues are particularly effective and reliable means to acquire information regarding immediate predation threats (Chivers and Smith 1998). An increasing number of aquatic organisms from a range of taxa have been shown to display risk recognition to chemical alarm cues from injured conspecifics (Chivers and Smith 1998; Wisenden 2000; Spivey et al. 2015; Lienart et al. 2016). In teleost fishes, epidermal damage-released chemical cues (alarm cues) are passively released following mechanical damage as would likely occur during a predation event, and therefore provide a reliable indicator of immediate predation risk (Wisenden and Millard 2001; Xia et al. 2017).

Notwithstanding that strong responses to chemical alarm cues are likely to accrue survival benefits for prey (Ferrari et al. 2010), adopting antipredator behaviors incurs some cost stemming from concurrent decreases in other fitness-related activities such as foraging and reproduction (Lima and Dill 1990; Skelly 1992; Brown and Smith 1996; Jones and Godin 2010; Spivey et al. 2015). Previous studies have suggested that guppies (*Poecilia reticulata*), in the first week after birth, did not exhibit risk recognition when exposed to chemical cues from conspecifics of the same size class (e.g. Xia et al. 2017). The absence of risk recognition in newly emerged larvae was suggested to reflect conflicting energy requirements as maximizing growth and development via foraging appeared to trump risk assessment (Xia et al. 2017). In the context of predator avoidance, ability to accurately assess the level of local predation risk is critical for prey. Since antipredator responses come with costs stemming from foregoing other activities, these responses should only be used when the organism perceives a threat of predation (Friesen and Chivers 2006). Optimal solutions to these cost-benefit trade-offs may drive selection

Address correspondence to J. Xia. E-mail: jigangxia@163.com.

on the opportunistic use of public, social information (Seppänen et al. 2007; Elvidge et al. 2010). Thus, how fish respond to chemical information from heterospecifics is an important ecological issue worthy of attention. For example, are there differences in antipredator behavior in response to conspecific versus heterospecific alarm cues? And, do these responses vary with ontogenetic shifts?

Generally, behavioral responses to alarm cues in aquatic species are examined with emphasis on the potential survival benefits accrued by conspecific receivers, in accordance with the hypothesis of taxonomic cue conservation and the prediction that behavioral responses to heterospecific cues should be weaker than responses to evolutionarily conserved, homologous cues (Chivers and Smith 1998; Brown et al. 2000). For example, Mitchell et al. (2012) found the magnitude of antipredator behavior in damselfishes elicited by alarm cues was proportional to the phylogenetic distance of the donor, corroborating earlier reports on salmonids (Mirza and Chivers 2001); within poeciliids, there is also evidence for cue conservation at the population level (Brown et al. 2009). Similarly, studies on gastropods and amphibians demonstrated ability to respond to cues in a threat-sensitive manner reflecting phylogenetic proximity (Schoeppner and Relyea 2005; Dalesman and Rundle 2010; Atherton and McCormick 2017). Conversely, three-spined stickleback (*Gasterosteus aculeatus*) showed no discrimination between alarm cues from conspecifics or heterospecifics, responding to both cues equally (FitzGerald and Morrisette 1992), and similarity in size of cue donor and cue receiver has been demonstrated as more important than relatedness in cyprinodontiformes (Elvidge et al. 2010; Elvidge and Brown 2015). Apart from these studies, heterospecific responses to alarm cues and their changes with ontogeny in fishes are still incompletely documented.

In contrast to the hypothesis of taxonomic cue conservation, we hypothesize that the response strategies of fish to chemical cues, a form of public information (Danchin et al. 2004), should be closely related to their ecological niches (Holt 2009). Specifically, magnitudes of response to alarm cues from heterospecifics and to conspecifics of differing ontogenetic stage, should increase with relative degree of niche overlap, and decrease with niche differentiation. In the context of public information theory, similarities in habitat usage and vulnerability to predators (prey guild membership; Scharf et al. 2000; Mirza et al. 2003) should increase the value of heterospecific cues relative to the values of cues from conspecifics of different life history stages (Elvidge and Brown 2015), particularly if those conspecifics are occupying different ecological niches as diet, habitat, and vulnerability to common predators shift with ontogeny (Harvey and Brown 2004). To test this hypothesis, we used 2 sympatric species of sunfish as our experimental models.

Sunfish of the family Centrarchidae are well known to ecologists and are commonly used model systems in aquatic ecology research due to their diversity and ubiquity in North America (Cooke and Philipp 2009). Bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish are 2 well-studied species that demonstrate niche shifts due to intense interspecific competition when they co-occur (Holt 1984). Despite their phylogenetic proximity as congeners, both species have different life history traits that may partially explain their coexistence (Robinson et al. 1993). In sympatry, adult bluegill are generalists that feed in the open water on zooplankton and insects, whereas adult pumpkinseed tend to reside in the benthos and specialize on crushing hard-bodied prey like snails (Osenberg et al. 1992; Robinson et al. 1993). Thus, the adults of each species likely experience different predation pressures from different suites of predators, with pumpkinseed more vulnerable to aquatic predators, and bluegill facing predation threats from both aquatic and avian

predators (McCartt et al. 1997). However, juveniles of both species overlap in their habitat use and are commonly found together in shallow, complex habitats associated with aquatic vegetation, suggesting that these species undergo niche differentiation from each other as well as niche shifts between juvenile and adult ontogenetic stages (Figure 1; Polis 1984; Osenberg et al. 1992).

Over eco-evolutionary timeframes, exposure to different sources of predation pressure should drive the development of different defense mechanisms and antipredator strategies. Based on established differences in habitat use, we developed and tested 4 hypotheses. First, adult bluegill and pumpkinseed will vary in their responses to conspecific and heterospecific alarm cues (Hypothesis 1). Second, pumpkinseed will demonstrate greater responses than bluegill to chemical alarm cues since they reside lower in the water column and are less vulnerable to avian predators (Hypothesis 2). Third, the 2 species are more ecologically similar in their early life history stages when both occupy spatially complex habitats associated with aquatic vegetation, so there should be no significant differences in antipredator behaviors in response to juvenile conspecific and heterospecific alarm cues since their ecological niches overlap to a large degree (Hypothesis 3). Fourth, there should be different patterns of response in both species to alarm cues from juvenile and adult donors since their ecological niches become more differentiated with ontogeny (Hypothesis 4).

MATERIALS AND METHODS

Fish collection and housing

Adult bluegill and pumpkinseed were collected via angling using live bait (earthworms *Lumbricus* spp.) and size 8 J-hooks from a boat on Lake Opinicon at the Queen's University Biological Station (Chaffey's Lock, ON, Canada) during late May to early July 2016. Only fish that were shallowly hooked (through the lip) were retained and held in species-specific coolers filled with lakewater (mean temperature 20.1 °C), that was changed via bucket at 15 min intervals. The fish were transferred to one of two 500-L circular, flow-through holding tanks continuously supplied with lakewater within 2 h of capture. Fish were held for at least 24 h prior to testing to allow them to acclimate to conditions under captivity. None of the retained fish died during transport or acclimation. After the acclimation period, only visibly healthy bluegill (69.0 ± 2.02 g, 13.2 ± 0.11 cm standard

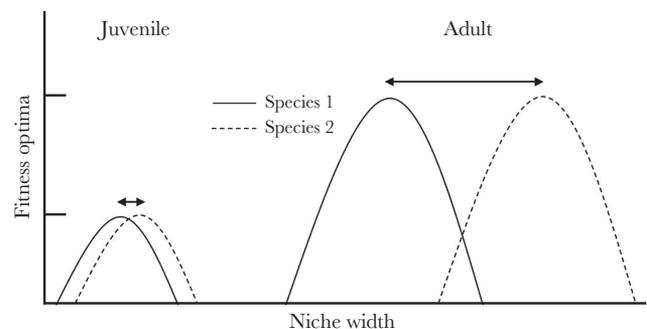


Figure 1

Niche differentiation between juvenile and adult life history stages in 2 hypothetical species. Similarities in habitat use, diet, and vulnerability to different suites of predators result in greater niche overlap between heterospecifics of similar, early life history stage than between conspecifics of different life history stages, potentially increasing the value of heterospecific information relative to conspecific information for predator avoidance behaviors in juveniles, and decreasing the value in adults.

length, $N = 75$) and pumpkinseed (86.3 ± 2.98 g, 13.7 ± 0.14 cm standard length, $N = 75$) were used as experimental fish and any individuals demonstrating injury or debilitation were released back to the lake. Once a fish had been assayed, it was measured (standard length and weight) and released back into Lake Opinicon in an area away from where fish collections occurred.

Alarm cue preparation

Two adult bluegill (14.6 cm and 15.8 cm standard length) and 2 adult pumpkinseed (14.4 cm and 14.7 cm standard length) captured via angling, as well as 18 juvenile bluegill (5.97 ± 0.16 cm standard length) and 14 juvenile pumpkinseed (6.53 ± 0.10 cm standard length) captured using a beach seine were used as alarm cue donors. Donors were euthanized via cerebral percussion followed by decapitation and lateral skin filets were immediately removed to produce alarm cues. In total, we collected 194.8, 233.2, 204.3, and 223.5 cm² of skin from adult bluegill, adult pumpkinseed, juvenile bluegill, and juvenile pumpkinseed, respectively. Skin tissue was mechanically homogenized in chilled lakewater and filtered through polyester floss to remove debris. The final concentrations were adjusted to 0.1 cm²/mL with lakewater. The 4 alarm cue solutions, as well as lakewater as a control treatment, were packaged in 20 mL aliquots and frozen at -20 °C until use.

Experimental protocol

Trials were conducted in 3 glass aquaria (60 cm length \times 30 cm width \times 30 cm height) with capacity of 45 L. Each tank was equipped with a single air stone attached to the side opposite the camera. An additional 2-m length of airline tubing was attached to the air stone through which chemical stimuli (20 mL) could be injected without disturbing the test fish. The tank was divided into 3 horizontal sections on the exterior of the tank to facilitate the recording of behavioral changes in time spent at the surface or on the bottom of the tank. Surface swimming and bottom-dwelling behavior were defined as those that located within 5 cm from the surface or bottom of the aquarium, respectively.

All trials were conducted indoors between 0900–1800 and access to the tank facility was restricted so as to not disturb the fish. Individual adult bluegill or pumpkinseed were introduced into the test tanks and allowed to habituate for 30 min prior to testing. Trials were divided into a 5 min pre-stimulus observation period and a 5 min post-stimulus observation period after a 20 s recovery interval following stimulus injection. A total of 150 trials were conducted, consisting of 10 treatments ($n = 15$ for each). All focal individuals were tested only once. During the pre- and post-stimulus observation periods, body posture (time spent with the dorsal fin $<30^\circ$, between 30° and 60° , or $>60^\circ$) and behavior (time spent still, frozen, at the surface, or on the bottom of the tank) of fish were video recorded. We defined “frozen” as remaining motionless with the dorsal fin held $>30^\circ$. In order to concisely present the experimental results, we considered behaviors and body postures as independent (though not mutually exclusive) classes of antipredator responses.

Statistical analyses

The recorded responses were divided into 2 categories based on whether the response was related to body posture (changes in time spent in 3 dorsal fin positions) or behavior (changes in time spent still, frozen, at the surface, or on the bottom of the tank). None of the response variables met the assumptions of normality (Shapiro–Wilks test, all $P < 0.05$), so they were rank-transformed for analysis as “aov” models (after Scheirer et al. 1976). The 2 multivariate responses were then

analyzed in factorial 2-way MANCOVAs with alarm cue type and focal species as fixed factors, and fish weight and length as linear covariates. The MANOVA models were then decomposed into univariate Anovas, and whenever one of the fixed-effects factors or their interaction term had a significant effect on a response, it was examined further with Tukey’s HSD post hoc test applied to a 1- or 2-way Anova (i.e. non-significant fixed effect factor[s], interaction term, or linear covariate[s] removed from the model). All analyses used R version 3.4.1 (R Core Team 2017) and figures were made using “gplots” (Warnes et al. 2016).

Ethical note

This work was conducted in accordance with the regulations of the Canadian Council on Animal Care under Carleton University protocol no. 104281 and Queen’s University protocol no. 1592. All experimental procedures were consistent with ASAB guidelines for the treatment of animals in behavioral research (<http://asab.nottingham.ac.uk/ethics/guidelines.php>). Collection of wild fish for research occurred under the authority of a Scientific Collection Permit issued to S.J.C. by the Ontario Ministry of Natural Resources and Forestry.

RESULTS

Dorsal fin postures

For the suite of dorsal fin (body) postures, alarm cue type was the only explanatory variable with a significant effect on the multivariate response, while differences in time spent with the dorsal fin $<30^\circ$ and $>60^\circ$ were individually influenced by alarm cue type (Table 1). Post hoc testing revealed significant differences in time spent with dorsal fins held $<30^\circ$ between every alarm cue type and the control

Table 1

MANOVA results and univariate differences in dorsal fin position (antipredator posture) in adult bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) exposed to chemical alarm cues

Multivariate response:

	Wilks' Lambda	df	P
Cue	0.754	4, 360.11	0.00011
Species	0.991	1, 136	0.74
Weight	0.986	1, 136	0.57
Length	0.993	1, 136	0.81
Cue \times Species	0.946	4, 360.11	0.81

Univariate responses:

	F	df	P
<i>Fin <30°:</i>			
Cue	7.45	4, 138	<0.0001
Species	0.42	1, 138	0.52
Weight	1.26	1, 138	0.26
Length	0.87	1, 138	0.35
Cue \times Species	0.75	4, 138	0.56
<i>Fin 30°–60°:</i>			
Cue	1.84	4, 138	0.12
Species	1.15	1, 138	0.29
Weight	0.34	1, 138	0.56
Length	0.13	1, 138	0.72
Cue \times Species	0.88	4, 138	0.48
<i>Fin >60°:</i>			
Cue	6.92	4, 138	<0.0001
Species	0.005	1, 138	0.95
Weight	0.006	1, 138	0.94
Length	0.31	1, 138	0.58
Cue \times Species	0.33	4, 138	0.86

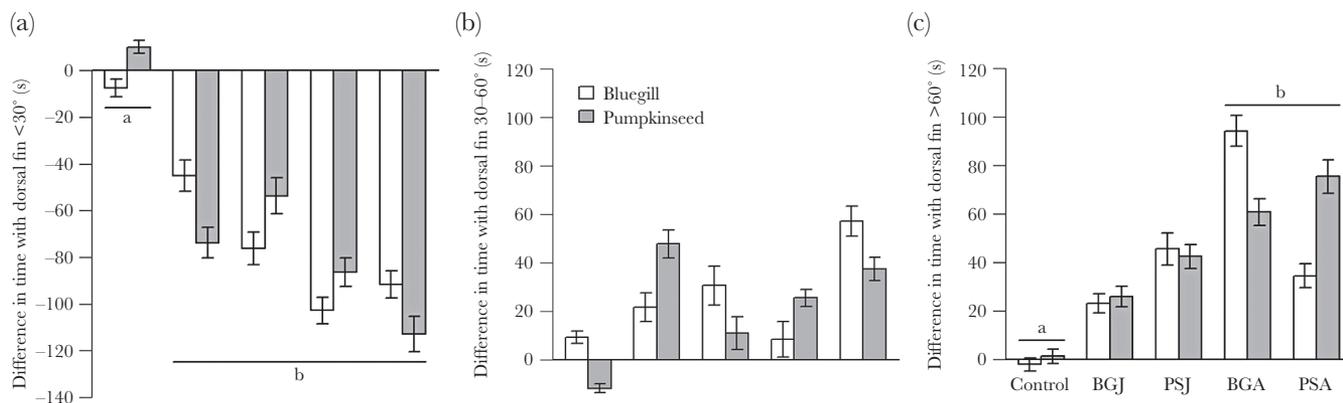


Figure 2 Mean (\pm SE) changes in time (s) spent with the dorsal fin (a) $<30^\circ$, (b) 30° – 60° , and (c) $>60^\circ$ to the anteroposterior axis of the body in adult bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) over 5 min pre- and post-stimulus observation periods. Stimuli consisted of lakewater (Control), or damage-released chemical alarm cues from juvenile (BGJ) or adult (BGA) bluegill or pumpkinseed (PSJ, PSA). Letters denote significant pairwise differences between alarm cue types (Tukey’s HSD, $P < 0.05$).

treatment. Focal fish decreased their time spent in the $<30^\circ$ posture when exposed to any alarm cues, but less so to juvenile versus adult cues. Decreases were greater in response to the cues of heterospecific versus conspecific juveniles, but this trend was reversed and conspecific adult cues elicited greater decreases than heterospecific adult cues (Figure 2a). There were no significant differences or trends in the time spent with dorsal fins held 30° to 60° (Figure 2b). Only the alarm cues from adult donors resulted in significantly more time spent in the $>60^\circ$ posture relative to the control, with conspecific cues eliciting greater responses than heterospecific cues (Figure 2c).

Antipredator behaviors

The multivariate antipredator behavioral response was significantly influenced by both focal species and alarm cue type, but not their interaction term, as was the univariate response of difference in time spent holding still, whereas time spent frozen was influenced by alarm cue type and time spent at the surface was influenced by focal species (Table 2). Bluegill demonstrated greater increases in time spent holding still in response to adult bluegill alarm cues than pumpkinseed, and there were similar but nonsignificant trends in response to the alarm cues from both juvenile and adult pumpkinseed. The only significant pairwise difference between cue types, however, was between adult bluegill alarm cue and the control (Figure 3a). Times spent frozen differed significantly between each alarm cue type and the control ($P < 0.05$), with the response pattern towards juvenile alarm cues demonstrating greater increases in time frozen to the heterospecific cue than the conspecific cue. This pattern was reversed in the responses to the adult alarm cues (Figure 3b). Bluegill demonstrated mean decreases in time spent at the surface in response to all treatments, while pumpkinseed only demonstrated decreases in response to juvenile pumpkinseed alarm cue (Figure 3c). Time on the bottom was not influenced overall by alarm cue type, and adults of both species again demonstrated inverse patterns of response, with greater responses to juvenile heterospecific cues and greater responses to adult conspecific cues (Figure 3d).

DISCUSSION

The use of information available in the environment is critical for organisms to respond appropriately to ever-changing conditions and enables adaptive behavioral plasticity. Differences in behavioral responses to risky conspecific and heterospecific information

Table 2 MANOVA results and univariate differences in movement and vertical positions (antipredator behavior) in adult bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) exposed to chemical alarm cues

Multivariate response:			
	Wilks’ Lambda	df	P
Cue	0.731	4, 413.07	0.00028
Species	0.921	1, 135	0.025
Weight	0.988	1, 135	0.81
Length	0.974	1, 135	0.47
Cue×Species	0.908	4, 413.07	0.66
Univariate responses:			
	F	df	P
<i>Time still:</i>			
Cue	2.92	4, 138	0.024
Species	6.41	1, 138	0.013
Weight	0.06	1, 138	0.80
Length	0.36	1, 138	0.55
Cue×Species	0.66	4, 138	0.62
<i>Time frozen:</i>			
Cue	7.72	4, 138	<0.0001
Species	2.67	1, 138	0.10
Weight	1.35	1, 138	0.25
Length	1.85	1, 138	0.18
Cue×Species	1.11	4, 138	0.35
<i>Time on surface:</i>			
Cue	1.63	4, 138	0.17
Species	3.23	1, 138	0.07
Weight	0.01	1, 138	0.91
Length	0.13	1, 138	0.72
Cue×Species	0.36	4, 138	0.84
<i>Time on bottom:</i>			
Cue	1.82	4, 138	0.13
Species	0.32	1, 138	0.57
Weight	0.004	1, 138	0.95
Length	0.75	1, 138	0.39
Cue×Species	1.23	4, 138	0.30

in fishes that undergo ontogenetic niche shifts are important for the basic understanding of intra- and inter-species relationships, co-evolution, and community trophic dynamics (Goodale et al. 2010). Our results demonstrate that behavioral response patterns to

chemical alarm cues in sunfish are highly ecologically plastic and are likely closely related to niche dynamics. To our knowledge, this study is the first demonstration of alarm cue responses in fish based to explicitly adopt a niche perspective.

The ability of prey to accurately assess their risk of predation is critical for survival and decision-making to balance trade-offs between risk avoidance and maximizing other fitness-related activities (Lima and Dill 1990). These trade-offs drive gradients of risk responsiveness that will differ between species depending on their life history characteristics (Brown and Smith 1996; Lima and Bednekoff 1999; Spivey et al. 2015). In this study, the behavioral responses of the experimental fish to alarm cues could be divided into 3 distinct grades, as follows: Grade I: change in habitat utilization between the relatively riskier surface or the relatively safer lower portion of the water column, potentially avoiding predators; Grade II: changes in activity levels, reducing their conspicuousness to foraging predators; Grade III: changes in body posture (time spent with the dorsal fin $>60^\circ$) as an active deterrent to predators (Smith 1997). A putative Grade IV may consist of active escape from predators (Godin 1997), although our experimental setup and protocol could not detect this.

Supporting the idea that fin posture is an antipredator response (Grade III) that will be influenced by the degree of risk perceived

by a focal fish (Smith 1997; Brown et al. 2009; Brown et al. 2011), adult bluegill and pumpkinseed both spent significantly more time with their fins held $>60^\circ$ in response to adult alarm cues, with these responses mediated by donor species such that conspecific cues resulted in greater responses than heterospecific cues. These observations supported Hypothesis 1 (each species will demonstrate different responses to conspecific vs. heterospecific cues) and Hypothesis 4 (different responses to juvenile vs. adult cues), but contradicted Hypothesis 2 (pumpkinseed will demonstrate greater responses than bluegill due to background predator exposure). Conversely, times spent with dorsal fins held $<30^\circ$ decreased, with heterospecific patterns of response similar to those in the $>60^\circ$ measure. This pattern was reversed in response to juvenile alarm cues, contradicting Hypothesis 3 (no difference in response to juvenile cues), with adults demonstrating trends towards less time spent with dorsal fins held $<30^\circ$ following heterospecific cue exposure. These observations suggest that the $<30^\circ$ posture could be associated with exploration or foraging behaviors, and the increases in time spent in the $>60^\circ$ posture in response to alarm cues may require compensatory behavioral mechanisms (Elvidge et al. 2014) following a period of predator deterrence associated with the adoption of the $>60^\circ$ posture that we did not capture in our 5 min observation periods.

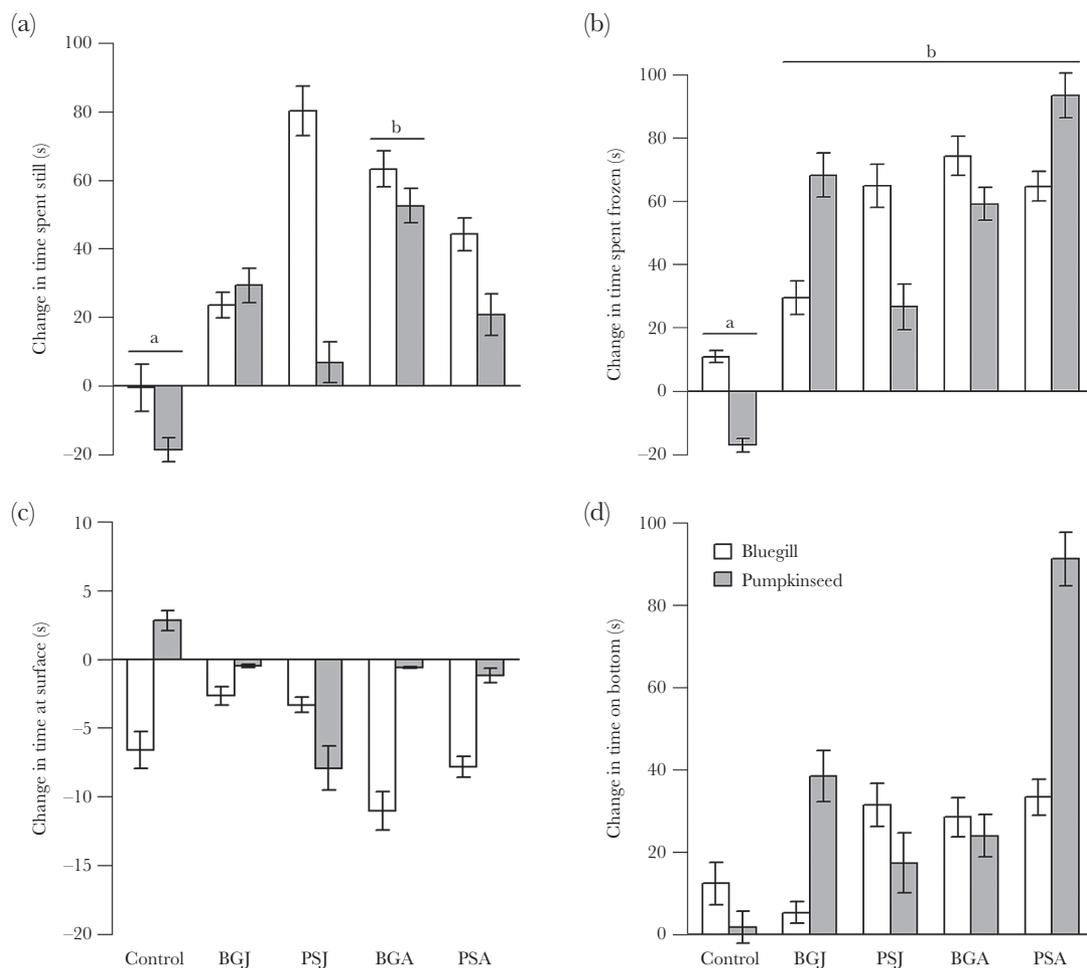


Figure 3

Mean (\pm SE) changes in time (s) spent (a) still, (b) frozen, (c) at the surface, and (d) at the bottom of the observation tanks in adult bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) over 5 min pre- and post-stimulus observation periods. Stimuli consisted of lakewater (Control), or damage-released chemical alarm cues from juvenile (BGJ) or adult (BGA) bluegill or pumpkinseed (PSJ, PSA). Letters denote significant pairwise differences between alarm cue types (Tukey's HSD, $P < 0.05$).

Both species significantly increased their time spent frozen in response to the different alarm cue types, whereas only the adult bluegill alarm cue elicited greater time spent still relative to the control. It is not clear why the alarm cues from heterospecific juveniles should elicit greater freezing responses than conspecific cues, but the trends in response to adult cues match the general predictions of heterospecific alarm signaling (Elvidge et al. 2010; Elvidge and Brown 2015). The interspecific difference in time spent at the surface, and the observation that adult pumpkinseed tended to spend more time at the bottom in response to their own alarm cues, likely reflects the natural history of these populations in Lake Opinicon, where adult pumpkinseed tend to be more benthic while adult bluegill tend to be more pelagic (Keast et al. 1978). For bluegill, decreasing time spent at the surface may indicate attempts at refuging in relatively safer benthic habitat, whereas for pumpkinseed, increasing their time spent at the bottom will also reduce their time spent in the riskier areas higher in the water column.

Although evidence exists for the conservation of alarm signals within taxonomic groups (Brown et al. 2001; Mirza and Chivers 2001; Golub and Brown 2003), the public availability of chemical cues in aquatic environments should favor selection on opportunistic eavesdropping by any receiver that can detect the cues (sensu Danchin et al. 2004). Fish in early life history stages often occupy similar habitats and ecological niches, and therefore are likely exposed to similar levels of risk from shared predators as members of a common prey guild. Thus, learned recognition of cues indicating risk from other prey guild members is essential for survival (Brown et al. 2011). Chivers et al. (1995) demonstrated that fathead minnows (*Pimephales promelas*) could recognize and avoid areas where Iowa darter (*Etheostoma exile*) alarm pheromone was detected and that this was a learned response. Likewise, Pollock et al. (2003) found that naïve fathead minnows do not respond to brook stickleback (*Culaea inconstans*) alarm cues; however, when fathead minnows and brook stickleback are raised together in the presence of predators, minnows display antipredator responses when exposed to stickleback alarm cues (Ferrari et al. 2010). Manassa et al. (2013) confirmed that naïve *Pomacentrus wardi* were capable of transmitting the recognition of a predator odor to another closely related damselfish (*Pomacentrus moluccensis*) and a phylogenetically distant species (*Apogon trimaculatus*) through the process of social learning. Moreover, Atherton and McCormick (2015) provided evidence that cinnamon clownfish (*Amphiprion melanopus*) embryos were not only able to detect and react to conspecific chemical alarm cues, but were also capable of using this information to learn about predation risk before they hatched. Presumably, the use of heterospecific alarm cues to assess predation risk constitutes a widespread phenomenon within communities of small fishes, proportional to the degree of niche overlap. Over the course of growth and development, however, shared predation risk should diminish as niche differentiation occurs and adults of both species encounter different predation risks from each other and from juvenile congeners. Consequently, there should be some adaptive value to forgetting (Brown et al. 2013) learned risky associations from earlier life history stages. Our observed changes in body posture (time spent with the dorsal fin >60°) in adult bluegill and pumpkinseed only occurred in response to adult alarm cues and not juvenile alarm cues, supporting this prediction.

To conclude, we have demonstrated that the response strategies of a teleost fish to external chemical cues reflects the overlap or differentiation of their ecological niches. These findings open new research avenues into the role of the ecological niche in shaping fish behavioral phenotypes and their responses to risky public information.

FUNDING

This study was supported by the National Natural Science Foundation of China (NSFC 31770442) and Youth Top-notch Talent Cultivation Program of Chongqing Normal University (14CSBJ08) to J.X., by NSERC to C.K.E. and S.J.C., and the Canada Research Chairs Program to S.J.C.

We thank C.H. Reid, I.J. Byerly, D. Dusevic, M.J. Lawrence, A.J. Zolderdo, A. Abrams, L. Kelly, and J.L. Brooks for their assistance with animal collection.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Xia et al. (2018).

Handling editor: Ulrika Candolin

REFERENCES

- Atherton JA, McCormick MI. 2015. Active in the sac: damselfish embryos use innate recognition of odours to learn predation risk before hatching. *Anim Behav.* 103:1–6.
- Atherton JA, McCormick MI. 2017. Kin recognition in embryonic damselfishes. *Oikos.* 126:1062–1069.
- Brown GE, Adrian JC, Smyth E, Leet H, Brennan S. 2000. Ostariophysan alarm pheromones: laboratory and field tests of the functional significance of nitrogen oxides. *J Chem Ecol.* 26:139–154.
- Brown GE, Ferrari CO, Chivers DP. 2013. Adaptive forgetting: why predator recognition training might not enhance poststocking survival. *Fisheries.* 38:16–25.
- Brown GE, Ferrari MC, Chivers DP. 2011. Learning about danger: chemical alarm cues and threat-sensitive assessment of predation risk by fishes. In: Brown C, Laland K, Krause J, editors. *Fish cognition and behaviour*. West Sussex, UK: Blackwell Scientific. p. 59–80.
- Brown GE, LeBlanc VJ, Porter LE. 2001. Ontogenetic changes in the response of largemouth bass (*Micropterus salmoides*, Centrarchidae, Perciformes) to heterospecific alarm pheromones. *Ethology.* 107:401–414.
- Brown GE, Macnaughton CJ, Elvidge CK, Ramnarine I, Godin JGJ. 2009. Provenance and threat-sensitive predator avoidance patterns in wild-caught Trinidadian guppies. *Behav Ecol Sociobiol.* 63:699–706.
- Brown GE, Smith RJE. 1996. Foraging trade-offs in Fathead Minnows (*Pimephales promelas*, Osteichthyes, Cyprinidae): acquired predator recognition in the absence of an alarm response. *Ethology.* 102:776–785.
- Chivers DP, Smith RJE. 1998. Chemical alarm signaling in aquatic predator-prey systems: a review and prospectus. *Écoscience.* 5:338–352.
- Chivers DP, Wisenden BD, Smith RJE. 1995. The role of experience in the response of fathead minnows (*Pimephales promelas*) to skin extract of Iowa darters (*Etheostoma exile*). *Behaviour.* 132:665–674.
- Cooke SJ, Philipp DP. 2009. *Centrarchid fishes: diversity, biology and conservation*. West Sussex, UK: John Wiley and Sons.
- Dalesman S, Rundle SD. 2010. Cohabitation enhances the avoidance response to heterospecific alarm cues in a freshwater snail. *Anim Behav.* 79:173–177.
- Danchin E, Giraldeau LA, Valone TJ, Wagner RH. 2004. Public information: from nosy neighbors to cultural evolution. *Science.* 305:487–491.
- Elvidge CK, Brown GE. 2015. Size-based differences determine the contextual value of risky information in heterospecific information use. *Anim Behav.* 102:7–14.
- Elvidge CK, Ramnarine I, Brown GE. 2014. Compensatory foraging in Trinidadian guppies: effects of acute and chronic predation threats. *Curr Zool.* 60:323–332.
- Elvidge CK, Ramnarine IW, Godin JG, Brown GE. 2010. Size-mediated response to public cues of predation risk in a tropical stream fish. *J Fish Biol.* 77:1632–1644.
- Ferrari MCO, Wisenden BD, Chivers DP. 2010. Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Can J Zool.* 88:698–724.
- FitzGerald GJ, Morrissette J. 1992. Kin recognition and choice of shoal mates by threespine sticklebacks. *Ethol Ecol Evol.* 4:273–283.
- Friesen RG, Chivers DP. 2006. Underwater video reveals strong avoidance of chemical alarm cues by prey fishes. *Ethology.* 112:339–345.
- Godin JGJ. 1997. Evading predators. In: Godin JGJ, editor. *Behavioural ecology of teleost fishes*. Oxford: Oxford University Press. p. 191–236.

- Golub JL, Brown GE. 2003. Are all signals the same? Ontogenetic change in the response to conspecific and heterospecific chemical alarm signals by juvenile green sunfish (*Lepomis cyanellus*). *Behav Ecol Sociobiol.* 54:113–118.
- Goodale E, Beauchamp G, Magrath RD, Nieh JC, Ruxton GD. 2010. Interspecific information transfer influences animal community structure. *Trends Ecol Evol.* 25:354–361.
- Hara TJ. 1992. Overview and introduction. In: Hara TJ, editor. *Fish chemoreception*. New York: Chapman and Hall. p. 1–12.
- Harvey MC, Brown GE. 2004. Dine or dash?: ontogenetic shifts in the response of yellow perch to conspecific alarm cues. *Environ Biol Fish.* 70:345–352.
- Holt RD. 1984. Spatial heterogeneity, indirect interactions, and the coexistence of prey species. *Am Nat.* 124:377–406.
- Holt RD. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. *Proc Natl Acad Sci USA.* 106:19659–19665.
- Jones KA, Godin JG. 2010. Are fast explorers slow reactors? Linking personality type and anti-predator behaviour. *Proc Biol Sci.* 277:625–632.
- Keast A, Harker J, Turnbull D. 1978. Nearshore fish habitat utilization and species associations in Lake Opinicon (Ontario, Canada). *Environ Biol Fish.* 3:173–184.
- Lienart GD, Ferrari MC, McCormick MI. 2016. Thermal environment and nutritional condition affect the efficacy of chemical alarm cues produced by prey fish. *Environ Biol Fish.* 99:729–739.
- Lima SL, Bednekoff PA. 1999. Temporal variation in danger drives anti-predator behavior: the predation risk allocation hypothesis. *Am Nat.* 153:649–659.
- Lima SL, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Can J Zool.* 68:619–640.
- Manassa RP, McCormick MI, Chivers DP. 2013. Socially acquired predator recognition in complex ecosystems. *Behav Ecol Sociobiol.* 67:1033–1040.
- McCartt AL, Lynch WE, Johnson DL. 1997. How light, a predator, and experience influence bluegill use of shade and schooling. *Environ Biol Fish.* 49:79–87.
- Mirza RS, Chivers DP. 2001. Are chemical alarm cues conserved within salmonid fishes? *J Chem Ecol.* 27:1641–1655.
- Mirza RS, Fisher SA, Chivers DP. 2003. Assessment of predation risk by juvenile yellow perch, *Perca flavescens*: responses to alarm cues from conspecifics and prey guild members. *Environ Biol Fish.* 66:321–327.
- Mitchell MD, Cowman PF, McCormick MI. 2012. Chemical alarm cues are conserved within the coral reef fish family Pomacentridae. *PLoS One.* 7:e47428.
- Osenberg CW, Mittelbach GG, Wainwright PC. 1992. Two-stage life histories in fish: the interaction between juvenile competition and adult performance. *Ecology.* 73:255–267.
- Polis GA. 1984. Age structure component of niche width and intraspecific resource partitioning: can age groups function as ecological species? *Am Nat.* 123:541–564.
- Pollock MS, Chivers DP, Mirza RS, Wisenden BD. 2003. Fathead minnows, *Pimephales promelas*, learn to recognize chemical alarm cues of introduced brook stickleback, *Culaea inconstans*. *Environ Biol Fish.* 66:313–319.
- R Core Team. 2017. R: A language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: <https://www.R-project.org/>.
- Robinson BW, Wilson DS, Margosian AS, Lotito PT. 1993. Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. *Evol Ecol.* 7:451–464.
- Scharf FS, Juanes F, Rountree RA. 2000. Predator size - prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Mar Ecol Prog Ser.* 208:229–248.
- Scheirer CJ, Ray WS, Hare N. 1976. The analysis of ranked data derived from completely randomized factorial designs. *Biometrics.* 32:429–434.
- Schoepfner NM, Relyea RA. 2005. Damage, digestion, and defence: the roles of alarm cues and kairomones for inducing prey defences. *Ecol Lett.* 8:505–512.
- Seppänen JT, Forsman JT, Mönkkönen M, Thomson RL. 2007. Social information use is a process across time, space, and ecology, reaching heterospecifics. *Ecology.* 88:1622–1633.
- Skelly DK. 1992. Field evidence for a cost of behavioral antipredator response in a larval amphibian. *Ecology.* 73:704–708.
- Smith RJE. 1997. Avoiding and deterring predators. In: Godin JGJ, editor. *Behavioural ecology of teleost fishes*. Oxford: Oxford University Press. p. 163–190.
- Spivey KL, Chapman TL, Schmitz AL, Bast DE, Smith AL, Gall BG. 2015. The alarm cue obstruction hypothesis: isopods respond to alarm cues, but do not respond to dietary chemical cues from predatory bluegill. *Behaviour.* 152:167–179.
- Warnes GR, Bolker B, Bonebakker L, Gentleman R, Liaw WHA, Lumley T, Maechler M, Magnusson A, Moeller S, Schwartz M, et al. 2016. *gplots: Various R programming tools for plotting data*. R package version 3.0.1. Available from: <https://cran.r-project.org/package=gplots>.
- Wisenden BD. 2000. Olfactory assessment of predation risk in the aquatic environment. *Philos Trans R Soc B-Biol Sci.* 355:1205–1208.
- Wisenden BD, Millard MC. 2001. Aquatic flatworms use chemical cues from injured conspecifics to assess predation risk and to associate risk with novel cues. *Anim Behav.* 62:761–766.
- Xia J, Cheng M, Cai R, Fu S, Cooke SJ, Elvidge CK. 2017. Ontogenetic changes in chemical alarm cue recognition and fast-start performance in guppies (*Poecilia reticulata*). *Ethology.* 123:916–923.
- Xia J, Elvidge CK, Cooke SJ. 2018. Data from: niche separation, ontogeny, and heterospecific alarm responses in centrarchid sunfish. *Dryad Digital Repository*. <http://dx.doi.org/10.5061/dryad.6vq5q30>.