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FEATURED STUDENT RESEARCH PAPER

Baseline activity and shoal type determine antipredator behaviors in bluegill from a southern Ontario lake

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Abstract

Shoaling is an evolved behavior in fishes that has several adaptive advantages, including allowing individuals to avoid predation through risk dilution. However, factors such as size disparity and the presence of heterospecifics may influence the behavior of individual fish within shoals following exposure to elevated predation risk. Using bluegill *Lepomis macrochirus* as a model species, we measured changes in area use, shoaling index, and movement of a focal individual in isolation, in single-species shoals with two conspecifics, or in mixed-species shoals with two congeneric pumpkinseed *L. gibbosus*. The experimental shoals were exposed to one of three chemical cues selected to present graded levels of risk: lakewater controls (lowest risk), Northern pike *Esox lucius* predator odor (kairomones; intermediate risk), or conspecific chemical alarm cues (highest risk). Within the individual bluegill, we found that the multivariate response of area use and post-stimulus activity (line crosses) of the focal fish was significantly influenced by pre-stimulus activity, but not by cue type or fish size. As univariate responses, post-stimulus activity varied positively with pre-stimulus activity. Post-stimulus activity was greater in single-species shoals compared to mixed-species shoals, and again varied positively with pre-stimulus activity. Contrary to predictions, bluegill did not demonstrate graded antipredator responses to the chemical cues. Our findings suggest that prey fish may alter their risk-aversive behaviors in response to chemical stimuli based on shoal composition and provide further insight into the role of intra-prey guild interactions in response to predators in co-occurring prey species.

Significance statement

When faced with predation, individuals in groups may experience lower levels of risk than solitary individuals. Using bluegill as a model organism, we examined how antipredator behaviors, in response to chemical cues indicating different levels of risk, varied between focal individuals as singletons and in single- and mixed-species shoals. Contrary to our prediction of graded stepwise responses indicative of differing levels of risk posed by the cues, we found that individuals with greater baseline activity levels demonstrated weaker antipredator responses independent of cue type, while fish in single-species shoals had higher post-stimulus activity levels than fish in mixed-species shoals. Our results suggest that studies examining changes in antipredator and shoaling behaviors will benefit from including ecologically relevant scenarios involving sympatric prey guild members.

Keywords Alarm cues · Centrarchidae · Kairomones · Prey · Risk avoidance · Risk dilution

Introduction

Of the many selective forces encountered by animals, the ultimate consequence of predation renders it one of the most powerful. Predation has an important role in shaping the behavior, morphology, and life history traits of prey species (Brown and Chivers 2005) that have evolved numerous antipredator behaviors, including the formation of groups

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(Mirabet et al. 2007; Grobis et al. 2013; Gil et al. 2017). Individuals can accrue benefits from these groups, including decreased probabilities of predation through risk dilution (Fitzgibbon 1990; Shier 2006; Hain and Neff 2009) while increasing efficiency of foraging and finding mates (Dyer et al. 2009). In fishes, formation of single- and mixed-species shoals is a common antipredator defense, occurring in ~ 10,000 species (Ward et al. 2002, 2008; Piyapong et al. 2011).

Numerous hypotheses address the functions of grouping in fishes. Formation of shoals may enhance individual survival by disrupting the hearing (Larsson 2012), vision (Tosh et al. 2009), and/or electrosensory systems (Larsson 2009) of predators, reducing their ability to successfully locate and capture prey. Shoaling may also increase the possibility of escape from predators through improved predator detection (Godin et al. 1988) and coordinated antipredator defenses (Pitcher 1983), including directional schooling movements as per the avoidance hypothesis (Grobis et al. 2013). Additionally, shoaling may reduce the odds of predation on participating individuals via risk dilution (Inman and Krebs 1987) and the confusion effect, where the creation of a large mass makes it difficult for predators to identify individuals (Lee-Jenkins and Godin 2010).

Fish behavior within shoals can be affected by shoal composition. For example, single-species shoals of Chinese bream *Parabramis pekinensis* and qingbo *Spinibarbus sinensis* differed in both swimming speed and activity, but these differences between species disappeared when heterospecifics were present (Tang et al. 2017). Additionally, juvenile European perch *Perca fluviatilis* display a positive relationship between boldness and group size, indicating that individuals in larger shoals may display less antipredator behavior than individuals in smaller shoals (Goldenberg et al. 2014). These results demonstrate that fish have the ability to alter their behavior to match that of nearby counterparts.

Another well-studied antipredator response in fishes is the recognition of chemical alarm cues (Brown et al. 2010; Elvidge et al. 2010; Wagner et al. 2016) and the odors or kairomones of potential predators (Zhao et al. 2006; Frommen et al. 2011). Alarm cues are chemicals that are released passively from the epidermis following mechanical damage and reliably indicate elevated predation risk to nearby conspecifics (Smith 1992; Pollock et al. 2006a; Wisenden et al. 2008). In addition, some fish can recognize the alarm cues of familiar heterospecifics belonging to the same prey guild (Pollock et al. 2006b). Predator odors, by contrast, are released inadvertently and can alert prey not only to the presences of predators but, in some cases, their recent diet (Ylönen et al. 2007). In fishes that have not been previously exposed to predator odors, individuals are able to learn and adapt their behavior after a number of days such that they decrease foraging and area use, while increasing the amount of time spent refuging (Brown and Smith 1998). These antipredator adaptations are sometimes transmissible from parents to offspring (Korpi and Wisenden 2001; Nelson et al. 2013). Exposure to both alarm cues and predator odors elicits common antipredator responses, including temporary reductions in activity and increased shoaling behaviors (Lautala and Hirvonen 2008; Stephenson 2016).

Although the shoaling behavior of fishes in response to predators has previously been examined (Lima and Dill 1990; Hager and Helfman 1991), there is further need to understand the factors influencing the behavior of individual fish within these shoals. Earlier studies have shown that fish can alter their behavior non-randomly within shoals based on color, body length, and ontogenetic stage (Hoare et al. 2000; Ward et al. 2002). Other studies suggest that only smaller individuals should demonstrate antipredator behaviors as they are more vulnerable to predation from gape-limited predators (Golub et al. 2005), and that fish will assort themselves according to body length (Croft et al. 2005). However, few studies have addressed how the presence of closely related heterospecifics in a shoal may alter the behavioral responses of individuals to chemical predator cues.

To address this question, we investigated the antipredator responses of focal bluegill *Lepomis macrochirus* when they were solitary or in shoals of three with either an additional two conspecifics, or two heterospecifics consisting of the sympatric congener, pumpkinseed *L. gibbosus*. These two species share similar habitats in their early life history stages (Xia et al. 2018) and share common predators ranging from birds, including the common loon *Gavia immer* and great blue heron *Ardea herodias*, to piscivorous fish, including largemouth bass *Micropterus salmoides* and Northern pike *Esox lucius* (Wilson and Godin 2009). The test fish were exposed to one of three chemical stimuli posing graded levels of risk (sensu Brown et al. 2009): lakewater control (lowest risk), predator odor or kairomones (intermediate risk), or chemical alarm cues (highest risk). We predicted that (1) bluegill would demonstrate graded antipredator responses proportional to the level of risk conveyed by the chemical cues, (2) bluegill in shoals would demonstrate greater antipredator responses when shoals were more similar in species composition and body size, (3) smaller individual bluegill would generally demonstrate greater antipredator responses than larger individuals in response to predator cues, and (4) relative pre-stimulus behavior would be correlated with post-stimulus behaviour.

Materials and methods

Study site and specimen collection

We conducted the study at the Queen's University Biological Station (QUBS) on Lake Opinicon (44°35′06″N, − 76°17′47″

W) in Elgin, Ontario, Canada. From 7 to 11 May 2017, we collected a total of 155 bluegill (mean total length + SE = 114 + 17.6 mm) and 60 pumpkinseed (mean total length + SE = 133 + 34.4 mm), by either beach seine netting or angling with size 8 barbless J-hooks baited with earthworm *Umbrica* spp. Any fish that were deeply hooked, bleeding, or showing any sign of impairment were not suitable for our study. Because they were considered viable, we immediately released them back into the lake as stipulated in the scientific collection permit issued by the Ontario Ministry of Natural Resources and Forestry. We transferred the bluegill and pumpkinseed via aerated coolers into separate floating net pens (~ 3.5m^3) in Lake Opinicon. Approximately 1 h before each block of trials, we moved test fish for that block from the net pens into the QUBS wet lab and introduced them into three glass aquaria. Upon completion of each block of trials, we released the test fish back into the lake.

Preparation of alarm cue and predator odor

We euthanized five bluegill (mean total length + SE = 129 + 11.5 cm) via cerebral percussion to generate the alarm cues used in this experiment. We removed skin fillets from both sides of the fish and mechanically homogenized and diluted them with lake water to a final concentration of 0.1 cm^2 of skin/ml. This method and concentration have previously been shown to elicit predictable alarm responses in shoals under laboratory and field conditions (Brown and Godin 1999; Wisenden et al. 2003; Brown et al. 2009). We kept one adult Northern pike (total length = 45 cm, weight = 650 g) captured via angling from the same area of the lake as the bluegill and pumpkinseed in an outdoor holding tank filled with ~ 200 L of water, food-deprived, for 24 h to generate the predator odor. We froze the alarm cues and predator odor in 60 mL aliquots and stored them at − 20 °C until needed.

Experimental apparatus and design

Each aquarium measured 60 cm (length) × 30 cm (width) × 30 cm (height) and was filled with lake water to a depth of 18 cm (~ 32.54 L volume). To reduce exterior interference, we covered three of the walls on each aquarium with white paper. We taped green construction paper on the outside of the bottom surface to mimic natural substrate and left the fourth wall uncovered to allow visual observations (Fig. S1). We surrounded the test aquaria with a curtain to minimize disturbance and monitored each of them with two remotely controlled GoPro™ cameras (GoPro, San Mateo, California, USA) placed in front of and above the aquaria to record the responses of focal individuals. We positioned the cameras to capture the entire width of the aquarium in their fields of view.

We used three shoal scenarios for the trials, consisting of a solitary bluegill, a single-species shoal of three bluegill, and a mixed-species shoal of one bluegill and two pumpkinseed. In each scenario, observations focused on a single bluegill, which was haphazardly chosen and identified on video prior to each experimental trial. Each focal bluegill, as well as any shoalmates, were tested only once, measured (total length), and then released back into the lake. Trials consisted of 5 min pre-stimulus observations followed by the injection of 20 ml of lake water (control), alarm cues, or predator odor via plastic syringes and 1.5 m pieces of standard aquarium airline tubing, ~ 3 cm below the water surface in the back-right corner of each aquaria (Fig. S1), and an additional 5-min post-stimulus observation. We used a random number generator to assign treatments. Due to logistical constraints, we placed an upper limit of 12 trials per treatment per shoal type (Table 1). To avoid any contamination from previous trials, we emptied and cleaned each tank before starting the next trial. The mean temperature of the lake water used in the aquaria, creation of alarm cue, and predator odor, and in the floating pens was 14 °C.

Measurement of antipredator responses

We reviewed the videos to evaluate the baseline behavior and responses of bluegill to each treatment in terms of shoaling, area use, and overall movement. As per previous antipredator experiments (Mathis and Smith 1993; Chivers et al. 1995), we measured the cohesiveness of the shoal (shoal index) every 15 s and assigned a score of 1–3 depending on the number of fishes which were within a body length of the focal fish, where 1 = the focal fish was alone and 3 = all three fish were shoaling. These values were summed at the end of the pre- (baseline) and post-stimulus (treatment) periods to give the trial a final score. To determine if the addition of alarm cues or predator odor-affected area use, we divided the back wall of the aquaria into three horizontal zones, measuring 30 cm × 6 cm and again noted the location of each fish at 15-s intervals. Area use scores ranged from 1 to 3 for single bluegill and from 3 to 9 for the shoals, with a score of 1 assigned if a fish was in the bottom zone and a score of 3 if a fish was in the top zone. Activity as expressed in the total number of horizontal line crosses by a focal fish during the observations was measured against a 2 × 4 grid of 15 cm × 15 cm squares on the underside

Table 1 The sample size used for each shoal type with respect to the treatments they received

	Treatments		
Shoal type	Control	Alarm cue	Predator odor
Single bluegill	12	9	11
Multiple bluegill	10	10	11
Mixed shoal	11	9	10

of the tanks. In addition, we recorded any aggressive interactions between the focal bluegill and its shoalmates and scored aggression as a binary variable with 0 = no aggression during a pre- or post-stimulus observation period, and 1 = at least one act of aggression in either observation period. To minimize observer bias, blinded methods were used when all behavioral data were analyzed.

Statistical analyses

Although homogeneity of error variances in the three linear behavioral response variables (differences in shoal index and area use, and line crosses post-stimulus) did not differ between shoal types or chemical cues (Levene's F test, all $P > 0.05$), the response variables did not meet omnibus assumptions of normality (Shapiro-Wilk test, all $P < 0.05$), so we rank-transformed them for analysis (Scheirer et al. 1976). We partitioned the data into two groups, consisting of (1) the single bluegill treatment or (2) the shoals of three bluegill or one bluegill and two pumpkinseed. For the single bluegill, we combined area use and line crosses into a multivariate response and analyzed against chemical cue type as a fixed-effects factor, and baseline activity level (line crosses pre-stimulus) and fish size (total length, mm) as linear covariates in a MANCOVA. For the shoals, we combined all three behavioral measures into a multivariate response and analyzed against shoal type and chemical cue as fixed-effects factors, and baseline activity level and total length range (mm) of the shoal (difference in total length between the largest and smallest shoal members) as linear covariates. Aggression in the shoals was analyzed in a logistic regression with shoal type and cue as fixed-effects factors and baseline activity and total length range as linear covariates. All analyses were conducted using R version 3.4.3 (R Core Team 2017) and the "car" library (Fox and Weisberg 2011), and Figs were constructed using base R and "gplots" (Warnes et al. 2016).

Data availability The datasets generated and analyzed during the current study are available in the Open Science Framework repository, https://osf.io/bnsh8/?view_only=2c163365f7a0436f8ba6410bd469d15b.

Results

Individual bluegill

The multivariate response (area use and line crosses) was significantly influenced by pre-stimulus activity level, but not cue type or fish size (Table 2). Individually, the difference in area use was independent of pre-stimulus activity level (Fig. 1a; Table 2), fish size (Fig. 1b; Table 2), and cue type, although trials with alarm cue and predator odor showed non-significantly increased area use compared to control trials (Fig. 1c; Tables 2 and S1). Post-stimulus activity (line crosses) was independent of cue type (Fig. 1d; Table 2), although there were trends towards increased activity in response to predator odor and non-significantly decreased activity in response to alarm cues relative to the control treatments (Table S2). Post-stimulus activity varied positively with pre-stimulus activity (Spearman's $\rho = 0.669$, $P < 0.0001$; Fig. 1e; Table 2) and was independent of fish size (Fig. 1f; Table 2).

Conspecific and mixed-species shoals

The multivariate response (shoal index, area use, and line crosses) was not influenced by cue type, shoal type, the interaction between cue and shoal, pre-stimulus activity, or size range of the shoal members (Table 2). Although there were no significant differences in shoaling index and area use when analyzed by cue or shoal type, when alarm cues were introduced, conspecific shoals were non-significantly more dispersed horizontally, but non-significantly less dispersed vertically, than mixed-species shoals (Fig. 2a, b, respectively;

Table 2 Full results of analyses of bluegill *Lepomis macrochirus* responses to chemical cues as singletons or in shoals of three fish, composed of three conspecifics or one bluegill and two pumpkinseed *Lepomis gibbosus*. Fish/shoal size refers to total length (mm) of focal fish (singles) or total length range (mm) of shoal members (shoals). Test statistics are Wilks' λ (MANCOVA) and F (ANCOVA). Italics with asterisk (*) denotes $P < 0.05$, bold and italics denote $0.1 > P > 0.05$

		Cue type			Shoal type			Cue/shoal			Pre-stimulus activity			Fish/shoal size		
	Response	Statistic	df	*P*	Statistic	df	*P*	Statistic	df	*P*	Statistic	df	*P*	Statistic	df	*P*
Singles	Multivariate	λ 0.755	4.52	0.114	–	–	–	–	–	–	0.705	2.26	*0.011**	0.941	2.26	0.455
	Line crosses	F 2.591	2.27	***0.094***	–	–	–	–	–	–	10.829	1.27	*0.0028**	0.270	1.27	0.608
	Area use	F 1.234	2.27	0.307	–	–	–	–	–	–	1.594	1.27	0.218	1.596	1.27	0.217
Shoals	Multivariate	λ 0.951	6.102	0.859	0.925	3.51	0.261	0.800	6.102	***0.072***	0.961	3.51	0.551	0.919	3.51	0.227
	Line crosses	F 0.146	2.53	0.662	4.185	1.53	*0.046**	0.700	2.53	0.501	0.770	1.53	0.384	2.017	1.53	0.161
	Area use	F 0.139	2.53	0.870	1.739	1.53	0.193	0.914	2.53	0.407	0.077	1.53	0.783	0.673	1.53	0.416
	Shoal index	F 0.271	2.53	0.764	0.005	1.53	0.942	2.701	2.53	***0.076***	0.506	1.53	0.480	3.027	1.53	***0.088***

Tables 2 and S3-S4). In addition, neither shoaling index nor area use varied with pre-stimulus activity (Fig. 2c, d; Table 2) or shoal size range (Fig. 2e, f; Table 2). Line crosses were independent of cue type but varied significantly with shoal type (Table 2), as conspecific shoals tended to demonstrate a greater number of line crosses (Fig. 2g). Line crosses also positively related to pre-stimulus activity levels (Spearman's $\rho = 0.679$, $P < 0.0001$; Fig. 2h; Table 2) and were independent of shoal size range (Fig. 2i; Table 2). Aggression was observed in only one out of 61 trials, in a mixed-species shoal exposed to bluegill alarm cue, so this measure was excluded from analysis and we did not perform a logistic regression.

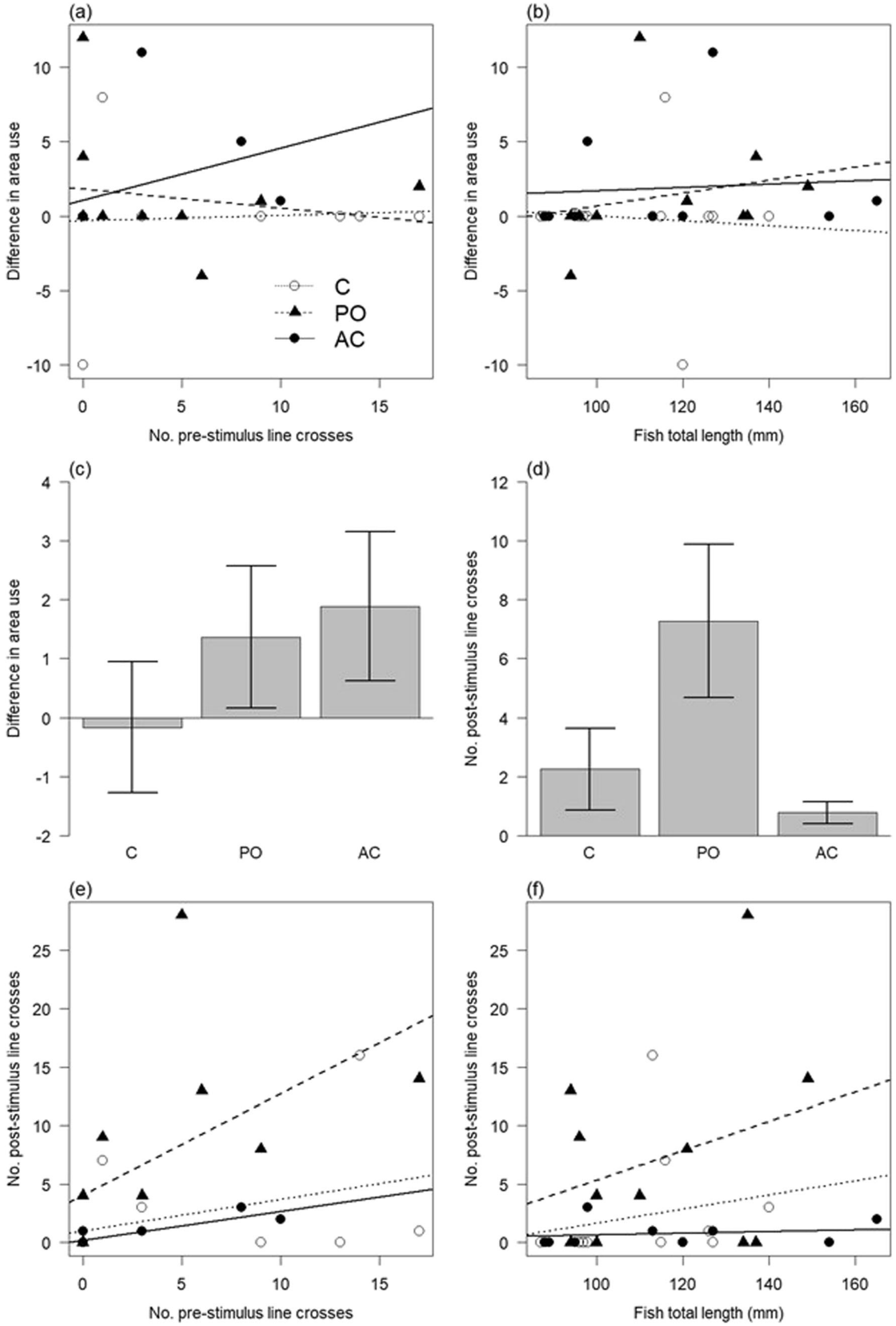
Discussion

In this study, we investigated the antipredator responses of bluegill when solitary and in single- and mixed-species shoals when exposed to alarm cues, predator odor, and lakewater controls. Our results suggest that behavioral patterns of solitary bluegill displayed during the immediate pre-stimulus period were the most important determinant of post-stimulus responses, and that in shoals, shoal composition appeared to have a greater influence on antipredator responses than cue type. In addition, neither body size of single bluegill nor the size range of the shoals influenced the magnitude of antipredator responses. These results highlight the importance of ecologically relevant heterospecific groupings to the behavioral strategies of prey individuals.

Single bluegill and bluegill in single- and mixed-species shoals did not demonstrate significantly different responses to the different chemical cues, in contrast to our a priori predictions of a graded, threat-sensitive pattern of response (Brown et al. 2009). However, single bluegill did demonstrate a response pattern in their activity levels consistent with the predicted values of the different cues to which they were exposed. Damage-released chemical alarm cues are reliable indicators of elevated predation risk, and immediate behavioral responses that mediate predation risk, such as cessation of conspicuous movement, confer survival advantages to individuals that detect them (Chivers and Smith 1998; Brown 2003). This was observed in our experiment as a decrease in overall activity by the single bluegill following exposure to alarm cues (Fig. 1d). Predator odors (kairomones), by contrast, indicate the presence of a potential predator but not necessarily that the predator currently poses a risk by actively foraging. Prey fish can also assess the recent diet of a potential predator based on the release of species-specific metabolites to determine whether the predator poses an immediate threat (Jachner 1997; Ferrari et al. 2010). The olfactory detection of a potential predator also provides prey the opportunity to locate and avoid the predator, which can manifest in increased activity levels associated with locating potential sources of risk (Smith 1997). Increased activity levels may also function as predator deterrents in some species (Brown et al. 1999), which is likely to occur with individuals seeking areas of refuge (Smith et al. 2009), explaining the increased number of lines crossed in the single bluegill experiments following exposure to predator odor (Fig. 1d).

Baseline differences between individuals generally predicted their antipredator responses, with individuals of greater baseline levels of activity during the behavioral trials maintaining higher activity levels following stimulus exposure. Although we only recorded two measurements per individual in the single bluegill treatment, the significant positive correlation between those measurements is indicative of repeatability (Biro and Stamps 2015) of activity as a behavioral measure in the fish we observed. Potential consequences for individuals arranged along shy-bold or more-or-less exploratory axes (Mazué et al. 2015) may include increased risk of predation, although relatively riskier strategies may also accrue greater fitness benefits for successful individuals (Lima and Dill 1990; Mittelbach et al. 2014). An example would be the tradeoff between sustained foraging rates in the presence of a predator, where it may be more beneficial for individuals to behave without displaying antipredator behavior in the face of predation (Angradi 1992). As previously noted, body size of the focal bluegill was independent of their behavioral responses, further supporting the notion that behavioral responses to risky cues are shaped by intrinsic (personality) factors rather than size.

The responses of focal bluegill in single- or mixed-species shoals did not differ significantly in response to cue type, but post-stimulus activity level differed between shoal type, with bluegill in single-species shoals demonstrating greater activity levels independent of cue than bluegill in mixed-species shoals. We also observed a tendentially significant interaction between cue and shoal types on the difference in shoaling index, with single-species shoals demonstrating trends towards increased cohesion following exposure to alarm cues, and mixed-species shoals towards decreased cohesion (Fig. 2a). Size range of the shoals (difference between the largest and smallest individuals in the shoal) also had a tendentially significant effect on shoaling index with smaller size ranges associated with greater shoal cohesion following cue exposure (Table 2; Fig. 2e). Collectively, our results can be supported by previous studies which found that the benefits of shoaling in response to predation risk are greatest when shoals are more similar in composition and size and decrease as differences between shoal members increase (Hoare et al. 2000; Ward et al. 2002). For example, shoals comprised of three species of cyprinids segregated by species upon exposure to a predator model (Allan and Pitcher 1986). Ultimately, dissimilarity of an individual within a shoal can increase prey recognition by a predator which can outweigh the "confusion effect" (Allen 1920). Alternatively, the greater post-


(a)
Difference in area use
10
5
0
-5
-10
0
5
10
15
No. pre-stimulus line crosses
C
PO
AC
(b)
Difference in area use
Fish total length (mm)
100
120
140
160
(c)
Difference in area use
4
3
2
1
0
-1
-2
C
PO
AC
(d)
No. post-stimulus line crosses
12
10
8
6
4
2
0
C
PO
AC
(e)
No. post-stimulus line crosses
25
20
15
10
5
0
No. pre-stimulus line crosses
(f)
No. post-stimulus line crosses
Fish total length (mm)

◀ **Fig. 1** Linear relationships between area use and **a** pre-stimulus line crosses and **b** fish size demonstrated by single bluegill *Lepomis macrochirus* in response to predator (Northern pike *Esox lucius*) odor (PO; triangles, dashed lines), alarm cues (AC; closed circles, solid lines), or a lakewater control (C; open circles, dotted lines). Mean (± SE) differences in **c** area use and **d** post-stimulus number of line crosses and linear relationships between post-stimulus line crosses and **e** pre-stimulus line crosses or **f** fish size

stimulus activity levels we observed in the single-species compared to the mixed-species shoals could be indicative of greater levels of cooperation among conspecifics. While increasing movement within a shoal may serve to enhance the confusion effect on predators, more distantly related shoalmates may be more likely to defect from a collective confusion strategy, leaving individuals with

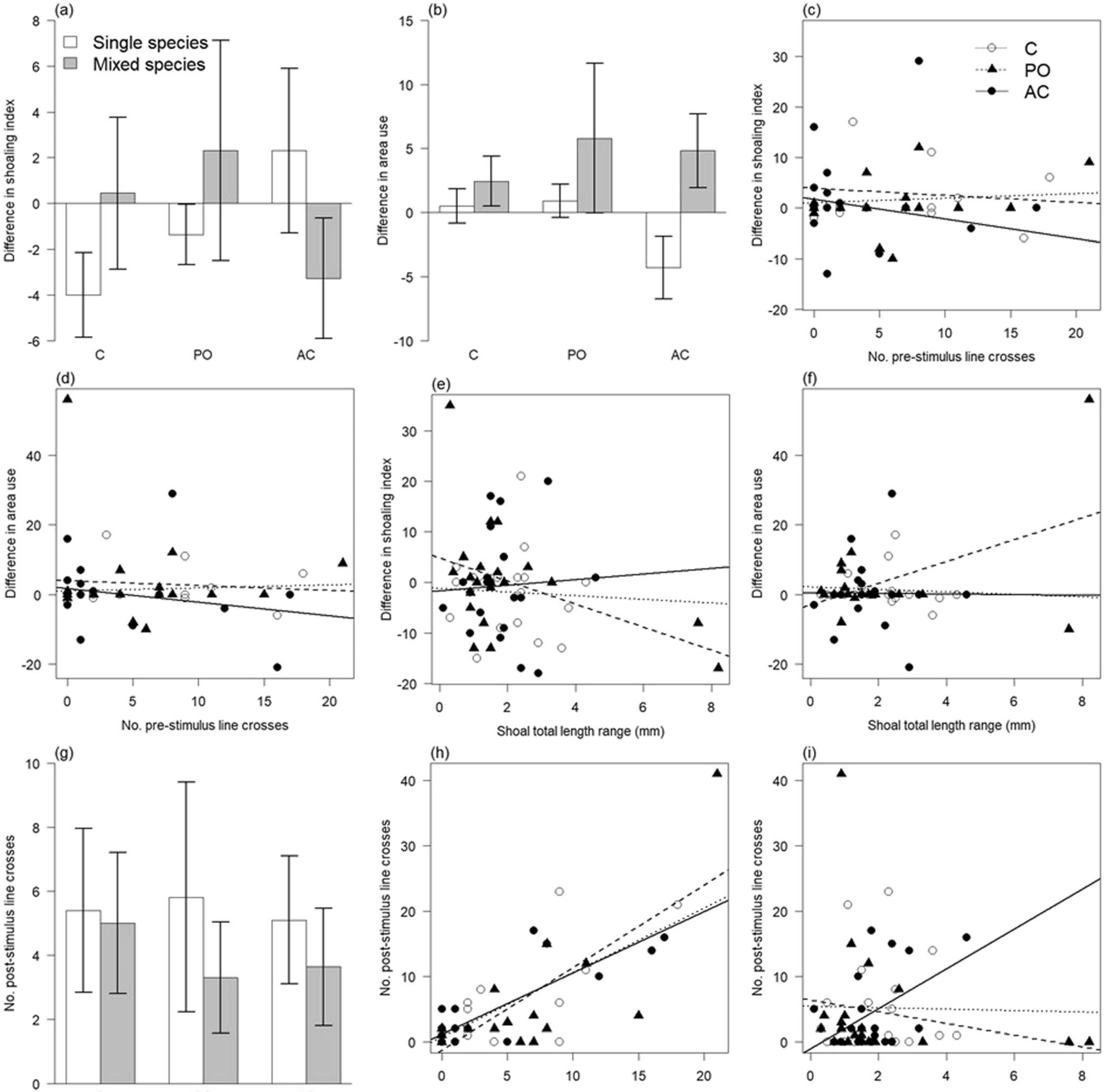


Fig. 2 Mean (± SE) differences in **a** shoaling index and **b** area use, and **g** post-stimulus number of line crosses demonstrated by focal bluegill *Lepomis macrochirus* in shoals of three bluegill (open bars) or mixed-species shoals consisting of one bluegill and two pumpkinseed *Lepomis gibbosus* (gray bars) to predator (Northern pike *Esox lucius*) odor (PO; triangles, dashed lines), alarm cues (AC; closed circles, solid lines), or a lakewater control (**c**; open circles, dotted lines), and linear relationships between shoaling index, area use, or line crosses and pre-stimulus activity levels (**c**, **d**, **h**) and size range of the shoal (**e**, **f**, **i**)

more conspicuous movement patterns to draw the attention of predators.

A caveat to our experiment was the fact we only used a single predator to create the predator odor cue, as the scent of a single individual may not be representative of the species. For example, since prey fish are able to determine the diet of a predatory fish through its odor, any ingestion by predatory fish may affect how prey perceive the threat (Ylönen et al. 2007). Although we kept the pike in a state of starvation after capture to remove the possibility of unwanted prey ingestion and changes to its odor, it is possible that the pike had fed shortly beforehand. If the pike in our experiment had fed on bluegill prior to capture, our test individuals may have perceived the predatory odor to be more of a risk than we intended, explaining why the difference between the intermediate- and high-risk cues was non-significant.

In conclusion, the results of this study show that intrinsic factors may better predict the antipredator responses of solitary bluegill rather than their physical characteristics. In the single bluegill experiments, we found that pre-stimulus activity levels significantly affected the multivariate response (area use and lines crossed) and post-stimulus activity. In the shoal experiments, line crosses were significantly influenced by shoal type and positively related to pre-stimulus activity, with this relationship more pronounced in single-species shoals. These results demonstrate the need to explore factors other than physical characteristics that may affect predator-prey interactions, to better understand the social workings within fish species. For example, individuals that are bolder in safe environments tend to also be more bold than shyer conspecifics in the presence of a predator (Smith et al. 2009). However, to our knowledge, no studies have yet investigated whether the behavior of an individual differs when exposed to predatory cues in isolation versus as part of a shoal. We encourage future studies linking fish personality with antipredator behavior to include ecologically relevant scenarios involving sympatric individuals.

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Author contributions Author contributions: All the authors conceived and designed the experiments. JIL, SMD, and WMT performed the experiments. CKE analyzed the data. JIL and CKE wrote and revised the manuscript; SMD and WMT participated in the editing and revision process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and all work reported herein was conducted in accordance with the guidelines set by the Canadian Council on Animal Care and specified in Carleton University Teaching Protocol no. 105370. Approval from an ethics committee was not required for this study.

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