



# Boldness and exploratory behaviors differ between sunfish (*Lepomis* spp.) congeners in a standardized assay

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## Abstract

The shy-bold behavioral continuum is an element of animal behavior which is often studied for its ecological relevance, particularly in the context of predation risk. How individuals respond to various predation cues is well studied at the individual level, but relatively little is known about how these responses can differ among closely related species. We exposed individual wild-caught juvenile bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) to kairomones of a common predator (Northern pike, *Esox lucius*), conspecific alarm cues, or a lake water control in a Z-maze trial commonly used to assess relative levels of bold and exploratory behaviors. Neither species exhibited any significant behavioral responses to either predation cue, but bluegill consistently displayed more bold-type behaviors than pumpkinseed. Although the lack of a behavioral response to predation cues in this study is equivocal, we identify clear differences in boldness between these two congeners and discuss the possibility of ecological niche differentiation driving these behavioral differences.

## Significance Statement

Closely related species exposed to similar predator guilds may differ in their behavioral responses to predation risk, particularly during different life-history stages. We exposed juveniles of two co-occurring sunfish congeners (*Lepomis* spp.) to chemical predation cues (predator kairomones or conspecific alarm cues) in a Z-maze assay to assess relative levels of bold and exploratory behaviors. Bluegill (*L. macrochirus*) were consistently bolder and more proactive than pumpkinseed (*L. gibbosus*). Ontogenetic ecological niche differentiation between these species may be the cause of these observed differences and life-history trajectories should be considered in the design of future research questions.

**Keywords** Alarm cue · Bluegill · Kairomone · Predation · Pumpkinseed

## Introduction

Individuals of many species are known to exhibit consistent inter-individual differences in behaviors, thus indicating the presence of different behavioral syndromes or “personalities” (Sih et al. 2004a, b; ale et al. 2007, 2010; Dall et al. 2012). Animal personalities both reflect and influence various ecological dynamics, notably plasticity in individual responses and short-term adaptability to environmental stressors (Sih et al. 2004a). Animal personalities have generally been studied in an eco-evolutionary context across five non-mutually exclusive behavioral axes consisting of shyness-boldness, exploration-avoidance, activity, sociability, and aggressiveness (Réale et al. 2007). The shy-bold axis is particularly relevant to how animals deal with risk and assess ambient risk levels, for example when faced

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with a foraging predator or a novel potential predator. In fishes, the shy-bold continuum has been a focus of behavioral studies due to its ecological relevance (Conrad et al. 2011; Mittelbach et al. 2014) and its potential relationship with susceptibility to angling and other methods of capture or harvesting (see Biro and Post 2008; Uusi-Heikkilä et al. 2008; Wilson et al. 2011, 2015; Keiling et al. 2020).

A variety of behavioral measures are used to evaluate individual shyness or boldness in fishes. In these situations, bolder individuals behave in a less risk-averse manner than more shy or timid (i.e. less bold) individuals (Toms et al. 2010; Conrad et al. 2011). Specifically, when faced with some form of risk, shyer individuals may reduce their swimming activity and general conspicuousness to avoid potential threats, whereas bolder individuals may do so to a lesser degree, and may even approach and inspect the threat to refine their assessment (Dugatkin and Godin 1992a, b; Wilson et al. 1993; Wilson and Godin 2009; Pellegrini et al. 2010; Ramsaran et al. 2021; Dos Santos et al. 2023). Intraspecific variation of shy-bold behaviors has been attributed to several factors such as individual body size (Brown and Braithwaite 2004; Brown et al. 2005, 2007b; Darby and McGhee 2019), habitat preference (Wilson and Godin 2009), environmental variability (Riesch et al. 2009; Biro et al. 2010; Frost et al. 2013), physiological status (e.g., metabolic rate and endocrine status; McKenzie et al. 2015; Bailey et al. 2022), and past experience with predation risk (Brown et al. 2005, 2007a, b, 2009, 2010, 2013; Magnhagen and Borcharding 2008; Archard and Braithwaite 2010; Magnhagen et al. 2012; Elvidge et al. 2016; Money et al. 2017; Des Roches et al. 2022).

Predation is a critical driving force of evolution, and its influence on fish personality is well documented (Smith and Blumstein 2008; Conrad et al. 2011; Lönnstedt et al. 2012). Selective forces driving behavioral phenotypes likely create a trade-off (Sih et al. 2004b; Brown and Chivers 2005; Smith and Blumstein 2008) where bolder individuals gain fitness benefits such as greater reproductive success and higher growth rates but suffer from higher mortality (Biro et al. 2004; Ward et al. 2004; Stamps 2007). These tradeoffs help explain the persistence of both bold and shy behavior phenotypes (Réale and Festa-Bianchet 2003; Biro et al. 2004; Ward et al. 2004; Stamps 2007; Hulthén et al. 2017). The presence of predators with different foraging methods could contribute to maintaining a balance of behavioral phenotypes, while differences in the relative magnitudes of certain predation pressures could favour the persistence of certain behavioral phenotypes over others. For example, shyer individuals have been shown to more susceptible to predation by ambush fish predators than bolder individuals (Blake et al. 2018), while bolder individuals have been

shown to be more susceptible to predation by aerial piscivores (Balaban-Feld et al. 2022).

The use of chemical cues is an important mechanism for prey to identify and respond to predation risk (Smith 1992; Mathis and Smith 1993a; Chivers et al. 1996; Burks and Lodge 2002; Mirza and Chivers 2002a; Wisenden and Chivers 2006; Ferrari et al. 2010). Predator kairomones, for example, are odours released by predators which can inform prey species about their presence. Kairomones can provide information about species, density, size, and proximity of predators (Mirza and Chivers 2002b; Kusch et al. 2004; Ferrari et al. 2006; Wisenden and Chivers 2006). Another chemical cue used by prey to identify predation risk are alarm cues, which are chemical signals released by damaged prey tissues (Smith 1992; Chivers et al. 1996; Ferrari et al. 2010). Much like kairomones from predators that co-evolved with prey, antipredator behavioural responses to alarm cues are innate (Berejikian et al. 2003; Hawkins et al. 2007; Hain and Neff 2009; Mitchell et al. 2012; Kopack et al. 2015) but can also be learned in situations with novel risk such as the introduction of an unfamiliar predator species (Mathis and Smith 1993b; Mirza and Chivers 2001, 2003a; Chivers et al. 2002; Mirza et al. 2003; Pollock et al. 2003; Darwish et al. 2005; Ferrari et al. 2005, 2005, 2007, 2008a; Brown et al. 2011). Naturally, alarm cues represent a greater perceived threat than kairomones as they indicate that an attack or predation event has recently occurred (Jordão 2004; Wisenden and Chivers 2006; Ferrari et al. 2010) although these cues may be used by prey together in an additive fashion (Burks and Lodge 2002; Ferrari et al. 2008a). Altogether, detection of these chemical cues provides fitness benefits for prey by allowing them to adjust behavior appropriately based on the severity of threats (Brown 2003; Wisenden et al. 2004; Wisenden and Chivers 2006; Ferrari et al. 2010; Landeira-Dabarca et al. 2019). These antipredator responses often include behaviours such as freezing or dashing, but can also induce bolder predator inspection behaviours (Chivers and Smith 1998; Brown and Godin 1999; Brown 2003; Pellegrini et al. 2010; Ramsaran et al. 2021). Interestingly, these behavioral responses show great interspecific and intraspecific variation (Chivers and Smith 1998).

Interspecific variations in personality, especially between congeners, have not been thoroughly investigated in literature. Nonetheless, behavioral responses to predation cues have been reported to differ between closely related fish species (Kiesel et al. 2012; Wilson et al. 2021), with niche separation (Xia et al. 2018), differential life history traits, and differential predation risk between species (Riesch et al. 2009; Li et al. 2022) often being implicated as potential causal factors. Indeed, behavioral differences can arise between species of the same trophic level when they are exposed to differential predation pressure (Kats and Dill

1998), and these responses can differ based on the species of predator and associated risks (Blake et al. 2018). However, it remains unclear to what degree personality, including shy-bold behaviors, may differ on average between species which are exposed to similar predators and predation risk.

Bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) are two congeners that occupy different ecological niches as adults where they coexist. Specifically, bluegill tend to spend more time foraging in pelagic areas than pumpkinseed, which forage more in benthic areas (Keast 1978a; Keast et al. 1978; Holt 1984; Mittelbach 1984). Nonetheless, the two species share similar thermal maxima (Becker and Genoway 1979) and juveniles of both species utilize similar shallow complex habitats and are often found together in these areas (Keast 1978a; Polis 1984; Osenberg et al. 1992). The objective of this study was to investigate how the kairomones of a common predator that has long co-evolved with both focal species (Northern pike, *Esox lucius*) and conspecific alarm cues affect shy-bold behaviors of wild-caught juvenile bluegill and pumpkinseed. We tested three hypotheses: (1) that predation cues would elicit bold and exploratory behaviors among both species as a predator inspection response (see Pellegrini et al. 2010; Ramsaran et al. 2021), (2) that both species would exhibit a greater behavioral response to alarm cues than to kairomones due to the greater risk associated with them, and (3) that the two species would vary in their behavioral response to predation cues.

## Methods

### Study site and species

This study was performed at the Queen's University Biological Station (QUBS) on Opinicon Lake, Ontario, Canada (44°34' N, 76°19' W) from August 9–18, 2023. Opinicon Lake is an 800 ha lake with an average depth of 2.5 m and

**Table 1** Body metrics of groups of juvenile bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. macrochirus*) used in Z-maze arena assays or as alarm cue donors

	Mass range (g)	Mean ± SD mass (g)	Total length range (mm)	Mean ± SD total length (mm)
Bluegill Z-maze	10.5–42.5	21.3 ± 8.5	90–139	113 ± 14
Pumpkinseed Z-maze	12.5–43.0	30.0 ± 7.8	94–139	122 ± 11
Bluegill alarm cue	NA	NA	90–117	114 ± 12
Pumpkinseed alarm cue	NA	NA	95–139	130 ± 8

Note Mass was not measured for fish used as alarm cue donors

a maximum depth of 11 m, supporting large populations of bluegill and pumpkinseed (Keast 1978a, b). Bluegill and pumpkinseed are closely related on a phylogeny, although there exists more closely related *Lepomis* species (Near and Kim 2021). Although these congeners occupy distinct ecological niches as adults (Keast 1978a; Xia et al. 2018), juveniles of both species are commonly preyed upon by Northern pike and black bass (*Micropterus* spp.), as well as a few avian predators (Keast 1978b, 1985).

Juvenile bluegill ( $n=64$ ; Table 1) and pumpkinseed ( $n=48$ ; Table 1) were captured from a boat via angling using size 8 baitholder hooks with crimped barbs and small chunks of earthworms (*Lumbricus* sp.). Fish were landed within 10 s of hooking and air exposure was minimized. Any deeply hooked fish (i.e., not hooked in the jaw) were released. Pumpkinseed and bluegill hooked in the jaw are known to have very high survival rates when angled (i.e., nearly 100%; Cooke et al. 2003). Once captured, fish were held in a 100 L cooler filled with lake water and equipped with a portable aerator (Frabill Inc., Chicago, IL, USA) until they could be transferred to holding facilities at QUBS (< 1 h). Fish were then held to acclimatize, unfed, for at least 24 h in one of two outdoor 500 L flow-through tanks (separated by species) each with their own continuous supplies of unfiltered lake water that flowed directly back into the lake. Water temperatures in mid August were stable at ~25 °C. All behavioral assays were completed within 96 h of capture.

### Chemical cue preparation

Water was drawn directly from Opinicon Lake and frozen in 60 mL aliquots in Whirl-Paks (Filtration Group, Austin, TX, USA) to serve as negative (process) controls as this water presumably contained negligible concentrations of predator cues. Three Northern pike (527–600 mm total length, 787–1052 g wet mass) were captured via angling from Opinicon Lake on August 9 to serve as predator odour (kairomone) donors. Kairomone collection followed Ramsaran et al. (2021) with the pike held in a covered outdoor 500 L flow-through tank supplied with lake water for 12.5 h before the water circulation was turned off for 12.5 h to let their kairomones accumulate. The pike were then released back into the lake and water was drawn from their holding tank, mixed to ensure homogeneity of kairomone concentration, and frozen in 60 mL aliquots by placing in a -20 °C freezer until use to preserve the chemical cues (Wisenden et al. 2009).

Damage-released chemical alarm cues from each sunfish species were used as positive controls as they convey a greater degree of predation risk than kairomones and thus widely known to elicit antipredator responses in prey

(Jordão 2004; Wisenden and Chivers 2006; Ferrari et al. 2010). Juvenile bluegill ( $n=7$ ; Table 1) and pumpkinseed ( $n=6$ ; Table 1) were euthanized via cerebral percussion and lateral skin filets were removed from both sides of each donor between the pectoral and caudal fins using a scalpel and fine forceps. Skin filets ( $\sim 240$  cm<sup>2</sup> per species) were each mechanically homogenized, filtered to remove debris, and diluted in chilled lake water to final concentrations of 1 cm<sup>2</sup> skin per 10 mL water ( $\sim 2.4$  L per species). The resulting alarm cues were packaged in 60 mL aliquots in Whirl-Paks and immediately placed in a  $-20$  °C freezer until use to preserve the chemical cues (Wisenden et al. 2009).

## Behavioral assay

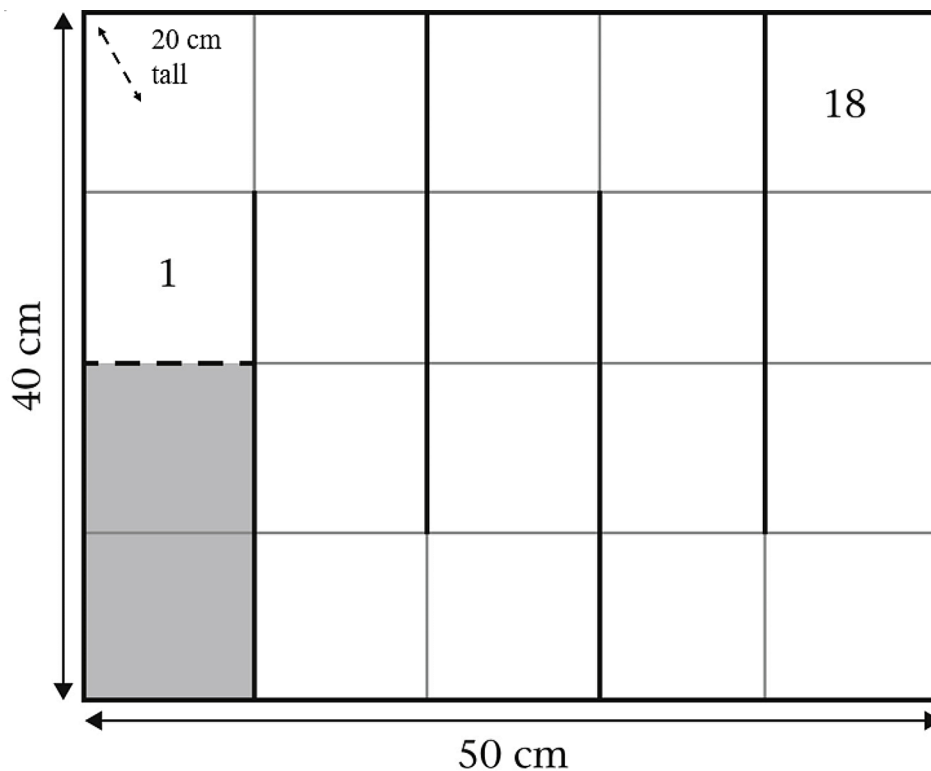
All behavioral assays were conducted during daylight hours in an indoor laboratory space at QUBS. Treatment groups (control, kairomone, or alarm cues) were sequentially alternated between assays to account for temporal variation in behaviors. The assay arena was thoroughly rinsed thrice with fresh lake water between trials before undergoing a complete water change to ensure no cues were leftover from previous trials. Focal bluegill were exposed to Northern pike kairomones, bluegill alarm cues, pumpkinseed alarm cues, and lake water controls ( $n=16$  each). Pumpkinseed were exposed to the same treatments except for bluegill alarm cues as a pilot study detected low overall rates of emergence from the acclimation chamber by pumpkinseed independent

of treatment (control: 25%; kairomone and pumpkinseed alarm cues: both 12.5%).

The Z-maze behavioral assay was set up as described by Lawrence et al. (2018) and Ramsaran et al. (2021), consisting of an arena (40 cm length, 50 cm width, 20 cm deep) filled with lake water to a depth of 6.6 cm (13.2 L). The bottom of the maze is divided with visible lines into 18 equal 10 cm x 10 cm squares numbered 1 (closest to acclimation chamber) to 18 (furthest from acclimation chamber; Fig. 1). A WiFi-enabled GoPro HERO5 camera (GoPro Inc., San Mateo, CA, USA) was mounted above the maze to record the trials using the companion smartphone app (<https://gopro.com/en/ca/shop/softwareandapp>). Individual fish were removed from their holding tank with a dip net and placed into the acclimation chamber as the 60 mL treatment aliquot was injected into the acclimation chamber. The fish was then allowed to acclimate for 5 min before the gate was opened using an overhead pulley system. Once the gate was raised, the focal fish was allowed 20 min to leave the acclimation chamber. If they failed to emerge, the outcome was recorded (binary: 0 = did not exit, 1 = did exit) and the trial was ended. If the focal fish did emerge (i.e. the head and pectoral fins crossed the gate threshold), a 5 min observation period was started.

Seven behavioral measurements were scored throughout the 5 min observation periods: (1) latency to exit the acclimation chamber (i.e. the time it took for the fish to exit once the gate was lifted); (2) the furthest square reached (from 0 to 18); (3) whether or not the fish finished the maze by

**Fig. 1** Schematic diagram of the Z-maze used for behavioral assays. The acclimation chamber (grey squares) is separated from the maze area (white squares) by a lifting gate (dotted line). Each square along the Z-maze is marked with light grey lines



reaching the furthest square; (4) the total number of lines crossed in the maze (between squares); (5) whether or not the fish re-entered the acclimation chamber; (6) the number of re-entries; and (7) total time spent in the maze (i.e. time spent outside of the chamber during the 5 min trials). For fish that did re-enter and/or finish the maze, their respective time(s) to do so from when they first exited the acclimation chamber were also recorded. After each individual trial was complete, the focal fish was weighed, measured (total length), and released back into the lake. It was not possible to record data without knowledge of treatment or species as our study involved visually identifiable focal species and logistical constraints resulted in a single experimenter (KAA) administering the treatments and transcribing the data.

### Statistical analyses

Binary response variables (whether or not a focal fish exited the acclimation chamber, finished the maze, and/or re-entered the acclimation chamber) were examined in generalized linear models (GLMs) with binomial error distributions against chemical cue type (treatment), species, and total length as predictor variables. For the subset of fish that did re-enter the acclimation chamber, the number of re-entries and the time it took from emergence until the first re-entry were analyzed with a GLM with Poisson error distribution and a linear (Gaussian) model, respectively, with the same structures as the binomial GLMs. For the subset of fish that finished the maze, the time it took to do so from initial emergence was also analyzed in a linear (Gaussian) model.

The remaining four behavioral responses recorded in trials where fish exited the acclimation chamber (latency to exit, furthest square reached, number of lines crossed, and time spent in the maze) were examined in a multiple analysis of covariance (MANCOVA) with the same structure as the GLMs. In general, the data were partitioned for analyses to prevent zero-inflation within behavioral responses. The GLMs were run using the ‘car’ package (Fox and Weisberg 2011). The linear response variables included in the MANCOVA were subsequently log-transformed for scaled principal component analysis (PCA) using the ‘factoextra’ (Kassambara and Mundt 2020) and ‘FactoMineR’ (Le et al. 2008) packages to identify discrete PC axes. Individual scores from principal components accounting for at least 5% of the variance were compared in an ANCOVA with species, treatment, and fish total length as predictor variables. All statistical analyses were performed in R version 4.2.1 (R Core Team 2022) and Figs were generated using the ‘ggplot2’ (Wickham 2016) and ‘cowplot’ (Wilke 2020) packages.

### Results

Whether or not a fish exited the acclimation differed significantly between species ( $\chi^2_{1,104} = 61.42, p < 0.001$ ) with more bluegill exiting than pumpkinseed (94% vs. 17%, respectively). However, the proportion of fish exiting the acclimation chamber did not differ significantly between chemical cue types ( $\chi^2_{3,104} = 0.23, p = 0.97$ ), nor was there a relationship between exiting and body length ( $\chi^2_{1,104} = 0.002, p = 0.96$ ) or in the interaction between chemical cue and species ( $\chi^2_{2,104} = 3.83, p = 0.15$ ).

Among the fish that did exit the acclimation chamber, subsequently re-entering it was not significantly influenced by chemical cue ( $\chi^2_{3,60} = 1.59, p = 0.66$ ), species ( $\chi^2_{1,60} = 2.04, p = 0.15$ ), or body length ( $\chi^2_{1,60} = 0.003, p = 0.96$ ), and there was no significant interaction between cue and species ( $\chi^2_{2,60} < 0.001, p > 0.99$ ). Similarly, among fish that re-entered the acclimation chamber at any point, there was no significant relationship between the number of re-entries and cue type ( $\chi^2_{3,53} = 0.69, p = 0.88$ ), species ( $\chi^2_{1,53} = 0.01, p = 0.91$ ), or body length ( $\chi^2_{1,53} = 1.18, p = 0.28$ ), and there was no significant interaction between cue and species ( $\chi^2_{2,53} = 0.17, p = 0.92$ ). The time it took for any fish to re-enter the acclimation chamber was also not significantly influenced by cue type ( $F_{3,53} = 0.44, p = 0.72$ ), species ( $F_{1,53} = 1.21, p = 0.28$ ), or body length ( $F_{1,53} = 0.88, p = 0.35$ ), and there was no significant interaction between cue and species ( $F_{2,53} = 0.44, p = 0.65$ ).

Whether or not a fish finished the maze (i.e., reached the terminus) did not differ significantly among chemical cue types ( $\chi^2_{3,60} = 2.89, p = 0.41$ ), but significantly more bluegill finished the maze than pumpkinseed ( $\chi^2_{1,60} = 4.09, p = 0.043$ ; 77% vs. 38%, respectively). There was also no significant relationship between finishing the maze and body length ( $\chi^2_{1,60} = 0.054, p = 0.82$ ), and there was no significant interaction between cue and species ( $\chi^2_{2,60} = 1.66, p = 0.44$ ). Among fish that did finish the maze, the time it took to do so was not significantly related to cue type ( $F_{3,42} = 0.29, p = 0.83$ ), species ( $F_{1,42} = 0.002, p = 0.96$ ), or body length ( $F_{1,42} = 0.26, p = 0.61$ ), and there was no significant interaction between cue and species ( $F_{2,42} = 2.46, p = 0.12$ ).

Among fish that exited the acclimation chamber, the multivariate response (latency to exit, furthest square reached, number of lines crossed, and time spent in the maze) did not differ significantly among chemical cue types but did differ significantly between species. The multivariate response also had no significant relationship with total length, and there was no significant interaction between cue and species (Table 2). The first two principal component axes of the multivariate Z-maze behaviors (PC1, PC2) accounted for over 97% of variance in responses, with PC1 accounting for 83.7% and PC2 for 13.5%. PC1 had positive loadings for

**Table 2** Multivariate responses of juvenile bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) in a novel Z-maze arena following exposure to Northern pike (*Esox lucius*) kairomones, bluegill alarm cues, pumpkinseed alarm cues, or lake water control

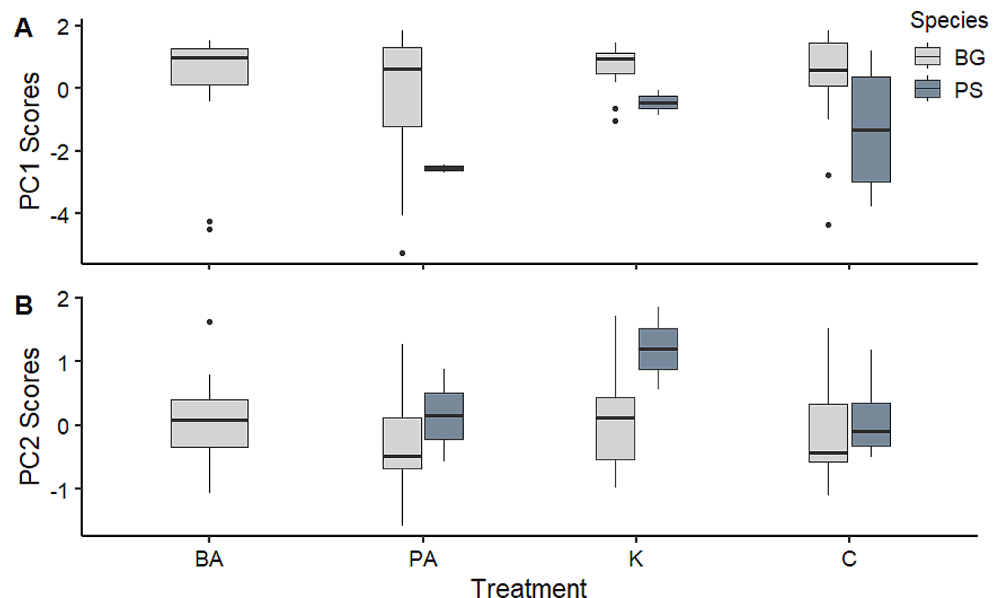
Response	Model Terms				
	Treatment	Species	Total length	Treatment × Species	
Multivariate	Pil-lai's trace	0.208	<b>0.200</b>	0.101	0.058
	F	1.10	<b>3.56</b>	1.59	0.44
	df	12,177	<b>4,57</b>	4,57	8,116
	P	0.36	<b>0.012</b>	0.19	0.90
Latency to exit	F	0.053	<b>10.17</b>	<0.01	0.87
	df	3,60	<b>1,60</b>	1,60	2,60
	P	0.98	<b>0.002</b>	0.99	0.42
Furthest square	F	1.89	<b>7.22</b>	0.003	0.64
	df	3,60	<b>1,60</b>	1,60	2,60
	P	0.14	<b>0.009</b>	0.96	0.53
Line crosses	F	0.16	<b>6.96</b>	2.38	0.33
	df	3,60	<b>1,60</b>	1,60	2,60
	P	0.92	<b>0.012</b>	0.13	0.72
Time in maze	F	0.84	3.71	0.11	0.49
	df	3,60	1,60	1,60	2,60
	P	0.48	0.59	0.74	0.62

Note Model terms with statistical significance (i.e.,  $P < 0.05$ ) are in bold font

**Table 3** Axis loadings of principal components of juvenile bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. macrochirus*) behaviors (latency to exit, furthest square reached, number of lines crossed, and time spent in the maze) in a Z-maze arena

Response	PC1	PC2	PC3	PC4
Latency	-0.74	0.67	0.02	0.01
Maze time	0.95	0.24	-0.16	-0.10
Square	0.98	0.14	-0.05	0.15
Lines crossed	0.97	0.14	0.21	-0.04

**Fig. 2** Individual principal component scores (PC) of the first two axes (A: PC1; B: PC2) of juvenile bluegill (BG; *Lepomis macrochirus*) and pumpkinseed (PS; *L. macrochirus*) behaviors in a Z-maze arena. The four behaviors included in the PCA were latency to exit the acclimation chamber, time spent in the maze, furthest square reached, and number of lines crossed. Central line depicts the median, boxes indicate the interquartile range (IQR), and whiskers extend to 1.5\*IQR. Outliers are denoted as black dots



time spent in the maze, furthest square reached, and number of lines crossed, with a negative loading for latency to leave the acclimation chamber (Table 3). Altogether, this pattern suggests that PC1 is representative of bold and exploratory behavior, as greater PC1 values are associated with quicker emergence, more time spent in the maze, and more exploration and activity within the maze. PC2 had a strong positive loading for latency to leave the acclimation chamber, and weaker positive loadings for the remaining three variables (Table 3). PC2 is likely representative of fish that quickly emerge from the acclimation chamber yet show little activity and exploration within the maze, or vice versa.

Individual PC1 scores did not differ significantly among chemical cue types ( $F_{3,60} = 1.21$ ,  $p = 0.32$ ), but did differ significantly between species ( $F_{1,60} = 5.18$ ,  $p = 0.026$ ) such that bluegill showed significantly more bold and exploratory behaviors (i.e., greater PC1 scores) than pumpkinseed (Fig. 2a). PC1 scores also had no significant relationship with body length ( $F_{1,60} = 0.02$ ,  $p = 0.90$ ), and there was no significant interaction between cue and species ( $F_{2,60} = 0.17$ ,  $p = 0.84$ ). PC2 scores did not differ significantly among chemical cues ( $F_{3,60} = 0.87$ ,  $p = 0.46$ ), between species ( $F_{1,60} = 3.51$ ,  $p = 0.07$ ), or with body length ( $F_{1,60} = 1.65$ ,  $p = 0.20$ ), and there was no significant interaction between cue and species ( $F_{2,60} = 0.95$ ,  $p = 0.39$ ; Fig. 2b).

## Discussion

Our study investigated how the boldness and exploratory behaviors of congeneric juvenile bluegill and pumpkinseed differed between species and with the presence of chemical cues indicating different levels of ecological risk (predator kairomones and conspecific alarm cues) using a Z-maze

arena assay. We observed bluegill to be significantly more likely to leave the acclimation chamber and subsequently explore the Z-maze than pumpkinseed. We also identified a potential boldness syndrome characterized by activity and exploratory behaviors (PC1: positive values associated with faster emergence from, and less time spent in the acclimation chamber, reaching farther squares, and crossing more lines). Contributions of individual behavioral measures to this boldness syndrome are similar to those identified in previous studies using Z-maze assays (see Ramsaran et al. 2021), indicating that the behavioral assays here were effectively able to capture variation in bold and exploratory behaviors among individuals. Our results found no statistically significant difference in any recorded behaviors between risky chemical cue treatments or with fish body size, but the considerable differences in bold and exploratory behaviors between our study species suggests that differing environmental pressures may have resulted in some ecological niche partitioning along the bold-shy axis in these populations of congeners.

There have been contradictory findings reported regarding shy-bold behavioral responses to perceived predation risk. For instance, several studies have found that various fishes, including bluegill, demonstrate bold and exploratory behaviors in response to elevated perceived predation risk (Pellegrini et al. 2010; Ramsaran et al. 2021), while others have reported that perceived predation risk does not affect the expression of shy-bold behavioral responses between individuals (Lawrence et al. 2019). Similarly, assessments of shy-bold behavioral patterns between habitats with varying levels of predation have been equivocal, with some reporting fish from high-predation environments to be bolder than fish from low-predation environments (Archard and Braithwaite 2010; Brown et al. 2005, 2007a, b, 2013; Magnhagen and Borchering 2008; Magnhagen et al. 2012; Elvidge et al. 2016; Money et al. 2017), while others report the opposite (Brown et al. 2009, 2010). Contrary to our predictions, the present study did not find differential shy-bold behavioral responses to potential predation cues. It is important to note that our study was limited to indirect predation cues, while in natural environments these fishes can also use visual and/or water movement cues to assess predation risk. Nonetheless, our results add to conflicting literature on this topic.

The contradicting results from the present study and that of Ramsaran et al. (2021), namely, that elevated risk was not associated with increased exploration and bolder responses in bluegill, were particularly surprising, given that both studies used the same Z-maze apparatus and protocol with fish collected from the same populations. Although Ramsaran et al. (2021) used bluegill that were slightly smaller (66–103 mm TL) than the fish assayed in our study (90–139 mm

TL), both size classes lie within the range of those that are preyed upon by Northern pike in Opinicon Lake (Keast 1978b), suggesting that body size was unlikely to be a causal factor in the observed differences between these two studies. Additionally, the absence of a distinct response to our positive control (conspecific alarm cues) while using similar or greater concentrations to other studies which induce behavioral responses in *Lepomis* spp. (see Xia et al. 2018) and other fishes (see Brown and Godin 1999; Mirza and Chivers 2003b; Brown et al. 2006, 2009, 2010; Ferrari et al. 2008a, b; Elvidge et al. 2010, 2013) suggests that if the fish in the present study were able to detect the conspecific alarm cues, they did not elicit a behavioral response that was measurable in our Z-maze assay (see Mirza and Chivers 2003b). In the present study, there may have been environmental variables which undermined previously reported bold-type responses to predator cues. Notably, the study of Ramsaran et al. (2021) occurred in May, when water temperatures (although not recorded) are typically lower than they are during mid-August (typically the warmest time of the year in north temperate waters), when our study was conducted. Indeed, environmental factors including water temperature have been noted to influence the personality and the shy-bold behaviors of fish (Biro et al. 2010; Frost et al. 2013), indicating that normal environmental variation could play a major role in driving such contradictory data within field-based behavioral studies.

Several studies have reported varying relationships between body size and boldness (Brown and Braithwaite 2004; Brown et al. 2005, 2007b). However, our results found no relationship between total length and any measured behavioral metric. Nonetheless, these results are consistent with other studies using *Lepomis* spp. which report no effect of body size on shy-bold behaviors (Moynes et al. 2020; Ramsaran et al. 2021), although both these studies and the present study used narrow size ranges of juvenile *Lepomis* spp. intended to limit each study to a single age class or cohort. The relationship between body size and boldness has been shown to decrease under high perceived predation risk (Meuthen et al. 2019) possibly as a result of larger prey animals acting in more risk-averse fashion consistent with the asset protection principle (Clark 1994). This could also explain why the results of the present study differ from Ramsaran et al. (2021). Nonetheless, our methodology allowed us to detect clear differences in bold and exploratory behaviors between the focal species.

Recent studies have shown that individual personality, especially behavioral traits associated with boldness, are associated with ecological niche and/or foraging decisions across various animals (von Merten et al. 2020; Boone et al. 2022; Gharnit et al. 2022), including fishes (Chipps et al. 2004; Robinson et al. 2007; Mittelbach et al. 2014;

Ólafsdóttir and Magellan 2016; Theódórsson and Ólafsdóttir 2022). The majority of this research focuses on the effects of bold-shy behaviors on individual foraging patterns or survival. However, the relationship between personality and ecological niche can also be examined at the population and/or species levels (Wilson 1998); nonetheless, relevant studies are somewhat under-represented (Ingle and Johnson 2014). Notably, bluegill and pumpkinseed have evolved to occupy separate ecological niches where they co-occur (Xia et al. 2018), with bluegill feeding more on pelagic zooplankton than do pumpkinseed, which tend to feed more on benthic invertebrates (Keast 1978a; Keast et al. 1978; Mittelbach 1984). As such, bluegill tend to spend more time higher in the water column away from structure (Werner et al. 1977; Keast 1978b) and are thus at a higher risk of predation to predatory fish and piscivorous birds (McCartt et al. 1997). Fish under more frequent risk from predators are known to exert less antipredator responses than those under less frequent risk in order to maintain sufficient levels of foraging activity (Foam et al. 2005; Mirza et al. 2006; Riesch et al. 2009). Consequently, our finding that bluegill were bolder on average than pumpkinseed could be explained by differential predator exposure due to evolutionary differences in their ecological niche, where bluegill may have evolved to become bolder on average than pumpkinseed which do not need to leave shelter to the same degree as bluegill to forage and experience lower predation threat near the benthos.

In summary, our study adds to contradicting findings regarding the effects of predation cues on shy-bold behaviors in fish but identifies clear differences in bold and exploratory behaviors between two co-occurring *Lepomis* spp. We propose that differences in ecological niche and foraging strategy are causal factors explaining this difference in shy-bold behaviors and thus predict that the observed behavioural differences would hold true in adults of these two species until they reach a size where predation risk by predatory fishes declines. The idea that ecological niche can dictate personality is one that is relatively underexplored in the literature. As such, we recommend that further research be conducted between additional species/populations and between ecotypes within species which have variable predation risk to due ecological niche differentiation to assess the degree to which ecological niche may affect shy-bold behaviors as well as other categories of behavioral temperaments.

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**Data availability** Raw data (annotated) and the R code used for analyses are publicly available through the Open Science Framework (<https://osf.io/6ac9m/>; DOI: <https://doi.org/10.17605/OSF.IO/6AC9M>).

## Declarations

**Ethics approval** Scientific Collection Permits were provided by the Ontario Ministry of Natural Resources and Forestry and Animal Care Permissions were provided by Carleton University (Permit 112698) under the auspices of the Canadian Council of Animal Care and the guidelines set forth by the Animal Behavior Society/Association for the Study of Animal Behaviour.

**Conflict of interest** The authors declare that they have no conflicts of interest.

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