

# Response of pumpkinseed sunfish to conspecific chemical alarm cues: an interaction between ontogeny and stimulus concentration

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**Abstract:** Recent studies have shown that juvenile centrarchids undergo ontogenetic shifts in their behavioural response towards conspecific and heterospecific chemical alarm cues based on threat-sensitive trade-offs between the benefits associated with predator avoidance and foraging. We conducted laboratory studies to test the hypothesis that the relative concentration of conspecific alarm cues provides relevant information, allowing individuals to maximize these trade-offs. Juvenile (<40 mm standard length) and subadult (>95 mm standard length) pumpkinseed sunfish (*Lepomis gibbosus*) were exposed to conspecific skin extracts at stock (undiluted) concentration or diluted 1:1 (50%), 1:3 (25%), or 1:7 (12.5%) with distilled water. Juvenile sunfish exhibited significant antipredator responses (relative to the distilled water controls) when exposed to conspecific skin extracts at a concentration as low as 25%. Juveniles exposed to 12.5% skin extract were not significantly different from the distilled water controls. Subadult sunfish exhibited significant antipredator responses only to the two highest concentrations. In response to the two lowest concentrations (25% and 12.5%), however, subadult sunfish exhibited significant foraging responses. These data demonstrate that the relative concentration of chemical alarm cues provides reliable information and allows individuals to accurately assess local predation risk and hence maximize potential trade-offs.

**Résumé :** Des études récentes ont montré que, chez les jeunes centrarchidés, il se produit des changements ontogéniques dans la réponse comportementale aux signaux chimiques d'alarme conspécifiques et hétérospécifiques; ces changements sont basés sur des compromis liés au risque entre l'évitement des prédateurs et la recherche de nourriture. Des études de laboratoire ont servi à vérifier l'hypothèse selon laquelle la densité relative des signaux d'alarme conspécifiques fournit une information pertinente qui permet aux individus de maximiser ces compromis. Nous avons exposé des crapets-soleil (*Lepomis gibbosus*) jeunes (<40 mm de longueur standard) et subadultes (>95 mm longueur standard) à des extraits de peau, soit à la concentration stock (non diluée), soit à des concentrations diluées à l'eau distillée, 1:1 (50 %), 1:3 (25 %) ou 1:7 (12,5 %). Les jeunes crapets ont de fortes réactions anti-prédateurs (par comparaison aux témoins traités à l'eau distillée) lorsqu'ils sont exposés aux extraits de peau de poissons conspécifiques à des concentrations aussi basses que 25 %. Les jeunes exposés à des extraits de peau à une concentration de 12,5 % ne réagissent pas différemment des jeunes témoins. Les crapets subadultes n'ont de réactions anti-prédateurs significatives qu'aux deux plus fortes concentrations. Cependant, en réaction aux concentrations les plus faibles (25 % et 12,5 %), les crapets subadultes ont des comportements significatifs de recherche de nourriture. Ces données montrent que les concentrations relatives de signaux chimiques d'alarme procurent des informations fiables qui permettent aux individus d'évaluer de façon précise le risque local de prédation et de maximiser ainsi les compromis potentiels.

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## Introduction

Prey individuals are continually faced with the conflicting demands of foraging and predator avoidance (Werner and Gilliam 1984; Lima and Dill 1990) and typically exhibit threat-sensitive trade-offs between the benefits associated with energy intake and antipredator responses (Helfman 1989; Lima and Dill 1990; Lima and Bednekoff 1999). A

variety of taxa, including many fishes, are able to maximize these trade-offs by undergoing size-dependent ontogenetic niche shifts (Werner and Gilliam 1984; Werner and Hall 1988; Keller and Moore 2000). In freshwater fishes, such as centrarchids, these ontogenetic shifts correspond to the size-dependent shift from invertebrate foraging to piscivory (Olson 1996; Mittelbach and Persson 1998). For example, juvenile largemouth bass (*Micropterus salmoides*) undergo major ontogenetic changes in habitat use, foraging strategies, and predation risk at a standard length of about 50–55 mm, typically within the first year of growth (Olson 1996). Juvenile sunfish (i.e., pumpkinseed sunfish (*Lepomis gibbosus*) and green sunfish (*Lepomis cyanellus*)) undergo a similar shift at around 85–90 mm, typically within the first 2 years of growth (Scott and Crossman 1973; Golub and Brown 2003). Natural selection should favour individuals that can reliably assess local predation risks, as this would

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allow them to maximize these trade-offs (Lima and Bednekoff 1999; Brown et al. 2002; Golub and Brown 2003).

A wide range of taxonomically diverse freshwater fishes, including centrarchids, have been shown to rely on chemical alarm cues to assess local predation risk (Chivers and Smith 1998; Wisenden 2000; Brown 2003). When detected by conspecifics and some sympatric heterospecifics, these chemical alarm cues can elicit dramatic, short-term increases in species typical antipredator responses (Chivers and Smith 1998; Smith 1999). Mounting evidence clearly demonstrates that individuals that respond to the presence of a chemical alarm cue with an increase in antipredator behaviour gain significant survival benefits (Mirza and Chivers 2000, 2001, 2003a, 2003b; Gazdewich and Chivers 2002). In addition, these chemical alarm cues provide reliable information regarding potential foraging opportunities to predators (Mathis et al. 1995; Brown et al. 2001a, 2002). Thus, the presence of alarm cues provides a valuable source of social information, allowing individuals to maximize threat-sensitive predator avoidance and foraging trade-offs (Brown et al. 2002; Golub and Brown 2003).

Juvenile centrarchids have recently been shown to exhibit a size-dependent shift in their behavioural response to heterospecific alarm cues (Brown et al. 2001a, 2002; Golub and Brown 2003), similar to the well-documented shift in habitat use (i.e., Werner and Hall 1988). Small, primarily invertebrate foraging largemouth bass and green sunfish exhibit increased antipredator behaviour when exposed to the alarm cues of some sympatric heterospecific prey guild members. However, larger piscivorous individuals increase their foraging response to the same cues (Brown et al. 2001a, 2002; Golub and Brown 2003). As small, invertebrate foragers, young-of-the-year bass and sunfish gain significant potential benefits associated with predator avoidance in response to the alarm growth rates through piscivory and at the same time experience reduced predator risk from gape-limited inshore predators. As a result, this trade-off favours foraging responses (Brown et al. 2002; Golub and Brown 2003).

Golub and Brown (2003) found that while juvenile green sunfish exhibit an ontogenetic shift in response to heterospecific alarm cues, there was no such shift in response to conspecific alarm cues. Regardless of size tested (40–120 mm), juvenile sunfish exhibited a significant antipredator response to conspecific skin extracts, suggesting that the antipredator benefits associated with conspecific alarm cues outweigh any potential foraging benefits (Golub and Brown 2003). Taken together, these data demonstrate that juvenile centrarchids use the chemical alarm cues of both conspecifics and heterospecifics as social information cues, allowing them to assess local predation risk (Brown 2003; Golub and Brown 2003).

Several researchers have shown that prey fish exhibit a nongraded response to conspecific alarm cues. For example, Brown et al. (2001b) found that fathead minnows (*Pimephales promelas*) exhibit a population-specific minimum behavioural response threshold to hypoxanthine-3-*N*-oxide (H3NO) (the putative Ostariophysan alarm pheromone) (Brown et al. 2000, 2001c, 2003). At concentrations of H3NO below this minimum threshold, there was no observ-

able (sensu Smith 1999) behavioural response. Increasing the concentration of H3NO above the minimum threshold did not result in increased response intensity. Brown et al. (2001d), however, demonstrated that while minnows did not exhibit an overt response to subthreshold concentrations of H3NO, they were attending to these cues. Minnows exposed to concentrations as low as 25% of the minimum behavioural response threshold were still able to acquire the recognition of a novel predator. Similar results have been demonstrated for juvenile rainbow trout (*Oncorhynchus mykiss*) (Mirza and Chivers 2003b). In addition, Lawrence and Smith (1989) found that fathead minnows increased exploratory behaviour in response to low concentrations of conspecific alarm cues. These results suggest that relative concentration of chemical alarm cues may provide individual prey with detailed information regarding local predation risk.

Given that pumpkinseed sunfish undergo major shifts in predation risk and foraging strategies within the first 1–2 years of growth (see above), it remains unknown if they will exhibit similar response patterns to decreasing concentrations of conspecific skin extracts. In addition, since larger individuals should be at lower risk from inshore predators (Golub and Brown 2003), it is also unknown if decreasing concentrations of conspecific alarm cues will represent qualitatively different information to juvenile versus subadult sunfish. We predict that smaller, juvenile sunfish should show consistent antipredator responses to conspecific chemical alarm cues until a minimum relative concentration is reached. Below this point, we predict no response. Conversely, we predict that larger, subadult sunfish should shift from an antipredator to a foraging response as the relative concentration of alarm cues decreases.

## Methods

### Fish collection and preparation of experimental stimulus

Two size classes of pumpkinseed sunfish, juveniles ( $36.7 \pm 0.5$  mm (mean  $\pm$  SE) standard length) and subadults ( $99.7 \pm 1.0$  mm standard length), were collected from the Lachine Canal in Montreal, Quebec, using beach seines. Each size class were held separately in 250-L holding tanks filled with dechlorinated tap water and equipped with a single airstone. A slow flow (approximately 2.5 L/min) of dechlorinated water was supplied to each holding tank. Both groups were fed daily ad libitum with commercial flake food and frozen brine shrimps (*Artemia* spp.)

We chose skin extract donors of an intermediate size class to control for the possibility that the production of the alarm cue may be linked to size. Skin extract solution was prepared from 12 donor pumpkinseed sunfish ( $57.7 \pm 4.3$  mm standard length). We chose this size of donor fish, as it was intermediate between our two size classes of test fish. In addition, Golub and Brown (2003) demonstrated that green sunfish ranging in size from 40 to 120 mm standard length exhibited consistent alarm responses to the skin extract of intermediate-sized donors. Fish were killed using a blow to the head (in accordance with Concordia University Animal Care Committee protocol AC-2002-BROW). Skin fillets were removed from both sides of the donor and placed im-

mediately into 50 mL of chilled, glass-distilled water. A total of 155 cm<sup>2</sup> was collected, homogenized, and filtered through glass wool (to remove any remaining tissue). The final volume was adjusted to 1520 mL using glass-distilled water, resulting in a concentration similar to that used by Golub and Brown (2003). The resulting solution was frozen in 25-mL aliquots at -20 °C until needed for trials. This solution was subsequently diluted with distilled water prior to running trials to produce one of the four different concentrations of stimulus: 100% (stock concentration), 50% (1:1), 25% (1:3), or 12.5% (1:7) dilutions of stock concentration.

### Test tanks

The experimental tanks consisted of 110-L glass aquaria equipped with a gravel substrate and a single airstone attached to an end wall. An additional length of tubing was attached to the airstone to allow for the injection of the experimental stimulus from behind a black viewing curtain so as not to disturb the test fish during trials. In addition, both sides of the tank were covered with white paper to maintain visual isolation from neighbouring tanks. Test tanks were kept on a 12 h light : 12 h dark cycle (dawn at 0630; dusk at 1830). Each tank was divided into five equal horizontal sections by drawing vertical lines on the exterior of the tank (to facilitate area use measurements; see below). All trials were recorded, for later analysis, using a digital video camera located behind the viewing curtain. All videotapes were coded such that all behavioural data were recorded blind to stimulus type (control versus experimental) and concentration.

### Experiment 1. Response of juvenile pumpkinseed sunfish

Shoals of four juvenile sunfish (matched for size) were placed into each of the test tanks and allowed to acclimate for a period of at least 24 h prior to testing ( $N = 10$  shoals for each treatment). Paired control and experimental trials consisted of a 10-min prestimulus and a 10-min poststimulus injection observation period. Prior to the prestimulus observation period, we withdrew and discarded 60 mL of water from the stimulus injection tube (to remove any residual water from the tube). An additional 60 mL was withdrawn and retained. Following the prestimulus observation period, 10 mL of distilled water (control) or conspecific skin extract at one of the four dilutions (experimental) was injected into the stimulus tube and slowly flushed into the tank with the retained 60 mL of water. Control trials were conducted between 0900 and 1200, and experimental trials were conducted between 1200 and 1500. Control trials were always carried out first to prevent any lasting response to the experimental stimuli obscuring a response to the control stimulus (Lawrence and Smith 1989; Hazlett 1997). The order of the experimental stimulus used was randomized.

During both the pre- and post-stimulus periods, three behavioural measures were recorded: (i) horizontal area use, (ii) time spent moving, and (iii) shoaling index. Horizontal area use was recorded every 15 s as the mean position of each fish within the test tank. Values for horizontal area use ranged from 1 (all fish in the section of the tank closest to the point of stimulus injection) to 5 (all fish in the section of the tank furthest away). Shoaling index was also recorded

every 15 s based on the proximity of each fish to its nearest neighbour. Shoaling index scores ranged from 1 (no fish within one body length of each other) to 4 (all fish within one body length of each other). Time spent moving was recorded as the total time that each of the four test fish spent swimming. The mean of the test shoal was used as the response variable. Decreased time spent moving and increased area use and shoaling index scores are indicative of an antipredator response in centrarchids (Brown and Brennan 2000; Golub and Brown 2003; Leduc et al. 2003). Increased time moving and decreased area use and shoaling index are indicative of a foraging response (Golub and Brown 2003).

### Statistical analysis

The difference between the pre- and post-stimulus observation periods was calculated for both control and experimental trials for each behavioural measure. We used these difference scores as the dependent variables in subsequent analyses. We examined the overall treatment effects using a repeated measures MANOVA, with skin extract concentration as the independent variable and control versus experimental observation as the repeated measure. Univariate repeated measures ANOVAs were used to further examine the effects of stimulus (distilled water versus skin extract) and concentration on individual behaviour patterns. All data were normally distributed; hence, no transformations were conducted on the data set prior to analysis.

### Experiment 2. Response of subadult pumpkinseed sunfish

The experimental protocol was as described above, with two exceptions. Groups of two subadult sunfish were placed in each test tank (matched for size,  $N = 10$  pairs for each treatment) instead of the groups of four used to test the juvenile size class. We tested pairs of subadults (rather than groups of four) to reduce the potential confounds of stress and (or) aggression within the test groups. As a result, distance between individuals (distance between the midpoint of the head of each individual) was calculated every 15 s instead of shoaling index. Horizontal area use and time spent moving were recorded, as in Experiment 1, except that each data point was based on the average of two (rather than four) individuals. The data were analyzed as described above. As in Experiment 1, all data were normally distributed.

## Results

### Experiment 1. Response of juvenile pumpkinseed sunfish

Our repeated measures MANOVA revealed significant overall repeated measures (distilled water control versus skin extract experimental stimuli;  $F_{[3,34]} = 19.53$ ,  $P < 0.0001$ ) and concentration ( $F_{[3,34]} = 11.42$ ,  $P < 0.0001$ ) effects. In addition, we found significant interaction between the two main effects ( $F_{[3,36]} = 6.41$ ,  $P = 0.001$ ).

Subsequent univariate repeated measures ANOVAs revealed significant interaction terms for time spent moving and shoaling (Table 1). While there was no significant interaction term for area use values, we did find significant dif-

**Table 1.** Results of multivariate and univariate repeated measures ANOVA for juvenile pumpkinseed sunfish (*Lepomis gibbosus*) exposed to distilled water controls versus conspecific skin extracts at varying concentrations.

	<i>F</i>	df	<i>P</i>
<b>Multivariate analysis</b>			
Repeated measure	19.53	3, 36	<0.0001
Concentration	11.42	3, 34	<0.0001
Repeated measure × concentration	6.41	3, 36	0.001
<b>Univariate analysis</b>			
Time spent moving			
Repeated measure	21.89	1, 36	<0.0001
Concentration	0.44	3, 36	0.73
Repeated measure × concentration	30.3	3, 36	0.042
Shoaling index			
Repeated measure	29.91	1, 36	<0.0001
Concentration	6.83	3, 36	0.0009
Repeated measure × concentration	2.92	3, 36	0.047
Area use			
Repeated measure	5.39	1, 36	0.026
Concentration	3.03	3, 36	0.042
Repeated measure × concentration	1.50	3, 36	0.23

ferences for both concentration and repeated measure effects (Table 1).

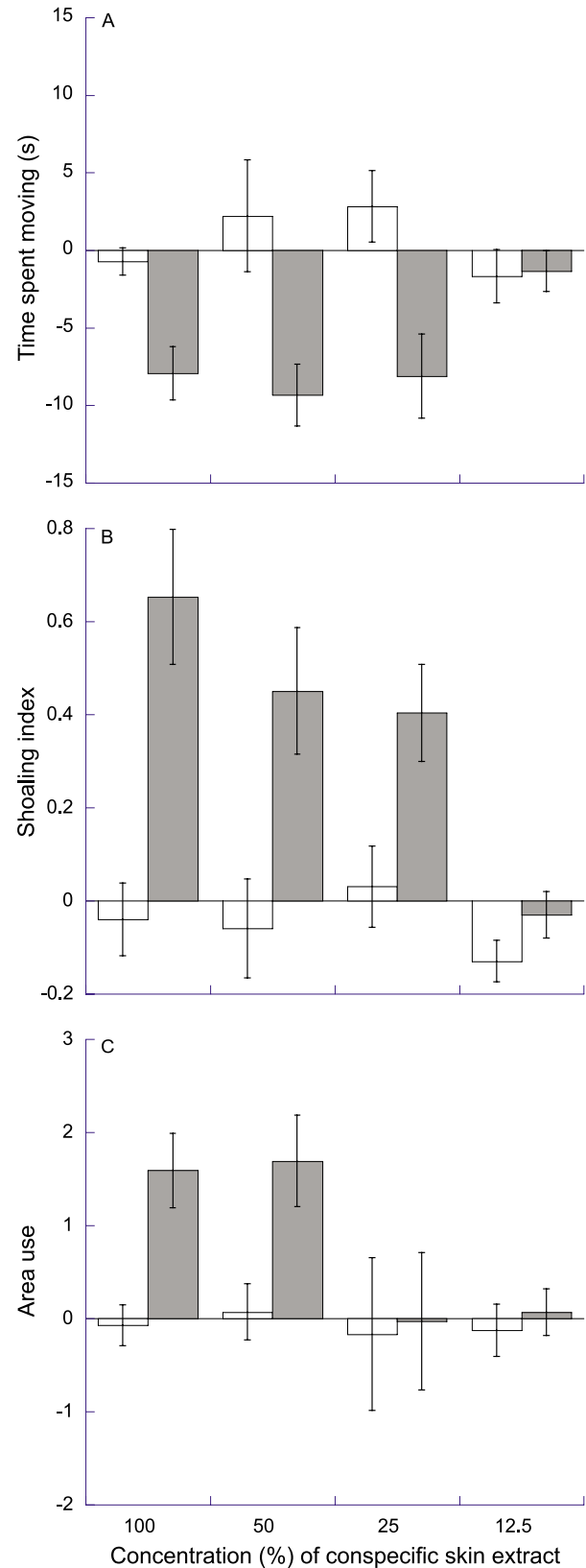
Juvenile pumpkinseed sunfish exhibited a significant increase in antipredator behaviour when exposed to conspecific skin extract as low as 25% dilutions. Juveniles significantly decreased time spent moving and increased shoal cohesion (relative to distilled water control) in response to conspecific skin extract as low as 25% (1:3 dilution) (Figs. 1A and 1B, Table 1). Juveniles also increased area use values (i.e., remained further away from the point of stimulus injection) when exposed to conspecific skin extract at the two highest concentrations (Fig. 1C, Table 1). When exposed to the lowest concentration (1:7 dilutions), juvenile sunfish did not exhibit any significant changes in behavioural response relative to the distilled water controls (Fig. 1, Table 1).

### Experiment 2. Response of subadult pumpkinseed sunfish

As with the juvenile cohort, our repeated measures MANOVA revealed significant repeated measures ( $F_{[3,34]} = 3.87$ ,  $P = 0.018$ ) and concentration ( $F_{[3,36]} = 4.90$ ,  $P = 0.0006$ ) effects as well as a significant interaction term ( $F_{[3,36]} = 19.47$ ,  $P < 0.0001$ ). Subsequent univariate comparisons revealed significant interaction terms (repeated measure × concentration) for time spent moving, shoal cohesion, and area use (Table 2), demonstrating that subadult pumpkinseed sunfish exhibited a concentration-dependent shift in the response to conspecific skin extracts, with individuals shifting from antipredator to foraging behaviour as the relative concentration of skin extract decreased.

Subadult sunfish exhibited significant decreases in time spent moving in response to 100% and 50% dilutions of conspecific skin extract but exhibited significant increases in time moving when exposed to 25% and 12.5% dilutions (Fig. 2A, Table 2). Subadult sunfish exhibited a significant

**Fig. 1.** Mean ( $\pm$ SE) change in time moving (A), shoaling index (B), and area use (C) for juvenile pumpkinseed sunfish (*Lepomis gibbosus*) exposed to varying concentrations of conspecific skin extract. Open bars, distilled water controls; shaded bars, sunfish skin extract;  $N = 10$  per treatment.



**Table 2.** Results of multivariate and univariate repeated measures ANOVA for subadult pumpkinseed sunfish exposed to distilled water controls versus conspecific skin extracts at varying concentrations.

	<i>F</i>	<i>df</i>	<i>P</i>
<b>Multivariate analysis</b>			
Repeated measure	3.87	3, 36	0.018
Concentration	4.90	3, 34	0.006
Repeated measure × concentration	19.47	3, 36	<0.0001
<b>Univariate analysis</b>			
<b>Time spent moving</b>			
Repeated measure	1.54	1, 36	0.22
Concentration	2.56	3, 36	0.07
Repeated measure × concentration	12.87	3, 36	<0.0001
<b>Distance to neighbour</b>			
Repeated measure	3.08	1, 36	0.088
Concentration	1.26	3, 36	0.30
Repeated measure × concentration	3.79	3, 36	0.018
<b>Area use</b>			
Repeated measure	7.54	1, 36	0.009
Concentration	0.76	3, 36	0.52
Repeated measure × concentration	2.92	3, 36	0.047

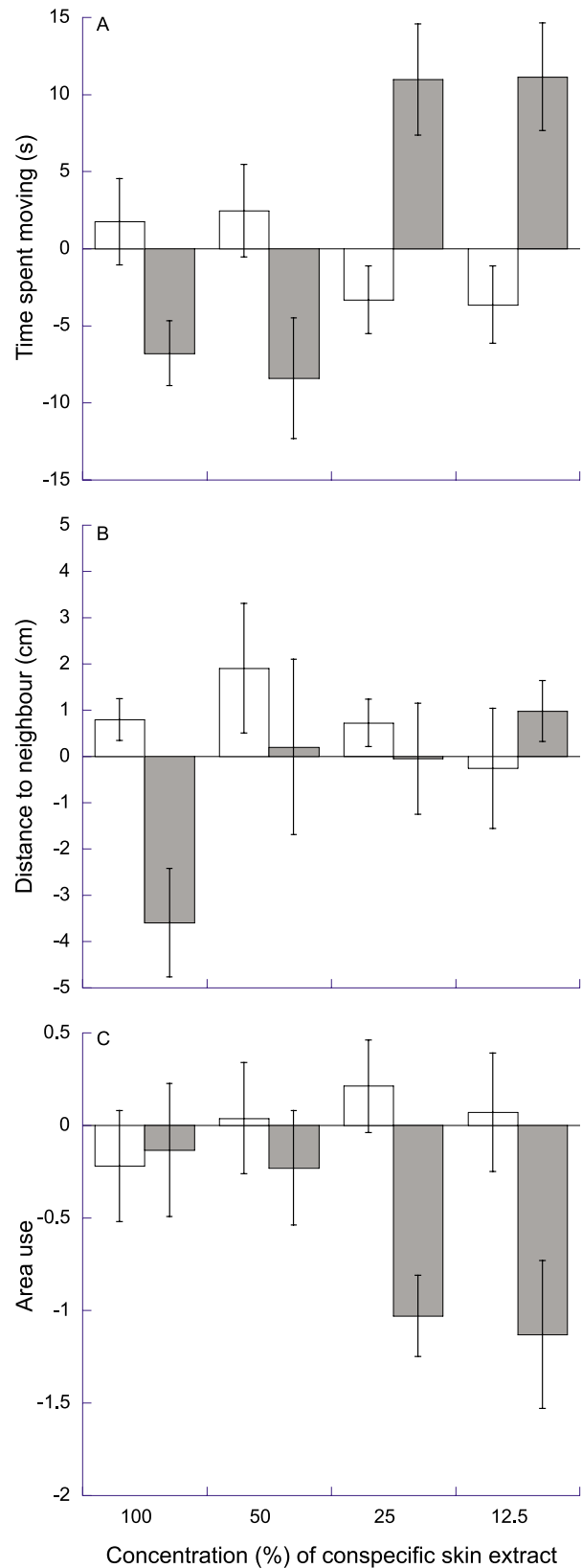
decrease in distance to neighbour (i.e., increased shoal cohesion) in response to 100% skin extract (Fig. 2B, Table 2). We observed no significant change in distance to neighbour (compared with distilled water control) for subadult exposed to 50%, 25%, or 12.5% dilutions of conspecific skin extracts (Fig. 2B, Table 2). Subadults exposed to 100% and 50% dilutions of skin extract exhibited no significant change in area use, whereas those exposed to the two lowest concentrations exhibited significant decreases in area use (i.e., moved towards the point of stimulus injection) (Fig. 2C, Table 2).

**Discussion**

These data clearly demonstrate a significant interaction between the effects of ontogeny and relative concentration on the behavioural response of pumpkinseed sunfish towards conspecific chemical alarm cues. Juveniles below the size at which small fishes can be included in the diet exhibited consistent antipredator responses when exposed to conspecific alarm cues as low as 25% (1:3 dilution). We found no significant difference between the lowest concentration and the distilled water controls for juvenile sunfish. Subadult sunfish, however, exhibited a dramatically different response pattern. We found significant antipredator responses (decreased time moving and increased shoal cohesion) for individuals exposed to the two lowest concentrations (25% and 12.5%). Taken together, these data suggest an ontogenetic shift in the use of conspecific chemical alarm cues by pumpkinseed sunfish. In addition, our results suggest that the relative concentration of alarm cues provides valuable information regarding local predation risk.

The ontogenetic shift in response to both conspecific and heterospecific chemical alarm cues has been argued to be regulated by a threat-sensitive trade-off between antipredator ( $B_{ap}$ ) and foraging ( $B_{for}$ ) benefits (Brown et al. 2002; Golub

**Fig. 2.** Mean ( $\pm$ SE) change in time moving (A), distance to neighbour (B), and area use (C) for subadult pumpkinseed sunfish exposed to varying concentrations of conspecific skin extract. Open bars, distilled water controls; shaded bars, sunfish skin extract; *N* = 10 per treatment.



and Brown 2003). For small invertebrate foraging individuals,  $B_{ap}$  would be high relative to  $B_{for}$ , favouring an anti-predator response. However, as individuals reach sufficient size to include prey fish in their diet,  $B_{for}$  should be expected to increase. At the same time,  $B_{ap}$  should decrease, as these large, subadult individuals will have outgrown the gape limitations of some inshore predators (Golub and Brown 2003). Our results suggest that relative concentration may provide information regarding local predation risk, hence allowing individuals to fine tune this trade-off.

Golub and Brown (2003) failed to detect any ontogenetic change in response to the skin extract of conspecifics. The undiluted treatment used in our experiment was deliberately adjusted to a concentration similar to that used in Golub and Brown (2003), and thus, the data showing that both size classes respond with antipredator behaviour at the highest concentration tested are consistent with the results of Golub and Brown (2003). The switch to a foraging response at lower concentration by subadults suggests that the relative concentration of alarm cue conveys qualitatively different information to individuals of this size class. Two nonmutually exclusive mechanisms may account for the observed response pattern by subadult sunfish in our study.

Initially, smaller prey fish, which could be consumed quicker, would be expected to release less alarm cue than larger prey items owing to quicker handling times and less tissue damage. As such, the relative concentration of an alarm cue may provide reliable indications of the size of prey items being consumed nearby. Alternatively, given that the relative concentration would decrease as a function of distance, lower concentrations might indicate that a predation event is occurring nearby but at a sufficient distance that the signal receiver is not at an immediate risk of predation. Thus, larger individuals that might gain some foraging benefits could be drawn to the source of the cue (sensu Mathis et al. 1995; Chivers et al. 1996) and continue foraging in a risk-sensitive manner.

It remains unknown, however, if this pattern of behavioural response would be found in other centrarchids. Pumpkinseed sunfish are opportunistic piscivores, with prey fish making up only approximately 40% of their diet (Scott and Crossman 1973). The proportion of fish in the diet of centrarchids ranges considerably, from as little as 10%–20% (i.e., smallmouth bass (*Micropterus dolmieu*) and rock bass (*Ambloplites rupestris*)) to 95%–100% (i.e., largemouth bass) (Mittelbach and Persson 1998). Presumably, as the proportion of prey fish in the diet increases, so should benefits associated with the use of chemical alarm cues as a source of foraging information (Brown et al. 2002). As such, we would predict that the degree of piscivory should significantly affect the presence and magnitude of the concentration–ontogeny interaction demonstrated here.

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