

# Production of chemical alarm cues in convict cichlids: the effects of diet, body condition and ontogeny

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While much is known regarding the role of chemical alarm cues in the mediation of predator–prey dynamics within aquatic ecosystems, little is known regarding the production of these critically important information sources. In a series of laboratory experiments, we tested the possible effects of diet, body condition and ontogeny on the production of chemical alarm cues in juvenile convict cichlids (*Archocentrus nigrofasciatus*, Cichlidae, Acanthopterygii). Juvenile cichlids were fed one of two diets, tubifex worms (*Tubifex* spp.) or brine shrimp (*Artemia* spp.). Shrimp fed cichlids grew longer and heavier and were in better condition than were tubifex fed cichlids. In Experiment 1, cichlids exhibited a stronger antipredator response to conspecific skin extracts from donors fed shrimp versus tubifex, regardless of test cichlid diet. In Experiment 2, juvenile cichlids were exposed to the skin extracts of high versus low condition donors fed either tubifex or shrimp diets. Cichlids exhibited a significantly stronger antipredator response to skin extracts of high condition donors, regardless of donor diet. Finally, in Experiment 3, juvenile cichlids were exposed to skin extracts of similar sized juvenile conspecifics, adult conspecifics, swordtail (*Xiphophorus helleri*) or a distilled water control. We found no evidence of an ontogenetic change in the production of alarm cues as cichlids exhibited similar intensity antipredator responses when exposed to juvenile and adult conspecific alarm cues. Taken together, these data suggest that individual diet may influence body condition with the consequence of influencing chemical alarm cue production in juvenile cichlids.

## Introduction

A wide variety of freshwater fishes rely on damage-released chemical alarm cues to detect and avoid potential predators (Chivers & Smith 1998, Brown 2003). These alarm cues are typi-

cally stored in the epidermis, and are released following mechanical damage to the skin, as would occur during predation events (Chivers & Smith 1998, Smith 1999). When detected by nearby conspecifics and some sympatric heterospecifics, these chemical alarm cues can elicit

dramatic, short-term increases in species-typical antipredator behaviour (Chivers & Smith 1998, Smith 1999, Brown 2003). In addition, chemical alarm cues can elicit a suite of long-term responses, including acquired recognition of novel predators and induced morphological and life history changes (Chivers & Smith 1998, Smith 1999). Responding to chemical alarm cues, either directly or as a recognizable component of a predator's dietary odour, has been shown to lead to increased survival during encounters with potential predators (Mathis & Smith 1993, Mirza & Chivers 2000, 2003a, 2003b, Chivers *et al.* 2002). In addition, senders of an alarm cue may benefit through increased escape probabilities as a result of the recruitment of secondary predators (Mathis *et al.* 1995, Chivers *et al.* 1996) or through kin selected indirect fitness gains (Brown & Smith 1994, Wisenden & Smith 1998, G. E. Brown & R. S. Mirza unpubl. data).

While much is known regarding the function of chemical alarm cues to both the senders and receivers, relatively little is known regarding the production mechanisms and associated energetic costs of chemical alarm cues. Fishes of the Superorder Ostariophysi possess specialized epidermal club cells, which produce and store the chemical alarm cue (Pfeiffer 1977, Smith 1992). Wisenden and Smith (1997, 1998) examined the effects of diet on epidermal club cell production in fathead minnows (*Pimephales promelas*). Their results demonstrate that minnows reared on a higher food ration were able to produce a greater number of club cells. These results suggest that there is an energetic cost associated with the production of chemical alarm cues and also suggest that diet (or diet quality) may directly influence the production of chemical alarm cues in freshwater prey fishes. Recently, Brown *et al.* (2001a) argued that the Ostariophysan alarm cue is produced from metabolic by-products associated with the degradation of proteins. The use of metabolic by-products is thought to be an energetically efficient method of cue production (Brown *et al.* 2001a). A similar production mechanism may also be selected in non-Ostariophysan fishes.

Ontogenetic stage may also have a significant impact on the production of chemical alarm cues. Mirza and Chivers (2002) exposed small (~4.0

cm) and large (~9.5 cm) brook charr (*Salvelinus fontinalis*) to the skin extracts of small versus large conspecifics. They report that for both size classes, individuals exhibited a significantly stronger antipredator response to like-sized donors. Harvey and Brown (2004) likewise exposed juvenile and adult yellow perch (*Perca flavescens*) to the skin extracts of juvenile versus adult donors and found no ontogenetic effect on the production of chemical alarm cues. Thus, the role of ontogeny in the production of chemical alarm cues remains equivocal.

Convict cichlids (*Archocentrus nigrofasciatus*, Cichlidae, Acanthopterygii) possess a damage-released chemical alarm cue, analogous to that seen in the well-studied Ostariophysan fishes (Wisenden & Sargent 1997, Alemadi & Wisenden 2002). However, unlike Ostariophysan fishes, cichlids do not possess the specialized epidermal club cells known to store the alarm cue (Pfeiffer 1977). As such, it remains unknown if factors such as diet quality, individual condition and/or ontogenetic stage have any influence on the production of damage-released chemical alarm cues in a non-Ostariophysan species.

The goal of this study is to examine the potential influence of diet type, body condition and ontogenetic stage on the production of chemical alarm cues by juvenile convict cichlids. In Experiment 1, we fed juvenile convict cichlids either a high or low quality diet to test for the possible effects of diet type on both the production of chemical alarm cues and the behavioural response of signal receivers. Differences in response of alarm cue receivers to the cues of donors fed high versus low quality diets could result from differences in donor body condition or a difference in diet quality, per se. In Experiment 2, we specifically test for the possible effects of stimulus donor condition, as separate from diet type. Finally, in Experiment 3, we test for the potential effects of ontogenetic stage on the production of alarm cues.

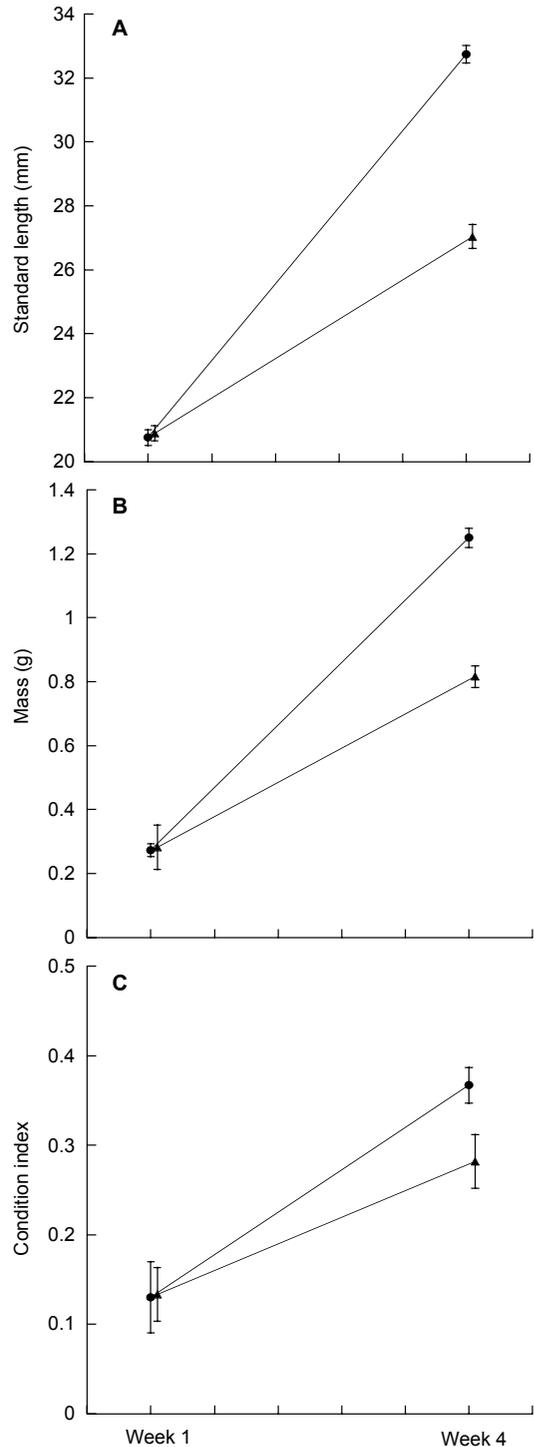
## Material and methods

For all three experiments, test and stimulus donor cichlids originated from our laboratory stock

population. Prior to testing, stock populations were held in 110-l glass aquaria, filled with continuously filtered, dechlorinated tap water (27 °C, pH 7.2), containing a gravel substrate, and were fed *ad libitum*, twice daily with commercial flake food. All stock tanks were held under a 12:12 light:dark cycle. Swordtails (*Xiphophorus helleri*) used in Experiment 3 (see below), were obtained from a commercial supplier and held in a 37-l glass aquarium under identical conditions as described above for convict cichlids.

As both Experiment 1 and 2 required test fish and/or stimulus donors fed different diets, we placed 30 juvenile cichlids into each of eight 37-l holding tanks, under identical conditions as described above. Four of these tanks were fed a high quality diet (previously frozen adult brine shrimp, *Artemia* spp.) and four were fed a low quality diet (re-hydrated tubifex worms, *Tubifex* spp.). According to the manufacturer's information, brine shrimp have similar crude protein levels and higher crude fat levels than tubifex worms. As such, we predicted that the brine shrimp diet was of higher quality than the tubifex diet.

Cichlids were fed daily for four weeks during this growth phase of the study. Each tank was fed the same mass of either shrimp or tubifex ( $1.05 \pm 0.04$  g, dry weight per day). At the initiation and completion of the growth phase, individual cichlids were measured (length to the nearest 0.5 mm and weight to the nearest 0.01 g). In addition, we calculated an allometric index of body condition ( $\text{weight}_g \times \text{length}_{\text{mm}}^{-1} \times 10$ ) for each fish. We compared the weight and length differences between fish fed different diets for starting and final measurements using unpaired *t*-tests with  $\alpha = 0.013$  to control for increasing Type 1 error rates (Sokal & Rohlf 1981). At the onset of the growth phase, there was no significant difference in mean length ( $t_6 = -0.26$ ,  $P = 0.80$ ; Fig. 1A), or weight ( $t_6 = -1.25$ ,  $P = 0.26$ ; Fig. 1B). However, cichlids fed the shrimp diet were longer ( $t_6 = 12.25$ ,  $P < 0.0001$ ; Fig. 1A), and heavier ( $t_6 = 12.74$ ,  $P < 0.0001$ ; Fig. 1B) than those fed tubifex worms at the completion of the growth phase. To test for a significant effect of the diet type on the condition index, we conducted an analysis of covariance using weight as the dependent variable, diet type as the independent variable and length as the covariate (García-Berthou 2001).



**Fig. 1.** Mean ( $\pm$  S.E.) (A) standard length, (B) mass, and (C) condition index ( $\text{mass} \times \text{length}^{-1} \times 10$ ) for juvenile cichlids fed shrimp (circles) and tubifex (diamonds) diets. Week 1 = initiation of growth phase, Week 4 = completion of growth phase.

We found a significant interaction between the diet type and length ( $F_{1,12} = 14.61$ ,  $P = 0.002$ ; Fig. 1C), indicating that cichlids fed shrimp were in better condition (heavier for length) than those fed tubifex worms.

## Experiment 1: Influence of diet quality

### Experimental stimuli

Skin extracts were collected from eight donor cichlids from each of the two diet treatments (mean  $\pm$  S.E. standard length =  $3.11 \pm 0.48$  and  $2.52 \pm 0.42$  cm for shrimp and tubifex diets respectively). Donor fish were killed with a blow to the head (in accordance with Concordia University Animal Care Protocol #AC-2002-BROW). We collected skin fillets from either side of donors and immediately placed them into 50 ml of chilled, glass-distilled water. We then homogenized the samples, filtered them through polyester floss (to remove any particulate matter) and adjusted the final volume with the addition of distilled water. We collected a total of 25.50 cm<sup>2</sup> (in 290 ml) and 25.62 (in 292 ml) of skin for shrimp- and tubifex-fed donors, respectively. Skin extracts were frozen in 15 ml aliquots until needed. As a control, we also froze 15 ml aliquots of distilled water.

### Experimental protocol

All observations were conducted in a series of 37-l test tanks, equipped with a single airstone and an additional length of tubing to allow for the injection of control and experimental stimuli from a distance of at least 2 m. The tanks contained a gravel substrate and were filled with dechlorinated tap water, but were not filtered. Temperature and lighting were identical to these in the holding tanks. We positioned the test tanks behind a black plastic viewing blind. All observations were videotaped for later behavioural analysis.

For each trial, we arbitrarily selected two cichlids from one of the holding tanks (tubifex-fed or shrimp-fed) and placed them into a test

tank 24 h prior to testing. All test (cue receiver) fish, regardless of diet treatment, were fed *ad libitum* with commercial flake food prior to testing, in order to reduce potential confounds of a foraging-antipredator behaviour trade-off (Smith 1981, Brown & Smith 1996, Brown & Cowan 2000). Trials consisted of paired control and experimental observations. For both control and experimental observations, we conducted a 10-min pre-stimulus and a 10-min post-stimulus injection observation period. Control and experimental observations were conducted on sequential days, with 24 h between. Order of presentation (control versus experimental stimuli) was randomized. Mean ( $\pm$  S.E.) standard length at testing was  $2.89 \pm 0.43$  and  $2.71 \pm 0.31$  cm (shrimp and tubifex diet respectively).

Prior to the pre-stimulus observations (for both control and experimental), we withdrew and discarded 60 ml of tank water through the stimulus injection tube (to remove any residual cues from the tube). We then withdrew and retained an additional 60 ml of water. Following the 10-min pre-stimulus observation period, we injected either 10 ml of distilled water (control trials) or 10 ml of either tubifex-fed or shrimp-fed cichlid skin extract (experimental trials) and slowly flushed it into the tank using the retained 60 ml of tank water. Each pair of cichlids was used only once.

During both pre- and post-stimulus observation periods, we recorded a suite of four behavioural measures typical of an antipredator response for juvenile cichlids (Wisenden & Sargent 1997). Vertical area use was recorded (every 15 s) as the position of each cichlid within the tank. Area use scores ranged from 2 (both cichlids near the substrate) to 8 (both cichlids near the water surface). Time spent moving was recorded as the total time each cichlid was swimming (expressed as a per capita value). Distance between individuals was recorded every 15 s as a measure of shoal cohesion. Finally, we recorded the total occurrence of aggressive interactions (chasing and biting). Decreased area use, time spent moving, distance to neighbour and aggressive interactions are indicative of an antipredator response in juvenile convict cichlids (Wisenden & Sargent 1997).

## Statistical analysis

For each behavioural measure, we calculated the difference between pre- and post-stimulus observation periods (post – pre), and used these difference scores as dependent variables for all subsequent analyses. We assessed the effects of stimulus donor diet and receiver diet using two-way repeated measures ANOVAs, with distilled water control versus skin extract experimental treatments as the repeated measure. We tested a total of 10 pairs of cichlids per treatment combination. Individual receivers were used only once.

## Experiment 2: Influence of body condition

### Test fish

Test fish were fed, *ad libitum*, twice daily with commercial flake food prior to the experiment. Mean ( $\pm$  S.E.) length at testing was  $2.84 \pm 0.12$  cm. Unlike Experiment 1, all test fish for Experiment 2 were fed the same diet (flake food).

### Stimulus preparation

We collected skin extract from tubifex and shrimp fed cichlids as described above. However, for each diet, we collected skin from high condition index and low condition index fish. Thus, we had a total of four stimulus types: (1) shrimp diet, high condition, (2) shrimp diet, low condition, (3) tubifex diet, high condition, and (4) tubifex diet, low condition. Donor cichlids were chosen such that the mean condition index was similar

for both diet treatments for high and low condition treatments (Table 1). The final concentration of each of the four skin extracts was the same as that used in Experiment 1 (Table 1).

## Experimental protocol

Pairs of cichlids were tested as described above for Experiment 1 with one exception. We did not use the distilled water control, as the results of Experiment 1 demonstrated that juvenile cichlids do not respond to the introduction of distilled water.

## Statistical analysis

As in Experiment 1, we calculated the difference between pre- and post-stimulus observation periods and used these difference scores as dependent measures in subsequent analyses. We tested the potential influence of donor diet and donor condition index using two-way ANOVAs, with donor diet (shrimp vs. tubifex) and donor condition (high versus low) as independent variables. We tested 10 pairs of cichlids for each treatment combination.

## Experiment 3: effects of ontogeny on alarm cue production

### Test fish

Test fish were of the same population, and were held under identical conditions as described in Experiment 1. Cichlids were likewise fed as described above. Mean ( $\pm$  S.E.) length at testing was  $2.86 \pm 0.09$  cm.

**Table 1.** Mean ( $\pm$  S.E. in parentheses) length (cm), weight (g), and condition index for skin extract donors and total area of skin collected and final adjusted volumes of stimuli used in Experiment 2.

	Length (cm)	Weight (g)	Condition index	Total skin area (cm <sup>2</sup> )	Final volume (ml)
Shrimp high condition	4.01 (0.09)	2.18 (0.11)	0.54 (0.03)	27.52	312
Shrimp low condition	3.07 (0.15)	0.70 (0.08)	0.23 (0.02)	19.70	224
Tubifex high condition	3.62 (0.12)	1.90 (0.07)	0.53 (0.02)	26.61	302
Tubifex low condition	2.63 (0.14)	0.56 (0.05)	0.21 (0.02)	19.87	226

Pairs of juvenile convict cichlids were exposed to skin extracts collected from similar sized conspecifics and from larger adult cichlids. In addition, we tested two control stimuli, distilled water and swordtail skin extract. While poeciliids possess an analogous chemical alarm cue system (Garcia *et al.* 1992, Brown & Godin 1999, Mirza *et al.* 2001), it is not recognized by juvenile cichlids, and therefore serves as a control for the odour of any injured prey fish.

### Stimulus preparation

We collected skin extracts from 11 juvenile (mean  $\pm$  S.E. length =  $2.99 \pm 0.11$  cm) and three adult ( $8.19 \pm 0.03$  cm) cichlids and from seven swordtails ( $5.38 \pm 0.44$  cm) as described above. We selected donors that had a similar weight to length ratio (i.e. condition index, *see* above). Mean condition index was 0.35 (range: 0.32–0.38) for the adult donors and  $0.33 \pm 0.04$  for juvenile donors. We collected a total of 29.46 cm<sup>2</sup> (in 221 ml) of juvenile cichlid skin, 27.53 cm<sup>2</sup> (in 312 ml) of sub-adult cichlid skin and 33.21 cm<sup>2</sup> (in 379 ml) of swordtail skin. The final concentrations of all three stimuli were the same as used in Experiments 1 and 2. Prior to

the preparation of skin extracts, adult and juvenile cichlid and swordtail donors were fed brine shrimp and commercial flake food ad libitum, twice daily. Skin extracts were frozen in 20 ml aliquots at  $-20$  °C until required. As a control, we also froze 20 ml aliquots of distilled water.

### Experimental protocol

Trials were conducted, as described above for Experiment 2. Pairs of cichlids were exposed to 10 ml of one of the four stimuli. Antipredator behaviour was recorded as above. We compared the change in behaviour in response to the four stimuli using one-way ANOVAs. We conducted a total of 10 trials for each of the four stimuli. Post-hoc multiple comparisons were made using Fisher's Protected Least Squared Differences.

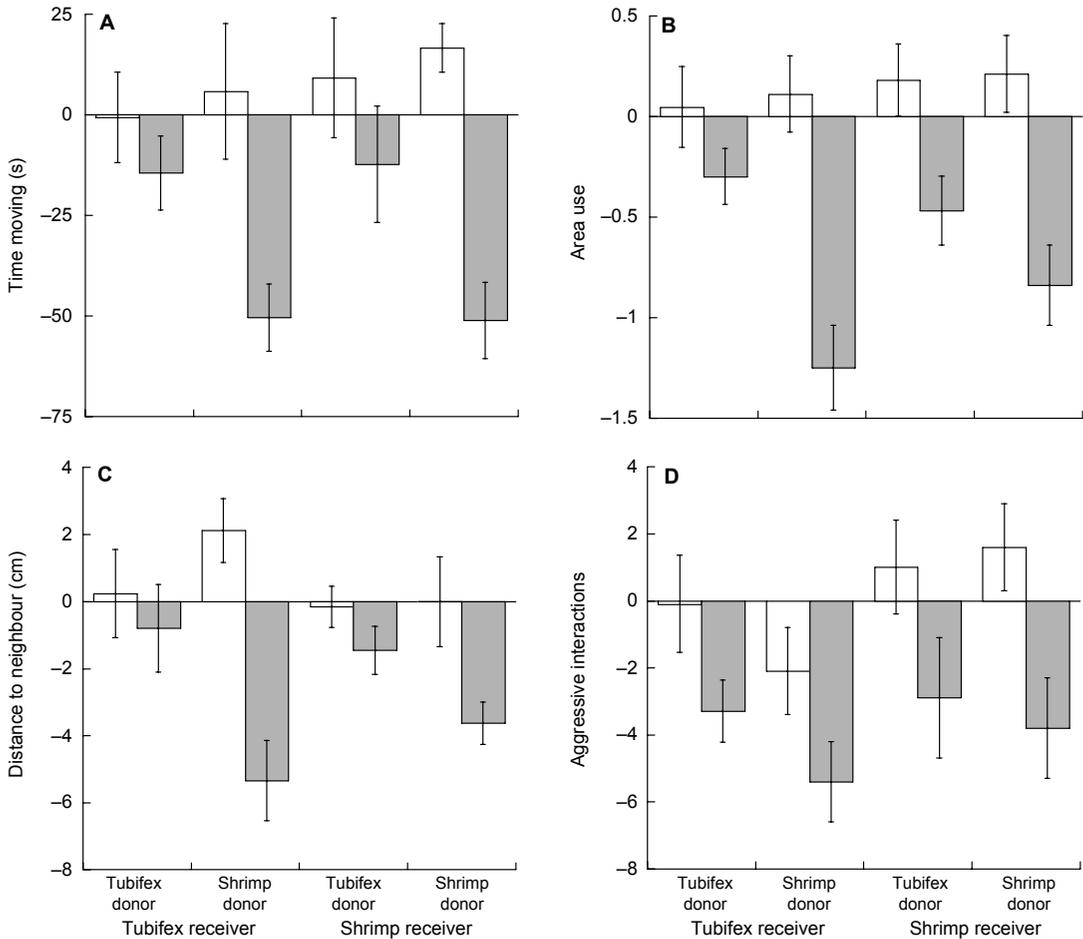
## Results

### Experiment 1: Influence of diet quality

For each of the four behavioural variables, we found a significant repeated measures effect (Table 2 and Fig. 2). In addition, for time spent

**Table 2.** Results of repeated measures ANOVAs for each response variable tested in Experiment 1. Repeated measure denotes control (distilled water) versus experimental (skin extract) trials, test diet denotes diet of test (cue receiver) cichlids and stimulus diet denotes diet of skin extract donors.  $N = 10$  per treatment combination.

		<i>F</i>	<i>df</i>	<i>P</i>
<b>Time moving</b>	Repeated measure	13.48	1,36	< 0.0001
	Repeated measure $\times$ test diet	0.19	1,36	= 0.66
	Repeated measure $\times$ stimulus diet	4.16	1,36	< 0.05
	Repeated measure $\times$ test diet $\times$ stimulus diet	0.007	1,36	= 0.93
<b>Area use</b>	Repeated measure	25.77	1,36	< 0.0001
	Repeated measure $\times$ test diet	0.001	1,36	= 0.97
	Repeated measure $\times$ stimulus diet	4.57	1,36	< 0.05
	Repeated measure $\times$ test diet $\times$ stimulus diet	0.89	1,36	= 0.35
<b>Distance to neighbour</b>	Repeated measure	20.74	1,36	0.0001
	Repeated measure $\times$ test diet	1.82	1,36	= 0.19
	Repeated measure $\times$ stimulus diet	9.96	1,36	< 0.01
	Repeated measure $\times$ test diet $\times$ stimulus diet	1.74	1,36	= 0.19
<b>Aggressive interactions</b>	Repeated measure	12.53	1,36	< 0.001
	Repeated measure $\times$ test diet	0.39	1,36	= 0.53
	Repeated measure $\times$ stimulus diet	0.13	1,36	= 0.72
	Repeated measure $\times$ test diet $\times$ stimulus diet	0.10	1,36	= 0.76

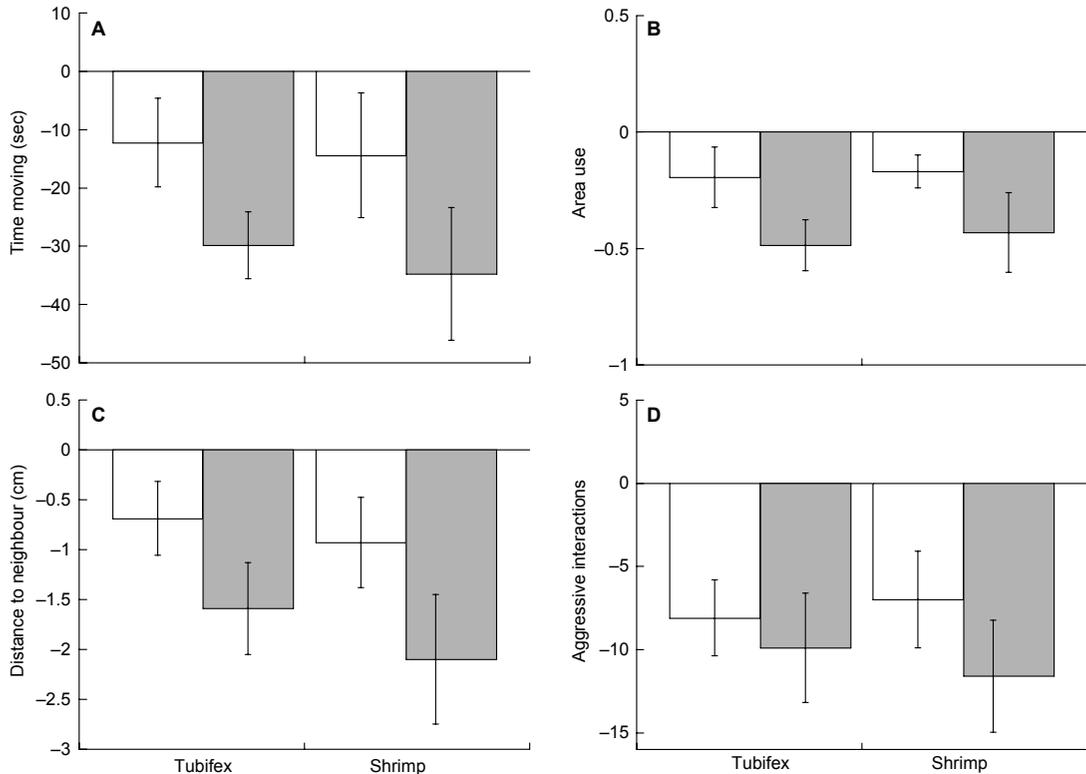


**Fig. 2.** Mean ( $\pm$  S.E.) change (post – pre) in (A) time moving (seconds), (B) area use, (C) distance to neighbour (cm), and (D) occurrence of aggressive interactions for juvenile cichlids fed either tubifex or shrimp diets (cue receivers) and exposed to a distilled water control (open bars) or tubifex or shrimp fed skin extract (cue donors, solid bars).  $N = 10$  per treatment combination.

moving, area use and distance between individuals, we found a significant interaction between stimulus donor diet and the repeated measures effect (Table 2 and Fig. 2). There was no significant interaction between the repeated measures effect and test fish diet for frequency of aggressive interactions (Table 2 and Fig. 2). Regardless of their own diet, juvenile cichlids exposed to shrimp diet skin extract exhibited significantly greater reductions in time moving, area use and distance to neighbour than those exposed to tubifex diet skin extracts. Test fish diet did not affect the response patterns. There was no significant interaction between treatment (repeated measures effect) and either skin extract or test fish

diet for the frequency of aggressive interactions (Table 2 and Fig. 2).

The results of Experiment 1 suggest that cichlids fed a high quality diet produce a chemical alarm cue that elicits a stronger antipredator response than that of cichlids fed a lower quality diet. A potential confound resulting from the growth phase is that the shrimp fed cichlids were in generally better condition than were cichlids fed tubifex. As a result, it is possible that the observed response patterns found in Experiment 1 may be due to overall body condition versus diet *per se*. We, therefore, conducted Experiment 2 to examine the effects of growth (body condition) on the production of chemical alarm cues.



**Fig. 3.** Mean ( $\pm$  S.E.) change in (A) time moving (seconds), (B) area use, (C) distance to neighbour (cm), and (D) occurrence of aggressive interactions for juvenile cichlids exposed to skin extract from low condition index (open bars) versus high condition index (solid bars) donors fed either tubifex or shrimp diets.  $N = 10$  per treatment combination.

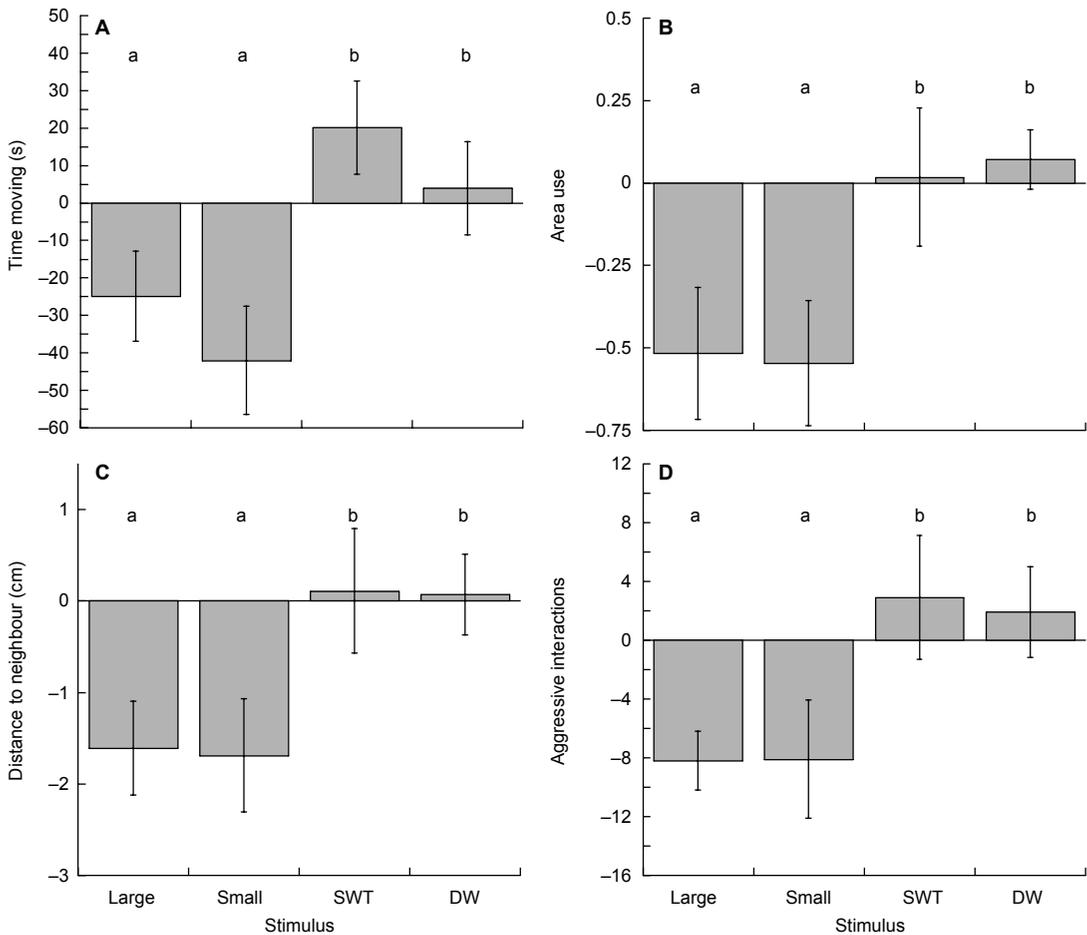
**Table 3.** Results of two-way ANOVAs for each response variable recorded in Experiment 2. Diet denotes diet treatment (shrimp versus tubifex) of stimulus donors, Condition denotes condition index (high versus low) of stimulus donors.  $N = 10$  per treatment.

	<i>F</i>	<i>df</i>	<i>P</i>
Time moving			
Diet	0.15	1,36	= 0.70
Condition	4.25	1,36	< 0.05
Diet $\times$ condition	0.02	1,36	= 0.88
Area use			
Diet	0.10	1,36	= 0.75
Condition	4.76	1,36	< 0.05
Diet $\times$ condition	0.01	1,36	= 0.91
Distance to neighbour			
Diet	0.56	1,36	= 0.46
Condition	4.32	1,36	< 0.05
Diet $\times$ condition	0.08	1,36	= 0.78
Aggressive interactions			
Diet	0.10	1,36	= 0.92
Condition	1.14	1,36	= 0.29
Diet $\times$ condition	0.22	1,36	= 0.64

## Experiment 2: Influence of body condition

For time spent moving, area use and distance to neighbour, we found significant effects of stimulus donor condition index, but no significant effects of stimulus donor diet (Table 3 and Fig. 3). As in Experiment 1, there was no significant difference in the frequency of aggressive interactions (Table 3 and Fig. 3). We found no significant interactions between stimulus donor diet and condition index for any of the response variables (Table 3). Juvenile cichlids exposed to the skin extract of high condition index donors, regardless of donor diet, exhibited significantly more intense antipredator behaviours than did those exposed to the skin extracts of low condition index donors.

The results of Experiment 2 demonstrate that the skin extract of fish of higher condition elicits a stronger antipredator response than does the skin extract of lower condition donors, regardless of the diet type. However, the growth phase



**Fig. 4.** Mean ( $\pm$  S.E.) change in (A) time moving (seconds), (B) area use, (C) distance to neighbour (cm), and (D) occurrence of aggressive interactions for juvenile cichlids exposed to sub-adult conspecific (Large), juvenile conspecific (Small), or swordtail (SWT) skin extracts or a distilled water (DW) control.  $N = 10$  per treatment combination. Different letters denote significant differences ( $P < 0.05$ ) based on Fisher's Protected Least Squared Differences.

of our study suggests that cichlids fed a higher quality diet (shrimp) had a higher growth rate than those fed a lower quality diet (tubifex). As such, differences in growth rate (ontogeny) rather than condition *per se* may account for the observed results. We, therefore, conducted Experiment 3 to address the question of the role of ontogeny in chemical alarm cue production.

### Experiment 3: Effects of ontogeny

For each of the four behavioural measures, we found a significant effect of stimulus type. Cichlids exposed to the same size and larger cichlid skin extract significantly decreased time spent

moving ( $F_{3,36} = 3.93$ ,  $P = 0.016$ ), area use ( $F_{3,36} = 3.32$ ,  $P = 0.031$ ), distance to neighbour ( $F_{3,36} = 3.11$ ,  $P = 0.039$ ), and the frequency of aggressive interactions ( $F_{3,36} = 3.08$ ,  $P = 0.039$ ) when compared with either distilled water or swordtail skin extract (Fig. 4). Moreover, there was no significant difference in the response to same size versus larger conspecific skin extracts (Fig. 4), suggesting that ontogeny does not account for the response patterns reported for Experiment 1 and 2.

### Discussion

Taken together, the results of these experiments strongly support the hypothesis that individual

diet significantly influences chemical alarm cue production in juvenile convict cichlids. Experiment 1 demonstrated that cichlids fed a higher quality diet (brine shrimp) produced an alarm cue, which elicited a significantly stronger anti-predator response in conspecifics. Experiment 2 demonstrated that the mechanism responsible for the observed difference in response intensity was overall condition. Cichlids of higher body condition (i.e. heavier for a given length) were able to produce either more alarm cue per cm<sup>2</sup> of skin or produced a chemical, which was more recognizable. Experiment 3 further supports this hypothesis by demonstrating that when body condition is held constant, the age of skin donors does not result in significant differences in chemical alarm cue production.

Two possible non-mutually exclusive mechanisms may account for the observed diet/body condition effects. Initially, higher quality diets, leading to better overall condition, may result in the production of more chemical alarm cue per area of skin. As a result, the skin extract of high condition donors would result in a higher functional concentration of chemical alarm cue. Such a mechanism assumes that the response to conspecific chemical alarm cues is graded (Brown 2004). There is, however, contradictory evidence regarding graded versus non-graded responses. Brown *et al.* (2001b) exposed shoals of fathead minnows to hypoxanthine-3-*N*-oxide (H3NO; the putative Ostariophysan alarm 'pheromone'; Brown *et al.* 2000, 2001a, 2003) at concentrations ranging between 6.7 to 0.1 nM. Minnows exhibited consistent antipredator behaviour responses when exposed to H3NO at concentrations of 0.4 nM and above. At concentrations below this point, there was no measurable change in overt antipredator behaviour (Brown *et al.* 2001b). In addition, they found that if chemical alarm cues above this threshold are detected, individuals respond in an 'all-or-nothing' fashion. Similar results have been shown for juvenile rainbow trout (*Oncorhynchus mykiss*; Mirza & Chivers 2003a) and pumpkinseed sunfish (*Lepomis gibbosus*; Marcus & Brown 2003). Conversely, Zhao and Chivers (2004), found evidence in support of a graded response in juvenile goldfish (*Carassius auratus*). Juvenile goldfish exhibited antipredator responses that decreased in intensity proportional

to the concentration of alarm cue, suggesting a true graded response pattern.

Alternatively, a higher quality diet may allow individuals to allocate more resources to alarm cue production. There is some indication in the literature that proteins may play a significant role as either recognizable alarm cues or as carrier compounds (Kasumyan & Ponomarev 1987). If individuals can accumulate a higher proportion of proteins in their diet from selectively foraging on higher quality food items, this may then result in an alarm cue that is more readily detected by signal receivers or is transmitted through the water column more readily. Brown *et al.* (2001b, 2003) argued that Ostariophysan alarm cues are produced from the metabolic byproducts of protein degradation. Such a mechanism would be energetically inexpensive, as the precursors to the alarm cue are readily available. If such a mechanism is operating in the cichlid alarm cue system, then increasing the overall quality or quantity of the diet (leading to an overall increase in body condition) may serve as a proximate mechanism accounting for our observed response patterns.

Previous studies have demonstrated significant trade-offs between hunger level and response to conspecific chemical alarm cues. When food-deprived for relatively short time periods (~24 hours), Iowa darters (*Etheostoma exile*) exhibited significant reductions in their response to conspecific alarm cues (Smith 1981). Likewise, fathead minnows (Brown & Smith 1996), finescale dace (*Phoxinus neogaeus*; Brown & Cowan 2000), and reticulate sculpins (*Cottus perplexus*; Chivers *et al.* 2000) fail to respond to conspecific alarm cues when food is deprived for periods of 24 to 48 hours. Thus, it could be argued that since the tubifex diet resulted in an overall poorer quality diet, test fish fed tubifex might be expected to be energetically stressed and hence, show a weaker response to alarm cues. This, however, is not the case in the current study, as we found no significant effect of test fish diet. If the tubifex fed test fish were energetically stressed (relative to shrimp fed test fish), we would expect to see a significant effect of test fish diet. However, cichlids fed tubifex or shrimp responded with similar intensities to the same donor-diet treatments. Likewise, Vilhunen and Hirvonen (2003) failed to find an effect of hunger level on the response

of juvenile Arctic charr (*Salvelinus alpinus*) to conspecific alarm cues.

Alemadi and Wisenden (2002) exposed juvenile convict cichlids within and just beyond the size range at which individuals would be defended by parents. They report that there was no difference in the response by smaller versus larger juveniles to the alarm cue of either size class tested. This suggests that even at an early developmental stage, juvenile cichlids are producing a recognizable alarm cue. Our results expand on this finding, demonstrating that there was no significant difference between juvenile and adult cichlids in the production of the alarm cue. Similar results have also been demonstrated for yellow perch (Harvey & Brown 2004).

It remains unknown if the cichlid alarm cue consists of some specific molecule or group of molecules (as in the Ostariophysan alarm 'pheromone' system; Brown *et al.* 2000, 2001a, 2003) or is some generalized cue. Cichlids lack the specialized epidermal club cells found in Ostariophysan fishes (Pfeiffer 1977), which might suggest a more generalized nature. However, we found no response to swordtail skin extract. Likewise, Wisenden and Sargent (1997) reported no response of juvenile convict cichlids to the skin extract of gambusia (*Gambusia affinis*) and Brown *et al.* (2003) found no response to hypoxanthine-3-*N*-oxide, the putative Ostariophysan alarm 'pheromone'. Combined, these results suggest that convict cichlids do not respond to the generalized cue of any injured prey fish, supporting the existence of a specialized cichlid alarm cue. To date, insufficient work has been conducted to address this question.

Responding to chemical alarm cues can significantly increase an individual's probability of surviving an encounter with a predator (Mathis & Smith 1993, Chivers *et al.* 2002, Mirza & Chivers 2003, G. E. Brown & R. S. Mirza unpubl. data). G. E. Brown and R. S. Mirza (unpubl. data) showed that individuals exhibiting a stronger antipredator response gain proportionally greater survival benefits. As such, a potential benefit associated with shoaling near conspecifics in good condition might be increased potential of detecting and responding to these critically important cues. Experiments are ongoing to directly test this hypothesis.

Size specific shoaling is well documented among prey fishes (Ward & Krause 2001), especially under conditions of increased perceived predation risk (Hoare *et al.* 2004). Preferentially shoaling with similar sized and/or morphologically similar individuals has been argued to reduce conspicuousness and hence predation risk (Landeau & Terborgh 1986, Theodorakis 1989, but *see* Mathis & Chivers 2003). In addition, differences in competitive ability would result in smaller individuals being out-competed by larger shoalmates (Peuhkuri 1997, Seppa *et al.* 1999, Kim *et al.* 2004). Individuals shoaling with similar sized or conditioned conspecifics versus smaller (Ward & Krause 2001) or those in lower body condition (Barber *et al.* 1998, Poulin *et al.* 1999) may gain additional benefit associated with stronger response intensities upon detection of conspecific alarm cues. Given the demonstrated survival benefits associated with responding to damage released chemical alarm cues (*see* above), selection should favour such size assortative shoaling under conditions of high predation risk (Hoare *et al.* 2004). Thus, in addition to the well documented direct benefits associated with size assortative shoaling, our current results suggest there may exist indirect benefits as well.

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