

# **RESPONSE OF JUVENILE GOLDFISH (*CARASSIUS AURATUS*) TO CHEMICAL ALARM CUES: RELATIONSHIP BETWEEN RESPONSE INTENSITY, RESPONSE DURATION, AND THE LEVEL OF PREDATION RISK**

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## **1. INTRODUCTION**

How prey animals balance their activities against antipredator demands has been a major issue in modern behavior ecology (Sih, 1984; 1992; Berejikian et al., 1999). Prey animals in nature experience a broad range of predation risk. Failure of prey animals to respond to predators results in a high risk of being attacked or captured. In contrast, prey that overrespond to a threat may waste time and energy that would otherwise be directed towards other activities (Sih, 1992; Lima and Dill, 1990; Chivers et al., 2001). Helfman (1989) proposed the threat-sensitive predator avoidance hypothesis to reflect this dynamic balance; the intensity of antipredator response of prey should reflect the level of predation threat (Helfman, 1989; Sih, 1992; Chivers et al., 2001; Chivers and Mirza, 2001).

Recent studies indicate that chemical cues provide a wealth of information for aquatic prey in assessing predation risk (Chivers and Smith, 1998; Kats and Dill, 1998; Chivers and Mirza, 2001). Aquatic media are well suited for chemical signals because a large number of compounds can dissolve in water, providing a large number of potential chemical signals for detection (Hara, 1994). Regarding chemical cues, the concentration of alarm cue that prey animals detect may be used to distinguish the degree of predation threat. The ability to exhibit threat-sensitive avoidance has important implications. By being able to differentiate between different cue concentrations, prey animals will not waste time and energy responding to predators that do not pose an imminent threat.

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In fishes, damage-released alarm cues are best characterized in the Superorder Ostariophysii (Smith, 1992). Goldfish, *Carassius auratus*, like other Ostariophysans, possess chemical alarm cues, which are released from epidermal club cells on injury and serve to warn nearby individuals of potential threat. When conspecifics detect the alarm cue, they respond with anti-predator behavior, including shoaling, reduced foraging, freezing or dashing (e.g., Wisenden and Smith, 1997; Chivers and Smith, 1998; Mirza and Chivers, 2003a, b). In this study we exposed juvenile goldfish to varying concentrations of conspecific alarm cue and quantified the behavioural response. We predicted that fish would respond with a greater intensity and duration to higher concentration chemical alarm cues than to lower concentration cues. The intensity and duration of the response to different chemical alarm cue concentrations has not been reported. Such studies are critical in understanding the ecological significance of responses to chemical alarm cues.

## 2. METHODS

We obtained juvenile goldfish from a commercial supplier in May, 2003. The fish were kept in 622-L artificial-stream tanks at about 14°C for 10 days prior to the experiment. Fresh dechlorinated water was introduced into the holding tank at a rate of 1 L/min. Fish were fed daily on commercial fish pellets.

Fish alarm cue was prepared from 8 small goldfish ( $4.23 \pm 0.18$  cm standard length (mean  $\pm$  SE)). Fish were humanely killed with a single blow to the head in accordance with guidelines set by the Canadian Council on Animal Care. A fillet of skin was removed from both sides of each fish and placed in 100 mL of chilled glass-distilled water. A total of 31.85 cm<sup>2</sup> of skin was collected which was then homogenized, filtered through filter floss to remove the larger particles, and then diluted in distilled water to make a final volume of 620 mL. This represented our base solution (1:1). We then diluted the appropriate volumes of the base solution with distilled water to create the following dilutions: 1:10, 1:100, 1:1000, and 1:5000. Stimulus solutions were pipetted into sample bags in 30-mL aliquots and frozen at -20°C until use. Distilled water was pipetted in 30-mL aliquots and frozen as well. During each behavioural trial (see below) we introduced 10 mL of either goldfish stimulus or distilled water.

We conducted a total of 90 trials, fifteen replicate trials in each of six treatments, five alarm cues (1:1, 1:10, 1:100, 1:1000, 1:5000) and one distilled water control treatment. Each trial consisted of three randomly chosen fish, thus a total of 270 juvenile goldfish were used. Each group of fish was used only once, and the order of the treatments was randomized.

The experimental set-up and procedure were similar to previous studies in our laboratory (e.g., Mirza and Chivers, 2003a, b). The trials were performed in 74-L aquaria (60 x 30 x 40 cm<sup>3</sup>). Each tank had two horizontal, 10-cm-long lines on the front of the tank which divided the tank into 3 vertical areas. Each of the test tanks contained a single airstone mounted in the centre of the end wall. A piece of airline was attached to the airstone.

Fish were allowed to acclimate in the test chambers for 48 h prior to trials. Each trial was 18 min in length and consisted of an 8-min pre-stimulus period and an 8-min post-stimulus period, with a 2-min stimulus-introduction period between the pre- and post-stimulus periods. Dye trials indicated that it took approximately 40 seconds for the

stimulus to disperse throughout the test tank. Prior to the prestimulus period, we removed and discarded 60 ml of water through the stimulus injection tube. We then removed and retained an additional 60 mL of water. After the prestimulus observation period, we injected either 10 mL of goldfish stimulus or distilled water and flushed it through with the 60 mL of the previously retained water. The tanks were drained and thoroughly rinsed after each use.

During both the pre- and post-stimulus periods we recorded shoaling index and vertical area use. Shoaling index was scored from 1 (no fish within one body length of another) to 3 (all 3 individuals within one body length of each other) and was recorded every 15 second. An area use score was also recorded every 15 seconds. The score was the sum of each fish's score (1 = the lower third of the water column, 2 = the middle third of the water column, and 3 = the upper third of the water column). A score of 3 would indicate that all fish were in the bottom third of the tank and a score of 9 would indicate that all fish were in the top third of the tank. The occurrence of freezing (fish drop to the bottom and remain motionless for at least 30 seconds) and dashing (a rapid burst of apparently disoriented swimming) (Chivers and Smith, 1998) behaviour was also recorded in each trial.

To assess the effect of variation in alarm cue concentration on the intensity of alarm responses, we calculated the average shoaling index and vertical area scores for each group of fish for both the 8 minute pre-stimulus and the 8 minute post-stimulus periods. Changes between the pre- and post-stimulus periods for each response variable were calculated as post-stimulus minus pre-stimulus. These differences were compared using a one way ANOVA followed by Tukey post-hoc tests (Zar, 1999). The occurrences of freezing and dashing were compared using a Chi-square test for independence (Zar, 1999).

In order to assess the effect of variation in alarm cue concentration on the duration of alarm responses, we calculated the average shoaling index and vertical area use score for each group of fish during the pre-stimulus period to determine a baseline level of response. Following exposure to the stimulus we calculated the shoaling index and vertical area scores for each group of fish for 1 minute intervals of the 8 minute post-stimulus period. We used repeated measures ANOVA to determine the effects of treatment on the response of the fish and whether there was a treatment by time interaction.

### 3. RESULTS

#### 3.1 Intensity of Alarm Response

Results from the one way ANOVA followed by Tukey post-hoc tests show that exposure to higher concentrations of alarm cue (1:1, 1:10, 1:100) caused a significant increase in shoaling and area use compared with lower concentrations of alarm cue (1:1000 and 1:5000) and distilled water (Figures 1 and 2). There were no significant differences between any two adjacent concentrations in both shoaling and area use behaviours (Figures 1 and 2).

Goldfish showed a greater amount of freezing and dashing as the concentration of alarm cues increased. Goldfish exhibited at least one period of freezing in all 15 trials when exposed to an alarm-cue concentration of 1:1 compared with 12 out of 15 trials to a

concentration of 1:10, 9 out of 15 trials to a concentration of 1:100, and 5 out of 15 trials to a concentration of 1:1000. There were no occurrences of freezing when fish were exposed to conspecific alarm cue concentrations of 1:5000 or distilled water ( $\chi^2 = 48.75$ ,  $df = 5$ ,  $p < 0.001$ ). Fish exhibited dashing in all 15 trials as well when exposed to alarm-cue concentration of 1:1 compared with 13 out of 15 trials to a concentration of 1:10 and 7 out of 15 times to a concentration of 1:100. There was significantly less dashing when fish were exposed to conspecific alarm cue concentrations of 1:1000 or 1:5000 or distilled water (4, 2, 1 respectively) ( $\chi^2 = 45.54$ ,  $df = 5$ ,  $p < 0.001$ ).

3.2 Duration of Alarm Response

Results from repeated measures ANOVA show there was a significant treatment effect and that there was a treatment by time interaction in terms of both shoaling index ( $F = 154.90$ ,  $p < 0.001$ , Figure 3) and area use ( $F = 233.05$ ,  $p < 0.001$ , Figure 4). At higher concentrations the responses were longer and stronger than those at lower concentrations.

4. DISCUSSION

In our experiment, we were able to determine the behavioural response intensity of juvenile goldfish exposed to different concentrations of conspecific alarm cues. Goldfish exposed to higher concentrations of alarm cues significantly increased shoaling and dashing more than goldfish exposed to lower concentrations of alarm cue, while those exposed to distilled water and the lowest concentration of alarm cues showed no response. These results indicate that responses to different concentrations of chemical cues are not generalized, but instead are graded to reflect the degree of threat.

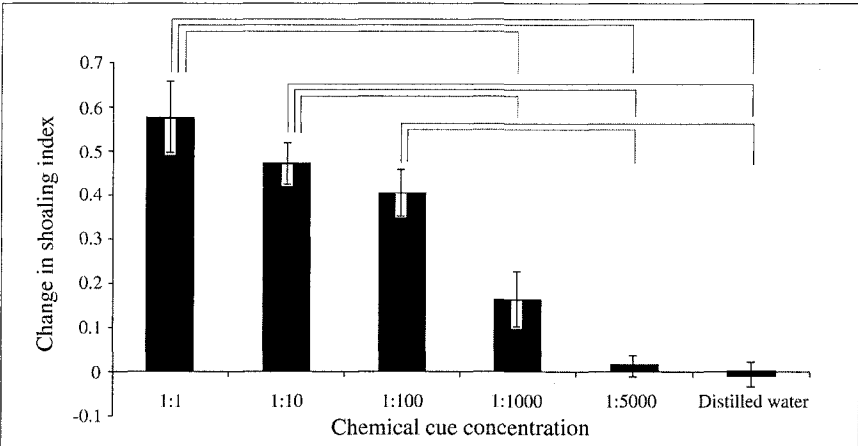
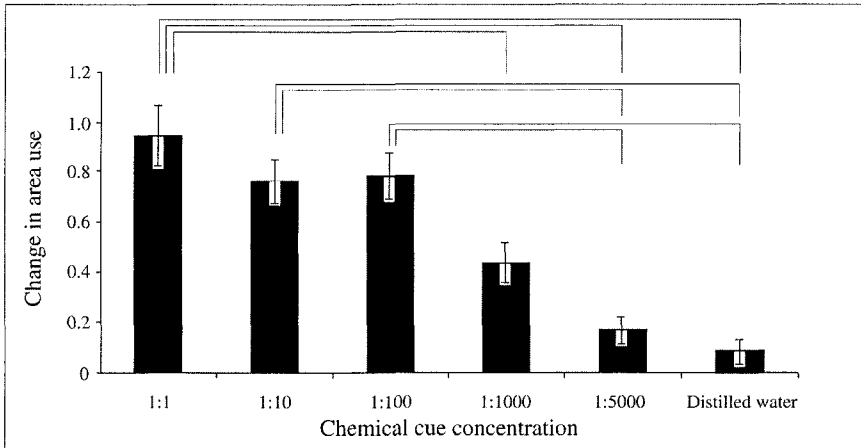
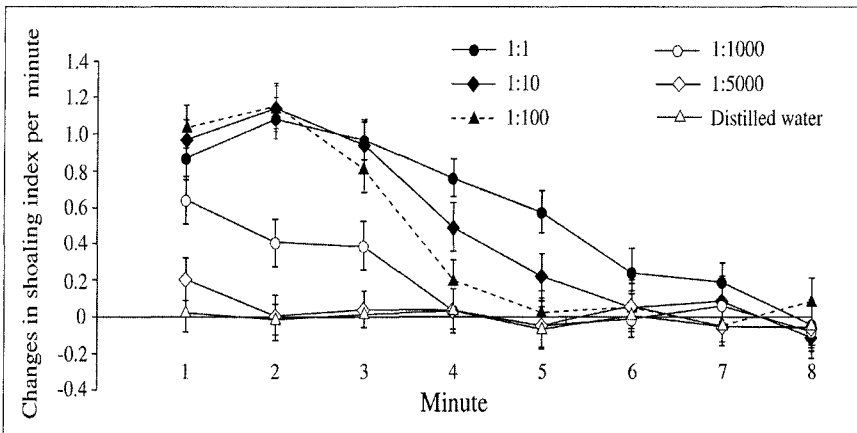


Figure 1. Mean ( $\pm$  SE) changes in the shoaling index of goldfish exposed to different concentrations of alarm cues. Lines over bars denote significance at  $p < 0.05$  (see text for details).

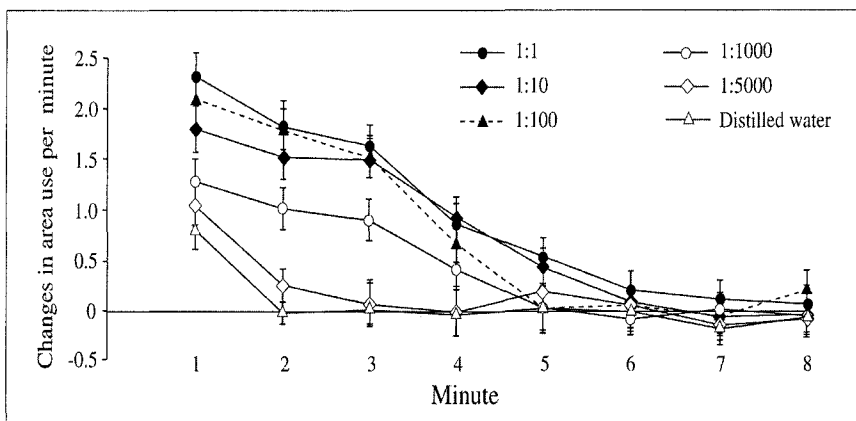


**Figure 2.** Mean ( $\pm$  SE) changes in the area use of goldfish exposed to different concentrations of alarm cues. Lines over bars denote significance at  $p < 0.05$  (see text for details).

Our data emphasize the importance of including a temporal component when measuring behavioural responses to alarm cues. Conclusions based on a minute by minute analysis clearly demonstrate that fish exposed to higher concentrations of alarm cue will exhibit a longer duration response than those exposed to lower concentrations of alarm cues. This provides another line of evidence that fishes exhibit an anti-predator response that matches the threat. Had we used the average response of the fish during the



**Figure 3.** Changes in shoaling-index score per minute (mean  $\pm$  SE) for goldfish exposed to chemical alarm cue of different concentrations and distilled water.



**Figure 4.** Changes in area use score per minute (mean  $\pm$  SE) for goldfish exposed to chemical alarm cue of different concentrations and distilled water.

8 minute post-stimulus periods, we would have missed this important temporal component, as have most previous studies of alarm cues (review Chivers and Smith, 1998). Reporting the average response would also miss the fact that fish returned to normal baseline activity within minutes of being exposed to the threat.

Combining information on the amount of alarm cue released by prey during a predation event with information on the active space of an alarm cue may indicate the distance at which animals can detect a nearby predation threat (Mirza and Chivers, 2003a). No studies have been conducted to determine the amount of alarm cue released during a predation event, but some authors have attempted to determine the active space of alarm cues. Lawrence and Smith (1989) calculated the active space of 1 cm<sup>2</sup> of fathead minnow, *Pimephales promelas*, skin as being greater than 58, 823 L. Likewise, Mirza and Chivers (2003a) found that juvenile rainbow trout, *Oncorhynchus mykiss*, showed a behavioral response threshold at 1 cm<sup>2</sup> skin in 134, 255 L of water. Our results suggest that 1 cm<sup>2</sup> of goldfish skin creates an active space of 122, 637 L. Mirza and Chivers (2003a) argue that these values are overestimates because they are calculated based on the final concentration that would be achieved if the stimulus was completely dispersed through the test tank. We also need to be cautious when drawing conclusions about the response thresholds of prey to alarm cues because the prey's physiological response threshold and its behavioural response threshold may be different. Both Brown et al. (2001) and Mirza and Chivers (2003a) provide evidence that prey fishes have the ability to use chemical alarm cues to assess their level of risk in the absence of an observable antipredator response. They found that prey exposed to concentrations of alarm cue below their behavioral-response threshold were still using the information to assess predation risk (Mirza and Chivers, 2003a). Notwithstanding the cautions outlined above, our data suggest that prey animals have the ability to detect predation-risk cues at a long distance.

Our data suggest that chemical alarm cues at different concentrations may provide important information for prey to assess and mediate their risk of predation. This general predation risk information is valuable even if information regarding the actual predator is lacking. It may be common for a prey animal to be alerted to the presence of a potential risk before the predator is detected. In this way prey animals are able to make an accurate assessment of their level of predation risk and to adjust the intensity of their antipredator response to reflect this risk. By responding to different intensity signals, the prey may obtain the advantage of alertness and lower susceptibility, thereby reducing their risk of mortality.

In nature prey animals might face sustained periods of risk when predators are abundant and in close proximity; in contrast, they might experience low risk when predators are sparse and wide ranging (Sih and McCarthy, 2002). Also, depending on the characteristics of the water body, it is possible that alarm cues could be diluted to lower concentrations as well as mixed with other odours from the environment, which may decrease the intensity of prey's antipredator response. Therefore, additional studies aimed at understanding the importance of graded responses to different concentrations of chemical cue in nature are needed.

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