Shoal choice in zebrafish, *Danio rerio*: the influence of shoal size and activity

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Shoaling fish are expected, in many cases, to gain fitness benefits from being in a larger shoal and previous experiments have shown that fish are indeed capable of choosing between shoals of different sizes. We investigated the influence of shoal activity on shoal size preference in the zebrafish. We gave test fish the choice between shoals of one to four stimulus fish, presented at two different water temperatures, and so differing in their activity levels. Where all stimulus fish were in water of the same temperature, test fish generally preferred the larger shoal. However, this preference could be reduced by presenting the larger shoal in colder water and so reducing its activity. We discuss these findings with reference to the factors that may influence shoal activity, the effect of temperature on shoaling behaviour and the mechanisms that may be used by fish to discriminate shoal size.

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Group living is a commonly observed phenomenon in nature and has been studied in a wide range of taxa. A number of costs and benefits of grouping have been identified including those relating to predation risk, foraging efficiency, territorial defence and reproductive success (Pulliam & Caraco 1984). The balance of costs and benefits for a given group member is expected to depend upon a multitude of factors including properties of both the individual and the group. One of these is group size. Fish in larger shoals are expected to suffer a lowered predation risk as a result of factors such as increased predator confusion (Landeau & Terborgh 1986), collective vigilance (Godin et al. 1988) and social information transfer (Mathis et al. 1995). There may also be foraging benefits to joining a larger group (Ryer & Olla 1992). Reflecting such advantages, laboratory studies have frequently shown association preferences of test fish for larger rather than smaller shoals (e.g. Krause et al. 1997; Reebs & Saulnier 1997).

Krause & Godin (1995), looking at the response of predatory cichlids, *Aequidens pulcher*, to shoals of guppies, *Poecilia reticulata*, found that attack frequency increased with increasing shoal activity. This suggests that activity as well as size might be a factor influencing predation risk for shoal members, an idea supported by the observation that shoaling fish may alter their activity levels in response to a perceived predation threat (e.g. Rehnberg & Smith 1988). Fish may have additional reasons for taking the activity level of a shoal into account. A given individual joining a shoal risks increased competition for food, which will depend both on shoal size and the nutritional status of the fish. Certain studies suggest that food deprivation may influence swimming speed in fish (Robinson & Pitcher 1989; Mikheev et al. 1992), and therefore activity levels in a shoal may be used as an indication of the nutritional status of its members (but see Krause et al. 1999). A shoal may also increase its swimming activity when anticipating food (Reebs & Gallant 1997) or encountering a food patch (Johansson & Leonardsson 1998).

No study has yet investigated whether fish take shoal activity into account when making a shoaling decision. We addressed this question by investigating the interacting effects of activity and size cues on shoal choice by the zebrafish. This tropical cyprinid forms small shoals in the wild (2–10 fish, personal observation) and has frequently been used in studies of fish behaviour (e.g. Bloom & Perlmutter 1977; Suboski et al. 1990).

METHODS

Experimental subjects (*N*=200) were the first-generation offspring of ca. 100 wild zebrafish which had been collected from Tribhuvan University campus, Rampur, Nepal in March 1996 and imported into the U.K. under a MAFF Tropical Fish Import licence. These fish had been transported in double-skin plastic bags containing oxygen over water and carried as hand luggage in the aeroplane; no fish died during transport. Eggs were obtained from group spawnings of these zebrafish in the laboratory

and offspring reared to adulthood en masse in 15-litre tanks (up to 35 fish/tank). Each tank contained a filter unit and gravel substrate; the temperature of the rearing unit was maintained at $25 \pm 1°C$ with a 14:10 h light:dark regimen. The fish were fed daily on Aquarian Tropical flake food. The fish were mixed between tanks frequently during rearing and also when being transferred to the experimental holding tanks; therefore we considered all experimental fish to be equally familiar with each other before the experiments. For the duration of the trials 'test' and 'stimulus' fish (mean standard length $\pm SD=25 \pm 1.5$ mm) were housed separately in 20-litre plastic tanks with a maximum stocking density of 2 fish/litre. Water temperature was held at $22 \pm 1°C$ and the fish were fed daily ad libitum on 'Aquarian' flake food. Since we could not reliably distinguish male and female zebrafish we used mixed-sex groups in the trials. Zebrafish come into breeding condition with a long day length and spawn at dawn (Westerfield 1993); we therefore minimized any influence of sexual behaviour on our results by adjusting the light cycle to 12:12 h light:dark and commencing trials 3 h or more after 'daybreak'.

Quantification of Fish Activity

In the first part of the study, we investigated whether we could manipulate zebrafish activity levels by varying water temperature (as in Krause & Godin 1995). We quantified fish activity at three water temperatures: 15, 20 and 25°C. These temperatures are within the range that a zebrafish might be expected to experience in the wild (personal observation).

We released an individual fish into a tank (14×20 cm and 20 cm high), lined with white polystyrene to increase contrast between the fish and the background, and filled to a depth of 11 cm with water that had been adjusted to the appropriate temperature. The fish was allowed 10 min to acclimate to the tank and water temperature. We then recorded its behaviour for 10 min with a video camera positioned directly above the tank. Fish movement was analysed with Ethovision Pro 95 motion detection software (Noldus Information Technology, Wageningen, The Netherlands). We used total distance moved over the 10-min observation period as our measure of fish activity.

We used four fish at each temperature treatment. All test fish were matched for size and fed in the stock tank immediately before a trial to control for any effect of hunger on activity levels; test fish were not reused. Treatment order was randomized.

Effect of Temperature upon Shoal Size Choice

In the second part of the study we examined the effect of shoal size and water temperature on zebrafish shoaling preferences. In each trial we gave a test fish the choice between a group of four fish, designated the 'reference shoal', and an alternative group of one, two, three or four

individuals. For half of the trials the reference shoal of four was presented in water at 25°C ('warm') and for the other half in water at 15°C ('cold'). The alternative shoal and the test fish were in water of 25°C for all trials.

The experimental apparatus comprised a test tank (30×20 cm and 25 cm high) with two smaller stimulus tanks (14×20 cm and 25 cm high), positioned at either end. Within the test tank we defined two 5-cm-wide preference zones adjacent to the side of each stimulus tank and marked by lines drawn on the tank wall. We considered a fish to be associating with a stimulus shoal when any part of its body was within the relevant preference zone. Each tank contained 2 cm of gravel substrate and was filled to a depth of 11 cm with water of the appropriate temperature immediately before each trial. A gap of ca. 5 mm was left between the sides of the test tank and each stimulus tank to minimize the development of any temperature gradient. The sides and back of the combined tank set-up were covered in brown paper. All observations were made from behind a hide to minimize disturbance to the fish.

We conducted trials as follows. We selected stimulus fish from the stock aquaria and placed them in the stimulus tanks containing water at the appropriate temperature. These fish were allowed to settle for 10 min. We then introduced a single test fish into a transparent cylinder placed in the centre of the test tank. After 10 min we released the fish by raising the cylinder using a remote pulley system. We observed the fish for a 10-min period and recorded how long it spent in each preference zone.

We did 64 trials, representing eight replicates for each of the eight treatments. Test and stimulus fish were fed in their holding tanks immediately before the experiments and carefully size matched within each trial. We used test fish once only throughout all trials; however, stimulus fish were selected from a pool of 80 individuals and returned to this pool after use. Treatment order and position of the stimulus shoals were randomized.

Occasionally (in ca. 5% of all trials), test or stimulus fish showed fright reactions such as freezing or predator evasion manoeuvres. When this was observed we aborted the trial and ran it with different individuals. Experimental fish did not exhibit adverse reactions to being moved to water of different temperatures other than those expected as a result of transferral between tanks. The stock holding temperature of 22°C was chosen so that all fish experienced a temperature change when introduced to the experimental apparatus.

Statistical Analyses

All statistical analyses were performed with SPSS for Windows, Release 9.0.0. Data sets were checked for normality (Kolmogorov–Smirnov one-sample test) and equality of variance (Levene's test) before analysis. All tests are two tailed.

Quantification of fish activity

The effect of water temperature on total distance moved was investigated with ANOVA. Data were log transformed to reduce heterogeneity among variances.
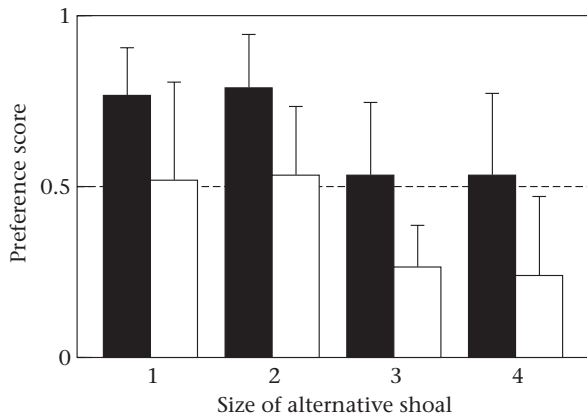
Figure 1. Mean+SD proportion of total shoaling time (preference score) that test fish spent with the reference shoal of four fish. The reference shoal was presented in either warm (■) or cold (□) water; size of the alternative shoal is shown. $N=8$ for each treatment.

Shoal size choice

For each trial we calculated total shoaling time, defined as the sum of the times that the test fish spent in each preference zone. We then generated a preference score by dividing time spent with the reference shoal of four fish by total shoaling time. Preference scores were arcsine transformed prior to analysis.

We used one-sample t tests to compare preference scores under each treatment to the mean preference score of 0.5 (arcsine transformed) that would be expected if the test fish allocated their time randomly to each shoal. A significant difference indicated an association preference for one of the two shoals under the treatment that we were considering. The overall effects of alternative shoal size and water temperature on preference score and total time shoaling were investigated with ANOVA.

RESULTS

Fish Activity at Different Temperatures

Temperature had a significant effect upon total distance moved (ANOVA: temperature: $F_{2,9}=7.902$, $P=0.01$), with fish being more active at higher temperatures.

Shoaling Preferences

Test fish visited both shoals in all trials (total shoaling time, mean $\pm$ SD$=441.0 \pm 72.3$ s). Fish within a preference zone typically swam to and fro against the glass adjacent to the stimulus shoal; time outside a preference zone was spent shuttling between shoals or, more rarely, swimming slowly around the tank. Significant association preferences for one of the two stimulus shoals were seen in four of the treatments (4 warm versus 1 warm: $t_8=4.14$, $P=0.004$; 4 warm versus 2 warm: $t_8=3.86$, $P=0.006$; 4 cold versus 3 warm: $t_8=-5.12$, $P=0.001$; 4 cold versus 4 warm: $t_8=-3.08$, $P=0.018$; Fig. 1). No significant preferences occurred in the remaining treatments (4 warm versus 3 warm: $t_8=0.37$, NS; 4 warm versus

4 warm: $t_8=0.27$, NS; 4 cold versus 1 warm: $t_8=0.25$, NS; 4 cold versus 2 warm: $t_8=0.5$, NS; Fig. 1).

Overall both water temperature and number of fish in the alternative shoal had a significant effect on proportion of shoaling time spent with the reference shoal (ANOVA: temperature: $F_{1,56}=23.01$, $P<0.001$; number: $F_{3,56}=8.12$, $P<0.001$; Fig. 1). There was no interaction between these two factors (ANOVA: $F_{3,56}=0.076$, $P=0.973$).

DISCUSSION

Our results clearly indicate that, in zebrafish, shoaling preference is influenced by the overall activity of alternative shoals. When choosing between shoals at the same temperature and differing in size by more than one fish, zebrafish spent more time with the larger shoal. However, when we manipulated shoal activity by changing the water temperature, the zebrafish tended to spend more time with the group that was expected to be more active overall, in terms of number of fish and swimming speed, even when it was smaller.

The zebrafish preferred to associate with more active shoals even though these might have been more conspicuous to predators. There may be several reasons for this result. First, both larger and more active shoals might also have been more conspicuous to the test fish. However, fish in all trials visited each alternative shoal at least once, meaning that they were aware of the presence of both shoals and able to choose between them. A second possibility is that zebrafish might simply have used shoal activity to gauge shoal size. Zebrafish in this experiment preferred to associate with larger groups and would be expected to gain fitness benefits by choosing to join them. Little work has been done to investigate the mechanism by which fish, or indeed any other animal taxa, assess group size; overall activity level of a shoal may be perceptually easier for a fish to assess than actual number of individuals.

A third explanation for the findings is that associating with more active fish may provide advantages over and above those provided by larger groups so that test fish were making a trade-off between group size and group activity. Shoals containing more active fish may, for example, find food patches more rapidly, be actively feeding or be more confusing to predators. Reebs & Gallant (1997) found that hungry golden shiners, *Notemigonus crysoleucas*, preferred to join more active, food-anticipatory shoals. Alternatively, a test fish might have been aware of its own swimming speed and avoided joining a slower-moving shoal where it risked an increased predation risk from the 'oddity effect' (Theodorakis 1989). This latter possibility could be addressed by repeating the experiment with test fish at 15°C.

Water temperature may have affected the appearance of the stimulus shoals in ways other than simply changing their activity levels. Weetman et al. (1998), for example, found that Trinidadian guppies, *Poecilia reticulata*, formed more closely knit shoals at higher water temperatures. Fish may well prefer to join a more

cohesive shoal, perhaps because this may give better protection from predators. However, this cannot explain our results where the alternative shoal consisted of a single fish, unless the cohesion of the group of four in cold water was reduced sufficiently that the test fish did not perceive them as an aggregated unit.

This study has shown for the first time that activity cues may be used by fish when making shoaling decisions. Fish may prefer to associate with more active individuals because this in itself provides fitness benefits. Alternatively, activity might be used as a cue by which a fish assesses shoal size. Further work is required to disentangle these possible explanations.

Acknowledgments

References

Bloom, H. D. & Perlmutter, A. 1977. A sexual aggregating pheremone system in the zebrafish, *Brachydanio rerio* (Hamilton-Buchanan). *Journal of Experimental Zoology*, **199**, 215–226.

Godin, J. -G. J., Classon, L. J. & Abrahams, M. V. 1988. Group vigilance and shoal size in a small characin fish. *Behaviour*, **104**, 29–40.

Johansson, F. & Leonardsson, K. 1998. Swimming speeds and activity levels of consumers at various resource and consumer densities under predation risk. *Canadian Journal of Zoology*, **76**, 76–82.

Krause, J. & Godin, J. -G. J. 1995. Predator preferences for attacking particular prey group sizes: consequences for predator hunting success and predation risk. *Animal Behaviour*, **50**, 465–473.

Krause, J., Rubenstein, D. & Brown, D. 1997. Shoal choice behaviour in fish: the relationship between assessment time and assessment quality. *Behaviour*, **134**, 1051–1062.

Krause, J., Hartmann, N. & Pritchard, V. L. 1999. The influence of nutritional state on shoal choice in zebrafish, *Danio rerio*. *Animal Behaviour*, **57**, 771–775.

Landeau, L. & Terborgh, J. 1986. Oddity and the 'confusion effect' in predation. *Animal Behaviour*, **34**, 1372–1380.

Mathis, A., Chivers, D. P. & Smith, R. J. F. 1995. Cultural transmission of predator recognition in fish: intraspecific and interspecific learning. *Animal Behaviour*, **51**, 185–201.

Mikheev, V. N., Pavlov, D. S. & Pakulska, D. 1992. Swimming response of goldfish, *Carassius auratus*, and the tetra, *Hemigrammus caudovittatus*, larvae to individual food items and patches. *Environmental Biology of Fishes*, **35**, 351–360.

Pulliam, H. R. & Caraco, T. 1984. Living in groups: is there an optimal group size? In: *Behavioural Ecology: An Evolutionary Approach*. 2nd edn (Ed. by J. R. Krebs & N. B. Davies), pp. 122–147. Oxford: Blackwell Scientific.

Reebs, S. G. & Gallant, B. Y. 1997. Food-anticipatory activity as a cue for local enhancement in golden shiners (Pisces: Cyprinidae, *Notemigonus crysoleucas*). *Ethology*, **103**, 1060–1069.

Reebs, S. G. & Saulnier, N. 1997. The effect of hunger on shoal choice in golden shiners (Pisces: Cyprinidae, *Notemigonus crysoleucas*). *Ethology*, **103**, 642–652.

Rehnberg, B. G. & Smith, R. J. F. 1988. The influence of alarm substance and shoal size on the behaviour of zebra danios, *Brachydanio rerio* (Cyprinidae). *Journal of Fish Biology*, **33**, 155–163.

Robinson, C. J. & Pitcher, T. J. 1989. The influence of hunger and ration level on shoal density, polarization and swimming speed of herring, *Clupea harengus* L. *Journal of Fish Biology*, **34**, 631–633.

Ryer, C. H. & Olla, B. L. 1992. Social mechanisms facilitating exploitation of spatially variable ephemeral food patches in a pelagic marine fish. *Animal Behaviour*, **44**, 69–74.

Suboski, M. D., Bain, S., Carty, A. E., McQuoid, L. M., Seelen, M. I. & Seifert, M. 1990. Alarm reactions in acquisition and social transmission of simulated-predator recognition by the zebra danio fish (*Brachydanio rerio*). *Journal of Comparative Psychology*, **104**, 101–112.

Theodorakis, C. W. 1989. Size segregation and the effects of oddity on predation risk in minnow schools. *Animal Behaviour*, **38**, 496–502.

Weetman, D., Atkinson, D. & Chubb, J. C. 1998. Effects of temperature on antipredator behaviour in the guppy, *Poecilia reticulata*. *Animal Behaviour*, **55**, 1361–1372.

Westerfield, M. (Ed.) 1993. *The Zebrafish Book: a Guide for the Laboratory Use of Zebrafish (*Brachydanio rerio*)*. 2.1 edn. Eugene, Oregon: University of Oregon Press.