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BRIEF COMMUNICATIONS

Influence of familiarity on shoaling behaviour in Texas and blacktail shiners

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The Texas shiner *Notropis amabilis* and the blacktail shiner *Cyprinella venusta* demonstrated statistically significant preferences for the side of the tank holding their familiar shoalmates, and significantly stronger shoaling behaviour with their familiar shoalmates, than with unfamiliar fish.

Key words: *Cyprinella venusta*; familiarity; *Notropis amabilis*; schooling behaviour; shoaling behaviour.

By living in groups, individual animals face both potential benefits (improved foraging, greater protection from predators) and costs (increased competition for resources, space and mates) in terms of Darwinian fitness (Alexander, 1974; Wilson, 1980). One of the best known and most complex grouping behaviours in nature is shoaling in fishes (Pitcher & Parrish, 1993). One of the benefits of living within schools (*i.e.* polarized shoals) is that individuals escape predation because members appear as many closely linked individual targets, but the similarities and synchronized movements of the targets make them difficult for a predator to track. This so-called 'confusion effect' makes it difficult for a predator to choose and focus its attack upon one target (Welty, 1934; Broadbent, 1965; Pitcher & Parrish, 1993). In order for the confusion effect to work, fish within a school must appear highly uniform and move in synchrony (Pitcher & Parrish, 1993). Individual variation in appearance (the 'oddity effect') is selected against because fish that differ in appearance from their schoolmates are more easily targeted and captured by predators (Landeau & Terborgh, 1986).

Recent studies on fish assortative schooling behaviour report that fishes prefer schooling with fishes with which they have schooled previously (*i.e.*

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'familiar' individuals) (Van Havre & Fitzgerald, 1988; Magurran *et al.*, 1995; Lachlan *et al.*, 1998). Chivers *et al.* (1995) found that schools of familiar fathead minnows *Pimephales promelas* Rafinesque were more cohesive and demonstrated more complex and potentially beneficial group behaviours, such as predator inspection. Prior research on the familiarity preference has involved three-spinned sticklebacks *Gasterosteus aculeatus* L. (Van Havre & FitzGerald, 1988), guppies *Poecilia reticulata* Peters (Griffiths & Magurran, 1997*a*), fathead minnows (Chivers *et al.*, 1995), European minnows *Phoxinus phoxinus* (L.) (Griffiths, 1997), brown trout *Salmo trutta* L. (Höjesjö *et al.*, 1998), Atlantic salmon *Salmo salar* L. (O'Connor *et al.*, 2000) and others (Krause *et al.*, 2000).

In this study, the role of familiarity in shoaling behaviour was investigated experimentally for the Texas shiner *Notropis amabilis* (Girard) and blacktail shiner *Cyprinella venusta* Girard, two previously untested cyprinids found in shallow, fast-moving rivers subject to seasonal flooding. Natural populations of both species were sampled from shoals occurring a minimum of 40 m apart in a 6 km section of the Guadalupe River, Kendall County, Texas, U.S.A. (29°57′ N; 98°42′ W). Baited traps were set for from 1–3 days to catch fishes in deeper, faster-moving portions of the river, whereas nets were used to catch individuals in slowly moving water along the edges as well as in isolated pools. Texas shiners were collected during October and November 2000; blacktail shiner, from January to March 2001, from shoals of *c.* 10 to 50 individuals. All experiments were performed prior to the spawning season for these species and no mating behaviours were observed.

The first experiment tested whether Texas shiner selectively shoal with familiar individuals. Sample groups of seven fish from six separately occurring shoals ($n = 42$) were placed in $46 \times 26 \times 21$ cm aquaria to form six experimental source groups. Glass panes wrapped in newspaper placed between the aquariums isolated groups visually. Aquariums were identically equipped with filters and plastic artificial vegetation. Fishes were fed daily with Tetramin fish food and were maintained at 25° C on a constant 12L : 12D photoperiod. Prior to experiments, fish were kept in the same aquarium for at least 18 days, after which time fish sharing the same tank were considered 'familiar' and those in separate tanks 'unfamiliar.'

The experimental protocol was based on a combination of techniques outlined in Griffiths & Magurran (1997*b*), Warburton & Lees (1996), Ranta *et al.* (1992*a, b*), Magurran *et al.* (1995) and Keenleyside (1955). A $75 \times 28 \times 30$ cm aquarium was partitioned three ways by two Plexiglas panes inserted 20 cm from each side, yielding a middle arena 35 cm wide. Each of the Plexiglas panes was made semi-permeable (*i.e.* permitting water movement but not fish movement) with *c.* 60 regularly spaced 5 mm holes. The water level in the tank was raised to 28 cm and each side was equipped with a Second Nature® Whisper™ filter, which moved water into the centre of the tank. To prevent shock to the experimental fishes caused by sudden changes in pH and temperature, both filters were run for 5 days prior to the experiment.

For each trial, two groups were compared. Source groups were selected in random order; all 15 possible combinations of groups were tested. Six fish were randomly chosen from each group, leaving one fish from each group to be used as focal fish. Two trials were conducted for each group combination, one with each focal fish. In each trial, a focal fish was measured for total length (L_T), held in a net in the middle of the experimental tank for 1 min to acclimate, and then gently released into the middle of the experimental tank. The side of the tank in which familiar shoals were placed was alternated between trials either by physically moving the group or by sequentially testing focal fish from opposing shoals. Focal fish were occasionally reused as stimulus fish, but not as focal fish.

Two measures of familiarity were tested simultaneously: the first examined whether an experimental fish preferred the shoal of familiar or unfamiliar fish. For each trial, total time (out of 300 s) spent on one side of the tank was recorded; the centre of the tank was designated by a thin strip of tape.

The second measure investigated the presence or absence of shoaling behaviour between the focal fish and the shoal nearest to it, and quantified the strength of shoaling behaviour. This procedure was similar to that of Chivers *et al.* (1995) in that it accounted for varying degrees of shoaling behaviour; however, it also attempted to differentiate shoaling from schooling behaviour. Every 30 s during 5 min trials, behaviour of the focal fish was scored. Fish were assigned a score of 0 ('weak') if they demonstrated no shoaling behaviour (characterized by skittering, lying motionless, or apparently ignoring the other fish), 1 ('moderate') for slight shoaling behaviour (focal fish facing or swimming parallel with other fish and occasionally following their movements), and 2 ('strong') for strong shoaling behaviour (constant apparent visual contact; maintaining a distance of within 4 cm of the group; and clear, consistent mimicry of other fish's movements). A score of 2 indicated schooling behaviour (*i.e.* a synchronized and polarized group movement) as opposed to shoaling (*i.e.* a disjointed group in close proximity; Pitcher, 1983). Because 30 s scores could not be treated as independent data points, total scores were averaged for periods during which the focal fish were on the side of the tank closest to the familiar group and for periods during which the focal fish were closer to the unfamiliar group. The relationships between strength of shoaling behaviour and familiarity were investigated with analysis of covariance (ANCOVA) using size of focal fish as a covariate, and side of tank (familiar *v.* unfamiliar) as a factor.

The protocol for the blacktail shiner study was identical to that for the Texas shiner, with the following exceptions. Sample groups of seven fish from four separately occurring shoals (for a total of 28) were collected from the Guadalupe River during February and March, 2001. The six possible between-groups comparisons were conducted. Because of the more limited number of fish that could be obtained for this experiment, six trials (as opposed to two with the Texas shiner) were conducted for each between-groups comparison. Focal fish from each group were reused on eight occasions in different between-groups comparisons. Fine differences in L_T and appearance were recorded and used to ensure that no focal fish was used more than once in any between-groups comparison.

Comparisons of total number of seconds spent close to familiar *v.* unfamiliar individuals indicated that for both Texas shiner and blacktail shiner, focal fish preferred shoaling with familiar individuals (Goodness of fit, one-sample z test, Texas shiner: $n = 30$, $P < 0{\cdot}001$; blacktail shiner: $n = 36$, $P < 0{\cdot}001$). In Texas shiner, focal fish spent on average 67% of their time with familiar fish [$196{\cdot}3 \pm 21{\cdot}8$ s (mean $\pm$ S.E., $n = 30$)]; in blacktail shiner, focal fish spent on average 66% of their time with familiar fish ($199{\cdot}0 \pm 16{\cdot}2$ s, $n = 36$). These values were significantly different from the expected value of 50% of time being spent on either side (χ^2, Texas shiner: d.f. = 29, $P < 0{\cdot}01$; blacktail shiner d.f. = 35, $P < 0{\cdot}01$).

Fish shoaled more strongly with familiar individuals in both species (ANOVA, Texas shiner: d.f. = 1 and 58, $P = 0{\cdot}002$; blacktail shiner: d.f. = 1 and 70, $P < 0{\cdot}001$). In the results for Texas shiner, the total number of occurrences of strong shoaling behaviour (*i.e.* schooling) was over three times higher with familiar individuals [with familiar = 0·986 ± 0·146 *v.* unfamiliar = 0·357 ± 0·120, mean ± S.E. value of strength of shoaling behaviour, $n = 30$; Fig. 1(a)]. For the blacktail shiner, the total number of occurrences of strong shoaling behaviour was nearly five times higher with familiar individuals [with familiar = 0·951 ± 0·113 *v.* unfamiliar = 0·247 ± 0·088, $n = 36$; Fig. 1(b)].

For Texas shiner, there were no significant differences in shoaling preference among tanks (ANOVA, d.f. = 11 and 18, $P > 0{\cdot}05$) or strength of shoaling behaviour (ANOVA, familiar side: d.f. = 11 and 18, $P > 0{\cdot}05$; unfamiliar side: d.f. = 11 and 18, $P > 0{\cdot}05$). Because six tests were conducted for every between-groups comparison for blacktail shiner and focal fish were reused across groups, there were concerns that tank effects might be present in the data; however,

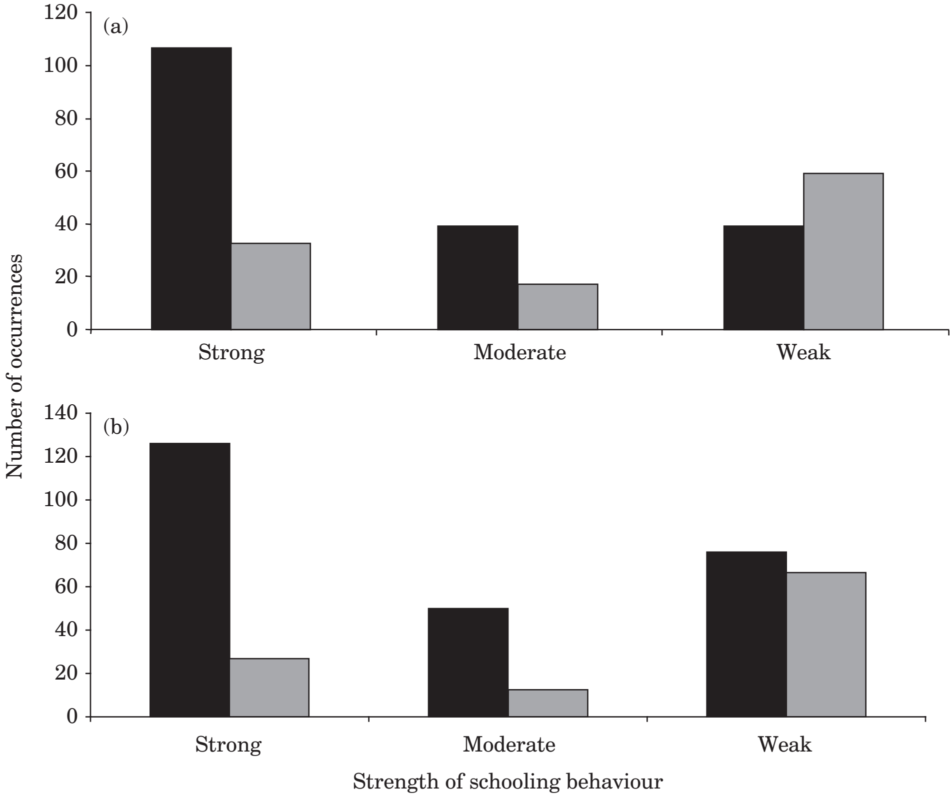

FIG. 1. Total number of occurrences for each of the three levels of shoaling behaviour (strong, moderate and weak) with familiar (■) and unfamiliar (□) conspecifics for (a) Texas shiner and (b) Blacktail shiner, representing observations taken every 30 s in each 5 min trial ($n = 30$ and 36, respectively).

ANOVA revealed no significant tank effects for shoaling preference (d.f. = 5 and 30, $P > 0{\cdot}05$) or strength of shoaling behaviour (familiar side: d.f. = 5 and 30, $P > 0{\cdot}05$; unfamiliar side: d.f. = 5 and 30, $P > 0{\cdot}05$).

There were no significant differences in L_T of fish among tanks; consequently, size played no apparent role in assortative shoaling observed [Texas shiner 355 ± 57 mm (mean ± S.E., $n = 42$); ANOVA, d.f. = 5 and 36, $P > 0{\cdot}05$; blacktail shiner 419 ± 61 mm ($n = 28$); d.f. = 3 and 24, $P > 0{\cdot}05$]. ANCOVA for Texas shiner indicated significantly different levels (*i.e.* *y*-intercepts) for strength of schooling behaviour with familiar as compared to unfamiliar fish as L_T of focal fish increased (d.f. = 1 and 53, $P < 0{\cdot}05$); however, regression analysis failed to uncover significant trends for increasing shoaling behaviour with focal fish L_T (ANOVA; familiar: d.f. = 1 and 28, $P = 0{\cdot}214$; unfamiliar: d.f. = 1 and 28, $P = 0{\cdot}732$). ANCOVA for blacktail shiner indicated significantly different rates of increase (*i.e.* slopes) for strength of shoaling behaviour with familiar as compared to unfamiliar fish as L_T of focal fish increased (d.f. = 1 and 68, $P < 0{\cdot}05$). This interaction was due to a significant relationship on the familiar side between the size of focal fish and shoaling behaviour (ANOVA, d.f. = 1 and 34, $P < 0{\cdot}05$) but not on the unfamiliar side (d.f. = 1 and 34, $P = 0{\cdot}510$).

The data indicate that both Texas and blacktail shiners distinguished conspecifics on the basis of previous interactions, and, when given the choice, spent significantly more time with familiar fish. This is in agreement with studies on guppies and three-spinned sticklebacks (Van Havre & Fitzgerald, 1988; Magurran *et al.*, 1995; Lachlan *et al.*, 1998). Moreover, strength of shoaling behaviour in both Texas and blacktail shiners is correlated with familiarity of experimental fish, in agreement with Chivers *et al.* (1995), who found that shoals of familiar fathead minnows were more cohesive than those of unfamiliar individuals.

The experimental setup differed somewhat from natural conditions in that fish were confined in groups for 18 days prior to testing. Periodic droughts, however, create isolated pools in the Guadalupe River lasting for months at a time. Under these conditions, isolated shoals of both species have been observed in the study populations; thus, familiarity preferences could develop in a similar fashion. Such preferences have been reported in wild populations of *P. reticulata* (Griffiths & Magurran, 1997*a*), and this study serves as further indication that familiarity preferences may develop even in environments subject to periodic flooding (*e.g.* guppies from streams subject to tropical storm deluges and the shiners of this study). This is perhaps an indication that some level of shoal cohesion is maintained even during extreme flood events, either through the utilization of refuges from fast-moving flood waters or post-flood regrouping.

In blacktail shiner, strength of shoaling behaviour with familiar individuals increased with L_T of the focal fish. If larger fish are more likely to lead shoals under natural conditions, the remaining group members may imitate the leaders' movements closely under experimental conditions as well.

Many antipredator responses of schools depend upon synchronized group movements (Pitcher & Parrish, 1993; Chivers *et al.*, 1995). In the present study, focal fish demonstrated the most distinct shoaling behaviour near familiar fish; thus, familiar fish may be able to maintain more cohesive movements in the presence of predators. In guppies, familiar fish may be more aware of each other's potential responses to predator stimulus (Magurran & Seghers, 1990; Milinski, 1991); in fathead minnows, schools composed of familiar individuals are more cohesive and demonstrate more complex and potentially beneficial behaviours, such as predator inspection (Chivers *et al.*, 1995).

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