ADULT THREE-SPINED STICKLEBACKS PREFER TO SHOAL WITH FAMILIAR KIN

by

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Summary

Theory predicts several advantages for animals to shoal with kin or familiars such as the evolution of altruistic behaviour or the reduction of competition because of more stable dominance hierarchies. In three-spined sticklebacks, *Gasterosteus aculeatus*, the influence of kinship and familiarity on shoaling decisions is ambiguous. We tested the potential for kin recognition of laboratory-bred adult, reproductively non-active sticklebacks in an experimental design in which a testfish was given the choice between two different shoals. One shoal consisted of its familiar full sibs while the other one was composed of fish unfamiliar and unrelated to the testfish. The time that testfish joined each group indicated that adult, reproductively non-active sticklebacks prefer to shoal with familiar relatives. Characteristics of the group such as measured by body mass, standard length, and body condition of its members did not significantly explain the shoaling preference for familiar kin.

Introduction

The ability to discriminate kin from non-kin provides a large range of benefits (Arnold, 2000; Ward & Hart, 2003). In mate choice, for example, individuals which recognise kin are able to avoid reproduction with related

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individuals and thus prevent the disadvantages of inbreeding. Brood-caring fish species where sneaker males occur, may be able to recognise and thus respond differently to their own and foreign offspring (Sargent, 1989). But the advantages are not limited to mate choice and reproduction. Shoaling with kin facilitates the evolution of altruistic behaviour because it may increase the inclusive fitness of an individual (Hamilton, 1964).

In sticklebacks, several studies focussed on the composition of shoals. Shoaling preferences were shown for groups consisting of conspecifics (Barber *et al.*, 1998 but see Keenleyside, 1955) or size-matched individuals (Ranta *et al.*, 1992; Barber, 2003). Dugatkin *et al.* (1994) found evidence for parasite-assortative shoaling in juvenile sticklebacks. The influence of familiarity and kinship on shoal choice in sticklebacks is equivocal. Juveniles showed a preference for shoals consisting of familiar siblings (Van Havre & FitzGerald, 1988; FitzGerald & Morrissette, 1992) or just familiar fish (Van Havre & FitzGerald, 1988). However, when testfish were confronted with odour cues alone no preference was found (Steck *et al.*, 1999). Peuhkuri & Seppa (1998) studied shoals of juvenile sticklebacks caught in the Baltic Sea using allozymes as genetic markers. They did not find any influence of kinship on shoal composition while Ward *et al.* (2002) observed stable partner associations in shoals of adult sticklebacks.

Almost all the above-mentioned studies were done with juvenile sticklebacks. Given that reproductively non-active adult sticklebacks in- and outside the breeding-season also occur in shoals (Bakker, 1994), theory predicts similar advantages for shoaling of adult fish as there exist for juveniles and thus for kin recognition. The aim of our study was to investigate the potential for kin recognition in adult reproductively non-active sticklebacks by studying whether they prefer to shoal with familiar full-sibs.

Methods

Experimental subjects

The sticklebacks used in the experiments were laboratory-bred, second and first generation offspring of anadromous fish which had been caught during their spring migration in 1998 and 2001, respectively, on the island of Texel, The Netherlands. They were tested in 2001 and 2002, respectively. We therefore will refer to them as 2001 and 2002 fish, respectively. The main data set was obtained using 2001 fish, and was supplemented with data of 2002 fish. The sample size of the latter was smaller due to logistic reasons such as a lack of individuals which were reproductively non-active during the experiments.

The 2001 fish had been bred and reared at the University of Bern, Switzerland (details in Mazzi *et al.*, 2002). Full-sib groups of maximally ten fish had been maintained under winter conditions (daylength 8L:16D, temperature 3-6°C) in 10 litre aquaria filled with tap water since December 1999 before they were transferred to the University of Bonn in October 2000. There they were kept in an airconditioned room under standardized summer conditions (daylength 15L:9D, temperature 14 ± 1°C) in 10-60 litre tanks depending on group size. The tanks were separated from each other by grey opaque partitions and supplied with a layer of gravel, half a clay flower pot and plants of *Vesicularia dubyana* Brotherus and *Vallisneria spiralis* L. to offer hiding places. Water in the tanks was cleaned and aerated through an inside filter, and a third of the water volume was replenished with tap water several times a week. The fish were fed daily *ad libitum* on frozen *Chironomus* larvae, *Tubifex*, *Artemia* or *Mysis*. Fish were food-deprived 24 hours before the test in order to reduce variation in swimming activity. In the experiments only adult, reproductively non-active fish were used, that is fish without any sign of developing breeding coloration or egg production. Fish were used only once. The experiments were performed in January 2001. At the time of the experiments, the 2001 fish were nearly two years of age. We tested fish from 13 different full-sib groups, 4 of them had been inbred during one generation, the rest had been outbred.

The 2002 fish had been bred and reared in a similar way as the 2001 fish. In the spring of 2001 wild-caught fish were bred in the laboratory. Fish were maintained and reared in tap water under standardized summer conditions (daylength 16L:8D, temperature 16 ± 1°C). Eggs from random mating pairs of wild-caught fish were taken out from their nests after fertilisation and placed in small one litre plastic aquaria where they were kept until hatching. Water was completely renewed every day, and dead eggs removed. After hatching fry were transferred to 10 litre plastic tanks. At an age of two month, the number of full-sibs in each group was reduced to 10 individuals. They were placed in 50 litre tanks where they reached adulthood. The aquaria were equipped in a similar way as for the 2001 fish. A third of the water volume was replenished once a week. At an age of about one year the fish were put under winter daylength (8L:16D, temperature 14 ± 1°C) during a period of four months. Fish were daily fed *ad libitum* with frozen *Artemia* and *Chironomus* larvae. Like in 2001 fish, 2002 fish were food-deprived 24 hours before the test. In the experiments only adult, reproductively non-active fish were used. All fish were used only once. At the time of the experiments (October 2002), the 2002 fish were on average about half a year younger than those of 2001. We tested fish from 5 different outbred full-sib groups.

Experimental design

Shoaling preferences were tested in a 1 m glass aquarium which was divided into three sections (25 cm, 50 cm, 25 cm) using a green mesh (mesh size 1 by 1 mm) (Fig. 1). This enabled the testfish to have visual and olfactory contact to each of the two stimulus groups. In the 2002 fish, the mesh partitions were replaced by partitions of transparent, perforated (diameter 4 mm and centres 20 mm apart) polyacryl. The tank was lit by two 30 Watt fluorescent lamps which were hung 1 m above the tank. The light was directed by cardboard such that only the tank was illuminated. Interactions of the fish with the environment of the aquarium were prevented by making the side and back walls of the tank opaque using matt black cardboard (2001) or grey plastic plates (2002). Before the front wall there was a matt black curtain with a hole in it for the camera. The bottom of the tank was covered by gravel. It was filled with one day old, aerated tapwater. In 2002 10% of the water in the experimental
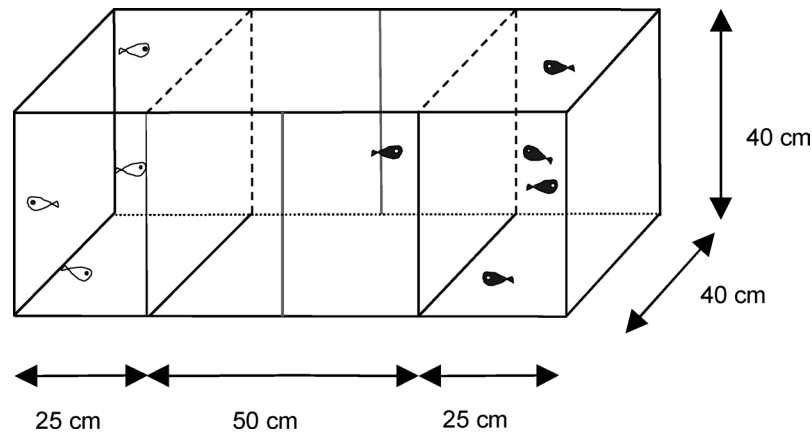
Fig. 1.   The glass aquarium used to measure shoaling preferences. In the left and right sections we placed two shoals of each four full-sibs. The testfish which was familiar and a full-sib to fish in one of the shoals, was placed in the middle section. The sections were separated by green mesh. Fish of the same coloration are familiar full-sibs. In front of the aquarium a webcam recorded fish movements. A line drawn on the front and back pane visually divided the middle section into two halves.

tank consisted of water in which one out of seven available perch, *Perca fluviatilis*, of about 20 cm standard length had been swimming for 7-10 days. This perch water was added to strengthen shoaling behaviour as a response to a predator's presence (Krause *et al*., 1998). The water temperature at the time of testing was 12°C and 15°C in 2001 and 2002, respectively. Aeration of the water was disconnected during the test.

In the left and right sections of the tank we placed four fish each of two different full-sib groups. The shoal fish were a random sample from their full-sib group. Shoals did not differ significantly in body mass, standard length and condition factor [100 × (mass in g)/(length in cm)^3; Bolger & Conolly, 1989] (Wilcoxon matched-pairs signed-ranks test, $N = 18$, all $z > -0.9$, all $p > 0.3$). The testfish, also caught randomly from one of the two full-sib groups, was placed in the middle section. The position of the relatives of the testfish alternated between tests. In 2001 the testfish was directly released in the middle section, whereas in 2002 the testfish was enclosed during two minutes in a perforated, transparent polyacrylic plastic cylinder (diameter 10 cm) which was placed in the middle of the tank. The testfish was released by raising the cylinder from a distance by a string. The test started after the testfish for the first time crossed a line drawn on the front and back walls that divided the middle section into two halves. We recorded fish movements for 30 minutes using a webcam (made by Creative, model CT6840) connected to a laptop computer. In order to minimize file size to 1-2 MB per min film recordings were made in black-and-white and at low but for our purpose sufficient resolution (2 frames per sec). After the recordings the standard body length and wet body mass of the fish were measured.

The digital film recordings were analysed afterwards. The time that the testfish spent in each halve of the middle section was quantified. Only when the total body length of the testfish had entered a particular halve, the time that it spent in that halve started to run. The observer was naïve with respect to the side where the relatives of the testfish were.

Statistical analysis

Because most data were not significantly normally distributed according to Kolmogorov-Smirnov tests, nonparametric statistics were applied. Given test probabilities are two-tailed throughout. Analyses were performed using SPSS 11.0.1 statistical package.

Results

Despite some differences in rearing and testing conditions, the time spent near relatives did not significantly differ between 2001 and 2002 fish (Mann-Whitney U test, $N_1 = 13$, $N_2 = 5$, $U = 30$, $p > 0.8$). The data of 2001 and 2002 were therefore pooled. Significantly more testfish stayed more than half of the test period near their familiar sibling groups (sign test, $N_1 = 15$, $N_2 = 3$, $p < 0.01$). Moreover, the testfish spent significantly more time near kin (mean % $\pm$ SD = 57.7 $\pm$14.4) than near non-kin (mean % $\pm$ SD = 42.3 $\pm$ 14.4) (Fig. 2; Wilcoxon matched-pairs signed-ranks test, $N = 18$, $z = -2.29$, $p = 0.021$). Similar results were obtained when the data of the two years were treated as replicates and the two p-values were combined
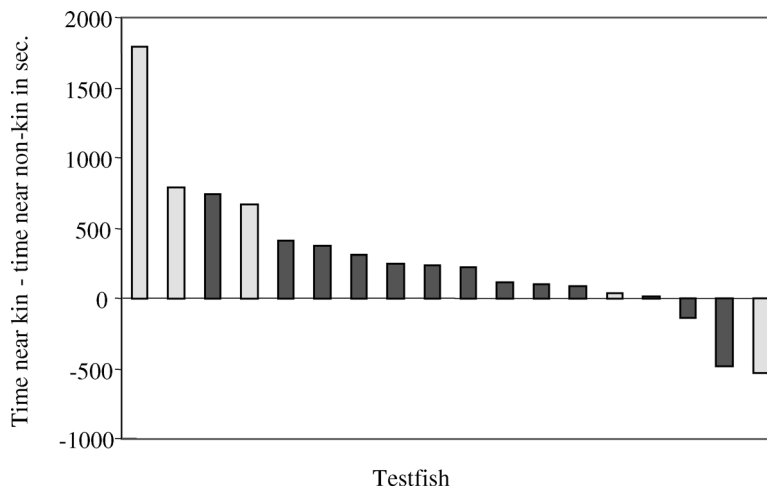

Fig. 2.   The time in seconds that testfish spent near the shoal of familiar kin relative to the time spent near the shoal of unfamiliar non-kin. Positive values indicate that the testfish spent more than half of the time near familiar full-sibs, negative values that it spent more than half of the time near unfamiliar non-kin. The test period was 1800 sec. The 18 testfish are arranged in order of decreasing preference for familiar kin. Bright bars mark the 2002 fish, dark bars the 2001 fish.

(Sokal & Rohlf, 1995, Fisher's test of combining probabilities: p-values of the sign test were 0.022 and 0.375 for 2001 and 2002, respectively, $\chi^2 = 9.60$, df $= 4$, $p < 0.05$; p-values of the Wilcoxon test were 0.046 and 0.138 for 2001 and 2002, respectively, $\chi^2 = 10.12$, df $= 4$, $p < 0.05$). Thus in addition to the pooled data, both the data of the fish tested in 2001 and the combined probabilities of the 2001 and 2002 fish gave significant results. The preference for familiar kin is thus robust.

Testfish alternated 38.5 (median; quartiles: 30.25 and 50.25; range 2-120) times between the two shoals. The number of changes did not significantly differ between fish which preferred their familiar relatives and those which did not (Mann-Whitney U test, $N_1 = 15$, $N_2 = 3$, $U = 21$, $p > 0.9$). There was also no significant difference between the data of 2001 and 2002 fish (Mann-Whitney U test, $N_1 = 13$, $N_2 = 5$, $U = 30.5$, $p > 0.8$). The median (quartiles) duration of individual visits to the shoal of familiar kin was 47.45 (38.7; 62.65) seconds and to the shoal of unfamiliar non-kin 33.74 (27.68; 42.05) seconds (Wilcoxon matched-pairs signed-ranks test, $N = 18$, $z = -2.59$, $p = 0.01$).

Body mass, size and body condition of its relatives in the group did not significantly correlate with the time that the testfish spent near its kin neither when they were expressed as group mean, variance of the group or difference between the testfish and the group nor as difference of the group means of the group of kin and those of non-kin (Table 1).

Discussion

Our experiments showed for the first time that adult, reproductively non-active sticklebacks are able to discriminate between familiar kin and unfamiliar non-kin and prefer to shoal with their familiar siblings. However, due to the experimental design used in our study we were unable to tell whether the effect was based on familiarity rather than kinship (but see Grafen, 1990). This will be a topic of further study now that we established that familiar kin can be distinguished from unfamiliar non-kin.

There are many advantages for fish that prefer to shoal with familiar individuals. For example, associating with familiar individuals can reduce the costs of competition because of more stable dominance hierarchies (Pusey & Packer, 1998). Höjesjö et al. (1998) showed that groups of familiar sea

TABLE 1. *Spearman rank correlation coefficients (r_s) of the time that testfish spent near the group of familiar kin and group characteristics*

Criterion of choice	r_s	p
Mean body mass	−0.252	0.314
Relative body mass	0.264	0.289
Difference in body mass	0.347	0.158
Variance in body mass	−0.131	0.604
Mean standard length	−0.242	0.334
Relative standard length	0.198	0.430
Difference in standard length	0.218	0.385
Variance in standard length	−0.088	0.730
Mean condition	0.324	0.189
Relative condition	0.009	0.971
Difference in condition	−0.058	0.819
Variance in condition	−0.321	0.195

Body mass, body size, and condition factor of the kin groups are expressed as means of the four fish in the group of kin, the value of the testfish relative to the mean value of the group, the difference of the means of the group of kin and those of non-kin, and as variances of the four fish in the group of kin. The sample size was 18, *p*-values are two-tailed.

trout, *Salmo trutta*, had more stable dominance ranks and a higher food intake. Chivers *et al*. (1995) found that familiar fathead minnows, *Pimephales promelas*, showed a greater shoal cohesion when confronted with pike odour or a pike model than groups composed of unfamiliar individuals. In addition, shoaling with familiar individuals facilitates the evolution of altruistic behaviour. Sticklebacks for instance preferentially joined individuals who had proven to be cooperative in the past (Milinski *et al*., 1990).

For shoals of closely related individuals there exist similar benefits. For example, in different salmonid species the level of aggression was lower when groups were composed of kin (Brown & Brown, 1993). Additionally to the direct benefits, individuals that shoal with kin can increase their inclusive fitness (Hamilton, 1964) by increasing the reproductive success of relatives rather than that of unrelated conspecifics.

One reason to prefer the group of relatives could be genetic and/or environmental variation in standard length, body mass or condition factor between full-sib groups. Three-spined sticklebacks prefer to shoal with individuals of similar size (Ranta *et al*., 1992; Barber, 2003; see Krause *et al*., 2000 for a review) probably because phenotypes that differ from the majority of

the group suffer a higher rate of predation, a phenomenon known as 'oddity effect' (Theodorakis, 1989). Furthermore, given that smaller fish are poorer competitors than larger ones (Krause, 1994) these fish should choose groups of similar body size to avoid competition with larger conspecifics (Krause, 1994). When testfish resembled the fish they lived with then they may prefer the group of relatives on the basis of phenotype matching (Brown *et al.*, 1993 but see Krause *et al.*, 2000) instead of kin recognition. However, we did not find a significant influence on shoaling preferences of the testfish's phenotype nor of the standard length, body mass or condition of both the familiar group of relatives and the unfamiliar, unrelated group.

In conclusion, adult, reproductively non-active sticklebacks preferred to shoal with familiar kin. The preference was not significantly related to visual, phenotypic traits of the shoal members such as body size, body mass, or condition. Further research has to reveal whether the shoaling preference is based on familiarity or relatedness.

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