

Nutritional state influences shoaling preference for familiars

Joachim G. Frommen*, Corinna Luz, Theo C.M. Bakker

Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, D-53121 Bonn, Germany

Received 1 May 2007; received in revised form 2 June 2007; accepted 3 June 2007

Abstract

Preferences for grouping with familiar individuals are shown in many animal species, including the three-spined stickleback (*Gasterosteus aculeatus*). Shoaling with familiars is advantageous because of more precise anti-predator behaviours or more stable dominance hierarchies. Additionally, associations with familiar individuals facilitate the evolution of altruistic behaviour. Thus, in situations of increased competition one might expect an increased preference for familiar fish. We gave single juvenile sticklebacks of different nutritional state the choice between shoals composed either of familiar or unfamiliar individuals. Satiated fish preferred to shoal with familiar individuals. A comparative analysis of 8 stickleback studies with 15 different tests using familiars showed that all tests gave similar results, i.e. sticklebacks of all age classes preferred to shoal with familiars in a non-sexual context. In contrast, hungry test fish did not prefer to shoal with familiar fish, but even showed a preference for the unfamiliar group. Because sticklebacks use early-life familiarity to recognize kin, the results suggest the avoidance of competition with relatives. To our knowledge, this is the first study showing an impact of nutritional state on social interactions with familiar individuals. © 2007 Elsevier GmbH. All rights reserved.

Keywords: Three-spined stickleback; *Gasterosteus aculeatus*; Hunger; Social interaction; Kin recognition

Introduction

Shoaling behaviour is a well-documented phenomenon in many fish species (Krause et al., 2000). Shoal members profit from an enhanced predator protection (e.g. Magurran, 1990), the possibility to find mating partners in the group (Wedekind, 1996), or an improved foraging efficiency (Pitcher et al., 1982). But there are also disadvantages which lead to a decreased fitness of group-living individuals. For example, groups attract predators more easily than single individuals (Botham et al., 2005), and fish joining a shoal increase their risk of getting infected by parasites (Poulin, 1999). Addition-

ally, shoal members experience a higher degree of competition for food when joining a shoal (Krause, 1994) and suffer a higher amount of general aggression than single fish (Krause and Ruxton, 2002). Thus, shoal choice is not random but influenced by an array of factors like body size (Ranta et al., 1992; Krause and Godin, 1994), group size (Krause et al., 1998), parasite load (Barber, 2003), predation level (Johannes, 1993; Brown and Warburton, 1997) or coloration (McRobert and Bradner, 1998; Modarressie et al., 2006).

A well-documented factor influencing shoal choice is the nutritional state of individuals. Hungry zebrafish (*Danio rerio*) for example preferred to shoal with satiated individuals, mainly because they had a significantly higher foraging success in groups composed of well-fed fish (Krause et al., 1999). Food-deprived banded killifish (*Fundulus diaphanus*) spent less time

*Corresponding author. Tel.: +49 228 735758; fax: +49 228 732321.
E-mail address: jfrommen@evolution.uni-bonn.de (J.G. Frommen).

shoaling than satiated ones (Hensor et al., 2003). In walleye pollock (*Theragra chalcogramma*) group cohesion decreased with declining food level and hungry fish showed an increase of risk-taking behaviour (Sogard and Olla, 1997). Metcalfe and Thomson (1995) found that European minnows (*Phoxinus phoxinus*) were able to assess whether a shoal was composed of good or bad competitors and that they showed a preference for shoaling with fish of low competitive ability. However, Lachlan et al. (1998) found no effect of hunger on shoaling decisions in guppies (*Poecilia reticulata*).

Familiarity influences shoaling decisions in many fish species like guppies (Griffiths and Magurran, 1997), climbing perch (*Anabas testudineus*) (Binoy and Thomas, 2006), rainbow fish (*Melanotaenia* spp.) (Brown, 2002), yellowfin tuna (*Thunnus albacares*) (Klimley and Holloway, 1999), and different cyprinids (Barber and Wright, 2001; Ward et al., 2003). The benefits of shoaling with familiars are multifarious. For example, it may lead to more stable dominance hierarchies and thus to a reduction of aggressive behaviour between the members of a shoal (Höjesjö et al., 1998; Gómez-Laplaza, 2005). Groups of familiar brown trout (*Salmo trutta*) showed an improved anti-predator behaviour (Griffiths et al., 2004). In addition, shoaling with familiar individuals may be the basis for the evolution of cooperation (Utne-Palm and Hart, 2000). Sticklebacks for instance preferentially joined individuals that had proven to be cooperative in the past (Milinski et al., 1990). Furthermore, sticklebacks found food faster and had a higher food intake when shoaling with familiar fish (Ward and Hart, 2005). Guppies accompanied by a familiar individual explored new surroundings faster than those accompanied by an unfamiliar one (Bhat and Magurran, 2006). Additionally, kinship is often estimated by familiarity, particularly in nesting species (Mateo and Holmes, 2004). Here, the individual has ample opportunity to become familiar with its nest mates' phenotypes in general, and with olfactory cues in particular. Later in life, it may use this familiarity to recognize related individuals (Mateo and Holmes, 2004).

Shoaling with relatives may increase an individual's inclusive fitness (Hamilton, 1964) by increasing the reproductive success of relatives, e.g. by facilitating the evolution of altruistic behaviour or reducing competition because of more stable dominance hierarchies. Brown and Brown (1993) showed that in different salmonid species, the level of aggression was lower when groups were composed of kin, which resulted in a higher growth rate. Gerlach et al. (2007) did not find such a reduction of aggressive behaviour between related zebrafish larvae. However, they also demonstrated an improved growth rate of larvae kept in groups of full siblings. Furthermore, predator defence like inspection

behaviour is expected to be more pronounced in groups of related individuals. If an individual dies while defending a group of relatives it gains indirect fitness if the related fish survive by this sacrifice (Hamilton, 1963; Waldman, 1988). On the other hand, when individuals in a group face a high amount of competition, kin-selected benefits may be negated (West et al., 2001, 2002). For example, when food is limited but predation is high, fish may benefit from the anti-predator behaviour of a group but may avoid competing for food with relatives. Thus, inclusive fitness may be improved by avoiding groups of kin.

Studies documenting an avoidance of kin other than in a mating context are scarce thus far. Kasuya (2000) showed that crawlers of the parthenogenetic mango shield scale (*Milviscutulus magniferae*) dispersed more frequently when the next neighbour was a sibling. Similar results were obtained in the common lizard (*Lacerta vivipara*) (Léna et al., 1998). Halverson et al. (2006) showed that in different habitats wood frog (*Rana sylvatica*) tadpoles preferred or avoided to group with kin. In fish, juvenile Atlantic salmon (*Salmo salar*) given the choice to shelter with kin and non-kin preferred the unrelated fish (Griffiths et al., 2003). However, the reasons for the avoidance of kin were not clear in most of those studies.

Three-spined sticklebacks form shoals during most of their life and are a great model system to study the influence of hunger and familiarity for two reasons. First, several studies have shown an influence of hunger on their social behaviour. Food-deprived individuals showed increased predator-inspection behaviour (Godin and Crossman, 1994). Sticklebacks given the choice between shoals of different sizes preferred the larger shoal, but this preference decreased with an increasing duration of food deprivation (Krause, 1993). Furthermore, Barber et al. (1995) found that satiated sticklebacks formed tighter shoals while hungry individuals spent more time out of visual contact with each other. Second, sticklebacks are known to differentiate between familiar and unfamiliar individuals (e.g. Barber and Ruxton, 2000) as well as between familiar or unfamiliar kin and unfamiliar unrelated fish (Frommen and Bakker, 2004, 2006; Frommen et al., 2007a, b). This ability gets lost when both kin and non-kin are familiar (Frommen et al., 2007a), indicating that learning of familiar cues and thus phenotype matching is the main mechanism of kin recognition in sticklebacks.

The aim of our study was to investigate whether juvenile sticklebacks adjust their preference for familiar fish in relation to their nutritional state. In a classical choice design, we therefore gave food-deprived or satiated test fish the opportunity to choose between shoals composed of familiar or unfamiliar individuals.

Materials and methods

Experimental subjects

Adult sticklebacks had been caught during their 2004 spring migration from a large, genetically heterogeneous (Heckel et al., 2002) population on the island of Texel, the Netherlands (Kemper, 1995) and were bred in the laboratory. Eggs were spawned in May and December 2004 and were taken out of the nests immediately after fertilization. Clutches were placed in 11 aquaria, two at a time. The aquaria were each divided into two compartments using a black mesh with a mesh size of about 1 mm allowing visual and olfactory contact. One clutch was placed in each compartment. Previous studies showed that stickleback fry of comparable age are able to become familiar with fish on the other side of the net (Frommen et al., 2007a). Thus, we got unrelated kin groups that were familiar with each other (Fig. 1). Single aquaria were divided from each other using opaque sheets of plastic. Fry hatched at an age of 9 ± 1 days. At an age of 36 days, groups were transferred to larger aquaria measuring $30 \text{ cm} \times 20 \text{ cm} \times 20 \text{ cm}$. Here, compartments were divided using perforated (hole diameter 1.7 mm), clear Plexiglas. Group sizes were reduced to 20 full siblings in each compartment. Fish were kept in an air-conditioned room under standardized summer light regime (day length 16L: 8D, temperature $15 \pm 1^\circ \text{C}$). The water in the small aquaria was changed daily, in the large aquaria weekly. Each compartment was aerated by an airstone. Fry were fed daily *ad libitum* with living *Artemia* nauplia. At an age of 12 weeks, we added frozen *Artemia*. We tested 34 hungry and 26 fed fish. All groups provided only one test fish; however, some groups provided the familiar group in one test and the unfamiliar group in another.

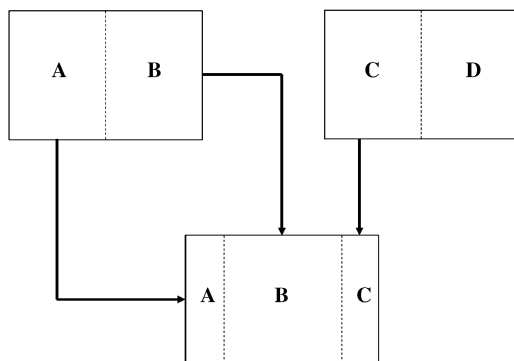


Fig. 1. Fish were kept in groups comprising kin and non-kin. Groups were separated by a sheet of perforated Plexiglas, allowing olfactory and visual contact. In the experiment, stimulus shoals comprised familiar non-siblings on one side and unfamiliar non-siblings of the same age on the other side. Hungry and satiated fish were fed 24 h and 30 min prior to testing, respectively.

Experimental design

Hungry fish were tested at an age of 57 ± 2 days, fed fish were 42 days older. Shoaling preferences were tested in a glass aquarium measuring $30 \text{ cm} \times 20 \text{ cm} \times 20 \text{ cm}$. It was divided into two stimulus sections (4.5 cm) on each side and a test section in the middle (21 cm) using perforated, clear Plexiglas. This enabled the test fish to have visual and olfactory contact to each of the two stimulus groups. The aquarium was filled with 1-day-old tap-water and lit by a 36 W fluorescent tube placed 10 cm above the aquarium. The water temperature at the time of testing was $17 \pm 1^\circ \text{C}$. Interactions of the fish with the environment of the aquarium were prevented by making the side and back walls of the aquarium opaque using grey plastic plates. Additionally, a black curtain was tightened around the test aquarium.

In the two stimulus sections, we placed seven randomly caught fish from each of two different full-sibling groups. Familiar stimulus shoals were taken out of one compartment of a rearing aquarium while the test fish was taken out of the second compartment. The unfamiliar stimulus fish were taken from a second aquarium (Fig. 1). In experiment 1, all fish had been food-deprived for 24 h while fish in experiment 2 were fed 30 min prior to testing with living and defrosted *Artemia ad libitum*. Thus, all fish in experiment 1 were hungry while all fish in experiment 2 were satiated. Fish used in experiment 1 were fed immediately after testing.

In both experiments, shoals had been size-matched by visual judgement. Because fish in experiment 1 were small and fragile we did not measure their standard body mass and standard length at the end of the experiments as we did for fish in experiment 2. However, we measured a random sample of 24 individuals which were kept under the same conditions, but not used in the experiments to obtain a rough overview of mass, size and condition of fish. The mass of these fish was $44.42 \pm 8.89 \text{ mg}$ (mean \pm SD), standard length was $15.63 \pm 1.03 \text{ mm}$ and condition factor (Bolger and Connolly, 1989) was 1.09 ± 0.1 . Familiar and unfamiliar shoals in experiment 2 did not differ significantly in mean body mass, standard body length and condition factor (Wilcoxon matched-pair signed-rank test, $N = 26$, all z between -1.397 and -0.978 , all $p > 0.16$). The mass of these fish was $116.37 \pm 22.83 \text{ mg}$ (mean \pm SD), standard length was $20.05 \pm 1.12 \text{ mm}$ and condition factor (Bolger and Connolly, 1989) was 1.42 ± 0.1 .

After introducing the stimulus shoals into the stimulus compartments, the test fish was transferred to a perforated, transparent plastic box (diameter $10 \text{ cm} \times 7 \text{ cm} \times 16.5 \text{ cm}$) which was placed in the middle of the test compartment. After 2 min, the box was lifted from behind the curtain by a string. As soon as the test fish crossed a line drawn on the front and back wall dividing the test compartment into two halves,

movements were recorded for 15 min using a web cam (Creative CT6840) placed in front of the aquarium and connected to a laptop computer behind the curtain. The position of the familiar group alternated between tests to circumvent side effects.

Literature survey of preferences for familiars of different ages

Due to logistical limitations, fish in the satiated control group were 42 days older than the fish in the food-deprived group. Thus, we cannot exclude age effects in this study. To differentiate between hunger and age effects, we conducted a literature search for studies dealing with the influence of familiarity on shoaling decisions in sticklebacks. From these studies, we calculated a preference index for familiar or unfamiliar fish by subtracting the percent of time that the test fish spent with the unfamiliar shoal from the percent of time the test fish spent with the familiar shoal. If necessary, preferences were estimated from graphs. Only studies using classical two-choice designs were used for analysis.

Data analysis

The time test fish spent in each half of the test compartment was quantified by analyzing the digital film recordings. As the time spent on each side is a good indicator of shoal choice in sticklebacks (e.g. Van Havre and FitzGerald, 1988; FitzGerald and Morrissette, 1992; Krause, 1993; Frommen and Bakker, 2004; Modarressie et al., 2006; Frommen et al., 2007a; Ward et al., 2007), and highly correlated with the time spent directly in front of each shoal in former experiments (Frommen et al., unpublished data), we did not use the less conservative measure of choice zones in front of each stimulus shoal. The observer was “blind” with respect to the side of the familiar groups.

All time variables were not significantly different from normal distribution according to Kolmogorov–Smirnov tests with Lilliefors-correction. Thus, parametric statistics were used. Some body characteristics did not reach normal distribution; in these cases, we used non-parametric statistics. Given test probabilities are two-tailed throughout. Analyses were performed using SPSS 11.0.1 statistical package.

Results

There was a significant difference ($\chi^2_1 = 6.818$, $p = 0.009$) in the number of hungry test fish which chose familiar ($N = 9$) and unfamiliar ($N = 24$) shoals, respectively. Hungry test fish spent significantly less time

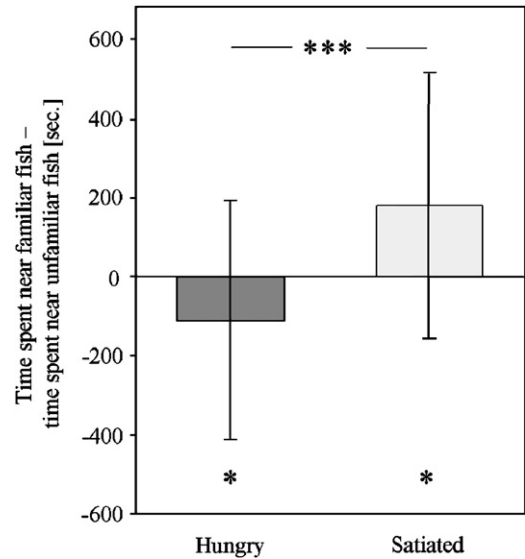


Fig. 2. Mean time in seconds that hungry ($N = 34$, dark bar) and fed ($N = 26$, light bar) test fish spent on the side of familiar fish minus mean time spent on the side of unfamiliar fish. A negative value implies a preference for the unfamiliar fish, while a positive value implies a preference for the familiar fish. Plotted are mean differences and standard deviations. Upper statistics indicate the difference between hungry and satiated fish, while the bottom statistics indicate deviations from a null expectation of zero. Each test lasted 900 s. * $p < 0.05$, *** $p < 0.001$.

on the side of familiar fish (mean time in seconds \pm S.D.: 394.6 ± 151.0) than near unfamiliar fish (505.4 ± 151.0) (one-sample t -test, $t_{34} = -2.139$, $p = 0.04$; Fig. 2).

The number of satiated test fish which chose the side of familiar fish ($N = 18$) differed significantly ($\chi^2_1 = 3.846$, $p = 0.05$) from that which chose unfamiliar fish ($N = 8$). Satiated test fish remained significantly longer on the side of familiar fish (540.3 ± 168.0) than near unfamiliar ones (359.7 ± 168.0) (one-sample t -test, $t_{26} = 2.733$, $p = 0.011$; Fig. 2). The number of hungry and satiated test fish which chose the familiar or unfamiliar shoal differed significantly ($\chi^2_2 = 10.315$, $p = 0.001$). Satiated test fish remained significantly longer near the familiar shoal than hungry fish (independent t -test, $t_{60} = -3.521$, $p < 0.001$; Fig. 2).

In the trials using satiated fish, neither size, mass nor condition of the test fish influenced the choice significantly (Pearson correlation, $N = 26$, all r_p between -0.188 and 0.114 , all $p > 0.56$). The same was true for the mean size, mass or condition of the fish in the familiar shoal, the variability of these traits in a shoal or their relationship to the characteristics of the test fish (Pearson correlation, $N = 26$, all r_p between -0.242 and 0.274 , all $p > 0.176$).

The literature survey revealed 8 studies with 15 different experiments dealing with the influence of

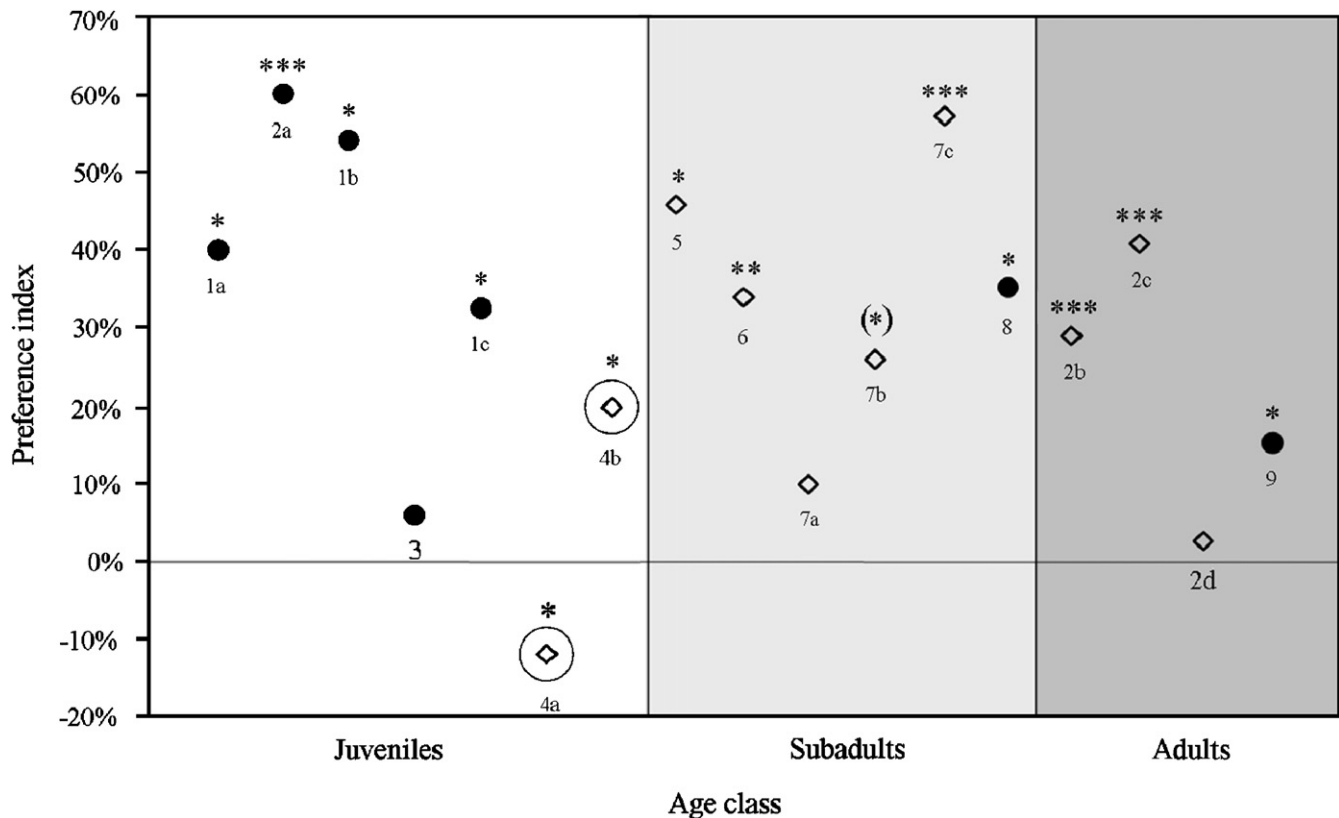


Fig. 3. Results of studies that investigated preferences for familiars in sticklebacks. Preferences are expressed as % association with familiars minus % association with unfamiliar, thus positive values indicate a preference for familiar fish. Age of juveniles ranged between 18 days (1a) and 99 days (4b), subadults ranged approximately between 6 and 8 months, adults approximately between 12 and 24 months. In all studies, test fish preferred the side of the familiar group (significant preferences are marked by asterisks: (*) $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The only exception is the study of hungry test fish (4a: this study), which significantly preferred the unfamiliar group. Fish in studies 1a–c, 2a, 3, 8 and 9 were familiar kin (filled circles), all others were familiar but unrelated (diamonds). The numbers refer to the following studies: 1a–c: juveniles, aged 18, 30 and 45 days, respectively (FitzGerald and Morrisette, 1992); 2a: juveniles, aged 18–20 days, 2b: adult females, 2c: adult females, kept apart for 10 days, 2d: adult males (Van Havre and FitzGerald, 1988); 3: juveniles, aged 43 days (Frommen et al., 2007a); 4a: hungry juveniles, aged 57 days; 4b: satiated juveniles, aged 99 days (this study); 5: subadults (Ward et al., 2004); 6: subadults (Ward and Hart, 2005); 7a–c: subadults, kept together for 7, 10 and 14 days, respectively (Ward et al., 2005); 8: subadults, aged 8 months (Frommen et al., 2007b); 9: adults aged between 18 and 24 months (Frommen and Bakker, 2004). The preferences in studies 1, 2, 5, 6 and 7 were estimated from graphs.

familiarity on shoaling decisions in sticklebacks in a non-sexual context (Fig. 3). Both younger and older fish as compared to the ones used in the present study preferred to shoal with familiars. Thus, in all age classes, test fish remained longer near the shoal composed of familiar fish; the only exception are the food-deprived test fish of the present study (Fig. 3).

Discussion

The influence of hunger as well as familiarity on shoaling decisions in fish has been amply discussed in recent studies (e.g. Krause, 1993; Barber and Ruxton, 2000). However, evidence for an influence of an individual's nutritional state on the social behaviour towards familiar individuals was lacking thus far. Our

study shows that the preference for familiar fish in juvenile sticklebacks depends on the nutritional state of the test fish. While satiated individuals preferred to shoal with familiars, hungry test fish chose the group of unfamiliar individuals. One benefit of shoaling with familiar individuals is the expression of altruistic behaviours. Utne-Palm and Hart (2000) found that sticklebacks which were familiar with each other showed reduced aggressive behaviour and a more equal distribution of food. Thus, one might have expected that hungry individuals in our study would show an increased preference for familiar fish. However, the opposite was the case.

Kin recognition in sticklebacks is triggered through social learning (Frommen et al., 2007a). Stickleback fry stay in the nest during the first days of their life (Wootton, 1976). During this time, they have ample

opportunities to get familiar with the phenotypic cues of other fry in the nest. These cues may be used as templates for kin recognition during the rest of their life (Frommen et al., 2007b). Fish in our experiments were reared together with non-sibs from hatching. Thus, the most plausible explanation is that sticklebacks assess fish in the familiar shoal as kin. Under food-deprived conditions, they might therefore avoid the familiar individuals to reduce competition with their assumed siblings (West et al., 2001, 2002). In contrast, satiated individuals preferred to shoal with the familiar fish, indicating that under conditions with a low level of competition advantages like an improved anti-predator behaviour (Griffiths et al., 2004) or a reduction of aggression and more stable dominance hierarchies (Höjesjö et al., 1998; Gómez-Laplaza, 2005) determine sticklebacks' shoaling decisions.

Alternatively, one might have expected such a response if we had tested individuals of low social rank, which thus tried to avoid the higher-ranking familiar fish (Gómez-Laplaza, 2005). However, test fish as well as stimulus fish were randomly caught out of their groups. Thus, it is quite unlikely that the test fish in the food-deprived treatment consistently were individuals of low social rank.

Another explanation could be that the hungry test fish knew about the poor nutritional state of the familiar group but not about that of the unfamiliar fish. Thus, it might be that the hungry test fish chose the unfamiliar group because of the possibility that its members might be weaker competitors. However, Metcalfe and Thomson (1995) found that European minnows were able to assess the competitive abilities of different shoals and preferred the poor competitors. If sticklebacks possess similar abilities, they should have been able to assess the nutritional state of both groups, noticing that the unfamiliar fish were of similar condition as the familiar ones.

The hungry test fish were about 57 days old while the satiated fish were 42 days older. The literature search revealed that all studies on familiarity effects in shoaling decisions of sticklebacks found a preference for the familiar group (see Fig. 3 for references). This was true for test fish which were younger as well as older than the fish used in this study. Fish in the literature survey were satiated to different levels but no study food deprived the fish before the test to the same extent as in the present study. Thus, it is most unlikely that the differences in preference for familiar fish were the result of age or size instead of nutritional state.

However, there might be another explanation. In all the above-mentioned studies, familiar juveniles which were younger than our test fish were also kin (Fig. 3). Thus, an alternative explanation might be that young fish (in contrast to older ones) are unable to recognize familiar non-kin and rather avoid everything that is

non-kin. However, if this was true, one would expect no deviation from the null expectation of zero, which contrasts with the findings of our study for the younger, hungry fish.

A further explanation could be that MHC class IIB expression patterns differ between stickleback families (Wegner et al., 2006). MHC genes are an important genetic component of the immune response of sticklebacks (e.g. Kurtz et al., 2004). If the risk of infection is higher in hungry fish, the food-deprived test fish might have avoided their assumed kin not because of the reduction of competition but because of the avoidance of fish with similar immune genes and thus similar susceptibility. Anyway, the results of the present experiments are one of the few examples of avoidance of relatives outside a mating context.

How sticklebacks recognise familiar individuals is unknown, but a major role of olfactory cues seems plausible (Thom and Hurst, 2004). Ward et al. (2004, 2005) showed that sticklebacks recognize individuals reared in the same habitat as well as those fed on the same food on olfactory cues only. Sticklebacks in our experiments were all kept under the same condition and fed the same food. Thus, diet and habitat cues should have played a minor role, suggesting that fish recognize each other by some kind of individual innate cue. MHC alleles might be such a cue (Thom and Hurst, 2004). Sticklebacks are able to “count” and compare MHC alleles of foreign individuals (Reusch et al., 2001). Maybe they are able to remember individual MHC cues and use them for the recognition of familiar fish.

Acknowledgements

This paper is dedicated to Klaus Peter Sauer on the occasion of his retirement as a full professor in Evolutionary Biology at the University of Bonn, Germany. We gratefully acknowledge his continuous scholarly, logistic, and financial support. We are grateful to Timo Thünken, Ricarda Modarressie and Ingolf Rick for discussions. Timo Thünken, Julia Schwarzer and two anonymous referees gave valuable comments on an earlier version of the manuscript. We thank Jan Hottentot for catching the sticklebacks and Peter Herold for help in building the set-up. All experiments complied with the current laws of Germany.

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