ORIGINAL ARTICLE

Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*)—familiarity, kinship and inbreeding

**Joachim G. Frommen · Marion Mehlis ·
Christina Brendler · Theo C. M. Bakker**

Abstract Shoaling with familiar kin is a well-known phenomenon. It has been described both for adult and for fry of three-spined sticklebacks (*Gasterosteus aculeatus*). However, evidence of preference for kin independent of familiarity is scarce. Furthermore, inbreeding effects have not been studied for shoaling preferences and there is a lack of studies about changes in individuals' ability to recognise kin during different phases of life history. We gave inbred and outbred, nonreproductive sticklebacks of different age the choice to shoal with a group of familiar siblings vs unfamiliar non-siblings and with unfamiliar siblings vs unfamiliar non-siblings. Subadult sticklebacks preferred to shoal with familiar kin over unfamiliar non-kin, but inbred and outbred individuals had similar preferences. When given the choice between unfamiliar siblings and unfamiliar non-sibs, adult outbred fish preferred the shoal of kin, while inbred fish behaved indifferently. Body characteristics of the group such as body mass, standard length and condition of its members did not significantly explain the shoaling preferences for kin. Thus, sticklebacks were capable of recognising familiar and unfamiliar kin. The latter capability was lost by inbreeding.

Keywords Phenotype matching · Fish · Sibling ·
Kin recognition · Shoaling

J. G. Frommen (✉) · M. Mehlis · C. Brendler · T. C. M. Bakker
Institute for Evolutionary Biology and Ecology,
University of Bonn,
An der Immenburg 1,
D-53121 Bonn, Germany
e-mail: jfrommen@evolution.uni-bonn.de

Introduction

Shoaling behaviour in fish has been a focus of behavioural studies for over 50 years (Keenleyside 1955). Shoaling fish benefit from enhanced antipredator protection (Magurran 1990), an improved foraging efficiency (Pitcher et al. 1982) or the possibility to find mating partners in the group (Wedekind 1996). On the other hand, individuals may experience a higher degree of competition when they join a shoal (Krause 1994) and increase their risk of getting infected by parasites (Poulin 1999). Thus, shoal choice is not random but influenced by a whole range of factors like body size (Ranta et al. 1992; Krause and Godin 1994), hunger level (Krause 1993), group size (Krause and Ruxton 2002) and competitive abilities (Metcalfe and Thomson 1995), parasite load (Ward et al. 2005a), predation level (Johannes 1993; Brown and Warburton 1997) or colouration (McRobert and Bradner 1998; Modarressie et al. 2006). Recent studies have shown that familiarity (Griffiths and Magurran 1997) and kinship (Arnold 2000; Behrmann-Godel et al. 2006) influence shoaling decisions in fish. Shoaling with familiars may lead to more stable dominance hierarchies, and thus, to a reduction of aggressive behaviour between the members of a shoal (Gómez-Laplaza 2005). In addition, shoaling with familiar individuals facilitates the evolution of altruistic behaviour (Utne-Palm and Hart 2000). Sticklebacks, for instance, preferentially joined individuals who had proven to be cooperative in the past (Milinski et al. 1990). Furthermore, groups of familiar individuals has shown an improved antipredator behaviour (Griffiths et al. 2004). For shoals of 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 and Brown 1993). In addition to direct benefits, individuals that shoal with kin

can increase their inclusive fitness (Hamilton 1964) by increasing the reproductive success of relatives. Recent work in sticklebacks have shown that adult, nonreproductive individuals prefer to shoal with familiar fish (Ward et al. 2005b) and with familiar siblings (Frommen and Bakker 2004). Whether the latter is based on kinship or on familiarity is not known. One aim of our study was to disentangle the influence of kinship and familiarity on the shoal choice of sticklebacks by giving single individuals the opportunity to shoal either with unfamiliar full-sibs or with unfamiliar non-sibs.

An ontogenetic shift in sociality has been shown for different animal taxa (MacPherson 1998; Krause and Ruxton 2002). Furthermore shoaling preferences may vary between habitats (Pitcher and Parrish 1993; Brown and Warburton 1997). Juvenile sticklebacks as well as non-reproductive adults preferred to shoal with familiar kin (FitzGerald and Morrissette 1992; Frommen and Bakker 2004). Both age classes mostly live in freshwater habitats which are often rather small (Wootton 1984). Thus the possibility of a reunion with a group once lost is high. At the age of a few months fish from our study population on the island of Texel, the Netherlands migrate from freshwater to the North Sea where they stay during the winter. Reaching adulthood they enter the freshwater again to spawn. During this time salinity of the water as well as food composition dramatically changes. Ward et al. (2005b) have found a strong influence of dietary and habitat cues on sticklebacks' ability to recognise familiar individuals while Fisher et al. (2006) demonstrated that alteration in the chemical environment disrupts the communication of swordtails (*Xiphophorus birchmanni*). Furthermore in the North Sea the possibility to meet again once the shoal has been lost will be much smaller than in freshwater. Peuhkuri and Seppä (1998) for instance found no higher relatedness of individuals within groups than between groups in a Baltic Sea population. Thus, it may be possible that subadult sticklebacks, which mostly live in seawater, do not have or do not express the capability to recognise familiar kin. We tested this by giving subadult sticklebacks the choice to shoal with either a group of familiar full-sibs or a group of unfamiliar non-sibs.

Inbreeding depression in fish is a well-studied phenomenon (Waldman and McKinnon 1993). Inbred rainbow trout, *Oncorhynchus mykiss*, for example, had more body deformations and a reduced fry survivorship (see Waldman and McKinnon 1993 for a review) while the specific growth rate of inbred Coho salmon, *Oncorhynchus kisutch*, was reduced (Gallardo and Neira 2005). Inbred stickleback eggs had a lower fertilization and hatching rate compared to outbred ones, and fewer inbred fish survived to the reproductive age (JGF, unpublished data). Much less is known about the influence of inbreeding on behavioural traits. In kin recognition, different scenarios seem plausible. Inbred kin should have more similar MHC haplotypes than outbred kin. If an individual treats all fish with MHC haplotypes similar to its own as kin, one might expect that inbred fish would have a more pronounced preference for kin. On the other hand, in inbred fish, the ability to recognise kin might be disturbed due to inbreeding degenerations. We aimed at studying the effect of inbreeding on shoaling behaviour by using both inbred and outbred fish.

Thus, our study had a threefold aim. First, is the recognition of familiar kin lost in subadult sticklebacks? Second, do sticklebacks discriminate kin independent of direct familiarity, that is, individuals they have never had contact with before? Third, is shoaling preference affected by inbreeding?

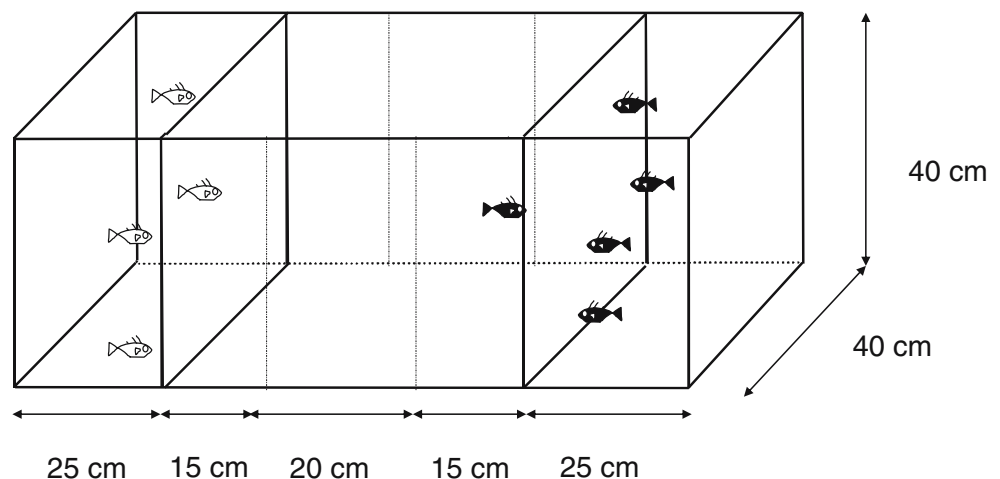## Material and methods

Experimental subjects

Sticklebacks used in the experiments were laboratory-bred outbred offspring of anadromous fish, which had been caught during their spring migration in April 2003 on the island of Texel, The Netherlands, and offspring of fish caught at the same location in April 2002, which had been inbred during one generation using brother–sister matings. Inbred and outbred eggs had been spawned in April and May 2003. Inbred eggs had a significantly reduced hatching and survival rate than outbred eggs (JGF, unpublished data). Clutches were taken out of the nests immediately after fertilization. Clutch sizes ranged between 40 and more than 100 eggs. Each clutch was divided into two subgroups, which were reared apart from each other. Thus, we got familiar and unfamiliar kin for each of the sib groups. At an age of 2 months, group sizes were reduced to 15 full sibs. At the time of the second experiment, group sizes ranged between five and 15 individuals. Till the end of experiment 1, fish were kept in an air-conditioned room under standardized winter light regime (day length, 8L:16D, temperature 17±1°C). Afterwards, the light regime was changed to summer conditions (day length, 16L:8D, temperature 17±1°C) for 5 months before winter conditions were reestablished. The rearing tanks contained 50 l of tap water and were separated from each other by grey opaque partitions. Thus, fish in each group were only familiar to each other but had no opportunity to get in contact with other groups. Water in the tanks was cleaned and aerated through an internal filter, and a third of the water volume was replenished with tap water once a week. The fish were fed daily ad libitum on frozen *Chironomus* larvae. In the experiments, only reproductively non-active fish were used;

hence, fish showed no sign to develop breeding coloration or egg production. In experiment 1, we tested subadult fish from 15 different full-sib groups; seven of them had been inbred during one generation, eight had been outbred. All individuals were used only once. In experiment 2, we tested adult fish from 30 different full-sib groups, 16 inbred and 14 outbred. All testfish were used only once while some groups provided the kin stimulus group in one test and the non-kin stimulus group in another. However, every single fish was used only once to avoid disturbing effects of prior experimental experience.

Experimental design

Subadult fish were tested in December 2003 at an age of about 8 months; adults about 16 months later in April 2005. Fish in 2003 and 2005 were members of the same kin groups. However, all testfish were used only once and unfamiliar fish never had contact to each other. Shoaling preferences were tested in a glass aquarium measuring 1 m which was divided into two stimulus compartments (measuring 25 cm each) on the right and left side and a testfish compartment (measuring 50 cm) in the middle (Fig. 1). Compartments were separated by perforated, clear Plexiglas. This enabled the testfish to have visual and olfactory contact to each of the two stimulus groups. Fifteen centimeters in front of each side compartment, a choice zone was marked on the front and back wall using permanent marker, leaving a neutral zone of 20 cm in the middle of the tank. A 30-W fluorescent tube placed above the tank lighted the setup. The tank was filled with 1-day-old tap water. The water temperature at the time of testing was 17±1°C. Interactions of the fish with the environment of the aquarium were prevented by making the side and back walls of the tank opaque using grey plastic plates. Additionally, a black curtain was tightened around the test tank.

In the left and right stimulus compartments of the tank, we placed four, randomly caught fish each of two different full-sib groups. Stimulus shoals consisted of subadult familiar full-sibs or unfamiliar non-sibs (experiment 1) and adult unfamiliar full-sibs or unfamiliar non-sibs (experiment 2). Size, mass and condition factor (calculated as $100 \times$ mass (g)/standard length (cm)^3; Bolger and Connolly 1989) of subadult fish varied between 2.7 cm, 0.23 g, 0.97 and 4.2 cm, 0.91 g, 1.61, and for adult fish, between 4.3 cm, 1.13 g, 1.03 and 6.1 cm, 3.35 g, 1.95. In both experiments, shoals did not differ significantly in mean body mass, standard body length and condition factor (paired t-tests; experiment 1: all t_{14} between -0.52 and 0.78, all $p > 0.45$; experiment 2: all t_{29} between -1.04 and 1.27, all $p > 0.2$). The position of the testfish' relatives alternated between tests. The testfish, also caught randomly, was placed in a perforated, transparent plastic cylinder (diameter, 10 cm), which was placed in the middle of the tank. After 2 min, the cylinder was lifted from behind the curtain by a string. Thereafter, fish movements were recorded for 30 min using a webcam (made by Creative, model CT6840) placed in front of the tank and connected to a laptop computer behind the curtain. At the end of the experiment, the standard body length and body mass of the fish were measured.

The digital film recordings were analysed afterwards. The time that the testfish spent in each choice zone was quantified. When the head of the testfish had entered a particular choice zone, time measurement started. The observer was naïve with respect to the side where the relatives of the testfish were.

Statistical analysis

All time variables were normally distributed according to Kolmogorov–Smirnov tests with Lilliefors correction. Some body characteristics were transformed (square, square root or reciprocal value) to make them normally distributed.

Fig. 1 The aquarium used to measure shoaling preferences. In the *left and right* stimulus compartments, we placed two shoals of each four full-sibs. The testfish which was a familiar full-sib (experiment 1) or an unfamiliar full-sib (experiment 2) to fish in one of the shoals was placed in the *middle section*. Perforated, clear Plexiglas separated the sections. Fish of the same coloration are full-sibs. In front of the aquarium, a webcam recorded fish movements. *Lines* drawn on the *front and back pane* visually divided the middle section into two choice zones and a neutral zone

40 cm

40 cm

25 cm   15 cm   20 cm   15 cm   25 cm

Thus, parametric statistics was used. Given test probabilities are two-tailed throughout. Analyses were performed using SPSS 11.0.1 statistical package.

Results

Experiment 1

The combined data of inbred and outbred fish showed that subadult testfish spent significantly more time near the shoal of familiar kin (mean time in seconds ± SD 1,023± 399) than near unfamiliar non-kin (492 s±366) (paired t-test, $t_{14}=2.75$, $p=0.016$, Fig. 2a). Inbred testfish alone preferred familiar kin (1,069 s±430) over unfamiliar non-kin (485 s±358), although this result was not statistically

significant (paired t-test, $t_7=2.12$, $p=0.072$, Fig. 2a). Outbred fish alone showed no significant preference for familiar kin (969 s±387) or unfamiliar non-kin (500 s± 402) (paired t-test, $t_6=1.63$, $p=0.155$, Fig. 2a). However, sample sizes for in- and outbred fish alone were small. The time inbred and outbred testfish spent near their familiar kin did not differ significantly (independent t-test, $t_{13}=0.47$, $p=0.65$, Fig. 2a). Furthermore, time inbred (246 s±139) and outbred (331 s±197) fish spent in the neutral zone did not differ significantly (independent t-test, $t_{13}=-0.976$, $p=0.347$). Neither body characteristics of the testfish (Pearson correlation, $N=15$, all r_p between -0.116 and 0.323, all $p>0.24$) nor the mean size, mass or condition of the shoaling fish, the variability of these traits or their relationship to the characteristics of the testfish influenced shoal choice significantly (Table 1).

Fig. 2 Mean time (±SD) test-fish spent near shoals of familiar kin and unfamiliar non-kin (**a**), and near shoals of unfamiliar kin and unfamiliar non-kin (**b**). Given is the time for all fish (*white bars*; $N=15$ and 30, respectively) and for inbred (*dark bars*; $N=8$ and 16, respectively) and outbred (*light bars*; $N=7$ and 14, respectively) fish separately. Each test lasted 1,800 s; *n.s.* Nonsignificant, *asterisk* *: $p < 0.05$, *asterisk* (*): $p < 0.1$
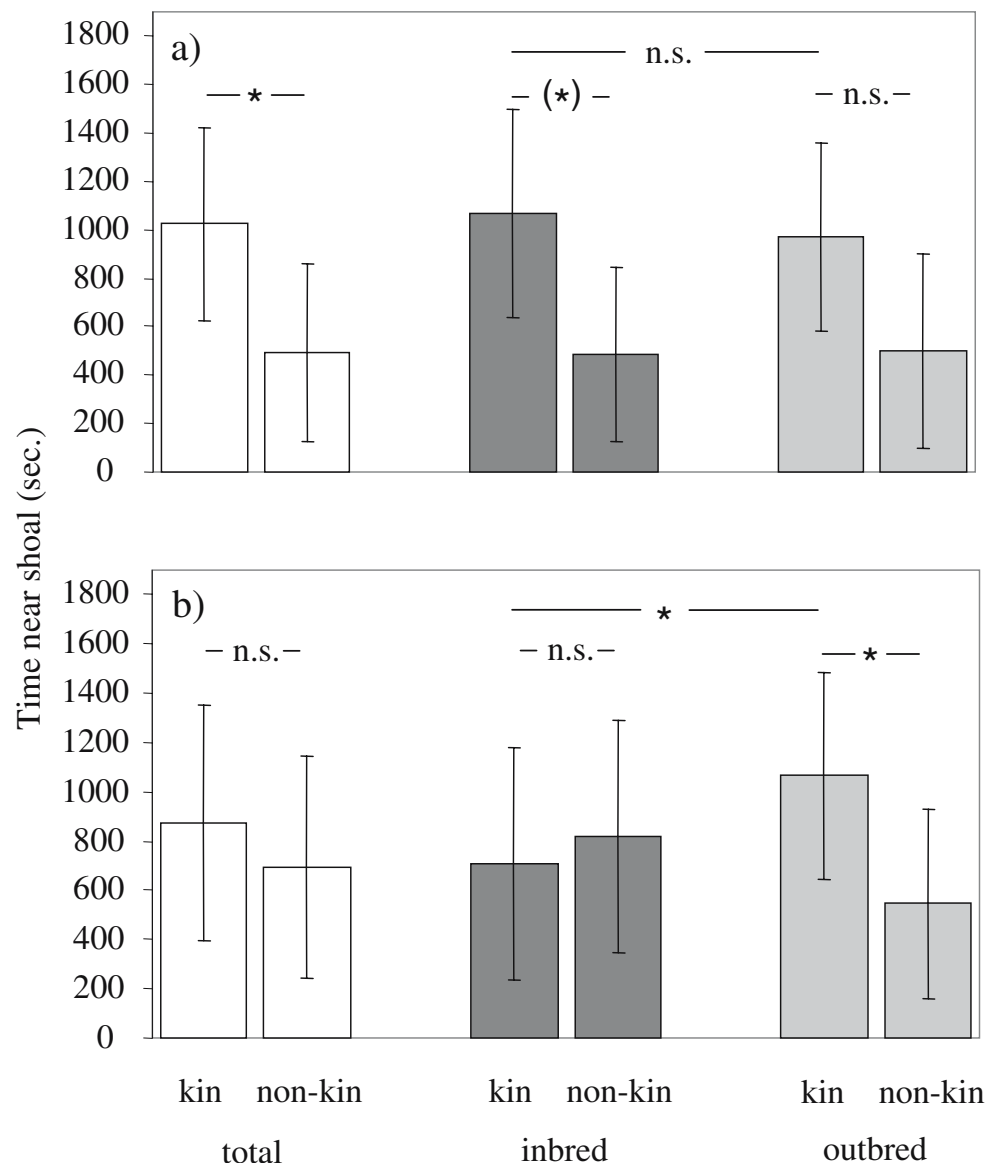
Table 1 Pearson correlation coefficients (r_p) of the time that testfish spent near the group of kin and group characteristics

Criterion of choice	Experiment 1		Experiment 2	
	r_p	p	r_p	p
Mean body mass	−0.119	0.672	−0.165	0.384
Relative body mass	0.383	0.158	0.133	0.484
Variance in body mass	−0.184	0.512	−0.255	0.174
Mean standard length	0.022	0.937	−0.269	0.15
Relative standard length	0.373	0.171	0.113	0.553
Variance in standard length	−0.138	0.624	−0.252	0.179
Mean condition	−0.284	0.305	0.19	0.314
Relative condition	0.110	0.697	−0.009	0.961
Variance in condition	0.396	0.144	−0.048	0.802

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 and as variances of the four fish in the group of kin. The sample size was 15 in experiment 1 and 30 in experiment 2; *p*-values are two-tailed.

Experiment 2

Inbred and outbred testfish combined showed no significant preference for either the shoal composed of unfamiliar kin (876 s±476) or unfamiliar non-kin (692 s±449) (paired *t*-test, $t_{29}=1.11$, $p=0.278$, Fig. 2b). Inbred testfish did not stay significantly longer near unfamiliar kin (710 s±470) than near unfamiliar non-kin (820 s±473) (paired *t*-test, $t_{15}=-0.47$, $p=0.644$, Fig. 2b) while outbred fish showed a significant preference for unfamiliar kin (1,066 s±420) over unfamiliar non-kin (547 s±385) (paired *t*-test, $t_{13}=2.45$, $p=0.029$, Fig. 2b). Outbred fish remained significantly longer near the group of siblings than inbred fish (independent *t*-test, $t_{28}=-2.17$, $p=0.038$, Fig. 2b). The time inbred (270 s±170) and outbred (186 s±150) fish spent in the neutral zone did not differ significantly (independent *t*-test, $t_{13}=1.42$, $p=0.167$). Like in experiment 1, neither body characteristics of the testfish (Pearson correlation, $N=30$, all r_p between −0.167 and 0.176, all $p > 0.35$) nor the mean size, mass or condition of the shoaling fish, the variability of these traits in a shoal or their relationship to the characteristics of the testfish influenced the choice significantly (Table 1).

Inbred testfish remained longer near familiar kin (experiment 1) than near unfamiliar kin (experiment 2). However, this result was not statistically significant (independent *t*-test, $t_{22}=-1.81$, $p=0.084$). Outbred testfish did not stay significantly longer near familiar than unfamiliar kin (independent *t*-test, $t_{19}=0.509$, $p=0.616$).

Discussion

The influence of kinship on shoaling decisions has been amply discussed in the fish literature (Ward and Hart 2003). Unfortunately, many studies have failed to disentangle familiarity effects and kinship (e.g. Frommen and Bakker 2004). Our results showed that subadult sticklebacks preferred to shoal with familiar siblings. Furthermore, adult outbred fish preferred unfamiliar kin over unfamiliar non-kin while inbred ones did not show such a preference, possibly as a result of inbreeding depression (but see Mazzi et al. 2004).

Using our experimental design, we cannot discriminate whether sticklebacks in experiment 2 recognise unfamiliar siblings on the basis of earlier experiences with kin (phenotype matching) or recognition alleles ("true" kin recognition) (Blaustein 1983; Grafen 1990). It is extremely difficult to rule out phenotype matching (Mateo 2004). Animals reared in isolation or in cross-fostered groups are still familiar with their own cues (self-referent phenotype matching) (Mateo and Johnston 2000). Male three-spined sticklebacks build nests in which one or more females deposit their eggs. The young stay for the first days of their life in the nest where they live in close association with other full-sibs and half-sibs (Wootton 1976). During this phase, they have got the opportunity to get familiar with the olfactory cues of their kin. These cues may be used later in life as a template to recognise relatives. Thus, phenotype matching is a plausible mechanism to explain kin recognition in sticklebacks.

Because sticklebacks from our study population migrate to the sea during autumn, their habitat structure changes from smaller rivers and ponds to large open areas. Thus, the possibility to meet a group again, once it is lost, should be low during winter. Additionally, not only habitat structures change but also environmental cues like water chemistry or food composition. Recent studies have shown that alterations in the chemical environment may disrupt the communication of swordtails (Fisher et al. 2006) and that dietary and habitat cues strongly influence sticklebacks' social decisions (Ward et al. 2005b). Thus, chemical recognition of familiar kin might be disturbed during the winter month. Therefore, it is conceivable that sticklebacks adjust their shoal choice on familiarity and/or kinship during early life and when they reach sexual maturity, while ignoring these factors during subadulthood. Contrary to this consideration, the results of experiment 1 show that subadult sticklebacks prefer to shoal with familiar kin, too. Thus, this ability is present during the whole life cycle of sticklebacks. However, we did not manipulate chemical cues of the sticklebacks' environment during the experiments. Hence, we cannot rule out that changes of the water chemistry may influence sticklebacks' shoaling decisions.

Inbred fish in experiment 1 remained longer near the shoal of familiar kin while they did not show a preference for unfamiliar kin in experiment 2. Therefore, one can conclude that direct familiarity (experiment 1) and phenotype matching (experiment 2) are two operating mechanisms influencing shoal choice in sticklebacks. While sticklebacks may use their short-term memory to recognise familiar individuals, they may use their long-term memory for phenotype matching. If this is true, the results of our study might suggest that in sticklebacks, inbreeding affects the long-term memory while the short-term memory remains unaffected. Furthermore, a shoal of familiar fish is easier to establish than a shoal of relatives. An individual that looses its familiar group might build up familiarity to formerly unknown fish quite fast while new fish cannot replace a lost group of kin. Thus, at least in open environments, recognising familiar fish might be more important in shoaling decisions while recognition of unfamiliar kin should play an important role in sticklebacks' mate choice (Frommen and Bakker 2006). Few other studies have addressed the impact of inbreeding on social behaviour in fish, thus far. For example, inbred and outbred stickleback females differed in their preference for symmetrical males (Mazzi et al. 2004) but not in their ability to avoid inbreeding via mate choice (Frommen and Bakker 2006). Inbred guppy (*Poecilia reticulata*) males performed different courtship behaviour than outbred ones (Farr 1983; Farr and Peters 1984) while Atlantic salmon (*Salmo salar*) fry with low degree of genetic diversity were less aggressive than more genetically diverse ones (Tiira et al. 2003; Gallardo and Neira 2005).

How sticklebacks recognise familiar and unfamiliar kin is unknown, but a major role of olfactory cues seems plausible (but see Steck et al. 1999). Ward et al. (2005b) showed that sticklebacks recognise individuals reared in the same habitat and those fed on the same food on olfactory cues only. Furthermore, sticklebacks were able to "count" and compare MHC alleles of foreign individuals and use this information in mate choice (Reusch et al. 2001). If this mechanism is also used in kin recognition, then one might expect that inbred fish would have a more pronounced preference for kin because inbreeding will have produced sibling groups with a reduced MHC-heterozygosity. When phenotype matching is the main mechanism of kin recognition, it may be easier to recognise inbred kin because they are more MHC-similar to each other. We did not, however, find a stronger preference for kin in inbred fish.

In conclusion, our study shows that sticklebacks are able to recognise familiar and unfamiliar kin and that this ability is influenced by inbreeding. Future investigations should focus on the question whether sticklebacks use phenotype matching or recognition alleles to recognise kin and which kinds of cues are used in the recognition process.

References

Arnold KE (2000) Kin recognition in rainbowfish (*Melanotaenia eachamensis*): sex, sibs and shoaling. Behav Ecol Sociobiol 48:385–391

Behrmann-Godel J, Gerlach G, Eckmann R (2006) Kin and population recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): can assortative shoaling drive population divergence? Behav Ecol Sociobiol 59:461–468

Blaustein AR (1983) Kin recognition mechanisms—phenotype matching or recognition alleles? Am Nat 121:749–754

Bolger T, Connolly PL (1989) The selection of suitable indices for the measurement and analysis of fish condition. J Fish Biol 34:171–182

Brown C, Warburton K (1997) Predator recognition and anti-predator responses in the rainbowfish *Melanotaenia eachamensis*. Behav Ecol Sociobiol 41:61–68

Brown GE, Brown JA (1993) Social dynamics in salmonid fishes—do kin make better neighbors? Anim Behav 45:863–871

Farr JA (1983) The inheritance of quantitative fitness traits in guppies, *Poecilia reticulata* (Pisces, Poeciliidae). Evolution 37:1193–1209

Farr JA, Peters K (1984) The inheritance of quantitative fitness traits in guppies, *Poecilia reticulata* (Pisces, Poeciliidae). 2. Tests for inbreeding effects. Heredity 52:285–296

Fisher HS, Wong BBM, Rosenthal GG (2006) Alteration of the chemical environment disrupts communication in a freshwater fish. Proc R Soc Lond B 273:1187–1193

FitzGerald GJ, Morrissette J (1992) Kin recognition and choice of shoal mates by threespine sticklebacks. Ethol Ecol Evol 4:273–283

Frommen JG, Bakker TCM (2004) Adult three-spined sticklebacks prefer to shoal with familiar kin. Behaviour 141:1401–1409

Frommen JG, Bakker TCM (2006) Inbreeding avoidance through non-random mating in sticklebacks. Biol Lett 2:232–235

Gallardo JA, Neira R (2005) Environmental dependence of inbreeding depression in cultured Coho salmon (*Oncorhynchus kisutch*): aggressiveness, dominance and intraspecific competition. Heredity 95:449–456

Gómez-Laplaza LM (2005) The influence of social status on shoaling preferences in the freshwater angelfish (*Pterophyllum scalare*). Behaviour 142:827–844

Grafen A (1990) Do animals really recognise kin? Anim Behav 39:42–54

Griffiths SW, Brockmark S, Höjesjö J, Johnsson JI (2004) Coping with divided attention: the advantage of familiarity. Proc R Soc Lond B 271:695–699

Griffiths SW, Magurran AE (1997) Schooling preferences for familiar fish vary with group size in a wild guppy population. Proc R Soc Lond B 264:547–551

Hamilton WD (1964) The genetical evolution of social behaviour 1. J Theor Biol 7:1–16

Johannes MRS (1993) Prey aggregation is correlated with increased predation pressure in lake fish communities. Can J Fish Aquat Sci 50:66–73

Keenleyside MHA (1955) Some aspects of the schooling behaviour of fish. Behaviour 8:183–248

Krause J (1993) The influence of hunger on shoal size choice by three-spined sticklebacks, *Gasterosteus aculeatus*. J Fish Biol 43:775–780

Krause J (1994) The influence of food competition and predation risk on size-assortative shoaling in juvenile chub (*Leuciscus cephalus*). Ethology 96:105–116

Krause J, Godin J-GJ (1994) Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae)—effects of predation risk, fish size, species composition and size of shoals. Ethology 98:128–136

Krause J, Ruxton GD (2002) Living in groups. Oxford University Press, Oxford

MacPherson E (1998) Ontogenetic shifts in habitat use and aggregation in juvenile sparid fishes. J Exp Mar Biol Ecol 220:127–150

Magurran AE (1990) The adaptive significance of schooling as an anti-predator defence in fish. Ann Zool Fenn 27:51–66

Mateo JM (2004) Recognition systems and biological organization: the perception component of social recognition. Ann Zool Fenn 41:729–745

Mateo JM, Johnston RE (2000) Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. Proc R Soc Lond B 267:695–700

Mazzi D, Künzler R, Largiadèr CR, Bakker TCM (2004) Inbreeding affects female preference for symmetry in computer-animated sticklebacks. Behav Genet 34:417–424

McRobert SP, Bradner J (1998) The influence of body coloration on shoaling preferences in fish. Anim Behav 56:611–615

Metcalfe NB, Thomson BC (1995) Fish recognize and prefer to shoal with poor competitors. Proc R Soc Lond B 259:207–210

Milinski M, Külling D, Kettler R (1990) Tit for tat: sticklebacks (*Gasterosteus aculeatus*) "trusting" a cooperating partner. Behav Ecol 1:7–11

Modarressie R, Rick IP, Bakker TCM (2006) UV matters in shoaling decisions. Proc R Soc Lond B 273:849–854

Peuhkuri N, Seppä P (1998) Do three-spined sticklebacks group with kin? Ann Zool Fenn 35:21–27

Pitcher TJ, Magurran AE, Winfield IJ (1982) Fish in larger shoals find food faster. Behav Ecol Sociobiol 10:149–151

Pitcher TJ, Parrish JK (1993) Functions of shoaling behaviour in teleosts. In: Pitcher TJ (ed) The behaviour of teleost fishes, 2nd edn. Croom Helm, London

Poulin R (1999) Parasitism and shoal size in juvenile sticklebacks: conflicting selection pressures from different ectoparasites? Ethology 105:959–968

Ranta E, Lindström K, Peuhkuri N (1992) Size matters when three-spined sticklebacks go to school. Anim Behav 43:160–162

Reusch TBH, Häberli MA, Aeschlimann PB, Milinski M (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. Nature 414:300–302

Steck N, Wedekind C, Milinski M (1999) No sibling odor preference in juvenile three-spined sticklebacks. Behav Ecol 10:493–497

Tiira K, Laurila A, Peuhkuri N, Piironen J, Ranta E, Primmer CR (2003) Aggressiveness is associated with genetic diversity in landlocked salmon (*Salmo salar*). Mol Ecol 12:2399–2407

Utne-Palm AC, Hart PJB (2000) The effects of familiarity on competitive interactions between threespined sticklebacks. Oikos 91:225–232

Waldman B, McKinnon JS (1993) Inbreeding and outbreeding in fishes, amphibians and reptiles. In: Thornhill NW (ed) The natural history of inbreeding and outbreeding: theoretical and empirical perspectives. University of Chicago Press, Chicago, pp 250–282

Ward AJW, Hart PJB (2003) The effects of kin and familiarity on interactions between fish. Fish Fish 4:348–358

Ward AJW, Duff AJ, Krause J, Barber I (2005a) Shoaling behaviour of sticklebacks infected with the microsporidian parasite, *Glugea anomala*. Environ Biol Fishes 72:155–160

Ward AJW, Holbrook RI, Krause J, Hart PJB (2005b) Social recognition in sticklebacks: the role of direct experience and habitat cues. Behav Ecol Sociobiol 57:575–583

Wedekind C (1996) Lek-like spawning behaviour and different female mate preferences in roach (*Rutilus rutilus*). Behaviour 133:681–695

Wootton RJ (1976) The biology of the sticklebacks. Academic, London

Wootton RJ (1984) A functional biology of sticklebacks. Croom Helm, London