Journal of Fish Biology (2007) **70,** 1295–1301
doi:10.1111/j.1095-8649.2007.01390.x, available online at http://www.blackwell-synergy.com

The tale of the bad stepfather: male three-spined sticklebacks *Gasterosteus aculeatus* L. recognize foreign eggs in their manipulated nest by egg cues alone

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(Received 28 March 2006, Accepted 8 December 2006)

The ability to discriminate between own and foreign eggs was investigated in brood-caring male three-spined sticklebacks *Gasterosteus aculeatus*. Males totally cannibalized clutches that contained both foreign and their own eggs significantly more often than sham-manipulated control clutches that only contained their own eggs. These results show that caring males are able to recognize the presence of foreign eggs by egg cues alone.

Key words: cannibalism; *Gasterosteus aculeatus*; kin recognition; paternal care; stickleback.

Extra-pair copulations and sneaked fertilizations as reproductive tactics have been described for a whole range of egg-laying species with paternal care (*e.g.* fishes: Taborsky, 1994; birds: Griffith *et al.*, 2002). In birds as well as in fishes, males which are uncertain about their paternity should reduce the amount of care for the brood (Xia, 1992). Kvarnemo (2006), however, argues that an increase of male care leads to a reduction of sperm competition and thus to an increase in paternity. Studies conducted to test this theory have generated mixed results (Rios-Cardenas & Webster, 2005).

Fishes have developed an impressive range of reproductive tactics. In brood-caring species there often exist not only territorial individuals but also satellite males or sneakers (Taborsky, 1994). Sneakers try to 'steal' fertilizations by spawning over foreign eggs immediately after the nest owner. Sneaking leads to a direct loss of fitness through a decreased number of eggs fertilized by the nest owner. Additionally, fitness also decreases indirectly through a waste of parental effort when their own and foreign eggs are treated equally. It would thus be advantageous to the nest owner to recognize foreign eggs and change his behaviour accordingly (Neff & Sherman, 2002). Results of studies dealing

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with this topic are ambiguous thus far: common gobies *Pomatoschistus microps* (Krøyer) (Svensson *et al.*, 1998) as well as fifteen-spined sticklebacks *Spinachia spinachia* (L.) (Östlund-Nilsson, 2002) did not reduce their brood-caring behaviour or increase the amount of filial cannibalism if the clutches had been fertilized by foreign males. In contrast, Sargent (1989) found that male fathead minnows *Pimephales promelas* Rafinesque discriminated against adopted eggs while spottail darters *Etheostoma squamiceps* Jordan distinguished between own and adopted clutches (Bandoli, 2002). Male pumpkinseed *Lepomis gibbosus* (L.) adjusted their brood care in relation to paternity (Rios-Cardenas & Webster, 2005) while males of the closely related bluegill *Lepomis macrochirus* Rafinesque did not differentiate between their own and foreign eggs, but exhibited a preference for related fry (Neff, 2003; Neff & Sherman, 2003). Males of the scissortail sergeant *Abudefduf sexfasciatus* (Lacepède) confronted with a potential sneaker reduced parental effort and increased filial cannibalism (Manica, 2004). In all those studies, however, males assessed their relatedness to eggs in their nest indirectly, *e.g.* by observing potential sneakers near their nest or actively adopting foreign eggs. A direct recognition of relatedness by egg cues alone is not reported thus far (Neff & Sherman, 2003; Bandoli, 2006).

Three-spined sticklebacks *Gasterosteus aculeatus* L. are an ideal fish to study male egg recognition for three reasons. First, sneaking occurs frequently (Largiadèr *et al.*, 2001). During the breeding season male three-spined sticklebacks establish small territories in which they build a tunnel-shaped nest. Females spawn their eggs into this nest where they are fertilized by the male. Directly after, or even before the nest owner, sneaking three-spined sticklebacks, which are also nest owners, try to creep through the nest and fertilize the eggs. The nest owner vigorously tries to prevent this intrusion (Wootton, 1984). Sneaking three-spined sticklebacks have been documented in several populations with up to 25% of nests containing eggs fertilized by sneakers (Largiadèr *et al.*, 2001). Sneaking has also been observed in the Texel population used in this study (Zbinden *et al.*, 2003; Bakker *et al.*, 2006). Sneaked fertilizations are sometimes followed by an attempt to steal some eggs by the sneaking male (van den Assem, 1967). Stolen eggs are carried to his own nest which becomes more attractive for females in this way (Goldschmidt *et al.*, 1993). Thus egg thievery may be another cause for the presence of foreign eggs in the nest. Second, male three-spined sticklebacks cannibalize their own eggs (Whoriskey & FitzGerald, 1985). Male parental care is energetically costly as expressed by a higher energy expenditure of caring males (Smith & Wootton, 1999). Therefore males may cannibalize part of their own eggs as an investment in future reproduction (Rohwer, 1978; Whoriskey & FitzGerald, 1985; Bakker & Mundwiler, 1994; Manica, 2002). Cannibalism can be total or partial when only few, mainly dead eggs are consumed (Wootton, 1976). Recognizing foreign eggs in the nest could be of advantage because males may thus avoid cannibalizing their own eggs but instead consume foreign offspring. Third, recent studies using three-spined sticklebacks from the Texel population have shown that adult individuals distinguish between familiar (Frommen & Bakker, 2004, 2006) as well as unfamiliar kin (Frommen *et al.*, 2007) and non-kin. Therefore three-spined sticklebacks from this population might also bear the potential for egg recognition.

Fish were caught on the island of Texel, the Netherlands during their spring migration in April 2005, and transported to Bonn, Germany. Females and males were kept together in large outdoor tanks (750 l). Prior to testing they were transferred to smaller aquaria containing 100–120 l of tap-water. Here the sexes were kept separately under summer conditions (16L:8D, 17° C). Holding tanks were equipped with filamentous algae as a hide, a filter (Hobby Aquaristik), and an air stone. Fish were daily fed with frozen *Chironomus* sp. larvae and *Artemia* sp.

Experiments took place in August 2005. Males showing breeding colouration were placed in separate tanks containing 25 l of tap-water, a Petri dish filled with sand placed near the back wall, 2 g of filamentous algae for nest building and an air stone. As male three-spined sticklebacks in the wild often reduce feeding during brood care (Wootton, 1976), males were kept under food-limited conditions. Therefore they were fed 30 frozen *Artemia* sp. every second evening (Feuth-de Bruijn & Sevenster, 1983). In order to stimulate nest building males were shown a receptive female every day. Males with finished nests were paired with a gravid female. The following day males were caught with a hand-net and placed in a 1 l box in a dark room in order to reduce stress to a minimum. Then the nest was taken out of the tank and opened carefully. Eggs were placed in a small dish filled with tap-water. The number of eggs was estimated by counting 30 eggs and weighing them to the nearest mg, then weighing the whole clutch and then estimating the total number of eggs (Bakker & Mundwiler, 1994). Clutch size in the exchange treatment (mean ± S.D. number of eggs 152 ± 73) and control treatment (164 ± 50) did not differ significantly (*t*-test, d.f. = 24, $P > 0{\cdot}05$). After counting, clutches were divided into two equal-sized halves. In the exchange treatment, one half was placed back into the original nest while the other half was placed in the nest of an unrelated male whose clutch was treated the same way before. In order to be able to distinguish between the eggs of different origin, one half of the clutch was dyed blue using a solution of Alcian blue (2 g 100 ml^{-1}) for up to 2 h following Kraak *et al.* (1997). This method did not harm the eggs (Kraak *et al.*, 1997). Own and foreign eggs were coloured blue alternately. After the manipulation of nest contents, the two nests were placed back into the males' tanks. Control nests were sham-manipulated, that is, treatment was conducted the same way, but here all eggs of one clutch were placed back into the original nest. One half of the clutch was dyed blue, too. Combining data of the exchange and control treatment revealed that dyeing eggs did not significantly affect the number of consumed eggs (Wilcoxon matched-pairs signed-ranks test, $n = 26$, $P > 0{\cdot}05$).

Before the males were transferred back to their tank, their wet body mass (M, mg) and standard body length (L_S, cm) was measured and the condition factor (K) was calculated as $K = 100ML_S^{-3}$ (Bolger & Connolly, 1989). Three males in the exchange treatment and two males in the control treatment did not re-accept their nest and were therefore excluded from analysis. All other males started brood-caring behaviour after being re-introduced suggesting that they re-accept their nests after manipulation. Seven days after the treatment, nests were taken out of the tank again and all remaining eggs were counted using a binocular microscope. All statistics were carried out using SPSS 12.0.

All *P*-values are two-tailed. After the experiments, adult fish as well as hatching fry were kept in the laboratory for further experiments.

Males with nests containing foreign eggs totally cannibalized their clutches significantly more often than males with clutches containing only their own eggs ($n_{exchange} = 9$ out of 13 nests, $n_{control} = 4$ out of 13 nests, *G*-test, d.f. = 1, $P < 0{\cdot}05$; Fig. 1). Cannibalism occurred in each trial. The median rate of egg cannibalism was high and similar in both treatments; exchange treatment: median = 100·0% (quartiles 57·95 and 100·0%), control treatment: median = 72·92% (51·59 and 98·44%) (Mann–Whitney *U*-test, $n_{exchange} = 13$, $n_{control} = 13$, $P = >0{\cdot}05$). The L_S, *M* and *K* of the males did not significantly correlate with the number of eggs eaten in both treatments (Spearman rank correlation, all $n = 13$, exchange treatment: all r between $-0{\cdot}282$ and 0·203, all $P > 0{\cdot}05$, control treatment: all r between 0·080 and 0·097, all $P > 0{\cdot}05$).

The question whether fishes adjust their brood care according to the amount of paternity is ambiguous thus far. While several studies show a decreased amount of brood care and a higher rate of filial cannibalism when the risk of sneaked fertilizations is high (Sargent, 1989; Bandoli, 2002; Manica, 2004; Rios-Cardenas & Webster, 2005) others fail to show such a connection (Svensson *et al.*, 1998; Östlund-Nilsson, 2002). The results of this study show that three-spined sticklebacks are able to recognize the presence of foreign eggs in their nests. Clutches were totally cannibalized significantly more often when they contained foreign eggs. When paternity is uncertain, three-spined sticklebacks may better cannibalize the clutch, gain energy and start anew. By doing so they may have better chances of future breeding cycles without foreign eggs.

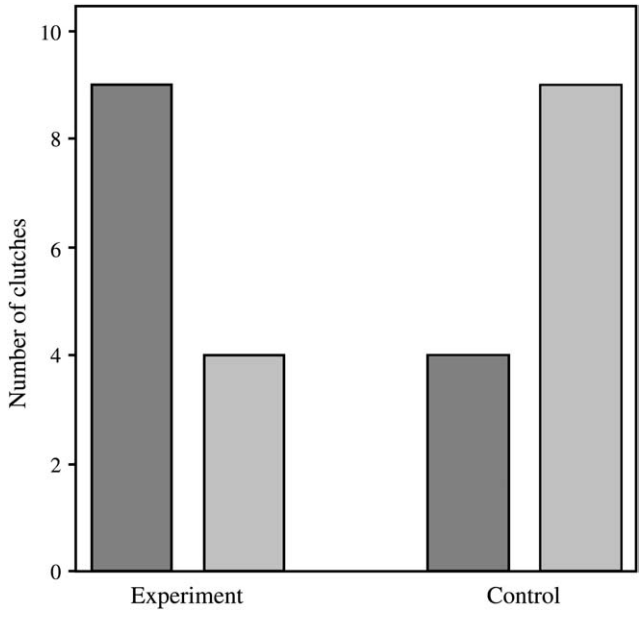

FIG. 1. Number of totally cannibalized ■ and partly cannibalized □ clutches in the exchange experiment and control treatment. Males totally cannibalized significantly more clutches when they contained foreign eggs (*G*-test). $P < 0{\cdot}05$.

There are two possible explanations for whole-clutch cannibalism. Males may consume all eggs as soon as they recognize that the nest contains foreign eggs or a certain proportion of foreign eggs. Alternatively, they may first consume all foreign eggs. After that, depending on the number of remaining eggs, they decide whether they also consume the remaining eggs or not. If there are too few eggs left, the male consumes them and starts to build a new nest. Former studies have shown that males tend to cannibalize small broods (van den Assem, 1967). Thus, males may cannibalize the whole clutch if their share of paternity is too small, but they may accept caring for foreign eggs if the ratio of foreign to their own eggs is low or if the total number of eggs is high. These explanations would also answer the question, why sneaking in three-spined sticklebacks occurs at all, when nest owners consume sneaked clutches.

While males in former studies assessed their relatedness to their eggs indirectly, *e.g.* by observing potential sneakers near their nest, fish in this study recognized foreign eggs by egg cues alone. The mechanism of egg recognition remains unknown thus far. Three-spined stickleback nests are tunnel shaped and thus relatively dark inside and hard to access, even for the nest owner. The eggs lie clumped on the bottom of the nest and it may be difficult to visually distinguish eggs of different fathers. Furthermore, when foreign eggs in the nest are the result of sneaking events, the eggs are from the same female and thus very similar in appearance. Therefore, olfactory cues are likely to play a major role. Three-spined sticklebacks use olfactory cues in mate choice (Reusch *et al.*, 2001), but Steck *et al.* (1999) found no evidence that juvenile three-spined sticklebacks are able to recognize siblings by olfactory cues alone. Thus, sibling and offspring recognition may be the outcome of two different mechanisms. While juveniles use at least partly visual cues to differentiate kin from non-kin, males are able to recognize their own eggs by olfactory cues alone.

The consumption of eggs or fry as an alternative feeding tactic has been shown for several fish species (Manica, 2002). Common gobies for example ate part of their eggs more often when they were kept under food-deprived conditions (Kvarnemo *et al.*, 1998). Three-spined sticklebacks in both the exchange and control treatment were kept under food-deprived conditions during the experiment. Thus, it might have been expected that males having a worse condition would consume more eggs than males of better condition. In both treatments M, L_S and K were not significantly correlated with egg consumption rate. Males must possibly be more severely food deprived for a longer time in order to evoke a relationship between K and egg consumption rate.

Summarizing, this study shows that male three-spined sticklebacks are able to recognize that their nests contain foreign eggs. The question whether they are able to distinguish between single eggs of their own and foreign eggs could not be answered in this study because too few clutches in the exchange treatment survived to give results of any validity.

We are grateful to R. Modarressie, J. Schwarzer, T. Thünken, I. Rick and C. Pollmann for discussions. T. Thünken, L. Engqvist, I. Ahnesjö and two anonymous referees gave valuable comments on a former version of the manuscript. We thank J. Strelau for caring and the Hottentot family for catching the sticklebacks and for hospitality.

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