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Proc. R. Soc. B 2010 **277**, 2627-2635 first published online 21 April 2010
doi: 10.1098/rspb.2010.0234

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To eat or not to eat: egg-based assessment of paternity triggers fine-tuned decisions about filial cannibalism

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Filial cannibalism occurs in many animal species ranging from insects to mammals, and is especially well described in teleost fishes. Numerous causes may lead to this behaviour, e.g. certainty of paternity. However, the cues males use to assess their paternity often remain unknown. One possible way to differentiate between own and foreign offspring is by using egg cues. Nevertheless, in egg-laying species, evidence for this is still scarce. In this study, male three-spined sticklebacks (*Gasterosteus aculeatus*), a fish with paternal care in which sneaking as well as filial cannibalism is common, were allowed to care for manipulated nests that contained different percentages of own fertilized eggs. After 7 days, embryo survival was determined. Furthermore, brood-caring as well as aggressive behaviour was measured daily. Clutches containing a higher proportion of foreign eggs were more likely to be completely cannibalized than clutches containing a lower proportion of foreign eggs, particularly when the clutch was laid early in the breeding season. However, the behavioural observations revealed no influence of paternity. The results show that paternity triggers filial cannibalism in sticklebacks and that males are able to evaluate their paternity using egg cues alone.

Keywords: three-spined stickleback; kin recognition; parent-offspring conflict; brood-care; aggression; oophagy

1. INTRODUCTION

Cannibalism is a well-documented phenomenon that has been described for many animal species (Polis 1981). Several forms of cannibalism can be distinguished (Rohwer 1978; Smith & Reay 1991), for example, hetero-cannibalism (also called ‘non-kin cannibalism’; Smith & Reay 1991) and filial cannibalism. While the former describes the eating of unrelated conspecifics, the latter describes the consumption of own offspring, either eggs or young. The occurrence of filial cannibalism has been shown not only in many fish species (see Manica 2002; Klug & Bonsall 2007 for review), but also in mammals (Elwood 1991), reptiles (Huang 2008), birds (Gilbert *et al.* 2005), crustaceans (Dumont & Ali 2004) and insects (Thomas & Manica 2003). Filial cannibalism is often associated with parental care (Manica 2002). At first glance, eating one’s own offspring appears to be counterintuitive, as it should decrease an individual’s fitness. However, brood-caring parents often face trade-offs (Trivers 1972), e.g. whether they should sacrifice part of their offspring to save the rest of the brood. In fishes, parental males that lose body condition during the breeding cycle might use parts of the clutch to gain nutrition (Mehlis *et al.* 2009). As a result, they might be

able to care for the surviving eggs or hatched fry better. In general, total and partial filial cannibalism can be differentiated. Both have different benefits in terms of current versus future reproductive success, and thus should be considered separately. Total filial cannibalism can only be an investment into future reproduction, while partial filial cannibalism might represent an investment into the present as well as future broods (Sargent 1992). The factors leading to filial cannibalism are numerous. In fishes, for instance, parent physical condition (Gomagano & Kohda 2008), food availability (Kvarnemo *et al.* 1998), time of egg laying during the course of the breeding season (Petersen & Hess 1991), brood size (Forsgren *et al.* 1996), age of the clutch (Lavery & Keenleyside 1990), sex of cannibals (Schwank 1986), availability of mates (Okuda & Yanagisawa 1996) or the presence of an egg predator (Chin-Baarstad *et al.* 2009) have been shown to be of importance.

One further factor that has been shown to influence cannibalistic behaviour is uncertainty of paternity (Xia 1992; Manica 2002). In many fish and bird species, sneaked fertilizations are common. Caring for foreign eggs is costly owing to several reasons. First, males invest energy in eggs that do not carry their genes, and second, by doing so, they invest less in their own offspring. Consequently, it should be adaptive when males adjust their brood-caring behaviour according to their

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paternity. Indeed, several studies have shown such adjustments (for example, birds: Davies *et al.* 1992; fishes: Neff 2003). The cues males use to estimate the amount of foreign eggs often remain unknown (Rios-Cardenas & Webster 2005). There is some evidence for indirect mechanisms. For example, the presence of a rival male sometimes induces cannibalistic behaviour (Manica 2004; Gray *et al.* 2007); however, other studies found contradictory results (Svensson & Kvarnemo 2007; Lissåker & Svensson 2008). Furthermore, the position of different clutches in the nest might be used to recognize eggs that were not fertilized by the nest owner (Sargent 1989). A direct mechanism to recognize own and foreign eggs is by cues from the eggs (e.g. olfactory or visual) themselves. Here, the ability to recognize kin might be a basic requirement to avoid the mistreatment of related individuals or eggs. Evidence for direct egg recognition is scarce thus far (but see Frommen *et al.* 2007; Green *et al.* 2008).

As they show intense paternal care, three-spined sticklebacks (*Gasterosteus aculeatus*) are a great model system to study filial cannibalism. Three-spined stickleback males are able to complete several breeding cycles within one breeding season (May–August). At the beginning, males get territorial and build a nest mostly composed of algae, in which several gravid females spawn. Afterwards, the male takes care of the eggs until they hatch, by fanning and cleaning the clutch (removing dead or infected eggs). Here, males adjust their fanning behaviour according to the number of received eggs, indicating their ability to adjust their brood-caring behaviour to the value of the brood (Van Iersel 1953; Bakker *et al.* 2006a). In addition, sneaked fertilizations are a common phenomenon in this species (Jamieson & Colgan 1992; Largiadèr *et al.* 2001; Le Comber *et al.* 2003). Furthermore, filial cannibalism occurs frequently (e.g. Van den Assem 1967; Belles-Isles & FitzGerald 1991; FitzGerald 1992; Frommen *et al.* 2007; Mehlis *et al.* 2009) and is more common late in the breeding season (Belles-Isles & FitzGerald 1991). In general, parental care is energetically costly (Smith & Wootton 1999) with more developed eggs needing more oxygen and, thus, care (Collins & Nelson 1993; Kamler *et al.* 1998). Consequently, males lose body condition during the breeding cycle (Sargent 1985; Smith & Wootton 1999). Male sticklebacks to some extent compensate this loss of body condition by cannibalizing part of their eggs (Mehlis *et al.* 2009). Here, it would be advantageous for the male to cannibalize mainly foreign eggs that were fertilized by a sneaker in order to minimize the loss of fitness. Indeed, a recent study demonstrated that stickleback males cannibalize their entire clutch more often when it contained 50 per cent in comparison to 100 per cent of own eggs (Frommen *et al.* 2007). However, whether males are able to more precisely estimate the percentage of foreign eggs in the nest and whether this influences egg cannibalism, brood-caring or aggressive behaviour has yet to be elucidated. The present study aimed to answer the question of how varying degrees of paternity influence the rate of total and partial filial cannibalism in male three-spined sticklebacks. Furthermore, brood-caring and aggressive behaviour of the males was quantified to determine whether they were influenced by the amount of own eggs in the nest.

2. MATERIAL AND METHODS

(a) *Experimental subjects*

About 500 three-spined sticklebacks from a heterozygous (Heckel *et al.* 2002), anadromous population were randomly caught during their spring migration in April 2006 on the island of Texel, the Netherlands, and brought to the Institute for Evolutionary Biology and Ecology in Bonn, Germany. Here, both sexes were kept together in a large outside tank (750 l), with air ventilation and a constant supply of tap-water at a flow rate of 3 l min⁻¹. Before the experiments started, all fish were fed daily with larvae of *Chironomus* spp. in excess.

(b) *Set-up*

The experiments were conducted between June and August 2006. Test tanks (length 40.5 cm × width 20.5 cm × height 25 cm) were placed in an air-conditioned room (temperature 17 ± 1°C) under standardized summer light regime (day-length 16 L:8 D). They contained 16.5 l of tap water, a sand-filled petri dish (Ø 9 cm), an airstone and 2 g of java moss (*Vesicularia dubyana*) for nest-building. A single nuptial-coloured male, randomly caught from the outside tank, was placed in each tank, before its standard length (SL_m), body mass (M_{m1}) and body condition (BC_{m1} = 100 M_{m1}/SL_m³, following Bolger & Connolly (1989)) were determined. To avoid interactions between the males, the tanks were separated from each other by grey opaque partitions. In order to stimulate nest-building, males were presented daily with a gravid female for 15 min in a transparent container (10 cm × 7 cm × 17 cm) in front of the tank (Frommen & Bakker 2006). Males that did not build a nest within 8 days were replaced by new ones after cleaning the whole tank.

(c) *Nest manipulations*

When nest-building was finished for at least 24 h, males were allowed to spawn with a randomly chosen female. Afterwards, the female was removed from the tank within 15 min. Females' body measurements were determined before spawning (SL_f, M_f and BC_f). Two hours after spawning, the male as well as its nest was removed from the tank. This time span was chosen to allow the egg shells to harden, thus minimizing the risk of destroying eggs by handling (Kraak & Bakker 1998). Males' body measurements (M_{m2} and BC_{m2}) were determined again. Adult fish's growth rate during one week under food-deprived conditions is negligible (Allen & Wootton 1982). Thus, SL was not measured again in order to reduce stress. Thereafter, the male was placed in a water-filled plastic box (16.5 cm × 10 cm × 10.5 cm) in a dark room to minimize stress during nest manipulation (Frommen *et al.* 2007). All eggs were removed carefully and placed in a small petri dish filled with tap water. Thirty-five eggs were counted and weighed to the nearest milligram. Then, the whole clutch was weighed and the total number of eggs in the clutch was calculated (Bakker & Mundwiler 1994; Frommen *et al.* 2007). Eggs of two clutches of similar age (spawning events maximally 2 h apart) were used for the exchange. Six different exchange treatments were conducted, in which 0, 20, 40, 60, 80 or 100 per cent of a male's own eggs were replaced by foreign eggs. The number of eggs in each nest before and after the manipulation did not differ significantly (Wilcoxon test, $n = 82$, $z = 0.113$, $p = 0.910$, average difference equals 0.3 eggs per exchange). To be able to distinguish the eggs

of different females, either foreign or own eggs, in alternated order between experiments, were dyed slightly blue by placing them in a solution of Alcian blue ($2 \text{ g } 100 \text{ ml}^{-1}$) for 30 min (Kraak *et al.* 1997). In several previous studies, this method did not influence egg survival or cannibalistic behaviour (Kraak *et al.* 1997, 1999a,b; Frommen *et al.* 2007). Afterwards, eggs were placed back into a male's nest, which was carefully repaired.

After manipulation, first the nest and then the male were returned to the tank and the 7-day-long experiment started. Ten males did not reaccept their nest, but rather destroyed it immediately and were therefore excluded from all analyses ($n_{\text{excluded males}}$: 0% own eggs: two, 20% own eggs: two, 40% own eggs: two, 60% own eggs: one, 80% own eggs: one and 100% own eggs: two). Eighty two males started brood-caring behaviour (fanning) after being reintroduced, suggesting that they reaccepted their nests after manipulation ($n = 13, 11, 12, 14, 12$ and 20 , respectively). One day before hatching (that is, 7 days after manipulation), nests were taken out of the tank and all remaining eggs were counted using a binocular microscope. The cannibalism rate was determined by counting the number of own and foreign eggs that had survived and comparing them with the initial number of eggs. Of 82 males, seven ($n = 1, 0, 2, 2, 1$ and 1 , respectively) destroyed their nest completely between the third and the fifth day of the experiment. Therefore, no further behavioural observations (see below) were made for these males. However, these males practised total cannibalism before destroying their nest and were therefore not excluded from analyses.

Three-spined sticklebacks often reduce foraging and food intake in the wild during brood care (Wootton 1976). Therefore, males were kept under a mild food limitation during the 7-day-lasting experiment. Starting at the second day of the experiment, males were fed with 30 frozen larvae of *Chironomus* spp. every second evening (Bakker & Mundwiler 1994; Frommen *et al.* 2007; Mehlis *et al.* 2009).

(d) Behavioural observations

Brood-caring and aggressive behaviour of all males was recorded daily between the second and sixth day of the experiment. To avoid time of day effects, the day was divided into five time sections, each lasting 2 h (time-section 1: 8.00–10.00 h, time-section 2: 10.00–12.00 h, time-section 3: 12.00–14.00 h, time-section 4: 14.00–16.00 h and time-section 5: 16.00–18.00 h). Brood-caring and aggressive behaviour were recorded only once for every male in each time section. For example, the recording of the brood-caring behaviour started on the second day in time-section 1, on the third day it took place in time-section 2, on the fourth day in time-section 3, on the fifth day in time-section 4 and on the sixth day in time-section 5. The recordings of the aggressive behaviour followed the same pattern. However, for one male, the recording of brood-caring and aggressive behaviour never took place on the same day in the same time section.

Brood-caring behaviour was recorded daily for 15 min. A webcam (Creative WebCam Live!) was placed 43 cm above the bottom of the tank. It was connected to a laptop, which allowed observation of the males without disturbing them. Although the installation of the webcam was carried out carefully to minimize stress, males sometimes showed fearful behaviour like hiding or freezing. Consequently, all males were recorded as soon as they started swimming.

Videos were analysed afterwards and the observer was naive with respect to the treatment the male belonged to and with respect to clutch age. To quantify the time the male spent near the nest, the duration in which at least its head was inside a circle around the petri dish ($\varnothing 11 \text{ cm}$) indicated on the laptop screen was measured. Additionally, the time males spent fanning was noted.

Aggressive behaviour was also recorded daily. A clear plastic box ($10 \text{ cm} \times 7 \text{ cm} \times 17 \text{ cm}$) filled with tap water was placed in the front of the tank. After 2 min, a neutral fish (a female, which had already spawned in this breeding season but now showing no sign of sexual behaviour or maturity) was introduced into the box and the behaviour of the male was recorded. Body measurements of the neutral fish (SL_{nf} , M_{nf} , BC_{nf}) were determined after the observations. Between treatments neutral fish did neither differ in mean SL_{nf} or BC_{nf} (Kruskal–Wallis test, d.f. = 5, both $\chi^2 \leq 8.960$, both $p \geq 0.111$) nor in SL_{nf} and BC_{nf} in relation to SL_{m} and BC_{m2} of the test fish (Kruskal–Wallis test, d.f. = 5, both $\chi^2 \leq 4.163$, both $p \geq 0.526$). Neutral fish were significantly larger and in poorer physical condition than test males (one-sample *t*-test, d.f. = 74; $t_{\text{SL}} = -6.187$ and $t_{\text{BC}} = 9.660$, both $p < 0.001$). Again, videos were analysed afterwards and the observer was naive with respect to the treatment the male belonged to and with respect to clutch age. For analysis, the time until the male first entered a rectangle ($10 \text{ cm} \times 7 \text{ cm}$) marked on the laptop screen in front of the neutral fish was measured (latency period). Afterwards, the time males spent within this rectangle was noted for 5 min. Additionally, the time males showed biting and bumping behaviour towards the neutral fish was recorded (Bakker 1986).

(e) Data analyses

Parametric statistics were used, as data did not significantly deviate from normal distributions according to Kolmogorov–Smirnov tests with Lilliefors correction. Some data were transformed (fanning rate, latency period, biting and bumping rate: square root; time near box: to the power of 1.535) to reach normal distribution. All given *p*-values were based on two-tailed tests.

To analyse the occurrence of total filial cannibalism, a 'generalized linear mixed effect model (GLMM)' with binomial error distribution was conducted in R 2.9.1 using the 'lmer' command of the 'lme4' library (Bates & Maechler 2009). The occurrence of total filial cannibalism (defined as yes or no) was used as a dependent variable. Explanatory variables were paternity (percentage of own eggs), breeding season (first day of the 7-day experiment during the course of the breeding season), egg number (initial egg number, directly after manipulation) and BC_{m2} . Additionally, an interaction between paternity \times breeding season was included. Female's eggs were given to one (in the treatment 0 and 100% own eggs), respectively, two (in the treatment 20, 40, 60 and 80% own eggs) males. In order to control for the origin of the eggs, females' identity was included as a random factor.

Both, time near nest and fanning rate (Pearson correlation, $n = 25$, $r_p = 0.654$, $p < 0.001$) and time near box and bumping and biting rate (Pearson correlation, $n = 25$, $r_p = 0.924$, $p < 0.001$) were strongly correlated. Therefore, a principal component analysis (PCA) was performed in order to obtain a one-dimensional variable of brood care (combined score of time near nest and fanning rate) and

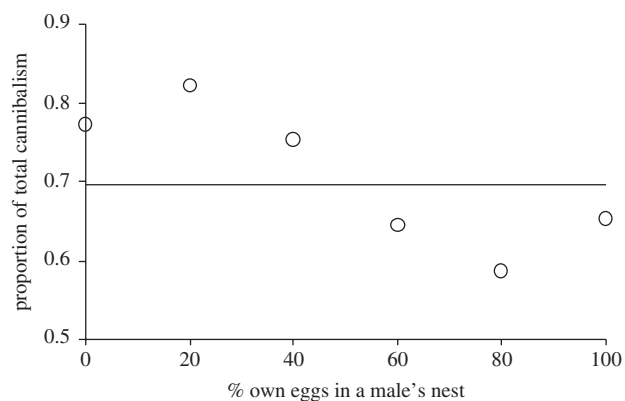


Figure 1. Observed proportion of total filial cannibalism in relation to the proportion of a male's own eggs in the nest. The line gives the overall proportion of total cannibalism. The occurrence of total filial cannibalism differed between the treatments, with males having a lower percentage of own eggs having a higher chance of total cannibalism.

aggression (combined score of time near box and bumping and biting rate). The PCA yielded a single significant factor for brood care (eigenvalue: 1.28; proportion of variance: 81.5%) and a single significant factor for aggression (eigenvalue: 1.37; proportion of variance: 93.6%). For the behavioural observations, 'linear mixed effect models (LME)' were conducted using the 'lme' command of the 'nlme' library (Pinheiro *et al.* 2009) of the R 2.9.1 statistical package. In total, three LME were conducted, in which brood care, aggression as well as the time males took to show a reaction towards the neutral fish (latency period) were used as dependent variables. In the case of brood care, explanatory variables were paternity, breeding season, egg number, SL_m , BC_{m2} , clutch age (day during the course of the 7-day experiment) and surviving eggs (percentage of total egg survival after the 7-day experiment). Furthermore, an interaction between paternity \times breeding season was included. In the case of aggression and latency period, SL_{nf} , BC_{nf} as well as an interaction between $SL_m \times SL_{nf}$ were additionally included. Owing to the fact that each behavioural trait was measured five times for every male, males' identity was included as a random factor in the analyses.

In all models, explanatory variables were stepwise removed in the order of statistical relevance. Tests of significance were based on likelihood-ratio tests that follow a χ^2 distribution. Explanatory variables that tended to be significant ($p < 0.1$) were left in the model.

3. RESULTS

(a) *Filial cannibalism*

Filial cannibalism occurred in all treatments. Total cannibalism (defined as less than 5% of eggs surviving) occurred in 57 out of 82 clutches; partial filial cannibalism occurred in 25 out of 82 clutches. The average proportion of total filial cannibalism was 69.51 per cent (figure 1).

(i) *Total filial cannibalism*

The occurrence of total filial cannibalism was significantly influenced by the percentage of own eggs in a male's nest

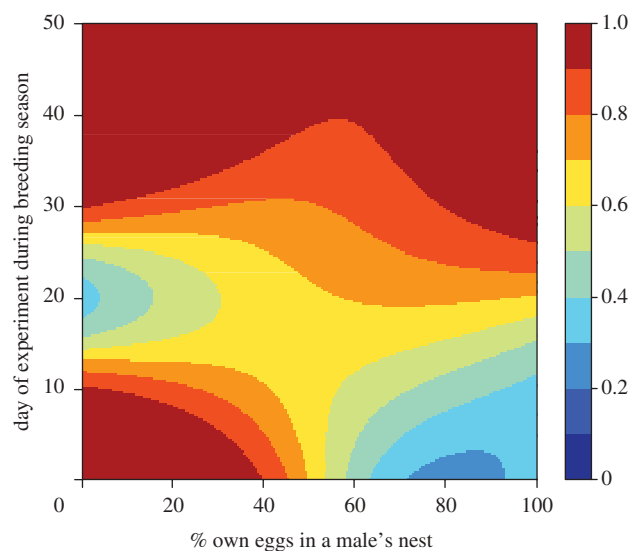


Figure 2. Estimated probability of total filial cannibalism in relation to paternity (percentage of own eggs) and day during the course of the breeding season. Different probability values are coded by different colours, clarified by the panel on the right (blue equates to low cannibalism rate; red equates to high cannibalism rate). The probabilities were estimated by the 'GLMM' presented in table 1, which also included non-significant quadratic terms of both breeding season and paternity and all possible interactions.

Table 1. Results of the 'GLMM' for total filial cannibalism in male three-spined sticklebacks ($n = 82$). See the text for details of the statistical analysis.

explanatory variables	Δ d.f.	χ^2	p
egg number	1	1.16	0.282
paternity \times breeding season	1	4.63	0.031
paternity ^a	1	5.93	0.015
breeding season ^b	1	0.35	0.555
BC_{m2}	1	6.71	0.010

^aThis test refers to the estimated effect of paternity at the beginning of the breeding season (first day of the experiment). Later on in the breeding season (last day of the experiment), this effect was weaker and statistically non-significant (Δ d.f. = 1, $\chi^2 = 2.67$, $p = 0.107$).

^bThis test refers to the estimated effect of breeding season for males with low paternity (0% own eggs). For males with many own eggs (100% own eggs), the effect was stronger and statistically significant (Δ d.f. = 1, $\chi^2 = 11.85$, $p < 0.001$).

(figure 1 and table 1). Furthermore, there was a significant interaction between the percentage of own eggs and the breeding season (table 1), showing that males adjusted their rate of total cannibalism early in the breeding season, but not towards its end (figure 2). Moreover, this interaction reveals that the risk of filial cannibalism changes with the ongoing breeding season, but only for males with many own eggs in their nest and not for males with few own eggs, where cannibalism was high throughout the experiments (figure 2). Finally, the occurrence of total filial cannibalism was influenced by BC_{m2} (table 1), males in better physical condition were less likely to cannibalize the whole clutch. Initial egg number did not significantly influence the occurrence of total filial cannibalism (table 1).

Table 2. Results of the 'LME' for behavioural observations ($n = 25$). See the text for details of the statistical analysis.

dependent variable	explanatory variables	Δ d.f.	χ^2	p
brood care (time near nest and fanning rate)	surviving eggs	1	<0.01	0.921
	paternity \times breeding season	1	0.10	0.748
	breeding season	1	0.06	0.807
	paternity	1	0.13	0.723
	egg number	1	0.24	0.627
	SL _m	1	2.14	0.144
	BC _{m2}	1	3.28	0.070
	clutch age	1	29.67	<0.001
aggression (time near box and bumping and biting rate)	SL _m \times SL _{nf}	1	0.36	0.547
	SL _{nf}	1	0.15	0.695
	paternity \times breeding season	1	1.01	0.314
	paternity	1	0.01	0.913
	egg number	1	0.87	0.350
	BC _{m2}	1	0.85	0.357
	surviving eggs	1	2.12	0.145
	breeding season	1	1.55	0.213
	clutch age	1	2.37	0.124
	BC _{nf}	1	1.92	0.166
	SL _m	1	3.34	0.068
latency	BC _{nf}	1	<0.01	0.937
	paternity \times breeding season	1	0.01	0.914
	BC _{m2}	1	0.26	0.614
	breeding season	1	0.67	0.412
	clutch age	1	1.02	0.312
	SL _m \times SL _{nf}	1	1.18	0.279
	SL _{nf}	1	0.98	0.323
	paternity	1	1.78	0.182
	surviving eggs	1	1.79	0.181
	SL _m	1	3.81	0.051
	egg number	1	4.50	0.034

(ii) Partial filial cannibalism

Partial filial cannibalism occurred in 25 cases. Treatments with 0 and 100 per cent own eggs were excluded in further analysis because in these treatments only foreign or own eggs, respectively, could be cannibalized by the males. No significant differences in percentage of partial cannibalism of own and foreign eggs could be found (Wilcoxon test, $n = 15$, $z = -0.852$, $p = 0.394$). However, there was a significant correlation between the percentage of own eggs in a male's nest and the relative probability of own egg cannibalism (Spearman correlation, $n = 15$, $r_s = -0.717$, $p = 0.003$), indicating that the relatively more own eggs a male had initially in his nest, the relatively more foreign eggs were cannibalized and *vice versa*.

(b) Behavioural observations

As it was impossible to determine the exact point of total cannibalism, only males that showed partial filial cannibalism ($n = 25$) were included in the analyses of behavioural observations. The results of the 'LME' showed that the percentage of own eggs in a male's nest did not influence brood-caring or aggressive behaviour (table 2). All results are shown in table 2, with only the statistically relevant issues are described below in detail.

Brood care was significantly influenced by clutch age (table 2). With increasing clutch age, males spent more time near their nest and they spent more time fanning. Additionally, brood care tended to be inversely related

to BC_{m2} (table 2). Males in a poorer body condition at the beginning of the experiment showed more intense brood-caring behaviour.

Aggression tended to be related to SL_m (table 2), with larger males showing more intense aggressive behaviour towards the neutral fish. Additionally, larger males attacked the neutral fish earlier than smaller males. Furthermore, the latency period was significantly related to egg number (table 2), with higher initial egg numbers leading to a slower reaction towards the neutral fish.

(c) Body measurements

Males from the six different treatments did not differ significantly in BC_{m1}, BC_{m2} or SL_m (Kruskal–Wallis test, d.f. = 5, all $\chi^2 \leq 5.876$, all $p \geq 10.318$). However, males tested late in the breeding season had a lower BC_{m1} (Pearson correlation, $n = 82$, $r_p = -0.374$, $p < 0.001$), while neither SL_m (Spearman correlation, $n = 82$, $r_s = 0.004$, $p = 0.970$) nor BC_{m2} (Spearman correlation, $n = 82$, $r_s = -0.128$, $p = 0.254$) were significantly correlated with the ongoing breeding season. In addition, females had a lower BC_f (Pearson correlation, $n = 82$, $r_p = -0.383$, $p < 0.001$) late in the breeding season. SL_f was not significantly correlated with the advancement of the breeding season (Spearman correlation, $n = 82$, $r_s = -0.003$, $p = 0.977$). Egg number was not significantly correlated with the course of the breeding season (Spearman correlation, $n = 82$, $r_s = -0.051$,

$p = 0.652$), but egg mass decreased with the ongoing breeding season (Pearson correlation, $n = 82$, $r_p = -0.443$, $p < 0.001$).

4. DISCUSSION

Sneaked fertilizations and extra pair copulations occur in many animal species (e.g. Taborsky 1994; Griffith *et al.* 2002). Therefore, it would be a huge advantage for brood-caring individuals to recognize own offspring in order to avoid an unprofitable investment in unrelated eggs or embryos. The present study shows that three-spined stickleback males are able to estimate the amount of foreign eggs in their nest and adjust their filial cannibalism rate accordingly. Indirect cues to assess paternity, such as the presence of rival males (Manica 2004; Rios-Cardenas & Webster 2005; Gray *et al.* 2007) or clutch position (Sargent 1989), were excluded. The only direct mechanism to estimate relatedness was through egg cues themselves (see also Frommen *et al.* 2007). In sticklebacks, visual cues might play a minor role in egg recognition. Nests are tunnel shaped and built on the substrate, thus, light intensity is rather limited. Furthermore, eggs are usually densely packed, making visual distinction of single eggs rather difficult. A previous study showed that kin recognition in sticklebacks is triggered by olfactory cues (Mehliss *et al.* 2008). Thus, it is plausible that also egg recognition is based on olfaction. The odour profile of an egg might change during its development; the odour of an unfertilized egg is completely based on maternal cues. With increasing development of the embryo, paternal genes might influence the odour, too, as they are now also expressed in the developing embryo. Therefore, it should be easier for a male to differentiate between own and foreign eggs when the embryos are older. This might explain the fact that seven males destroyed their nest completely between the third and the fifth day of the experiment, although they accepted the nest initially. By contrast, a study on bluegill sunfish (*Lepomis macrochirus*) showed that males were only able to recognize own offspring after hatching (Neff 2003). Here, the author argues that the males might use urinary cues of the hatched fry to distinguish them, which were absent before hatching.

Filial cannibalism occurred in all clutches, with a high frequency of total cannibalism. Initial egg number did not influence this cannibalistic behaviour. One has to realize, however, that males guarded only one clutch in the experiment, which is on the low end of the natural range. In the wild, males often collect several clutches from different females, thus a nest might contain more than thousand eggs (Kynard 1978; Kraak *et al.* 1999a; Bakker *et al.* 2006b). In teleost fishes, small broods face a higher risk of being cannibalized (Ochi 1985; Schwank 1986; Forsgren *et al.* 1996; Okuda & Yanagisawa 1996; Lindström & Sargent 1997; Lissåker & Svensson 2008). This was also shown for three-spined sticklebacks (Van den Assem 1967). Thus, low egg numbers could be an explanation for the high occurrence of total filial cannibalism in the present study.

Besides the overall high amount of males consuming their complete clutch, two explanatory variables described the occurrence of total filial cannibalism: paternity and body condition of the male (BC_{m2}).

Additionally, a significant interaction between paternity and time in the breeding season shows that paternity played a more prominent role early in the breeding season, while the occurrence of total cannibalism over all treatments was more general in later summer. This occurrence of total cannibalism related to paternity can be interpreted in two different ways. First, after a male had cannibalized all foreign eggs, only own eggs remained. In nests with a high percentage of foreign eggs, the number of remaining eggs was small. In these cases, the costs of brood caring might have outweighed the benefits (Clutton-Brock 1991), leading the male to also consume the remaining own eggs and to start a new breeding cycle. Owing to the fact that males in this experiment cared for only one clutch, this mechanism might explain the current results. An alternative explanation is that the males were not able to differentiate between single own and foreign eggs, because single eggs might have not produced enough olfactory cues. However, males might have been able to recognize that their clutches contained foreign eggs through a combination of cues of all eggs. If this amount was too high, they cannibalized the complete clutch.

At first glance, fathers of the nests, which were only partially cannibalized, did not appear to discriminate between single own and foreign eggs. However, in this sample, males that had a higher percentage of own eggs in their nest cannibalized relatively more foreign eggs. This might indicate that males indeed were able to distinguish between single own and foreign eggs. On the other hand, males that had relatively more foreign eggs in their nest cannibalized more own eggs. Therefore, it is also possible that males are not able to distinguish between single own and foreign eggs but rather cannibalized all eggs that smelled different compared with the major part of the eggs in their nest. However, if this was true, one would have expected a low amount of total cannibalism in the experiments where the males' nests contained no own eggs, which was not the case. Using our dataset, we are not able to definitely elucidate whether sticklebacks are able to discriminate between single eggs, leaving this issue open for further experiments.

The probability of total filial cannibalism increased at the end of the breeding season. Such an increase has been described for several fish species (e.g. Petersen & Hess 1991; Marconato *et al.* 1993), including the three-spined stickleback (Belles-Isles & FitzGerald 1991). In the present study, it might be explained in different ways. First, when males were placed into the tank, they had a significantly lower body condition (BC_{m1}) later in the breeding season, and males with a lower body condition had a higher probability to cannibalize the whole clutch. As the males were kept under mild food limitation during the experiment, it is possible that they used the clutches as a source of energy. Indeed, a recent study found that male sticklebacks maintain their body condition by cannibalizing eggs (Mehliss *et al.* 2009). Consequently, cannibalism of the total clutch might be an investment in future reproduction. Second, body condition of the females (BC_f), as well as egg mass, decreased in the course of the breeding season. Egg mass is known to be an indicator of egg quality in sticklebacks (Fletcher & Wootton 1995). Bigger eggs result in bigger larvae, which might have a higher chance of survival

(Kraak & Bakker 1998). In mate choice experiments, males preferred females that laid bigger eggs (Kraak & Bakker 1998). In the present study, males might have cannibalized the low-quality clutches, hoping to receive more and better eggs in future matings.

Males that are uncertain about paternity should change their brood-caring behaviour accordingly (Xia 1992). In this way, they could minimize an unprofitable investment in foreign eggs or embryos. In contrast to this assumption, three-spined stickleback males in the present study did not adjust their brood-caring or aggressive behaviour according to the amount of foreign eggs in their nest. An explanation might be that males made 'all-or-nothing' decisions. When they decided that the reproductive value of the clutch was too low to care for it, they totally cannibalized it and gained nutritional benefits (Mehlis *et al.* 2009). However, when they decided to care for their eggs, they showed normal brood-caring behaviour.

Males invested more energy at the end of the experiment, i.e. they spent more time fanning with increasing age of the clutch. Providing oxygen by fanning is essential for the survival and the development of the embryos (Van Iersel 1953; Bakker *et al.* 2006a), with older clutches needing more oxygen than younger ones (Reebs *et al.* 1984; Collins & Nelson 1993; Kamler *et al.* 1998). Brood care was also influenced by the males' body condition (BC_{m2}). Males showing a lower body condition at the start of the experiment, spent more time near their nest and fanned more. At first look this appears counterintuitive; an explanation could be that the chance to start a second breeding cycle is low for a male in bad physical condition. Thus, once a male in bad physical condition had made an all-or-nothing decision and decided not to cannibalize the whole clutch, it invests all its energy into the current brood.

In general, larger males were more aggressive than smaller ones. They spent more time close to the box, showed a higher biting and bumping rate and they reacted faster towards the neutral fish (although these results only approached significance, see table 2). This finding contrasts to some former studies on sticklebacks from other populations, which found no relationship between body size and aggressive behaviour (Van den Assem 1967; Sargent & Gebler 1980; Bakker 1986). However, studies on stickleback species pairs revealed that larger benthic morphs are more aggressive than smaller limnetic ones (Larson 1976). Finally, the more eggs a male received, the longer it took until it showed an aggressive reaction towards the neutral fish. Probably, these males were more cautious when the neutral fish appeared, because for these males the loss might be greater.

In summary, this study shows that male three-spined sticklebacks are able to assess the amount of foreign eggs in their nests by egg cues alone, and that this ability allows them to adjust precisely their cannibalistic behaviour. Brood-caring and aggressive behaviour was not affected by relatedness, maybe because of all-or-nothing decisions. Furthermore, cannibalism was related to time effects, which might be best explained by a decrease in egg quality and male body condition at the end of the breeding season.

This study adhered to the Association for the Study of Animal Behaviour/Animal Behaviour Society guidelines for

the Use of Animals in Research. All experiments complied with the current laws of Germany.

We are grateful to Timo Thünken and the 'Bakker' research group for discussion. Ingolf Rick, Andrea Manica and an anonymous referee gave thoughtful comments on an earlier version of the manuscript. We thank Jan Hottentot for catching the sticklebacks.

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