Female Choice and Paternal Care in the Fifteen-Spined Stickleback, *Spinachia spinachia*

BY

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Abstract


In the fifteen-spined stickleback, Spinachia spinachia, males provide females with direct benefits by fanning, cleaning and guarding the offspring. Males announce their parental skills through intense body shakes during courtship. Females preferred to mate with more intensely shaking males. As a result, females got better fathers for their offspring, as such males achieved a higher hatching success. Not only did male behavioural cues attract females, but males also used their nests as extrabodily ornaments. The nest is held together with shiny secretional threads consisting of a glycoprotein. Females chose to spawn in nests with more secretional threads. A likely reason for this is that the threads are metabolically costly for the male to produce and the amount of secretion indicates a male’s nutritional status, which is of great importance as parental duties are energetically costly. Moreover, females preferred nests built high up in the vegetation, as such nests were safer from egg predators. Competition with other males for females favoured males building higher nests than did their neighbours, probably because females preferred high nests. Male-male interactions, such as sneaking and egg stealing, caused decreased paternity among males in nature as assessed by a microsatellite analysis. Males adjusted their paternal effort according to their previous investment in the brood, but not according to paternity. Thus, female choice is based on multiple cues and results in better paternal care. Males invest in courtship, male-male competition, nest construction and paternal care, the outcome determining their success in mate attraction.

Key words: Fifteen-spined stickleback, Spinachia spinachia, Female choice, Paternal care, Nest building, Ornament, Fanning.

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LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-V.


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INTRODUCTION

Sexual selection

Why is sexual reproduction maintained in many organisms, when the alternative, asexual reproduction, allows passing your entire genome to your offspring? Probably the big advantage of recombination, in combination with genetic repair mechanisms, can account for much of the prevalence of sexual reproduction (for a review see Halliday 1994). A key question still is why males in many sexually reproducing species develop colourful structures that will make them more vulnerable to predators. While animals may adapt to survive better through natural selection, sexual selection can work in the opposite direction by favouring conspicuous traits, such as bright coloration.

During the last decades, female choice in particular has been in focus as one of the mechanisms behind the development of male secondary sexual traits (for a review see Halliday 1983, Andersson 1994, Andersson and Iwasa 1996). Today, sexual selection is viewed as a strong evolutionary force with widespread consequences for different morphological traits, behaviours, mating systems and life histories.

There are two forms of sexual selection: intrasexual selection and intersexual selection. Intrasexual selection is defined as competition within one sex for mating opportunities with the opposite sex, while intersexual selection is when one sex shows a preference while mating with members of the opposite sex. In general, intrasexual selection occurs as competition between males, and intersexual selection is normally driven by female choosiness for a mating partner.

Thus, males are most often the sex on which the impact of sexual selection is strongest (see for example Berglund et al. 1996). This is explained by the so-called Bateman gradient, where male reproductive success is only limited by the times he can mate, while female fecundity does not increase if she mates with a large number of males (Bateman 1948, see also Jones et al. 2000 for sex-role reversed species). Basically, this is due to the anisogamous reproduction of most organisms, i.e., some individuals (males) have many small and cheap gametes (sperm), while others (females) have few large ones (eggs). However, sex roles (i.e., which sex competes) may be dynamic due to differences in reproductive investment between the sexes in other things than gametes, such as parental care. Such differences may cause the potential reproductive rate of males to creep below that of females (see for example Berglund et al. 1989, Berglund and Rosenqvist 1990, Clutton-Brock and Vincent 1991, Clutton-Brock and Parker 1992, but see Okuda 1999). This will shift the operational sex ratio towards a female-bias (Kvarnemo and Ahnesjö 1996), causing females to compete for access to mates.

In species with conventional sex roles (males compete), males may offer females only genes (indirect benefits), or resources which will increase female fecundity or immediate fitness (direct benefits). Here I will briefly summarise these two mechanisms by which female choosiness may have evolved:

Indirect benefits

According to the "Fisher runaway process" females may seek genes for their offspring improving their sons' attractiveness. The daughters will inherit the taste for this arbitrary trait, resulting in a coevolution between taste and trait. This makes the process self-reinforced until natural selection counteracts it (Fisher 1930, elaborated by: O'Donald 1980, Lande 1981, Kirkpatrick 1982, Pomiankowski et. al. 1991). While the runaway process produces arbitrary traits which will only make the sons more attractive, “indicator models” assume that males
with conspicuous traits offer females genes which will increase the viability of both sons and daughters (first suggested by Fisher 1915). Zahavi (1975, 1977) developed the early ideas from Fisher (1915) and Williams (1966) in his handicap model, where he proposed that a trait must carry costs, like for example lower survival for the bearer, so that only high quality males can afford to develop the trait. Thereby the trait would work as an honest signal of quality for the females to choose from. An important development of the original handicap theory is the "revealing handicap" theory, in which the ornament reveals the male’s resistance against parasites (Hamilton and Zuk 1982, for a review Sorci et al. 1997). Here, an arms race between hosts and the parasites, in which the hosts must constantly evolve new resistance towards new forms of parasites, explains how the genetic variation for male quality (health) can be maintained.

Direct benefits

Direct selection on mating preferences arises whenever the preferences affect the fecundity or survival of the female (reviewed in Halliday 1983, Andersson 1994, Kirkpatrick and Ryan 1991). This may happen when males provide resources to females or offspring (Kirkpatrick 1985, Knapp and Kovach 1991, Forsgren 1997), when there is a cost of searching for mates (Parker 1983, Alatalo et al. 1987, Slagsvold et al. 1988, Reynolds and Côte 1995), where there is selection against inbreeding (Parker 1992), where males differ in sperm fertility (Halliday 1976, Halliday and Houston 1978, Eberhard 1985, Robertson 1990), where males give females food during courtship (Thornhill 1976, 1980), or where diseases and ectoparasites are transmitted (Borgia and Collis 1990).

This thesis (paper I, II and III) has focused on direct benefits for the female, where the fifteen-spined stickleback male provides care for the offspring by nest building, guarding and fanning the eggs. In this fish the male has no conspicuous breeding coloration but has evolved a conspicuous courtship behaviour (I) and a nest that attracts females (II, III). A nongenetic model suited for fish with paternal care predicted that males should develop secondary sexual characters such as courtship, intense coloration and/or nest quality to inform the female about the quality of his paternal care (Perrin 1995). For animals in general, a variation in paternal quality may explain the evolution of ornaments in males through genetic models, where the male trait is heritable but not his paternal quality (Heywood 1989, Hoelzer 1989, Price et al. 1993). Motro (1982) and Wolf et al. (1997), on the other hand, modelled a situation where an epigamic trait evolves because it reveals a heritable component of male parental quality. Thus, there are different opinions on whether offspring harbour genes for paternal quality, or if paternal quality more depends on environmental factors.

Parental care

Parental care may be a sexually selected trait if used to attract mates. If so, males should be more reluctant to perform parental duties, and maybe even desert broods if the number of potential mates increases. Moreover, males may increase their parental effort when they have a higher degree of paternity. In fact, there are many ways in which sexual selection and parental care may interact (for a review, see Reynolds 1996, Westneat and Sargent 1996). How intrasexual selection can interfere with male fifteen-spined stickleback paternity, and how paternal care may change according to the level of paternity, are questions dealt with in paper IV and V, respectively. Moreover, not only can sexual selection influence paternal care, paternal care may also influence intersexual selection if the level of paternal care provides females with information on which to base their preferences. Accordingly, females may choose males that are better fathers (Knapp and Kovach 1991, Forsgren 1997), but
theoretically this preference may also select for male paternal skills, so the argument may be circular.

While caring for the offspring, parents increase the fitness of the offspring, but at the cost of the parent's ability to invest in other offspring, now or in the future (Trivers 1972, Clutton-Brock 1991). In teleost fish parental care take many forms: paternal care, maternal care and biparental care. In 78% of all fish families no care is provided, but among those who do provide care, paternal care prevails (Breder and Rosen 1966, Blumer 1979, Gross and Shine 1981). Reasons for this include (for reviews, see Gross and Sargent 1985 and Clutton-Brock 1991): (1) if females lay their gametes first, only they have the possibility to desert the brood and "force" the male to stay with the offspring (the "gamete order hypotheses", Trivers 1972, Dawkins and Carlisle 1976). (2) In fish with external fertilization male paternal confidence should be higher and paternal care should therefore evolve (the "Paternity confidence hypothesis", Trivers 1972, Alexander 1974). (3) Male territoriality and external fertilization should predispose for paternal care (the "Association hypothesis", Williams 1975, Ridley 1978). (4) The benefits of parental care are similar to either sex, but the costs are typically larger for females, as their reproductive success more often is dependent on body size (Williams 1975, Baylis 1981, Gross and Shine 1981, Gross and Sargent 1985). None of the above hypotheses provide a satisfactory explanation to the prevalence of paternal care in fishes, however (reviewed in Clutton-Brock 1991). (5) Paternal care may be a sexually selected trait, attractive to females. Here I suggest that a male, by using the nest or by advertising parental quality as an extrabodily ornament, announces direct benefits to the female and hence gains a higher reproductive success than males not announcing this. Thus, a nest may function as a sexually selected trait, in effect an extrabodily ornament.

Parental care patterns in teleost fish are extremely diverse, ranging from simple egg guarding to full viviparity (Breder and Rosen 1966). Parental activities in fish include behaviours like guarding, nest building, substrate cleaning and fanning of eggs (Blumer 1979, Keenleyside 1979). Fanning serves two purposes: cleaning and oxygenating the eggs. The latter may be especially important in nest building species, where the nest itself reduces water circulation.

Benefits of parental care

Egg guarding in many fish may be essential for egg survival: egg cannibalism is common in fish and is performed both by females and males (Belles-Isles and FitzGerald 1993, Mori 1995). In addition, eggs are also attractive to other species, as eggs are high quality food. Eggs that are not fanned have a slower rate of development and are deformed more frequently (Zoran and Ward 1983). Furthermore, hatching success may increase with fanning activity (see paper I). Hatching success may also be affected if the eggs are put in a nest or not. In the peacock wrasse (Symphodus tinca), where care is facultative, hatching success is more than four times greater if females place their eggs in a nest under the care of a male than outside a nest with no care provided (Warner et al. 1995).

Energetic constrains of parental care

Many studies in fish have described costs associated with parental care, but only few have done so from a life history trade-off perspective (but see Jones and Reynolds 1999). Yet, many parental activities are costly (for a review see Clutton-Brock 1991, Smith and Wootton 1995), like for example fanning, depend on food intake (Townshend and Wootton 1985). A high fanning activity can cause a reduction in body fat and nonpolar lipids (FitzGerald et al. 1989, Coleman and Fischer 1991). Mass loss as a consequence of a reduced food intake during paternal care has been found in many species of fish: parental activities often shorten

Nest building may carry a considerably cost in fish, especially so in the stickleback family (Gasterosteidae) with their elaborate nests. The fifteen-spined stickleback is the largest species in this family, and exhibits by far the largest production of secretional threads (Hentschel 1979). The threads are made up by a protein, synthesised by kidney tubuli cells that transforms into protein secreting cells during the breeding period (Hentschel 1977, 1979). Proteins are energetically expensive to synthesise, suggesting a substantial cost for the males to produce the nest building protein (paper III).

Male-male interactions may reduce paternity through sneaking and egg stealing. Alternative reproductive behaviours (sneaks) are often performed by males inferior in competition over territories or females. I deal with this in paper IV. Sneaking may be a fixed life-history strategy (for a review see Gross 1984) or a conditional tactic, where males may switch between different reproductive tactics depending on circumstances (Magnhagen 1992, Wirtz 1978, Kodric-Brown 1986). The costs of being cuckolded (loss of paternity and a counterproductive investment in another's offspring) should provide strong selection for evolving defences against sneakers. Conversely, the benefits to sneakers, fertilizations without costs for parental care, should result in a strong selection favouring this parasitic reproductive behaviour. It is therefore unclear what, if any, balance may be achieved for the frequency of reproductive parasitism (Barnard and Sibly 1981). Furthermore, in the stickleback family males steal egg clutches from each other (van den Assem 1967, Wootton 1971, Li and Owings 1978, Mori 1995).

While the advantage of sneaking is easily understood, it is more difficult to explain the evolutionary benefit of stealing eggs from other males. I deal with this question in paper IV. Eggs may attract females, as found in many studies (Ridley and Rechten 1981, Marconato and Bisazza 1986, Unger and Sargent 1988, Knapp and Sargent 1989, Sikkel 1989, Kraak and Videler 1991, Goldschmidt et al. 1993, Forsgren et al. 1996). There may be several reasons for this: (1) the female reduces the chance of her eggs being eaten by a simple dilution effect (Rohwer 1978), (2) the chances of egg survival might increase with brood size if males provide more care to larger broods (Coleman et al. 1985, Sargent 1988), (3) a male with eggs may be more attractive because he demonstrates that he is prepared or able to care for the eggs (Ridley 1978), or (4) a female may copy the choices of other females to reduce her search costs or acquire high quality mates (Ridley 1978, Losey et al. 1986, Kraak 1996). Two or more of these mechanisms may of course work in concert. Moreover, stolen eggs may serve as an extra food supply, reducing the consequences of male filial cannibalism.

The fifteen-spined stickleback

The fifteen-spined stickleback is a marine or brackish water fish, unique to Europe. Its geographical distribution encompasses the Gulf of Biscay, the British Isles, the Norwegian Sea, and the Baltic Sea, where the 4‰ salinity isocline sets its distribution limit (Johnsen 1944, Gross 1978). In this species the sexes are monomorphic and no extravagant character or sexual coloration are obvious in any of the sexes. Females may grow slightly larger than males, usually around 135 mm and 119 mm, respectively, in the population I have studied.
However, specimens reaching 188 - 191 mm have been found in Scotland and Denmark (Johansen 1944, Kaiser and Croy 1991). The life span of the fifteen-spined stickleback is presumably one year (Jones and Hynes 1950), but since this conclusion is based on specimens only reaching 117 mm, Kaiser and Croy (1991) have suggested that the fifteen-spined stickleback may live more than one year.

The fifteen-spined stickleback is regarded as a primitive member of the Gasterosteidae (Wootton 1976, McLennan et al. 1988, McLennan 1993). During the reproductive period in the spring, the fish inhabits shallow eelgrass (Zostera marina) meadows and Fucus belts. The male builds a nest in macro algae, mainly Ascophyllum nodosum, Fucus vesiculosus and Halidrys siliquosa, using epiphytic algae as nest material (personal observation). The male completes the nest by wiring secretional threads (Hentschel 1977, 1979, De Ruiter and Mein 1982, see Fig. 1 in paper III) around the nest material to tie it together into a ball- or oblong shape. The width of the nest is between 2 and 8 cm and the length between 3 and 15 cm (personal observation). After one or more females have spawned in the nest, the male alone cares for the eggs until they hatch (Prince 1885, Sevenster 1951, Wootton 1976). Females may lay between 200 to 600 eggs per spawning. After the eggs are laid, the male is occupied primarily with fanning the eggs with his pectoral fins to oxygenate the embryos (Fig. 1), although he also guards, cleans and repairs the nest for about 20 days until the eggs hatch (Sevenster 1951, Wootton 1976). In contrast to the more intensively investigated three-spined stickleback (Bell and Foster 1994), few studies have been carried out on the reproduction of the fifteen-spined stickleback (but see Prince 1885, Sevenster 1951, Potts 1984, Potts et al. 1988, Östlund 1995).

Figure 1. A male fifteen-spined stickleback is fanning his nest with the pelvic fins, thereby creating a water current over the eggs (illustration from Hanström and Johnels 1965).
GENERAL METHODS

The investigation was performed from early May to July in 1993-1999 at Klubban Biological Station, Fiskebäckskil (58°15'N, 11°28'E), on the West Coast of Sweden. Fifteen-spined sticklebacks were collected locally from a depth of 1 to 6 m in Fucus and Zostera vegetation. Fish were collected mainly by snorkeling, hand trawling and boat-trawling. The males were placed singly in 50 l aquaria while females were placed together (approximately 10) in 250 l aquaria. All aquaria were continuously supplied with water pumped from sea, temperature (11 - 16 °C) and light followed natural cycles. All studies, except IV and partly II, which were based on field studies, were conducted in in- or outdoor 50-170 l aquaria or outdoor tanks (1,300 l).

Fish were fed twice a day with minced mussel meat, food pellets, frozen or live mysid shrimps, and live Artemia (in paper III, however, feeding conditions were different). I gave males a mixture of epiphytic and filamentous algae for nest building, along with a plastic plant on which to attach their nest.

In all mate choice experiments males were separated with an opaque wall to avoid male-male interactions. Female choice was estimated by separating the female from the males by a transparent screen (I and partly in II) and assessing how many times the female swam towards each male. The most frequently inspected male was considered chosen. In some experiments males were leashed in order to avoid male interactions. This was done by gently placing a 3 mm wide cling wrap around the body, close to the pelvic fins of the fishes. I then knitted a thin fishing line (Ø = 0.10 mm) to the cling wrap and attached the other end of the line to a little piece of cork which in turn was connected with the same type of line to the top of an adjacent aquarium wall. By leashing the males I was able to exclude male-male interactions and the females were able to inspect the males and their nests closely. Leashing stickleback males does not negatively affect courtship or mating (Östlund-Nilsson and Nilsson 2000) and I assumed that females had chosen the males in whose nests they spawned.

For the genetic analyses of paternity following nest-raiding (IV), the males, together with their nests, were collected by snorkeling. Males and eggs were returned live to the laboratory and samples were taken to the University of Georgia for genetic assay.

In the statistical analyses values given are means ± S.D. Data were checked for normality and statistical tests were chosen accordingly. Percentages/proportions were arcsine√x-transformed.

RESULTS AND DISCUSSION

When females benefit directly (I)

Female mate choice in paternally caring fish species is likely to be for direct benefits to the offspring. Often, paternal quality is related to male body size, as bigger males more efficiently may defend their offspring. Female choice for larger males has been demonstrated in many fish species with paternal or biparental care (Downhower and Brown 1980, Noonan 1983, Keenleyside et al. 1985, Bisazza and Marconato 1988, Côte and Hunte 1989, Rogers and Barlow 1991). However, females may also choose males on paternal competence (i.e. hatching success) over male-male competitive ability (Forsgren 1997). Moreover, a male’s courtship may be an honest indicator of his parental quality (Knapp and Kovach 1991).

In this study on the fifteen-spined stickleback, I first wanted to find out whether males differed in their competence as fathers and if females preferred better fathers. In order to estimate paternal quality I examined fanning behaviour and egg hatching success. I studied the fanning behaviour of the male because it is likely to be important for egg development.
rate (van Iersel 1953, Zoran and Ward 1983) and hatching success (Sargent and Gebler 1980, Zoran and Ward 1983). I also investigated if the males’ body size and courtship revealed any paternal skills to the females to base their choice upon.

Do females choose males on paternal skills?

One female at a time was introduced into a large outdoor tank (1,300 l) to choose between two males differing in size. The males were enclosed with their nest, each in a big transparent plastic bag (40 l). An opaque screen was placed between the mates. The female's position (on either side of the screen) was recorded fifteen times, with at least 30 min between samplings. The male that received more female visits was scored as preferred. After the mate choice experiment was finished each male was given another female to mate with. After mating, the males were kept individually and followed until eggs hatched (i.e. about 3 weeks). I observed the males for 10 min every second day to measure duration of each fanning bout (fanning bout duration) and duration between fanning bouts (pausing time) with a stopwatch. A fanning bout was considered to start when the male’s pelvic fins began to beat, and to end when the male stopped fanning and began swimming around in the aquaria.

Fanning bout frequency (i.e. number of fanning bouts + pauses per hour) was positively correlated to hatching success (# of hatched fry/# of eggs laid by a female; Fig. 2). The mean fanning bout duration correlated negatively with hatching success ($r^2=0.40, N=17, P=0.007$). Thus, males fanning with shorter but more frequent bouts enjoyed a higher hatching success. Females preferred males, that were subsequently found to provide shorter fanning bouts during the paternal phase ($N=12$ pairs, preferred: $20.9±3.1$ s, non-preferred: $24.2±4.0$ s, paired-t test, $t=2.51, P=0.015$).

I found no relationship between male length and mean fanning bout duration ($r^2=0.074, N=23, P=0.20$) or hatching success ($r^2=0.042, N=15, P=0.44$), nor did females prefer longer over smaller males ($N=13$ male pairs, preferred males: $116.0±7.8$ mm, non-preferred males: $118.9±8.7$ mm, paired test, $t=0.52, P>0.05$). Thus, male size seemed irrelevant for parental care and female choice in this population. A higher fanning bout frequency may increase hatching success by more quickly counteracting sudden oxygen drops in the nest. Obviously males who fanned often with short bouts hit the optimal trade-off between these activities.

![Figure 2. The linear regression between fanning bout frequency (bouts/h) when males provide parental care and their subsequent hatching success ($r^2=0.34, N=17, P=0.01$).](image)
How do males signal parental skills?

In a second experiment I wanted to find out if and how the males signal their competence as fathers. This experiment was performed on different males than those in the above experiment. After the males had finished nest building they were moved in pairs, along with their nests, to an experimental aquarium (110 l). The males in a pair were matched to differ only in mean displacement fanning bout duration (i.e. males fanning the empty nest; mean ± S.D. for short bout duration: 11.2±8.0 s and for long: 22.8±3.4 s; t=4.76, N=13, P=0.0005). An opaque wall separated the two males, and the female was separated from the males by a transparent wall. I recorded male courtship behaviours, i.e. the male’s displacement fanning bout duration and frequency of body shakings, during female inspections of the male. When a male shakes his body he bends in an S-posture, with the head up, and shakes very fast for about a second. I assessed female choice by recording how many times the female swam towards each male, and the most frequently inspected male during the 1-h observation period was considered chosen. After the experiment was finished, both males were moved back to their individual 50-litre aquaria, each provided with a female for spawning. Thereafter the males were kept individually until the eggs hatched. During this time mean fanning bout duration and hatching success were measured as previously.

In a second experiment I wanted to find out if and how the males signal their competence as fathers.

I found no correlation between displacement fanning bout duration during courtship and hatching success (r²=0.21, N=12, P=0.12). However, I found that males with a high frequency of body shakes during courtship enjoyed a significantly higher hatching success (Fig. 3). Furthermore, there was also a significant negative correlation between male shaking frequency and the mean fanning bout duration (r²=0.26, N=23, P=0.01). Females did not show a significant preference for males with a lower displacement fanning bout duration during courtship (paired t=2.0, N=12, P=0.07), but females were attracted to males shaking more (61±55 versus 22±16 shakes/h, paired t=2.6, N=12, P=0.03).

![Figure 3. The linear regression between male body shakes frequency during courtship and hatching success (r²=0.32, N=17, P=0.02).](image)

**Nests for safety and nests as ornaments indicating male condition (II, III)**

In the fifteen-spined stickleback the male's nest is quite conspicuous, being exposed high above the surrounding vegetation and constructed with many bright shiny protein (tangspiggin) threads. In the stickleback family (Gasterosteidae) the fifteen-spined stickleback
is the largest species, and exhibits by far the largest production of secretional threads (Hentschel 1979). The threads consist of a protein synthesized in kidney tubuli cells, which during the breeding period transform into protein secreting cells (Hentschel 1977, Hentschel 1979). The protein must be metabolically expensive to synthesize and therefore its rate of production could be related to the food intake of the animal.


In the fifteen-spined stickleback, females prefer males with a higher courtship rate. In paper II and III, I wanted to investigate if nest characters may influence female choice. I wanted to examine if females choose males that position their nests high in the macroalgae, above the surrounding vegetation, or if they preferred males with nests close to the bottom concealed among vegetation. A related question was if potential egg predators like shore crabs find nests built close to the bottom faster than those built higher up in the macroalgae? Moreover, I also carried out a field study where I asked the following question: Does male-male competition affect the positioning of the nest in the surrounding vegetation?

Do females prefer nests built high up (II)?

I made males build nests low or high in a plastic plant by obstructing building in either the upper or the lower parts of the plant. All high nests were about 14 cm above the bottom, and all low nests were about 1 cm from the bottom. Twelve pairs of males were matched as closely as possible in standard length, nest volume and nest age. The males were moved to an outdoor experimental tank (1,300 l) along with their nests and placed on either side of an opaque wall. In this experiment the males were not enclosed in aquaria but instead leashed with a very thin fishing line. Hereby, the female was allowed to interact closely with the males and could freely inspect their nests. Male mobility was restricted so that they could not see each other. Female choice was assessed by where she spawned, or rated by the same procedure as described previously, if spawning did not occur.

Females clearly preferred males with nests built higher up in the vegetation over males with low nests (12 out of 12 females chose high nests, two-tailed binomial P<0.001). High nests were safer, because they were less likely to attract egg predators such as shore crabs (*Carcinus maenas*). In fact, in another experiment without any guarding males, I found that shore crabs began to eat eggs in low nests before they attacked high ones in eight out of eight trials (two tailed binomial P= 0.008).

Field study of nest position and closest neighbour distance (II)

I measured the height of nests over the bottom and over the surrounding vegetation while snorkeling. I also measured the distance to the closest neighbouring nest. I did this at 7 localities not more than 1.3 km apart all in the vicinity of Klubban Biological Station.
I found that male-male competition might further explain why males build nests high up: the closer the distance to another nest, the higher the males built their nests (Fig. 4a). Furthermore, larger males had more distantly positioned neighbours, that is, such males occupied larger territories than did smaller males (Fig. 4b). There was no significant relationship between how high the nest was positioned and male length (Fig. 4c).

Are tangspiggin threads costly to produce? (III)

In species where the male offers parental care, an ornament (morphological or behavioural) may reflect his ability to do so (Hoelzer 1989, Wolf et al. 1997). In paper III I wanted to investigate if the amount of tangspiggin a male produces and attaches to his nest functions as an extra-bodily ornament, indicating the condition of the male. Male pairs were matched for standard length and wet mass and then placed in 50 l aquaria. I randomly let one of the males within each pair have a high food ration and the other male a low food ration. I ran 17 male pairs for 12 days, whereupon I stopped the experiment. The males in the low-food group were given food twice a day every third day and the males in the high-food group were supplied with food twice every day. Each male was supplied with a plastic plant to attach filamentous red algae for nest building. Each male was given the same amount of a mixture of two species of algae (Polysiphonia fibrillosa and Ceramium rubrum) to exclude a possible effect of alga availability on tangspiggin production. On the 12th day I collected all tangspiggin threads and nest material, including tangspiggin threads found on the bottom of the aquarium.

Well-fed males produced significantly more tangspiggin (on average 34.4 ± 37.9 µg dry mass / h • g wet mass fish, n = 16) than did starved males (13.0 ± 15.2 µg dry mass / h • g wet mass fish, n = 15) during a 12 day period (Wilcoxon Signed Rank Test, tied-Z = -2.97, P
To estimate which proportion of a male's resting metabolic rate that is devoted to produce tangspiggin, I measured the resting metabolic rate of individual males in a closed respirometer as described by Nilsson (1996). I then calculated the tangspiggin production cost in terms of oxygen consumption, assuming that tangspiggin has a similar amino acid composition and glycosylation degree as the spiggin produced by three-spined sticklebacks (Jakobsson et al., 1999, see III). I found that the high-food group used a significantly larger fraction of their resting metabolic rate (7.4 ± 8.1 %) for tangspiggin production than the low-food group (2.8 ± 3.3 %; paired t-test on transformed data; t = 3.83, n=15 pairs, P = 0.0021).

The resting rate of O₂ consumption was virtually identical between the two different treatments (4.21 ± 1.25 and 4.12 ± 0.77 μmolO₂/h • g wet mass fish, respectively, n=6 and n=5 for the high food and low food groups, respectively, Mann-Whitney U-test, tied-Z=0.00, P>0.999, Fig. 5b). Thus, starved males were unable to divert enough energy to produce this protein.

Do females choose males with more secretional threads in their nests (III)?

I used new males and females in this experiment; females choosing between the two males in a pair. The males within a pair were similar in standard length and wet mass. When the males had begun building a nest I let them adopt nests of different tangspiggin content from other males. One of the males in each pair had three times as much tangspiggin in his adopted nest as the other. After accepting their new nests the males were transferred along with their adopted nests to outdoor experimental aquaria (each 170 l). After the males had acclimatized I leashed them as in the previous experiment and placed an opaque wall between them. I observed the females until mating occurred (but not for longer than 1 h). I found that six out of six females chose to spawn with the male with more tangspiggin in his nest (two tailed binomial P=0.032).
Male-male interactions: sneaked fertilizations and egg-stealing (IV and V)

Male-male interactions may spur the evolution of armaments or ornaments (Berglund et al. 1996), but may also reduce male fitness through cuckoldry or egg theft from neighbouring nests (Mori 1995). The advantage of sneaking to a sneaker is easily understood, but it is more difficult to explain the evolutionary benefit of stealing eggs from other males. One explanation for egg stealing is that in some species females prefer males with eggs in their nests (Rohwer 1978).

In my population of the fifteen-spined stickleback I had noticed frequent interactions between the males, in the field as well as in the laboratory. By developing and using microsatellite markers in association with my collaborators, I now wanted to (1) document the frequency of egg theft in a wild of a population of fifteen-spined sticklebacks, (2) reveal the identity of the sneaker male (3) place these findings in a spatial context by analysing nest positions.

Furthermore, I also wanted to investigate if males suffering from sneaking are able to adjust their paternal effort to their degree of paternity, or if the previous investment in the clutch matters more for paternal effort.

Finally, I also examined if females prefer males that have more eggs in their nests, since this might explain egg stealing.

The genetic analysis of sneaking and egg-stealing (IV)

Six microsatellite markers were developed and employed to assay a total of 1307 embryos from 28 nests. Males, with or without nests, were collected, marked and their position on a map was recorded. All nest-holding males in the local area were genotyped for two to six loci. Analysis of male and embryo genotypes revealed that five of the 28 nests (18% including nests without and with a nest-holding male) contained progeny from sneaked fertilizations, and that four of the 24 nests (17%) with nest-holding males contained stolen egg clutches. However, only 66 of 1307 (5%) of the total number of eggs assayed came from sneaked fertilizations. Every sneaker male, however, fertilized on average 40% of the eggs in a clutch, suggesting that sneaking is beneficial to males adopting this strategy. Comparisons of the composite DNA genotypes of nest-holding males against the inferred sneakers indicated that one nest holder had sneaked a nest 7 meters from his own. This suggests that sneaking in this species can be a conditional, facultative strategy in which nest holding males engage opportunistically (Foster 1994).

The effects of paternity on paternal care (V)

Here I wanted to investigate how sneaking and egg-stealing affect paternal care. Male parental effort was estimated by measuring fanning frequency and predator defence, to see how this correlated with the level of paternity. Fanning behaviour was measured for ten minutes every third day. A total mean for each male during three weeks (until the eggs hatched) was calculated. Predator defence was measured three times during this period. The first measurement was taken on the day after mating, the second after twelve days, and the last when the fry started to hatch (day 19 - 21). I used two species of nest predators: goldsinny wrasse (Ctenolabrus rupestris) and shore crabs (Carcinus maenas). Male attack rate towards the predators was measured 40, 30, 20 and 10 cm from the nest. The predators were moved by me, I let 6 hours pass between introducing either predator, and I randomly changed the order by which the two species of predators were introduced (crab or wrasse first). I manipulated the paternity among the males in the following way:
1) **Full paternity males.** These males mated alone with a female. 2) **Shared paternity males.** Males that shared paternity with another male. This was accomplished by introducing another male (a “sneaker”) into a male’s aquarium when he was about to mate with the female. 3) **No paternity males.** These males had no paternity at all. To achieve this I switched the entire clutches between two freshly mated males.

When comparing males with different degrees of paternity, I found no significant differences in fanning bout duration (the time from when a male begins to move his pelvic fins till he stops; one-way ANOVA, \( F(2, 26)=0.025, P=0.97 \)), fanning bout frequency (i.e. number of fanning bouts + pauses per hour; one-way ANOVA, \( F(2,26)=0.14, P=0.87 \)), or hatching success (% eggs hatched; one-way ANOVA, \( F(2,27)=0.021, P=0.98 \)).

However, while the intensity of predator defence against goldsinny wrasses did not vary with the degree of paternity (two-way ANOVA, \( F_{2,71}=1.10, P=0.34, \) Fig 6a), the defence intensity increased significantly over time as eggs developed (two-way ANOVA, \( F_{2,71}=18.6, P<0.0001, \) Fig 6a).

![Graph](image)

**Figure 6.** Attack frequency by male fifteen-spined sticklebacks towards two potential egg predators, goldsinny wrasse (a) and shore crabs (b), in relation to the age of the eggs. The white bars represent males with no paternity, the striped bars shared paternity and the black bars full paternity. With both predators, attacks increased with clutch age but had no significant relationship with degree of paternity.

The same pattern was found with shore crabs: while defence did not vary with paternity (two-way ANOVA, \( F_{2,71}=1.14, P=0.33, \) Fig 6b), it increased as eggs grew older (two-way ANOVA, \( F_{2,71}=8.97, P=0.0003, \) Fig 6b).
Thus, there was no relationship between the degree of paternity and fanning activity, hatching success or nest defence. However, males adjusted their level of defence to the amount of energy and time already invested in the clutch.

*Can* female preference for more eggs explain egg stealing in this species (V)?

I let females choose between three males with different clutch sizes in their nests. The males did not differ in mass or length. In each replicate, the three males had their newly built nests removed, whereupon they were each given a nest from another male to adopt. The size and age of all three adopted nests were the same. After each male had mated with a female in the adopted nest, I removed the eggs completely from one male. I mixed and distributed the eggs from the other two males so that one of them received half a normal clutch and the other a clutch 1.5 times the size of a normal egg clutch (which is about 2 g). I placed the males in a large (1,300 l) outdoor tank. An opaque screen separated the males. I leashed each male (as in study II and III). After the female had seen all the males in the tank I observed her for 30 minutes. I considered a male to be preferred if she spawned with him (N=8), or, if spawning did not occur within the predetermined 30 minute observation period, if he received a more intensive female courtship that the other males (N=5).

The result showed that females preferred males with reduced clutches over males with enlarged clutches (two-tailed binomial test: P=0.022).

In conclusion, female choice is unlikely to be a driving force behind egg-stealing in this species. In the absence of a female preference for egg-filled nests, another reason to why males steal eggs could be that they use eggs as a food supply. Although the present data suggest that males can not recognise their own eggs, it is still possible that they can recognise stolen eggs by how they position them in the nest.

**GENERAL CONCLUSIONS**

In the fifteen-spined stickleback males provide females with directs benefits in the form of paternal care for offspring. By being choosy as to which male to mate with, females gain better skilled and nourished fathers, along with safer nests for her eggs. I have found that fanning of eggs is important in this species as this correlates with hatching success. Males that fan in shorter bouts, but more often, hatch more eggs. Moreover, a male signals his fanning skills to the female not by his body size but by shaking his body intensively during courtship. Body shakes correlate with fanning skills, and may therefore signal the level of parental investment (I).

Not only male characters are important to the female in her choice, but also male nest-building skills affect the choice. I have found that males use their nest as an extrabodily ornament, signalling the condition of the male and the safety for the eggs (II and III). Tangspiggin (as I named the nest building secretion produced by the males) is partly a sexually selected character: the shiny threads attract females to the newly built nest. Females also chose nests built high, rather than low, above the surrounding vegetation. Females may gain several direct benefits from these preferences: males producing more tangspiggin are in better condition and may provide better care for the eggs, and a nest constructed with more tangspiggin may also be a safer nest. Moreover, the height of the nest also indicates safety for the eggs. Competition with other males for females favours males building higher nests than did their neighbours, probably because females prefer high nests.

Male-male interactions in this species do not only influence the position of the nests, but may also reduce paternity for males through stolen fertilizations and eggs. The frequency of these "parasitic" male behaviours was assessed using microsatellite markers, and nest
raiding was found to be common among males (IV). Moreover, sneak matings were performed by males with nests of their own. There is a substantial fitness cost to males victimised by sneakers, as males do not recognise their own eggs. I found that males valued clutch age (i.e., time and energy invested into the offspring) more than the degree of paternity, as only the former affected the level of care provided. Moreover, females showed no preference for more eggs in the nests, so this can not explain why the nesting males steal eggs from one another (V). I suggest that males use the stolen eggs as food, and recognise the stolen eggs by their position in the nest.

Thus, female choice is based on multiple cues and result in better paternal care. Males invest in courtship, male-male competition, nest construction and paternal care, the outcome determining their success in mate attraction.

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REFERENCES


Sevenster, P. 1951. The mating of the sea stickleback. Discovery (London), 12, 52-56.


