Sexual selection in *Pomatoschistus* – nests, sperm competition, and paternal care

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Cover photographs
Front: *Pomatoschistus minutus* male guarding his nest. © Anders Salesjö
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Abstract
Sexual selection arises through variation in reproductive success. This thesis investigates different aspects important in sexual selection, namely nest building, sperm competition, paternity and paternal care, and their mutual interrelationships. In the studied species, the sand goby (Pomatoschistus minutus) and the common goby (Pomatoschistus microps), sperm competition did arise when small males, so called sneakers, sneaked into other males nests and released sperm. They seemed to use female behaviour as their prime cue for a sneaking opportunity. However, also nest-holders, both with and without eggs, were found to fertilize eggs in the nests of other males.

Clearly, nest-holding males tried to prevent other males from spreading their sperm in their nests, since they showed aggression towards such males. A nest building experiment indicated that the small nest-openings found in the sneaker male treatment were sexually selected through protection against sneaking or by female choice. Yet, no behavioural or genetical support for the hypothesis that the nest functions as a physical or visual defence, or that sneaker males prefer to sneak upon nests with wide nest-openings, were found in the other studies. Still, individual nest-holding males showed a higher mucus preparation effort inside the nest in the presence of a sneaker male than when alone. In close relatives, such mucus contains sperm, suggesting an importance in sperm competition. However, the mucus may also have pheromone and anti-bacterial functions and may constitute a mating effort, as found in other gobies. Both a behavioural and a mate choice experiment suggested that the males were not less eager to spawn in the presence of a sneaker male.

Sneak intrusion did not affect nest defence, fanning or filial cannibalism, nor had paternity an effect on filial cannibalism. This and various life history aspects, together with the fact that the parasitic male only fertilized a fraction of the clutches, would predict females to ignore sneaker males. This was also the case, as the presence of sneaker males was found not to affect female spawning decision. Still, several females spawned in two nests, which coincided with parasitic spawnings, suggesting a cost of disturbance for the females and thus a substantial cost to the nest-holding males in terms of lost mating success. However, females paid attention to other traits in their choice of mate since spawning was associated with sand volume of the nest, but not with nest-opening width. Also, female (but not male) courtship was correlated with partial clutch filial cannibalism, indicating that females are able to anticipate future male cannibalism.

In a partial correlation of nest opening, sand volume, male courtship display, displacement fanning and male size, a large number of traits were correlated both positively and negatively with regard to how we may expect them to be appreciated by females. For instance, males which fan well also build large nests or display intensely (but not both). Together with all the other results of this thesis, this shows the entangled selection pressures working on breeding animals, as well as the different male and female tactics employed to maximize their reproduction.
LIST OF PAPERS


V. Singer, A., Svensson, O., Kvarnemo C. & Lindström K. Genetic mating patterns studied in pools with manipulated nest site availability in two populations of *Pomatoschistus minutus*. *Manuscript*. 
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INTRODUCTION

When a male is going to reproduce, several different things may have an effect on his breeding success. First, in order to be able to breed, the male will often have to compete with other males for access to breeding sites and females. In some species it is the other way around and females are competing over access to males. In male-male contest, the largest male, or the one in the best condition, is most likely to win. Motivation, i.e. how important it is for the male to win, in terms of fitness, can also be of importance. However, the females should choose mates that will provide good genes to her offspring. Furthermore, if the male is providing care to the offspring, she also gains from choosing a good father (reviewed in Andersson, 1994).

When a male gets access to a female and breeds, the sperm from other males may be present (reviewed in Birkhead & Møller, 1998). The sperm from two or more males are then competing over the fertilization of the eggs (or egg). This competition between the sperm of different males was suitably termed “sperm competition” by Parker in 1970. In communally spawning fish, it is quite obvious that sperm from several males are competing. However, in both mammals and birds the females often copulate with more than one male, with sperm competition as a consequence. Sneakers, satellites and other types of alternative mating behaviours are also common in several groups of animals. Such males interfere with a breeding pair, copulate with the female or release sperm to fertilize some of the eggs, and leave (reviewed in Taborsky, 1994).

It is not enough to fertilize the eggs. The offspring must survive. In most animal species the parents just leave the offspring after fertilization. However, in many species, one or both parents provide care for their offspring. Parental care is an investment into offspring after fertilization that increases its survival through protection and promotion of its development. In mammals it is common that the female alone takes care of the young while in fish it is often the other way around, the male takes care of the eggs alone. In birds, however, biparental care is the most common; both parents take part in the incubation of the eggs and feeding of the young (reviewed in Clutton-Brock, 1991).

Parental care is a costly part of reproduction (reviewed in Magnhagen, 1991; Smith & Wootton, 1995). Therefore, the parents should try to minimize those costs. In biparental species, one of the parents can desert and leave all the care to its mate. In fish species with paternal care, filial cannibalism is common. The male is eating parts of its own clutch arguably to increase its fitness. It has been suggested that this behaviour increases the chances of raising the present clutch as well as to increase the likelihood of
breeding again (reviewed in Manica, 2002). Furthermore, a male should avoid caring for unrelated young. He can do this by defending his paternity as well as providing care in accordance to his paternity. There are several ways for a male to defend his paternity. He can, for example, defend the breeding site and guard his mate. Mating plugs, antiaphrodisiacs and sperm trails are more spectacular examples (reviewed in Birkhead & Møller, 1998)

**Sexual selection**

In his theory of sexual selection, Darwin (1871) proposed a mechanism to explain the evolution of secondary sexual traits such as the peacock's tail and the deer's antlers. Sexual selection arises from variance in mating success, caused by some individuals having an advantage over others in acquiring mates, while natural selection arises from variance in survival and fertility. Sexual selection, according to Darwin, can work in two ways: 1) By favouring the ability of individuals of one sex, usually males, to compete directly with each other for fertilizations. This is often called intra-sexual selection and may lead to evolution of traits such as weaponry and large body size and 2) by favouring traits in one sex, usually the males, to attract the other sex, usually the females. This is often called inter-sexual selection, although the selection still is within one sex, the sex competing to be chosen, as pointed out by Andersson & Iwasa (1996). Inter-sexual selection may lead to evolution of ornamentations such as the peacock's tail or courtship displays. Furthermore, sexual selection can also work between mating and fertilization. The main form of this post-mating competition is sperm competition (Birkhead & Møller, 1998). However, the exploration of “cryptic female choice” (females “choosing” which sperm will fertilize her eggs) has just recently been started (Eberhard, 1998). In some cases there is even a sexual selection after fertilization. This happens when a mate induces abortion or commits infanticide to promote its own reproductive success.

**Intra-sexual selection**

*Pre-mating competition*

One sex is often limiting the reproduction of the other sex that must then compete over the other (Emlen & Oring, 1977; Clutton-Brock & Parker, 1992). In most animal species the competition before mating within one sex usually acts as male-male competition (reviewed in Andersson, 1994; Andersson & Iwasa, 1996). However, in some animals
e.g. many pipefishes, the sex-roles are reversed. In these animals the females compete over the males since they are the limiting sex (reviewed in Vincent et al., 1992).

Post-mating competition – Sperm competition
The main form of male-male competition between mating and fertilization is sperm competition (Birkhead & Møller, 1998). Sperm competition (as defined above) is a remarkably powerful selective force, which has led to a wide range of adaptations in both sexes. The idea that a female mainly mates with one male only has proven wrong for most species, and, consequently, a lot of recent research has been focused on sperm competition in insects, birds, mammals and other animal groups (Birkhead & Møller, 1998). Parker et al. (1996) made a distinction between sperm competition risk and sperm competition intensity. Risk concerns the question “will there be a competition between two or more ejaculates or not”, whereas intensity concerns the question “how intense will the competition between two or more ejaculates be”. Recent research has shown that sperm production is not necessarily cheap, as often previously assumed, and must be included in fitness costs (Birkhead & Møller, 1998).

Sperm competition is common in fish (Blumer, 1979; Stockley et al., 1997). Also in territorial species where the male spawns with one female and guards the brood, sperm competition can be intense since it is common that parasitically spawning males interfere with the spawning pair and fertilize eggs (Taborsky, 1994; Gross, 1984; Stockley et al., 1997).

Alternative mating strategies and tactics
All individual males do not necessarily have the same means to reach the “goal” of fertilizing the eggs, and may therefore use different mating strategies or tactics. For example, a male may compete for a territory, attract a female and spawn with her, while an alternative route to fitness is to interfere with as many spawning pairs as possible, in each case trying to fertilize some of the eggs, and leave. A strategy is a genetically based program and a tactic is a phenotype given by the strategy. Phenotypic diversity can be divided into three different categories: alternative strategies, mixed strategy and conditional strategy (Gross, 1996). As the genes give an alternative strategy, the different phenotypes result from genetic polymorphism. All different alternative strategies are expected to have the same average fitnesses. In both the mixed as well as the conditional strategies, all individuals have the same genetic program i.e. they are monomorphic. In
the mixed strategy, all tactics are expected to have the same average fitnesses. Which tactic an individual employs is probabilistic e.g. the male may fight with a probability of 0.3 and sneak with a probability of 0.7. Natural selection will select for the optimum proportion in every particular population. In the conditional strategy the individual “chooses” tactic depending on its individual relative status. The average fitnesses of the different tactics are not necessarily equal. However, at the switch point between two tactics the fitnesses are the same. The “choice” distinguishes the conditional strategy from the mixed and alternative strategies. Conditional strategies are very well documented. However, there is no documented case of a mixed strategy and only a few candidates of alternative strategies (Shuster, 1992; Shuster & Wade, 1991; Shuster & Sassaman, 1997; Ryan et al., 1992; Lank et al., 1995).

*Sneakers, streakers, satellites and pirates*

Alternative reproductive tactics, such as sneaking, have evolved in many fish species where reproductive success depends on aggression and competition between males (Dominey, 1981; 1984). Often smaller males, which are less likely to successfully defend a nest and attract a female, spawn parasitically and take advantage of another male’s investment into secondary sexual characters, territory defence, paternal care et cetera. Such parasitic males have been called sneakers, streakers, satellites, female mimics and a lot of other things. However, there are differences between these types of parasitic spawners. A sneaker male is a male that sneaks into a male’s territory and releases sperm. A satellite is tolerated by the territorial male and sometimes even helps with territorial defence. A streaker does “kamikaze”-rushes through a male’s territory and releases sperm, and a female mimic mimics a female and is perceived as such by other males. Female mimics are often called satellites (Taborsky, 1994). Even successful males with a territory and therefore mating opportunities may steal fertilizations from other territory owners (van den Assem, 1967; Avise et al., 2002; Karino & Nakazono, 1993; Magnhagen, 1998). They thereby resemble bird systems in which already paired males constantly seek extra pair copulations (reviewed in Birkhead & Møller, 1992). Taborsky (1994) lists eighteen teleost species from six families in which males have been documented to leave their territories to spawn parasitically in neighbouring nests, which suggests that such behaviour may be quite common. These males may even be dominant over the nest-holding males that remain in their nests (van den Berghe, 1988; Mboko & Kohda, 1999; Magnhagen, 1998). Van den Berghe (1988) termed such dominant
parasitically spawning peacock wrasse (*Symphodus tinca*) males “pirates”. These pirates circumvented nest construction, guarding and care by temporarily taking over successful nests, spawned and left the care to the original nest holders.

Sneakers and satellites may have completely different life histories as compared to the dominant territory holding males (Gross, 1984). In many species, males may become sneakers before they are large enough to become territory owners (Wirtz, 1978; Dunbar, 1982), or they may be making the “best of a bad job”.

**Inter-sexual selection**

*Direct benefits*

Females often select males on the basis of the material resources they can offer, such as nuptial feeding, parental care and territory quality (reviewed in Andersson, 1994). If there are hazards during mating such as predation risk on the female or harassment by other males, females may choose safe mating. In addition, transmission of diseases and parasites may be avoided by choosing an obviously healthy male. Moreover, if there is a high risk of predation of the offspring, females may gain from choosing large males granted these are good at protection (Andersson, 1994). Hoelzer (1989) proposed that epigamic traits could evolve not only by a Fisherian process of run-away selection (Fisher, 1930, see below) or through selection for “good” genes (Zahavi, 1975; Hamilton & Zuk, 1982) but also as a way of advertising non-heritable components of parental quality. Then, if the paternal care will influence the viability or fecundity of the offspring the male should honestly display the quality and quantity of care that he is going to provide. Female choice of good fathers has now been demonstrated in different taxa, including fish (Andersson, 1994). For example, Forsgren (1997a) experimentally showed that large and dominant sand goby males (*Pomatoschistus minutus*) that are successful in male-male competition are not better in taking care of the eggs. Females did not prefer those dominant males, but the males that later proved to be the best fathers. Finally, if filial cannibalism (eating its own progeny) is common, females should select males to reduce the risk of cannibalism, for example by choosing a male that already has eggs in his nest (Lindström, 2000; Manica, 2002)

*Indirect benefits*

Some elaborate characters have evolved through male-male competition. Yet, many ornamental traits have most certainly evolved by females selecting males to obtain
indirect genetic benefits for their offspring. There are several different hypotheses to explain extravagant male ornaments. Fisher (1930) argued that by a self-reinforced runaway process that leads to more extreme male ornamentation and more choosy females, male ornaments arise simply because they are attractive to females. Zahavi (1975; 1977) argued that the peacock's tail is a handicap in the day-to-day survival. He suggested that females choose males with handicap traits just because they are handicapping. The handicap is a true signal of quality, showing the male's ability to survive in spite of it. If this is heritable, then the "goodness in surviving" will be passed to the offspring. Thus, females will select for “good” genes by choosing males that display honestly, signalling their genetic quality. In the classical paper “Heritable true fitness and bright birds: A role for parasites?” Hamilton and Zuk included parasite resistance in this framework (Hamilton & Zuk, 1982). There are also other theories to explain why females choose ornamented males. The sensory bias mechanism suggests that a new male trait may evolve, if it fits an already existing bias in the female sensory system (reviewed in Ryan & Kedd-Hector, 1992). For example, if females feed on red food items, red ornamented males, as a side effect, may catch their attention, and obtain higher mating success. These mechanisms are not mutually exclusive and it is unlikely that any of these alone can explain why mate preferences have evolved.

Parental care
Within the animal kingdom, fishes show the greatest variation in parental care. Parental care, or investment into offspring after fertilization, ranges from simple burial of eggs to internal gestation and live bearing (Gross & Sargent, 1985). The two main functions of parental care in fish are protection of the offspring and promotion of their development (e.g. Keenleyside, 1978; 1991). Traditionally, the evolution of parental care is explained in terms of natural selection; the fitness benefits of the care, mainly gained from the protection of the offspring and promotion of their development, exceeds the costs such as higher predation risk on the parent, reduced body condition, reduction in growth, and loss of breeding opportunities (Magnhagen, 1991; Smith & Wootton, 1995). Sexual selection has also been suggested to promote the evolution of parental care. Females may choose males that provide care (Trivers, 1972; Ridley & Rechten, 1981; Tallamy, 2000), and when paternal care has evolved it may be reinforced by females choosing good fathers (Liker & Szekely, 1997; Owens et al., 1994).
Paternity assurance and paternal care

Intuitively, natural selection should favor males that avoid caring for unrelated young. However, if and when to reduce or increase care must be seen in the light of life history decisions and trade-offs concerning costs and benefits with increasing or decreasing parental care. Some assumptions must be fulfilled to even suggest an effect of paternity, for example availability of information on paternity or variation in the risk of cuckoldry between breeding attempts (Neff & Sherman, 2002; Sheldon, 2002). Most studies have been focused on biparental birds (Sheldon, 2002), which differ from fishes with exclusive paternal care by the fact that there is a conflict within the pair over the feeding rate of the young (Trivers, 1972). However, in teleost fish there is one stunning example, namely the bluegill sunfish, *Lepomis macrochirus*. Males care for their offspring in relation to their perceived paternity (amount of sneaking) before hatching, whereas after hatching, when they are able to assess their paternity by chemical cues (Neff & Sherman, 2003), they care for their offspring in accordance with their true paternity (Neff & Gross, 2001; Neff, 2003a). Also in the scissortail sergeant, *Abudefduf saxatilis*, care is positively correlated with paternity (Manica, 2004). On the other hand, in other species of fish, no such pattern has yet been found (van den Berghe et al., 1989; Östlund-Nilsson, 2002) despite the fact that a wide variety of teleosts have been shown to recognise kin or offspring (Loiselle, 1983; McKaye & Barlow, 1976; Loekle et al., 1982; FitzGerald & van Havre, 1987; Brown & Brown, 1996).

Filial cannibalism

Although fitness benefits are expected to accrue to the parent from the care, there are also costs of parental care. Parental care, such as pregnancy, egg carrying and incubation, can make the parent more conspicuous and more exposed to predation (reviewed in Magnhagen, 1991). Brooding often prevents or reduces feeding by the parent (e.g. *Pomatoschistus microps*, Magnhagen, 1986), and results in reduced body condition (e.g. *Oxylebius pictus*, (DeMartini, 1987) and *S. tinca* (van den Berghe, 1992)). Decreased condition may then lead to increased risk of death through disease or starvation (e.g. *Cottus gobio*, Marconato & Bisazza, 1986) (reviewed in Smith & Wootton, 1995). One possibility for parents to reduce the parental costs is to cannibalize their own progeny, called filial cannibalism. For a long time filial cannibalism was considered to be a maladaptive pathological behaviour, or behaviour difficult to fit into evolutionary theory,
but nowadays it is regarded to enhance the cannibal's lifetime reproductive success (reviewed in Manica, 2002).

Filial cannibalism is often divided into two different types: whole clutch cannibalism and partial clutch cannibalism. Whole clutch cannibalism should occur when the reproductive value of the clutch is outweighed by the cost of parental care, i.e. when the clutch is too small. This is argued to be so because the cost of parental care (limited feeding opportunities during brood guarding and energy spent on fanning) does not at all or only slightly with brood size, whereas the benefit of parental care (number of young raised to independence) does (e.g. Rohwer, 1978; Petersen & Marchetti, 1989). Examples of fishes which sometimes completely cannibalise smaller clutches are the threespined stickleback, *Gasterosteus aculeatus* (van den Assem, 1967), the painted greenling (DeMartini, 1987), the Cortez damselfish, *Stegastes rectifraenum* (Petersen & Marchetti, 1989), the sand goby, *P. minutus* (Forsgren et al., 1996a) and the fantail darter, *Etheostoma flabellare* (Lindström & Sargent, 1997).

While whole clutch cannibalism denotes a termination of care, partial clutch cannibalism does not. In an evolutionary model developed to explain the occurrence of partial filial cannibalism in fishes, Rohwer (1978) argued that males that are able to perform several brood cycles in the course of a breeding season may dramatically increase their chances of re-nesting by eating parts of their clutches. According to Rohwer, the general conditions favouring the evolution of filial cannibalism are 1) that the male can guard multiple clutches, 2) that his foraging is restricted by the demands of egg guarding, and, most importantly, 3) that he benefits more than he loses from egg eating. In both the common goby *P. microps* (Kvarnemo et al., 1998) and in the Cortez damselfish *S. rectifraenum* (Hoelzer, 1992), there is strong evidence for fed males eating fewer eggs than starved ones. However, filial cannibalism is still debated and other hypotheses include that eggs contain essential nutrients lacking in other diets, removal of unfertilized and malformed eggs, maladaptation and mistakes (Manica, 2002).

Filial cannibalism and female choice
Whenever filial cannibalism is prevalent, natural selection should select females avoiding males that would later cannibalize their eggs. Moreover, if a female spawns in a nest that already contains eggs, there will be a dilution effect reducing the risk of her eggs being eaten (Rohwer, 1978; Lindström, 2000). This has been shown in several species of fish e.g. the three spined stickleback, *G. aculeatus* (Ridley & Rechten, 1981), the fantail
darter, *E. flabellare* (Knapp & Sargent, 1989), the river bullhead, *C. gobio* (Marconato & Bisazza, 1986; Bisazza & Marconato, 1988), the common goby (Reynolds & Jones, 1999) and the sand goby (Forsgren et al., 1996a). Furthermore, Rohwer (1978) argued that displacement fanning (courtship fanning) has evolved in species in which females are unable to see the eggs inside the nest and therefore choose actively fanning males. Females may also avoid cannibalism by choosing males in good condition. Female choice has been shown to be correlated with egg survival in both the sand goby (Forsgren, 1997a) and in the bicolor damselfish, *Stegastes partitus* (Knapp & Kovach, 1991). In the latter, display intensity was negatively correlated with filial cannibalism. In Svensson et al. (2004), however, the display rate of sand goby males tended to be positively correlated with cannibalism, and in Svensson and Forsgren, (2003) common goby females showed no preference for fed males although these males had a higher mating success. Furthermore, the nest may also give the female information on male condition and filial cannibalism (Kvarnemo et al., 1998; Barber et al., 2001).

**The nest**

Many species of animals build nests (Hansell, 2000). The nest may protect the offspring from adverse environmental conditions, such as extreme temperatures, wind or water (e.g. Hostache & Mol, 1998; Hansell, 2000). Another important effect is protection against predators. The nest can protect the offspring as well as the parents from being discovered by predators (Lindström & Ranta, 1992; Jones & Reynolds, 1999a; Hansell, 2000). A second protective mechanism may be that the nest *per se* makes it easier for a parent to defend itself and the offspring, as for example in *Reticulitermes speratus* termites (Matsuura, 2002) and nest sealing in Bucerotidae hornbills (Kemp, 1995).

In birds, the evidence for an influence of predation on the choice of nest sites is extensive (Hansell, 2000). For example, in the pied flycatcher, *Ficedula albicollis*, females prefer males with nest boxes high up in the trees, which are the nest boxes that suffer least from predation (Alatalo et al., 1988). The same is also true for fishes. For example the convict cichlid, *Archoecentrus nigrofasciatus* (Lavery, 1991), and the sticklebacks *G. aculeatus* (Sargent & Gebler, 1980; Kraak et al., 1999) and *Spinachia spinachia* (Östlund-Nilsson, 2000) prefer to spawn in safe nests.

On the other end, males may invest in constructions that are exclusively display sites. Those are ornaments that indicate the physical or genetical quality of the male (Hamilton & Zuk, 1982), and are completely independent of the care of eggs and young.
Examples are bowerbirds (Ptilonorhynchidae) (Hansell, 2000) and many Lake Malawi cichlids (McKay, 1991). In a cichlid from the *Copadichromis eucinostomus* species complex, males with skewed (asymmetric) bower heights had higher parasite load than males with less skewed bower heights (Taylor et al., 1998). In species with paternal care the nest may also provide the female with information on the quality of the care the male is going to provide (Hoelzer, 1989). In birds, nest building behaviour is often used in the courtship and the nest has been shown to correlate with paternal care (Moreno et al., 1994; Möller et al., 1995; Soler et al., 1995). Furthermore, similar to bowers, the nest may also function as an ornament (Soler et al., 1998; Jones & Reynolds, 1999a). In fish, nest building has been shown to correlate negatively with immune stress (enlarged spleen) (Barber et al., 2001) and positively with experimentally manipulated body condition (Kvarnemo et al., 1998; Jackson et al., 2002). Therefore, the nests of fishes probably provide the female with information on several aspects of male quality.

Finally, a well-built nest may be easier to protect against nest take-over or sperm competition due to sneaker male intrusions, and might thus be sexually selected through male-male competition. Defence against nest take-overs may actually be the primary function of nest sealing in the hornbill *Bycanistes subcylindricus* (Kalina, 1989). However, the importance of a nest as a physical defence to prevent conspecific males from stealing fertilizations has been virtually unexplored. Furthermore, a sneaker male or a male without a nest site may also find it harder to discover a cryptic nest, as suggested for the three spined stickleback, *G. aculeatus* (Sargent & Gebler, 1980). Consequently, nest constructions can be sexually selected, in addition to being naturally selected. The mode of selection is also likely to vary over time, with stronger sexual selection before egg-laying shifting to natural selection after egg-laying.

**METHODS**

**Study species**
The study species of my thesis are the common goby, *Pomatoschistus microps* (Krøyer) in paper I and the sand goby *Pomatoschistus minutus* (Pallas) in paper II-V. These closely related (Huyse et al., 2004) small bottom dwelling fishes have similar appearance, habits and ecology (Miller, 1986). The main differences are that the common goby is smaller than the sand goby, and more often found on shallow muddy areas, whereas the sand goby is found on shallow sand bottom areas. Males and
females of both species are cryptic. However, the males have black stripes on the body and on the fins. The sand goby also have blue coloration in the anal and dorsal fin. Both are short lived and reproduce repeatedly during only one season. The breeding season for the common goby is between end of May and beginning of August while the breeding season for the sand goby is between end of April and beginning of July (Magnhagen, 1999).

Males of both species build nests by covering empty mussel shells with sand, or mud, and attract females by courtship display including erect fins and tail beats. Females show their spawning interest by a characteristic hovering movement and sand goby females also by blackened eyes. The male alone tends for the eggs until hatching. During each of several brood cycles the male may receive eggs from several females and each female lay several clutches over the season (reviewed in Magnhagen, 1999). In the common goby, sneak mating has been thoroughly investigated in aquaria by Magnhagen (1998; 1995; 1994; 1992), and in a field study on the sand goby, half of all nests contained eggs fertilized by another male than the nest-holding male and one tenth of all eggs were fertilized parasitically (Jones et al., 2001a). When dissecting all sand goby males in catches early and late in the season, small males with rudimentary breeding coloration were found to be sexually mature, with huge testes but only rudimentary seminal vesicles (involved in mucus secretion), thereby differing from males in breeding coloration. No immature males were found (C. Kvarnemo & O. Svensson, unpublished). This type of small males with large gonads exists in other populations of the common goby, but has not been found in the study population of the present thesis (Magnhagen, 1992; Magnhagen, 1999).

**General methods**

The experiments were conducted in June and July 1995 and May and June 2000 - 2003 at Klubban Biological Station (58°15´N, 11°28´E) or at Tjärnö Marin Biological Laboratory (58° 52´ N, 11° 10´ E) both on the west coast of Sweden as well as on Tvärminne Zoological Station (59°50´N, 23°15´E) (fig 1). The fish were caught in nearby bays to respective station. For storage we used 130-L aquaria. In experiments, we used indoor aquaria with a thin layer of sand on the floor. Exceptions are the first experiment of **paper III** in which very little sand was used and **paper V** in which we used outdoor pools with a diameter of 1.5m. Clay flowerpots cut into halves were used as standardised nest sites except in **paper II** in which we used natural mussel shells. The aquaria and
pools were provided with continuously renewed seawater and temperature followed natural conditions.

**Figure 1.** The location of the three field station where the studies have been performed. Reproduced from (Forsgren et al., 1996b) with the insertion of Tjärnö.
THE PAPERS

Paper I: Parental behaviour in relation to the occurrence of sneaking in the common goby

In this experiment, the common goby males were able to spawn with or without a sneaker male present. After a female was released into the aquarium, the fish were observed until spawning finished. It was noted, whether or not successful sneaking occurred. We also measured the time the sneaker stayed in the nest. The day after spawning, the egg area was drawn and the male was left to care for his eggs for four days. The day before hatching (i.e. five days after spawning), fanning rate and nest defence towards a netted dogwhelk, *Hinia reticulata*, and a dead shore crab, *Carcinus maenas*, was measured, and finally the egg area was drawn again to measure filial cannibalism.

There was no difference between males that experienced sneaking and those that did not in any of the measured behavioural traits that relate to paternal care (table 1). The time the sneaker stayed in the nest was not correlated with those traits either. The lack of correlation between sneaking and care could be because the males were not able to assess paternity or that the cost of decreased care in terms of mortality or quality of the offspring would have been higher than the benefits of decreased care or of cannibalising some of the eggs.

This was among the first studies which tackled predictions on paternity and care experimentally, and the first that did it on fish (Neff & Sherman, 2002).

Table 1. Estimates of parental care in male common gobies from the two different treatments without and with sneaking, respectively. Fanning time was measured during ten minutes. Defence score was measured as the defence against a dead shore crab and a netted dogwhelk. The differences were tested with t-test. Percentages were arcsine ($\sqrt{x}$) -transformed prior to testing. Defence score was tested with Mann-Whitney nonparametric test.

<table>
<thead>
<tr>
<th>Male trait</th>
<th>Mean ±SE not sneaked</th>
<th>Mean ±SE sneaked</th>
<th>t/z</th>
<th>df/n°:n¹</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial brood loss area (cm²)</td>
<td>0.58±0.33</td>
<td>0.34±0.23</td>
<td>0.56</td>
<td>17</td>
<td>0.58</td>
</tr>
<tr>
<td>Partial brood loss (%)</td>
<td>11.6±3.7</td>
<td>9.9±3.3</td>
<td>0.26</td>
<td>17</td>
<td>0.80</td>
</tr>
<tr>
<td>Fanning time (s)</td>
<td>309±33</td>
<td>270±57</td>
<td>0.61</td>
<td>15</td>
<td>0.51</td>
</tr>
<tr>
<td>Defence score</td>
<td>7.5±0.64</td>
<td>7.8±0.46</td>
<td>0.29</td>
<td>10:9</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Paper II: Sexually selected nest-building - *Pomatoschistus minutus* males build smaller nest-openings in the presence of sneaker males

In this paper males built nests under five different sperm competition and egg predator regimes and the nest opening width and time to nest building was measured. The five treatments were:

1. Sneaker males: the nest-building male was accompanied by two smaller, potential sneaker males, to increase the perceived risk of sneaking.
2. Nest-building males: the nest-building male had visual contact with two other nest-building males in the adjoining aquaria on each side to increase the impression of male–male competition. The males seemed to consider these males behind the glass as opponents, as they showed aggressive colourations, displayed vigorously and performed tail beats towards each other.
3. Egg-predators: the nest-building male was accompanied by one shore crab and one netted dogwhelk, to increase the perceived risk of future egg-predation.
4. *P. microps* males: the nest-building male was accompanied by two common goby males, as a control to the possibility that nest holders would see the sneaker males as egg-predators or nest-site competitors.
5. Alone: the male was allowed to build his nest alone, to have a situation with no risk of sneaking, nest-site competition over females or egg-predation.

Males with sneaker males present built the smallest nest-openings, smaller than males held alone or with common goby males (which may prey upon eggs and compete over nest-sites but not compete over fertilizations). Males with visual access to other nest-building males tended also to build smaller openings than males held alone or with common goby males. Males with egg-predators present built nests with openings not differing significantly from any other treatment (fig 2). Thus, our results indicate that the small nest-openings found in the sneaker male treatment are sexually selected through protection against sneaking or by female choice. Across treatments, time span before a male started to build his nest also explained variation in nest-opening width; males starting late built larger nest-openings (table 2).

Female choice for well-built nests has been studied to some extent before. However, to our knowledge, this is the first study showing males to modify their nests as a response to male-male competition.
Figure 2. Nest opening width in five treatments in, measured 48 hours into the experiment, and analysed with time to nest building as a covariate (ANCOVA). The figure shows mean ± SE. Duncan’s post hoc test, **p<0.01, *p<0.05, O p<0.10.

Table 2. R-values of a partial correlation, with nest opening measured 24 hours after the first traces of nest building (which on average was 60 hours after the start of the experiment). 61 observations were used in this computation.

<table>
<thead>
<tr>
<th></th>
<th>Male length</th>
<th>Shell width</th>
<th>Time to nest building</th>
<th>Nest opening width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start date</td>
<td>0.052</td>
<td>-0.114</td>
<td>-0.106</td>
<td>0.110</td>
</tr>
<tr>
<td>Male length</td>
<td>0.154</td>
<td>0.144</td>
<td>0.179</td>
<td></td>
</tr>
<tr>
<td>Shell width</td>
<td>-0.029</td>
<td></td>
<td></td>
<td>0.168</td>
</tr>
<tr>
<td>Time to nest building</td>
<td></td>
<td></td>
<td></td>
<td>0.455***</td>
</tr>
</tbody>
</table>

Significant r-value ***P<0.001

Paper III: Sand goby sneakers tune in on females but nothing else – A study using manipulated nest-opening size and genetic paternity analysis

The aim of this study was to experimentally test if the opening of the sand goby nest functions as a physical and visual defence against sneaker males. Furthermore, we also wanted to correlate male behaviour, female behaviour, sneaker male behaviour,
genetically determined paternity and filial cannibalism. In the first experiment (physical defence) males were provided plastic nests (fig 3a) and very little sand and were therefore not able to change the shape of the nest. All fish were free to spawn. After observation, the egg area was drawn and the male was left to care for his eggs for nine days. Thereafter the egg area was drawn again to measure filial cannibalism. In the second experiment (visual defence), the nests simulated natural nests. However, the males were not given access to them and were therefore not able to change their shape, and no fish was free to spawn. In the second experiment we measured sneaker male preference, and the set-up was thereafter repeated with a female instead of a sneaker male measuring female preference for wide and tight nest openings (fig 3b).

(a)

(b)

Figure 3. a) The artificial nests used in experiment 1 (nest-opening as a physical defence). The nests were made of PVC rings with ceramic tiles on top. The tight nest-opening is 14 mm while the wide is 25 mm. b) The set-up in experiment 2 (nest-opening as a visual defence) used in both the sneaker male preference trial and the female preference trial. The aquarium was divided into three compartments. In the middle compartment (A), a small sneaker male, or a female, was able to choose between two nests with large or small openings. If the focal fish was observed in the middle section (within the area marked with dotted lines) it was considered to show no preference. In the sneaker male preference trial each vial (B) contained a ripe female and a male in breeding colour, whereas in the female preference trial each vial only contained one male in breeding colour.
The outcome of this experiment was that we found no effect of nest-opening. Probably, it does not function as a physical defence against sneaker males. However, we cannot conclude yet that it does not have a concealment function, at least not over longer distances. The main cue for sneaker males was female behaviour, since they performed more sneaking behaviour when the females courted the nest-holding males whereas male courtship had no effect. In the second experiment, sneaker males preferred the side of the aquaria where the females were visible most often, but again, male behaviour was irrelevant. The paternity analysis revealed that the sneaker males indeed do fertilize eggs and the fertilization success was positively correlated with the total time they managed to stay in the nests (fig 4). However, filial cannibalism was not affected by sneak intrusions or paternity. On the other hand, female courtship was negatively correlated with future partial filial cannibalism.

![Graph](image)

**Figure 4.** There was a significant positive correlation between the total time the sneaker male stayed in the nest and the genetic paternity of the sneaker male

**Paper IV. The importance of sperm competition risk and nest appearance for male behaviour and female choice in Pomatoschistus minutus**

In this study, the aim was to test experimentally 1) if individual males during increased sperm competition risk (the presence of a sneaker male) reduce courtship or change it
from “spectacular” displays into a less visual form of courtship namely displacement fanning. Furthermore, we wanted to test 2) if male mucus preparation effort was increased during increased sperm competition risk. The mucus of gobiids has been found to protect the eggs from bacterial infection (Fishelson, 1991), to contain pheromones (Colombo et al., 1980; Locatello et al., 2002) and spermatozoa (Marconato et al., 1996). Due to the latter, a role in sperm competition has been suggested (Ota et al., 1996). We also wanted to test 3) if female spawning decision and male mating success was affected by sperm competition risk and 4) if high nest-building effort measured as volume of sand over the nest and small nest-opening width coincides with later male mating success. In addition we 5) performed a partial correlation on the sand volume of the nest, nest opening width, male courtship display, displacement fanning and male size, all of which are traits that have been suggested to be cues important for female spawning decision. The experimental set-ups are described in fig 5.

Figure 5. a) The set-up in experiment 1, the male behaviour experiment. The behaviour of the nest-holding male recorded while being sequentially exposed to different risk of sperm competition. The vial marked ♀ contained a gravid female and the other vial contained either a sneaker male (s♂) during the first filming which was changed for an empty vial (e) for the second filming, or in the opposite order, whereas some males were exposed to empty vials both times. b) The set-up in experiment 2, the mating success experiment. The female was allowed to spawn with either of two nest building males, one of which was exposed to a sneaker male in a vial (s♂) and one exposed to an empty vial (e). Differences in nest attributes between the two males were noted.
Interestingly, when males in the male behaviour experiment were exposed to a sneaker male they increased the numbers of bouts of mucus trail preparations i.e. by rubbing their anal-urogenital area towards the ceiling (fig 6). In three gobiid species that have been examined, such mucus trails contain spermatozoa, which are slowly released for hours (Ota et al., 1996; Marconato et al., 1996; Scaggiante et al., 1999; Rasotto & Mazzoldi, 2002), and a role in sperm competition has been suggested (Ota et al., 1996). Thus, most likely, the nest-holding males of the present study increased their sperm trail effort as a consequence of a perceived increase in sperm competition risk. However, the mucus may also have pheromone and anti-bacterial functions and constitute mating effort, as found in other gobies. Also, the male was engaged in as many less cryptic behaviours (fanning, being outside the nest and displaying) when there was a sneaker male present compared to when there was not, suggesting that they were not hiding or less eager to spawn.

**Figure 6.** Nest mucus trail preparation in the male behaviour experiment. Pair-wise comparisons of individual males held alone first and then with a sneaker male present or vice versa. Eight of the 0-0 lines and three of the 0-1 lines are overlapping.
In the mating success experiments, females spawned as often in the nest with a sneaker male present as they did in the nest next to an empty vial. Therefore, it seems that females did not avoid nor prefer sneaker males and males were not less eager to spawn in the presence of sneaker males. However, the volume of sand the male had put on his nest was positively associated with female spawning decision, while nest-opening width was not.

In a partial correlation of nest-opening, sand volume, male courtship display, displacement fanning and male size, a large number of traits were correlated (table 3). Our main result from that analysis is that these traits are correlated both positively and negatively with regard to what is expected to be preferred by females. Therefore, if only measuring one or few of those traits, there is a risk of drawing the wrong conclusion.

Table 3. Partial correlation on male attributes in the male behaviour experiment. Sand volume is the amount of sand the nest is built of, displacement fanning was measured during 90 min of filming and courtship display was measured as displaying time during the first five minutes of displaying.

<table>
<thead>
<tr>
<th></th>
<th>Nest opening width</th>
<th>Male length (mm)</th>
<th>Displacement fanning (s)</th>
<th>Display (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand volume of the nest</td>
<td>-0.510**</td>
<td>0.041</td>
<td>0.388*</td>
<td>-0.394*</td>
</tr>
<tr>
<td>Nest opening width</td>
<td>-0.222</td>
<td>0.155</td>
<td>-0.356(*)</td>
<td></td>
</tr>
<tr>
<td>Male length (mm)</td>
<td></td>
<td>-0.074</td>
<td>-0.394*</td>
<td></td>
</tr>
<tr>
<td>Displacement fanning (s)</td>
<td></td>
<td></td>
<td>0.414*</td>
<td></td>
</tr>
</tbody>
</table>

**p<0.01, *p<0.05, (*)p<0.1

Paper V: Genetic mating patterns studied in pools with manipulated nest site availability and two populations of Pomatoschistus minutus

In this paper, we investigated experimentally if parasitic spawning, for example sneaking, is affected by nest site availability. We used outdoor pools with a diameter of one and a half meters. Each experimental pool contained four potentially nest-building males, four females and nest sites in shortage (two nest sites), or in excess (six nest sites). Both treatments were conducted at two different sites that differ in natural nest site availability (Forsgren et al., 1996b). The study sites were Klubban Biological Station at the Swedish west coast, with marine waters and an excess of nest sites and at Tvärminne Zoological Station at the Finnish coast of the Baltic Sea, with brackish water and a shortage of nest sites (fig 1). Microsatellite-DNA analyses were performed to reveal parentage and hence parasitic spawning.
There was no difference between the two treatments in the number of eggs fertilized by another male than the nest-holder or in the number of males that were exposed to or performed parasitic spawning (fig 7). Nest-holding males were larger than non-nest-holding males (fig 8). However, non-nesting males and nest-holders, both with and without eggs, were found to spawn parasitically. Furthermore, several females spawned in two nests, which coincided with parasitic spawnings, suggesting a disturbance and thus a substantial cost to the nest-holder of parasitic spawnings in terms of lost mating success.

**Figure 7.** The proportion (mean ± SE) of nests suffering from parasitic spawning in each treatment at each site.

**Figure 8.** In each pool, nest-holding males were larger in size (body length, mean ± SE mm) than males that did not hold a nest site, regardless of study site or nest site availability treatment.
DISCUSSION
The aim of this discussion is to compare the results of the five papers of the present thesis. It will of course be very *Pomatoschistus* oriented and only key articles of other organisms are cited. See the five individual papers for a more complete discussion with regard to other studies.

**Sneakers, streakers, satellites or pirates**
Sneak, streak or pirate, what do the common goby and the sand goby do? In my opinion there is a size dependent gradient, from small obligate sneaker males, via facultative sneaking over something that best can be called “aggressive sneaking”, to “weak” piracy among large males. I have not yet seen any true van den Berghe piracy (1988) carried out by dominant males without nests, only parasitic spawning by dominant males. In other species, parasitic spawning males have the appearance of females (so called female mimics) and have also been proven to be regarded as such by the males (Gonçalves et al., 1996; Okuda et al., 2003; Neff & Gross, 2001). However, the aggression the nest-holding males show towards the small males clearly shows that this is not the case in the common goby (unpublished data from paper I, Magnhagen, 1994) or the sand goby (paper III; paper IV).

In Magnhagen’s (1992) “Alternative reproductive behavior in the common goby, *Pomatoschistus microps*: an ontogenic gradient”, males which first were placed with a nest-holding male, were later allowed to built a nest of their own. The smallest males sneaked but did not build nests, intermediate males both sneaked and built nests, whereas large males only built nests. The very largest males did not build nest at all but stole nests. However, two different types of “sneaking” were described: 1) pale males which tried to slip into the nest unnoticed and 2) aggressive males with black coloration which fought with the nest-holding male. The coloured aggressive males were larger than the pale. The sneaker males in paper I was of the same size as the pale sneaking males which did not build nests. Furthermore, in Magnhagen (1998) and Svensson and Forsgren (2003) nest-holding common goby males were spawning parasitically. In Magnhagen’s study, some males even evicted the nest-holding male for a while and therefore behaved like pirates, although a pirate with a nest of his own. In paper I, all common goby males that tried to sneak were successful at entering the nest. One male first tried to sneak into the nest, but when failing to do so he started to show aggressive colours and tried to fight himself into the nest. When also that failed he dug himself in from behind. However, the
majority of the sneaker males were cryptic throughout the observations (data not presented in paper I).

In the sand goby, the pattern is similar. Furthermore, several microsatellite-DNA studies have proven parasitic males to fertilize eggs (paper III, paper V, Jones et al., 2001b; Jones et al., 2001a; Malavasi et al., 2001). In addition, we have found a true sneaker male morph with large testes among small males that only show rudimentary breeding coloration (C. Kvarnemo and O. Svensson, unpublished data). Furthermore, exactly as in the grass goby Zosterisessor ophiocephalus (Scaggiante et al., 1999) and in the black goby Gobius niger (Rasotto & Mazzoldi, 2002), they were sexually mature, with huge testes but only rudimentary seminal vesicles (involved in mucus secretion), thereby differing from males in breeding coloration. Such males have not been found among common gobies in our study population, although they reportedly occur in other populations (Magnhagen, 1992; Magnhagen, 1999). In paper II no extensive observations were performed, but at least some of the sand goby sneaker males were of a size large enough to build nests and may therefore also have been regarded as nest-site competitors. In the first experiment of paper III, the same type of sneaker males was used as in paper II. The sneaker males were smaller than the nest-holders and most of them were pale and tried to slip into the nest. However, a few showed aggressive colouration. For example, one sneaker male first displayed towards the female then towards the male and then entered the nest. The nest-holding male pulled him out by his peduncle. In the second experiment in paper III, we used even smaller males, some with only rudimentary breeding coloration. We know from the above mentioned study (C. Kvarnemo & O. Svensson, unpublished) that such males are sexually mature: when dissecting all sand goby males in catches taken early and late in the season, no immature males were found. We used such small males since they were predicted not to build nests but only to spawn parasitically. Those males behaved in a very cryptic, still vigilant, way and showed clear interest in the females. Therefore, the same type of small sneaker males was also used in paper IV. In neither of these set-ups were the sneaker males allowed to actually sneak, but in the summer 2004 I also observed such males with rudimentary breeding coloration to sneak. In paper V, we were primarily interested in nest building males and if nest-holders spawn parasitically with each other. Therefore, the smallest males were avoided. In that study we found that all types of males certainly spawn parasitically, including males without nest, males with nest but without eggs and males with eggs. Thus, nest-holding males do spawn parasitically and the type of piracy that
Magnhagen (1998) described for the common goby also exists in the sand goby. However, since the fish were not observed in paper V, we use the term “parasitic spawners” for all of them.

**Patterns of parasitic fertilization**

*Paper III* shows that sneaker males managed to sneak both before and during mating. The same was true in *paper I* (unpublished data). Importantly, our microsatellite DNA analyses show that sneaker males could fertilize eggs even when only entering the nest before spawning. This is possible since sand goby sperm are motile in sea water for at least two hours (O. Svensson & H. Elofsson, unpublished data) and, as mentioned before, goby males attach sperm-containing mucus by rubbing their anal-urogenital area towards the ceiling of the nest. In *paper III* and in Magnhagen (1992), not only the nest guarding males but also the sneaker males were observed upside down in the nest, rubbing their anal-urogenital towards the ceiling.

In half of the cases in which a sneaker was observed to enter the nest, parasitic fertilization was successful, and in no case had eggs been fertilised without us having observed a sneak entry. This was true even in the cases where spawning occurred after our observation had ended. Furthermore, the time the sneaker male stayed in the nest was correlated with fertilisation success. Therefore, sneak entry in combination with the duration of the sneaking was a good estimate of paternity.

**The Pomatoschistus nest**

In both the common goby and the sand goby, the obvious reason to build a nest is to protect the offspring from predation, as an exposed egg mass would be eaten within minutes. In *paper IV*, females spawned significantly more often in the nests covered by most sand, whereas nest-opening width had no-effect on female mate choice. Consistently, in *paper III*, manipulated nest-openings of nests with controlled sand volume had no effect on female choice or behaviour. A similar result has earlier been found in the common goby, in which females prefer to spawn in manipulated nests with thick cover of sand and small nest-openings that were also discovered by egg predators less often (Jones & Reynolds, 1999a). Even though the nests were not manipulated in *paper IV*, female choice of a thick sand cover is very likely. Nest-building in *Pomatoschistus* is affected by condition (Kvarnemo et al., 1998; Jackson et al., 2002) and dissolved oxygen (Lissåker et al., 2003) and, therefore, fulfils parts of the essential
assumptions for sexually selected ornaments (Kotiaho, 2001). Also paper II suggests that nest-building is condition dependent since time to nest-building was correlated with nest-opening width 24 hours after the first traces of nest-building. Similarly, in the common goby a large nest-opening and nests that were not covered before spawning has been shown to be associated with lower future hatching success, due to less filial cannibalism (Kvarnemo et al., 1998). Barber et al. (2001) also found that in the three spined stickleback, G. aculeatus, variation in nest appearance was correlated with the time to nest-building, as well as immunological traits and secondary sexual characters. Direct benefits by choosing a well covered Pomatoschistus nest also include reduced adult predation by birds (Lindström & Ranta, 1992). Thus, sand cover may be a more reliable estimate of male and nest quality, than the highly variable nest opening width. This may explain why females seemed to pay attention to this one nest character but not the other, even though the two were highly correlated. However, egg predation pressure does not make males to decrease their nest openings, neither before mating (paper II) nor when males are protecting eggs (Jones & Reynolds, 1999b).

In Paper II males build smaller nest-openings when held with smaller males compared to when held alone suggesting that a small nest-opening has importance for protection against sneaker males. This could also suggest that the nest-holding males reduced the size of the nest-openings because they perceived the smaller sneaker males as a threat not only as cuckolders, but also as competitors for females, for nest-sites or as future egg predators. However, the results from our five-treatment experiment indicate that the nest holders did not primarily regard these sneakers to be egg predators or nest-site competitors, because they did not react by reducing the nest-opening in the presence of a potential egg predator and nest-site competitor, namely common goby males. It is likely that the benefit of a small nest-opening before mating would be low in terms of reducing the risk of future egg-predation, both because egg-predation cannot occur when there are no eggs in the nest, and because males often have to rebuild the narrow opening after spawning anyway. Consequently, assuming that common gobies are not much more severe egg-predators than sand gobies, it is unlikely that the reason for building the extra-small nest-openings in the presence of small sand gobies is to decrease the risk of egg-predation. Similarly, common gobies are probably more severe nest-site competitors than small sand gobies that are known to be poor nest-site competitors (Magnhagen & Kvarnemo, 1989). Therefore, since the nest-holding sand gobies did not even reduce their nest-openings in the presence of common gobies, it is unlikely that they reduced their
nest-openings to prevent the smaller sand goby males from taking over their nests. However, it is possible that they perceived an increased competition over the choice of the females (see paper IV). Furthermore, we did not measure nest volume of sand, which may further explain the results.

In paper III, we found no behavioural or genetical support for the hypothesis that the nest functions as a physical defence, concealment, or that sneaker males prefer to sneak upon nests with wide nest-openings. Most sneak entries occurred when the male was occupied with courtship, chasing the other sneaker male or nest building. However, it is still possible that the nest opening works as a concealment over larger distances or in other circumstances. Paper V showed that nest holding males were larger regardless of nest site availability. However, nest site availability had no effect on any measurement of parasitic spawning. The same was true in Jones et al.’s (2001b) field study on the same two sand goby populations. However, in the common goby, (Borg et al., 2002) and in the freshwater goby Rhinogobius sp. OR (N. Okuda, personal communication Okuda et al., 2003), the shape of territory or breeding site availability has been shown to affect mating tactics.

Why do not all individuals build large nests?
In both Pomatoschistus and birds, there is evidence for advantages of building a large nest. Soler et al. (1998) stressed the question: “Why don’t all individuals build a large nest?” They came up with three different explanations supported by data on birds. First, large nests are more conspicuous, and as such suffer more from predation. However, in the common goby and the sand goby, the data suggest that the well-built nests suffer less from predation (Jones & Reynolds, 1999a; Lindström & Ranta, 1992). Secondly, there can be a cost of nest-building in terms of adult predation. This may very well be important in both gobies, although never tested. When males are moving sand upon the nest by tail beats they are conspicuous, at least to the human eye. The third suggested cost is the carrying of nest material, which is likely in Pomatoschistus as well (Kvarnemo et al., 1998; Lissåker et al., 2003). Finally, and maybe most importantly, in fish, a male has to fan the eggs to provide oxygen-rich water to the developing eggs, whenever the eggs are spawned in a nest that limits the water exchange around them. After spawning, a nest with a small nest opening may be very costly to the male in terms of energetic expenditure on fanning. It is very likely that nest-guarding males have to trade off the
benefits from a small nest opening against the increased fanning costs, and that such a
trade-off is particularly important for energetically limited males.

**Paternal care and paternity**

*Filial cannibalism*

Filial cannibalism is as strange phenomenon in which the parent eats its own progeny.
Strange since the first adaptive explanation is more than twenty years old (Rohwer, 1978)
and still there is no consensus, probably due to several reasons to cannibalize (Manica,
2002). In paper I, we found that clutches that suffered from partial cannibalism were
smaller than those that did not, and that the largest clutches never suffered from
cannibalism. We proposed that males do not eat when their nest is full or close to full
since they will not try to attract additional spawning females to the nest. Forsgren et al.
(1996a) obtained the same result in the sand goby, even though their significant negative
correlation becomes a trend when full clutch cannibalism is removed from the data-set.
Those two studies are the only ones showing partial clutch cannibalism to decrease with
clutch size (Manica, 2002) and even the opposite has been found (Okuda et al., 2004). On
the other hand full clutch cannibalism is clearly a threshold in which the smallest clutches
are consumed (Manica, 2002). Consistently, in paper III, the smallest clutches tended to
be consumed (in paper I no clutch was completely cannibalised), which has also earlier
been shown in both the common goby (Kvarnemo et al., 1998) and the sand goby
(Lissåker et al., 2003). In paper III, female display towards males and their later
hatching success were positively correlated, suggesting that females are able to assess
future cannibalism. This is in concordance with Forsgren (1997a), where females
preferred to spawn with males, which later proved to have high hatching success.
However, in paper III, male display was not correlated with future cannibalism, and in
Svensson et al. (2004) sand goby male display tended to be *positively* correlated with
cannibalism, suggesting that male display may be an unreliable cue for females to base
their mate choice on. In Svensson and Forsgren, (2003) common goby females showed
no preference for fed males (only visual cues available), even though these males later
had a higher mating success.

*Paternity assurance and paternal care*

In paper I we measured paternal care by filial cannibalism, nest defence and fanning and
in paper III only by filial cannibalism. In both paper I and paper III, paternity
assurance (sneak intrusion) had no effect on the level of paternal care (although the p-value was quite low in paper III). In paper III we also measured paternity with microsatellite DNA which had no influence on care. Our result is consistent with studies on the fifteen-spined stickleback *S. spinachia* (Östlund-Nilsson, 2002) and *S. tinca* (van den Berghe et al., 1989). On the other hand, Neff and colleagues have in both experimental and field studies found correlations between male care and paternity in the bluegill sunfish, *L. macrochirus* (Neff & Sherman, 2003; Neff, 2003a; Neff & Gross, 2001; Neff, 2003b). Also in the scissortail sergeant, *A. saxatilis*, experimental intrusion by non-nesting males affected care (Manica, 2004). Full clutch cannibalism is clearly a threshold response such that small clutches are consumed (Manica, 2002). Results by Okuda and Yanagisawa (1996a; 1996b) suggest that this threshold is not universal but individual. Therefore, in theory sneaking may push a male with a small clutch over the threshold. However, the clutches in paper I (no full clutch cannibalism) and paper II (full clutch cannibalism only tended to be correlated with clutch size) may have been too large to get an effect of sneaking. In birds, which often differ by providing biparental care, numerous studies have shown decreased care as an outcome of decreased paternity assurance, whereas many others have found no effect. Also theoretical models give different outcomes and some assumptions must be fulfilled to even suggest an effect of paternity on male care (Neff & Sherman, 2002; Sheldon, 2002).

**Sperm competition – the male perspective**

What does the nest holding *Pomatoschistus* males do to prevent parasitic spawning? First, they are very aggressive towards the sneaker males (unpublished data from paper I, paper III, paper IV; Magnhagen, 1994) which is a clear equivalent of mate guarding in other taxa (see Birkhead & Møller, 1998). Interestingly, in the male behaviour experiment in paper IV, the individual males increased their numbers of bouts of mucus trail preparations, when they were exposed to a sneaker male. As mentioned earlier, this mucus may contain spermatozoa. Thus, most likely, the nest-holding males increased their mucus trail effort as a consequence of a perceived increase in sperm competition risk (see the “Female choice and male courtship” section for an alternative explanation). Nest-holding males also stayed for a longer time completely inside their nest when the sneaker males were present. However, we have no reason to believe that they were hiding. First, the male was engaged in as many non-cryptic behaviours (fanning, being outside the nest and displaying) when there was a sneaker male present compared to
when there was not. Second, if males experiencing increased sperm competition risk were reluctant to spawn, we would have expected fewer spawnings in the presence of sneaker males in the mating success experiment. However, this was clearly not the case. This is in contrast with males of the common goby (Magnhagen, 1998), the Mediterranean wrasse *Symphodus ocellatus* (Alonzo & Warner, 1999) and the three-spined stickleback, *G. aculeatus* (Le Comber et al., 2003). However, in contrast to all those species (Alonzo & Warner, 1999; Rowland et al., 1991; Borg, 1985; Magnhagen, 1998; Borg et al., 2002) sand goby males have been documented not to be choosy (Kvarnemo & Forsgren, 2000) and at the study site females do not need to compete over males (Forsgren et al., 1996b). The adaptive significance of the nest as protection against sneaker males is discussed in “The *Pomatoschistus* nest” section of this discussion.

**Sperm competition – the female perspective**

Both common goby (*paper I*, Magnhagen, 1998) and sand goby females (*paper III*) court sneaker males. However, in *paper III*, sneaker males clearly used female behaviour against the nest-holding male as their main cue for sneaking opportunities whereas male behaviour had no effect. In *paper IV*, female spawning decision was not affected by the presence of sneaker males. However, female ignorance is what may be predicted. First, it is hard to see any large benefits of sneaking to the female: fertilization rate is close to 100% in the sand goby whether or not there are sneaker males present (personal observation). Second, the pelagic larval stage (low philopatry) and huge population size and therefore most likely outbred population make inbreeding avoidance or genetically variable offspring unlikely benefits. On the other hand, two of our females split their clutches between the two nests. In *paper V*, female clutch splitting was highly correlated with males losing paternity to other males. Therefore, spawning interruption is a likely female cost of parasitic spawnings. Except for the disturbance, it is hard to see any large female costs of sneaking: when a sneaker male manages to enter the nest, the preferred nest-holding male still fertilizes the majority of her clutch (*paper III*, Malavasi et al., 2001) and the males do not increase their rate of filial cannibalism (eating their own progeny) or reduce fanning and nest defence when experiencing sneak intrusion or lost paternity (*paper I; paper III*). Furthermore, due to the lack of female preference for sneaker males in *paper IV*, we have no reasons to believe that sneakers are of extraordinary genetic quality by having “good” or exclusive sneaker genes although we cannot rule out this possibility yet.
Female choice and male courtship

Sand goby females are known to use multiple cues in their choice of mate; courtship (Forsgren, 1997b), nest attributes (paper IV, see the “The Pomatoschistus nest” part of this discussion), displacement fanning (Pampoulie et al., 2004), male size (Forsgren, 1992; Kvarnemo & Forsgren, 2000), breeding colouration (Forsgren, 1992) and sound during nest inspection (Lindström & Lugli, 2000) are all documented choice criteria or at least used in courtship whereas olfactory cues as in the black goby, G. niger (Colombo et al., 1980) are waiting to be tested. Different cues may have different contents and provide information about male condition and forthcoming care as well as advertises indirect genetic benefits. The different cues may also be serial and one trait may be important to get a female to inspect the nest, while the final decision may be taken after nest inspection. This is exemplified by the bitterling, Rhodeus sericeus, in which the initial decision is based on male behaviour and colour, whereas the final decision is based on whether the mussel (in which the female places the eggs) already contains eggs or not, which in turn affects hatching success (Candolin & Reynolds, 2001). However, different contents of cues do neither explain, suggest nor exclude correlations.

In the partial correlation (paper IV), on sand volume of the nest, nest opening width, male length, displacement fanning during 90 min (no eggs in the nest) and courtship display during the first five minutes of displaying, the numbers of significant correlations are astonishing. Thus, our main result is that these traits are correlated, and that they are so both positively and negatively with regard to what is expected to be preferred by females. Therefore, if only measuring one of the traits, there is a risk of drawing the wrong conclusions. This result also suggests that males are able to evaluate their own qualities and then might be able to use their best means to court the females (i.e. different courtship tactics). Different courtship tactics is a feasible explanation for the otherwise contradicting result that males which fan well also build large nests or display intensely (but not both). We found a negative correlation between male size and courtship, a correlation that has been found in other studies as well (Reynolds, 1993; Magnhagen, 1998). The trade offs between traits and their opposing, coinciding or even manipulative messages are probably quite complicated e.g. by differing in different predation and light regimes (Reynolds, 1993). For a discussion of possible causalities, see the discussion of paper IV.

A feasible, but less likely, alternative explanation of our result in paper IV is that the increased mucus preparation effort was a reaction to a perceived increase in mating
competition. Yet, spawning *G. niger* males react with aggression but not with increased mucus preparation on foreign mucus trails (and they do not react at all on trails from sneaker males suggesting those to be chemically silent) (Locatello et al., 2002). Still, the mucus may contain sexual pheromones which attract females (Colombo et al., 1980). Consistent with this, the frequency of anal-urogenital rubbing in *Z. ophiocephalus* has been shown to increase in the presence of females (Ota et al., 1996). The mucus may also protect the eggs from infection (Fishelson, 1991) and females may gain direct benefits from choosing a nest well prepared with mucus. In this context, it is also possible to hypothesise “good” gene or condition indicator mechanisms. However, if the main purpose of mucus trail preparation was to signal one or several of these traits to attract females, the males would have been expected to increase their courtship display and displacement fanning effort as well, which they did not. Furthermore, if there was a strong female preference for mucus-prepared nests, we would have predicted females in the mating success experiment (paper IV) to spawn most often in the nest next to the sneaker male (provided that also these nest-holding males increased mucus preparation when exposed to sneakers), which they did not.

**CONCLUSIONS**

Parasitic spawning was important in the mating systems of the study species and males reacted on sneakers with aggression and increased mucus trail preparation inside their nests when exposed to sneaker males. When building in the presence of when sneakers, sand goby males build smaller nest openings compared to when they were alone or in the company of common goby males. However, no behavioural or genetical support for the hypothesis that the nest functions as a physical or visual defence, or that sneaker males prefer to sneak upon nests with wide nest-openings, were found. Sneaker males, on the other hand, seem to tune in on females courting nest-holding males, but nothing else. Neither male nor female spawning decision was affected by the presence of sneaker males, and sneak intrusion or paternity was not taken into account into paternal care. Several females spawned in two nests, which coincided with parasitic spawnings, suggesting a cost of disturbance for the females and thus a substantial cost to the nest-holding males in terms of lost mating success. In contrast to the presence of sneaker males, the sand volume of the nest seems to be important in female mating decision and males which fan well also build large nests or display intensely (but not both) suggesting different male mating tactics. Regardless of nest site availability, also nest holding males,
both with and without eggs, spawned parasitically which has implications for the opportunity for sexual selection in the species.

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