Lise Cats Myhre

Effects of the Social and Physical Environment on Mating Behaviour in a Marine Fish

Thesis for the degree of Philosophiae Doctor

Trondheim, November 2012

Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
Department of Biology

NTNU – Trondheim
Norwegian University of
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Tight lines,

Lise Cats Myhre
(aka. FiskeLise)

Trondheim, November 2012

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LIST OF PAPERS

1. **Lise Cats Myhre**, Karen de Jong, Elisabet Forsgren and Trond Amundsen. 2012. Sex roles and mutual mate choice matter during mate sampling. *American Naturalist* 179: 741 – 755.

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DECLARATION OF CONTRIBUTION

1. The study was planned and the methods developed by all authors. LCM conducted most of the field work with significant help of a field assistant and some help from KdJ, EF and TA. LCM analysed the data and the paper was written by LCM, EF and TA with comments from KdJ.
2. The study was initiated by LCM and developed in discussions with EF and TA. LCM executed the experiment, analysed the data and wrote the paper with contributions from EF and TA.
3. LCM, SW and TA planned the project. SW developed the personality test protocol, in discussion with TA and EF. LCM executed the experiment with some help from SW. LCM analysed the data and wrote the paper with contributions from EF, SW and TA.
4. SW and TA planned the study together with IM. IM executed the experiment with help from a field assistant. IM analysed the data with help from SW and the paper was written by IM, LCM and TA with contributions from SW.

INTRODUCTION

According to Darwin (1871) sexual selection results from non-random variance in fitness due to differential ability to obtain mates and to reproduce. Today, the definition of sexual selection includes both pre-mating (see below) and post-mating processes (e.g., sperm competition and cryptic female choice, Parker 1970, Birkhead and Møller 1993). It is common to distinguish between intra-sexual selection and inter-sexual selection. In the pre-mating phase intra-sexual selection arises when members of the same sex compete with each other for access to the opposite sex (or for breeding opportunities or resources, Clutton-Brock 2007, 2009). This can lead to the evolution of, for instance, large body size and weaponry in the competing sex (Andersson 1994). Inter-sexual selection by mate choice occurs when members of one sex preferentially mate with certain individuals of the opposite sex. Mate choice can lead to the evolution of extravagant ornaments like bright colours and long tails, but also complex vocalizations and distinct courtship behaviours (Andersson 1994, Kraaijeveld et al. 2007).

How individuals search and sample potential mates, and the behavioural dynamics within and between the sexes is important for understanding sexual selection processes. A number of strategies have been proposed for how an individual can search for and select a mate. Most mate sampling strategies can roughly be classified as either best-of-*N* or threshold strategies (Janetos 1980, Wittenberger 1983), but also more complex mate sampling strategies have been proposed, taking repeated sampling, simultaneous sampling, cognitive abilities, and uncertainties in the decision process into account (e.g., Luttbeg 1996, Hutchinson and Halupka 2004, Wiegmann and Angeloni 2007, Wiegmann et al. 2010, Castellano and Cermelli 2011).

Mate search is determined by a balance of costs and benefits. The benefit is obvious, acquiring the highest quality or 'best' mate possible. However, the 'best' mate might differ from individual to individual, and hence, the optimal strategy for obtaining this mate might also differ (Wiegmann and Angeloni 2007). Choosiness reflects the effort an individual is prepared to invest in finding and assessing mates (Jennions and Petrie 1997, Widemo and Sæther 1999). Thus, the cost of choice should influence choosiness (Crowley et al. 1991, Johnstone et al. 1996, Johnstone 1997, Kokko and Johnstone 2002, Gowaty and Hubbell

2009). Potential costs include time and energy spent searching for mates, deteriorating breeding prospects for species with a short breeding period, predation risk, harassment by the opposite sex, and lost mating opportunities (Real 1990, Reynolds and Gross 1990, Kokko and Monaghan 2001). Empirical studies agree with theory and report less mate discrimination under increased cost of travel (e.g., Milinski and Bakker 1992, Booksmythe et al. 2008), increased predation risk (e.g., Forsgren 1992, Godin and Briggs 1996) and less time before the end of the mating period (e.g., Backwell and Passmore 1996).

An individual's choosiness might also depend on the physiological condition or quality of that individual, as well as on environmental and social factors (Parker 1983, Jennions and Petrie 1997, Cotton et al. 2006, Candolin and Salesto 2009). Under circumstances where both sexes are choosy (mutual mate choice, Johnstone et al. 1996, Johnstone 1997) and there is high variance in mate quality in both sexes, assortative mating based on mate quality could occur (Parker 1983, Johnstone et al. 1996, Holveck and Riebel 2010).

Many factors can affect the strength of sexual selection. In order to understand the selection processes in nature, and to avoid an overly simplistic view of animal mating systems, we need knowledge of the factors and processes that lead to variation in sexual selection (Ahnesjö et al. 2008), and how this variation relates to the social and physical environment. There are many ways to measure the strength of sexual selection. In this thesis I relate to the following two: (1) opportunity for selection**,** a measure of the standardised intra-sexual variation in reproductive success (Crow 1958, Wade 1979, Wade and Arnold 1980, Shuster and Wade 2003). This measurement determines the maximum strength of sexual selection (Jones 2009), and (2) selection differentials, the mean trait of breeding individuals compared to the mean trait of all the individuals of one sex in the population (Lande 1979, Lande and Arnold 1983).

The social environment

An individual's social environment includes conspecifics (and heterospecifics) that the individual interacts with. The social environment could therefore be influenced by population size, density, sex ratio, mate availability and levels of competition. Sexual selection acting on males and females is influenced by social interactions between individuals (e.g. Oh and Badyaev 2010).

One important aspect of the social environment in a mating context is the strength of mating competition. The strength of mating competition has implications for the behaviour, reproductive success and productivity of females and males (e.g. Forsgren et al. 2004). One factor proposed to determine mating competition is the ratio of males and females ready to mate, the operational sex ratio (OSR: Emlen and Oring 1977). If mature females are a limiting resource for male reproduction, male-male competition will dominate (e.g. Emlen and Oring 1977, Clutton-Brock and Parker 1992, Kvarnemo and Ahnesjö 1996, 2002). If, on the other hand, ready-to-mate females are more abundant than ready-to-mate males (female-biased OSR), females are expected to compete for matings (Vincent et al. 1992). Sex roles are defined according to which sex competes most for mates (Vincent et al. 1992). Accordingly, conventional sex roles are generally found in populations with a male-biased OSR and reversed sex roles in populations with a female-biased OSR (but see Kokko and Jennions 2008).

Traditionally, the fact that male mating competition predominates in many species has been explained by sex differences in investment in the offspring caused by anisogamy (Trivers 1972). Males can produce more gametes than females and therefore increase their reproductive success by mating with as many females as possible (Bateman 1948, Andersson 1994). Females on the other hand, which have a greater investment in each egg, should be more careful in their choice of mating partners. Gametes are only part of the investment into offspring in many animals. Parental investment is defined as any effort that raises offspring survival at the expense of the parent's ability to invest in other offspring (Trivers 1972). Parental investment includes behaviours such as brooding, feeding the young and predator defence. A sex difference in parental investment often leads to a sex difference in the frequency with which each sex can engage in reproductive events (Kvarnemo and Ahnesjö 1996, Parker and Simmons 1996). The potential reproductive rate (PRR: the potential offspring production per unit time; Clutton-Brock and Parker 1992) can be used to predict the direction of mating competition and affects the OSR because it determines the availability of females and males that are ready to mate (Clutton-Brock and Vincent 1991, Clutton-Brock and Parker 1992, Kvarnemo and Ahnesjö 1996, Parker and

Simmons 1996, Ahnesjö et al. 2001). But also other factors, such as adult sex ratio and the cost of breeding have been proposed to affect the strength of mating competition (Kokko and Monaghan 2001, Kokko and Johnstone 2002, Kokko and Jennions 2008, but see Simmons and Kvarnemo 2006). It is important to note that competition and mate choice do not need to reflect opposite sex roles, individuals of one sex can both compete and exert mate choice (Jones and Hunter 1993, Owens et al. 1994, Amundsen 2000, Amundsen and Pärn 2006).

The social environment does not necessarily need to be fixed. It could change due to, among other factors, changes in density, dispersal, adult sex ratio, OSR, and sex biased mortality. For example, the strength of mating competition might vary not only among species, but also spatially and temporally within species as a result of variable OSR (Emlen and Oring 1977, Clutton-Brock and Parker 1992, Kvarnemo and Ahnesjö 1996, Kvarnemo and Ahnesjö 2002), changes in food availability (e.g. Gwynne and Simmons 1990, Kvarnemo and Simmons 1999), or nest availability (e.g. Forsgren et al. 1996, Borg et al. 2002). Processes of sexual selection could be affected by the sexual dynamics both within and between the sexes due to variation in mating competition (Forsgren et al. 2004, de Jong et al. 2009), and density (Kokko and Rankin 2006). However, as pointed out by Johnstone (1997), most theoretical models of mate search have ignored the effects of competition and mutual mate choice. To fully understand mating dynamics and sexual selection, we clearly need a better understanding of mate sampling and the choice and competition processes involved under variable social environments.

The physical environment

The physical environment a species inhabits includes abiotic factors like temperature, minerals, structural complexity, altitude, currents, wind, salinity, and turbidity, as well as biotic factors such as food availability, predation risk, and the spatial distribution of resources and shelter. Many of these factors can influence both internal and external processes important in a mating context. In the aquatic habitat, for example, temperature might affect timing of maturation and oogenesis in fish (Bromage et al. 2001, Levy et al. 2011), while currents might affect the cost of mate searching (Milinski and Bakker 1992, Booksmythe et al. 2008). The transmission of signals could be affected by turbidity (reviewed in van der Sluijs et al. 2011), and the strength of competition among individuals could be affected by the spatial distribution of resources essential for mating (e.g., food for nuptial gifts: Gwynne and Simmons 1990, nests and territories: Forsgren et al. 1996, Borg et al. 2002).

Physical elements in the environment, ranging from the features of the landscape, to smaller objects such as plants, to very fine structure such as grains of sand, make up the structural complexity of habitats. Habitat complexity might affect the amount of shelter available for the animals living in the habitat. It might also influence communication by visual or other means, which could have impacts on mate and competitor detection (Hibler and Houde 2006, Candolin et al. 2007), both of which could have implications for the strength of sexual selection.

Moreover, the environment an individual inhabits might not be constant, but can change over time and space. These changes could occur relatively fast, even within one breeding season. Changes in the physical environment could influence the direction and strength of sexual selection through effects on the environmental potential for polygamy (Emlen and Oring 1977). This includes encounter rate of mates and competitors, the spatial distribution of resources, and the duration of the breeding season (a long breeding season gives time to obtain more mates), which might all have implications for sexual interactions between the sexes.

Given the tremendous natural (and human-induced) variation in how habitats are structured, it is both surprising and unfortunate that effects on mate search, and on intra- and inter-sexual interactions are little studied in regard to habitat variation. Also, how resources necessary for breeding (e.g. nests) are distributed in the physical environment might have pronounced effects on the behaviour within and between the sexes. This is also surprisingly little studied (but see Reichard et al. 2009, Saraiva et al. 2009).

Individual variation in behaviour

Individual differences in behaviour have often been assumed to be non-adaptive variation that surrounds an adaptive population mean (Dall et al. 2004). However, these individual differences are often not random, but consistent, even in different contexts (e.g. Huntingford 1976). Animal personalities are defined as consistent differences between individuals in their behaviour across time and context (e.g. Gosling 2001, Réale et al. 2007). The existence of personalities have been documented in many animals (for review see Gosling 2001). Variation in the environment has been proposed as an explanation for the evolution and persistence of animal personalities (Dall et al. 2004, Smith and Blumstein 2008, Schuett et al. 2010). Different personality types might constrain an individual's response to both biotic and abiotic factors, or different personalities might be selected for under different circumstances (Sih et al. 2004, 2012, Schuett et al. 2010). However, little is known about how personality relates to behaviours important in sexual selection (but see Schuett et al. 2010, Colléter and Brown 2011).

AIMS OF THE THESIS

There is a need for increased knowledge on how variation in the environment affects sexual selection. The main objective of this thesis is to investigate how variation in the social and physical environment affects the mating behaviours of females and males, and how this, in turn, affects sexual selection. I also investigate if male personality relates to male mating behaviour. Reproductive dynamics are central aspects of an organism's life, which also have consequences on the population level.

I especially aim to:

- Investigate how variation in the social environment affects female and male behaviour during female mate sampling (Paper 1).
- Determine if variation in the physical environment affects female and male mating behaviours, reproductive success and the opportunity for selection (Papers 2 & 4).
- Explore if there is a relationship between male personality and male mating behaviours, and examine whether the expression of behaviours for certain types of males co-varies with the structural complexity of the physical environment (Paper 3).

To investigate these topics I used the two spotted goby, *Gobiusculus flavescens,* as a model species. It has unusual sex roles that switch from conventional to sex role reversed over the breeding season (Forsgren et al. 2004). In the early part of the breeding season there are typically many ready-to-mate males and a shortage of ready-to-mate females. As the breeding season proceeds, mating-ready females become plentiful, and female mating competition predominates (Forsgren et al. 2004). This makes the species highly suitable for studying how the social environment (the strength of mating competition) affects behaviours during mate search under natural conditions.

The ecology of the two-spotted goby also makes it a good model species for investigating the effects of variation in the physical environment on processes of sexual selection. It inhabits environments ranging from gravel-dominated bays with almost no structure to structural complex kelp forests. Additionally, clusters of filamentous algae grow on the existing macro alga, making the habitats more structurally complex as the season progresses. Male two-spotted gobies also need to occupy a resource that can be used as a nest to be able to reproduce (e.g. typically empty mussel shells or brown alga in the wild; Gordon 1983, Amundsen and Forsgren 2001, Svensson 2006). The occurrence of nests can be highly variable in nature, from highly aggregated to dispersed. This makes it possible to study effects of habitat complexity and nest distribution on sexual selection within natural limits for this species. In addition, parasitic spawning is rare in this species (Mobley et al. 2009), making it possible to estimate reproductive success for males without using genetic analyses.

MODEL SPECIES: The two-spotted goby

Ecology

The two-spotted goby, *Gobiusculus flavescens* (Fabricius), is a small (adult total length 35-55 mm), marine fish that normally lives for only one year (Johnsen 1945). It is sexually dimorphic during the breeding season (fig. 1). Females develop round and orange bellies as gonads and eggs mature, but the belly colouration varies among ready-to-mate females (Amundsen and Forsgren 2001, Svensson et al. 2005, 2009). Males have iridescent blue spots along the lateral line and alternating blue and dark red fields on their enlarged dorsal fins.

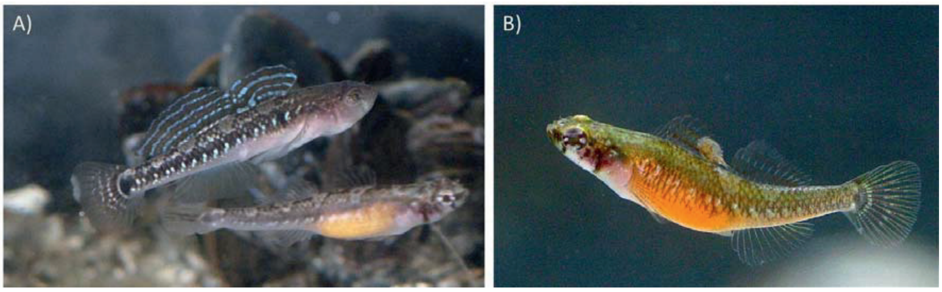

Figure 1: (A) A male two-spotted goby making a fin display to afemale in the background, and (B) a female two-spotted goby making a sigmoid display (with an ecto-parasitic copepod attached to the first dorsal fin). Photographs by A, Elisabet Forsgren, B, Trond Amundsen.

The two-spotted goby is very common along the rocky shores of Western Europe (Collins 1981) and can be found from northwest Spain to Vesterålen in northern Norway (Miller 1986). It inhabits a range of habitats in the shallow algal vegetation (0-15 m depth) in protected and moderately exposed areas. The species is often associated with macro algae vegetation such as sugar kelp, *Saccharina latissima* (fig. 2A), *Laminaria* spp., and *Saccorhiza polyschides* (Wheeler 1980, Svensson 2006), which by themself creates a highly structured environment. In addition, as the breeding season progress, the habitat becomes even more structured by clusters of filamentous algae and the flourishing of an invasive species *Saragassum muticum* (fig. 2B). The two-spotted goby is also common in gravel-dominated bays where the environment is more open, only with patches of various algae (*Fucus* spp.) creating some structure. Two-spotted gobies are semi-pelagic (Wheeler 1969). They often form quite stationary, loose, large groups in close association to the algae vegetation (Svensson et al. 2000), and seek shelter among the algae when threatened by predators (Utne et al. 1993, Utne and Aksnes 1994). Their diet usually consists of copepods, particularly pelagic species, the larvae of crustaceans and small worms (Wheeler 1969, Berg 1979, Costello et al. 1990). Two-spotted gobies can be very numerous along the Scandinavian coasts and have an important role in the plankton-planktivore-piscivore food chain here. Together with copepods and codfish, gobies make up what is perhaps the most important food chain in many coastal environments in Norway (Fosså 1991, Giske et al. 1991, Hop et al. 1992, 1993).

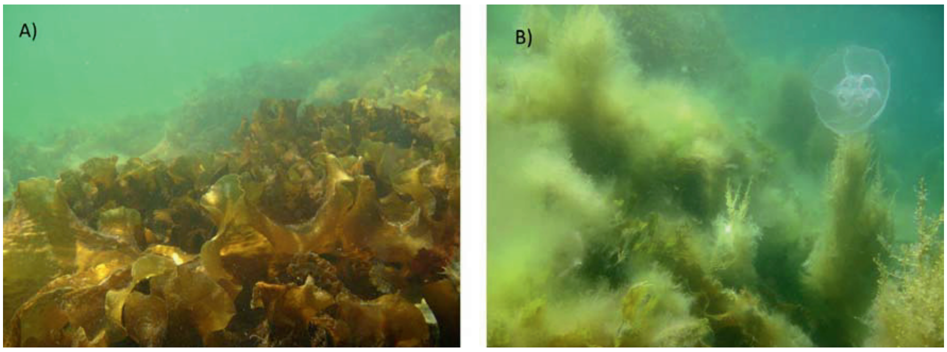

Figure 2: Natural two-spotted goby habitat can be variable depending on season and exposure. (A) Sugar kelp early in the breeding season, and (B) another, more sheltered locality late in the season with clusters of filamentous algae growth on the existing macro algae and an invasive species *Saragassum muticum* flourishing (bottom right in picture B), making the habitat more structurally complex. Photographs by Trond Amundsen.

Reproductive biology

The species is a substrate brooder where males provide all the parental care. Breeding males are stationary and take up a nest in shallow waters (< 5 m depth) in mussel shells, on kelp leaves, or in crevices (Gordon 1983, Amundsen and Forsgren 2001, Svensson 2006). Females deposit clutches of typically 1000-1500 eggs (Pélabon et al. 2003, Svensson et al. 2006) in a single layer in a male’s nest and the male fertilizes them. Males may simultaneously care for clutches from several (2-6) females (Gordon 1983, Mobley et al.

2009). Males tend the eggs by guarding, fanning and cleaning them until hatching (Skolbekken and Utne-Palm 2001, Bjelvenmark and Forsgren 2003). The eggs hatch after one to three weeks, depending on water temperature (Skolbekken and Utne-Palm 2001, Svensson et al. 2006). Both sexes can reproduce repeatedly during their single breeding season (Collins 1981, de Jong 2011). Consequently, the choices made during their only breeding season define their whole lifetime reproductive success.

Both sexes display distinct courtship behaviours, and both can initiate courtship. Males use a suite of courtship displays. A male will typically start to court a female by first swimming up to the female while erecting his dorsal and anal fins (fin display, fig. 1A). When laterally close to the female he may then quiver/vibrate his body (quiver display). This can happen repeatedly before the male tries to lead the female towards his nest by swimming with undulating body movements (lead display) (Amundsen and Forsgren 2001, Pélabon et al. 2003). Females court males by bending their body to a sigmoid shape (sigmoid display, fig. 1B), seemingly to show off their distended orange belly (Amundsen and Forsgren 2001).

Agonistic behaviour occurs most often within the sexes, but can also sometimes occur between the sexes (mostly males chasing females). Males are generally side-by-side when they compete visually by raising their fins (fin display) and tilting the head slightly downwards. Male-male agonistic behaviour also includes chases which might involve biting if physical contact occurs (Forsgren et al. 2004, Wacker et al. 2012). Such chases appear to function in defence of territories or nest sites. Females rarely perform competitive chases, likely because they do not defend physical resources. Instead, females compete by showing sigmoid displays to other females (Forsgren et al. 2004).

Mate choice has been demonstrated in both sexes of the species in laboratory studies (Amundsen and Forsgren 2001, Borg et al. 2006). Females have been found to prefer large males early in the season, but show little discrimination late in the season (Borg et al. 2006). Males, on the other hand, seem to pay little attention to female size (Pélabon et al. 2003), but show a strong preference for females with more orange-coloured bellies (Amundsen and Forsgren 2001), although, male mate preference is found to be affected by male size (Amundsen and Forsgren 2003).

METHODS

In this chapter I will briefly explain the methods used in this thesis. For detailed descriptions of the methods see the individual papers.

Study area

The laboratory and field work for this thesis was based at the Sven Lovén Centre for Marine Sciences (SLC) in Kristineberg in the period 2008-2011. The research station is situated at the mouth of the Gullmarsfjord (58^{o} 15' N, 11^{o} 27' E) in Bohuslän, on the west coast of Sweden (fig. 3).

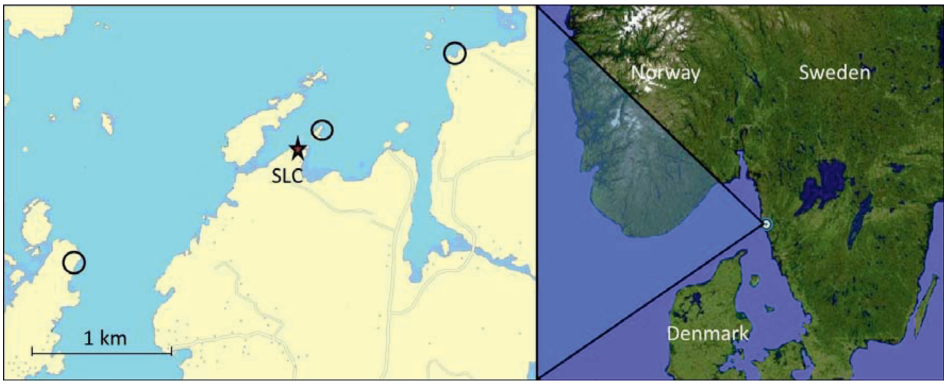

Figure 3: The study area on the west coast of Sweden. The research station (SLC) where the laboratory studies (Paper 2, 3 and 4) were carried out is marked by a star. The fish for the laboratory studies were collected from areas less than 2 km from the research station. The circles indicate the study sites where the female mate sampling study was conducted (Paper 1). Maps were redrawn from kart.gulesider.no.

Fish collection and marking

Fish were caught with hand held dip nets while snorkelling in the shallow waters (< 5 m depth) around islands < 3 km from the research station. Healthy-looking individuals were selected and transported back to the research station by boat in large, covered, plastic containers. At the laboratory the fish were kept in single sex aquaria. All fish were fed twice a day *ad libitum* with freshly hatched *Artemia* nauplii.

For individual recognition of the fish, both in the field and in the laboratory, they were marked with Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Shawn Island, Washington). Before being marked, the fish were anaesthetized with a solution of di-phenoxy-ethanol and seawater (120 µl:1 litre). The fish were marked with one to three thin colour stripes, each 4-5 mm long, next to the dorsal fins. After marking, each fish was put in a cup of fresh seawater and regained normal swimming behaviour within 5 minutes. No change in behaviour was detected after marking.

Behavioural observations

When observing fish, both in the field and the laboratory, the observer always tried to stay as still as possible and sudden movements were avoided. The distance between the observer and focal fish were 0.5-3 meters, depending on water transparency (field), and where in the tank the fish was swimming (laboratory). Behavioural data were continuously recorded during the observations using a notepad and a stopwatch.

During observations we recorded every female-male encounter and behaviours performed and received within a range (2-3 body lengths) from the focal fish. For both sexes we noted courtship behaviour (females: sigmoid displays directed at males, males: fin displays directed at females, quiver displays, and lead swim), agonistic behaviour (females: sigmoid displays directed at other females, males: fin displays directed at other males, and chases), as well as nest entries of both sexes. For Papers 2 and 3 we also noted where in the tank the sexual interactions occurred and female movement patterns.

Field observations

Field observations (Paper 1) were done by snorkelling. The presence of an observer did not seem to influence the natural behaviour of the fish. However, recordings were not started until the fish assumed normal swimming after release (typically within < 5 minutes). Observations lasted for an average of 32 minutes.

Laboratory experiments

While field studies allow a high degree of realism, experimental manipulation of important parameters are often only feasible in the laboratory. By using large mesocosm tanks we increased realism by giving the fish space to move and allowing natural social groupings of the fish. In Papers 2-4 we used 2x2 m grey tanks with a water depth of approximately 35-40 cm. The tanks were kept indoors under controlled lighting, with a continuous flow of seawater (from 7 m depth) and water temperatures following the natural sea-temperature.

In all our laboratory studies we provided artificial nests for the males. The nests consisted of a PVC-tube (80 mm long and 13 mm inner diameter), with an acetate sheet inside, attached to a rock with a rubber band for stability. A nest-tube can hold eggs from approximately four females (Bjelvenmark and Forsgren 2003, Forsgren et al. 2004). The inside area of the nest-tube is very similar to natural mussel nests in our study area. Both in the lab and the field two-spotted gobies readily spawn in our artificial nests (e.g. Amundsen and Forsgren 2001, Forsgren et al. 2004, Svensson et al. 2006, de Jong et al. 2009, de Jong 2011). These artificial nests were used to track males' reproductive success over the duration of the experiments. We recorded every morning and evening which males had obtained a nest and the egg cover in the nest. Regardless of treatment, we always kept some structural elements in the tanks. In addition to the artificial nests they were also equipped with plastic plants.

Focal observations were conducted at least one day after the initiation of a trial. The fish need some time to acclimatize in the tank before they establish a breeding population. For both Paper 2 and 3 focal females were released into the experimental tanks at a later stage than the initial males and females. This was done because (1) we wanted to record behaviours from an already established breeding population, (2) when releasing fish into a tank where the existing fish are calm they exhibit less stress behaviour, and (3) males would focus their interest on the newly released females.

Manipulation of the physical environment

In papers 2 and 3 we manipulated the habitat complexity in the tanks, to be either simple or complex (fig. 4A, B). This was done by adding opaque (white) plexi-glass dividers in one of the treatments to increase the structural complexity. The dividers were formed in such a way that they did not preclude movement across the tanks, the fish could both swim under, over and slip past the edges of the dividers. The bottoms of the tanks in both treatments were marked in sections with tape to ease the recordings of female movement.

In paper 4 we manipulated the distribution of nests to be either dispersed or aggregated (fig. 4C, D). Nests were either placed in the central part of the tanks with the nest openings 10 cm apart, or along the sides 60 cm apart. The number of nests and the distribution of artificial algae were kept constant.

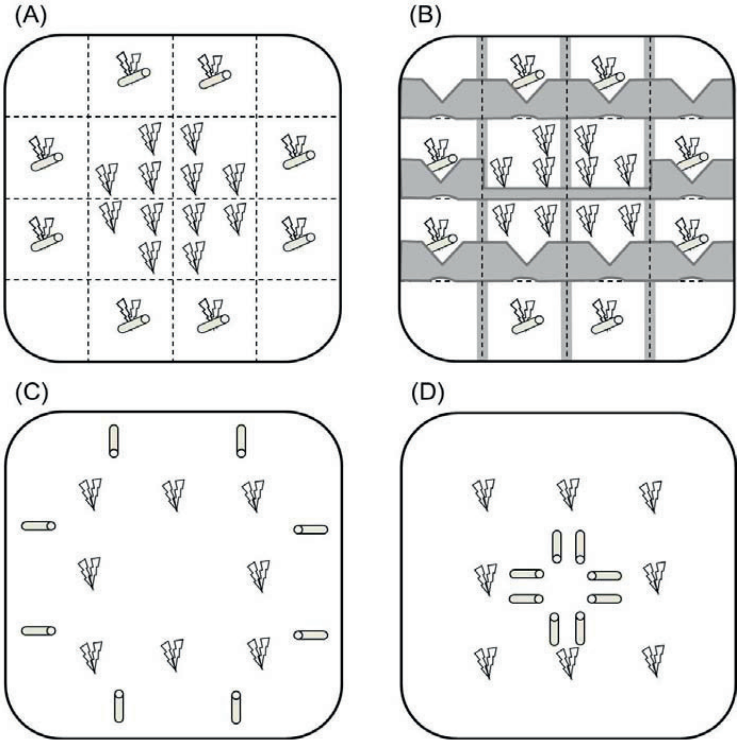

Figure 4: Design of the experimental set-ups of manipulations of habitat complexity and nest distribution. The habitat complexity (Paper 2 & 3) was manipulated to be either (A) open, with only artificial nests and plants as structural elements, or (B) structurally complex, where long dividers across the tank (grey area) reduced visual range and contact among the fish. The dividers had the same shape for both dimensions. The nest distribution (Paper 4) was manipulated to be either (C) dispersed with the nests being 60 cm apart or (D) aggregated with the nest in the centre of the tank and 10 cm apart.

Personality assay

We conducted an 'emergence-test' to assess male personality on all males used in Paper 2 before they were assigned to a treatment. This test measures an individual's propensity to leave a safe area (i.e. refuge) and enter an unknown or less safe area. This is a standard personality test used in fish and is mostly interpreted to reflect an individual's boldness (e.g., Brown and Braithwaite 2004, Brown et al. 2005, Scharnweber et al. 2011). The choice of leaving or not leaving shelter (as in the 'emergence test') is one that two-spotted gobies face regularly in their natural environment, where they move between the open water and the kelp forest. In Paper 3, we separate between 'bold' and 'shy' individuals as those that emerged from shelter within 30 minutes, and those that did not, respectively, after a 30 min acclimatization period.

MAIN RESULTS

Paper 1: Sex roles and mutual mate choice matter during mate sampling

A drastic change has previously been documented in the social environment over the breeding season for two-spotted gobies (Forsgren et al. 2004). However, little is known about how this change in the social environment affects the natural behaviour of females and males during mate sampling. To investigate this we released and observed individually marked ready-to-mate females (one by one) in their natural habitat (for release sites see fig. 3) in the early and late breeding season (predominately male and female mating competition, respectively). The females were followed by snorkelling until mating or until they were lost out of sight of the observer.

We found females to engage in substantial mate sampling, visiting a median of 5.5 males (range 1 – 40, $N = 28$) before mating. Mate sampling females typically swam in one main direction along the algal vegetation, visiting males. Females rarely turned back to re-visit males, indicating that they used some kind of threshold strategy when mate sampling. We found encounter rates with males and the number of males sampled before mating to be affected by the social environment. Females experienced a three times higher male encounter rate per minute and visited more males before mating during the early- compared to the late breeding season. Also, the number of courtship events before mating was higher in the early compared to late season. As expected, we found female mating competition to increase from the early to the late season (fig. 5A) and female agonistic displays occurred often when multiple females courted the same male in the wild. Interestingly, as female mating competition changed, the sexual dynamics between the sexes also changed dramatically. Females initiated a lower proportion of the courtship events under low mating competition (early season) than they did under high mating competition (late season) (fig. 5B). In accordance with this, females also terminated a higher proportion of the courtship events in the early than in the late season (fig. 5C). Courtship initiation can be interpreted as a reflection of sexual eagerness, whereas courtship termination could be interpreted as a sign of choosiness. Our results suggest that females became more eager to mate and less discriminating as the season progressed and there were fewer available mating options. Males, on the other hand, likely became more discriminating as the season progressed since they got more ‘mating willing’ females to choose from.

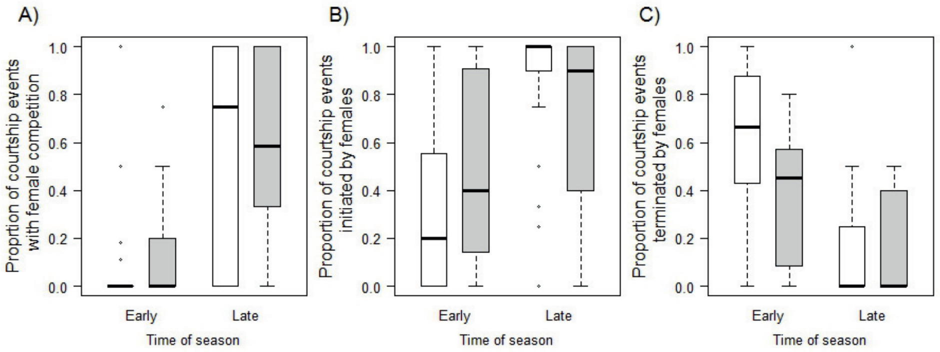

Figure 5: Sexual interactions during mate sampling in the two-spotted goby early and late in the breeding season. Proportion of courtship events (A) that involved multiple females courting the same male, (B) initiated by females, and (C) terminated by females. Open boxes, mate-sampling females that did not mate during observations (early N = 41, late N = 47), shaded boxes, females observed until mating (early N = 18, late N = 10). Boxplot details: the thick lines represent the median, the top and bottom of the boxes represent the seventy-fifth and twenty-fifth percentiles, and the dashed error bars extend to the most extreme data point ≤ 1.5 times the interquartile range from the box. Outliers are shown as separate data points.

Paper 2: Effects of habitat complexity on mating behaviour and mating success

To determine if a more structurally complex environment affected female and male mating behaviours and the opportunity for selection we conducted a laboratory based experiment where we manipulated the physical environment in large tanks to either be open (without visual/physical barriers, fig 4A) or structured (with visual/physical barriers, fig. 4B). Eight males and eight females were introduced to the tanks with either simple or complex habitat and allowed to breed. At day two, we introduced focal females to the already established breeding population and recorded their mate search behaviour in addition to behaviours from encountered males. Male reproductive success (egg cover in the nest) was recorded over the duration of the experiment.

We found female movement patterns to be constrained under increased habitat complexity. The females swam around less (fig. 6A) and visited fewer unique male nest sections per minute during their mate search in the complex compared to the open habitat. In the complex habitat the females also experienced a lower courtship rate (fig. 6B), were courted by fewer different males before mating, and females had a longer search time before mating. Furthermore, in complex habitats fewer females experienced direct male intra-sexual mating competition (agonistic behaviours and simultaneous courtships) during their mate search (fig. 6C). Our results suggest that female mate searching and male courtship of females is reduced in more complex environments. Although we found no effect of habitat complexity on male reproductive success or mating skew, we detected a selection for larger males in the open habitat, whereas length had no effect on the likelihood that a male got mated in the complex habitat. This suggests that the sexual selection pressure might have been more relaxed in the complex compared to the open environment.

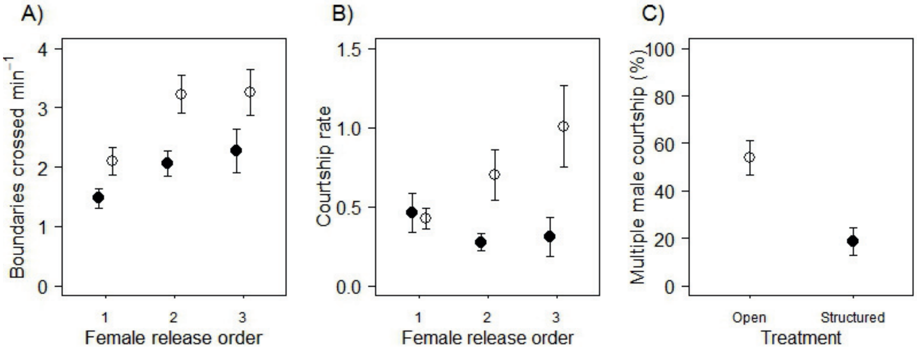

Figure 6: The effects of habitat complexity (open environment: open dots; complex environment: filled dots) on (A) number of section boundaries crossed per minute, (B) the number of courtship events per minute, and (C) the percentage of females that experienced several males courting them simultaneously in each environment. Each point represents mean ± 1SE. Analyses in A and B are separated by female release order. For discussion of effects of release order see Paper 2.

Paper 3: How does personality relate to mating behaviour?

To explore the relationship between male personality and mating behaviours we first assessed individual male personalities using a standard personality assay, the emergence test. The personality assay was conducted before the initiation of the experiment. Both

Papers 2 and 3 are based on data from the same experiment. After the personality assay, males were introduced to the tanks with either a simple or a complex habitat (fig. 4A, B) and allowed to breed. Male mating behaviours were recorded during interactions with focal females released at day two. We recorded identity and behaviour of all the males that interacted with these females. In this way we only recorded male behaviour in a mating context. Also, male reproductive success was recorded over the duration of the experiment.

We found boldness, as quantified in an emergence-assay, to relate to several aspects of mating behaviour. Bold males showed a higher propensity to court encountered females, especially in the complex environment where shy males courted a much lower proportion of females encountered than the bold males in the same environment (fig. 7A). This suggests that the physical and social environment should be taken into consideration when interpreting behaviours in relation to personality. Independent of environment treatment, bold males had a higher proportion of courtship events close to their nest and were more likely to mate during observations (fig. 7B). Accordingly, at the end of the experiment bold males had more eggs in their nest than shy males (fig. 7C). These findings show that personalities expressed in a standardized personality assay are reflected in sexual behaviours and realized mating success and, hence, could have fitness consequences.

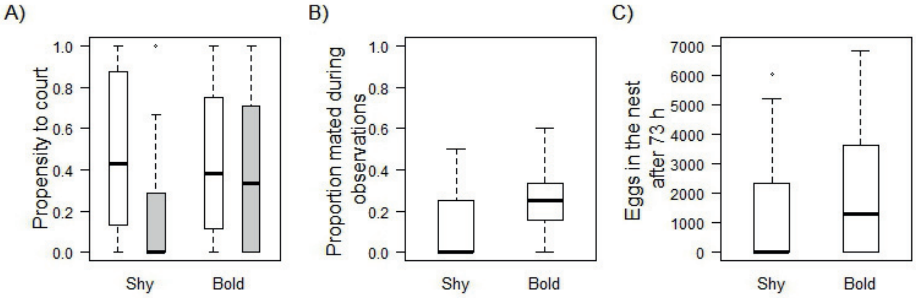

Figure 7: Male personality in relation to (A) propensity to court in the open (open bars, N_{shy} = 36, N_{bold} = 83) and complex (shaded bars, N_{shy} = 30, N_{bold} = 87) environment, (B) proportion of all shy (N = 73) and bold (N = 186) individuals that mated during observations, and (C) number of eggs in the nest at the end of the experiment for all individuals. Boxplot details: see fig. 5.

Paper 4: Nest distribution affects behaviour and mating success

In this study we tested how the distribution of a breeding resource (nests) affected mating competition, male mating success and the opportunity for selection. This was done by manipulating the distribution of nests to be either aggregated or dispersed in large tanks (fig. 4C, D). We introduced 8 males and 16 females into each tank. Focal observations of each male were conducted for 10 min per male on day one and two of the experiment. During focal observations we recorded agonistic and courtship behaviour of both sexes. Additionally, we recorded nest occupancy and male reproductive success every morning and evening of the experiment.

We observed behavioural differences between the treatments. When nests were aggregated, a higher proportion of the males that had acquired a nest displayed agonistic behaviours (fig. 8A) and females courted a higher proportion of the nest holding males compared to when nests were dispersed (fig. 8B). This suggests that both sexes experience higher intra-sexual competition when nests are aggregated.

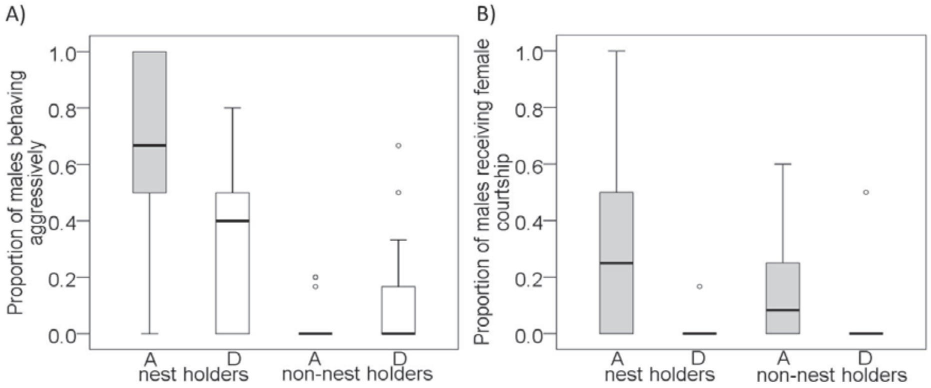

Figure 8: Behavioural differences between treatments wh2ere nest distribution was either aggregated (A, shaded bars) or dispersed (D, open bars) for nest holders (N_A = 11, N_D = 13) and non-nest holders (N_A = 14, N_D = 14). Proportion of two-spotted goby male nest holders and non-nest holders that (A) behaved agonistically, and (B) was courted by females during observations on day two. Boxplot details: see fig. 5.

The aggregation of nests led to a lower proportion of males occupying nests, lower proportion of mated males, and lower male reproductive success during all stages of the experiment. Consequently, there was a higher mating skew (opportunity for selection) when the nests were aggregated. Resource (nest) distribution pattern seems important for an individual's ability to monopolize the resources and the opportunity for selection (i.e. variation in reproductive success).

DISCUSSION

In this thesis I have shown that changes in both the social (Paper 1) and physical (Paper 2, 3 & 4) environment affect the mating behaviour of both females and males (fig. 9). I found that when the environment changes, the encounter rate of both potential mates and competitors also change. Courtship behaviour, female movement pattern, time to mating, and the level of agonistic behaviour were all influenced by the social and/or the physical environment. These are all important mating behaviours affecting sexual selection processes. Thus, my research has demonstrated that changes in the environment could lead to changes in essential mating behaviours, which again could lead to a greater variation in the strength of sexual selection under variable or shifting environments.

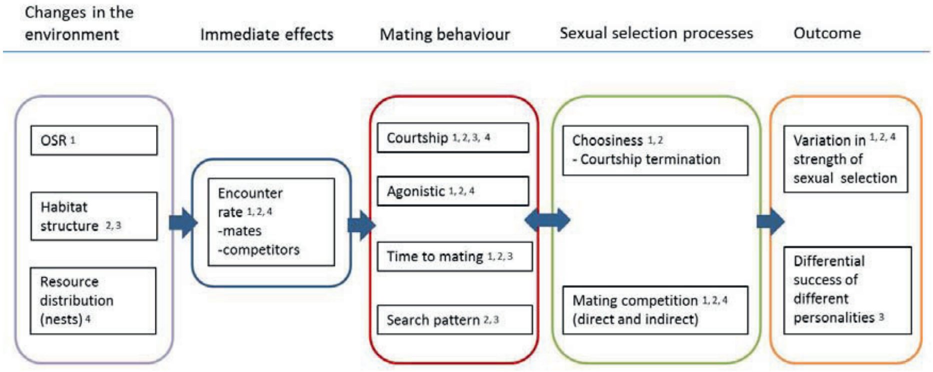

Figure 9: A simplified schematic presentation of how some environmental changes can affect sexual selection processes, and hence, cause variation in the strength of sexual selection. The arrows in the figure indicate the main processes and effects discussed in the text. However, most factors are interrelated in some way. Numbers refer to the different papers (1 = Paper 1, etc.).

In many empirical studies of sexual selection, classical mate-choice setups are used where an individual can choose between, but not freely interact with, two or several individuals (e.g. Berglund 1994, Amundsen and Forsgren 2001, Engström-Öst and Candolin 2007, Heuschele et al. 2009, Sundin et al. 2010). In this thesis, however, I investigated effects of the environment on sexual selection in nature (Paper 1) and under more natural conditions (Paper 2-4). Thus, my studies allows for more realistic interpretations of what is happening in nature: from the immediate effects on the encounter rate and how this in turn affects behaviours which could have implications for mating and reproductive success, and hence, the strength of sexual selection (fig. 9). Also, when focusing on the processes, rather than the patterns, of selection, the underlying mechanism of selection becomes more evident (Wade and Kalisz 1990).

Environmental effects on social interactions

Mate search

I found the immediate effect of changes in both the social (Paper 1) and physical (Papers 2 & 4) environment to be on the encounter rates of potential mates and competitors. In territories of male threespine sticklebacks (*Gasterosteus aculeatus*), a reduction in structural complexity was also found to increase female encounter rates (Candolin and Voigt 2001). Theory predicts mate encounter rate to influence effort invested in mate search (Kokko and Wong 2007). However, there is likely a reciprocal relationship between mate search patterns and encounter rates of prospective mates (and competitors). The movement pattern of individuals in search of a mate influence encounter rate, and encounter rate of prospective mates influence the mate search pattern. My results indicate that the physical complexity of the environment affected female movement patterns (Paper 2). Female movement was restricted in the complex environment and females experienced a lower mate encounter rate in this environment (Paper 2). Mate locating behaviour has also been found to differ between habitats of disturbed, open forest, and intact, dense forest in a butterfly (Bonte and Van Dyck 2009). In many fish species with paternal care, including the two-spotted goby, males are constrained from leaving their territory/nest. Females are therefore the more active sex in mate searching. However, I found males to initiate courtship more often in the central part of the tanks (away from their nests) in the complex compared to in the open environment, which is environments with low and high encounter rates, respectively (Paper 2). This suggests that males also adjust their effort in locating mates according to encounter rates of females. The number of potential mates assessed during mate search has implications for mate choice, and thus the operation of sexual selection (Jennions and Petrie 1997, Benton and Evans 1998).

I found female two-spotted gobies to show extensive mate searching in the wild, visiting several (median 6, range 1 – 40) males before mating (Paper 1). This high number of males visited is generally higher than observed in the few mate sampling studies conducted on fish (sapphire devil, *Chrysiptera cyanea*: Gronell 1989, beaugregory damselfish, *Stegastes leucostictus*: Draud et al. 2008, sand goby, *Pomatoschistus minutus*: Forsgren 1997, peacock blenny, *Salaria pavo*: Fagundes et al. 2007). However, many males sampled does not necessarily mean a high level of female mate discrimination. My findings revealed that if males are choosy, females may have to extend their mate search to find a 'willing male' (Paper 1). Thus, the classical assumption that males are always available as a mating partner is not always valid. Furthermore, if males more readily accept high quality females, such females may have a less extensive mate search than low-quality ones. This is in contrast to the general expectation that high quality females can afford a more extensive search (e.g. Cotton et al. 2006). Unfortunately, we do not have the data to tell how individual female quality relates to mate search in two-spotted gobies. This could be an interesting topic for future research.

Courtship

A high encounter rate of potential mates gives a higher opportunity to court and a higher chance of being courted (Kokko and Rankin 2006, de Jong et al. 2012). I found courtship rates to be affected by both the physical (Paper 2) and social (Paper 1) environment. This was probably due to the immediate effects of the social environment experienced. Choosiness is predicted to be influenced by encounter rates of potential mates and competitors (Crowley et al. 1991, Kokko and Monaghan 2001, Servedio and Lande 2006, Gowaty and Hubbell 2009, de Jong et al. 2012). I found support for this prediction in Paper 1. Females terminated more courtship interactions when they experienced a high mate encounter rate and there was little female intra-sexual competition (Paper 1). Under these conditions there was probably also a low cost of being choosy (no lost mating opportunities) (e.g. Real 1990, Reynolds and Gross 1990, Berglund 1995).

A recent model by de Jong et al (2012) suggests that when competitors become abundant and potential mates scarce, individuals respond by becoming more eager to court. I found support for this prediction: fewer mating options and higher intra-sexual competition induced females to court more (Paper 1 & 4). When nests were aggregated fewer males acquired a nest (Paper 4), thus, fewer males were available for mating. Fewer available mates could lead to higher female intra-sexual competition (Paper 1 & 4; Ahnesjö et al. 2001, Forsgren et al. 2004). Similar results were found in the peacock blenny (Saraiva et al. 2009). Also, when nests were aggregated, females visiting a male might simultaneously be exposed to many mating-ready-males which could induce more female courtship.

Interestingly, I also found that personality influenced eagerness to court. Compared to shy males, bold males courted a higher proportion of encountered females, especially in the complex environment (Paper 3). Engaging in courtship is risky (e.g. Magnhagen 1991), and emerging into open spaces (as in the emergence-test) is also risky. Similar response to these two types of situations is suggestive of a consistent personality difference in risk-taking. The complex environment might be perceived as more risky by the fish since they had less overview of the tank and could not see potential predators hiding nearby. Therefore boldness could have a greater effect on behaviour in complex environments.

Agonistic behaviours

As with courtship, agonistic behaviour might depend on the rate of encounters of potential competitors (de Jong et al. 2012). There is a greater scope for direct competition when nests/resources are aggregated (as in Paper 4; Emlen and Oring 1977), under high densities (Reichard et al. 2004), biased OSR/high mating competition (as in Paper 1; Jirotkul 1999) or in environments without visual obstructions (as in Paper 2; Hibler and Houde 2006). When the distribution of nests was aggregated a higher proportion of the males behaved agonistically (Paper 4). Males might have invested more in nest defence and in maintaining a territory around the nest, excluding other males from neighbouring nests when nests were aggregated, and thus, excluding other males from the mating game (Kokko and Rankin 2006). In Paper 2 I found habitat complexity to affect male competition. During mate search, more of the females experienced simultaneously courtship and male-male aggression (Paper 2). Male-male competition could ensure honesty of male signals (Candolin 2000). It could also help females to compare males: two-spotted goby females need to compare males directly to be able to discriminate between males of different size (Å.A. Borg, E. Forsgren and T. Amundsen, unpublished data). However, courtship interference could also constrain females in choosing freely between potential mates (Qvarnström and Forsgren 1998, Kangas and Lindström 2001, reviewed in Wong and Candolin 2005).

The results of my studies (Papers 1 – 4) indicate that the mate choice and competition processes are affected by the physical and social environment. These effects seem to mainly occur due to the immediate effects the environment has on the social structuring of individuals, their behaviour, and the rates of encounters between potential mates and competitors.

Environmental effects on mating success

Time to mating

The physical environment affected time until mating (Paper 2 & 4). I found that females took a longer time before mating in the complex compared to open environment (Paper 2). My results also show that it took longer time before females got to mate when nests were aggregated compared to when nests were dispersed (Paper 4). A prolonged time period before (or between) matings might seem insignificant. However, in this short lived species with only a single breeding season, even a short delay could have severe fitness consequences through negative impacts on life time reproductive success. In cases of high female intra-sexual competition, females have been observed to lose their eggs (late breeding season, personal observations). If females fail to obtain a mate and spawn, an entire batch of eggs is lost since her eggs will be overripe within a few days (e.g. Kjørsvik et al. 1990, Legendre et al. 2000) and she will miss an entire breeding cycle.

High levels of male mating competition have been found to affect spawning rate (zebrafish, *Danio rerio*: Spence and Smith 2005) and longevity (sand gobies: Lindström 2001), and are thus likely to affect fitness of males. I found male-male competition to be promoted in open habitats (Paper 2), and when breeding resources were aggregated (Paper 4). Also, the nests of males contained less eggs when nests were aggregated than when nests were dispersed

(Paper 4). Continuous agonistic interactions at the expense of courtship could ultimately lead to lost mating opportunities or no matings (e.g. Santangelo et al. 2002, Reichard et al. 2004, Spence and Smith 2005).

My results show that personality also affected time until mating. Bold males mated faster with a newly released female compared to the shy males (Paper 3). This was probably due to bold males courting more often and courting closer to their nests (Paper 3). There could be a trade-off between boldness, longevity and reproduction (i.e. a ‘pace of life’ syndrome, Réale et al. 2009). Bold males may take higher risks and die younger but mate quicker compared to shy males who take lower risks, experience longer time between matings, but survive longer.

Potential for selection

I found the distribution of nests to affect the potential for sexual selection (Paper 4). This is in accordance with findings on bitterlings, *Rhodeus amarus* (Reichard et al. 2009). There was a stronger mating skew when the nest distribution was aggregated – matings were more evenly distributed among males when nests were dispersed (Paper 4). Other characteristics of the physical environment that potentially could influence sexual selection are the complexity of the habitat and turbidity. I found no difference in mating skew among male two-spotted gobies due to habitat complexity (Paper 2). Others have found that matings are more evenly distributed among individuals under limited visibility, leading to a lower potential for selection (three-spined stickleback: Candolin 2004, sand goby: Järvenpää and Lindström 2004). One explanation for the different results could be that the temporal distribution of mates differed between studies. In Paper 2 females arrived relative synchronously, which could affect males ability to monopolize mates (Emlen and Oring 1977, Lindström and Seppä 1996).

Selection on traits

Habitats usually cover a range of structural variability, also when it comes to complexity and light environments that might affect detectability of nearby conspecifics. I found positive selection on male size in the open environment, but not in the complex environment (Paper 2). This selection could have resulted both from female choice and male competition (Borg et al. 2006, Wacker et al. 2012), both likely affected by the environment (Paper 2, discussed above). Other studies have also found the strength of sexual selection on several traits to be relaxed under reduced visibility (e.g. Seehausen et al. 1997, Candolin 2004, Järvenpää and Lindström 2004, Candolin et al. 2007, Wong et al. 2007, Heuschele et al. 2009). Communication systems used in mate choice (visual, chemical, acoustic and electric) are often influenced by changes or disturbances in the environment (birds: Brumm and Slater 2006, fishes: van der Sluijs et al. 2011). Also costs and benefits of certain sexually selected traits might vary under different environmental conditions (Wong et al. 2007, Candolin and Heuschele 2008).

As I have discussed above, aggregation of nests induced competitive behaviour between males. Competitive ability could be affected by body condition or size in the two-spotted goby (Wacker et al. 2012). I did not, however, find any selection on these traits due to the distribution of nests (Paper 4). However, the test has limited power due to limited sample size and should be interpreted with caution. Reichard et al. (2009) documented selection on traits important for sperm competition and dominance in bitterlings when oviposition sites were aggregated. This raises the question if other traits than condition and length could be important for competitive superiority in the two-spotted goby.

CONCLUSIONS

In this thesis I have shown that:

(1) Two-spotted goby females have extensive mate search. Mate searching females revisited males rarely, and the majority mated with the last male visited. This mate search pattern fits with some kind of threshold sampling strategy. Mutual mate choice probably influenced the number of males sampled and does not necessarily reflect high sexual selection on males. The social environment influenced sexual interactions during mate sampling. Females were more choosy and initiated fewer courtship interactions under low compared to high female intra-sexual competition (Paper 1).

(2) The physical environment affected mating behaviours. A more complex habitat lowered the courtship rate, made it more difficult to compare males, and made females take longer time in finding a suitable mate. Male competition was also affected by habitat complexity. More of the females experienced male competition in open compared to complex habitats. The effects of habitat complexity on mate choice and competition processes likely led to selection on male length in the open but not in the complex environment (Paper 2).

(3) Also, the physical spacing of nests had implications for mating behaviours. The level of competition experienced by both sexes was higher when nests were aggregated than dispersed. Males showed more agonistic behaviour and females courted more when nests were aggregated. Also the number of males acquiring a nest and that got to mate was lower when nests were aggregated compared to when they were dispersed. As a consequence, the opportunity for selection was higher when nests are aggregated (Paper 4).

(4) Personality (‘boldness’) affected behaviours that had implications for male mating and reproductive success (Paper 3). Bold males were more likely to court, courted closer to their nest, and had a higher mating success. Although some relationships between boldness and behaviour were stronger in the complex environments, there were no significant effects of environment - personality interactions on mating behaviour.

In summary, my studies show that changes in the social (Paper 1) and physical (Paper 2, 3 & 4) environment affected mating behaviours in the two-spotted goby. The observed differences in behaviours are likely due to the social structuring and the encounter rates of potential mates and competitors, both of which I found to be affected by the environment. Mating behaviours both affect and reflect choosiness and intra-sexual competition, two of the main processes affecting the strength of sexual selection.

The physical characteristics of habitats vary greatly in nature. As described earlier, the complexity of an environment might stem from features of the landscape, substrate, rocks, crevices, algae and plants. This could also affect the distribution of resources necessary for breeding, such as nest sites. At the same time, human (and natural) impacts might alter habitat complexity and the distribution of resources needed for breeding. Therefore it is important to take the social and physical environment into account when interpreting or predicting sexual selection. Mating behaviour is an important aspect of an organism's life. Population is the unit that responds to changes in selection. However, it is important to have knowledge about the underlying behaviour and processes for the observed changes in selection. Also, knowledge about relationships between environmental factors and sexual selection may allow us to predict consequences of natural and human-induced alterations of the environment.

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PAPER 1

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Sex Roles and Mutual Mate Choice Matter during Mate Sampling

Lise Cats Myhre,[1] Karen de Jong,[1] Elisabet Forsgren,[2] and Trond Amundsen[1,*]

1. Department of Biology, Norwegian University of Science and Technology, NO 7491 Trondheim, Norway; 2. Norwegian Institute for Nature Research, P.O. Box 5685 Sluppen, NO 7485 Trondheim, Norway

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abstract: The roles of females and males in mating competition and mate choice have lately proven more variable, between and within species, than previously thought. In nature, mating competition occurs during mate search and is expected to be regulated by the numbers of potential mates and same-sex competitors. Here, we present the first study to test how a temporal change in sex roles affects mating competition and mate choice during mate sampling. Our model system (the marine fish *Gobiusculus flavescens*) is uniquely suitable because of its change in sex roles, from conventional to reversed, over the breeding season. As predicted from sex role theory, courtship was typically initiated by males and terminated by females early in the breeding season. The opposite pattern was observed late in the season, at which time several females often simultaneously courted the same male. Mate-searching females visited more males early than late in the breeding season. Our study shows that mutual mate choice and mating competition can have profound effects on female and male behavior. Future work needs to consider the dynamic nature of mating competition and mate choice if we aim to fully understand sexual selection in the wild.

Keywords: mate sampling, mutual mate choice, sex roles, female competition, sexual selection, *Gobiusculus flavescens*.

Introduction

Darwin (1859, 1871) identified intrasexual competition (mostly between males) and mate choice (mostly by females) as the two main processes of sexual selection. Competition for mates is normally strongest in males (“conventional sex roles”). In some species, however, female mating competition predominates (“reversed sex roles”), and in yet other species mating competition regimes are temporally or spatially variable (“dynamic sex roles”; Darwin 1871; Emlen and Oring 1977; Berglund et al. 1986; Gwynne and Simmons 1990; Vincent et al. 1992; Kvarnemo and Ahnesjö 1996, 2002; Berglund and Rosenqvist 2003; Forsgren et al. 2004). Theory suggests that the strength and direction of mating competition is determined by the operational sex ratio (OSR, or ratio of sexually active males to females; Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996, 2002) and by sex-specific costs of breeding (Kokko and Monaghan 2001; Kokko and Johnstone 2002). The OSR may also affect choosiness in both sexes, even if this link is less straightforward than that between OSR and competition (Owens and Thompson 1994). In nature, mating competition occurs at the time when, typically, females search for suitable mates and males try to attract mates. Despite this, few studies have investigated how variation in sexual competition, as reflected in OSR variation, affects male and female behaviors during mate sampling (but see Dale et al. 1992). Instead, previous mate-sampling work has typically considered the searching female as the only player in the game. However, the dynamics of male and female mating behaviors can be affected by female-female competition and by male mate choice. To our knowledge, no previous study has analyzed the dynamics of competition and choice behavior of both females and males during mate search and how these are linked to variation in OSR. This is unfortunate, as spatial and temporal variation in mating competition appears more widespread than previously thought and can sometimes lead to a complete shift in sex roles (e.g., Kvarnemo and Ahnesjö 2002; Amundsen 2003; Ahnesjö et al. 2008). Both theoretical (e.g., Johnstone et al. 1996; Bergstrom and Real 2000; Kokko and Monaghan 2001; Chenoweth et al. 2006; Servedio and Lande 2006) and empirical (e.g., Jones and Hunter 1993; Cunningham and Birkhead 1998; Kraak and Bakker 1998; Amundsen 2000; Bonduriansky 2001; Amundsen and Pärn 2006; Chenoweth et al. 2007; Kraaijeveld et al. 2007; Clutton-Brock 2009) studies suggest mutual mate choice to be widespread. Likewise, competition is no longer a male-only phenomenon; competition within both sexes has been shown to be common in species with both conventional and reversed sex roles

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[*] Corresponding author; e-mail: trond.amundsen@bio.ntnu.no.

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(e.g., Petrie 1983; Berglund and Rosenqvist 2003; Amundsen and Pärn 2006; LeBas 2006; Clutton-Brock 2007, 2009; Rosvall 2011). While female-female competition is expected to restrict female mate search (Dale et al. 1992), mutual (male) mate choice is expected to extend female mate search, as some "wanted males" may reject certain searching females. Hence, when female-female competition and/or mutual mate choice apply, mate-sampling dynamics and consequent sexual selection could be significantly more complex than the simplified "female samples–male accepts" situation.

A crucial factor for the operation of sexual selection by mate choice is the number of potential mates that are assessed ("sampled") by mate-searching individuals (Jennions and Petrie 1997; Benton and Evans 1998). Because mate search is costly, any female will assess only a subset of males during mate search (Real 1990; Crowley et al. 1991; Luttbeg 1996). However, much work on sexual selection has implicitly assumed that if females are choosy, all males are available as potential partners. If mate sampling is in reality limited, such assumptions may lead to erroneous conclusions regarding the strength of sexual selection. Despite considerable theoretical analysis (Parker 1978, 1983; Janetos 1980; Wittenberger 1983; Real 1990, 1991; Luttbeg 1996, 2002; Wiegmann et al. 1996, 1999, 2010*a*, 2010*b*; Wiegmann and Angeloni 2007; Castellano and Cermelli 2011), empirical knowledge on mate sampling is still scant, taxonomically biased (toward birds; Gibson and Langen 1996; Jennions and Petrie 1997; Amundsen 2003), and lagging far behind theory. A classical issue has been whether a mate-searching female should sample a given number of males before choosing the best ("best-of-*N*" or pool comparison) or whether she should instead sample males sequentially until finding one that exceeds a certain threshold quality ("threshold" or "sequential search" models; Janetos 1980; Wittenberger 1983; Real 1990; Wiegmann et al. 1996). The initial models have been criticized for being too simplistic, however, and recent models have included more realistic assumptions regarding memory, assessment error, information processing, and search costs (e.g., Luttbeg 1996, 2002; Wiegmann et al. 1996, 2010*b*; Wiegmann and Angeloni 2007; Castellano and Cermelli 2011). As pointed out by Johnstone (1997), however, models of single-sex discrimination may be misleading when mutual mate choice applies, and most theoretical analyses of mate search have ignored the effects of competition. If we are to fully understand mating dynamics and sexual selection, we clearly need a better understanding of mate sampling and the choice and competition processes involved.

Here, we investigate how sex role variation affects male and female behavior during female mate sampling in a small marine fish, the two-spotted goby (*Gobiusculus flavescens*). The two-spotted goby model system is uniquely suitable for testing how reproductive behavior is affected by variation in mating competition because of its dramatic change in OSR and complete shift in sex roles (from conventional to reversed) over the course of the breeding season (Forsgren et al. 2004). Early in the breeding season, there is an excess of mating-ready males, whereas late in the season few males remain, but ready-to-mate females are plentiful (Forsgren et al. 2004). Thus, a mate-searching female is expected to experience little intrasexual competition and many mating opportunities early in the season but strong female-female competition and fewer mating options late in the season. Two-spotted gobies of both sexes, like many other small littoral fishes, can breed repeatedly over the course of their single breeding season (females may spawn up to at least six consecutive broods; K. de Jong and L. R. Graña, unpublished data). Individual fish may therefore experience a dramatically changed competitive situation from their first to their last breeding.

Among fishes with parental care, the majority have male care, with guarding and fanning of eggs laid on a substrate being the most common form of care (Blumer 1982; Gross and Sargent 1985; Clutton-Brock 1991; Gross 2005; Mank et al. 2005). The two-spotted goby is such a substrate-breeding, territorial fish, with males performing uniparental care. However, paternal care per se does not cause sex role reversal. In fact, the vast majority of fishes with uniparental male care have conventional rather than reversed sex roles (Forsgren et al. 2002). This is likely because males can often accept and care for several egg clutches at the same time, implying that their potential reproductive rate is normally higher than that of females (Clutton-Brock and Vincent 1991; Vincent et al. 1992). Sex roles may, however, become reversed if the OSR is skewed toward females. This may, for example, be the case in certain populations of peacock blennies *Salaria pavo* due to nest site shortage (Almada et al. 1995) and in the late part of the breeding season of two-spotted gobies when the adult sex ratio is strongly female biased (Forsgren et al. 2004). Two-spotted gobies display mutual mate choice, with either sex potentially rejecting mating offers at any point of time, depending on circumstances. It is not known what causes the dramatic reduction in male density that regularly occurs over the breeding season in two-spotted gobies. However, potential explanations include higher predation on conspicuous, territory-defending males than on shoaling females and high male costs of competition or parental care (Lindström 2001; Forsgren et al. 2004). When, as in two-spotted gobies, males defend a spawning site, females may base their choice not only on characteristics of the male but also on the quality of the spawning site (Kodric-Brown 1983; Bisazza and Marconato 1988; Candolin and Reynolds 2001) and on whether or not there are already eggs in the nest (Reynolds and Jones 1999). In such species, territoriality also severely constrains male range, and females are the ones most active in mate search regardless of sex roles. We therefore observed male and female mating behaviors by following focal females during mate search. To our knowledge, mate choice and mating competition during mate search in a dynamic sex role system have never been investigated before.

We compared male and female mating behaviors between periods early and late in the breeding season. This represents conditions of predominant male and female mating competition, respectively (Forsgren et al. 2004), and allowed us to test predictions from sex role theory regarding sex differences in mating behavior during female mate sampling. With respect to mating competition, we predicted (1) initiation of courtship mainly by males early in the breeding season and by females late in the breeding season, reflecting which sex faces the strongest competition, and (2) consequently more cases of simultaneous female courtship and female-female aggression late in the season. With respect to mate choice, we predicted (3) termination of courtship mainly by females early in the breeding season and by males late in the breeding season, reflecting a reduction in mating opportunities for females and an increase in mating opportunities for males, and (4) fewer female rejections after nest inspection late in the season, reflecting reduced female choosiness.

Methods

Study Species

The two-spotted goby is a small (total length [TL] mostly 35–55 mm), sexually dimorphic, marine fish that is common along the rocky shores of western Europe (Collins 1981). Both sexes normally live for only 1 year (Johnsen 1945) but can reproduce repeatedly during their single breeding season (Collins 1981). During breeding (April–July in our study area), two-spotted gobies inhabit the shallow algal zone (0–5-m depth), often associated with beds of brown algae such as sugar kelp (*Saccharina latissima*; Svensson 2006). Foraging individuals often occur in large shoals (Svensson et al. 2000). By contrast, breeding males are typically stationary (Forsgren et al. 2004), defending a small area around a nest site, whereas mating-ready females often aggregate in small groups. The species is a substrate brooder, with females depositing clutches of typically 1,000–1,500 eggs (Pélabon et al. 2003; Svensson et al. 2006) in mussel shells, on kelp leaves, and sometimes under rocks (Gordon 1983; Amundsen and Forsgren 2001; Svensson 2006). In fishes, once a female has developed mature eggs and ovulated, she has a limited time window of at most a few days in which to spawn at each breeding cycle (Mollah and Tan 1983; Kjørsvik et al. 1990; Legendre et al. 2000). Once eggs have been deposited and fertilized, male two-spotted gobies defend the eggs and care for them by fanning and cleaning them until hatching (after 1–3 weeks, depending on water temperature; Skolbekken and Utne-Palm 2001; Bjelvenmark and Forsgren 2003). Males often simultaneously care for clutches from several (typically 2–6) females (Gordon 1983; Mobley et al. 2009).

Both sexes display conspicuous visual ornamentation and distinct competition and courtship behaviors (Amundsen and Forsgren 2001; Pélabon et al. 2003; Forsgren et al. 2004). Males compete by visual fin displays, showing off their erect colorful fins at close range, and by chases that may involve biting if physical contact occurs (Forsgren et al. 2004). Such chases appear to function in defense of territories or nest sites. Females rarely make competitive chases. Instead, females compete by visual, sigmoid, displays to other females, typically when multiple females court the same male (Forsgren et al. 2004). Courtship may be initiated by either the male or the female. Males attract females to their nest with a suite of courtship displays: (1) fin display, erecting their dorsal and anal fins, (2) quiver display, swimming laterally to the female while quivering their body, and (3) lead display, swiftly approaching the female before swimming with undulating body movements toward the nest (Amundsen and Forsgren 2001; Pélabon et al. 2003). Females court males by bending their body to a sigmoid shape, displaying their distended orange belly (Amundsen and Forsgren 2001). Mate choice has been demonstrated in both sexes of the species in laboratory studies (Amundsen and Forsgren 2001; Borg et al. 2006). Females have been found to prefer large males early in the season but do not show such a preference late in the season (Borg et al. 2006). Males, on the other hand, seem to pay little attention to female size (Pélabon et al. 2003) but show a strong preference for females with more orange-colored bellies (Amundsen and Forsgren 2001). Females develop more orange bellies as eggs mature, but there is also extensive variation in belly coloration among ready-to-mate females (Amundsen and Forsgren 2001; Svensson et al. 2009*b*). Orange belly coloration in females partly reflects gonad carotenoid concentration (Svensson et al. 2006, 2009*b*).

Study Sites

The study was conducted during spring/summer 2008 at the mouth of the Gullmarsfjord (58°15′N, 11°27′E) on the west coast of Sweden and was based at the Sven Lovén Centre for Marine Sciences (SLC) at Kristineberg. Data were collected from April 21 to May 31 (early breeding season; strong male but little female competition) and from June 16 to July 11 (late breeding season; strong female but little male competition).

We chose two main study localities for observations of female mate sampling, Pittlehuvud (58°14′31″N, 11°24′59″E) and Öddehålet (58°15′22″N, 11°27′57″E). We alternated observations between the two localities. A few fish were observed at a third locality, Råttholmen (58°15′3″N, 11°26′55″E), when prevailing winds prevented work at the main localities. Most of the observations (90%) were made by L. C. Myhre and K. Olsson; the remaining observations (10%) were made by E. Forsgren, K. de Jong, and T. Amundsen.

General Procedures

Females were caught with dip nets while snorkeling around islands <2 km from the SLC and brought to the laboratory by boat. We selected healthy-looking females with round, clearly distended, bellies (i.e., likely to be ready to spawn; Amundsen and Forsgren 2001; Pélabon et al. 2003; Svensson et al. 2009*b*) but did not select among these mature females with respect to individual belly coloration. We recorded female TL (to the nearest 0.5 mm) on a grid and wet body mass (BM; to the nearest 0.01 g) using a digital balance. There were no significant differences between females observed early ($N = 171$) and late ($N = 183$) in the breeding season with respect to TL (early: mean = 47.1 ± 0.2 mm, range = 40.0–52.0; late: mean = 47.0 ± 0.2 mm, range = 38.0–54.0; $t_{352} = 0.46$, $P = .65$) or BM (early: mean = 0.96 ± 0.01 g, range = 0.60–1.64; late: mean = 0.98 ± 0.01 g, range = 0.55–1.60; $t_{352} = -0.99$, $P = .32$). However, analyses of female "roundness" (standardized residuals from the regression of female TL and BM) showed that females were on average slightly less round early (mean residuals = −0.15 ± 0.09) compared to late in the breeding season (mean residuals = 0.14 ± 0.06; $t_{352} = -2.72$, $P = .007$).

Females were anesthetized with a solution of diphenoxyethanol and seawater (120 μL : 1 L) before being marked with Visible Implant Elastomer (VIE) tags (Northwest Marine Technology, Shawn Island, Washington). VIE tagging is widely used (e.g., Buckley et al. 1994) and has been shown not to affect mortality or behavior in other gobiids (Malone et al. 1999; Griffiths 2002). The method has previously been successfully adopted in two-spotted gobies (de Jong et al. 2009). Each female was marked dorsally with three thin color stripes, each 4–5 mm long. VIE-tagged females were kept in a storage aquarium for 1–2 days before release and were fed daily ad lib. with freshly hatched *Artemia* nauplii. Only three out of 410 marked females died while kept in the laboratory. None of the marked females seemed to be negatively affected by the VIE tags as judged from subsequent behavioral observations, and there was no evidence of infections caused by tagging.

Observational Protocol

Females to be released for observation were collected from other localities than the release site, to ensure they had no prior knowledge of the males they would encounter. Females were transported by boat to the study sites and were kept in floating holding pens until released. One female at a time was released at a randomly chosen spot within the locality. Mate sampling and courtship (performed and received) were observed from a distance of 0.5–2.0 m while snorkeling. The presence of the observer did not seem to interfere with the females' natural behavior, in line with previous work showing that two-spotted gobies can be observed at close range while performing natural behaviors (field: Forsgren et al. 2004; lab: Amundsen and Forsgren 2001; de Jong et al. 2009). Recording started once females assumed normal swimming after release (typically within <5 min).

Behavioral data were continuously recorded using an underwater notepad and a stopwatch. Focal females were frequently within visible range of one or more males and were recorded to "visit" if they came within 15 cm of a male, irrespective of whether courtship (by either party) occurred. At some visits, either the male or the female swam swiftly and distinctly toward the other (an "approach"). However, females often visited males without either party distinctly approaching the other or courting. If the focal female courted, or was courted by, a male she visited, we recorded this as a "courtship interaction" and noted which sex initiated courtship. The sex that first terminated sexual display or ignored sexual display was recorded as the sex that "terminated courtship." Courtship initiation and termination are crucial parameters to assess mating eagerness (initiation) and mate rejection (termination) and how these respond to changes in mating competition in males and females. We also recorded whether the visited male was simultaneously courted by several females and any cases of female-female agonistic behavior (sigmoid displays at other females). These two parameters would reflect the strength of intrasexual female competition. Mating was considered to have occurred if a focal female followed a male into his nest or disappeared with a male into the algal vegetation and remained there for more then 10 min. Sometimes, however, females followed males to their nest and briefly inspected the nest without staying to mate. Such rejections should reflect some degree of choosiness and were therefore recorded. Nest inspections were typically brief (mean = 58 s, range = 6–283 s, $N = 37$).

We recorded a "revisit" if a focal female swam away

(>1.5 m) and was out of visual range from a male before returning to the same male, whether or not other males were visited in the interim. Search time was defined as the time from commencement of normal swimming after release until mating. Mating defined the "end point" of a mate-sampling session and can reflect decisions by both parties because both males and females can be choosy in this system. With marked mate-searching females being the focal individuals of our study, and males abundant yet not individually marked, the continuous recording of female behavior and movements precluded recording how many females visited each male and detailed characteristics of males or their nests.

In total, we released 354 females (220 at Öddehålet, 128 at Pittlehuvud, and 6 at Råttholmen; the difference in numbers of females between the two main localities mainly reflects suitability of weather conditions). When released, females either swam down to the kelp vegetation to rest for some time or immediately commenced normal swimming in the shallows (0–3-m depth). Of the females released, 142 were out of sight before they showed any mate-sampling behavior, typically shortly after release. If a female could be continuously observed but did not show any mate-sampling behavior (i.e., not approaching or courting any male and not responding to male courtship) within the first 30 min (87 females), we terminated the observation. This leaves a sample of 125 females that showed mate-sampling behavior. All future analyses refer to these females, which were observed until they were out of sight or observations had to be terminated for logistical reasons ($N = 97$; mean observation time: 32.9 ± 2.0 min, range = 6.3–112.1, $N = 96$) or until they mated ($N = 28$; mean time until mating: 26.9 ± 3.8 min, range = 2.8–82.8, $N = 27$). Thus, our analyses are based on a large sample of females that performed mate sampling but did not mate during observations (early season $N = 43$, late season $N = 54$) and a smaller sample of females that mated during the period of observation (early season $N = 18$, late season $N = 10$). The behaviors of females that did not mate while being observed were largely similar to the behaviors of those that mated during observations, indicating that these females continued sampling and mated at some point after they were out of sight of the observer. We observed mate-sampling females for an average of 31.6 ± 1.8 min, with no significant difference in observation time between early (33.7 ± 3.2 min, $N = 59$) and late (29.6 ± 1.7 min, $N = 64$) season observations (ANOVA: $F_{1,121} = 0.002$, $P = .97$). In two instances, time was not recorded because the stopwatch failed.

Statistical Analyses

Data were analyzed using R (ver. 2.13.1; R Development Core Team 2011). Proportional data were analyzed using generalized linear mixed models (GLMM) with binomial error distribution of the residuals with a logit link; count data were analyzed using GLMM with Poisson error distribution of the residuals with a log link. We checked for overdispersion, and, if the model was overdispersed, we corrected for this by refitting the model with a random effect on the individual level. As detailed above, the data set for mate-sampling females comprised one group of females that mated during observations and a larger group that mate sampled but did not mate while being observed. We tested whether this factor as well as time of season had any effect on the results by including the two factors and their interaction as fixed factors in the model and locality as random factor. We also tested females that mated during observations and those that did not mate during observations separately, and we have separated the two groups in the figures. We tested the fit of the models using likelihood ratio tests, and removed nonsignificant variables. Intercepts and estimates are given on log or logit scale, depending on the error distribution used, and are presented ±1 SE. Medians are presented with range or interquartile range (IQR); means are presented ±1 SE.

Results

Mate-sampling females usually swam in one main direction along the shore line ~15–50 cm above the algal vegetation. In the early part of the season, they would mostly swim solitarily, whereas sampling females often joined one or more round females, sometimes forming small shoals, late in the season. Often, several males were simultaneously in sight of the observer, and thus likely of the sampling female, in particular early in the season. However, territorial males ("stationary males"; Forsgren et al. 2004) appeared generally to stay closer to the algal vegetation than did roaming males, and it may thus have been harder for the females to observe or assess several territorial males simultaneously. When a searching female came close to a territorial male, the male might or might not approach her; if he did approach the female, he often also made fin displays. Females did also actively approach males. Responses by males and females to opposite-sex courtship were highly variable. A courted female might ignore or actively avoid the male (swim on), follow him at close range, and/or respond with courtship displays. Similarly, a male courted by a female might ignore courtship (no change in behavior) or respond with courtship displays. In cases where mutual sexual behaviors (approach, courtship) occurred, the male might lead the female toward the nest, adopting a "lead display." The female might then "inspect" (enter) the nest and might either stay to spawn ("mating") or leave the area after one or more nest inspections. Females that visited several males sometimes temporarily interrupted sampling by joining feeding shoals.

Courtship Initiation and Termination

Focal females experienced courtship interactions (displays by the male, themselves, or both) with a median of 2 males (range = 0–37, $N = 125$), with significantly more courtship interactions early (median = 4, range = 0–37, $N = 61$) compared to late in the breeding season (median = 2, range = 0–7, $N = 64$; GLMM: intercept [early] = 1.43 ± 0.11, estimate [late] = 0.47 ± 0.17, $z = -5.81$, $P < .001$).

Over the whole season, females initiated on average 57% ± 4% of all courtship interactions. As predicted from sex role theory, mate-sampling females initiated a smaller proportion of courtship interactions early compared to late in the breeding season. This pattern was statistically significant among females not observed until mating (GLMM: intercept [early] = −0.64 ± 0.15, estimate [late] = 1.48 ± 0.30, $z = 7.16$, $P < .001$; fig. 1*A*) but not in the smaller sample of females observed until mating (GLMM: intercept [early] = −0.20 ± 0.37, estimate [late] = 0.88 ± 0.71, $z = 1.54$, $P = .12$; fig. 1*A*). There was a significant interaction between time of season and whether a female mated or not during observations (GLMM: intercept [early, unmated] = −0.65 ± 0.16, estimate [late, mated] = −2.15 ± 0.54, $z = -2.77$, $P = .005$): females observed mating initiated relatively more courtship interactions than those not mating during observations early, but not late, in the season (fig. 1*A*).

As predicted, females terminated a much higher proportion of courtship interactions (i.e., rejected males) early compared to late in the season. This pattern was highly statistically significant for the larger sample of females that did not mate during observations (GLMM: intercept [early] = 0.40 ± 0.27, estimate [late] = −1.47 ± 0.30, $z = -6.31$, $P < .001$; fig. 1*B*) but not for the smaller sample of females observed mating (GLMM: intercept [early] = −0.57 ± 0.20, estimate = −1.20 ± 0.51, $z = -1.25$, $P = .21$; fig. 1*B*). The seasonal contrasts for females observed mating and those not mating during observations were largely similar (fig. 1*B*). Despite this, there was a significant interaction effect of time of season and whether a female mated during observations (GLMM: intercept [early, unmated] = 0.39 ± 0.24, estimate [late, mated] = 1.63 ± 0.59, $z = 2.10$, $P = .036$) on courtship termination. Early in the season, females observed mating terminated fewer courtship interactions than those not mating during observations; such a pattern was not evident late in the season (when females rarely terminated courtship; fig. 1*B*).

When analyzing all visits by females to males, and not only those that involved courtship, we found that females courted a much smaller proportion of visited males early (13.6% ± 2.9%, $N = 61$) compared to late in the breed-

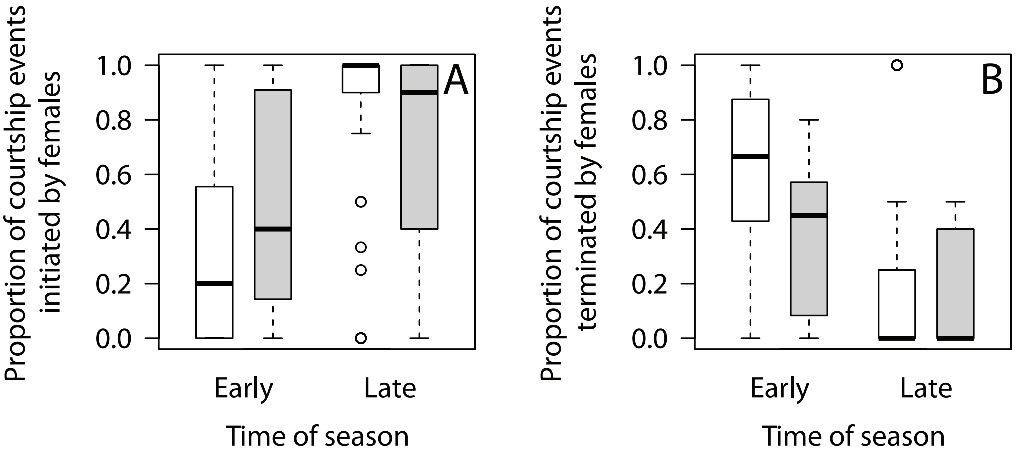

Figure 1: Sexual interactions during courtship in two-spotted gobies (*Gobiusculus flavescens*) early and late in the breeding season. The proportion of courtship interactions (*A*) initiated and (*B*) terminated by females (open boxes, mate-sampling females that did not mate during observations [early $N = 41$, late $N = 47$]; shaded boxes, females observed until mating [early $N = 18$, late $N = 10$]). The thick lines represent the median, the top and bottom of the boxes represent the seventy-fifth and twenty-fifth percentiles, and the dashed error bars extend to the most extreme data point ≤1.5 times the interquartile range from the box. Outliers are shown as separate data points.

ing season (33.3% ± 3.8%, $N = 64$; GLMM: intercept [early, unmated] = −2.16 ± 0.28, estimate [late, mated] = −0.95 ± 0.28, $z = 5.55$, $P < .001$). Females that mated during observations courted a higher proportion (40.4% ± 5.9%) of males than did females not mating during observations (20.0% ± 2.7%; estimate [mated] = −0.96 ± 0.25, $z = 4.81$, $P < .001$).

There was an interaction effect of time of season and whether or not females were observed until mating on the propensity for visited males to court the focal female (GLMM: intercept [early, unmated] = −1.10 ± 0.15, estimate [late, mated] = 3.52 ± 0.56, $z = 4.35$, $P < .001$). The propensity for visited males to court mate-sampling females not observed until mating was much higher early (median = 1, IQR = 0.67–1) compared to late in the season (median = 0, IQR = 0–0.71; GLMM: intercept [early] = 1.10 ± 0.15, estimate [late] = −0.94 ± 0.27, $z = 7.46$, $P < .001$). However, males showed a similarly high propensity to court females that mated during observations early (median = 0.83, range = 0.17–1) and late in the season (median = 0.90, range = 0.33–1; GLMM: intercept [early] = 0.69 ± 0.31, estimate [late] = 1.12 ± 0.49, $z = 0.88$, $P = .38$).

A higher proportion of females inspected nests without mating early (19 of 61 [31%] females) compared to late in the breeding season (8 of 64 [13%]; $\chi^2 = 5.4$, df = 1, $P = .021$). Among females observed mating, 13 out of 18 early-season females (72%) mated at the first nest they entered, whereas the other five inspected two to four nests before mating. Nine out of 10 late-season females (90%) mated with the owner of the first nest entered; one inspected two nests. The proportion of females mating with the nest holder of the first nest entered was not significantly different between early and late in the breeding season ($\chi^2 = 0.38$, df = 1, $P = .53$). In two instances, a female initially seemed to reject a male upon nest inspection but later mated in the same nest.

Female-Female Competition

Simultaneous courtship to a male by several females is an indication of strong female-female mating competition. Such simultaneous courtship was rare early in the season (26 of 339 courtship interactions [8%]) but occurred frequently in the late part of the breeding season (71 of 125 courtship interactions [57%]). On a per-female basis, the proportion of courtship interactions involving more than one simultaneously courting female was affected by an interaction between time of season and whether or not the female mated during observations (GLMM: intercept [early, unmated] = −3.64 ± 0.42, estimate [late, mated] = −6.30 ± 0.66, $z = -4.06$, $P < .001$). However, both classes of females experienced far more simultaneous female courtship late compared to early in the season (females not observed until mating: median early = 0%, median late = 75%, GLMM: intercept [early] = −3.63 ± 0.41, estimate [late] = 0.34 ± 0.46, $z = 8.57$, $P < .001$; females observed mating: median early = 0%, median late = 58%, GLMM: intercept [early] = −1.68 ± 0.48, estimate [late] = −0.34 ± 0.48, $z = 2.80$, $P = .005$; fig. 2*A*). When multiple females courted the same male simultaneously, they often (at 22 of 82 [27%] such interactions) performed agonistic sigmoid displays toward other females. The likelihood of female-female agonistic displays appeared to be a function of competition rather than of time of season because agonistic female displays occurred at similar frequencies at simultaneous display cases early (6 of 26 cases) and late (16 of 56 cases) in the season (GLMM: $z = 0.45$, $P = .65$). Notably, males were never observed to perform courtship if simultaneously courted by more than five females (fig. 2*B*).

Female Mate Sampling

Including data from the whole season, mate-sampling females visited a median of 7 males (range = 1–74, $N = 125$). Females not observed until mating visited a median of 8 males (range = 1–74, $N = 97$), whereas the subset of females that mated during observations visited a median of 5.5 males (range = 1–40, $N = 28$; fig. 3*A*). Thus, females observed mating visited significantly fewer males than did females not observed mating (GLMM: intercept [early, unmated] = 2.28 ± 0.42, estimate [mated] = 1.93 ± 0.18, $z = -1.98$, $P = .048$). Early in the breeding season, females visited males at a rate about three times as high as they did late in the season, irrespective of whether they mated during observations or not (GLMM: intercept [early] = 3.55 ± 0.40, estimate [late] = 2.53 ± 0.11, $z = -8.98$, $P < .001$; fig. 3*B*). Nevertheless, females spent a similar length of time before mating early (28.8 ± 5.3 min, range = 3–83 min, $N = 17$) and late (23.7 ± 4.7 min, range = 8–53 min, $N = 10$) in the season (GLMM: intercept [early] = 3.12 ± 0.23, estimate [late] = 2.96 ± 0.29, $z = -0.57$, $P = .57$). Females that were not observed until mating were observed for a similar length of time early and late in the season (early: 35.7 ± 3.9 min, range = 6.3–112.1, $N = 42$; late: 30.7 ± 1.8 min, range = 9.0–66.5, $N = 54$; GLMM: intercept [early] = 4.31 ± 0.42, estimate [late] = 5.19 ± 0.07, $z = 13.5$, $P < .001$). As a result, females visited about three times as many males early as they did late in the season, both those observed mating (GLMM: intercept [early] = 2.22 ± 0.21, estimate [late] = 1.32 ± 0.38, $z = -2.36$, $P = .018$) and those not observed until mating (GLMM: intercept [early] =

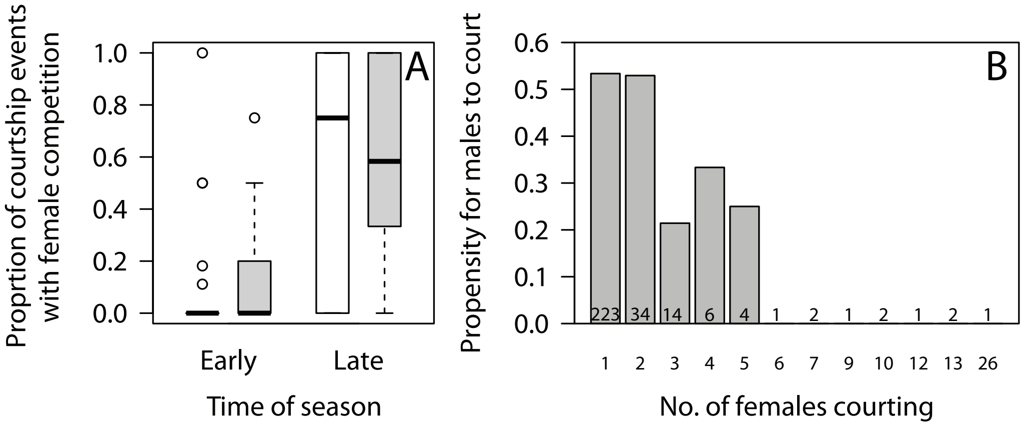

Figure 2: Extent and effect of simultaneous courtship of the same male by multiple females in two-spotted gobies (*Gobiusculus flavescens*). *A*, Proportion of courtship interactions that involved multiple females courting the same male early and late in the breeding season. Open bars, females not mating during observations (early $N = 41$, late $N = 47$); shaded bars, females observed until mating (early $N = 18$, late $N = 10$). The thick lines represent the median, the top and bottom of the boxes represent the seventy-fifth and twenty-fifth percentiles, and the dashed error bars extend to the most extreme data point ≤1.5 times the interquartile range from the box. Outliers are shown as separate data points. *B*, Propensity of a male two-spotted goby to court in relation to how many females were simultaneously courting the male. The numbers in the bars represent the total number of observations where focal females courted.

2.34 ± 0.40, estimate [late] = 1.11 ± 0.16, $z = 7.59$, $P < .001$; fig. 3*A*).

Females only infrequently revisited males, and there was no significant difference in the proportion of females that revisited males early (8 of 61 females [13%]) and late (12 of 64 females [19%]) in the breeding season ($\chi^2 = 0.38$, df = 1, $P = .54$). Among the eight females that revisited males early in the season, the majority (6) had not visited any other male in the interim. Late in the breeding season, most revisits (8 of 12) were to males that had been unresponsive at the first visit, and none of these cases involved visits to other males in the interim. Almost all of the females that mated (26 of 28) did so with the last male sampled (no revisit). Of the remaining two females, one (early season) was courted simultaneously by two males and mated with the one first encountered; the other (late season) initially rejected a male and then visited another male (no courtship by either party) before returning to mate with the initially rejected male. Eight of the 28 mating females mated with the first male that courted them; for three this was the first male visited, whereas the other five had visited other males without being courted.

Discussion

We observed a dramatic change in female and male behavior during mate sampling over the breeding season. Early in the season, males were much more eager to engage in courtship than were females. Late in the season, however, females initiated the vast majority of courtship interactions. Females were more choosy early compared to late in the breeding season, whereas males were more choosy late compared to early in the season. These findings support the hypothesis that a reduction in male density over the breeding season increases female mating competition and gives males an opportunity to be selective. Both of these factors seem to affect female mate sampling, leading to less discriminate females and fewer males sampled when female competition is strong.

Our results come from a large sample of mate-sampling females, of which a minority mated during our typical 30 min of continuous observation, whereas the majority were out of sight of the observer before mating. We found that the main patterns of behavior, despite some interesting minor differences discussed below, were similar for the two groups. The sample sizes for the two groups, however, imply a much higher statistical power for females that we were unable to observe until mating. Thus, in a number of analyses where numerical contrasts between early- and late-season data are similar for the two groups, only the larger sample of females not observed until mating revealed a significant effect. Given the higher power, we base our general inference on these results in such cases.

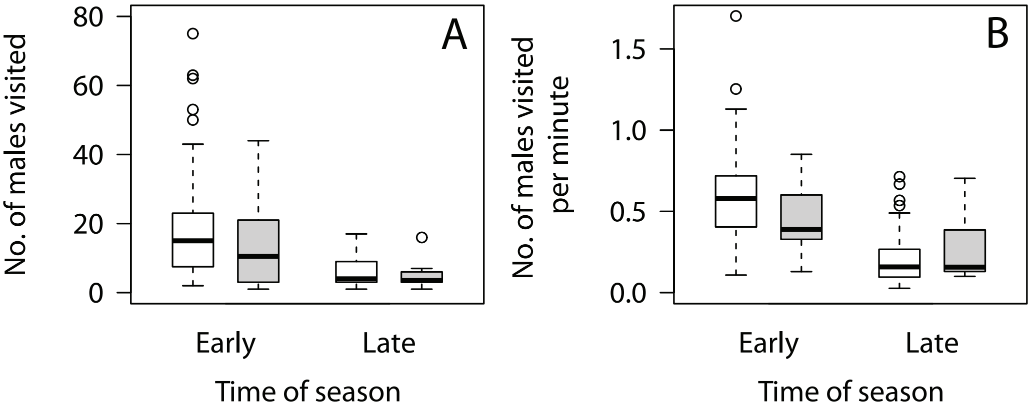

Figure 3: Extent and rate of mate sampling by female two-spotted gobies (*Gobiusculus flavescens*). *A*, Number of males visited early and late in the breeding season for mate-sampling females that did not mate during observations (open bars; early $N = 43$, late $N = 54$) and females observed until mating (shaded bars; early $N = 18$, late $N = 10$). *B*, Visitation rate to males (no. males/min) during female mate sampling early and late in the breeding season for females that did not mate during observations (open bars; early $N = 42$, late $N = 54$) and females observed until mating (shaded bars; early $N = 17$, late $N = 10$). The thick lines represent the median, the top and bottom of the boxes represent the seventy-fifth and twenty-fifth percentiles, and the dashed error bars extend to the most extreme data point ≤1.5 times the interquartile range from the box. Outliers are shown as separate data points.

Sex Role Dynamics

Late in the season, when males were in short supply, females initiated the vast majority of courtship interactions. As active courtship by females is not considered in most mate-sampling models, and not often reported in empirical works on mate choice (but see Fagundes et al.'s [2007] work on sex-role-reversed peacock blennies), such a predominance of female courtship is remarkable. By contrast, females initiated only about a quarter of courtship interactions early in the season, when there was little female competition. These results suggest a strong increase in female eagerness to mate as the breeding season progressed. A similarly marked change was observed for courtship termination, which likely reflects choosiness. Early in the season, females terminated about 50% of courtship interactions, but they nearly never terminated courtship interactions late in the season. At this time of the season, males terminated the majority of courtship interactions. Taken together, this shows that late-season females were eager to mate and apparently less discriminating, but their mating offers were often rejected by visited males. We suggest that the change in female behavior was caused by higher search costs (fewer males available, reduced encounter rate; Crowley et al. 1991; Kokko and Monaghan 2001; Gowaty and Hubbell 2009) and increased female mating competition (female-biased OSR; Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). The fact that, late in the season, courtship interactions were frequently initiated by females and terminated by males contrasts with the classical assumption that males are always available as mating options to females, but it fits a dynamic view of sex roles and mate choice (Parker 1983; Kvarnemo and Ahnesjö 1996; Johnstone 1997; Bergstrom and Real 2000; Kokko and Monaghan 2001; Servedio and Lande 2006).

Female-female competition increased markedly over the season, as males decreased in number. Late in the season, the majority (about 75%) of courtship interactions involved multiple courting females, something that nearly never occurred in the early part of the season. Late in the season, females almost always joined any courtship interaction they came near (L. C. Myhre, personal observation). When females were simultaneously courting the same male, they often engaged in intrasexual agonistic behaviors. Work on birds has found increased mating competition to increase female-female aggression (Kempenaers 1994) and female copulation frequency (Fiske and Kålås 1995) and to restrict mate search (Dale et al. 1992). Female two-spotted gobies likely experienced an increased cost of lost mating opportunities (Johnstone 1997) late in the breeding season, as more females were competing for the few males still present.

Mutual Mate Choice

We have previously documented that both sexes exhibit mate preferences in the two-spotted goby (Amundsen and Forsgren 2001; Borg et al. 2006). Both males and females may reject mating offers at any time of the season. However, the degree to which each sex is choosy may vary over time, determined by sex-specific costs and benefits (Crowley et al. 1991; Johnstone et al. 1996; Johnstone 1997; Kokko and Johnstone 2002; Gowaty and Hubbell 2009). Male rejection would inevitably force females to extend their mate search. This was frequently observed late in the season. Due to strong female competition, a typical courtship interaction late in the season involved multiple females, suggesting that males would pay a small cost from being choosy (Svensson et al. 2009*a*). In two-spotted gobies, a consequence of male choosiness (and a female-biased OSR) seems to be that females become less choosy and instead become competitive.

The rate at which females visited males dropped very significantly over the breeding season, as male density declined. The reduction in visitation rate was evident both for those females that mated during observations and for those that did not. Female two-spotted gobies visited about three times as many males early (when sex roles are conventional) as they did late in the breeding season (when sex roles are reversed). Search time until mating did not, however, differ between females observed early and late in the season, suggesting that the reduction in number of males sampled was caused by a lower density of males. The reduction in density of mating-ready males may have been even greater than the visitation rate implies, because, late in the season, many males were apparently unresponsive to courtship (see above), and some may have had full nests (Forsgren et al. 2004). Thus, sexual selection on males by female choice becomes weaker over the season, because the number of males sampled affects the strength of sexual selection (Andersson 1994; Benton and Evans 1998). Early in the season, females inspected nests without mating more often than they did later on. This may reflect a reduced female choosiness late in the season, when fewer males are available.

It has been an implicit assumption in mate-sampling studies that choice is exerted only by females and that any mating-ready male is available as a potential partner to the searching female. However, few if any studies have explicitly identified which sex actually terminates courtship interactions. This is unfortunate in light of the accumulating evidence for temporally or spatially variable sex roles (e.g., Gwynne and Simmons 1990; Gwynne et al. 1998; Forsgren et al. 2004; Shibata and Kohda 2006) and for mutual mate choice to occur even in systems with conventional sex roles (e.g., Cunningham and Birkhead 1998; Amundsen 2000; Bonduriansky 2001; Amundsen and Pärn 2006; Clutton-Brock 2007, 2009; Kraaijeveld et al. 2007). In two-spotted gobies, male choice is clearly important and may affect sexual selection both directly (acting on females) and indirectly via a negative effect on female choosiness (relaxing selection on males). A seemingly high number of males sampled does not necessarily imply strong sexual selection on males if males rather than females frequently reject their potential partner, as they often do late in the season. In the two-spotted goby, sexual selection is likely to be stronger on males early in the season and stronger on females later in the season. Given that mutual mate choice appears to be more widespread than traditionally thought, similar complex effects of mutual mate choice on mating behavior, mate sampling, and sexual selection may exist in a range of species.

Our study has taken advantage of the fact that, in two-spotted gobies, a marked change in OSR and mating competition takes place over the 3–4-month breeding season (Forsgren et al. 2004). During the course of the breeding season, both male and female two-spotted gobies have the potential to reproduce repeatedly because a single breeding cycle (spawning until hatching in males, egg development in females) takes only 1–3 weeks (Skolbekken and Utne-Palm 2001; Bjelvenmark and Forsgren 2003). This allowed us to compare situations of high and low mating competition for each of the sexes within the same breeding season and population and thus provided a unique opportunity to test predictions from sex role theory. However, this “natural experiment” also means that conclusive causality cannot be established as unambiguously as in a controlled laboratory experiment: maybe the progression of the breeding season rather than mating competition per se affects female and male behaviors? First, time of season might affect behavior by making individuals more exhausted and less vigorous with time. Second, an animal might become less choosy toward the end of the season, because time is running short, as suggested by Janetos’s (1980) “last chance tactic.” Third, sea temperatures increase over the season, which might lead to overall higher levels of activity (Kvarnemo 1998). None of these scenarios would, however, fit the opposite behavioral changes over the season by the two sexes (less courtship by males, more courtship by females; greater choosiness by males, less choosiness by females). Moreover, for two-spotted goby females, time is running short at every breeding cycle, when she has developed mature eggs and has only a brief time window to search for and mate with a suitable male (because fish eggs quickly overripen after ovulation; e.g., Mollah and Tan 1983; Kjørsvik et al. 1990; Legendre et al. 2000). This applies throughout the breeding season and is likely much more important to the female than season as such. Janetos’s (1980) idea that late-season females would run short of time and thus be less choosy have received some support from studies of pied flycatchers *Ficedula hypoleuca* (Hovi and Rätti 1994). However, it is questionable whether, in a short-lived repeated spawner, choosiness should decrease toward the end of the season. As argued by Crowley et al. (1991), residual reproductive value is lower when prospects for further breeding are poor, reducing the cost of being choosy. Taking all of this into account, we are not able to envision a scenario based on seasonal effects only (independent of mating competition) that could explain the observed behavioral dynamics. Moreover, recent experimental work in the laboratory has demonstrated that two-spotted gobies respond behaviorally to differences in mating competition in parallel trials (de Jong 2011). Hence, several lines of evidence suggest that the effects observed in this study are not due to time of season as such. This does not, however, exclude the possibility that other factors, in addition to sex role variation, may affect the behavior of one or the other sex. One such factor may be mate quality, which could change independently in males and females over the season (Parker 1983; Owens and Thompson 1994). However, it is very unlikely that mate quality changes could explain the major patterns observed.

Mate-Sampling Tactics

Female two-spotted gobies actively sampled among males, visiting usually five or six males, and typically having courtship interactions with two, before mating. The vast majority of females observed until mating mated with the last male visited. About 30% (8 of 28) mated with the first male that courted them, three of these with the first male visited. These patterns fit with some of the suggested models, including the fixed threshold variants pure threshold and last chance option (Janetos 1980), the one-step decision process (Janetos 1980), and Real's (1990) sequential search model; the latter two are considered variants of the same logic by Jennions and Petrie (1997). Mating with the last male also fits Dombrovsky and Perrin's (1994) optimal stopping rule, but this tactic is incompatible with mating with the first male, as some females do. Wittenberger's (1983) sequential comparison tactic does not fit mating with either the last or the first male. Our results are not in line with best-of-*N* tactics or random mating (Janetos 1980; Parker 1983). Thus, we can exclude several suggested sampling tactics, but our results are still compatible with two fixed threshold tactic variants, with Real's sequential search model, and with its simpler predecessor, the one-step decision tactic. As emphasized by more recent theoretical contributions (e.g., Wiegmann et al. 1999, 2010*a*, 2010*b*; Luttbeg 2002; Wiegmann and Angeloni 2007; Castellano and Cermelli 2011), however, the regulation of female mate sampling is likely to involve far more dynamic factors than captured by the early models. These include factors that are not easily quantified empirically and for which we have no information. Male quality, and its variation, is one factor that could not be analyzed because marking all males at the study sites would not have been feasible. Despite these limitations, we conclude that two-spotted goby females appear to employ some sort of sequential search, or threshold, tactic, as has also been suggested for other fishes (Forsgren 1997; Fagundes et al. 2007; Draud et al. 2008). There was no evidence to suggest that females employed different sampling tactics early and late in the breeding season. However, the fact that females sampled fewer males late in the season, and that they often initiated but rarely terminated courtship interactions at this time, together suggest a lowered female mate acceptance threshold. Such a lowered threshold is further supported by males seemingly being of lower rather than higher quality late in the season: breeding males are generally smaller at this time (de Jong 2011; S. Wacker et al., unpublished data), and both males and females are increasingly infected by parasites as the season progresses (L. C. Myhre, K. de Jong, E. Forsgren, and T. Amundsen, personal observations). Notably, if male quality and female acceptance thresholds are proportionally reduced as a function of season, the fraction of males that meet the threshold criterion would not be affected.

In most species studied so far, the typical female samples fewer than five males. It is also commonplace that a significant fraction of females mate with the first male visited (Gibson and Langen 1996, Jennions and Petrie 1997); in fact, most studies on fishes, amphibians, and insects (but not birds) suggest that the typical female visits only one male before mating (Arak 1988; Forsgren 1997; Reid and Stamps 1997; Murphy and Gerhardt 2002; Fagundes et al. 2007; Draud et al. 2008). Most mate-sampling studies in the wild have been carried out on birds, with only a few studies on fish and other taxa. This is unfortunate because mate-sampling studies provide valuable knowledge on the action of sexual selection in nature (Gibson and Langen 1996; Benton and Evans 1998). In the two-spotted goby, the median number of males visited is one of the highest recorded so far, matched only by fiddler crabs *Uca annulipes* (Backwell and Passmore 1996), great reed warblers *Acrocephalus arundinaceus* (Bensch and Hasselquist 1992), and pronghorn antelopes *Antilocapra americana* (Byers et al. 1994). In particular, some female two-spotted gobies showed extremely extensive mate search, visiting up to 74 males (and having courtship interactions with at most 37 males). Even if observations varied in duration and these high values were for females observed for 2–3 times the mean observation time, the range between these values and females mating with the first male visited show highly variable search behavior among females. Notably, mate sampling was more extensive early in the season; all instances of females visiting 50 or more males occurred during this period, and the typical female visited 10–15 males (fig. 3*A*). While the high numbers of males visited by some females likely reflect a high male density in the population (Forsgren et al. 2004), leading to high encounter rates (Kokko and Rankin 2006), the extensive variation among females suggests considerable individual variation in costs and/or benefits of mate search (Wiegmann and Angeloni 2007).

A high breeding density, as seen in two-spotted gobies, may allow females to perform some visual assessment without making close-range visits. Such potential long-range assessment is hard to verify empirically. However, the fact that mating was always preceded by courtship interactions, and that these interactions took place at close range, suggests that mate assessment in two-spotted gobies mainly occurs during close visits. Previous work on the species has found females to discriminate among males on the basis of size and courtship (Borg et al. 2006), which can hardly be done at long range.

A general problem in mate-sampling studies (including our study) is the difficulty of observing all females until they mate. When only a subset of the females can be observed until mating, the numbers of males sampled by mating females will underestimate the true population mean. This problem is hard to eliminate in empirical research and could lead to an underestimation of mate sampling not only for single species but also for the general patterns (Gibson and Langen 1996; Jennions and Petrie 1997). In our study, the fact that most mate-sampling females did not mate during our ca. 30 min of observation suggests that the recorded values (typically 5–6 males visited, and courtship interactions with 2 males) significantly underestimate mate sampling in the species. Early in the season, females that mated during observations were more likely than other mate-sampling females to initiate courtship interactions, whereas late in the season female mating often occurred after the relatively rare cases of male courtship during that period. These findings suggest that females that mated during observations were a nonrandom sample that were either particularly eager to mate (early in the season) or experienced favorable mating opportunities (late in the season) and thus probably had less extensive mate searches than the typical female. This is particularly noticeable because the recorded extent of mate sampling is high compared to other fish species and, indeed, most animals studied (Gibson and Langen 1996; Forsgren 1997; Jennions and Petrie 1997; Fagundes et al. 2007; Draud et al. 2008).

Conclusions

This study is, to our knowledge, the first analysis of mate sampling and related mating behaviors in a system exhibiting both conventional and reversed sex roles. It is also the first where mutual mate choice during mate sampling has been empirically investigated. In the two-spotted goby, OSR and sex roles shift over the breeding season, drastically changing the number of mating opportunities for both sexes. We found that females became less selective and faced stronger mating competition later in the season. As predicted, males initiated the majority of courtship interactions early, whereas females typically initiated (and males terminated) courtship late in the season. Our results emphasize the importance of considering mutual mate choice in mate sampling and sexual selection. As shown in this study, mate choice, mating competition, and sexual selection can vary dramatically within a species. In order to understand the process of sexual selection, and how it is affected by behaviors of males and females, studies of competition and choice in both sexes are needed across a range of taxa and social systems. Our study demonstrates that if only one sex (the female) is assumed to make mating decisions during mate sampling, crucial information regarding the process of sexual selection may go undetected.

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Left, female two-spotted goby displaying its colorful, egg-filled belly, as is regularly done during female courtship. By the end of the breeding season, most courtship is by females to males. This female also carries an ectoparasitic copepod on its dorsal fin. *Right*, goby fish inhabit kelp forests along rocky shores like this Nordic archipelago. Photographs by Trond Amundsen.

PAPER 2

Effects of habitat complexity on mating behavior and mating success in a marine fish

Running title: Habitat complexity and mating behavior

Lise Cats Myhre[1*], Elisabet Forsgren[2], Trond Amundsen[1]

1. Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway
2. Norwegian Institute for Nature Research (NINA), P. O. Box 5685 Sluppen, NO-7485 Trondheim, Norway
* Corresponding author, email: lise.myhre@bio.ntnu.no
Other authors email: trond.amundsen@bio.ntnu.no, elisabet.forsgren@nina.no

ABSTRACT: The environments animals inhabit vary greatly in structural complexity, both naturally and as a consequence of human disturbance. Structural complexity might affect communication by visual and other means, impair detection of potential partners and affect sexual selection processes. Previous studies on shallow water fishes suggest that sexual selection can be relaxed when visibility is reduced. Here, we test whether habitat complexity affects mate search, mate choice and the opportunity for sexual selection in the two-spotted goby, *Gobiusculus flavescens*, a marine fish with paternal care. In 2x2m tanks, we established environments with low or high habitat complexity, and introduced a mixed-sex group of fish (8 males, 8 females), which were allowed to breed. Two days later, we released additional (focal) ready-to-mate females in the tanks and observed female mate search and mating behaviors of both sexes. For females, habitat complexity negatively affected rate of movement, encounter rate with males, courtship rate and time until mating. For males, habitat complexity resulted in fewer cases of multi-male simultaneous courtships. Additionally, fewer courtship interactions were interrupted by male-male aggression in the complex habitat. However, these clear behavioral effects did not appear to affect the mating skew among males. Despite the absence of a difference in the opportunity for selection between treatments, we detected positive selection for male length in the open but not in the structurally complex environment. The results indicate that habitat complexity affects mating behaviors of both females and males and that a more structurally complex habitat might relax sexual selection.

Keywords: sexual selection, mate sampling, female choice, environmental change, habitat structure, *Gobiusculus flavescens*, two-spotted goby

Introduction

In nature, habitats vary greatly in complexity. Within the same species, populations may sometimes inhabit widely different habitats. The same population may also experience dramatic temporal changes in its habitat, both between and within years. Finally, within populations, individuals may inhabit different habitat types. Such differences in habitat complexity could have profound effects on animal communication and behavior. For example, the expression and transmissibility of signals could be affected by environmental factors (e.g., Moyaho et al. 2004, Brumm and Slater 2006, van der Sluijs et al 2011). A simple habitat (e.g., open fields, clear waters) might permit the transmission of visual signals over long distances, whereas a physically and structurally complex habitat (e.g., dense vegetation, rocky environments) will impede visibility and decrease visual range. The complexity of a habitat can affect a range of behaviors, such as movement patterns (e.g., Longepierre et al. 2001, Orpwood et al. 2008), aggressiveness (e.g., Carfagnini et al. 2009, Kadry and Barreto 2010, Danley 2011), and foraging behavior (e.g., Ryer et al. 2004, Andruskiw et al. 2008, Michel and Adams 2009). The physical complexity of a habitat might also influence behavioral processes important for sexual selection, such as species recognition, mate choice and intra-sexual competition. For instance, a structurally complex habitat might relax intra-sexual competition by making it difficult for animals to detect competitors (e.g., Hibler and Houde 2006). Structural complexity might also relax mate choice by making it harder for animals to locate or assess potential mates. Thus, animals might adapt their behavior to the structure of the environment they inhabit. For instance, calls used for mate attraction often differ between open and densely vegetated habitats (birds: Morton 1975, Boncoraglio and Saino 2007, Smith et al. 2008, mammals: Peters and Peters 2010). Additionally, male mate-locating behavior in an insect has been found to vary between different forest structures (Bonte and Van Dyck 2009). If an increased habitat complexity causes females (or males) to sample fewer potential mates before mating, then this lesser sampling should lead to weaker sexual selection (Jennions and Petrie 1997; Benton and Evans 1998). Among shallow water fishes, more complex habitats are associated with reduced competitive interactions among males (Hibler and Houde 2006), increased male courtship intensity (Dzieweczynski and Rowland 2004, Candolin et al. 2007), increased female inspection of males (Hibler and Houde 2006, Candolin et al. 2007) and a decreased mating skew (Candolin 2004).

The marine coastal environment contains habitats ranging from very simple (pure sand/gravel bottom) to very complex (rugged rocky shores, complex algal vegetation, etc.). Both natural and anthropogenic influences could have effects on habitat structural complexity. Along Scandinavian coasts, for instance, habitat complexity can be affected by the increased growth of filamentous algae as a result of natural seasonal growth, increased nutrient concentrations (Rosenberg et al. 1996) and the removal of top predators, which can decrease the abundance of grazers through cascading trophic effects (Eriksson et al. 2009). It has been documented that the increased growth of fast-growing seasonal filamentous algae reduces visibility and alters habitat complexity (Larsson et al. 1985, Pihl et al. 1995, Rosenberg et al. 1996). In addition to clusters of filamentous algae on existing macro algae, the invasive macro algae *Sargassum muticum* (Thomsen et al. 2006), contributes to increased structural complexity. In the more extreme cases, the growth of filamentous algae has "taken over" and suppressed the original kelp-forest, thus creating carpets of filamentous algae (Moy et al. 2008) and habitats with almost no structure. Thus, environmental changes currently observed in marine coastal environments of Scandinavia (Karlsson 2007; Moy et al. 2008) might both increase and decrease the complexity of a habitat. Similar changes seem to occur in coastal waters around the World. Likewise, the structural complexity of global marine environments is highly variable, from uniform sandy bays with little vegetation to coral reefs of sometimes extreme structural complexity.

Visibility in aquatic environments might be similarly affected by increased turbidity, which can be caused by natural or human-induced phytoplankton blooms or pollution. Increased turbidity has been found to reduce male mating skew (Järvenpää and Lindström 2004), impair male mate choice (Sundin et al. 2010, Lindqvist et al. 2011) and might, in a worst-case scenario, lead to the breakdown of reproductive isolating mechanisms between species (e.g. Seehausen et al. 1997).

In order to understand the operation of sexual selection and other important processes in nature, it is crucial to know how these processes are affected by environmental variability. This study aims to determine if a more structurally complex environment affects female and male mating behaviors and sexual selection. We used a small marine fish, the two-spotted goby (*Gobiusculus flavescens*), as a model species. Two-spotted gobies inhabit and breed in a range of coastal habitats. They are abundant in moderately exposed environments in fjords, bays and around islands and skerries of all sizes, but seem to be less common in the most exposed outer localities, and at very sheltered, inshore localities. Typically, their habitat is dominated by large macroalgae, usually dominated by *Laminaria* spp. and *Saccharina latissima* (Wheeler 1980, Svensson 2006), which by themselves create a highly structured environment, but also with a range of other algae growing either on the substrate or on the kelp. The species might also seem to prefer some natural structure, as one often find shoals and individuals in crevices, depressions and other somewhat less exposed locations. However, the species is also common in and near gravel-dominated bays, where the environment might be partly much more open, often only with various algae (e.g. *Fucus* spp.) creating some structure. The light environment is variable depending on weather conditions and physical structure (darker in crevices and in the shadows of rocks and algae). Water transparency varies naturally in accordance to time of season, algal-blooms, weather and other conditions (personal observations, all authors). The two-spotted goby is a keystone species in the coastal ecosystem, and constitutes an important food source for juvenile cod (*Gadus morhua*) (Fosså 1991, Giske et al. 1991, Salvanes and Nordeide 1993). Changes in the coastal environment are therefore likely to influence this species.

To test how habitat complexity affects mating behavior and sexual selection in two-spotted gobies, we conducted an experiment in which the habitat complexity (spatial structure) was manipulated as either low (open habitat) or high (spatially structured habitat) in large, indoor tanks. Individually marked, mixed-sex groups of gobies was released into the tanks, and were allowed to interact freely. We allowed the initial group of fish two days in the tank before additional females was released for focal observations. Along with male reproductive success (monitoring of egg batches), both female and male mating behaviors were observed.

We predicted that a structured environment (complex habitat) would affect behaviors in the following two ways: (1) because of difficulty in detecting males, females would sample fewer males before mating, and (2) because of a reduction in the visual contact between males, male-male competition would be relaxed, which would lead to less male courtship interference. As a result of these predicted responses we expected eggs to become more evenly distributed among males (lower mating skew and lower potential for sexual selection) in the complex habitat.

Methods

Model species

The two-spotted goby (*Gobiusculus flavescens*) is a small (adult mostly 35-55 mm), sexually dimorphic, marine fish. This species is quite common along the rocky shores of W Europe (Collins 1981). Two-spotted gobies are semi-pelagic (Wheeler 1969) and often form large shoals near the algal vegetation (Svensson et al. 2000). In nature, fish density varies between localities and with time of season (Forsgren et al. 2004). On a local scale there is often large variation within localities as fish can occur from single individuals up to shoals including several hundred individuals (Svensson et al. 2000). During the breeding season, many males are relatively stationary, which is like to reflect them having and defending a nearby nest or nest-site (Forsgren et al. 2004, de Jong 2011). Females, by contrast, mostly occur as parts of shoals. Such shoals are relatively unstable (Svensson et al. 2000), both in terms of size and composition of individuals, and seem not to have any strong spatial association. Breeding-ready females often encounter stationary males in multi-female groups (Myhre et al. 2012). Both males and females might occur as parts of sometimes very large (many hundred or more individuals) feeding shoals, typically in the open water just outside the algal vegetation. Two-spotted gobies often seek shelter among the algae when threatened by predators (Utne et al. 1993, Utne and Aksnes 1994). The species typically live for only one year (Johnsen 1945), but have a polygamous mating system where both sexes can reproduce repeatedly during a breeding season (Collins 1981, Mobley et al. 2009, K. de Jong, L. Rodrigues-Graña, unpublished data). The species is a substrate brooder with paternal care. Breeding males take up a nest in mussel shells, on kelp leaves, or in crevices (Gordon 1983, Amundsen and Forsgren 2001, Svensson 2006). Females typically deposit clutches of 1000-1500 eggs (Pélabon et al. 2003, Svensson et al. 2006) in a male's nest and can successively lay several batches of eggs in the nest of different males over the breeding season, at intervals likely ranging from 1 to several weeks. Males might simultaneously care for clutches from several (2-6) females (Gordon 1983, Mobley et al. 2009) and they tend the eggs by guarding, fanning and cleaning them until hatching (Skolbekken and Utne-Palm 2001, Bjelvenmark and Forsgren 2003). Only one or a few days after hatching of the eggs, a male may again engage in courtship and take care of new clutches laid by attracted females (in the lab: Eriksen 2007).

Two-spotted gobies exhibit dynamic sex roles (Forsgren et al. 2004, Myhre et al. 2012). At the start of the breeding season, mating competition is strongest among males (conventional sex roles), whereas later in the season, mating competition is stronger among females (reversed sex roles, Forsgren et al. 2004). Both sexes display visual ornamentation and distinct courtship behaviors during the breeding season (Amundsen and Forsgren 2001, Pélabon et al. 2003, Forsgren et al. 2004; for definitions see table 1). During their mate search, ready-to-mate females actively search for males and typically visit several males before mating (Myhre et al. 2012). The males, if interested, begin courting the female and attempt to lead the female to the nest; the female might then respond with courtship displays and follow the male to his nest (Myhre et al. 2012). Both sexes may initiate courtship, and mate choice has been demonstrated in both sexes (Amundsen and Forsgren 2001, Borg et al. 2006, Myhre et al. 2012). Males prefer females with more orange belly coloration (Amundsen and Forsgren 2001), and females prefer large males early in the breeding season (Borg et al. 2006).

General procedures

The experiment was conducted between 11 May and 21 June 2010 at the Sven Lovén Centre for Marine Sciences, Kristineberg (58° 15’ N, 11° 27’ E), on the west coast of Sweden.

The fish were collected around islands up to 2 km from the research station by snorkelers, using hand-held dip nets. The fish were separated by sex in the field and brought back to the laboratory by boat. Females and males were collected from different localities to ensure that they had no familiarity with each other. In the laboratory, the males were placed individually into aquaria (25x30x30 cm, LxWxH) and acclimatized for approximately 40 hours. The females were kept together for two days (up to 35 females in 60x40x35 cm aquaria) before being used in the experiment. Females used for focal observations were held in an aquarium (35x35x35 cm) for two additional days, with five fish in each aquarium. All of the fish were fed *ad libitum* twice daily (morning and evening) with *Artemia* sp. nauplii. Aiming to include only individuals that were ready to mate, we selected healthy-looking males and females with a high “belly roundness”, which is an indicator of gonad maturity (Svensson et al. 2006).

All of the fish used in the experiment were marked individually with Visible Implant Elastomer (VIE) tags (North-West Marine Technology, Shawn Island, Washington), as described by de Jong et al. (2009). Using four colors (blue, red, green and orange), each male was marked in one of two possible locations. The females were given two marks, in two of four possible locations, using three colors (blue, red and green; 54 combinations). Both before and after the trials, we measured the total body length (TL) for each fish to the nearest 0.5 mm. The TL measurements was conducted using a measuring board, and we determined the wet body mass (BM, to an accuracy of 0.01 g) using a Mettler digital balance. These measurements allowed us to calculate a rough estimate of how many females had laid their eggs during the experiment. The females (introduced on day two) that had had lost weight (> 0.03 g) by the termination of the experiment were judged likely to have spawned. The females that likely spawned lost a median of 12.2 % (range 4 - 29 %) of their BM.

All of the males were tested in a "personality assay" before they were used in the experiment. These results will be published elsewhere.

Experimental design

The treatments were designed to test whether visual obstructions induced by a spatially structured (complex) environment affected mating behaviors and sexual selection. The experiment was conducted indoors under controlled light and water flow conditions. We created two treatments that consisted of an open and a structured (spatially complex) environment in 200x200 cm grey tanks with a water depth of ca 35 cm. Two sets of replicates of each treatment were run in parallel (four tanks), which added up to a total of 16 replications of each treatment. Each trial was run for three days, and we let the fishes interact freely during that time. All of the fish were randomly assigned (by flipping a coin) to a treatment. Neither females nor males differed in TL or BM between treatments (table 2), and the sizes represented the natural range of sizes in the field. The size of the males ranged from 37.8 ± 0.3 mm to 47.8 ± 0.5 mm (smallest and largest fish, respectively, in each trial), the within-trial size range did not differ between treatments ($t_{29.21} = -0.06$, $P = 0.95$), and the coefficient of variation was very similar between the treatments (structured: CV = 0.091, open: CV = 0.085).

For both treatments, we used white tape to mark the bottom of the tanks in sections (~ 50x50 cm). The tape facilitated the recordings of female movements during the observations. We provided each tank with eight artificial nests and 20 plastic plants. The nests were placed next to one plant, approximately 20 cm from the wall, and twelve plants were placed in the four central sections of the tank, approximately 10 cm apart (fig. 1). The open environment had only the artificial nests and plants as structural elements in the tank (fig. 1A). Thus, the fishes in this treatment could potentially see most of the other fish in the tank the majority of the time. The structurally complex environment treatment (hereafter termed structured environment) had the same basic setup with respect to the artificial nests and plants, but also included six opaque (white) plastic dividers across the length and width of the tank (fig. 1B). These dividers created partly separated sections (~ 50x50 cm, which matched the division of the marked sections) around the artificial nests, but they did not preclude movement between the sections. Around the nest sections the dividers were 40 cm high with a “V” section removed. The bottom of the “V” was 20 cm above the bottom of the tank so the fish could swim through. We also cut an approximately 2 cm high “arch” under each “V” in the bottom of the dividers. Hence, the fish could swim both below and above the dividers and slip past the edges. To create a more open area where the fish could shoal, the dividers were only 10 cm high in the central sections (see fig.1B). The dividers hampered visual contact between the fish in the tank, and are thus likely to have made the detection of both potential mates and competitors more difficult.

We used a two-phase design for the experiment. First, we established a breeding population in each tank, and allowed the males and females time to acclimatize to the laboratory conditions and interact freely and spawn (no behavioral observations at this stage). Two days later, we released additional (focal) females and observed the mating behavior of these females and any males they interacted with during their mate search. Apart from the data on male mating success over the course of the experiment, all data in the study are based on these behavioral observations.

For the first phase (the establishment of breeding populations), individually marked males and females were released in the middle of each tank (sex ratio males:females 8:8) at ca 14:00 h on day 0 (hereafter called ‘initial’ females and males). An even sex ratio was chosen so that males would take up nests and mating could take place. Additionally, this ratio was chosen so that all or most males would have room for additional clutches in their nests at the time of focal female introduction (second phase). We provided PVC nest tubes (80 mm long and 13 mm inner diameter, lined with an acetate sheet) capable of holding clutches from approximately four females (Bjelvenmark and Forsgren 2003, Forsgren et al. 2004). The second phase began on day 2 between 10:00 h and 14:00 h (approximately 44 hours after the initial males and females were introduced to the tank). We released five marked ready-to-mate females (see details below) and observed behaviors (performed and received) for three of these females (focal females). We released two non-focal females just before the release of the first focal female to avoid a situation where the first of these focal females would be the only ready-to-mate female in the tank at that time. The primary reason for observing females introduced at a later stage than the initial females was to simulate a situation where the females would have no prior knowledge of the males and would sample mating options in a natural environment of already breeding males and females. The fact that the focal females were introduced to an already acclimatized group of fish that performed natural breeding behaviors also seemed to minimize stress in focal females, with the result that most of them display natural mate search behavior almost immediately upon release. The trials were terminated on day 3 (after 15:00 h), by which time most of the females had spawned. All of the fish and nests were collected at the end of the trial (approximately 73 h after the trial initiation). The fish were measured (as described above) and fin clipped before being released back into the sea. The egg content of each nest was photographed to allow for the counting of the number of eggs. The eggs were counted as an estimate of male reproductive success at the end of the experiment.

The tanks had a continuous flow of sea water (from 7 m depth). The water transparency was high and did not restrict visual range in any of the treatments. The water temperature followed the natural sea temperatures and ranged from 10.5 to 16.8 degrees Celsius during the experiment (the temperature was not recorded for the first two replications). The light schedule followed a natural summer light regime for that latitude, with a light:dark ratio of 17:7.

Observational protocol

Because females are the sex that actively searches for mates (Myhre et al. 2012), females were chosen for the focal observations. On the morning before the release of the focal females, typically 4-5 of the males held a nest, and approximately three of these males had eggs in their nest (fig. 5). In 7 (open: 3, structured: 4) out of 32 trials, one of the males in a tank had a full nest prior to the release of focal females, which left 7 out of 8 males available for mating. Most (6-7 out of 8 in most trials) of the initial females had already spawned at the time of the focal female introduction. This figure was calculated from the number of nest holders with eggs and the mean nest fullness (fig. 5), and we assumed one spawning to typically fill up 25% of a nest (Bjelvenmark and Forsgren 2003). We first released two non-focal and then three focal females in each trial. The focal females were released one at a time, and they were observed until mating (see table 1) or for 30 minutes if mating did not occur. The mean observation time was 18.4 minutes (range 0.3 – 30.0 minutes). In addition to female movement between sections, all of the behaviors (table 1) performed and received within a radius of 10 cm from the focal female were recorded. We flipped a coin to randomly determine in which treatment, out of a pair of treatments run in parallel, we would first release the focal females. Thus, we randomly determined where the observations began. The observations were alternated between the treatments. All of the females were released in the middle of the tank. At the time of their release, the females sometimes immediately laid down on the bottom, but began swimming normally within < 3 minutes (mean 45 ± 12 sec., one female stayed 15 minutes on the bottom). The behavioral recording did not begin until normal swimming commenced. In total, we observed 96 focal females (48 for each treatment). The focal females did not differ significantly in either TL or BM between the treatments (table 2).

As a measure of male success, every morning (around 8:00 h) and evening (around 19:00 h) we recorded which males were holding a nest, the position of the nest and the percentage of nest area covered with eggs (nest fullness, in 10 % increments).

Statistical analyses

The data analyses were performed using R v. 2.13.1 (R Development Core Team 2011). We used generalized linear mixed models (GLMM) to analyze the data with the appropriate error distributions (proportion data: binomial with a logit link; count data: Poisson with a log link). When analyzing the female mate search, we included ‘tank’ as a random effect and ‘treatment’, ‘release order’ and the interaction between these effects as fixed effects. We checked for over-dispersion. If the model was over-dispersed, we then fitted the model again by adding a random effect at the individual level. We tested the fit of the models using a likelihood ratio test (LRT), and removed non-significant variables. For the analysis of the position of events in the tank, we entered ‘male identity’, ‘female identity’ and ‘tank’ as random factors. Estimates are given as a contrast to the intercept and on a log or logit scale depending on the error distribution used, ± 1 SE. The release order affected the observation time of the focal females (the mean observation time, first focal: 14 ± 2 min, second focal: 23 ± 2 min, and third focal: 18 ± 2 min; GLMM: intercept (first) 6.05 ± 0.22, estimate (second) 0.77 ± 0.25, $z = 3.07$, $P = 0.002$, estimate (third) 0.29 ± 0.25, $z = 1.14$, $P = 0.25$), regardless of the treatment ($z = 1.56$, $P = 0.12$). Thus, to make analyses more comparable among the females, most of the analyses are focused on how many times an event occurred per minute. The time until mating during the 30 min observations was analyzed using a survival analysis with a constant hazard assumed.

To quantify variation in egg acquisition among males, we used the cm^2 of the nest that was covered with eggs, and we calculated the opportunity for selection (I) as the variance in reproductive success divided by the square of mean reproductive success (Wade 1979, Wade and Arnold 1980) over time for each treatment and replicate. At termination of each replicate the number of eggs was counted (from photos) and used to calculate I. To test whether male traits (TL and condition) affected reproductive success, we analyzed the relationships between these traits and reproductive success and then tested if the male selection differentials differed from zero. The means are presented ± 1 SE; medians are presented with the range.

Results

Space use

After having been released in the center of the tank, the focal females in the open environment typically swam to the peripheral sections where the males had their nests. On average, it took the females less than a minute (mean time 40 ± 10 sec., $N = 47$) to reach the peripheral sections. By contrast, females in the structured environment usually remained in the center of the tank for an average of approximately five minutes (the mean time until reaching the peripheral sections was 292 ± 53 sec., $N = 46$; GLMM: intercept (open) = 2.19 ± 0.31, estimate (structured) = 2.73 ± 0.44, $z = 6.25$, $P < 0.001$). While in the central sections, the focal females typically formed loose shoals together with the initial females. The positions of the initial females were not systematically recorded, but these females (which had mostly spawned) often appeared to shoal in the central (more open) part of the tank in the structured treatment. In the open environment, these females would often shoal across the full tank.

Both release order and treatment had an effect on the movement of focal females (number of section-boundaries crossed per minute; LRT: treatment $P = 0.003$, release order $P < 0.001$). Thus, we performed separate tests in relation to the release order of the females. The focal females in the structured environment moved around in the tank less actively (crossed fewer section boundaries per minute) than did the females in the open environment (two sample t-test, first female: $t_{26.98} = 2.20$, $P = 0.037$, second female: $t_{26.21} = 2.97$, $P = 0.006$, and third female: $t_{29.86} = 1.82$, $P = 0.08$, fig. 2A). Compared to the focal females of the open environment, the focal females in the structured environment visited a lower number of unique male nest sections per minute (open: median 0.37, range 0.13 – 3.33, structured: median 0.17, range 0 – 1.53; GLMM: intercept (open) = 3.73 ± 0.12, estimate (structured) = -0.87 ± 0.18, $z = -4.91$, $P < 0.001$).

The likelihood that a courtship event (i.e., courtship by the male, the female or both) took place in one of the four central sections of the tank (see fig. 1) was much higher in the structured environment (150 of 235 (64 %) courtship events) than in the open environment (96 of 368 (26 %) courtship events; GLMM: intercept (open) = -1.20 ± 0.31, estimate (structured) = 1.61 ± 0.45, $z = 3.62$, $P < 0.001$, $N = 597$).

Female mate search

The focal females ($N = 48$ in both treatments) were frequently in contact with males during their mate search. Because the release order affected the female search time, our analyses are based on the rates of events per time unit. The release order and treatment had a significant interaction effect on the encounter rate (number of males encountered per minute; LRT: $P = 0.010$). Thus, we conducted separate tests in relation to the release order of females. The male encounter rate for the first released focal female did not differ between the open and structured environment ($t_{18.29} = -0.95$, $P = 0.36$, fig. 2B). However, the second and third focal females had lower encounter rates with males in the structured environment than in the open environment (second focal female: $t_{29.20} = 2.33$, $P = 0.027$, third focal female: $t_{28.21} = 2.17$, $P = 0.039$, fig. 2B).

Compared to the focal females that mated in the open environment, the focal females that mated during observations in the structured environment experienced approximately half as many courtship events (i.e., encounters that included courtship) before mating (open: mean 5.69 ± 1.15, $N= 32$, structured: mean 2.70 ± 0.49, $N = 23$; GLMM: intercept (open) = 1.39 ± 0.16, estimate (structured) = -0.63 ± 0.26, $z = -2.41$, $P = 0.016$). Of all of the courtship events recorded, only 12 out of 597 such events were by a focal female courting an unresponsive male. We recorded 585 courtship events that included male courtship (open: 359, structured: 226), and only 65 courtship events including female courtship (open: 34, structured: 31). When both females that mated and those that did not mate during the observations were included, the proportion of encounters that included courtship tended to be lower in the structured environment (median 0.32) than in the open environment (median 0.55; GLMM: intercept (open) = -0.21 ± 0.30, estimate (structured) = -0.73 ± 0.42, $z = -1.73$, $P = 0.08$). Accordingly, focal females experienced courtship events with fewer males in the structured (median 2 males, range 0 – 5) environment than they did in the open environment (median 3 males, range 0 – 7; GLMM: intercept (open) = 1.09 ± 0.09, estimate (structured) = -0.31 ± 0.13, $z = -2.28$, $P = 0.023$). The release order and treatment had a significant interaction effect on the courtship rate (number of courtship events per minute; LRT: $P = 0.014$). We therefore conducted separate tests to account for the release order of the females. The courtship rate for the first released focal female did not differ between the open and the structured environment ($t_{23.11} = -0.27$, $P = 0.79$, fig. 2C). However, when compared to the females in the open environment, the second and third focal females in the structured environment experienced a lower male courtship rate ($t_{18.42}$ = 2.56, $P = 0.019$ and $t_{21.40}$ = 2.45, $P = 0.023$, respectively, fig. 2C).

During their mate search, the focal females inspected the nests of males without staying to spawn in 42 out of 98 (43%) cases. The median nest inspection lasted for 16 seconds (range 1 – 304 sec, inter quartile range 8 – 54, $N = 40$). The number of nest inspections per focal female did not differ between the treatments (open: median 1, range 0 – 4, and structured: median 1, range 0 – 3; GLMM: $z = -1.40$, $P = 0.16$, $N = 42$). However, if a female made a nest inspection, the likelihood for her to mate in that nest was higher if there were eggs in the nest (GLMM: slope (egg) = 1.72 ± 0.46, $z = 3.73$, $P < 0.001$). The treatment had no effect on this result (GLMM: $z = 0.57$, $P = 0.57$).

Compared to the open environment, focal females searched for a longer time before mating during observations in the structured environment (survival analysis: intercept (open) ± SE = 7.29 ± 0.17, estimate (structured) ± SE = 0.56 ± 0.27, $z = 2.04$, $df = 1$, $P = 0.042$, $N = 96$, fig. 3). Only 23 of 48 (48%) females mated during observations in the structured environment (mean time until mating ± SE = 10.2 ± 1.8 min., range 1.0 – 28.0 min) compared to 32 of 48 (67%) in the open environment (mean time until mating ± SE = 9.5 ± 1.6 min., range 0.3 – 29.5 sec.; GLMM: $z = 0.43$, $P = 0.67$). Almost all of the females released on day two (153 of 160, 96%) mated during the 1.5 days between the release and termination of the experiment.

Male-male competition

Compared to the focal females in the open environment, fewer of the focal females in the structured environment were simultaneously courted by several males (GLMM: intercept (open) = 0.19 ± 0.35, estimate (structured) = -1.82 ± 0.55, z = -3.31, $P < 0.001$, fig. 4A). In line with this result, when compared with the focal females in the open environment, fewer focal females in the structured environment experienced male-male aggression during courtship (GLMM: intercept (open) = -0.99 ± 0.32, estimate (structured) = -1.16 ± 0.57, $z = -2.03$, $P = 0.043$, fig. 4B).

Male reproductive success

Except for fewer nest holders the evening after the introduction of focal females in the structured environment, we found no differences between the treatments for any measures of male success or opportunity for selection (table 3, fig. 5).

The selection differentials (i.e., the mean trait values for breeding males compared to the mean trait of all the males in the population, here: tank) indicated no selection for male length (t_{15} = 0.69, P = 0.50) in the structured environment. However, in the open environment, we found a nearly significant positive selection for length (Wilcoxon signed rank test: V = 106, P = 0.052, fig. 6A), and a statistically significant selection for length after the removal of an extreme outlier (V = 104, P = 0.013, N = 15, fig. 6A). We did not detect any selection on male body condition in either the open (one-sample t-test: t_{15} = 0.44, P = 0.67) or the structured (t_{15} = -0.45, P = 0.66) environment (fig. 6).

Discussion

Habitat complexity had significant impacts on the mating behavior of both males and females. When compared to females in the open environment, the females in the structured environment were slower to begin exploring, moved around less, had lower male encounter rates, experienced courtship interactions with fewer males, and experienced a longer search time before mating. Fewer females also experienced multi-male courtship and male-male aggression in the structured environment. The opportunity for selection, which was estimated from the variation in reproductive success, was unaffected by the treatment. Despite this finding, we found evidence of selection on male length in the open, but not in the structured, environment. Thus, habitat complexity might relax sexual selection by affecting the mating behaviors of both males and females.

The effects of habitat complexity on female mating behavior

We found that the order of release for the focal females significantly affected what they experienced during their mate search (see fig. 2). We typically detected a greater interest by the males for the second and third focal females compared to the first. An inevitable consequence of the chosen design was that density changed during the course of the experiment. However, the changes in density were modest (range 4 – 5.25 fish/m^2) and are unlikely to have affected the observed behaviors. The changes in density of the two sexes also affected the operational sex ratio, but again to a modest degree that cannot explain the major behavioral effects seen. Also, the differences between treatments were minimal (see fig. 5). The greater interest by males for the second and third focal female might rather be due to a lag in the male's recognition of the presence of mating-ready females, which might cause the sexual activity of the males to increase as time elapsed and as more females were released for observations. Such a scenario is in line with a recent study on guppies (*Poecilia reticulata*) in which males responded to their recent social environment, rather than to immediate stimuli (Jordan and Brooks 2012). Because there was little or no difference between the two environments for the first-released female, but clear patterns for the two later-released females, we base our general inference on the patterns revealed by the later-released females.

Compared to females in the open environment, the females in the structurally complex environment were slower to move out of the central parts of the tank and start exploring the environment. The females in the complex environment also moved around less, crossed fewer section boundaries, and visited fewer unique male nest sections per minute. These findings suggest that habitat complexity constrain female movement and the detection of males, both of which could affect sexual selection.

Compared to the focal females in the open environment, the second and third-released focal females in the structured environment had a lower male encounter rate and experienced a lower rate of courtship events (number of courtship events per minute observed). A lower courtship rate is inevitable if the females experience a lower encounter rate. However, also the proportion of encountered males that courted the focal females tended to be lower in the structured environment compared to the open environment. This indicates that the lowered courtship rate is not only a direct result of a lower encounter rate. Also in three-spined sticklebacks (*Gasterosteus aculeatus*) it has been found that female encounter rate was lower for males in less open territories (Candolin and Voigt 2001). If lower male encounter rates lead to overall fewer males visited (as is likely in the wild), our results suggest that sexual selection by mate choice would be weakened by increasing habitat complexity.

Fewer of the females mated within the 30 min observation time in the structured environment, but most (96%) of the females had mated by the end of the experiment. A structurally complex environment might make it harder to detect males (and for the males to detect females). Thus, it might take longer to find a suitable mate. Some previous studies have found that females spend more time evaluating a male when the visibility is poor (guppy: Hibler and Houde 2006, sticklebacks: Candolin et al. 2007, Engström-Öst and Candolin 2007). In order to discriminate between males of different size, female two-spotted gobies seemingly need to see both males at the same time (Å.A. Borg, E. Forsgren and T. Amundsen, unpublished data). Thus, a structurally complex environment could hamper female choice by making it difficult to compare males. Regardless of the treatment, females were more likely to stay and spawn in nests if there were eggs present. This suggests that females prefer males with eggs in their nest, as found in many other fishes (e.g., Jamieson 1995, Forsgren et al. 1996, Reynolds and Jones 1999). To summarize, when compared to the females in the open environment, the focal females in the structured environment swam around less, encountered interested males less frequently, had courtship events with fewer of the males and experienced fewer opportunities for directly comparing males. These findings suggest that it was harder for the females to choose between males in the structured than in the open environment, and that their mate search process was slower.

The effects of habitat complexity on male mating behavior

Two-spotted goby males compete over nests, a resource necessary for breeding (i.e., to be qualified to mate; Ahnesjö et al. 2001), and for access to mates (Forsgren et al. 2004, de

Jong et al. 2009, Wacker et al. 2012). Compared to the open environment, fewer females in the structured environment experienced simultaneous courtship by several males, and there were fewer male-male aggressive interactions during courtship. It might have been more difficult for the males in the structured environment not only to detect females, but also to detect and join on-going courtship events. Thus, a more structurally complex environment appears to reduce male-male competition by means of courtship interference. Reduced courtship interference as a consequence of visual obstructions has also been found in guppies (Hibler and Houde 2006). Male-male interactions could help females assess male qualities, but male dominance may also constrain females from choosing freely between potential mates (Qvarnström and Forsgren 1998, Kangas and Lindström 2001, reviewed in Wong and Candolin 2005).

In the open environment, males did not have to leave their nest sections to attract females because the females frequently visited these sections. However, in the structurally complex environment, a higher proportion of courtship events took place in the central part of the tank, away from the males' nests, where the females appeared to be primarily shoaling. The results suggest that when males detect where females are gathered, the males go there to seek out a potential mate. This finding is consistent with another study which indicated that male two-spotted gobies spend less time in their nests when the encounter rate with ready-to-mate females is low (de Jong 2011).

The effects of habitat complexity on the scope for sexual selection

We found no difference among the treatments in the number of males that received eggs or in the opportunity for selection. Previous studies have found that the mating skew decreases under increased algal cover (three-spined sticklebacks: Candolin 2004) and with more turbid conditions (sand gobies, *Pomatoschistus minutus*: Järvenpää and Lindström 2004). In fishes, the time window from when a female has ovulated until she needs to spawn is, at most, only a few days (Mollah and Tan 1983, Kjørsvik et al. 1990, Legendre et al. 2000). We introduced two non-focal and three focal females during a relatively short time period (< 3 h). In the related sand goby, the duration of a spawning event is approximately one-two hours and our impression is of a similar duration for two-spotted gobies (personal observations). Thus, if superior males were all engaged in spawning, females would face a choice between either postponing spawning or mating with an inferior male. Hence, the rather synchronous release of females might explain why we did not detect any difference in the opportunity for selection between treatments. Synchronous arrival of females is generally considered to reduce the potential for males to monopolize females (e.g., Emlen and Oring 1977, Grant et al. 1995, Lindström and Seppä 1996). Unlike the situations in the wild, the females of this study were confined to the tank and had no other mating options.

Comparing the males that mated (i.e., had eggs in their nest) to all of the males in each tank, we detected a positive selection for male length in the open environment (after removing an outlier). However, we did not detect such selection in the structured environment. A positive selection for male length could have resulted from female choice or from male competition (e.g., Censky 1997, Howard et al. 1998, Hagelin 2002, Schütz and Taborsky 2011), both of which are important in two-spotted gobies (Borg et al. 2006, Wacker et al. 2012, T. Amundsen and J. Bjelvenmark, unpublished data). There is evidence that habitat complexity affected both mate choice and male competition processes (discussed above). These effects seem to a large extent to occur because habitat structure affects the social structuring of individuals, their behavior, and the rates of male-female and male-male encounters (e.g., Oh and Badyaev 2010). Hence, when investigating potential effects of habitat structure on animal behavior, these must be analyzed in a social rather than individual perspective.

Conclusion

Habitat complexity strongly affected the mating behaviors of both females and males. In the complex habitat, females generally moved around less, experienced fewer male encounters and less courtship, and took longer to mate. For males, a complex habitat appeared to hamper their detection of searching females and also of other males engaged in courtship, which resulted in less frequent multi-male courtship events and less frequent male-male courtship interference. From a female point of view, it appears that habitat complexity makes it harder to detect potential mates. It might also be more difficult to visually compare alternative mates, partly because females cannot simultaneously see neighboring nest-holding males, but also because the females more rarely experience two or more males courting at the same time. From a male perspective, the structure appears to reduce male-male competition. Thus, a structurally complex habitat might weaken sexual selection through effects on both male and female behavior. Consistent with this finding, and despite the treatments' lack of effect on the opportunity for sexual selection, we found a positive selection for male size in the open, but not in the structured, environment.

In conclusion, our results suggest that variation in habitat complexity, which occurs naturally in most species but can also result from human impacts, significantly affects central processes of sexual selection. This is an insight of wide-ranging implication and relevance, given that sexual selection is a major force in shaping animal behavior and morphology, and important for the reproductive potential of populations. Knowing how habitat structure affects sexual selection will be important in predicting animal responses to environmental change. Also, habitat structure should be taken into account when interpreting results from studies on sexual selection in the wild (or in the laboratory). More research is needed to reveal the importance of habitat complexity for the processes of mate choice and intra-sexual mating competition across animal taxa.

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Table 1: Behaviors recorded during observations of focal female two-spotted gobies (*Gobiusculus flavescens*).

Behavior	Definition
Courtship:	
Sigmoid display	Female bending to a sigmoid shape, displaying distended orange belly
Fin display	Male erecting dorsal and anal fins
Quiver display	Male quivering his body
Lead display	Male swimming with undulating body movements towards nest
Agonistic behavior:	
Male-male fin display	Males lining up side-by-side, erecting dorsal and anal fins
Chase	Darting towards another individual, often with extended fins
Other definitions:	
Male encounter	Focal female < 2 body lengths from a male
Mating	Focal female staying >10 min in a male's nest
Search time	Time from commencement of normal swimming until entering nest for mating If no mating occurred within 30 min, the search time was set at 30 min
Courtship event	Courtship by the male, the female, or both
Nest inspection	Female entering a male's nest

Table 2: The characteristics of female and male two-spotted gobies (*Gobiusculus flavescens*) used to test the effect of habitat complexity on mating behavior. Total length (mm) and body mass (g) are given as the mean ± 1 SE (range). Differences between the treatments, open and structurally complex (structured), are tested with a two sample t-test. Males and initial females are those introduced to the tank on day zero, the non-focal females were introduced on day two but were not observed, and the focal females are those fish that were released and observed on day two. See Methods for further explanations.

		Open	Structured	*t*	*df*	*P*
Initial females	*N*	128	128			
	Length	41.83 ± 0.25 (35.0 – 48.5)	41.51 ± 0.25 (36.0 – 49.0)	0.89	253.68	0.37
	Body mass	0.666 ± 0.011 (0.387 – 0.979)	0.652 ± 0.011 (0.408 – 1.033)	0.92	253.74	0.36
Males	*N*	128	128			
	Length	42.01 ± 0.31 (35.0 – 52.0)	42.42 ± 0.34 (35.5 – 53.5)	-0.87	252.17	0.38
	Body mass	0.614 ± 0.014 (0.349 – 1.091)	0.635 ± 0.015 (0.321 – 1.193)	-0.99	252.37	0.32
Non-focal females	*N*	32	32			
	Length	42.56 ± 0.48 (36.0 – 48.5)	43.19 ± 0.59 (36.5 – 49)	-0.91	61.94	0.36
	Body mass	0.728 ± 0.022 (0.414 – 0.979)	0.749 ± 0.025 (0.456 – 0.980)	-0.62	60.92	0.55
Focal females	*N*	48	48			
	Length	43.18 ± 0.45 (35.0 – 50.5)	43.90 ± 0.42 (36.5 – 48.5)	-1.18	93.29	0.24
	Body mass	0.761 ± 0.023 (0.423 – 1.205)	0.793 ± 0.021 (0.459 – 1.089)	-1.01	93.02	0.32

Table 3: Tests for the effect of habitat complexity on nest ownership, mating success and mating skew in male two-spotted gobies (*Gobiusculus flavescens*) over time. Focal females were added between 42 and 53 hours after the trial initiation. The tests are between an open and a structurally complex environment and performed with either the Welch two-sample t-test or the Wilcoxon rank sum test. Significant differences are indicated in bold. § = estimated by the variation in the number of eggs in the nest at the end of the experiment.

	Hours after trial initiation					
	18 h	29 h	42 h	53 h	66 h	73 h
No. of nest holders	$W = 101.5$ $P = 0.32$	$W = 139$ $P = 0.69$	$t_{24.37} = 1.19$ $P = 0.24$	$t_{28.10} = 2.37$ $\mathbf{P = 0.025}$	$W = 161.5$ $P = 0.19$	$W = 130$ $P = 0.95$
No. of nest holders with eggs	$W = 94$ $P = 0.19$	$W = 130.5$ $P = 0.94$	$t_{28.53} = 1.05$ $P = 0.30$	$W = 135$ $P = 0.80$	$W = 127.5$ $P = 1.00$	$W = 131.5$ $P = 0.91$
Mean nest fullness	$W = 0.67$ $P = 0.93$	$W = 107.5$ $P = 0.64$	$W = 90.5$ $P = 0.16$	$W = 116.5$ $P = 0.68$	$W = 106.5$ $P = 0.43$	$W = 117$ $P = 0.70$
Opportunity for selection (*I*)	$W = 75$ $P = 0.54$	$W = 124$ $P = 0.89$	$W = 117.5$ $P = 0.71$	$W = 120$ $P = 0.78$	$t_{28.38} = 0.29$ $P = 0.78$	§$t_{29.73} = 0.38$ $P = 0.71$

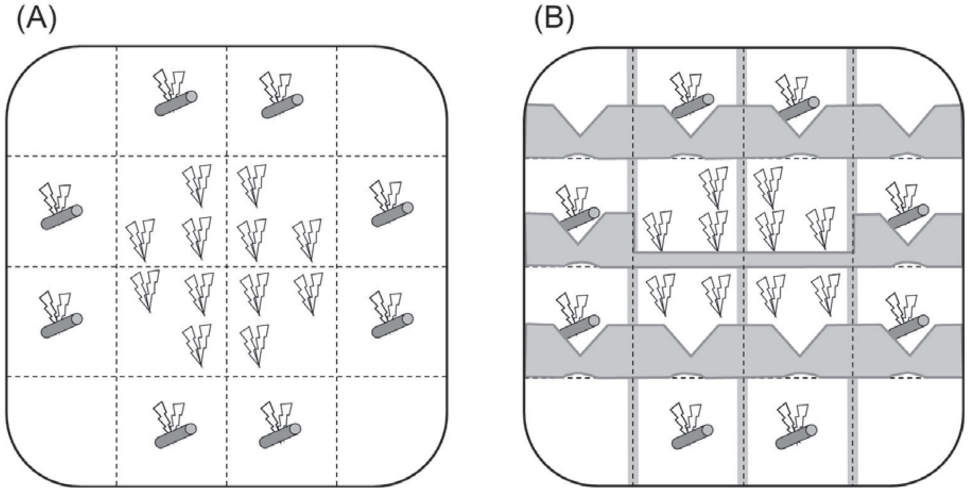

Figure 1: Design of the experiment to test for effects of habitat complexity on mating behavior and mating success in two-spotted gobies (*Gobiusculus flavescens*). (A) The open environment treatment with only plastic plants and eight artificial nests. (B) The structurally complex environment treatment, where opaque plastic dividers across the tank (grey area) reduce the visual range and contact among the fish. The dividers had the same shape for both dimensions. The thin dotted lines illustrated where we marked the tank in sections. The dark grey tubes are the artificial nests. See Methods for further details.

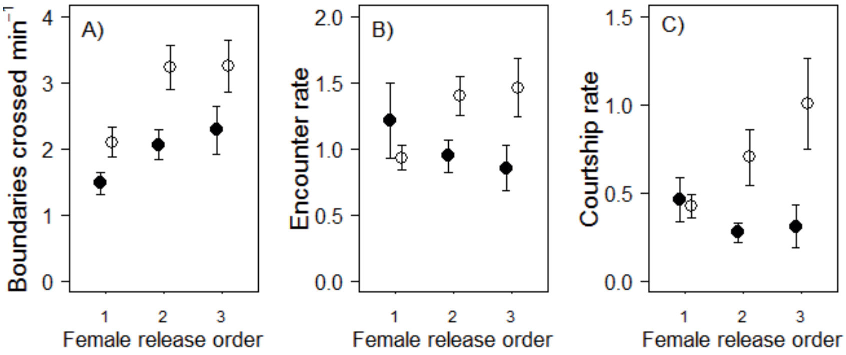

Figure 2: The effects of habitat complexity (open environment: open dots; structured environment: filled dots) and the release order of the focal female two-spotted gobies (*Gobiusculus flavescens*) on the number of A) section boundaries crossed per minute, B) male encounters per minute, and C) courtship events per minute. Each point represents the mean ± 1 SE (*N* = 16, for each point).

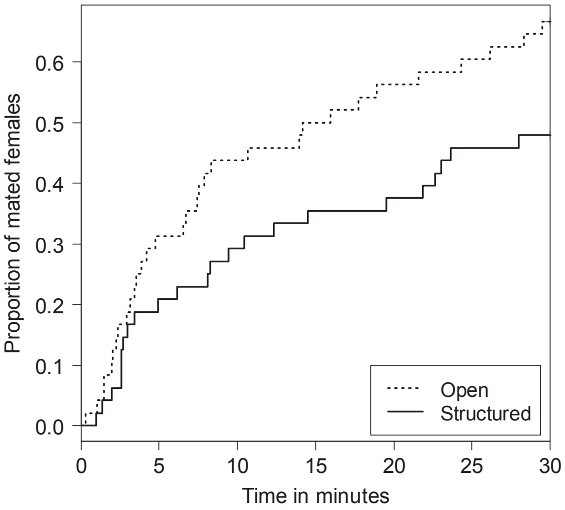

Figure 3: The effects of habitat complexity on the proportion of focal female two-spotted gobies (*Gobiusculus flavescens*) that mated over time (*N* = 48 for both open and structured environment) during 30 min observations (0 = commencement of normal swimming).

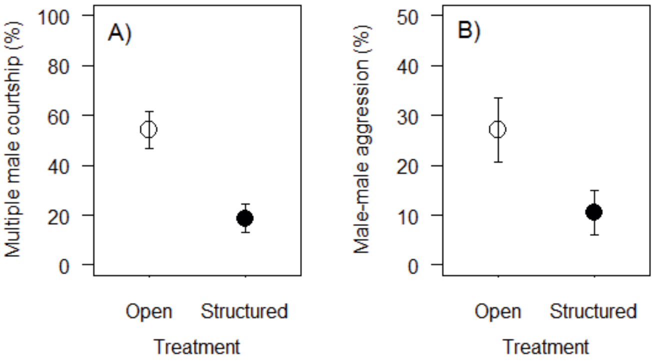

Figure 4: The effect of habitat complexity on multiple-male courtship and male-male aggression during courtship events. The percentage (mean ± 1 SE, *N* = 48 for both treatments) of focal female two-spotted gobies (*Gobiusculus flavescens*) that experienced courtship events in the open (open dots) and structured environment (filled dots) in which A) several males courted them simultaneously, and B) a courting male showed aggressive behavior toward other males.

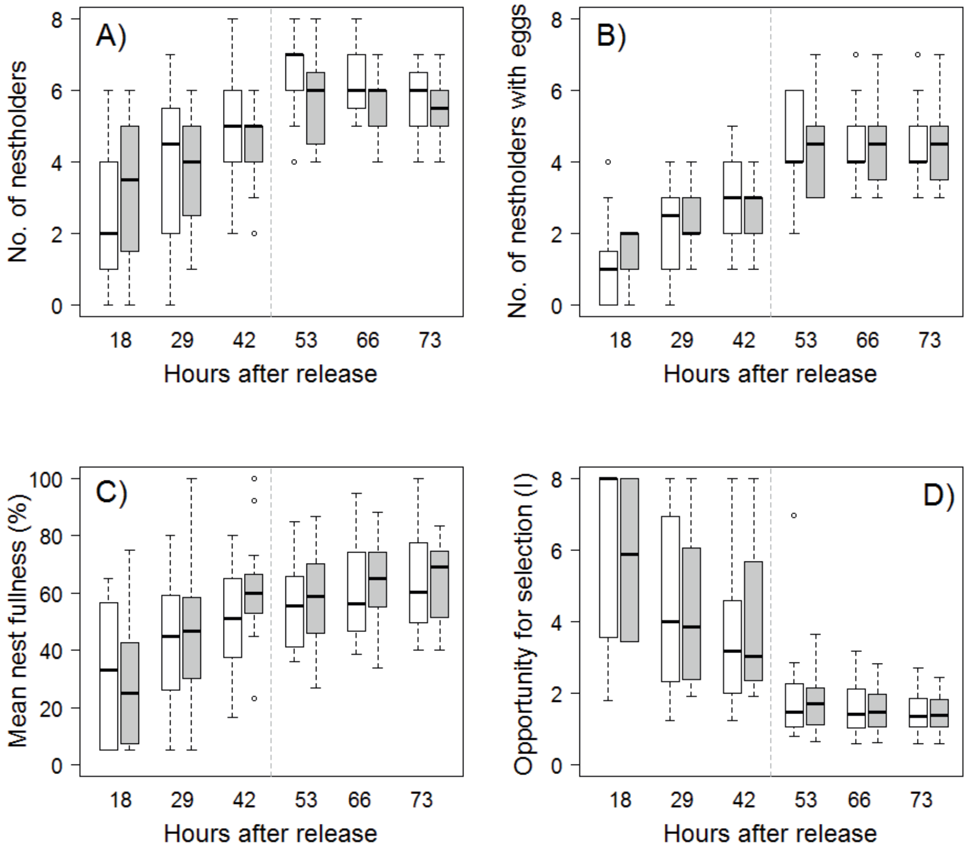

Figure 5: The effects of habitat complexity on nest ownership, mating success and mating skew in male two-spotted gobies (*Gobiusculus flavescens*) over time for an open environment (open bars, N = 16) and a structurally complex environment (shaded bars, N = 16). A) The mean number of nest-holders, B) the mean number of nest-holders with eggs, C) the mean nest fullness (%) for males with eggs (after 18 hours: open N = 10, structured N = 13, for the other observation times $N_{open} = N_{structured}$ = 16) and D) the opportunity for selection (I) in egg cover (cm^2) for all males. The dashed grey line represents the time where we introduced focal females for observations in the tanks. The thick lines in the boxes represent the median for each distribution, while the top and bottom of the boxes represent the 75th and 25th percentiles. The dashed error bars extend to the most extreme data point ≤ 1.5 times the interquartile range from the box. Outliers are shown as separate data points.

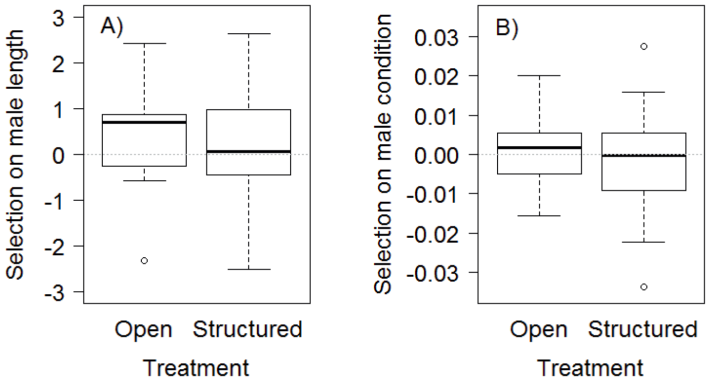

Figure 6: The effect of habitat complexity on the selection for male total length and male body condition in two-spotted gobies (*Gobiusculus flavescens*). The selection differentials (the mean trait for breeding males compared to the mean trait of all the males in the tank, *N* = 16 for each environment) for A) male length (mm) and B) male condition (the residuals from the regression between male length and weight). For boxplot details, see figure 5.

PAPER 3

How does personality relate to mating behaviour? An experiment with two-spotted gobies

Running title: Boldness and mating behaviour

Lise Cats Myhre[a]*, Elisabet Forsgren[b], Sebastian Wacker[a], Trond Amundsen[a]

[a]Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway
[b]Norwegian Institute for Nature Research (NINA), Postbox 5685 Sluppen, NO-7485 Trondheim, Norway
*Corresponding author, e-mail: lise.myhre@bio.ntnu.no
Telephone: +47 73596220, Fax: +47 73596100
Other authors e-mail: elisabet.forsgren@nina.no, wacker@bio.ntnu.no, trond.amundsen@bio.ntnu.no

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ABSTRACT: Animal personalities have received much attention the recent years. To understand how personality might potentially affect fitness, it is important to increase our knowledge and understanding of the relationships between personality and sexual behaviours. In this study, we investigated how 'boldness' relates to mating behaviour and mating success in male two-spotted gobies (*Gobiusculus flavescens*), a small marine fish with paternal care. We assessed boldness using a standard personality assay (emergence test) and thereafter analysed mating behaviour and mating success in mesocosm tanks. We exposed males to either open or complex environment to investigate the interactive effects of personality and environment on mating behaviour and mating success. Males were housed together with females at an even sex ratio, and allowed to breed. After two days, we released additional ready-to-mate females into the tanks and observed the mating behaviours of males during encounters with these females. We found no evidence for 'boldness' to relate to aggression during courtship interruptions or with the likelihood of becoming a nest holder. However, we found a positive relationship between boldness and the likelihood of courting encountered females, particularly in the complex environment. Moreover, 'bold' males performed more of their courtship near the nest and obtained matings faster than 'shy' males. The mating success of bold males was higher than that for shy males, which resulted in the nests of bold males generally containing more eggs than those of shy males. Our results indicate that personality might affect reproductive success and fitness by affecting male courtship behaviour.

Keywords: personality, boldness, mating behaviour, mating success, reproductive success, two-spotted goby, *Gobiusculus flavescens*

Animal personality (Gosling 2001), also referred to as temperament (Réale et al. 2007), behavioural syndromes (Sih et al. 2004b) and coping styles (Koolhaas et al. 1999), is defined as consistent differences between individuals in their behaviour across time and context (e.g., Gosling 2001; Réale et al. 2007). In animals, personality is commonly divided into five behavioural axes (sensu Réale et al. 2007): (i) shyness-boldness, (ii) exploration-avoidance, (iii) activity, (iv) aggressiveness, and (v) sociability.

In recent years, animal personalities have been found to relate to several traits of ecological importance (reviewed in Réale et al. 2007; Sih et al. 2004a; Sih et al. 2012). For instance, personality has been found to co-vary with dispersal (e.g., Cote & Clobert 2007; Dingemanse et al. 2003; Rasmussen & Belk 2012), dominance (e.g., Colléter & Brown 2011; Dingemanse & de Goede 2004), and foraging (e.g., Bergvall et al. 2011; David et al. 2011; Nannini et al. 2012). Personality might also relate to an animals' reproductive success (Smith & Blumstein 2008). For example, personality traits of fish have been found to co-vary with male fertilisation success (zebrafish, *Danio rerio*: Ariyomo & Watt 2012), and hierarchy position, and thereby, with reproductive success (rainbowfish, *Melanotaenia duboulayi*: Colléter & Brown 2011). Bold bighorn sheep rams (*Ovis canadensis*) were found to survive longer than shy rams and to experience higher reproductive success later in life (Réale et al. 2009). Although there are several examples of relationships between personality and reproductive success (e.g., Ariyomo & Watt 2012; Colléter & Brown 2011; Réale et al. 2009; Smith & Blumstein 2008), the relationship between personality and specific mating behaviours remains poorly understood (but see Godin & Dugatkin 1996; Magellan & Magurran 2007). Knowledge about the links between personality traits and behaviour can further our understanding of the effects of personality on fitness and the evolutionary background for personality differences.

Relationships between personality traits and behaviour might vary between environments (e.g., Brown et al. 2005; Dingemanse et al. 2007). Dingemanse et al. (2007) found that the behavioural correlations between aggressiveness, activity and exploratory behaviour in sticklebacks (*Gasterosteus aculeatus*) only existed in ponds where piscivorous predators were present. Likewise, the fitness consequences of different personality traits might depend on environmental factors and thereby contributing in maintaining personality variation within populations (Dingemanse et al.

2004; Réale & Festa-Bianchet 2003). Although studies of animal personalities have received much attention in recent years (Conrad et al. 2011; Dingemanse & Réale 2005; Gosling 2001; Réale et al. 2007; Sih et al. 2004a; Sih et al. 2004b; Sih et al. 2012; Smith & Blumstein 2008), few studies have investigated how personality relates to reproductive success under different environmental conditions (but see Dingemanse et al. 2004), and furthermore, how personality is related to mating behaviours.

In this study, we investigated whether male two-spotted gobies (*Gobiusculus flavescens*) differ in their personalities, and if so, whether their personality relates to mating behaviour, and consequentially, to mating success. The two-spotted goby is a small, marine, substrate-brooding fish. In other species with a similar biology to the two-spotted goby, personality has been found to correlate with risk-taking behaviour in relation to predators (e.g., sticklebacks: Huntingford 1976; convict cichlids, *Amatitlania nigrofasciata*: Jones & Godin 2010; fathead minnows, *Pimephales promelas*: Pellegrini et al. 2010). The two-spotted goby inhabits and breeds in the kelp forests along the rocky shores of Western Europe (Collins 1981; Wheeler 1980) and seeks shelter among the algae when threatened by predators (Utne & Aksnes 1994; Utne et al. 1993). Juvenile cod (*Gadus morhua*) represents one of the main predators of this species (Fosså 1991; Salvanes & Nordeide 1993). Two-spotted goby males take up and defend a nest in brown algae or empty mussel shells (Gordon 1983; Mobley et al. 2009), and provide paternal care until their eggs hatch (Bjelvenmark & Forsgren 2003; Skolbekken & Utne-Palm 2001). Potentially, in the two-spotted goby, the personality type might affect a male's propensity to leave the safer algal environment for the open water column to compete for mates, which in turn might affect mating success. The environment that is inhabited by the two-spotted goby can vary from relatively open to structurally complex, both between and within populations and locations. This means that any personality-environment interaction might result in variable mating behaviours both within and between populations.

To investigate potential personality differences between the males, we assessed a central and ecologically relevant aspect of male personality in a standardised assay, the emergence test, which evaluates the willingness to leave shelter. Thereafter, individually marked fish were released into large tanks together with females at an even sex ratio. To analyse the relationships between male personality and mating behaviours, we observed males during encounters with ready-to-mate females, as well as with any other males at such encounters. The study was performed in tanks with either an open or a structurally complex environment. The physical environment has been shown to affect mating behaviours in this species (Myhre et al. manuscript) and can affect the extent of mating competition in other species (Dingemanse et al. 2004). The aim of this study was to test whether personality relates to mating behaviours and mating success in males and to explore any interactions between personality and the environment in regards to mating.

METHODS

This study was conducted at the Sven Lovén Centre for Marine Sciences at Kristineberg, on the west coast of Sweden, from 11 May to 21 June 2010.

Study species

The two-spotted goby (*Gobiusculus flavescens*) is a small (adult mostly 35-55 mm), sexually dimorphic, marine fish. It is semi-pelagic (Wheeler 1969) and often forms large shoals close to the substatum and around fronds of algae (Svensson et al. 2000). The species is often associated with kelp forests that are dominated by *Laminaria* spp. and *Saccharina latissima* (Svensson 2006; Wheeler 1980). However, there are local variations in habitat complexity, vegetation and bottom substrate. In nature, fish density varies according to localities and time of season (Forsgren et al. 2004). On a local scale, the density can range from single individuals up to shoals of several hundred individuals (Svensson et al. 2000). Two-spotted gobies live in a habitat where they can easily (quickly) move between the open water column and the kelp forest. When threatened by a predator, they typically seek shelter (Utne & Aksnes 1994; Utne et al. 1993) but ultimately leave shelter to forage and to attract mates (Myhre et al. 2012; Utne et al. 1993). During the breeding season, females actively search for mates and visit males sequentially (Myhre et al. 2012). Males also leave their nest to look for mates at this time. The males attract females to their nests via a suite of the following courtship displays: fin (erecting dorsal and anal fins), quiver (quivering the whole body) and lead displays (swimming with undulated body movements towards his nest)

(Amundsen & Forsgren 2001; de Jong et al. 2009; Forsgren et al. 2004; Pélabon et al. 2003). Males exhibit agonistic behaviours via male-male fin displays (males lining up side-by-side, erecting their dorsal and anal fins) and chases (darting towards another individual, often with extended fins) (Forsgren et al. 2004; Wacker et al. 2012). Females court by bending their bodies to display their orange and distended bellies (sigmoid display) (Amundsen & Forsgren 2001). Both sexes can initiate courtship (e.g., Amundsen & Forsgren 2001; de Jong et al. 2009; Forsgren et al. 2004). The females can respond to male courtship with courtship and by following the male to his nest. In the nest, females deposit clutches of typically 1000-1500 eggs (Pélabon et al. 2003; Svensson et al. 2006). Males might simultaneously care for clutches from several (2-6) females (Gordon 1983; Mobley et al. 2009). The two-spotted goby normally lives for only one year (Johnsen 1945), but both sexes can reproduce repeatedly during their single breeding season (Collins 1981; de Jong 2011, K. de Jong, L. Rodrigues-Graña, unpublished data).

General procedures

The fish were caught by snorkelers with hand-held dip nets near the research station and transported to the laboratory by boat in covered plastic containers. For the experiments, we used only males that appeared to be ready-to-mate (see Forsgren et al. 2004) and that did not harbour visually detectable parasites, and females with distended bellies (an indication of gonad maturity, Svensson et al. 2009). The males were placed into separate containers for transport to the laboratory. At the laboratory, the males were transferred to individual aquaria (25 x 30 x 30 cm, L x W x H) placed in a thermo-constant room (16°C) and were acclimated for approximately 40 hours prior to being subjected to a personality assay (described below). The females were kept together in large aquaria (60 x 40 x 35 cm, up to 35 females) for two days before being used in the experiment. We measured the total length of all of the fish (TL, to the nearest 0.5 mm) using a measuring board, and determined wet body mass (BM, to the nearest 0.01 g; after careful blotting of the fish) using a Mettler digital balance. Males were measured after the personality assay. All of the fish used in the experiment were marked individually using Visible Implant Elastomer (VIE) tags (North-West Marine Technology, Shawn Island, Washington), as described by de Jong et al. (2009). Using four colors (blue, red, green and orange), each male was marked at one of two possible locations next to the dorsal fins. The females were given two marks, each at two of four possible locations, using three colors (blue, red and green; 54 combinations). All fish regained normal swimming behaviour within 10 min after this procedure and we could not detect any adverse effects of marking. The fish was next randomly assigned for treatment. All of the fish were fed twice per day (morning and evening) *ad libitum* with *Artemia* sp. nauplii. The males were fed at two to five hours prior to the start of the personality assay.

Neither the length nor mass of the males and females differed between environment treatments (table 1). We used males of a range of sizes to mimic the natural populations. The length of the males ranged on average from 37.8 ± 0.3 mm to 47.8 ± 0.5 mm (smallest to largest in each trial) and the within-trial size range was similar between treatments ($t_{29.21} = -0.06$, $P = 0.95$).

Personality assay

To assess male personality, we conducted an 'emergence-test' that measured an individual's propensity to leave a safe area (i.e., refuge) and to emerge into an unknown or less safe (open) area. This is a standard personality test used in fish, and is mostly interpreted to reflect individual 'boldness' (e.g., Brown & Braithwaite 2004; Brown et al. 2005; Scharnweber et al. 2011). The choice of leaving or not leaving shelter (as in the 'emergence test') is one that two-spotted gobies, as well as many other fishes, regularly face in their natural environment. Their willingness to leave shelter might reasonably be considered to reflect some kind of boldness. The personality test aquaria (75 x 25 x 30 cm) were painted brown on all four sides to minimise reflections and external influences during the experiment. Each aquarium included two environments (see fig. 1): a sheltered area (i.e., refuge) with gravel and plastic plants (25 x 25 cm), and an open area with no structural elements (50 x 25 cm). The latter was assumed to be perceived as a risky area by the fish. An opaque divider separated the two environments when the male was first introduced into the sheltered area. After 30 min of acclimation, the divider was lifted approximately 10 cm, allowing the fish to enter the open area. We left the room immediately after lifting the divider and video-recorded the activity in the larger (open) section for 30 min from when the divider was lifted.

From the recordings we calculated the following parameters for each fish: (i) the emergence time, measured as the time (s) for the emergence of the snout from the shelter, (ii) total number of trips out of the shelter, (iii) the mean trip time (out of shelter), (iv) the maximum distance moved from the shelter (in 5 cm intervals) and (v) the mean maximal distance from the shelter per trip. If a given male did not emerge from the shelter within 30 min, we assigned it the maximum score for emergence time (1800 s), and a minimum score of zero for all of the other measurements.

We log-transformed (x + 1) the behavioural variables (except for the measurements regarding distance from the shelter) before standardising the variables to produce a mean value of zero and a standard deviation of one. We then performed a principal component analysis (PCA) that included varimax rotation (table 1). The absolute loadings of the behavioural variables on principal component 1 (PC1) were generally similar throughout, and only component 1 exhibited a high eigenvalue (> 1) in all cases (see table 2a). This finding suggests that our behavioural variables reflected differential expression of the underlying trait. We interpret the willingness to leave shelter as an expression of boldness, and therefore, we will refer to it as ‘boldness’ hereafter.

To validate the emergence test as an expression of consistent individual differences in behaviour, we had previously (in 2009) tested another (smaller) set of males twice using the same personality assay, with 48 h between each test (trials 1 and 2). They were treated and subjected to identical conditions as the males evaluated in 2010. We tested for individual consistency in the responses between the trials on these males. The PC1 scores from trial 1 (table 2b) and from trial 2 (table 2c) were strongly correlated ($r = 0.58$, $t_{38} = 4.44$, $N = 40$, $P < 0.001$) and the PC1 structure (i.e., factor loadings) was very similar for the two trials. Thus, we established that the behaviour measured was generally consistent within individuals, and as such, was indicative of individual personality. The presentation of the PCA in table 2 illustrates the relationship between the variables recorded and the similarity with the consistency test between the different groups of males.

Experimental design and observation protocol

We aimed to test whether personality correlated with mating behaviours and mating success. We also aimed to test whether the physical environment affects the relationships between personality and mating. We therefore designed two treatments, an open and a structurally complex environment, and we tested for a potential interaction between treatment and male personality.

Open and structurally complex environment treatments (hereafter referred to as "open" and "complex" environments) were established in indoor tanks (size 2 x 2 m) with water depths of approximately 35 cm. We provided each tank with 20 plastic plants and 8 PVC nest tubes (each 80 mm long with a 13 mm inner diameter, lined inside with an acetate sheet, attached to a rock). Each nest was capable of containing clutches from approximately four females (Bjelvenmark and Forsgren 2003, Forsgren et al. 2004). The tanks were marked in sections (50 x 50 cm) with tape to facilitate the recordings of the locations. The open environment had only the artificial nests and plants as structural elements in the tank (fig. 2). The fish in this environment could therefore see most of the other fish in the tank most of the time. The complex environment had the same basic setup in regards to nests and plants, but included six opaque plastic dividers across the length and width of the tank. These dividers created sections (50 x 50 cm) that were largely visually isolated (matching the division of the marked sections) around the artificial nests (fig. 2). The dividers did not preclude movement between sections as the fish could swim both under and over the dividers. The dividers did however, limit the visual contact between the fish in the tank, and thus, likely impaired the detection of potential mates and competitors. The set-up was also used for another study that tested how habitat complexity affects female mate search and mating behaviour, as well as male mating success (Myhre et al. manuscript).

The tanks had continuous flow of seawater from 7 m depth. The water temperature followed natural sea temperatures and ranged from 10.5 to 16.8 °C during the experiment (temperature was not recorded for the two first replicates). The light schedule followed a naturally regimented summer schedule for that latitude (light:dark 17:7 h). Two replicates were performed in parallel at any one time - two of each treatment. In total, we performed 16 replicates of each treatment.

We used a two-phase design for the experiment. First, we established a breeding population in each tank, allowing the males and females the time required to acclimate to the laboratory conditions, to interact freely, and to spawn (no behavioural observations at this stage). Two days later, we released focal females into the tanks and observed them as they sampled the males. At this stage, typically 4-5 of the males in the tank held a nest, and approximately three of these males had eggs in their nest. In 7 (open: 3, complex: 4) out of 32 trials, one of the males in a tank had a full nest, which left 7 out of 8 males available for mating. Furthermore, most of the initial females had spawned at this stage, and therefore, males would focus their interests on the newly released females. We recorded natural mating behaviours in encounters between focal females and the individual males. We also recorded the mating success of males (whether they had eggs in their nest, and how many) at various stages of the experiment.

The first phase was initiated at 1-3 h after the personality assay was conducted. Individually marked males and females (sex ratio 8:8) were released together in the middle of the tanks at ca. 14:00 h (day 0). Every morning at approximately 8:00 h and every evening at approximately 19:00 h, we recorded which nest each particular male attended. As a measure of male reproductive success, we visually estimated the percentage of the nest area covered with eggs (nest fullness, in 10 % increments). The second phase was initiated on day 2, approximately 44 hours after the initiation of the trial, when we sequentially released five marked ready-to-mate females. We conducted focal observations on three of these five released females. The focal females were released and observed one at a time. We released two non-focal females just before the release of the first focal female to make sure that the focal females were not the only spawning-ready females in the tank at the time of observation, as well as to minimise the difference in social environment between the three consecutive focal females. The females were observed searching for mates until mating or for 30 min if mating had not already occurred. We collected data on the individual male behaviours at encounters with (< two fish lengths ≈ 10 cm from) mate-searching focal females and noted in which section the encounters occurred. This observation protocol was aimed to collect data on the male-female interactions and on female mating in relation to boldness of males, as well as any behavioural interactions with other males during the male-female encounters. The protocol did not record male-male aggression unless it was in the context of male-female interactions. We recorded the mating behaviour from 236 out of 256 males. The remaining 20 males never encountered any focal females during observation. On the afternoon of day 3, we terminated the replicates (after 15:00 h, 73 h after trial initiation), by which time most of the females had spawned. We collected all of the nests and photographed the egg clutches for the counting of eggs that would provide an estimate of reproductive success of the individual males. The eggs within a nest could be from one or several females. The estimated nest fullness (visually assessed; see above) was highly correlated with the number of eggs in a nest at the termination of the trials (r_s= 0.95, $S = 24234.23$, N = 144, $P < 0.001$).

Statistical analyses

All of the analyses were conducted in R version 2.14.0 (R Development Core Team 2011). For the linear (LMM) and the generalised linear mixed models (GLMM), we used the function *lmer* from the *lme4* package (Bates et al. 2011). Due to truncation of the PC1 scores (because many of the males never left the shelter during the emergence test), we analysed all personality data in two different ways: (1) using PC1 score on a continuous scale, and (2) as a factor with two levels (males that emerged vs. those that did not, hereafter referred to as 'bold' vs. 'shy'). Our results indicated that the main personality difference observed was between the males that emerged and those that did not. The two types of analyses (linear, with 'boldness' represented by PC1, and dichotomous with 'bold'/'shy' as described) produced similar relationships with mating but with generally stronger and clearer patterns for the simpler 'bold'/'shy'-based analyses. Thus, it might seem that the main personality difference is reflected in this dichotomy. For reasons of brevity, we therefore only report these latter analyses.

In all mixed models, we tested if the observed behaviours could be explained by 'boldness', the environment treatment or by the interaction term (fixed effects). When analysing the male behaviours per event, we included 'male identity' and 'female identity' as random effects (intercept). We included 'tank' as a random effect (intercept, N = 32) in the analyses of the behaviours summarised on a per male basis. For the analyses regarding male reproductive success, we used mixed models with the corresponding appropriate error structure. Each male was considered as one observation (N_{male} = 256), and we used the experimental tank as a random effect (intercept, N_{tank} = 32) unless otherwise specified. The fixed effects (the interaction term and the environment treatment) were sequentially removed until $P < 0.10$ in a likelihood ratio test (based on the maximum likelihood, ML). However, we always retained 'boldness' in our models. We do not elaborate on the effects of environment as such, as this is addresses in a separate article (Myhre et al. manuscript). We checked all of the models for over-dispersion, and if the model was over-dispersed we refitted the model with a random effect on the individual level. The estimates and slopes (± 1 SE) are given as contrasts to the intercept. The P-values from the final linear mixed models were obtained using the function *pvals.fnc* from the *languageR* package (Baayern 2011). Means are presented ± 1 standard error (SE), medians are presented with the inter-quartile ranges (IQR). Boldness did not affect the likelihood that a male was observed in interactions with focal females (z = -0.72, P = 0.47), and the number of observed males did not differ between treatments (z = 0.34, P = 0.74).

The propensity to perform particular behaviours (e.g., aggression or courtship) was calculated as the number of occurrences an event happened relative to all occurrences. To calculate an estimate of male courtship intensity we first log-transformed (x + 1) the frequency of the three different types of courtship behaviours, as well as the number of male nest entries, prior to standardising them (mean of zero and a standard deviation of one). Next, we conducted a PCA analysis of these variables, with varimax rotation. We only included data from the female-male encounters that included male courtship (N = 579). The PC1 explained 67 % of the observed variance and had an eigenvalue of 2.68. The variables had similar loadings on PC1: fin (0.53), quiver (0.49), and lead display (0.52), as well as nest entries (0.45).

Ethical note

All procedures were carried out with permission from the Göteborg ethical committee for laboratory animals (license no. 166-2008). In accordance with the ethical permission, all fish were released back into their natural environment at the end of the trials. No fish were kept in the laboratory for longer than six days, and the fish appeared to be in good physical state at the time of release. Male-female aggression was not frequent even if occurred. Male aggression (apart from posturing) typical takes the form of swift chases but these chases very rarely result in physical contact - the subdominant individual typically retreats quickly. We did not detect any physical injuries resulting from agonistic behaviour during observations or at the end of the experiment.

RESULTS

Personality assay

Out of the 256 males assayed in 2010, 73 (29 %) did not emerge from the shelter within 30 min. The median latency period to emerge was 14.7 min (IQR 4.9 – 30 min). The males made a median of 3 (IQR 0 – 9, maximum 33) trips out of the sheltered area, and for those males leaving the shelter, the average trip lasted for a median of 18 s (IQR 11 - 40 s, maximum 1773 s, $N = 183$). The mean maximal distance from the shelter during the individual trips was a median of 10 cm (IQR 0 – 25 cm), and the median maximal distance during the trial was 20 cm (IQR 0 – 50 cm). Overall, the males that emerged early during the trials made more frequent, longer, and more distant trips away from the shelter. These results indicate that boldness-related behaviours vary substantially between males.

Neither male length (TL, Welch two-sample t-test: $t_{159.8} = 1.05$, $P = 0.30$), body mass (BM, $t_{157.9} = 1.14$, $P = 0.25$), nor condition (the residuals from the regression between TL and BM on a log-log scale, $t_{124.7} = 0.24$, $P = 0.81$) differed between the bold and the shy individuals (i.e. individuals that did or did not leave shelter)

Mating behaviour and boldness

Seventy-three males (in 21 trials) engaged in courtship with a female when there was also another male within two fish lengths from the female. Twenty-nine (40%) of these males exhibited aggressive behaviour towards the other males that were present (thereby interrupting their courtship of the female). Twelve of the males exhibited agonistic fin displays and 17 chased other males. Boldness did not relate with an individual’s propensity to exhibit agonistic fin displays (GLMM: $z = -0.53$, $P = 0.60$) or to chase (GLMM: $z = -0.15$, $P = 0.88$, fig. 3A). The environment (i.e., treatment) elicited no effect and was removed from the models.

The bold males exhibited a significantly higher propensity to court females that they encountered than did the shy males (GLMM: intercept (shy, open) ± SE = -0.59 ± 0.31, estimate (bold) ± SE = 0.39 ± 0.14, $z = 2.70$, $P = 0.007$, estimate (complex) ± SE= -0.70 ± 0.41, $z = -1.71$, $P = 0.09$, fig. 3B). Although the interaction term was nonsignificant (*P* = 0.74), the graphs suggested that this result was predominantly attributed to the boldness effect in one of the environments. When we analysed the relationship between boldness and courtship separately for the two environments, we found that the bold males exhibited a significantly higher propensity to court the females in the complex (GLMM: intercept (shy) = -1.33 ± 0.28, estimate (bold) 0.45 ± 0.21, *z* = 2.17, *P* = 0.030) but not in the open environment (GLMM: intercept (shy) = -0.55 ± 0.37, estimate (bold) = 0.35 ± 0.20, *z* = 1.74, *P* = 0.08).

We detected no difference between the bold and shy males in courtship intensity (i.e., the frequency of courtship displays during encounters that included male courtship, LMM: *t* = 1.13, *P* = 0.26). In fact, the variance within males (SD = 1.50, the residual variance) was larger than the variance between individuals (males: SD = 0.37 and females: SD = 0.62, the random effects). The environment elicited no effect and was thus removed from the models.

We recorded whether courtship occurred near the nest of the courting male (in his "nest section") or elsewhere. The bold males exhibited a significantly higher proportion of courtship events at their nests (median 0.15, IQR 0 – 0.50) compared to the shy males (median 0, IQR 0 – 0.25; GLMM: intercept (shy, open) = -1.53 ± 0.30, estimate (bold) = 0.90 ± 0.28, *z* = 3.18, *P* = 0.001, estimate (complex) = -0.54 ± 0.29, *z* = -1.86, *P* = 0.06).

Mating success and boldness

Fifty-three males (21 %) mated with focal females during the maximally 30 min of observation. The likelihood of mating was significantly higher for the bold compared to the shy males (GLMM: intercept (shy) = -2.96 ± 0.36, estimate (bold) = 0.81 ± 0.40, *z* = 2.05, *P* = 0.040, fig. 4). The environment elicited no effect in the models and was thus removed.

We found no relationship between boldness and the likelihood for males to hold a nest at any stage of the experiment (table 3). The likelihood to be mated did not relate with boldness at 18 h after the initiation of the trial (before the release of focal females), but tended (0.05 < *P* < 0.10) to be related to boldness at 53 h (a few hours after the introduction of focal females) and at 73 h after trial initiation (table 3). Surprisingly, among the males that had mated at 18 h after trial initiation, the nests were significantly fuller for the shy compared to the bold males (table 3). This relationship disappeared as more males mated during the experiment (table 3). At the end of the experiment, bold males had significantly more eggs in their nest compared to shy males (GLMM: intercept (shy) = 0.14 ± 0.80, estimate (bold) = 2.68 ± 0.94, $z = 2.85$, $P = 0.004$, fig. 5, analysis including all males). The environment elicited no effect and was thus removed from the model.

DISCUSSION

The study provides novel insights into the relationships between personality, mating behaviour and mating success. Based on an "emergence from shelter" personality assay, the most informative distinction appeared to be between those fish that left the shelter ("bold") and those that did not ("shy"). Boldness was not related with size or condition of the males. The bold males were, however, more likely than the shy males to court females they encountered. This effect was particularly strong when the environment was structurally complex. The bold males more often courted females near their own nest, and had a higher mating success during the focal observations. By the end of the experiment, the bold males tended to more often be mated, and they had significantly more eggs in their nests. We did not, however, detect any effects of boldness on the likelihood of becoming a nest-holder or of fighting off other males during courtship interactions with females.

Boldness and courtship

The fact that bold males exhibited a higher propensity to court could either be because they are more active or more risk-taking (i.e., bold). As we only observed males in a sexual context, we cannot distinguish between boldness and general activity. However, there is no obvious link between activity and courtship, as active males need not be eager to court. Engaging in courtship is risky - it increases conspicuousness and takes attention away from predator vigilance (Magnhagen 1991). In nature, two-spotted gobies are subjected to a constant risk of predation, potentially causing them to be constantly alert to predation even when there is no predator present (as in our experiments). In terms of exposure to predation, venturing into open spaces and engaging in courtship represents both risky and potentially rewarding behaviour. Our study shows that individuals responded similarly to these two types of risky situations, suggesting consistent personality differences in risk-taking.

The difference in courtship propensity between the bold and shy males was particularly marked in the complex environment, where the shy males appeared very reluctant to court. The complex environment might be perceived as more dangerous because the fish do not have a complete overview of the tank and cannot see predators that might be hiding nearby, behind a visual obstruction. In such situations, the differences in boldness might have greater effects on behaviour.

Boldness did not relate with courtship intensity. In fact, we found a larger variation in courtship intensity within rather than among individuals. Male courtship intensity has been found to relate to male state (parasite load; Pélabon et al. 2005) and partner attractiveness (female colouration; Amundsen & Forsgren 2001). Once courtship is initiated, it is possible that factors other than boldness are more important in explaining courtship variation.

We found that the bolder males exhibit a significantly higher proportion of their courtship events near their nests (in the 50 x 50 cm section around the nest). Courtship near the nest might more likely result in mating, which might partially explain why the bolder males had a higher mating success during observations (see below).

Boldness and aggression

This study was not aimed to investigate male-male aggression, except in the context of courtship. We found no indication that boldness affects the likelihood that courting males aggressively related to other males nearby. As courtship interruptions were infrequent, this analysis has limited power. Yet, this result might suggest that risk-taking is not strongly related to aggression. In the three-spined sticklebacks a link between boldness and aggression have been found in some but not all populations (Dingemanse et al. 2007).

We found no relationship between boldness and the likelihood of becoming a nest holder. This might fit the lack of a relationship between boldness and aggression, as the latter is likely to be important in nest acquisition.

Boldness and mating

We found a higher probability for the bold males to mate with females during observations (i.e., the bold males got mated quicker). Although the fish were confined to the same space for longer periods of time with limiting mating options for the females, at the end of the experiment, the bold males still exhibited an almost significantly higher probability of being mated compared to the shy males. The positive relationship between boldness and mating success is likely related to their higher propensity to court, but it could also be affected by female preference for bold males. Whether female two-spotted gobies show a preference for bolder males remains unknown. Other studies have, however, found a female preference for a certain personality type (guppy: Godin & Dugatkin 1996), sometimes depending on their own personality (zebra finch, *Taeniopygia guttata*: Schuett et al. 2011).

At the end of the experiment, the bold males had more eggs in their nests on average than did the shy males. Our results show that personality traits (boldness) can be significantly related to reproductive success and fitness, suggesting that this personality dimension is reproductively important.

Eighteen hours after the initiation of the trials, before the focal female observations, the very few shy males that had mated had fuller nests compared to the mated bold males. However, this pattern disappeared as more males mated, and at the end of the experiment, there was no difference between the shy and bold mated males in the acquired egg area (the proportion of the nest covered with eggs). This result suggests that the overall more eggs received by bold males is simply a reflection of more bold males having mated. Boldness has also been found to relate positively with reproductive success in other species (fish: Ariyomo & Watt 2012; Colléter & Brown 2011; mammals: Smith & Blumstein 2008), whereas exploration and aggression elicited no or little effect for reproductive success in males (Smith & Blumstein 2008).

Conclusion

We found that the bolder males were more likely to engage in courtship (especially in complex environments), to court near their own nests, and were more likely to mate during observations. These findings show that personalities as expressed in a standardised boldness/risk-taking assay are reflected in sexual behaviours and mating success. Interestingly, the spatial structure of the environment influenced the relationship between boldness and the propensity to court, as the shy males in the complex environment courted a lower proportion of females than did the shy males in the open environment. The bold males also obtained more eggs in their nests by the end of the experiment. Our results show that boldness reflects aspects of personality of crucial importance for mating and reproduction, and that variation in personality can relate to variation in reproductive success and can hence have consequences for overall fitness. More studies are needed to investigate whether other behavioural axes relate in the two-spotted goby, and whether other personality dimensions relates with behaviours that are centrally important for mating.

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Table 1: The mean total length and wet body mass (± SE) of the females (N_{open} = 208, N_{complex} = 208) and the males (N_{open} = 128, N_{complex} = 128) used in the experiment for the open and complex environment treatments. The difference between the treatments is tested with a two-sample *t*-test.

	Open environment	Complex environment	*t*	df	*P*
Female					
Total length (mm)	42.3 ± 0.2	42.3 ± 0.2	-0.24	413.97	0.81
Body mass (g)	0.698 ± 0.009	0.699 ± 0.010	-0.12	412.65	0.91
Male					
Total length (mm)	42.0 ± 0.31	42.4 ± 0.34	0.87	253.17	0.38
Body mass (g)	0.614± 0.014	0.635 ± 0.015	-0.99	252.37	0.32

Table 2: The results from the principal component analyses of the behaviours recorded in an emergence-from-cover personality assay in male two-spotted gobies (*Gobiusculus flavescens*) for (a) males used in this study, (b) males used to test consistency in behaviour, trial 1, and (c) the same males, trial 2. Loadings, eigenvalues and explained variance are given for the emerging axes.

	a) Males used in this study		b) Consistency test, trial 1		c) Consistency test, trial 2	
	N = 256		*N* = 40		*N* = 40	
	PC1	PC2	PC1	PC2	PC1	PC2
Behaviour						
Emergence time	-0.39	-0.76	0.41	-0.71	0.42	-0.63
No. of trips	0.44	0.28	-0.46	0.28	-0.44	-0.60
Mean time per trip	0.46	-0.21	-0.46	-0.29	-0.46	0.22
Max distance from shelter	0.48	-0.19	-0.47	-0.11	-0.46	-0.41
Mean distance from shelter per trip	0.45	-0.51	-0.43	-0.55	-0.46	0.18
Eigenvalue	3.95	0.55	4.30	0.47	4.11	0.40
% Variance explained	78.91	10.97	86.05	9.44	82.18	7.95

Table 3: The median mating success (and inter quartile range, IQR) at 18, 53 and 73 hours after the initiation of the trials for male two-spotted gobies categorised into shy (*N* = 73) and bold (*N* = 183). The likelihood to be a nest holder and to be a mated male was tested by GLMM with binomial error distribution with a logit link. The nest fullness was tested by GLMM with poisson error distribution and log link. Tank (N_{shy} = 29, N_{bold} = 32, if not otherwise specified) was included in all of the models as a random factor (intercept). The interaction term between boldness and environment was included in the initial models, but as there were no significant interaction effects of environment, it was removed. The focal observations were conducted approximately 44 hours after initiation of the trials.

	Hours after trial initiation								
	18			53			73		
	Shy	Bold	Test	Shy	Bold	Test	Shy	Bold	Test
Proportion holding a nest	0.0 (0.0 – 0.50)	0.37 (0.16 – 0.57)	*z* = 1.06, *P* = 0.29	0.75 (0.50 – 1.0)	0.78 (0.67 – 0.86)	*z* = 1.61, *P* = 0.11§	0.75 (0.50 – 1.0)	0.72 (0.60 – 0.83	*z* = 0.71, *P* = 0.48
Proportion mated (i.e., with eggs)	0.0 (0.0 – 0.25)	0.17 (0.0 – 0.26)	*z* = 0.43, *P* = 0.67	0.50 (0.25 – 0.75)	0.57 (0.50 – 0.72)	*z* = 1.67, *P* = 0.092	0.50 (0.25 – 0.74)	0.56 (0.50 – 0.75)	*z* = 1.93, *P* = 0.054
Nest fullness (%) among mated	50 (45 – 50) N_{tank} = 9 N_{male} = 10	11 (5 – 42) N_{tank} = 22 N_{male} = 29	*z* = -2.21, *P* = 0.028	60 (31 – 86) N_{tank} = 22 N_{male} = 35	57 (49 – 67) N_{tank} = 32 N_{male} = 109	*z* = 1.08, *P* = 0.28	70 (41 – 88) N_{tank} = 22 N_{male} = 35	64 (56 – 73) N_{tank} = 32 N_{male} = 112	*z* = 0.96, *P* = 0.34

§ Significant effect of the environment (*z* = -2.26, *P* = 0.024)

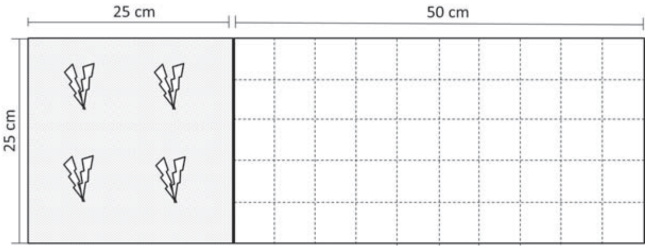

Figure 1: The aquarium configuration for the male two-spotted goby (*Gobiusculus flavescens*) personality ('boldness') assay. The thick line represents an opaque divider that during acclimation separated the sheltered (left) and the open (right) sections of the tank. The bottom of the sheltered section was covered with gravel, included 4 artificial algae and the light was dimmed in comparison to the open section. The bottom of the open section was white with a 5x5 cm grid to facilitate the recordings of the male movement. This section contained no structural elements.

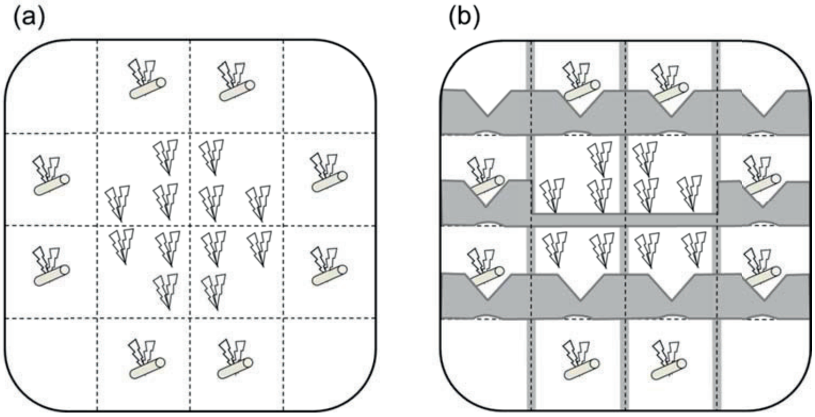

Figure 2: The experimental set-up of the two treatments involving (a) an open environment and (b) a complex environment, into which mixed sex groups of two-spotted gobies (*Gobiusculus flavescens*) were introduced. The tanks of both treatments were marked in sections (~ 50 x 50 cm) on the bottom with tape (thin dotted lines). Each tank contained artificial PVC nests (as illustrated by the shaded tubes) and artificial plants. The complex environment also had opaque plastic dividers (shaded) that obstructed vision, but did not preclude movement between sections. The dividers had the same shape for both dimensions.

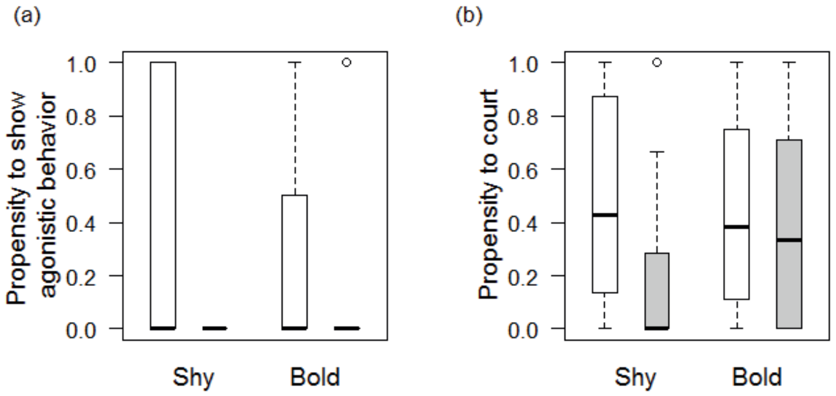

Figure 3: The male two-spotted goby (*Gobiusculus flavescens*) behaviour in relation to boldness in the open (white boxes) and complex (shaded boxes) environment treatments. (a) The propensity to show aggressive behaviour to another male during courtship with a focal female in the open (N_{shy} = 14, N_{bold} = 36) and the complex (N_{shy} = 5, N_{bold} = 18) environments. (b) The propensity to court females (proportion of encounters that included male courtship) in the open (N_{shy} = 36, N_{bold} = 83) and the complex (N_{shy} = 30, N_{bold} = 87) environments. Boxplot details: the thick lines in the boxes represent the median for each distribution, whereas the top and bottom of the boxes represent the 75th and 25th percentiles, respectively. The dashed error bars extend to the most extreme data point ≤ 1.5 times the interquartile range from the box. Outliers are shown as separate data points.

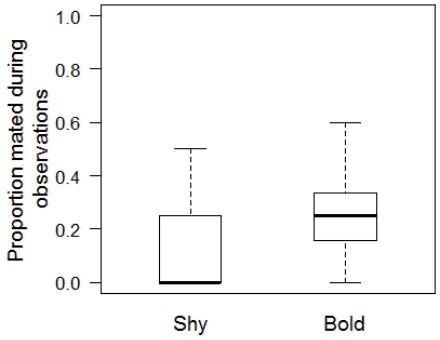

Figure 4: The proportion of shy and bold male two-spotted gobies (*Gobiusculus flavescens*) that mated with the focal females within the 30 min of observation per tank. For boxplot details see figure 3.

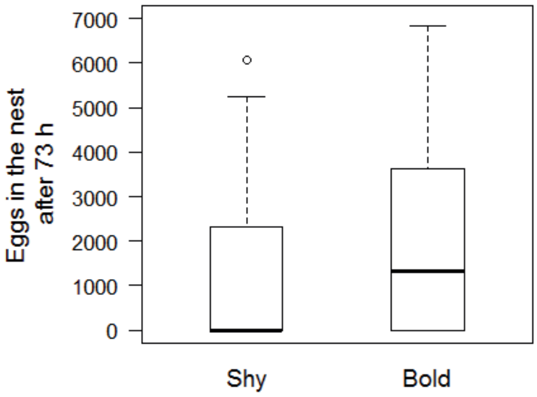

Figure 5: Reproductive success (i.e., number of eggs) of all of the shy ($N = 73$) and the bold ($N = 186$) male two-spotted gobies (*Gobiusculus flavescens*) at the end of the experiment. For boxplot details, see figure 3.

PAPER 4

Nest distribution affects behaviour and mating success in a marine fish

Running title: Nest distribution and mating success

Isabel Mück[a,b,*] Sebastian Wacker[a], Lise Cats Myhre[a], Trond Amundsen[a]

[a]Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway
[b]Current address: Institute of Evolution and Ecology (Animal Evolutionary Ecology), University of Tübingen, D-72076 Tübingen, Germany
Tel.: +49 – 7071 - 2974603, Fax: + 49 - 7071 - 29 5634
*Corresponding author, e-mail: isabel.mueck@biologie.uni-tuebingen.de

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ABSTRACT: The distribution of breeding resources, such as nest sites, can have a pronounced impact on a population by affecting the proportion of individuals that succeed to breed and hence the variation in reproductive success. Aggregation of important resources can lead to resource monopolisation by a limited number of individuals, and thus affect the intensity of sexual selection. In this study, we tested how nest distribution (dispersed vs. aggregated) affects: (1) mating behaviour, (2) male nest occupation and mating success, and (3) reproductive success and the opportunity for selection. We used the two-spotted goby (*Gobiusculus flavescens*), a small marine fish with a resource-based mating system, as our model species. When nests were aggregated, a larger proportion of the males behaved aggressively, fewer males succeeded in occupying a nest, fewer males became mated, and those males that mated received fewer eggs from spawning females. These effects resulted in a higher variance in reproductive success, and hence a higher opportunity for selection (I_{rs}), in the aggregated treatment. We suggest that the results are a direct consequence of males defending a territory around their nest, preventing competitively inferior males from breeding. However, we found no significant selection differentials for body length or condition of males in either treatment. Our results support the hypothesis that aggregation of essential resources like nests promotes resource monopolisation. In species facing highly clumped nesting resources in the wild, monopolisation may negatively impact population productivity but could lead to strong selection on traits that promote male competitive ability.

Keywords: *Gobiusculus flavescens*, mating competition, nest distribution, resource monopolisation, sexual selection, two-spotted goby

Introduction

Darwin (1871) described two major processes of sexual selection: intra-sexual selection (usually male-male competition), and inter-sexual selection (usually female choice). In mating systems characterised by male resource defence and territoriality, intra-sexual selection may determine a male's access to resources critical for reproduction (Emlen and Oring 1977; Kodric-Brown 1983; Searcy and Yasukawa 1983; reviewed in Andersson 1994). Such resources can be territories, suitable sites for building a nest, or physical structures that can be used or modified for nesting. The monopolisation of resources essential for mating involves the exclusion of competitors from these resources, and thereby also from mating (e.g. Ahnesjö et al. 2001). Consequently, resource monopolisation could have implications for sexual selection on male traits (Darwin 1871; Emlen and Oring 1977; Shuster and Wade 2003; but see Klug et al. 2010a).

Both the availability and distribution of breeding resources can vary greatly among environments. The two factors may have significant impacts on the intensity of competition for successful mating (Grant 1993; Reynolds 1996). Highly aggregated breeding resources are more likely to be economically defendable (Grant 1997), and therefore also more likely to be monopolised by a fraction of individuals in a population (Emlen and Oring 1977).

It is well established that the abundance or scarcity of breeding resources may affect animal mating dynamics. Typically, when breeding resources are scarce, male-male competition for these resources is strong, whereas when resources are abundant, resource competition is relaxed and males instead compete more directly for females and female choice becomes more important (fishes: Nellbring 1986; Forsgren et al. 1996; birds: Forero et al. 1996; Jacot et al. 2009; Strubbe and Matthysen 2009; forest marsupials: Banks et al. 2011). In fishes a scarcity of suitable nesting structures has been found to increase mating competition (common goby, *Pomatoschistus microps*: Borg et al. 2002), reduce reproductive success (sand goby, *Pomatoschistus minutus*, and common goby: Nellbring 1993), and increase sexual selection (sand goby: Forsgren et al. 1996; Lindström 2001). However, very few studies on fish have tested how the spatial distribution of breeding resources affects mating behaviour and mating success (but see Reichard et al. 2009; Saraiva et al. 2009). This is unfortunate, because many substrate-brooding fishes use structures in the environment for depositing eggs, and such structures are rarely uniformly distributed. Nesting structures may be mussels (used by many gobies), crevices in rocks (used by, for instance, temperate blennies), coral structures (used by, for instance, damselfishes, blennies and gobies), or crevices in macroalgal vegetation (used by two-spotted gobies and some other species). The spatial distribution of breeding resources of these sorts can be highly variable, both within and between species, but the effects of such variability on breeding behaviour and reproductive success are poorly known. In peacock blennies (*Salaria pavo*), however, nest aggregation has been found to negatively affect the proportion of males that succeed in breeding, despite no detected effect on male aggressive or courtship behaviour (Saraiva et al. 2009). Females, on the other hand, were more active in courtship when males were aggregated (Saraiva et al. 2009). Peacock blennies naturally breed in rock crevices but reproduce mainly in holes in bricks in Saraiva et al.'s study population. In european bitterlings (*Rhodeus amarus*), a species depositing its eggs in live mussels, aggregation of host mussels resulted in a higher variation in reproductive success, and hence a higher opportunity for selection. However, no study has so far tested the effect of nest aggregation on male aggression, male success in nest occupation and mating, the opportunity for selection arising from variation in reproductive success, and realised selection on male traits. Such studies are needed if we are to fully understand how the distribution of breeding resources affects behaviour, selection and population productivity.

The aim of this study was to address how nest distribution (dispersed vs. aggregated) affects: (1) male mating behaviour (i.e. male agonistic and courtship behaviours), (2) male nest occupation success, (3) male mating success, (4) variation in reproductive success and hence opportunity for selection, and (5) selection on male size and condition. We used the two-spotted goby (*Gobiusculus flavescens*), a small substrate-brooding marine fish, as the model species. Like many other substrate-brooding fishes, two-spotted gobies use natural structures for depositing and caring for eggs, rather than constructing a nest. Like many other gobies, two-spotted gobies seem to prefer mussels as their nesting substrate (Brevik 2007). While benthic gobies inhabiting finer sediment bays typically modify their nesting mussels by depositing sand to hide the nest and produce a smaller opening, the semi-pelagic two-spotted goby do normally not modify its nesting structure. The species typically breeds in blue mussels (*Mytilus edulis*; other mussels can also be used), in crevices in holdfasts or on the leaves of kelp (typically *Saccharina latissima* and *Laminaria digitata* in our study area), and sometimes under stones. Two-spotted gobies mostly inhabit semi-exposed areas affected by considerable wave action and typically with little fine sediment. In this environment, it is unlikely that nest modification is feasible and we have never observed the species to modify any substrate or structure used for breeding. In the present study, the term 'nest' is used to describe a substrate used for breeding, in accordance with previous work on the species (e.g. Amundsen and Forsgren 2001; Forsgren et al. 2004; Wacker et al. 2012). Breeding resources, and in particular the apparently preferred blue mussels (Brevik 2007), can vary considerably in spatial distribution. For instance, in some places mussel banks provide many potential nesting sites at short distance from each other. Competition for nests is common in substrate-brooders, including the closely related sand goby (Lindström 1988; Magnhagen and Kvarnemo 1989; Lehtonen and Lindström 2004) and common goby (Magnhagen 1994, 2006; Borg et al. 2002), and has been observed both in the lab and in the wild in two-spotted gobies (personal observations).

To test for effects of nest aggregation, we kept the number of nests constant but manipulated their distribution to be either dispersed or aggregated in experimental populations held in mesocosm tanks. We predicted that aggregation of nests would lead to: (1) increased male-male competition, (2) fewer males obtaining a nest and hence breeding, and (3) a higher variation in reproductive success among males. We also tested whether nest aggregation affected realised selection on male size and condition.

Methods

The study was conducted from 19 June to 12 July in 2010 at The Sven Lovén Centre of Marine Sciences at Kristineberg, on the western coast of Sweden (58^{o} 15' N, 11^{o} 27' E).

Study species

The two-spotted goby (*Gobiusculus flavescens*) is highly abundant along the western coast of Europe (Collins 1981), and a keystone species in coastal ecosystems of Scandinavia (Fosså 1991; Giske et al. 1991; Hop et al. 1992, 1993). During the breeding season, this small (adult total length 3-6 cm), semi-pelagic fish inhabits the shallow algal zone (ca. 0-5 m depth) along moderately exposed rocky shores. The reproductive season lasts from May to July in our study area. Two-spotted gobies live for only one year throughout most of its range (Johnsen 1945), but both sexes may reproduce repeatedly over the course of their single breeding season (Eriksen 2007; de Jong 2011). Densities of stationary males (likely to be nest-holders) and breeding-ready females vary greatly over the breeding season, and between localities (Forsgren et al. 2004; Myhre et al. 2012). Males may attract several females to spawn during a single breeding attempt (mostly 2-6; Mobley et al. 2009). Each female typically lays a clutch of 1000-1500 eggs (Pélabon et al. 2003; Svensson et al. 2006). Unlike in some other goby species (e.g. Magnhagen 1992; Jones et al. 2001a, b; Singer et al. 2006), parasitic male spawning is very rare in two-spotted gobies (Mobley et al. 2009). The near-absence of sneaking allows precise quantification of reproductive success without adopting genetic analyses. After spawning, the male cares for the eggs (by defence, fanning and cleaning) until hatching, which normally occurs after 1-3 weeks depending on water temperature (Skolbekken and Utne-Palm 2001; Bjelvenmark and Forsgren 2003; Svensson et al. 2006). Males may defend areas encompassing more than one nest, but have never been observed to mate or care for eggs in more than one nest at a time.

The two-spotted goby is sexually dimorphic, and visual ornamentation plays an important role during the courtship of both sexes. Typically, the male initiates courtship with a fin display, erecting his colourful dorsal fins, often followed by vibrating his body (quiver) perpendicularly to the female before swimming to his nest with undulating body movements (lead swim) (Amundsen and Forsgren 2001; Pélabon et al. 2003). Courtship may be interrupted by any of the two parties at any stage. Ready-to-spawn females carry round (egg-filled), orange bellies (Amundsen and Forsgren 2001; Svensson et al. 2006), which they bend in a sigmoid display towards males during courtship (Amundsen and Forsgren 2001; Myhre et al. 2012). Agonistic interactions among males usually start with visual fin displays, but also include chases (fast approaches towards another male) (Forsgren et al. 2004; de Jong et al. 2009; Wacker et al. 2012). Males involved in agonistic interactions often change their colour and become darker than usual (personal observations).

Fish collection and husbandry

All fish were caught with hand-held dip nets while snorkelling < 3 km from the research station, and were brought to the laboratory by boat. At the laboratory, fish were separated by sex and location of collection and stored in 60 litre aquaria for an acclimatisation period of 24-48 h before being used in a trial. Storage aquaria were equipped with a 1-2 cm layer of gravel and 2-3 artificial plants, and had a continuous flow of sea-water. The fish were fed twice a day *ad libitum* with *Artemia* spp. nauplii, and were released into their natural habitat after the experiments.

Experimental design

The aim of the study was to investigate how mating behaviour and mating success of two-spotted goby males is affected by the distribution of breeding resources (nest sites). To achieve this, we established two treatments with a high yet realistic difference in nest distribution: (1) dispersed (nest openings facing the tank centre 60 cm apart, Fig. 1a) and (2) aggregated (nest openings facing the tank centre 10 cm apart, Fig. 1b). Both treatments were arranged in 2x2 m grey PVC tanks with a water depth of ca. 40 cm and a continuous flow of sea-water (from 7 m depth) of ambient temperature (range 15 °C mid-June to 19 °C mid-July). In each tank, we entered eight males and 16 females. The tanks allowed the fish to exhibit natural reproductive and social behaviours, and as such could be considered 'mesocosms'. Each tank had eight artificial nests, matching the number of males in the tank. The artificial nests were 80 mm long PVC tubes with a 13 mm inner diameter, attached to a stone, and open in both ends. Previous studies have shown that males readily occupy such artificial nests (in the laboratory: e.g. Amundsen and Forsgren et al. 2001; Svensson et al. 2006; de Jong et al. 2009, in the wild: Forsgren et al. 2004; de Jong 2011). One artificial nest can accommodate eggs from approximately four females (Bjelvenmark and Forsgren 2003; Forsgren et al. 2004). With this design, males could compete over nests, or over females. Nest competition would occur if male territoriality prevented other males from establishing in nearby nests. Competition for females would occur if more than half of the males succeeded in establishing nest ownership; then there would be fewer females ready to spawn than the males could accommodate eggs in their nests. As the experiment was conducted over a period of nearly a month, we conducted sets of two trials, one of each treatment, in parallel in order to avoid order effects on treatment differences. We switched treatment between tanks for each set of trials in order to balance any tank effects. In total, we conducted 14 replicates of each treatment. The replicates ran for three days.

Selection of experimental fish

The total length (TL) of all fish was measured to the nearest 0.5 mm, using a measuring board. The wet body mass (BM) was measured on an electronic scale to the nearest 0.01 g. The measures were taken in the morning (between 08:30 and 10:30) just before the fish were allocated to experimental treatment. Females were selected for the experiment based on the roundness of their bellies (an indicator of gonad maturity, Svensson et al. 2006), which was assessed on a scale from 1 (slim) to 3 (very round) (e.g. Forsgren et al. 2004). Only very round females (roundness of 3) were used, to ensure readiness to mate. For each set of trials (one of each treatment), 16 males were selected at random from the holding tanks. These 16 males were divided into eight pairs of fish that were similar in size. From each of these pairs, it was decided by the flip of a coin which individual should be used in each treatment. By this procedure, the two treatments had populations of males that were randomly selected yet had a very similar size range. Neither male (aggregated: 42.5 ± 1.5 (SD) mm, mean range 39.8 – 45.3 mm, dispersed: 42.7 ± 1.4 mm, mean range 40.3 – 45.0 mm; t_{222} = -0.32, P = 0.75) nor female TL (aggregated: 42.39 ± 2.67 mm, mean range 36.0-50.50 mm, and dispersed: 42.53 ± 2.29 mm, mean range 35.0-49.50 mm; t_{446} = -0.62, P = 0.54) differed between treatments (values refer to mean TL for each trial, and ranges reflect the mean for the smallest and largest fish in trials, respectively). Similarly, BM did not differ between treatments for males (aggregated: 0.63 g ± 0.16 g, mean range 0.35 – 1.06 g, dispersed: 0.63 g ± 0.17 g, mean range 0.36 – 1.22 g; t_{222} = 0.16, P = 0.87) or females (aggregated: 0.727 ± 0.14 g, mean range 0.419-1.253 g, dispersed: 0.725 ± 0.13 g, mean range 0.397-1.200 g; t_{446} = 0.14, P = 0.89). Male condition was calculated as the residuals from the regression of mass on length (e.g. Amundsen and Forsgren 2003). To individually recognise males in a tank, they were marked with visible implant elastomer (VIE) tags (North-West Marine Technology, Shawn Island, Washington) in one of two dorsal positions. Each male had a unique combination of position and colour (red, green, yellow or orange) for the tag (see de Jong et al. 2009 for details of the marking procedure). Previous studies on gobies, including the two-spotted goby (de Jong et al. 2009; Myhre et al. 2012), have not detected any negative impact of VIE-tagging on behaviour (Whiteman and Côté 2004) or survival (Malone et al. 1999; Griffiths 2002). After marking, females and males were simultaneously released to the experimental tanks at around 10:00 h. During the experiment, one male and three females died (all in the aggregated treatment) and had to be replaced.

Behavioural observations

We observed each male for 10 minutes after one day (d1) and after two days (d2) in the experimental tanks. The observations were made between 9:00 and 15:00 h. The order of observation was randomised with respect to treatment and male identity. During observations we recorded all agonistic (fin displays, chases) and courtship (fin displays, quivers, lead swims) behaviours displayed by males, and any courtship (sigmoid displays) by females directed at males. We used the data from the first nest check (after 24 hours) for d1 and data from the third nest check (after 48h) for d2 to determine which males were nest holders and which were not.

Nest ownership and mating success

We recorded nest ownership of males and male mating success twice each day of the experiment except for the first day. Nest checks were made in the morning (08:30 – 09:00) and in the evening (18:00 – 20:30) (i.e., approximately 24, 36, 48 and 60 h after trial initiation). For each nest we noted whether a male was present in the nest, and the identity of any such male. A male was considered to occupy a nest (hereafter termed ‘nest holder’) if he was observed inside a nest at inspection. He was also considered a nest holder if, at a certain inspection, he was observed < 10 cm from a nest and had been observed inside this nest at a previous nest inspection. Only a minor fraction of males (~5 %) were assigned as nest holders by the latter method. Males not observed inside nests were considered ‘non-nest holders’. At each check, we visually estimated egg cover in the nest (% nest fullness) to the nearest 5 %. To disturb the fish as little as possible, sticks with a small mirror attached were used to inspect the nests. To assess the number of ready-to-mate females at any stage of the experiment (as an increasing fraction of the females had spawned), we scored the roundness of all 16 females at each nest check.

Data analyses

Data were analysed using PASW Statistics 18.0 for Windows. Tests between the two treatments were performed using Mann-Whitney U-tests unless otherwise noted. Analyses of behaviours are mostly expressed in terms of the proportion of individuals expressing a particular behaviour at a given stage. For these analyses, aggressive fin displays and chases were pooled to produce a variable termed “agonistic behaviours”, and fin displays, quivers and lead swims directed at females were pooled to produce “courtship”. Because relatively few males had established as nest holders after one day of experiment (d1), in particular in the aggregated treatment, analyses of behaviours are only presented for d2. Data related to behaviour and reproduction were analysed on a per tank basis, as the data within a tank were not independent. Reported sample sizes represent the number of trials with data relevant for the test or graphical presentation in question, for each treatment. Means are presented ± 1SD and medians with inter-quartile ranges.

The opportunity for selection (I_{rs}) describes the upper limit to selection that is possible given a certain variance in reproductive success (Wade 1979; Shuster and Wade 2003). We calculated the opportunity for selection as the variance of the nest fullness divided by the square of mean of the nest fullness for each tank (Wade 1979; Shuster and Wade 2003). We calculated selection differentials (Arnold 1983; Arnold and Wade 1984) for male body length and condition as the difference between the trait values for mated males, and for all males in the population. The proportion of ready-to-mate males to females was used to calculate the operational sex ratio (OSR; the fraction of ready-to-mate males of all ready-to-mate individuals; Kvarnemo & Ahnesjö 1996) in each tank.

Results

Mating behaviour

A significantly higher proportion of nest holders performed agonistic behaviours (i.e. chases or fin displays) in the aggregated than in the dispersed treatment ($Z = -2.26$, $P = 0.026$, Fig. 2a). However, the rate of aggressive behaviours during agonistic encounters by nest-holders did not differ between treatments (aggregated: median = 2.00, range 1 – 5.5, IQR 2.3; dispersed: median = 1.00, range 1- 4.67, IQR 2.67; $Z = -0.74$, $P = 0.49$).

Only few non-nest holders behaved aggressively towards other males (Fig. 2a). Thus, in the aggregated treatment, nest-holding males were significantly more likely to behave aggressively than were non-nest holders (nest holders: median = 0.67, IQR 0.50; non-nest holders: median = 0.00, IQR 0.04; $Z = -3.55$, $P = 0.001$). In the dispersed treatment, the pattern was similar yet less clear and only near statistical significance (nest holders: median = 0.40, IQR 0.50; non-nest holders: median = 0.00, IQR 0.21; $Z = -1.99$, $P = 0.061$).

In both treatments, only few courtship displays were recorded during the 10 min of observation for each male (Fig. 2b). However, the proportion of nest holders performing courtship displays tended to be higher in the aggregated treatment than in the dispersed treatment ($Z = -2.10$, $P = 0.072$, Fig. 2b). Notably, nest-holding males of the aggregated treatment were more often courted by females than were males of the dispersed treatment ($Z = -3.01$, $P = 0.011$, Fig. 2c). Very few non-nest holding males were courted by females.

Nest occupation and mating success

In the aggregated treatment, a significantly smaller proportion of the males were recorded to occupy a nest (be a ‘nest-holder’) than in the dispersed treatment. This pattern was evident at all stages of the experiment (24 h: $Z = -2.87$, $P = 0.007$; 36 h: $Z = -2.93$, $P = 0.003$; 48 h: $Z = -2.55$, $P = 0.011$; 60 h: $Z = -2.40$, $P = 0.016$, Fig. 3a). In line with this, a smaller proportion of males were recorded as mated (i.e. had eggs in their nest) in the aggregated than in the dispersed treatment (24 h: $Z = -2.94$, $P = 0.009$; 36 h: $Z = -2.89$, $P = 0.004$; 48 h: $Z = -2.52$, $P = 0.012$, 60 h: $Z = -1.99$, $P = 0.050$, Fig.

3b). Among those males occupying a nest ('nest-holders'), however, there was no difference in the proportion that became mated between the two treatments, at any stage of the experiment (24 h: Z = - 0.65, N_A = 3, N_D = 11, P = 0.56; 36 h: Z = - 1.04, N_A = 9, N_D = 13, P = 0.36; 48 h: Z = - 0.36, N_A = 11, N_D = 13, P = 0.73, 60 h: Z = - 0.22, N_A = 12, N_D = 13, P = 0.85, Fig. 3c). However, except at the very start of the experiment, nest fullness was higher in the aggregated than in the dispersed treatment (24 h: Z = -1.42, N_A = 2, N_D = 9, P = 0.22; 36 h: Z = -2.34, N_A = 6, N_D = 12, P = 0.018; 48 h: Z = -2.64, N_A = 10, N_D = 13, P = 0.006; 60 h: Z = - 2.32, N_A = 11, N_D = 13, P = 0.018, Fig. 3d). By the end of the experiment, 30 out of 40 (75 %) nest holders had mated in the aggregated treatment and 47 out of 63 (75 %) in the dispersed treatment. The combination of fewer males becoming mated and smaller clutch sizes caused an even greater contrast in productivity when non-mated males (empty nests) were included and mean nest fullness compared between the two treatments (aggregated: median = 16 %, IQR 29, N_A = 14; dispersed: median = 38 %, IQR 24, N_D = 14; Z = - 2.79, P = 0.004).

Operational sex ratio

Nest distribution had a strong effect on the numbers of males and females ready-to-mate (Table 1). As only males controlling a nest can mate, the difference in nest ownership between treatments affected the number of males ready to mate. At the same time, the number of females ready to mate decreased as they mated with males, at rates different between the treatments. Overall, this caused relatively fewer mating-ready males but more mating-ready females in the aggregated than in the dispersed treatment (Table 1), implying a relatively more female-biased operational sex ratio in the aggregated treatment (Fig. 4).

Opportunity for selection and selection differentials

As a consequence of the greater variation in mating success when nests were aggregated, there was a significantly higher opportunity for selection (I_{rs}) in the aggregated than in the dispersed treatment (Z = - 2.17, P = 0.030, Fig. 5a). This could lead to stronger selection on any male trait affecting mating success. To test whether male body length and condition were under selection we calculated selection differentials on these traits. However, we did not detect positive (or negative) selection on male length or condition in any of the two treatments Mated males of each treatment did not differ significantly in body length or in condition from an average male for this treatment (one-sample t-tests: aggregated: body length: t_{10} = 1.18, P = 0.26, condition: t_{10} = - 0.78, P = 0.45, N_A = 11; dispersed: body length: t_{12} = 1.54, P = 0.15, condition: t_{12} = - 0.31, P = 0.76, N_D = 13; Fig. 5b, c). There was no differences in the selection differentials for body length or condition between treatments (two-sample t-test: body length: $t_{17.9}$ = 0.13, P = 0.90, condition: $t_{19.0}$ = - 0.45, P = 0.66, Fig. 5b, c).

Discussion

Our results clearly show that nest aggregation increases male-male aggression, thereby reducing male nest occupation rate and mating success. In turn, these effects cause a higher opportunity for selection when nests are aggregated than when they are dispersed. These results demonstrate that the distribution of nesting resources can have profound effects on processes of sexual selection.

Mating behaviour

As predicted, nest aggregation resulted in more male-male aggressive behaviours, as a higher proportion of males performed agonistic acts (aggressive visual displays or chases) in the aggregated than in the dispersed treatment. This effect is likely caused by more frequent close encounters between males, and males more frequently getting sufficiently near each other's nests as to be considered a threat. Our results clearly show that males spend more time competing when nests are aggregated. When the distribution of a breeding resource is aggregated, the result could be both more competition for the resource as such, and a greater scope for direct competition for mates among neighbours. In our study, we cannot strictly disentangle if the effect of nest aggregation on male aggression is induced by competition for nests, for mates, or both.

Males performed only infrequent courtship to females during observations, and the difference between treatments in courtship was not statistically significant. The much more frequent occurrence of male-male than male-female interactions suggests that males gave priority to defence of breeding resources over mate attraction. Defence of territories or nest sites have been found to take priority over mate attraction also in several other fish species (three-spine sticklebacks, *Gasterosteus aculeatus*: Candolin 1997; beaugregory damselfish, *Stegastes leucostictus*: Santangelo et al. 2002; European bitterlings, *Rhodeus amarus*: Reichard et al. 2004; Smith et al. 2006; zebra fish, *Danio rerio*: Spence and Smith 2005).

Female courtship was, like male courtship, relatively infrequent. However, a higher proportion of males was courted by females in the aggregated than in the dispersed treatment. Given that fewer males became nest owners when nests were aggregated, the finding suggests that nest aggregation *per se* induces more female courtship. This could be because any female attracted to a male that is part of a nesting aggregation will simultaneously be exposed to a number of other mating-ready males, or because females face stronger intra-sexual selection due to the more strongly female-biased operational sex ratio resulting from aggregation (Forsgren et al. 2004, Fig. 5). A similar effect of nest aggregation on operational sex ratio and female courtship has been found in the peacock blenny (Saraiva et al. 2009). The fact that some females also courted non-nest holding males either suggest that females are sometimes unaware of the nest-ownership status of males, or that males can engage in mate attraction before establishing nest ownership (see Wacker et al. 2012).

Nest occupation and mating success

The proportion of males recorded as nest holders increased over the course of the experiment in both treatments. As predicted from theory, however, the proportion of nest holders was markedly lower in the aggregated than in the dispersed treatment at all stages. These results indicate that nest aggregation promotes nest monopolisation, likely because dominant males defend a territory around their own nest large enough to restrict other males from establishing in nearby nests. Our findings support Emlen and Oring's (1977) hypothesis that aggregation of important resources leads to resource monopolisation by a limited number of individuals within a population. Similar to our results, nest aggregation also limited success in getting a nest in peacock blennies (Saraiva et al. 2009). Defence of breeding territories commonly occurs among substrate-brooding fishes (e.g., peacock blenny, *Salaria pavo*: Almada et al. 1995; cichlid fish, *P.*

tropheops, P. maylandia: Genner et al. 1999; painted goby, *Pomatoschistus pictus*: Amorim and Neves 2008; rose bitterling, *Rhodeus ocellatus*: Smith 2011).

Matching the effect on nest ownership, and again as predicted from theory, the proportion of males that became mated was negatively affected by nest aggregation. This effect was evident at all stages of the experiment. Given that a male had established nest-ownership, however, the likelihood of becoming mated was unaffected by treatment. This suggests that one main effect of nest aggregation is to reduce the likelihood for a male to obtain a nest, with reduced mating success as a consequence. The lower success in establishing nest-ownership fits with the more frequent male aggression when nests are aggregated.

As fewer males became nest-holders when nests were aggregated, one might have expected each of these males to mate with more females and hence get more eggs in the nest. This was, however, not the case. Instead, nest fullness (% of nest covered by eggs) was lower in aggregated nests. Our findings suggest that males in the aggregated treatment were either prevented from attracting multiple females because they had to engage in resource defence, or that spawning was more frequently interrupted (by competing males or females) when nests were aggregated. Interruption of courtship or spawning is not uncommon in substrate-brooding fishes (e.g. Itzkowitz 1974; Spence and Smith 2005). The closer individuals are together, the greater is the scope for direct competition for mates, hence increasing the intensity of intra-sexual competition (Kokko and Rankin 2006). Disputes over resources and the aggressive behaviours involved may easily lead to loss of mating opportunities (e.g. Huntingford and Turner 1987; Reichard et al. 2004; Spence and Smith 2005). Interestingly, none of the two other similar studies that we know of (Reichard et al. 2009; Saraiva et al. 2009) found a negative effect of nest aggregation on nest fullness. One possible explanation for this discrepancy might be that dominance relationships are more stable in European bitterlings and peacock blennies than in two-spotted gobies, with limited courtship interruption and competition going on once a male has obtained a nest.

In our experiment, the fish of each experimental population were faced with only one option when it comes to nest distribution. This was deliberate, in order to reveal the consequences of nest aggregation *per se*. In nature, however, animals are more likely to be faced with a mosaic of aggregated and dispersed nesting sites, of varying quality. For instance, a two-spotted goby male may be faced with a choice of trying to gain ownership of a high-quality mussel that is part of a mussel bank already occupied by one or more other males, or rather to search for a less favourable nesting site elsewhere (e.g. on kelp). In such cases, the optimal strategy for a male will depend on his competitive abilities, and the relative benefit of breeding in a high-quality nest given he succeeds in establishing nest-ownership. Hence, the distribution of nesting resources should effect individual decision-making, and thereby the structuring of the breeding population. For instance, we may expect to find phenotypic differences between males holding high-quality, aggregated nests and lower-quality, dispersed nests. In species facing highly clumped nesting resources in the wild, monopolisation may negatively impact population productivity but could lead to strong selection on male traits promoting competitive ability.

Effects of nest aggregation on selection

We found a higher opportunity for selection when nests were aggregated, reflecting a greater variation among males in reproductive success. This was probably due to fewer males acquiring nests in the aggregated treatment. A higher opportunity for selection is in line with Emlen and Oring's (1977) hypothesis that sexual selection should be stronger when resources are clumped (but see Klug et al. 2010a). Hence, there should be greater scope for selection on any male trait affecting competition for nests and/or mates in the aggregated treatment, even if the opportunity for selection need not be reflected in realised selection on specific traits (Sutherland 1985, 1987; Koenig and Albano 1986; Westneat 2006; Klug et al. 2010b). Two candidate traits for competitive ability would be size (length) and body condition. However, we found no significant selection on male length or condition in any of the treatments. These analyses suffer from limited power, as one often needs large sample sizes to detect on-going selection on phenotypic traits, and should be interpreted with caution. They do, however, raise the question whether traits other than size and condition may be more important in determining competitive superiority in two-spotted gobies. Interestingly, in European bitterlings, stronger positive selection on body size was found with dispersed than with aggregated nests (Reichard et al. 2009).

Conclusion

In conclusion, the results show that, in two-spotted gobies, nest aggregation promotes nest monopolisation, and prevents competitively inferior males from breeding. Aggregation of nests induced more aggressive interactions among males, with the result that fewer males managed to obtain a nest. In consequence, fewer males succeeded in mating, and those that mated also had a lower reproductive success. The result was a significantly higher opportunity for selection when nests were aggregated, but we were unable to detect any realised phenotypic selection on male size or condition. To our knowledge, this is the first experiment to test how nest aggregation affects behavioural interactions, nest occupation, mating and reproductive success, and selection in the same study. Given the great variability in the spatial distribution of nesting resources both within and between species, it is important to understand how resource distribution affects reproduction and selection. The few studies that exist so far have yielded partly different results; studies across a range of species and taxa are therefore required before any general conclusions can be made.

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Table 1: Comparison of numbers of mating-ready males and ready-to-mate females, and consequent operational sex ratio (OSR) between nest distribution treatments at nest checks (24h, 36h, 48h and 60h) throughout the experiment. $N_A = N_D = 14$ trials. P-values are from Mann-Whitney U tests between treatments.

Nest check (h)	Treatment	Males median	range	IQR	*P*	Females median	range	IQR	*P*	OSR *P*
24	aggregated	0.0	0 – 3	0.25	0.014	14.5	6 - 16	2.00	0.14	0.008
	dispersed	1.0	0 – 3	1.50		13.5	5 - 16	4.25		
36	aggregated	1.0	0 – 3	2.00	0.008	14.0	5 - 16	2.25	0.039	0.001
	dispersed	2.5	0 – 5	1.25		8.5	5 - 16	5.50		
48	aggregated	2.0	0 – 4	2.25	0.039	8.5	1 - 14	7.25	0.036	0.015
	dispersed	3.0	0 – 6	2.25		4.5	1 - 14	5.00		
60	aggregated	3.0	0 – 5	2.25	0.10	6.0	1 - 14	6.50	0.035	0.030
	dispersed	4.0	0 - 6	3.00		3.0	0 - 14	3.75		

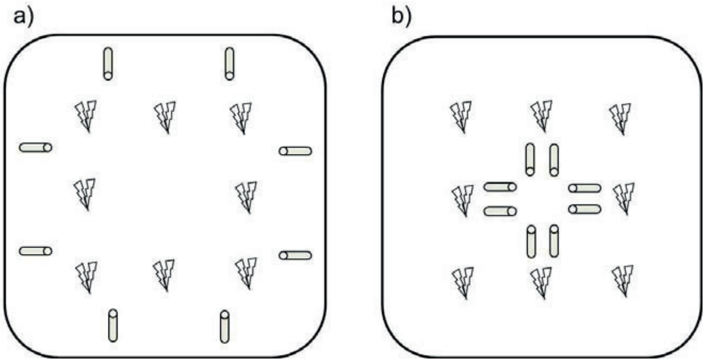

Figure 1: Experimental set-up to test how nest distribution affects male behaviour, nest occupation success, nest fullness and the opportunity for selection in the two-spotted goby (*Gobiusculus flavescens*). Each mesocosm tank (2 x 2 m, depth 40 cm) contained eight artificial nest tubes (open) and eight artificial plants (shaded). The nests were distributed to be either (**a)** dispersed (nest entrances 60 cm apart) or (**b)** aggregated (nest entrances 10 cm apart)

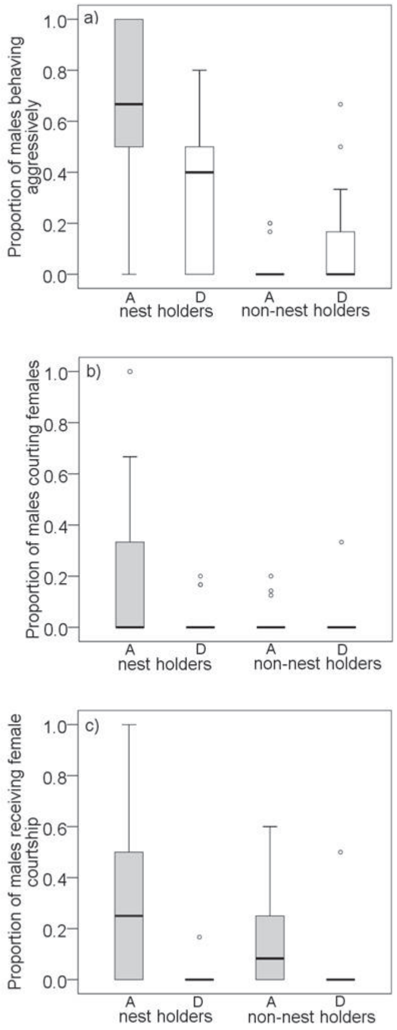

Figure 2: Mating behaviour of male and female two-spotted gobies in relation to nest distribution (A = aggregated, shaded bars; D = dispersed, open bars) and nest-ownership of males (nest-holders: $N_A = 11$, $N_D = 13$; non-nest-holders: $N_A = 14$, $N_D = 14$; N representing no. of trials with individuals belonging to a certain category). (**a**) Proportion of males behaving aggressively to other males (by visuals displays or chases), (**b**) proportion of males courting females (by fin displays, quivers or lead swims), and (**c**) proportion males being courted females (sigmoid displays). Boxplot details: the horizontal lines represents the median, the top and bottom of the box the seventy-fifth and twenty-fifth percentiles, error bars represent the most extreme data point ≤ 1.5 times the inter quartile range from the box. Outliers are shown as separate data points

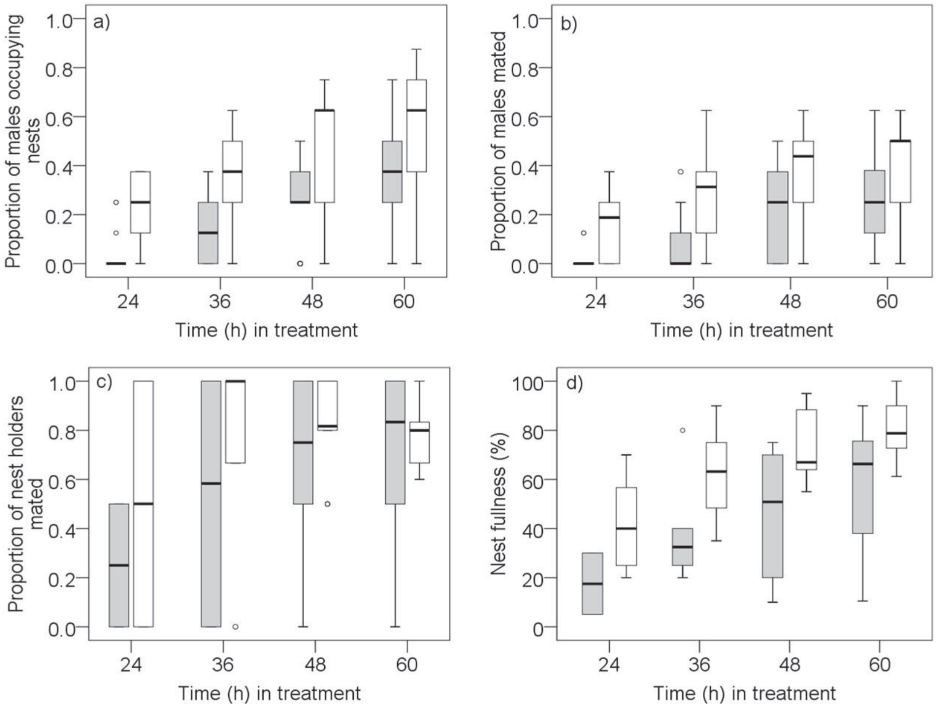

Figure 3: Nest occupation and mating success of two-spotted goby males in relation to nest distribution (A = aggregated, D = dispersed). (**a**) Proportion of males occupying a nest, (**b**) proportion of males that was mated, (**c**) proportion of nest-holding males that were mated, and (**d**) nest fullness (% of nest covered by eggs) based on mean egg cover of mated males in each trial. All parameters were recorded at several stages during the experiment, as indicated on the x-axis. Panels (a) and (b) are based on all trials ($N_A = N_D = 14$); for panels (c) and (d) sample sizes vary between stages and are given with the tests in the text. Boxplot details: see Fig. 2

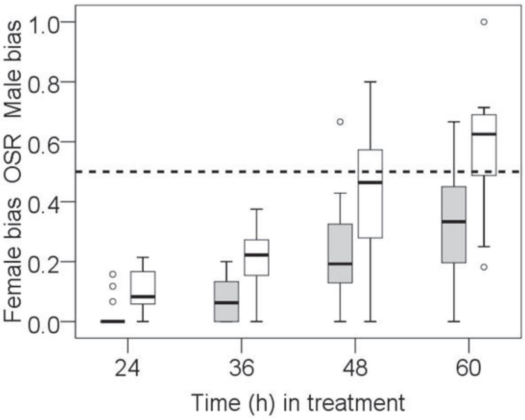

Figure 4: The effect of nest distribution on the operational sex ratio (OSR) of two-spotted gobies (shaded boxes: aggregated, N_A = 14; open boxes: dispersed, N_D = 14 open boxes: dispersed). The OSR expresses the proportion of mating-ready males as a fraction of all mating-ready individuals (Kvarnemo and Ahnesjö 1996). Boxplot details: see Fig. 2

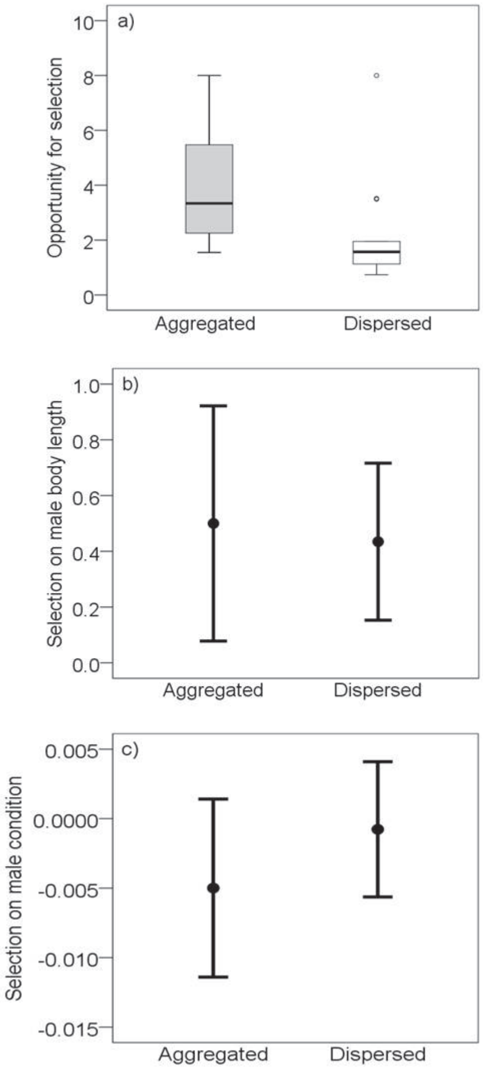

Figure 5: Effects of nest distribution on selection on males in two-spotted gobies (shaded boxes: aggregated, $N_A = 11$; open boxes: dispersed, $N_D = 13$). (**a)** Opportunity for selection; box plot details: see Fig. 2), (**b**) selection differentials (mean ± 1 SE) on male body length (mm), and (**c**) selection differentials (mean ± 1 SE) on male body condition (g).

Doctoral theses in Biology
Norwegian University of Science and Technology
Department of Biology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos Zoology	Breeding events of birds in relation to spring temperature and environmental phenology
1978	Egil Sakshaug	Dr.philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation
1984	Eivin Røskaft	Dr. philos Zoology	Sociobiological studies of the rook *Corvus frugilegus*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos Zoology	Biochemical genetic studies in fish
1985	John Solem	Dr. philos Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains
1985	Randi E. Reinertsen	Dr. philos Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds
1986	Bernt-Erik Sæther	Dr. philos Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach
1986	Torleif Holthe	Dr. philos Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna
1987	Helene Lampe	Dr. scient Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires
1987	Olav Hogstad	Dr. philos Zoology	Winter survival strategies of the Willow tit *Parus montanus*
1987	Jarle Inge Holten	Dr. philos Botany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient. Zoolog	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction
1988	Hans Christian Pedersen	Dr. philos Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care
1988	Tor G. Heggberget	Dr. philos Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure
1988	Marianne V. Nielsen	Dr. scient Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*)
1988	Ole Kristian Berg	Dr. scient Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.)
1989	John W. Jensen	Dr. philos Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth
1989	Helga J. Vivås	Dr. scient Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*
1989	Reidar Andersen	Dr. scient Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture
1990	Bengt Finstad	Dr. scient Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season
1990	Hege Johannesen	Dr. scient Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams
1990	Tor Jørgen Almaas	Dr. scient Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues
1990	Magne Husby	Dr. scient Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*
1991	Tor Kvam	Dr. scient Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway
1991	Jan Henning L'Abêe Lund	Dr. philos Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants
1991	Trond Nordtug	Dr. scient Zoology	Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway

1991	Odd Terje Sandlund	Dr. philos Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism
1991	Nina Jonsson	Dr. philos	Aspects of migration and spawning in salmonids
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks
1992	Arne Vollan Aarset	Dr. philos Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*.
1993	Kjetil Bevanger	Dr. scient. Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)
1994	Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994	Morten Bakken	Dr. scient Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*

1994 Arne Moksnes	Dr. philos Zoology	Host adaptations towards brood parasitism by the Cockoo
1994 Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1994 Torbjørn Forseth	Dr. scient Zoology	Bioenergetics in ecological and life history studies of fishes.
1995 Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions
1995 Hanne Christensen	Dr. scient Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*
1995 Svein Håkon Lorentsen	Dr. scient Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition
1995 Chris Jørgen Jensen	Dr. scient Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995 Martha Kold Bakkevig	Dr. scient Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport
1995 Vidar Moen	Dr. scient Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations
1995 Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden
1996 Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae
1996 Ola Ugedal	Dr. scient Zoology	Radiocesium turnover in freshwater fishes
1996 Ingibjørg Einarsdottir	Dr. scient Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines
1996 Christina M. S. Pereira	Dr. scient Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation
1996 Jan Fredrik Børseth	Dr. scient Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics
1996 Gunnar Henriksen	Dr. scient Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region
1997 Gunvor Øie	Dr. scient Bothany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae
1997 Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters
1997 Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming
1997 Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture
1997 Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher
1997 Torgeir Nygård	Dr. scient Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors

1997 Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway
1997 Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry
1997 Rolv Lundheim	Dr. scient Zoology	Adaptive and incidental biological ice nucleators
1997 Arild Magne Landa	Dr. scient Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation
1997 Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*
1997 Jarle Tufto	Dr. scient Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997 Trygve Hesthagen	Dr. philos Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997 Trygve Sigholt	Dr. philos Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997 Jan Østnes	Dr. scient Zoology	Cold sensation in adult and neonate birds
1998 Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins
1998 Thor Harald Ringsby	Dr. scient Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998 Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998 Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity
1998 Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro
1998 Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach
1998 Bente Gunnveig Berg	Dr. scient Zoology	Encoding of pheromone information in two related moth species
1999 Kristian Overskaug	Dr. scient Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999 Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999 Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway
1999 Ingvar Stenberg	Dr. scient Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999 Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis

1999 Trina Falck Galloway	Dr. scient Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999 Marianne Giæver	Dr. scient Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999 Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*
1999 Ingrid Bysveen Mjølnerød	Dr. scient Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999 Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999 Stein-Are Sæther	Dr. philos Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999 Katrine Wangen Rustad	Dr. scient Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999 Per Terje Smiseth	Dr. scient Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*)
1999 Gunnbjørn Bremset	Dr. scient Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999 Frode Ødegaard	Dr. scient Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999 Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000 Ingrid Salvesen	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000 Ingar Jostein Øien	Dr. scient Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race
2000 Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000 Sigbjørn Stokke	Dr. scient Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000 Odd A. Gulseth	Dr. philos Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000 Pål A. Olsvik	Dr. scient Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000 Sigurd Einum	Dr. scient Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001 Jan Ove Evjemo	Dr. scient Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001 Olga Hilmo	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems

2001	Ingebrigt Uglem	Dr. scient Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr.philos Biology	Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster*
2002	Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears
2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt
2004	Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
2004	Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar

2004	Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*
2004	Børge Moe	Dr.scient Biology	Energy-Allocation in Avian Nestlings Facing Short-Term Food Shortage
2005	Matilde Skogen Chauton	Dr.scient Biology	Metabolic profiling and species discrimination from High-Resolution Magic Angle Spinning NMR analysis of whole-cell samples
2005	Sten Karlsson	Dr.scient Biology	Dynamics of Genetic Polymorphisms
2005	Terje Bongard	Dr.scient Biology	Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period
2005	Tonette Røstelien	ph.d Biology	Functional characterisation of olfactory receptor neurone types in heliothine moths
2005	Erlend Kristiansen	Dr.scient Biology	Studies on antifreeze proteins
2005	Eugen G. Sørmo	Dr.scient Biology	Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations
2005	Christian Westad	Dr.scient Biology	Motor control of the upper trapezius
2005	Lasse Mork Olsen	ph.d Biology	Interactions between marine osmo- and phagotrophs in different physicochemical environments
2005	Åslaug Viken	ph.d Biology	Implications of mate choice for the management of small populations
2005	Ariaya Hymete Sahle Dingle	ph.d Biology	Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia
2005	Anders Gravbrøt Finstad	ph.d Biology	Salmonid fishes in a changing climate: The winter challenge
2005	Shimane Washington Makabu	ph.d Biology	Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana
2005	Kjartan Østbye	Dr.scient Biology	The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation

2006	Kari Mette Murvoll	ph.d Biology	Levels and effects of persistent organic pollutans (POPs) in seabirds Retinoids and α-tocopherol – potential biomakers of POPs in birds?
2006	Ivar Herfindal	Dr.scient Biology	Life history consequences of environmental variation along ecological gradients in northern ungulates
2006	Nils Egil Tokle	ph.d Biology	Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus*
2006	Jan Ove Gjershaug	Dr.philos Biology	Taxonomy and conservation status of some booted eagles in south-east Asia
2006	Jon Kristian Skei	Dr.scient Biology	Conservation biology and acidification problems in the breeding habitat of amphibians in Norway
2006	Johanna Järnegren	ph.d Biology	Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity
2006	Bjørn Henrik Hansen	ph.d Biology	Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway
2006	Vidar Grøtan	ph.d Biology	Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates
2006	Jafari R Kideghesho	ph.d Biology	Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania
2006	Anna Maria Billing	ph.d Biology	Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction
2006	Henrik Pärn	ph.d Biology	Female ornaments and reproductive biology in the bluethroat
2006	Anders J. Fjellheim	ph.d Biology	Selection and administration of probiotic bacteria to marine fish larvae
2006	P. Andreas Svensson	ph.d Biology	Female coloration, egg carotenoids and reproductive success: gobies as a model system
2007	Sindre A. Pedersen	ph.d Biology	Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor* - a study on possible competition for the semi-essential amino acid cysteine
2007	Kasper Hancke	ph.d Biology	Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae
2007	Tomas Holmern	ph.d Biology	Bushmeat hunting in the western Serengeti: Implications for community-based conservation
2007	Kari Jørgensen	ph.d Biology	Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens*
2007	Stig Ulland	ph.d Biology	Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry
2007	Snorre Henriksen	ph.d Biology	Spatial and temporal variation in herbivore resources at northern latitudes
2007	Roelof Frans May	ph.d Biology	Spatial Ecology of Wolverines in Scandinavia
2007	Vedasto Gabriel Ndibalema	ph.d Biology	Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti National Park, Tanzania

2007	Julius William Nyahongo	ph.d Biology	Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania
2007	Shombe Ntaraluka Hassan	ph.d Biology	Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania
2007	Per-Arvid Wold	ph.d Biology	Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning
2007	Anne Skjetne Mortensen	ph.d Biology	Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios
2008	Brage Bremset Hansen	ph.d Biology	The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem
2008	Jiska van Dijk	ph.d Biology	Wolverine foraging strategies in a multiple-use landscape
2008	Flora John Magige	ph.d Biology	The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania
2008	Bernt Rønning	ph.d Biology	Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, (*Taeniopygia guttata*)
2008	Sølvi Wehn	ph.d Biology	Biodiversity dynamics in semi-natural mountain landscapes. - A study of consequences of changed agricultural practices in Eastern Jotunheimen
2008	Trond Moxness Kortner	ph.d Biology	"The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations"
2008	Katarina Mariann Jørgensen	Dr.Scient Biology	The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation
2008	Tommy Jørstad	ph.d Biology	Statistical Modelling of Gene Expression Data
2008	Anna Kusnierczyk	ph.d Bilogy	*Arabidopsis thaliana* Responses to Aphid Infestation
2008	Jussi Evertsen	ph.d Biology	Herbivore sacoglossans with photosynthetic chloroplasts
2008	John Eilif Hermansen	ph.d Biology	Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania
2008	Ragnhild Lyngved	ph.d Biology	Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning
2008	Line Elisabeth Sundt-Hansen	ph.d Biology	Cost of rapid growth in salmonid fishes
2008	Line Johansen	ph.d Biology	Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution
2009	Astrid Jullumstrø Feuerherm	ph.d Biology	Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease

2009	Pål Kvello	ph.d Biology	Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas
2009	Trygve Devold Kjellsen	ph.d Biology	Extreme Frost Tolerance in Boreal Conifers
2009	Johan Reinert Vikan	ph.d Biology	Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches
2009	Zsolt Volent	ph.d Biology	Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter
2009	Lester Rocha	ph.d Biology	Functional responses of perennial grasses to simulated grazing and resource availability
2009	Dennis Ikanda	ph.d Biology	Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania
2010	Huy Quang Nguyen	ph.d Biology	Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments -Focus on formulated diets
2010	Eli Kvingedal	ph.d Biology	Intraspecific competition in stream salmonids: the impact of environment and phenotype
2010	Sverre Lundemo	ph.d Biology	Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe
2010	Iddi Mihijai Mfunda	ph.d Biology	Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. Tha Case of Serengeti Ecosystem, Tanzania
2010	Anton Tinchov Antonov	ph.d Biology	Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis
2010	Anders Lyngstad	ph.d Biology	Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation
2010	Hilde Færevik	ph.d Biology	Impact of protective clothing on thermal and cognitive responses
2010	Ingerid Brænne Arbo	ph.d Medical technology	Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans
2010	Yngvild Vindenes	ph.d Biology	Stochastic modeling of finite populations with individual heterogeneity in vital parameters
2010	Hans-Richard Brattbakk	ph.d Medical technology	The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits
2011	Geir Hysing Bolstad	ph.d Biology	Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy
2011	Karen de Jong	ph.d Biology	Operational sex ratio and reproductive behaviour in the two-spotted goby (*Gobiusculus flavescens*)
2011	Ann-Iren Kittang	ph.d Biology	*Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS:– The science of space experiment integration and adaptation to simulated microgravity
2011	Aline Magdalena Lee	ph.d Biology	Stochastic modeling of mating systems and their effect on population dynamics and genetics
2011	Christopher Gravningen Sørmo	ph.d Biology	Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in *Arabidopsis thaliana*

2011	Grethe Robertsen	ph.d Biology	Relative performance of salmonid phenotypes across environments and competitive intensities
2011	Line-Kristin Larsen	ph.d Biology	Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment
2011	Maxim A. K. Teichert	ph.d Biology	Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density
2011	Torunn Beate Hancke	ph.d Biology	Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology
2011	Sajeda Begum	ph.d Biology	Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh
2011	Kari J. K. Attramadal	ph.d Biology	Water treatment as an approach to increase microbial control in the culture of cold water marine larvae
2011	Camilla Kalvatn Egset	ph.d Biology	The Evolvability of Static Allometry: A Case Study
2011	AHM Raihan Sarker	ph.d Biology	Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh
2011	Gro Dehli Villanger	ph.d Biology	Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals
2011	Kari Bjørneraas	ph.d Biology	Spatiotemporal variation in resource utilisation by a large herbivore, the moose
2011	John Odden	ph.d Biology	The ecology of a conflict: Eurasian lynx depredation on domestic sheep
2011	Simen Pedersen	ph.d Biology	Effects of native and introduced cervids on small mammals and birds
2011	Mohsen Falahati-Anbaran	ph.d Biology	Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis*
2012	Jakob Hønborg Hansen	ph.d Biology	Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance
2012	Elin Noreen	ph.d Biology	Consequences of diet quality and age on life-history traits in a small passerine bird
2012	Irja Ida Ratikainen	ph.d Biology	Theoretical and empirical approaches to studying foraging decisions: the past and future of behavioural ecology
2012	Aleksander Handå	ph.d Biology	Cultivation of mussels (*Mytilus edulis):*Feed requirements, storage and integration with salmon (*Salmo salar*) farming
2012	Morten Kraabøl	ph.d Biology	Reproductive and migratory challenges inflicted on migrant brown trour (*Salmo trutta* L) in a heavily modified river
2012	Jisca Huisman	ph.d Biology	Gene flow and natural selection in Atlantic salmon
2012	Maria Bergvik	ph.d Biology	Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus*
2012	Bjarte Bye Løfaldli	ph.d Biology	Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*.
2012	Karen Marie Hammer	ph.d Biology.	Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia
2012	Øystein Nordrum Wiggen	ph.d Biology	Optimal performance in the cold

2012	Robert Dominikus Fyumagwa	Dr. Philos.	Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania
2012	Jenny Bytingsvik	ph.d Biology	Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs
2012	Christer Moe Rolandsen	ph.d Biology	The ecological significance of space use and movement patterns of moose in a variable environment
2012	Erlend Kjeldsberg Hovland	ph.d Biology	Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters