Karen de Jong

Operational Sex Ratio and Reproductive Behaviour in the Two-spotted Goby *(Gobiusculus flavescens)*

NTNU – Trondheim
Norwegian University of
Science and Technology

Ideas are like fish eggs
some hatch, some don`t

To Kalle, to life.

And to Rein, because ”Behind every successful woman there is a substantial amount of coffee.” (Stephanie Piro) and you made most of that coffee.

Acknowledgements

I have much enjoyed these years in Trondheim and as a PhD-student, and there are many people I would like to thank. I will try to mention the ones that were most important.

First I will thank my mother, who showed me what women are capable of, even when they don't get the same chances. She also showed me that there is no need to rush life, it is never too late to decide what you really want to do. Then my father, who unfortunately is no longer around, but who introduced me to the joy of living and studying life; and my brother, for being my brother. I would also like to thank my friends and other family, for being there when I needed you.

My supervisors: Trond Amundsen for his interest in all I do and his endless questions that, whilst not always convenient during fieldwork, demonstrate his expertise and commitment; Elisabet Forsgren, for her enthusiasm and brilliant ideas; and both of them for the enormous amount of work they invested in improving my writing.

Next I have to thank Sebastian, for jointly surviving that first intense summer; for tolerating me as -demanding- chief during the second and third and as a colleague in the fourth and fifth; for five years of great collaboration and so much more. Lise, for being the best first master student anyone could wish for and for being a great colleague and friend. Line and Geir, for being great office mates and friends, for patiently repeating their questions when I didn't hear the first time, and for always being willing to answer mine. Lines stapler, for creating some order in my papers. Claudia, for forcing me to exercise in autumn when Trondheim turns dark. Ivar, for always having time to answer stupid questions about statistics. Jon, for endless, enjoyable discussions and Ani, for the same. Laura, who lured me away from behaviour into a world of unimaginable small eggs and cute larvae, of which eventually nothing was included in this thesis, for great cooperation and company. Andreas, for welcoming me to Trondheim and leaving a thesis that grew to be our "Bible" of methods and a field site packed with functional gadgets. Jorunn, Camilla and Marte, who proved to me in Sweden that Norwegians are truly insane, and who made my first Goby summer certainly the

Table of contents

List of papers

I **Karen de Jong,** Sebastian Wacker, Trond Amundsen & Elisabet Forsgren.

Do operational sex ratio and density affect mating behaviour? An experiment on the two-spotted goby[1]

(2009; Animal Behaviour 78:1229-1238; doi:10.1016/j.anbehav.2009.08.006)

II **Karen de Jong**, Elisabet Forsgren, Hanno Sandvik & Trond Amundsen.

Are we measuring mating competition correctly?[2]

(Manuscript)

III **Karen de Jong**, Trond Amundsen & Elisabet Forsgren.

Operational sex ratio affects male courtship propensity, but not courtship frequency, in the two-spotted goby[3]

(Manuscript)

IV **Karen de Jong**, Lise Cats Myhre, Sebastian Wacker, Elisabet Forsgren & Trond Amundsen.

Seasonal variations in reproductive behaviour of the two-spotted goby, a fish with dynamic sex roles[4]

(Manuscript)

---

[1]This study was initiated by TA and EF, the methods were developed by all authors, experimental work was done by SW and KdJ, analyses and writing were done by KdJ with comments from the other authors.

[2]This study was based on an idea by KdJ and developed in discussions with EF and TA, the literature search was done by KdJ, the meta-analysis by KdJ and HS, and KdJ wrote the manuscript with comments from the other authors.

[3]This study was initiated by KdJ, the design was further developed in discussions with TA and EF, and the study was executed, analysed and written up by KdJ with comments from TA and EF.

[4]This study was based on ideas by TA and KdJ, the design developed by KdJ in discussion with the other authors, the field work done by LCM, SW and KdJ, analyses were done by KdJ with help from SW, and the manuscript was written by KdJ with comments from the other authors.

Abstract

Mating competition is an important process in sexual selection and can be expressed as complex courtship rituals and agonistic behaviours. The strength of mating competition is likely affected by mate availability. The operational sex ratio, the sex ratio of ready-to-mate individuals, reflects mate availability, and is therefore expected to affect competitive behaviour. This thesis explores the effect of operational sex ratio on competitive mating behaviour in the two-spotted goby (*Gobiusculus flavescens*). In a field population of two-spotted gobies at the west coast of Sweden, sex roles switch from conventional (males competing for females) to reversed (females competing for males), within a reproductive season. This coincides with a change in the OSR from male-biased to female-biased.

In a laboratory study, we found an unexpected effect of OSR on courtship frequency (Paper I). Males courted more in a female biased OSR. Other competitive behaviours were not affected by OSR or overall density. In paper II, we argue that courtship frequency is affected by the frequency of encounters with potential mates as well as the motivation to court. Thus, frequency may not be the correct measurement of mating competition. We provide a simple model that explains the relation between OSR, encounter rates, courtship propensity and courtship frequency. The model shows that studies measuring courtship frequency could find opposite results to studies measuring courtship propensity. A meta-analysis shows that this is indeed the pattern that exists in the literature (Paper II). A second laboratory study measured male courtship behaviour both as frequency and propensity in two OSR-treatments that did not differ in density or adult sex ratio (Paper III). This study found that male courtship propensity was higher in a male biased OSR than in a female-biased OSR, as expected. Courtship frequency, on the other hand, did not differ between treatments. Moreover, courtship propensity was associated with the OSR at the time of observation, while courtship frequency was associated with encounter rates with potential mates.

Paper IV explores how reproductive behaviour changes over the breeding season in a natural population. Early in the season, more of the nests were occupied and males attending a nest early were larger (in 2008) or in better condition (in 2007) than males attending a nest late in the season. Brood size was associated with male size and nest size early in the season, but only with nest size late in the season. Late in the season, males received more visits from ready-to-mate females in one of the years (2008), but this did not result in more eggs in their nest (both years). Brood size was smaller and brood survival lower late than early in the season; brood survival was also associated with brood size (only data for 2008). These results indicate that males competed for breeding opportunities early in the season, when male density is high, while lower quality males postpone breeding to later in the season. This leads to the surprising suggestion that females are competing for low quality breeding opportunities late in the season. Thus, female breeding decisions warrant further investigation.

My main conclusions from this thesis are that male propensity to court is affected by OSR in the expected direction, but encounter rate is related to encounter rates rather than OSR. Thus, changes in frequencies of behaviour may be hard to interpret. Furthermore, knowledge of the biology of natural populations is crucial to the design and interpretation of laboratory experiments.

Synopsis

Introduction

Sexual Selection

Sexual selection is the second of Darwin's great theories (Darwin 1871), which he suggested to explain the existence of ornaments, such as songs, conspicuous colours and elaborate physical traits in -most often- males. These ornaments seem to elude natural selection, because they do not have any obvious survival value and even appear to hinder survival by being conspicuous, unpractical or both (Darwin 1859; Endler 1980, 1983). The question is then, why such an ornament would evolve despite the fact that, in most cases, they only increase mating success in one sex, while natural selection acts against them. Darwin (1871) suggested that these ornaments could have evolved because they would give individuals an advantage in the competition for mates, i. e. sexual selection.

Competition for mates is a driving force in sexual selection, although there can also be selection on other processes, such as resource competition and sperm competition. The definition of competition I use here includes direct intra-sexual for mates through agonistic behaviour, but also competition that results from mate choice, such as elaborate traits or courtship displays that are used to attract potential mates (sensu Andersson 1994). In many species, an individual needs resources to obtain matings, such as nests or territories in birds and fish. Thus, when studying mating competition, it is important to realise that not all individuals may be qualified to mate (Kvarnemo and Ahnesjö 1996, 2002). If females mate multiply, there could also be post-mating competition among the sperm of several males for eggs to fertilize (sperm competition; Parker 1970, Birkhead & Møller 1993). Behavioural competition for mates could be expressed as agonistic behaviour, courtship behaviour or alternative mating tactics (Andersson 1994). These behaviours will directly affect an individuals` mating success through the monopolization of mates (agonistic behaviour), by influencing mate choice by the other sex (courtship) or by increasing mating success directly (by tactics such as sneaking or coercion).

In many species males are the more competitive sex, while females are more choosy (conventional sex roles; Darwin 1871, Andersson 1994). Traditionally this has been explained by the differences in parental investment that result from anisogamy. Since males invest less in sperm than females in eggs, males are unlimited in how many females they can fertilise and should therefore compete for the available females (Trivers 1972). However, since then, females have been found to compete for males in some species (reversed sex roles), whilst in some other species, sex

roles have been shown to be dynamic (Gwynne and Simmons 1990, Simmons & Bailey 1990, Vincent et al. 1992, Andersson 2005). Even in species with conventional sex roles, females have been found to compete for males as well and males could be choosy (Gwynne 1991, Owens & Thompson 1994, Amundsen 2000, Clark & Grant 2010, but see Kokko & Johnstone 2002). In addition, sex roles could vary over time and between populations (Gwynne 1990, 1991, Almada et al. 1995, Forsgren et al. 2004) Thus, the strength of mating competition is not fixed, but is likely to be affected by other factors than anisogamy as well.

Both the spatial and temporal distribution of potential mates can affect the strength of mating competition. In general, if potential mates are clumped together, but become ready-to-mate one by one, there is the largest scope for competition, because one individual could then potentially mate with all potential mates (Emlen & Oring 1977). Thus, how strong mating competition becomes depends on how many same-sex individuals are both ready-to-mate and at a particular location. The spatial component of this argument is expressed in population density. The closer individuals are together, the greater the scope for direct competition for mates and for mate choice, because individuals are easier to compare and the cost of mate choice is low (Kokko & Rankin 2006). Thus, we expect increased competition with higher density, unless competition becomes too costly and therefore breaks down (Emlen & Oring 1977, Grant et al. 2000).

The temporal distribution of mates is expressed in the operational sex ratio (OSR; the sex ratio of ready-to-mate individuals at a given time; Emlen 1976). The OSR is expected to affect mating competition because it reflects how many potential mates are available for each same-sex competitor. Population operational sex ratio is affected by the sex ratio of adult individuals in a population, but also by sex differences in the potential reproductive rate (Clutton-Brock & Vincent 1991, Clutton-Brock & Parker 1992, Kvarnemo & Ahnesjö 1996, 2002; Figure 1). While changes in adult sex ratio will affect the operational sex ratio directly, changes in potential reproductive rates will have a delayed effect on the operational sex ratio (Kvarnemo & Merilaita 2006). The potential reproductive rate is the population mean of how many offspring each of the sexes would be able to produce if they would have unlimited access to mates (Clutton-Brock & Vincent 1991, Clutton-Brock & Parker 1992). In most cases, males are expected to have a higher potential reproductive rate than females, because sperm is cheaper to produce than eggs. Thus, a male could potentially fertilize many females in a short period and gain offspring from each of them, while a female would not gain from mating more often before she develops new eggs (Bateman 1948), although females may benefit from multiple matings (Jennions & Petrie 2000, Hosken & Stockley

2003). However, in some species, females have the higher reproductive rate (Vincent et al. 1992), and sex differences in potential reproductive rate could be affected by temperature or food availability (Kvarnemo 1994, 1996, Figure 1)

Not only the current availability of mates could affect mating competition, also future prospects and a trade-off between investment in current offspring or the chance to compete for new matings may affect how willing an individual is to compete for matings (Queller 1997, Kokko & Jennions 2008). Mortality costs of breeding have been suggested to affect mating competition both directly and via a change in operational sex ratio (Kokko & Monaghan 2001, Kokko & Jennions 2008; Figure 1). These additional factors would mean that a switch in sex roles does not necessarily happen at an equal OSR. Nevertheless, the OSR is expected to affect the strength of mating competition within a sex (Kokko & Monaghan 2001).

Correlations between variations in OSR and variation in mating competition have been found in several species (Kvarnemo & Ahnesjö 1996, Forsgren et al. 2004). However, there are as yet no clear patterns emerging from controlled experiments, even though many studies have collected valuable data on how OSR relates to mating behaviours. Few of the previous studies have investigated mating competition both in the field and in the laboratory. Limited knowledge of mechanisms in the field may decrease the relevance of laboratory set-ups and complicate the interpretation of results from laboratory experiments.

This thesis aimed to test for an effect of OSR on mating competition in males and females of the two-spotted goby, a species in which the strength of mating competition in both sexes has been found to co-vary with OSR in a field population (Forsgren et al. 2004).
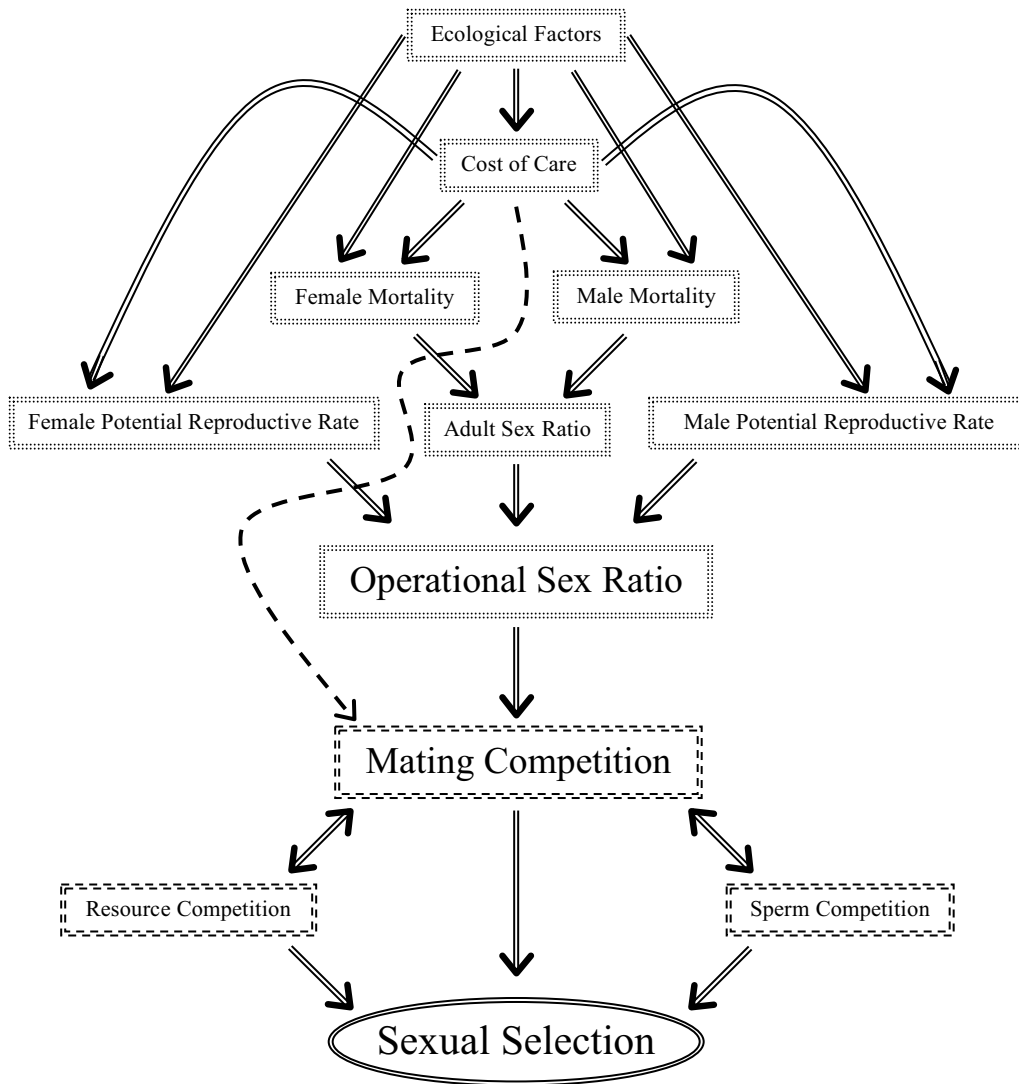
Figure 1. Schematic overview of factors that could affect mating competition and, ultimately, sexual selection. This thesis focuses on the relation between OSR and mating competition, these are therefore in larger boxes. Note that the relative thickness of the lines does not reflect importance of the effects.

Aim

The aim of this thesis was to explore the relationship between operational sex ratio and competitive behaviour. Paper I aimed to test whether there was a causal effect of OSR or density on mating behaviour in both sexes. Paper I awoke the suspicion that laboratory experiments often did not use the most appropriate measure of mating competition, in contrast to field studies. Paper II therefore aimed to document the use of measures of mating competition and analyse whether the use of different measures led to different results. Paper III tested the effect of OSR on both male courtship frequency and male courtship propensity in relation to the encounter rate with ready-to-mate females. The aim of paper IV was to explore the reproductive background of the two-spotted goby and to describe how reproductive behaviour changes over the season in a fish with dynamic sex roles.

Model system: the two-spotted goby (*Gobiusculus flavenscens*)

Why study a small brown boring fish?

Most divers and snorkelers off the coast of Europe will not first of all remember having seen shoals of two-spotted gobies. However, although this is probably true for most organisms, the closer you look the more interesting these little not-so-very-brown-after-all fish become. In contrast to most other species, and even most closely related gobies, both male and female two-spotted gobies are ornamented (Amundsen & Forsgren 2001, Svensson et al. 2009; Figure 2).

Male ornaments and courtship were described by Guitel (1895), who was impressed by the stamina of the in his eyes "petit joli homme". Males have iridescent spots along the mid-line of their body, and brightly coloured fins (Figure 2A; Guitel 1895, Amundsen & Forsgren 2001). It is interesting to note that Guitel (1895) did not note that females develop round orange bellies with egg development (Figure 2B; Smitt 1892, Svensson et al. 2006), nor did he describe female courtship behaviour. The fact that ornaments differ between males and females and that they are emphasized in courtship, suggests that sexual selection may be acting on both sexes (Amundsen & Forsgren 2001, 2003).
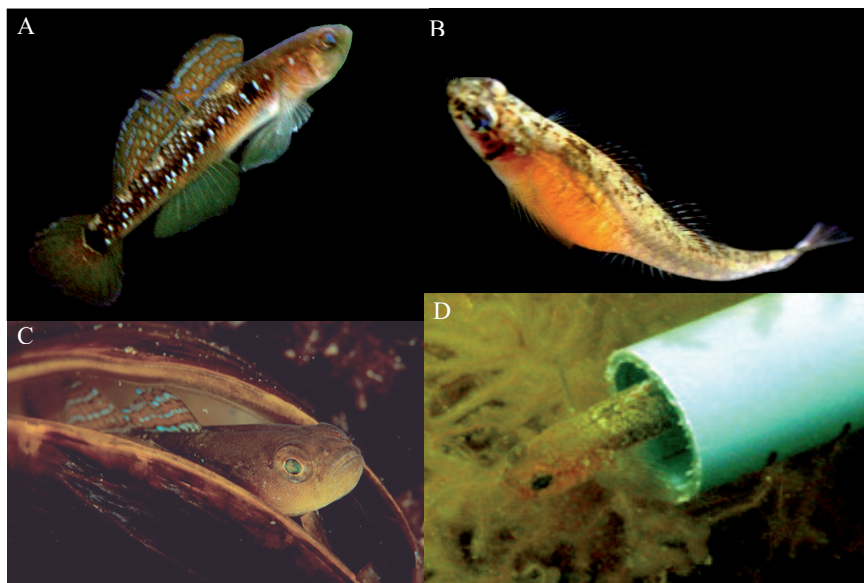
Figure 2. The two-spotted goby (*Gobuiusculus flavescens*). A male in fin display (A) and a female (B). Below, the male is shown in a natural (C: mussel shell) and an artificial (D: PVC-tube) nest. Images: Lise Cats Myhre (A-B), Anders Salesjö (C) & Karen de Jong (D).

Reproductive biology

The two-spotted goby is a small (3 - 6 cm) marine fish that lives along the coast of Europe, from northwest Spain to northern Norway (Miller 1986). In our study area at the west-coast of Sweden, the breeding season lasts from April to July, although the actual onset of breeding can vary from early to late April and the end of the breeding season can vary from early to late July and even early August (personal observations). During the breeding season, sexually mature individuals inhabit shallow waters along rocky shores (Miller 1986). Non-breeding individuals often form semi-pelagic foraging shoals (Miller 1986; Svensson et al. 2000). Breeding males defend a nest in empty mussel shells (e. g. *Mytilus edulis*), in brown algae (typically *Saccharina latissima* and *Laminaria digitata*), or in crevices. Females visit males to spawn and generally lay all eggs from one clutch in the same nest (Skolbekken & Utne-Palm 2001), but can develop up to five clutches over the season (Rodriguez-Graña & K. de Jong, unpublished data). Males provide all parental care, defending the nest and cleaning and aerating the eggs until they hatch (Skolbekken & Utne-Palm 2001). In the field, males have been found to care for clutches of up to six different females simultaneously (Mobley et al. 2009).

Courtship and agonistic behaviour

A male typically starts courtship by swimming towards the female and raising his fins (fin display; Figure 2A), this can be followed by vibrating his body perpendicular to the female (quiver). He then may swim to the nest waving his tail in a very typical manner (lead swim), while the female may or may not follow. He often enters the nest before he swims back to the female if she did not follow him into the nest (Amundsen & Forsgren 2001, Pélabon et al. 2003). This sequence may be (partially) repeated several times. Females may follow a male closely and bend their body in a S-shape, presenting her round, orange belly to the male (sigmoid display; Amundsen & Forsgren 2001, Borg et al. 2006). Females can show sigmoid displays to a male for several seconds, alternating the direction of the bend (personal observations).

Agonistic behaviour in males can consist of fin displays to males and females. These fin displays are slightly different from courtship displays (personal observations). Males are generally side-by-side when they raise their fins and have their head turned slightly downwards, while the head is more levelled during courtship fin displays (personal observations). Males may also chase and attack other males. Female agonistic behaviour consists of sigmoid displays to other females, chases (Forsgren et al. 2004), and pushing each other aside with the head (personal observations).

Methods

In this chapter I will elaborate more on the methods than there is generally space for in articles for publication. In particular, I will focus on how the methods I used in the laboratory relate to the biology of the two-spotted goby in its natural habitat. For details on the methods for each study however, I refer to the respective papers in this thesis.

Study area

Both the laboratory and the field work for this thesis were carried out between 2006 and 2009 at the Sven Lovén Centre for Marine Sciences in Kristineberg. The station is situated on the west coast of Sweden (Figure 3A) at the mouth of the Gullmar fjord (58.15°N, 11.27°E).
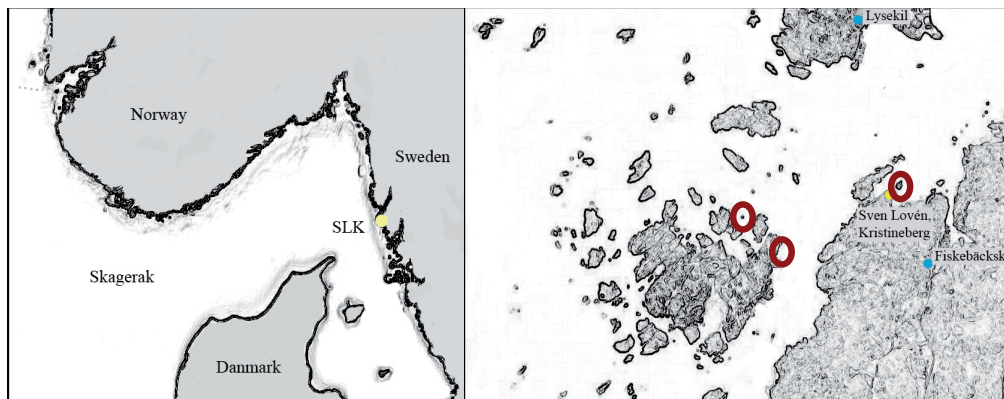
Figure 3. Study area on the west coast of Sweden. SLK is the Sven Lovén Centre for Marine Sciences at Kristineberg, where the laboratory experiments were carried out (Paper I & III). Two-spotted gobies were caught all around the islands and along the coast up to three kilometres from the station. Circles indicate where artificial nests where placed for paper IV. Maps were redrawn from Google Earth.

Fish collection, husbandry and marking

All experimental fish were caught by snorkelers with hand held nests (for details see Svensson 2006) in shallow waters (> 5 m) at the mouth of the Gullmar fjord (Figure 3). They were transported to the laboratory in large, covered, plastic containers and stored upon arrival in large single sex aquaria (up to 60 fish per 60 l aquarium). All fish were measured on a measuring board (Figure 4A) and weighed in a cup of pre-weighted seawater (Figure 3B). Both males (Paper I, III, IV) and females (Paper I) were marked for individual identification by injecting visible implant elastomer (VIE; Northwest Marine Technology TM, www.nmt.us) of different colours subcutaneously (Figure 4C & D), after anesthetization with 2-phenoxyethanol. All fish regained normal swimming behaviour within 10 min after this procedure. This method of tagging has been shown to not influence mortality or behaviour in other gobies (Malone et al. 1999; Griffiths 2002; Whiteman & Côté 2004). No change in behaviour could be detected after marking. Additionally, two males and four females that were kept in the laboratory for three months in 2007, courted, spawned and the male took care of the eggs. In Paper IV, we did not find any evidence for an effect of our manipulations (catching, handling and marking) on male nest attendance or fate of the attended brood.

Figure 4. Handling of the two-spotted gobies used in the experiments of this thesis. Measuring was done on a millimetre grid (A), weighing in a cup with pre-weighed water (B) and marking by subcutaneous injection of elastomer (D). C depicts a marked male. Images: Isabel Mück (A, B & C), Karen de Jong (D).

Experimental aquaria (Paper I & III)

All aquaria were provided with a continuous flow of surface water from the Gullmar fjord. Aquaria had a layer of gravel on the bottom and contained several plastic plants to provide shelter. Light came from windows and additional artificial light sources set to reflect a typical Swedish summer with 6 hours dark and 18 hours light.

In paper I, we used a standard laboratory set-up where all individuals were in constant visual contact (Figure 5A). However, we divided the aquarium in single-sex compartments to prevent the OSR changing over time, due to females spawning and males receiving eggs. Thus, there was no free interaction between the sexes. In paper III, we used a novel design, where male nest sites were partly shielded from the rest of the aquarium (Figure 5B). Thus, females could visits males to spawn and males could swim out of their compartment into the central compartment. Free interaction between the sexes was possible, but there was no constant visual contact.
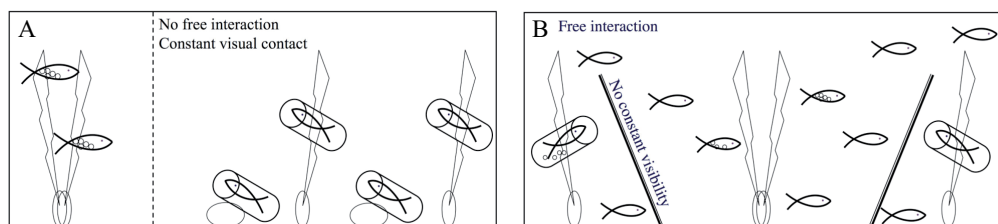
Figure 5. The laboratory designs of paper I (A) and paper III (B); two experiments to test for an effect of OSR on courtship behaviour in the two-spotted goby.

Analyses of behaviour

In the analyses for papers I and III we used a set of courtship and agonistic behaviours (see model species for a description) previously described in both field (Forsgren et al. 2004) and laboratory studies (Amundsen & Forsgren 2001, Pélabon et al. 2003). Both male courtship and agonistic displays exist of several different behaviours that can vary in frequency between displays, which complicates the quantification of behaviour. In addition, it is not obvious that any behaviour interpreted by us as, for example, courtship behaviour, is intended as such by the animal performing the behaviour. A principal component analysis (PCA) is a good way to address this problem, PCA is not a novel method, but because I think PCA is a rather underused method in the study of mating competition, I will spend some time here explaining the advantages. A PCA gathers all behaviours that are expressed in a similar situation, and thus correlated, on one component. A score on this

component then reflects the actual strength of courtship. All components in a PCA will be perpendicular to each other and therefore independent. This has the advantage that tests of treatment effects on the component scores will be independent as opposed to testing treatment effects on all the different behaviours separately. In addition, using PCA circumvents the problem how to weigh different behaviours if these are added to arrive at a total courtship frequency.

Here, I compare the Principal Component Analyses from paper I with a principal component analyses done on the ten minutes direct observation per male that were done for paper III, although they were not included in the paper for reasons of clarity and conciseness. Note that all courtship behaviours are similarly correlated to the first component in both experimental designs (Table 1). Thus, they are likely to indeed reflect courtship. Agonistic behaviour to females falls out on the second component in paper III and was not observed in paper I (Table 1). Coercion and harassment of females have been shown to be alternative mating tactics in, for example, guppies (Farr 1980). However, because this kind of behaviour was not seen in the design for paper I, and, in contrast to courtship behaviours, was directed to both ready-to-mate and to other females, I deem it more likely that this kind of agonistic behaviour to females reflects nest defence. Females have been seen to eat eggs during field observations (personal observations).

In paper III, I defined three different kinds of fin displays, courtship fins, agonistic fins and undefined fins. Fin displays have proven to be difficult to assess in the past and often gave different results than stronger courtship behaviours (Amundsen & Forsgren 2001, Pélabon et al. 2003). They can be shown in both agonistic behaviour and courtship, and previously, they were largely classified on the basis of the sex of the receiving individual. The Principal Component analyses for the data from paper III shows that careful assessment by a trained observer can discriminate between the two (Table 1).

Comparing the PCA loadings from the two experiments further suggests that agonistic behaviour differed markedly between the experiments, while courtship behaviour was similar (Table 1). In the design for paper I, males could interact freely with each other, but not with females (Figure 5A), while in the design for paper III, females could interact freely, and males did not have visual contact if they were in their own compartments (Figure 5B). This may suggests that agonistic behaviour is more dependent on physical interaction than courtship behaviour.

Synopsis

Table 1. Two Principal Component Analyses on the behaviours scored during observations for Paper I and III. Loadings for male behaviours and time spent in different areas on three and four components in two different studies (Paper I & III). Loadings range from 0 to 1. Loadings lower than 0.2 are omitted for the sake of clarity. Some behaviours load on more than one component, therefore the highest loading for each behaviour is given in bold. The highest loadings for courtship behaviours are also highlighted in green.

Components	Paper I (Day 4)			Paper III			
	PC1	PC2	PC3	PC1	PC2	PC3	PC4
Mate inspection							
Follow female				.	.	**.755**	.
Undefined fin display				.357	.	**.630**	.223
Courtship							
Courtship fin display	**.911**	.	.	**.962**	.	.	.
Quiver	**.928**	.242	.	**.960**	.	.	.
Lead swim	**.856**	.304	.	**.924**	.	.	.
Enter nest	**.711**	.346	.308	.248	**.539**	.	.
Fast approach	**.528**	.	.	**.595**	.269	-.386	
Agonistic to female							
agonistic fin to female				.	**.676**	.392	.
chase to female				.	**.796**	.	.
attack on female				.	**.662**	.	.
Agonistic to male							
Agonistic fin display	.	**.604**	.	.	.	.	**.744**
Chase	.371	**.704**	.				
Attack	.	**.806**	.				
Place in aquarium							
Scans in association area	.383	.	**-.774**				
Scans at nest	.256	.	**.838**				
Visits to neutral				.	.	**.591**	.580
Visits to other nest holder				.	.	.	**.792**
Variance explained	36%	18%	15%	25 %	15 %	13 %	13 %

Operational sex ratio and density in the laboratory

Most laboratory studies manipulate operational sex ratio through the adult sex ratio, while ascertaining that all individuals are ready-to-mate. This has the advantage that it is easy to assess mate availability (Paper I). However, it does not always reflect changes in operational sex ratio in the field, because these may be caused by a change in the potential reproductive rate (Okuda et al. 1999) or the number of qualified individuals (e. g. Gwynne 1984, Kvarnemo & Ahnesjö 2002) rather than a change in the adult sex ratio. Alternatively, only the density of one sex may change (Forsgren et al. 2004). In addition it has been suggested that sex ratio may affect mating competition differently than OSR (Kokko & Jennions 2008). In paper III we therefore changed operational sex ratio solely by changing the number of round ready-to-spawn females, keeping the total number of females constant. This partly reflects the situation in the field, where late in the season, most of the females are ready-to-mate (Forsgren 2004). However, in the field, male density also decreased over the season. It proved hard to create operational sex ratios that are as female biased as they are in the field, without creating an unrealistically high overall density in the aquarium. Incorporating space for eggs in the nest in the calculation of OSR (see Forsgren et al. 2004), the sex ratio in paper III was between 0.26 and 0.33 in the experiment, while it decreased to below 0.2 in the field (Forsgren et al. 2004).

Another issue with operational sex ratios in the laboratory is that they inevitably change over time, if individuals are allowed to mate. Only in long-term experiments where individuals are allowed to recover after each mating event (e.g. Kvarnemo et al. 1996), is this not a problem. In paper I we prevented the operational sex ratio from changing by blocking physical interaction between males and females. This allows for longer acclimatisation to the setup and may have increased the general frequency of all behaviours compared to paper III. However, inserting a divider also prevents certain interactions between males and females and may thus affect courtship behaviour. In the principal component analyses (Table I), we show that courtship behaviour did not differ between the treatments in the association of the separate behaviours. Thus at least the relation of the different behaviours is similar without physical contact. However, an individual male's perception of the operational sex ratio may depend on how easy it was to get females to spawn. Therefore I decided not to prevent physical interactions between the sexes in the experiment in paper III.

Nest availability in the field and the laboratory

Both in the field and in the laboratory experiments we used PVC-tubes as nest sites. Two-spotted gobies spawn readily in these nests in the laboratory and in the field (Svensson 2006, Forsgren et al. 2004). The standard size used in laboratory experiments (Paper I, III, Pélabon et al. 2003 etc) is 8 cm by 13 mm, which results in 32 cm inner area where females can lay their eggs. In paper IV, we also used a larger size (9 cm by 19 mm) to assess if larger males would prefer larger nests. Two-spotted gobies can use everything that has a smooth surface to lay eggs. In one case I recorded spawning on a small plastic square we used to mark artificial nests (personal observations). Naturally, two-spotted gobies use crevices in rocks, the stem and base of the leaves of sugar kelp and mussels as nest sites. There is some evidence that two-spotted gobies prefer blue mussels over algae (Brevik 2007). This implies that our artificial nests may be preferred over algal nests as well, especially late in the season when filamentous algae are abundant. On the other hand, a study on female mate sampling that shared one of the sites in 2008 (L. C. Myhre, K. de Jong, E. Forsgren & T. Amundsen, unpublished data) found that only 7 of 23 females followed late in the season once passed a male in an artificial nest, suggesting that the majority of the males had natural nests even in late season in 2008.

It is not clear whether there is competition for nests in this species, but since they can use algae, crevices and shells, it is hard to imagine that nests could be limiting in this system. On the other hand, females do inspect nests before they spawn, and have been seen to refuse males after nest inspection (L. C. Myhre, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). Hence, there could be competition for high quality nest sites. However, because we aimed to test the effect of OSR on mating competition in the laboratory experiments, we minimized nest competition by providing enough nests for each male.

Abstracts of the four papers included in this thesis:

Below, I include the abstracts of the four papers to give an overview of the research that was done before I discuss the main results.

Abstract paper I

Males generally compete for females through courtship and aggression (conventional sex roles). However, sex roles are reversed in some species and can even vary between populations or over time within a single species. The operational sex ratio (OSR), the sex ratio of ready-to-mate individuals, is a central factor proposed to explain the strength and direction of mating competition. Density can both act as a cue for OSR and have its own effects on competition. In the two-spotted goby, *Gobiusculus flavescens*, a seasonal switch in sex roles coincides with a change in OSR from male to female biased and a decrease in male density. This dynamic system provides an ideal opportunity to test the effects of OSR and density on competitive behaviour experimentally. In an aquarium experiment, we created two different sex ratios at two different densities and observed courtship and agonistic behaviour in both sexes. We changed sex ratio to equality after 4 days of treatment, to test for a lag in the behavioural response. Contrary to our predictions, males courted more after prolonged exposure to a female-biased sex ratio, although we also found a tendency for a positive effect of male density on male courtship frequency. We did not find any other effects of treatment on either male or female behaviour. Furthermore, we did not find evidence for a lag in the response to a change in density or sex ratio. We conclude that OSR and density did not affect individual frequencies of behaviour in the expected direction.

Abstract paper II

Central to sexual selection theory is the question why one sex usually competes more for mates than the other sex. Theory predicts that the abundant sex in the sex ratio of ready-to-mate individuals (OSR) will compete most. In accordance, the OSR matches the strength and direction of mating competition in field populations of several species. However, experimental tests have provided inconclusive results, possibly because experiments generally measure the frequency of courtship and agonistic behaviour. Frequency is affected by the number of potential mates or opponents encountered, as well as by the level of mating competition. The propensity to behave at each encounter, generally used in field studies, should reflect a behavioural response, but is used in very few experiments. In two simple models, we show that courtship frequency can respond differently from courtship propensity to a change in OSR. Agonistic frequency and propensity respond similarly, but not identically, and an increase in frequency could merely reflect an increase in encounters with competitors. In a meta-analysis we show that studies measuring courtship frequency indeed produce different results from studies measuring courtship propensities. We do not claim originality for the idea that encounter rate affects frequencies of behaviour, but stress its importance in the design of future experiments. We provide suggestions how to conclusively test OSR theory.

Abstract paper III

The operational sex ratio (OSR; the sex ratio of ready-to-mate individuals) was proposed to affect the strength of mating competition in 1976. The suggestion has received correlational support from field studies, but results from controlled experiments have not matched patterns found in the field. To date, most experiments measure frequencies of competitive behaviour. However, frequencies could be affected by the number of encounters with mates and competitors, as well as by the level of competition. The propensity to compete at each encounter should better reflect the behavioural response. Here, we adopt an experimental design that allows for recording encounter rates and propensities to compete. In a laboratory experiment we changed the OSR via the number of ready-to-mate females, without changing the adult sex ratio. We provided structured aquaria with male compartments at each end that were partly closed off from the central compartment, where we entered the females. This allowed us to record all visits of males and females to focal males. As predicted, we found that males in a male-biased OSR were more likely to court visiting ready-to-mate females, while total courtship frequency was not affected by OSR. These results corroborate a recent model that predicts that if OSR affects courtship propensity, this does not necessarily result in a similar effect on courtship frequency. Moreover, males spent less time in their nests and interacted more in a male-biased OSR. Our results provide the first conclusive experimental evidence that a difference in OSR alone affects competitive mating behaviour.

Abstract paper IV

To interpret results of experimental studies, we need to understand the ecology of our model organism. The two-spotted goby has proven useful as a model system in a variety of topics, but knowledge about the reproductive biology in natural populations is still relatively scant. In this study we describe the reproductive behaviour of male two-spotted gobies breeding in artificial nests in the Gullmar fjord on the west coast of Sweden. We placed 10 artificial nests at one location in 2007 and 45 artificial nests at each of two locations in 2008. We inspected nests regularly over the season and marked, measured and weighed males that attended the nests. Our results indicate that males cared for relatively synchronous broods and did not generally attend the same nest longer than the developmental period of one brood. Our results also indicate that males competed for breeding opportunities early in the season, when male density is high. Early in the season, more of the nests were occupied and males attending a nest early were larger (in 2008) or in better condition (in 2007) than males attending a nest late in the season. In addition, males attending a nest were in better condition than males in feeding shoals (only data for 2007). Late in the season, males received more visits from ready-to-mate females in one of the years (2008), but they did not have fuller nests (both years). Brood size was smaller late than early in the season and was associated with nest size. Early in the season brood size was also associated with male size. Brood survival was associated with brood size and lower late than early in the season.

Main results and discussion

How to measure mating competition: frequency versus propensity (Paper II)

A discussion about how to measure behaviour may seem better placed in the method section of this thesis. However, how to measure mating competition is not a trivial issue. In Paper II we suggest that, while the frequency of competitive behaviours is widely used as a measure of mating competition (Figure 6), it may not actually measure the behavioural response to competition. We argue that frequency is a compound measure of the motivation to behave and the number of opportunities an individual gets to express a certain behaviour.

The problem with measuring changes in the frequency of a behaviour is that a frequency captures not only how likely an animal is to behave in a certain way, but also how many opportunities an animal gets to behave. For example, if a male is very interested in mating, and would court each female he sees, but does not encounter a single female, he will not court and thus have a courtship frequency of 0. Similarly, if a male would defend each female with vigour against any competitors, but he hardly ever encounters a male, the frequency of agonistic behaviours would be low. We were not the first to realize that encounter rates may affect the frequency of certain behaviours (e.g. Ward & Fitzgerald 1988) and several studies have attempted to correct for the number of individuals in the experiment is various ways (Jirotkul 1999, Jivoff & Hines 1998, Debuse et al. 1999, Grant et al. 2000, Silva et al. 2010). However, because OSR and encounter rates may not be linearly related (see OSR and encounter rates) this is not a solution and could even lead to seemingly positive results in the absence of any response.
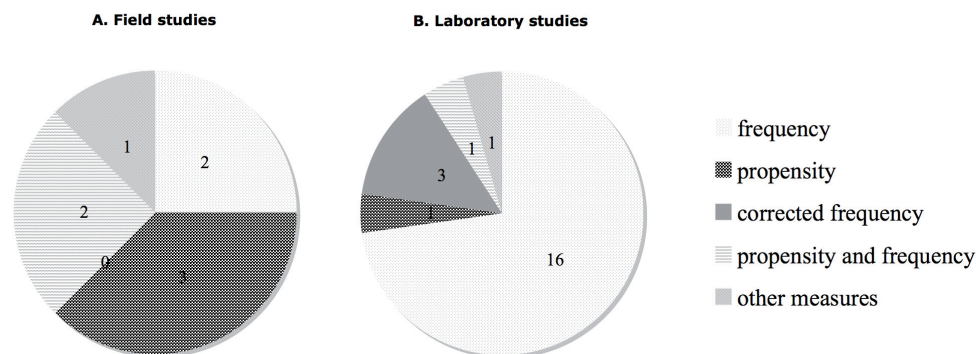
Figure 6. The number of field studies (A) and laboratory studies (B) reviewed in Paper II that report different measures of courtship behaviour in relation to an effect of OSR on mating competition.

In a simple graphical model, we show that, if courtship behaviour is affected by encounter rates with potential mates, this could lead to a decrease in courtship frequency when the OSR becomes more biased towards the own sex, even if the propensity to court each encountered potential mate increases (Figure 7A). To test if this pattern is observable in the literature, we did a meta-analysis on all studies that tested the effect of either propensity, frequency, or both. We found that male courtship frequency overall significantly decreases when OSR becomes male biased, while male courtship propensity was not significantly affected by OSR (Figure 8). We did not find enough studies on female behaviour to warrant further analyses. Therefore, I only discuss male courtship and agonistic behaviour here. However, since the mechanisms are likely to be similar, everything I state below is likely to be valid for female behaviour as well.

The overall effect of OSR on courtship propensity was not significantly different from zero in the meta-analyses. However, sample size was low ($N = 4$; Berglund 1994, Borg et al. 2002, Forsgren et al. 2004, Faleiro et al. 2008) and three of four studies find an increase in male courtship propensity when OSR becomes more male biased (Figure 8). The one study that did not find an effect of OSR simply states that males of the long-snouted seahorse always initiate courtship behaviour, regardless of sex ratio treatment (Faleiro et al. 2008). We can therefore not conclude that OSR does not affect the propensity to court from the meta-analyses. Male courtship frequency, on the other hand, decreased significantly when OSR became more male biased, as predicted by our model (Figure 7A & 8). Moreover, the few field studies we found that reported decreased courtship in an OSR bias towards the own sex, all measured courtship frequencies (Ward & Fitzgerald 1988, Okuda 1999, Takahashi 2000).
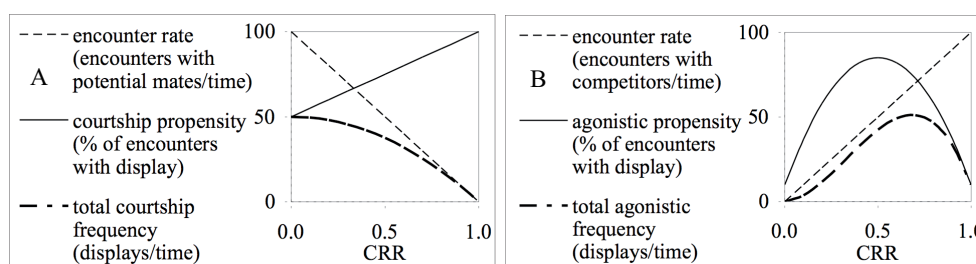
Figure 7. A simple model showing that the relation of the frequency of behaviours, encounter rates and the propensity to behave to OSR (as CRR: competitors/(competitors + potential mates)). The model is taken from Paper II, but adjusted to show what would happen in case of a dome-shaped relation between OSR and agonistic behaviour. The model shows that an effect of encounter rates on the frequency to behave could lead to: A: a decrease in courtship frequency even if courtship propensity increases; B: a dome shaped relation between OSR and agonistic behaviour for both measures, but a decrease in propensity could be masked by an increase in the encounter rate (in this example between a CRR of 0.5 and 0.75).

The propensity of agonistic behaviour is expected to increase with an increasing OSR bias towards the own sex, at least until agonistic behaviour becomes too costly and breaks down (Emlen & Oring 1977). Thus, we expect a dome-shaped relation of agonistic propensity with OSR (Grant et al. 2000). The frequency of agonistic behaviours is likely to increase when the OSR becomes biased towards the own sex, because of the increased numbers of encounters with competitors (Figure 7B). This increase could continue, even if the propensity to behave is already decreasing, although the frequency will in the end break down when agonistic propensity becomes very low (Figure 7B). This does not mean that it is not problematic to use agonistic frequency as a measure of mating competition. The frequency of agonistic behaviours may not reflect a response of the animal (Paper II), although they likely affect energy budgets. This may seriously compromise the conclusions we can draw from studies on agonistic behaviour. It would mean that we do not have conclusive evidence that animals adjust their behaviour to the social circumstances from studies that find an increase in the frequency of agonistic behaviours in an OSR bias towards the own sex, because the increase in frequency could just be caused by an increase in the encounter rate. A decrease in the agonistic frequency on the other hand, often found at high densities (Michener & McLean 1996, Grant & Foam 2002, Clark & Grant 2010), should reflect a decrease in the propensity, because the encounter rates are likely to be high. However, to reliably measure when this break down happens, we would have to measure propensities, because high encounter rates may mask a decrease in the propensity to behave. Thus, it should always be beneficial to develop designs that allow for distinction between the effects of OSR on encounter rates and the behavioural response.

Figure 8. The number of studies on male courtship (A) or female courtship (B) behaviour, that report frequency (black) or propensity (grey) measures grouped by the effect of OSR (as CRR: competitors/(competitors + potential mates)) they found. Note that there are very few studies on female behaviour.

Male courtship behaviour and operational sex ratio (Paper I & III)

In both paper I and paper III we tested the effect of OSR on male courtship behaviour. In paper I, our setup was such that individuals were in constant visual co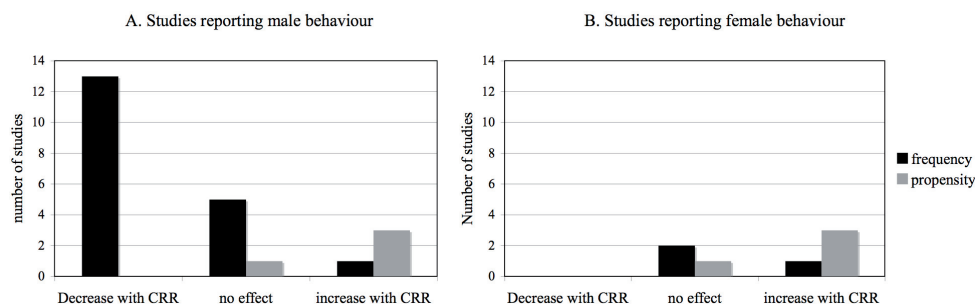ntact, but the sexes were prevented from physical interaction. We manipulated OSR via the adult sex ratio, and ensured that all individuals were ready-to-mate). We measured the courtship frequency in four different treatments 2:4, 4:8, 4:2 and 8:4 (males:females). In the design of paper III on the other hand, male nest sites were partly shielded from the rest of the aquarium, but the sexes were allowed to interact freely so that females could visits males to spawn. In this experiment we created a male biased and a female biased OSR-treatment by varying the number of ready-to-mate females, while the adult sex ratio was kept at 2:10 (males:females) in both treatments. Both males were provided with a nest site. In the female biased treatment, we added 10 ready-to-mate females and in the male biased treatment, we added 2 ready-to-mate females and eight other females. We measured both male courtship frequency and his propensity to court each ready-to-mate female that visited his compartment.

In paper I, male courtship frequency was higher in the male biased OSR, while in paper III courtship frequency did not differ between the treatments (Figure 9A & 10B). A decrease in male courtship frequency because of a switch to other competitive behaviours, as has been suggested for other species (e.g. Grant & Foam 2002, Jirotkul 1999, Shine et al. 2003) is not very likely. In paper I, we did not find evidence for a negative correlation between courtship and agonistic behaviour in the principal component analyses. In addition, agonistic behaviour did not increase in the male-biased treatments (Figure 9B). A switch to sneaking behaviour is not likely either, since no such behaviour has been observed in the field or the laboratory (personal observation, E. Forsgren, T. Amundsen, L. C. Myhre, S. Wacker, personal communication) and a study on the genetic mating system found a very low incidence of sneaking in field nests (Mobley et al. 2009). In addition, in a path analyses in paper III, we show that courtship frequency was not associated with current OSR, but was significantly associated with the number of encounters with potential mates. These results corroborate the general pattern found in the meta-analysis and follow the predictions from our simple model (Paper II, Figure 7 & 8). Within aquaria, we found that males that received more visits courted more, as expected. On the other hand, between aquaria, we found a negative relation between the number of visits by ready-to-mate females and male courtship frequency. Thus, the interactions between courtship frequency, courtship propensity and encounter rates may be rather complex (Paper III). At any rate, these results show that the courtship frequency is not the best measure of a behavioural response to OSR.
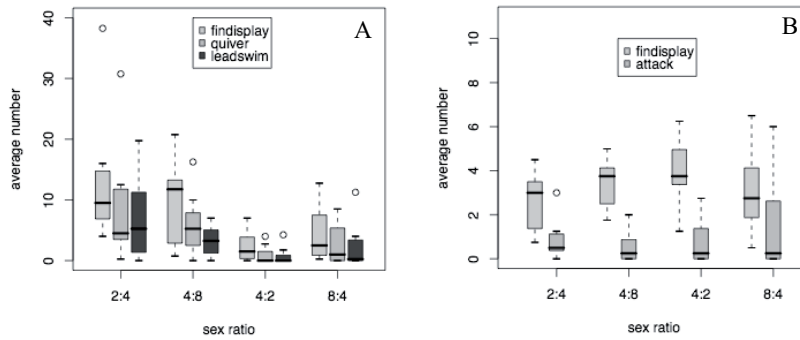
Figure 9. Courtship frequency (A) and agonistic frequency (B) of male two-spotted gobies in four sex ratio treatments (males:females) in a traditional laboratory design with constant visual contact, but no free interaction between the sexes (Paper I). Graphs are box plots showing the median and 25th-75th percentiles in the box and lines represent minima and maxima. Circles denote outliers.
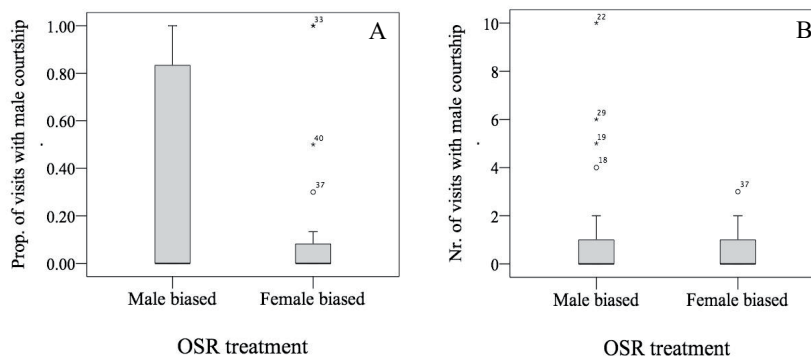
Figure 10. Courtship propensity (A) and courtship frequency (B) of male two-spotted gobies in two OSR-treatments in a novel experimental design that allowed free interaction between the sexes, but no constant visual contact (Paper III). Graphs are box plots showing the median and 25th-75th percentiles in the box and lines represent minima and maxima. Stars and circles denote outliers.

Synopsis

In paper III, the propensity to court was higher in the male biased treatment. In addition, the path analysis showed that current OSR, not nest fullness or mate encounter rate (the number of female visits to a male) was significantly associated with courtship propensity. To our knowledge, only three studies to date have used a measure of courtship propensity to test a causal link of OSR on courtship behaviour. Berglund (1994) found that male latency to court was shorter in a male biased OSR in the pipefish *Syngnathus typhle*. Faleiro and collaborators (2008) found that males initiated courtship regardless of sex ratio in the long-snouted seahorse (*Hippocampus guttulatus*). In the field, Borg et al. (2002) showed that an experimental manipulation of nest density affected both OSR and the proportion of courtship events initiated by each of the sexes in the common goby (*Pomatoschistus microps*). Thus, three of four studies have now found a decrease in male courtship propensity in a female biased OSR. These findings are in line with theoretical predictions (Emlen & Oring 1977, Kvarnemo & Ahnesjö 1996, Kokko & Monaghan 2001, Kokko & Jennions 2008) and findings from field studies (Almada et al. 1995, Borg et al. 2002, Forsgren et al. 2004, Cratsley & Lewis 2005). However, further studies are urgently needed to test if this is a general pattern, especially on those species where a relation between OSR and mating competition has been found in the field.

Male agonistic behaviour and operational sex ratio

While courtship behaviour has mainly been studied in fish (paper II), the relation between OSR and agonistic behaviour has been studied in a broader range of taxa (e.g. Amhibians: Emlen 1976, Verrell 1983, Tejedo 1988, Birds: Colwell & Ohring 1988, Insects: Gwynne & Simmons 1990, Krupah & Sih 1993, Thomas & Manica 2005, Mammals: Michener & McLean 1996). In controlled experiments, the frequency of male-male agonistic behaviour has often been found to increase with male density and a more male-biased OSR (e.g. Gwynne & Simmons 1990, Enders 1993, Grant et al. 1995, Kvarnemo et al. 1995, Quinn et al. 1996, Jirotkul 1999, Head & Brooks 2006, Smith 2007). However, if this is simply caused by an increase in the number of male-male encounters (e.g. suggested in Smith 2007), these results are not evidence of a behavioural response (Paper II).

In a set-up where all individuals were in constant visual contact (paper I), we found no effect of OSR or density on the frequency of male agonistic behaviour (Figure 9A). This means that there was no increase in the propensity to behave in the male biased treatment, because any effect of an increase in the number of encounters should only enhance a potential increase in propensity (Figure 7B). On the other hand, agonistic behaviour has been shown to break down at high male densities and strongly male biased OSR`s and thus may show a dome-shaped relationship to the level of

competition (Emlen & Oring 1977, Grant et al. 2000, Klug et al. 2008). Thus, there may have been a decrease in the propensity to behave agonistically, which was masked by the higher male-male encounter rate (Figure 7B). Overall, male-male agonistic behaviour was not very frequent in this experiment (Figure 9B). This could suggest that a breakdown had already happened, due to a high male density. Although males have been seen to nest at similar distances in the field (personal observations), the density in the two-male treatment was markedly higher than typical natural densities (16.7 compared to 0.56 - 0.07 males/m^2; Forsgren et al. 2004).

In paper III, in a design where male compartments were partly shielded from the central compartment and each other, we found even fewer agonistic male-male interactions and none of the escalated behaviours such as chases and attacks. In this case, agonistic male-male interactions were only observed in the male-biased treatment. The design of paper III may have been experienced by the males as a very low male density. Especially in the female biased treatment, males spent most of their time in their nests, where they had no visual contact with the other male. Regrettably, the setup did not allow for testing the propensity to behave agonistically directly, because male visits to another male's compartment were very rare (Paper III). However, these results suggest that the number of agonistic male-male encounters can be affected by the density of ready-to-mate females, as well as by male density.

Female competitive behaviour, operational sex ratio and sex roles

To date, there are few studies on the relation between operational sex ratio and mating competition in females (Paper II; Figure 8). Regrettably, this thesis has not succeeded in studying male and female competitive behaviour at the same time. We did not focus on female behaviours in paper III, but instead focused on males. Paper I provides some information on the frequency of female behaviours, which did not differ between treatments. However, the interactions were few, especially for female agonistic behaviour, limiting the power of the tests (Paper I). Moreover, we may not have created a skewed enough female bias in the experiments to establish strong female-female competition. Nevertheless, we did create female biased situations, both in sex ratio (two females to each male, paper I) and in OSR (five females to each male, with space in the nests for, on average, eight females to spawn, paper III). In the experiment for paper III, I recorded female behaviour in the male compartment on video, although I did not mark the females for individual recognition. However, the overall low number of courtship and agonistic behaviours did not warrant further investigation. Females may not have competed more strongly, because the operational sex ratio was not female-biased enough, because they respond to different cues, or because OSR does not affect

female mating behaviour. One explanation could be that although mating competition affects the level of competition within a sex, the actual switch in sex roles seen in the field may not occur at equal OSR, because other factors influence when this switch happens (Kokko & Monaghan 2001, Clark & Grant 2010).

Reproductive success in the wild (Paper IV)

In Paper IV, we investigate how male reproductive success varies over the season in the two-spotted goby. We placed ten artificial nests out in the field at one location in 2007, and forty-five artificial nests of different sizes at each of two locations in 2008. We marked attending males at several occasions and inspected all nests for eggs and an attending male at regular intervals. We found that early in the season attending males were larger than late in the season in 2008, and in better condition in 2007. Early in the season, nest holders were also in better condition than shoaling males in 2007 (no data for 2008). We only found evidence for nest take-overs early in the season in 2008 (no data for 2007). These data show that competition for nests may be an important factor in mating success early in the season, when male density is high (Forsgren et al. 2004).

In 2008, we also estimated brood size, brood survival (if a brood potentially hatched) and male reproductive success (the total area of potentially hatched eggs). Both brood survival and male reproductive success was lower late than early in the season, and we saw more dead eggs in the nest (Figure 11). It may thus be advantageous to breed early in the season for two-spotted gobies, as is the case for many temperate species (Fairbairn 1977, Hendersson et al. 1988). In contrast, we expected brood size to be larger late in the season, when females are competing for males (Forsgren et al. 2004). Although we did find that males received more female visits late than early in the season, brood size was smaller late than early in the season of 2008, and nest fullness did not differ between the different parts of the season in both years. This may partly reflect a higher brood reduction late in the season, because we did not inspect nests daily. However, in 1999, nest fullness was higher late in the season (Forsgren et al. 2004). Thus, there may be variation in years in differences in mating success between early and late in the season.

Brood size was correlated to nest size over the whole season. Because nests are on average only half-full, I think that this is more likely to be due to female choice for large nests than due to limited nest space. Early in the season, males in large nests were significantly larger than males in smaller nests in 2008. Such a correlation could be caused by competition for the larger nests (Lindström 1992, Andersson 1994, Wong et al. 2008), or by small males actively preferring smaller nests

(Kvarnemo 1995, Takegaki et al. 2008). The difference in size of attendant males between smaller and larger nests disappeared at mid season, suggesting that males were competing for large nests. However, not only the larger nests were occupied late in the season, when many nests were empty, suggesting that not all males prefer large nests.

Within the smaller nests, male size was correlated to brood size, but only early in the season. This suggests that the female preference for larger males, found in a laboratory experiment (Borg et al. 2006), is realized mating success in the field. Late in the season, females did not prefer large males (Borg et al. 2006), which also corresponds to the findings of Paper IV, although our sample size was low late in the season, so we may not have had enough power to detect an effect. Male reproductive success, as the area of potentially hatched eggs, was higher early than late in the season, but was not significantly associated with nest size or male size.

Overall, these data suggest that males compete for breeding opportunities early in the season, and that they compete for nests and mates at the same time (Forsgren et al. 2004). Nest size is likely to be the most important factor in mating competition in both parts of the season. Possibly as a result of this competition, high quality males breed early and lower quality males breeding late, when competition is relaxed. This should be considered when asking questions about effects on male and female behaviour in this species, because it would mean that there is a substantial decrease in reproductive success over the season for both sexes.
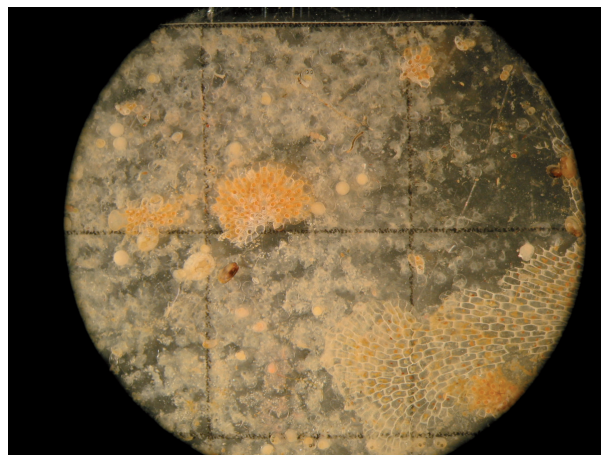
Figure 11. Picture of a brood just after hatching that was attended by a two-spotted goby male in the late part of the breeding season in 2007. (gridlines are 1 cm apart) Note that many of the eggs are dead (opaque) and there are patches of on-growth in between the eggs. Image: Karen de Jong.

Operational sex ratio and encounter rates (Paper I, III, IV)

The relation between OSR and encounter rates is likely not so straight forward as we simplistically assumed in Paper II, because mate search behaviour of both sexes may depend on density (Kokko & Wong 2007). This increases the urgency of disentangling the effects of OSR on encounter rates and the propensity to behave. It may also explain the different effects of OSR on courtship frequency between our constant visual contact design (Paper I) and our free interaction design (Paper III), because the different designs may have led to different encounter patterns. In paper I, all individuals were in constant visual contact, preventing the measurement of encounter rates. In paper III, unexpectedly, the number of visits by ready-to-mate females to males did not differ between the treatments. This suggests that the ten ready-to-mate females in the female biased treatment visited males less often than the two ready-to-mate females in the male biased treatment. Females may become less choosy in female biased OSR (Balshine-Earn 1996, Jirotkul 1999), as was indeed suggested by the finding that females only prefer larger males early in the season, when OSR is male-biased (Borg et al. 2006) and in the field, females visit fewer males per time late than early in the season and terminated fewer courtship events (L. C. Myhre, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). However, males received more female visits late than early in the season in the field (Paper IV), although the difference (0.3 vs. 0.5 per minute) was less than what would be expected from the marked change in OSR (Forsgren et al. 2004).

For male-male encounters, we found that the number of visits from other males decreased over the season in the field (Paper IV), as expected from the decrease in density found previously (Forsgren et al. 2004). However, in paper III, males spend less time in their nest in a male biased OSR and correspondingly engaged in more male-male interactions (Paper III). Thus, also male-male encounter rates may not only depend on male density, but also on mate availability. Importantly, local density and OSR, likely reflected in encounter rates, may have different effects than or interact with population density and OSR (Reichard et al 2008, Casalini et al. 2010).

Sexual selection and the value of OSR

There has recently been some discussion in the literature on the value of OSR to predict the level of mate monopolization and sexual selection (e.g. Shuster 2009, Klug et al. 2010). Therefore I would like to briefly explain my opinion on this issue. First of all, sexual selection is not only affected by mating competition, but also by resource competition, sperm competition and mate choice (Figure 1). These processes may be additive in their effect on behaviour and sexual selection (Alonso-

Pimentel & Papaj 1999), but they could also interact. Nest competition can decrease mating competition in the sex that is competing for resources and decrease competition in the opposite sex (Almada et al. 1995, Forgren et al. 1996, Borg et al. 2002, Heinsohn 2008). Sperm competition can increase the variation in reproductive success that is caused by variation in mating success, or decrease it (Jones et al. 2001). Choosiness could also occur in the competing sex and increase competition for the best mates (Owen & Thompson 1994). Thus, we cannot simply look at the effect of OSR or other population measures on variation in reproductive success, without observing what happens in the population, which traits are important and which processes of sexual selection are in action. This thesis showed that OSR affects male competitive behaviour (Paper III). It also suggests however, that in two-spotted gobies, competition for nests may be important as well, and that mating success of nest holding males is not necessarily higher even if both mating and nest competition should be relaxed (Paper IV).

There is no a priori to expect the degree of mate monopolization to increase with the rate of competitors to potential mates (Klug et al. 2010). If this ratio becomes too high, it may become too costly to defend mates and no individual will be able to defend more than one mate (Kokko et al. 1998, Shuster and Wade 2003, Fitze & leGaillard 2008). The potential for mate monopolization will to a large extend affect behavioural mating competition. Whether an individual is likely to invest in competitive behaviours, depends on the cost-benefit ratio of a certain behaviour. Thus when the potential for mate monopolization is high, individuals are likely to invest in courtship and agonistic behaviour, while when direct competition becomes too costly, individuals may switch to alternative mating tactics (Shine et al. 2003, Jirotkul 1999, Magellan & Maguran 2007). Lower quality individuals may postpone mating until they are either big enough to compete (Warner 1984, Berglund 1991) or competition is less intense (Paper IV).

Operational sex ratio is thus neither a predictor of the strength of sexual selection or a measure of mating competition. However, it gives us a simple competitor-to-resource ratio for mating competition at any given time and place (Kvarnemo & Ahnesjö 1996, 2002, Grant et al. 2000). The total OSR over the whole breeding season is thus not a very useful measure (Shuster 2009). In stead, it should interest us how variations in OSR over time and between populations within species affects competition for mates. This would allow us to test whether and how these differences result in variation in sexual selection on certain traits.

Future directions

OSR and mating competition

This thesis showed OSR affected the propensity to court. To my knowledge, there are only four experimental studies to date that report a measure of courtship propensity (all on fish: Berglund 1994, Borg et al. 2002, Faleiro et al. 2008, Paper III), and only one that quantified encounter rates in the same experiment (Paper III). Hence, there is an urgent need for more studies on the effect of operational sex ratio on the propensity of agonistic and courtship behaviour in a variety of taxa. Depending on patterns of movement and mate search of both sexes, it will be different for each model species what setup would provide the opportunity to test propensities of behaviour. Thus, knowledge of the natural behaviour of a species is needed for the design of such experiments. It may therefore be more complicated to test propensities in the laboratory than the beguilingly simple setup with different numbers of individuals in a simple aquarium. However, as discussed above, interference from such simple designs may be accordingly difficult.

There are very few studies on female mating competition in relation to OSR. Females may compete for males, even in species with conventional sex roles (Clutton-Brock 2007). Regrettably, this thesis has not changed this pattern, because overall, we saw very little competitive behaviour in females compared to males. This may suggest that females respond to different cues than males. Another reason to focus on males in paper III was that females were swimming around together and spent most of their time in the central part of the aquarium. As a result, it was much harder to quantify separate encounters for females in this design. Thus, studies on female behaviour may have to use different designs than studies on male behaviour.

Female mating competition and reproductive success

In paper IV, we show that reproductive success is lower late than early in the season. Therefore it is not surprising that males are competing for early breeding opportunities. However, females are competing for males late in the season. This is interesting, because there should be strong selection on females to breed early as well, especially because our data suggest that brood survival and male quality is higher early in the season. One explanation could be that males and females could use different cues to time the onset of reproduction (Ball & Ketterson 2008), and therefore, variations between years may cause a mismatch between peak mating for males and females. However, female density does not increase over the season (Forsgren et al. 2004). Thus, it is not likely that

females start breeding later than females. A more likely explanation for why the OSR is male biased early in the season could be found in the differential effect of ecological factors on potential reproductive rates of both sexes (Kvarnemo 1994, 1997). Females could be limited in their reproductive rate early in the season by the low temperature and by the availability of food, since in the Gullmar fjord, likely food sources are limited and temperatures are low (< 10 C) in April and early May (Lindahl & Hernroth 1988). In the closely related sand goby, temperature was found to affect the OSR via sex difference in potential reproductive rate (Kvarnemo 1994, 1996). However, OSR was male biased in warmer waters, opposite of what would be expected in the two-spotted goby. It would be interesting to test how potential reproductive rates in males and females of the two-spotted goby change over the season, which ecological factors are involved and how these affect mating competition.

Conclusions

The most important conclusions to be drawn from this thesis are:

1. Operational sex ratio affects male mating competition in the two-spotted goby.

2. Frequencies of competitive behaviours reflect both the motivation of an individual and the number of encounters with potential mates and competitors, and are as such not ideal measurements of mating competition.

3. Spatial behaviour of individuals are affected by the density of both competitors and potential mates, which renders it impossible to predict how frequencies reflect the propensity to behave without knowledge of the encounter rates.

4. Knowledge on the behaviour of study species in the wild is of the utmost importance for the design and interpretation of laboratory experiments.

References

Ahnesjö, I., Forsgren, E. & Kvarnemo C. 2008. Variation in sexual selection in fishes. In: Fish behaviour (ed. Magnhagen, C., Braithwaite, V. A., Forsgren, E & Kapoor, B. G.). Science Publishers, Enfield, NH, pp. 303-335.

Almada, V. C., Gonçalves, E. J., Oliveira, R. F. & Santos, A. J. 1995. Courting females - ecological constraints affect sex-roles in a natural-population of the blenniid fish *Salaria pavo*. Animal Behaviour 49, pp. 1125-1127.

Alonso-Pimentel, H. & Papaj, D. R. 1999. Resource presence and operational sex ratio as determinants of copulation duration in the fly *Rhagoletis juglandis*. Animal Behaviour 57, pp. 1063-1069.

Amundsen, T. 2000. Why are female birds ornamented? Trends in Ecology & Evolution 15, pp. 149-155.

Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. Proceedings of The National Academy of Sciences of the United States of America 98, pp. 13155-13160.

Amundsen, T. & Forsgren, E. 2003. Male preference for colourful females affected by male size in a marine fish. Behavioral Ecology and Sociobiology, 54, pp. 55-64.

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton, NJ.

Andersson, M. 2005. Evolution of classical polyandry: three steps to female emancipation. Ethology 111, pp. 1-23.

Ball G. F. & Ketterson, E. D. 2008. Sex differences in the response to environmental cues regulating seasonal reproduction in birds. Philosophical Transactions Of The Royal Society B. 363, pp. 231-246.

Balshine-Earn, S. 1996. Reproductive rates, operational sex ratios and mate choice in St. Peter's fish. Behavioral Ecology and Sociobiology 39, pp. 107-116.

Bateman, A. J. 1948. Intra-sexual selection in Drosophila. Heredity 2, 349-368.

Berglund, A. 1991. Egg competition in a sex-role reversed pipefish: subdominant females trade reproduction for growth. Evolution 45, pp. 770-774.

Berglund, A. 1994. The operational sex-ratio influences choosiness in a pipefish. Behavioral Ecology 5, pp. 254-258.

Birkhead, T. & Møller, A. P. 1993. Female control of paternity. Trends in Ecology & Evolution 8, 100-104.

Brevik, C. 2007. Reirplassvalg hos tangkutling *Gobiusculus flavescens*. Thesis (MSc), NTNU, Trondheim, Norway.

Borg, Å. A., Forsgren, E. & Magnhagen, C. 2002. Plastic sex-roles in the common goby - the effect of nest availability. Oikos 98, pp. 105-115.

Borg, Å. A., Forsgren, E. & Amundsen, T. 2006. Seasonal change in female choice for male size in the two-spotted goby. Animal Behaviour 72, pp. 763-771.

Casalini, M., Reichard, M. & Smidt, C. 2010. The effect of crowding and density on male mating behaviour in the rose bitterling (*Rhodeus ocellatus*). Behaviour 147, pp. 1035-1050.

Clark, L. & Grant, J. W. A. 2010. Intrasexual competition and courtship in female and male Japanese medaka, Oryzias latipes: effects of operational sex ratio and density. Animal Behaviour 80, pp. 707-712.

Clutton-Brock, T. H. & Parker, G. A. 1992. Potential reproductive rates and the operation of sexual selection. Quarterly Review Of Biology 67, pp. 437-456.

Clutton-Brock, T. H. 2007. Sexual selection in males and females. Science 318, pp. 1882-1885.

Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and the potential reproductive rates of males and females. Nature 351, pp. 58-60.

Colwell, M. A. & Oring, L. W. 1988. Sex-ratios and intrasexual competition for mates in a sex-role reversed shorebird, Wilson phalarope (*Phalaropus tricolor*). Behavioral Ecology and Sociobiology 22, pp. 165-173.

Cratsley, C. K., and S. M. Lewis. 2005. Seasonal variation in mate choice of *Photinus ignitus* fireflies. Ethology 111, pp. 89-100.

Darwin, C. 1859. On the Origin of Species. Cramercy Books, Random House, New York.

Darwin, C. 1871. The descent of Man, and Selection in Relation to Sex. Appleton, New York.

Debuse, V. J., Addison, J. T. & Reynolds, J. D. 1999. The effects of sex ratio on sexual competition in the European lobster. Animal Behaviour 58, pp. 973-981.

de Jong, K., Wacker, S., Amundsen, T. & Forsgren, E. 2009. Do operational sex ratio and density affect mating behaviour? An

experiment on the two-spotted goby. Animal Behaviour 78, pp. 1229-1238.

Emlen, S. T. 1976. Lek organization and mating strategies in the bullfrog. Behavioral Ecology and Sociobiology 1, pp. 283-313.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. Science 197, pp. 215-223.

Enders, M. M. 1993. The effect of male size and operational sex-ratio on male mating success in the common spider-mite, *Tetranychus urticae* Koch (Acari, Tetranychidae). Animal Behaviour 46, pp. 835-846

Endler, J. A. 1980. Natural-selection on color patterns in *Poecilia reticulata*. Evolution 34, pp. 76-91.

Endler, J. A. 1983. Natural and sexual selection on color patterns in Poeciliid fishes. Environmental Biology of Fishes 9, pp. 173-190.

Fairbairn, D. J. 1977. Why breed early? A study of reproductive tactics in *Peromyscus*. Canadian Journal of Zoology 55, pp. 862-871.

Faleiro, F., Narciso, L. & Vicente, L. 2008. Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? Aquaculture 282, pp. 33-40.

Farr, J. A. 1980. Social behavior patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae): an experimental study of the effects of intermale competition, female choice, and sexual selection. Behaviour 74, pp. 38-91

Fitze, P. S. & Le Galliard, J. 2008. Operational sex ratio, sexual conflict and the intensity of sexual selection. Ecology Letters 11, pp. 432-439.

Forsgren, E., Amundsen, T., Borg, Å. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles in a fish. Nature 429, pp. 551-554.

Forsgren, E., Kvarnemo, C. & Lindström, K. 1996. Mode of sexual selection determined by resource abundance in two sand goby populations. Evolution 50, pp. 646-654.

Grant, J. W. A. & Foam, P. E. 2002. Effect of operational sex ratio on female-female versus male-male competitive aggression. Canadian Journal Of Zoology 80, pp. 2242-2246.

Grant, J W. A., Bryant, M. J. & Soos, C. E. 1995. Operational sex-ratio, mediated by synchrony of female arrival, alters the variance of male mating success in Japanese medaka. Animal Behaviour 49, pp. 367-375.

Grant, J. W. A., Gaboury, C. L. & Levitt, H. L. 2000. Competitor-to-resource ratio, a general

formulation of operational sex ratio, as a predictor of competitive aggression in Japanese medaka (Pisces: Oryziidae). Behavioral Ecology 11, pp. 670-675.

Griffiths, S. P. 2002. Retention of visible implant tags in small rockpool fishes. Marine Ecology Progress Series 236, pp. 307-309.

Gwynne, D. T. 1984. Sexual selection and sexual differences in Mormon crickets (Orthoptera: Tettigoniidae, Anabrus simplex). Evolution 38, pp. 1011-1022.

Gwynne, D. T. 1985. Role-reversal in katydids: habitat influences reproductive behavior (Orthoptera: Tettigoniidae, *Metaballus sp.*). Behavioral Ecology and Sociobiology. 16, pp. 355-361.

Gwynne, D. T. 1990. Testing parental investment and the control of sexual selection in katydids: the operational sex ratio. The American Naturalist 136, pp. 474-484.

Gwynne, D. T. 1991. Sexual competition among females: What causes courtship-role reversal?. Trends In Ecology & Evolution 6, pp. 118-121.

Gwynne, D. T. & Simmons, L. W. 1990. Experimental reversal of courtship roles in an insect. Nature 346, pp. 172 - 174.

Guitel, F. 1895. Observations sur les mïurs du *Gobius ruthensparri*. Archives de Zoologie Experimentale et Génerale 3, pp. 63-288.

Head, M. L & Brooks, R. 2006. Sexual coercion and the opportunity for sexual selection in guppies. Animal Behaviour 71, pp. 515-522.

Heinsohn, R. 2008. The ecological basis of unusual sex roles in reverse-dichromatic eclectus parrots. Animal Behaviour 76, pp. 97-103.

Henderson, P. A., Holmes, R. H. A. & Bamber, R. N. 1988. Size-selective overwintering mortality in the sand smelt, *Atherina boyeri* Risso, and its role in population regulation. Journal of Fish Biology 33, pp. 221-233.

Jennions, M. D. & Petrie, M. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews 75, pp. 21-64.

Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. Animal Behaviour 58, pp. 287-294.

Jivoff, P. & Hines, A. H. 1998. Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. Animal Behaviour 55, pp. 589-603.

Jones, A. G., Walker, D., Kvarnemo, C., Lindström, K. & Avise, J. 2001. How cuckoldry can decrease the opportunity for

sexual selection: Data and theory from a genetic parentage analysis of the sand goby, *Pomatoschistus minutus*. Proceedings Of The National Academy Of Sciences Of The United States Of America 98, pp. 9151-9156.

Klug, H., Heuschele, J., Jennions, M. D. & Kokko, H. 2010. The mismeasurement of sexual selection. Journal of evolutionary biology 23, pp. 447-462.

Klug, H., Kontax, C. I., Annis, S. & Vasudevan, N. 2008. Operational sex ratio affects nest tending and aggression in male flagfish *Jordanella floridae* Goode & Bean. Journal of Fish Biology 72, pp. 1295-1305.

Kokko, H. & Jennions, M. D. 2008 Parental investment, sexual selection and sex ratios. Journal of evolutionary biology 21, pp. 919-948.

Kokko, H. & Johnstone, R. 2002. Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. Philosophical Transactions of the Royal Society B. 357, pp. 319-330.

Kokko, H. & Monaghan, P. 2001. Predicting the direction of sexual selection. Ecology Letters 4, pp. 159-165.

Kokko, H. & Rankin, D. J. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philosophical Transactions Of The Royal Society B. 361, pp. 319-334.

Kokko, H. & Wong, B. B. M. 2007. What determines sex roles in mate searching?. Evolution 61, pp. 1162-1175.

Kokko, H., Lindström, J., Alatalo, R. V. & Rintamäki, P. T. 1998. Queuing for territory positions in the lekking black grouse (*Tetrao tetrix*). Behavioral Ecology 9, pp. 376-383.

Krupa, J. J. & Sih, A. 1993. Experimental studies on water strider mating dynamics: spatial variation in density and sex ratio. Behavioral Ecology and Sociobiology 33, pp. 107-120.

Kvarnemo, C. 1994. Temperature differentially affects male and female reproductive rates in the sand goby: consequences for operational sex ratio. Proceedings of the Royal Society B. 256, pp. 151-156.

Kvarnemo, C. 1995. Size-assortative nest choice in the absence of competition in males of the sand goby, *Pomatoschistus minutus*. Environmental Biology of Fishes 43, 233–239.

Kvarnemo, C. 1996. Temperature affects operational sex ratio and intensity of male-male competition: Experimental study of sand gobies, *Pomatoschistus minutus*. Behavioral Ecology 7, pp. 208-212.

Kvarnemo, C. 1997. Food affects the potential reproductive rates of sand goby females but not of males. Behavioral Ecology 8, pp. 605.

Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. Trends In Ecology & Evolution 11, pp. 404-408.

Kvarnemo, C. & Ahnesjö, I. 2002. Operational sex ratios and mating competition. In: Sex ratios: concepts and research methods (ed. Hardy, I. C. W.), Cambridge University Press, Cambridge, pp 366-382.

Kvarnemo, C. & Merilaita, S. 2006. Mating distribution and its temporal dynamics affect operational sex ratio: a simulation study. Biological Journal Of The Linnean Society 89, pp. 551-559.

Kvarnemo, C., Forsgren, E., & Magnhagen, C. 1995. Effects of sex ratio on intra-and inter-sexual behaviour in sand gobies. Animal Behaviour 50, pp. 1455-1461.

Lindahl, O. & Hernroth, L. 1988. Large-scale and long-term variations in the zooplankton community of the Gullmar fjord, Sweden, in relation to advective processes. Marine ecology progress series. Oldendorf 43, pp. 161-171.

Lindström, K. 1992. Female spawning patterns and male mating success in the sand goby, Pomatoschistus minutus. Marine Biology 113, 475–480.

Magellan, K. & Magurran, A. E. 2007. Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. Animal Behaviour 74, pp. 1545-1550.

Malone, J. C., Forrester, G. E. & Steele, M. A. 1999. Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. Journal of Experimental Marine Biology and Ecology 237, pp. 243-253.

Mobley, K. B., Amundsen, T., Forsgren, E., Svensson, P. A. & Jones, A. G. 2009. Multiple mating and a low incidence of cuckoldry for nest-holding males in the two-spotted goby, *Gobiusculus flavescens.* BMC Evolutionary Biology 9:6.

Michener, G. R. & McLean, I. G. 1996. Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. Animal Behaviour 52, pp. 743-758.

Miller, P. J. 1986. Gobiidae. In: Fishes of the North-eastern Atlantic and the Mediterranean Volume 3 (ed. Whitehead, P. J. P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J. & Tortonese, E.). UNESCO, Paris, pp. 1019-1085.

Okuda, N. 1999. Sex roles are not always reversed when the potential reproductive rate is higher in females. American Naturalist 153, pp. 540-548.

Owens, I. P. F. & Thompson, D. B. A. 1994. Sex differences, sex ratios and sex roles. Proceedings of the Royal Society B. 258, pp. 93-99.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in insects. Biological reviews of the Cambridge Philosophical Society 45, pp. 525-568.

Parker, G. A. 1974. Assessment strategy and evolution of fighting behavior. Journal of Theoretical Biology 47, pp. 223-243.

Pélabon, C., Borg, Å. A., Bjelvenmark, J., Forsgren, E., Barber, I. & Amundsen, T. 2003. Do male two-spotted gobies prefer large fecund females? Behavioral Ecology 14, pp. 787-792.

Queller, D. C. 1997. Why do females care more than males? Proceedings of the Royal Society B. 264, pp. 1555-1557.

Quinn, T. P., Adkison, M. D. & Ward, M. B. 1996. Behavioral tactics of male sockeye salmon (Oncorhynchus nerka) under varying operational sex ratios. Ethology 102, pp. 304–322.

Reichard, M., Smidt, C. & Bryja, J. 2008. Seasonal change in the opportunity for sexual selection. Molecular Ecology 17, pp. 642-651.

Shibata, J. & Kohda, M. 2006. Seasonal sex role changes in the Blenniid *Petroscirtes breviceps*, a nest brooder with paternal care. Journal of Fish Biology 69, pp. 203-214.

Shine, R., Langkilde, T. & Mason, R. T. 2003. The opportunistic serpent: Male garter snakes adjust courtship tactics to mating opportunities. Behaviour 140, pp. 1509-1526.

Silva, K., Vieira, M. N., Almada, V. C. &. Monteiro, N. M. 2010. Reversing sex role reversal: compete only when you must. Animal Behaviour 79, pp. 885-893.

Shuster, S. M. 2009. Sexual selection and mating systems. Proceedings of the National Academy of Sciences 106, pp. 10009-10016.

Shuster, S., & Wade, M. 2003. Mating Systems and Strategies. Princeton Univercity Press, Princeton, NJ.

Simmons, L. & Bailey, J. W. 1990. Resource influenced sex roles of zaprochiline tettigoniids (Orthoptera: Tettigoniidae). Evolution 44, pp. 1853-1868.

Skolbekken, R. & Utne-Palm, A. C. 2001. Parental investment of male two-spotted goby, *Gobiusculus flavescens* (Fabricius). Journal of Experimental Marine Biology and Ecology 261, pp. 137-157.

Smith, C. C. 2007. Independent effects of male and female density on sexual harassment, female fitness, and male competition for mates in the western mosquitofish Gambusia affinis. Behavioral Ecology and Sociobiology 61, pp. 1349-1358.

Svensson, P. A. 2006. Female coloration, egg carotenoids and reproductive success: gobies as a model system. Thesis (PhD), NTNU, Trondheim, Norway.

Svensson P. A., Barber, I. & Forsgren, E. 2000. Shoaling behaviour of the two-spotted goby. Journal of Fish Biology 56, pp. 1477-1487.

Svensson, P. A., Pélabon, C., Blount, J. D., Surai, P. F. & Amundsen, T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens, Gobiidae*)? Functional Ecology 20, pp. 689-698.

Svensson, P. A., Blount, J. D., Forsgren, E., & Amundsen, T. 2009. Female ornamentation and egg carotenoids of six sympatric gobies. Journal of Fish Biology 75, pp. 2777-2787.

Tagegaki, T., Matsumoto, Y., Tawa, A., Miyano, T. & Natsukari, Y. 2008. Size-assortative nest preference in a paternal brooding blenny *Rhabdoblennius ellipes* (Jordan & Starks). Journal of Fish Biology 72, pp. 93-102.

Takahashi, D. 2000. Conventional sex roles in an amphidromous *Rhinogobius* goby in which females exhibit nuptial coloration. Ichthyology Research 47, pp. 303-306.

Tejedo, M. 1988. Fighting for females in the toad bufo-calamita is affected by the operational sex-ratio. Animal Behaviour 36, pp. 1765-1769.

Thomas, L. K. & Manica, A. 2005. Intrasexual competition and mate choice in assassin bugs with uniparental male and female care. Animal Behaviour 69, pp. 275-281.

Trivers, R. L. 1972. Parental investment and sexual selection. In: Sexual Selection and the Descent of Man 1871-1971 (ed. B. Campbell). Heinemann, London, pp. 136-179.

Verrell, P. A. 1983. The influence of the ambient sex-ratio and intermale competition on the sexual-behavior of the red-spotted newt, *Notophthalmus viridescens* (Amphibia, urodela, salamandridae). Behavioral Ecology and Sociobiology 13, pp. 307-313.

Vincent, A. C. J., Ahnesjö, I., Berglund, A. & Rosenqvist, G. 1992. Pipefishes and seahorses - are they all sex-role reversed. Trends in Ecology and Evolution 7, pp. 237-241.

Ward, G. & Fitzgerald, G. J. 1988. Effects of sex-ratio on male-behaviour and reproductive success in a field population of threespine sticklebacks (*Gasterosteus aculeatus*) (Pisces, gasterosteidae). Journal of Zoology (London) 215, pp. 597-610.

Warner, R. R. 1984. Deferred reproduction as a response to sexual selection in a coral reef fish: a test of the life historical consequences. Evolution 38, pp. 148-162.

Whiteman, E. A. & Côté, I. M. 2004. Dominance hierarchies in group-living cleaning gobies: causes and foraging consequences. Animal Behaviour 67, pp. 239-247.

Wong B. B. M., Lehtonen, T. & Lindström, K. 2008. Male nest choice in sand gobies, *Pomatoschistus minutus*. Ethology 114, pp. 575-581.

Paper I

Do operational sex ratio and density affect mating behaviour? An experiment on the two-spotted goby

Karen de Jong [a,*], Sebastian Wacker [a,b,1], Trond Amundsen [a], Elisabet Forsgren [a,c,2]

[a] *Department of Biology, Faculty of Natural Sciences and Technology, Norwegian University of Science and Technology, Trondheim*
[b] *Department of Behavioural Biology, University of Münster, Germany*
[c] *Norwegian Institute for Nature Research, Trondheim*

Males generally compete for females through courtship and aggression (conventional sex roles). However, sex roles are reversed in some species and can even vary between populations or over time within a single species. The operational sex ratio (OSR), the sex ratio of ready-to-mate individuals, is a central factor proposed to explain the strength and direction of mating competition. Density can both act as a cue for OSR and have its own effects on competition. In the two-spotted goby, *Gobiusculus flavescens*, a seasonal switch in sex roles coincides with a change in OSR from male to female biased and a decrease in male density. This dynamic system provides an ideal opportunity to test the effects of OSR and density on competitive behaviour experimentally. In an aquarium experiment, we created two different sex ratios at two different densities and observed courtship and agonistic behaviour in both sexes. We changed sex ratio to equality after 4 days of treatment, to test for a lag in the behavioural response. Contrary to our predictions, males courted more after prolonged exposure to a female-biased sex ratio, although we also found a tendency for a positive effect of male density on male courtship frequency. We did not find any other effects of treatment on either male or female behaviour. Furthermore, we did not find evidence for a lag in the response to a change in density or sex ratio. We conclude that OSR and density did not affect individual frequencies of behaviour in the expected direction.

© 2009 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Sexual selection drives the evolution of many striking traits and can conflict with natural selection (Darwin 1871). One of the driving forces behind sexual selection is the competition for mating opportunities (Darwin 1871; Andersson 1994). Mating competition is generally stronger in one sex than in the other, leading to fixed sex roles. These can be either conventional, where males compete for females, or reversed, where females compete for males (Trivers 1972; Andersson 1994). In some species, mating competition varies over time or between populations and dynamic sex roles have been found (insects: Gwynne 1984, 1985; fishes: Forsgren et al. 2004; Shibata & Kohda 2006). Such systems provide unique opportunities to study the factors affecting the strength of mating competition in both sexes.

It is generally agreed that selection pressures promoting competition are similar for both sexes. Mating competition arises if the availability of the opposite sex limits the production of offspring (Emlen & Oring 1977; Clutton-Brock & Vincent 1991). The level of competition in each sex should therefore depend on the ratio of ready-to-mate males to ready-to-mate females, known as the operational sex ratio (OSR; Emlen 1976; Emlen & Oring 1977). The OSR is modified by two principal factors: first, the adult sex ratio (ASR) or, more precisely, the sex ratio of qualified-to-mate individuals in a population (Ahnesjö et al. 2001) and second, the sex difference in potential reproductive rate (PRR; Clutton-Brock & Vincent 1991), defined as the maximum number of offspring that an average parent can produce per unit time if access to mates was not limited (Clutton-Brock & Vincent 1991; Clutton-Brock & Parker 1992). The higher the PRR, the sooner an individual is ready to mate again. The ASR and PRR in their turn could be influenced by differences in parental investment (Trivers 1972) and costs of reproduction (Kokko & Monaghan 2001), although a difference in relative costs of reproduction between the sexes could also affect competition directly (Trivers 1972; Emlen & Oring 1977; Kokko & Monaghan 2001).

Same-sex and opposite-sex densities are inherently correlated with OSR and may thus be used as a cue to changes in OSR, and

* Correspondence: K. de Jong, Department of Biology, Faculty of Natural Sciences and Technology, Norwegian University of Science and Technology, 7491 Trondheim, Norway.
E-mail address: karen.de.jong@bio.ntnu.no (K. de Jong).
[1] S. Wacker is currently at the Department of Biology, Faculty of Natural Sciences and Technology, Norwegian University of Science and Technology, 7491 Trondheim, Norway.
[2] E. Forsgren is currently at the Norwegian Institute for Nature Research (NINA), 7485 Trondheim, Norway.

hence which behaviour to adopt. On the other hand, same-sex density could also affect behaviour directly, for example by increasing the level of competition simply by increasing the encounter rate of competitors, while opposite-sex density could, for example, lead to the aggregation of potential mates, affecting the opportunity for monopolization (Kokko & Rankin 2006). It is therefore interesting to distinguish between the effects of OSR and density of each of the sexes on mating behaviour. Teasing apart these effects can be difficult, but can be achieved statistically as an interaction effect of OSR and total density.

The OSR correctly predicts existing sex roles in several species (e.g. Vincent et al. 1994; Thomas & Manica 2005; Sogabe & Yanagisawa 2007). Furthermore, field studies have revealed that OSR correlates well with changes in the strength and direction of mating competition and associated behaviours within species in a wide range of taxa, such as birds (Colwell & Oring 1988), fish (Vincent et al. 1994; Borg et al. 2002; Forsgren et al. 2004) and rodents (Schwagmeyer & Brown 1983; but see Davis & Murie 1985; Michener & McLean 1996). While these field studies clearly show a link between OSR and competitive behaviour, causality is often easier to demonstrate in laboratory manipulations, especially when there are confounding factors that may affect behaviour and covary with OSR, such as food availability (Kim & Choe 2003; Kolluru et al. 2007), time of season (Goldman et al. 2004), temperature (Denoël et al. 2005; Silva et al. 2007) and density of same-sex or opposite-sex individuals (Spence & Smith 2005; Smith 2007).

Until now, surprisingly few laboratory studies have investigated the effect of OSR on competitive behaviour while controlling for confounding factors, and the results are inconsistent (e.g. Kvarnemo et al. 1995; Grant et al. 2000; Spence & Smith 2005; Head et al. 2008). A potential explanation for the variation in these results may be that different species react differently to a change in the OSR. For example, males can resort to more sneaking and less courtship in highly competitive situations, as shown in, for example, the garter snake, *Thamnophis sirtalis parietalis* (Shine et al. 2003) and the Amur bitterling, *Rhodeus sericeus* (Mills & Reynolds 2003). A combination of field and laboratory studies in a range of species would thus be needed to assess whether these are differences between species or between situations.

Most empirical work on mating competition has focused on the more competitive sex (but see Kvarnemo et al. 1995), justifiably, because the behavioural changes in the dominant sex can be expected to be more obvious and easier to test. However, OSR theory explains the behaviour of both sexes by the same processes and changes in sex roles may depend on an interaction between the behavioural changes in both sexes. Additionally, the behaviour of one sex could be strongly related to the behaviour of the other sex. Hence, there is a need for studies that analyse behavioural responses simultaneously in the two sexes.

What cues animals use to assess OSR is not known, nor whether there is a time lag in the behavioural response to a change in the OSR. In the field, local OSR may fluctuate on an hourly basis, while the overall population OSR may be fixed or change more slowly, rendering it advantageous to delay the response to a sudden change in OSR or to adopt Bayesian updating (McNamara & Houston 1980; Stephens & Krebs 1986). Such a lag could make it harder to find effects of experimental treatments, especially when the treatment time is short or when OSR changes during treatment. Such a change in OSR could, for instance, occur if free interaction between the sexes results in mating, removing one or both mated individuals from the pool of ready-to-mate individuals.

Two-spotted gobies, *Gobiusculus flavescens*, are an excellent model system for the study of factors controlling the strength of mating competition. Unlike in most species, sex roles are not fixed. Instead, they switch from conventional to reversed during

a breeding season, coinciding with, although slightly lagging behind, a change in OSR from male to female biased, caused by a dramatic decrease in male density (Forsgren et al. 2004). This suggests an unusual flexibility in the behavioural patterns of both sexes, possibly caused by changes in OSR or male density. Furthermore, both males and females have ornaments, indicating that sexual selection is acting on both sexes (e.g. Amundsen & Forsgren 2001; Svensson et al. 2009). In this study we experimentally tested the effects of OSR and density on competitive behaviour (courtship and aggression) in both sexes and over several days. A previous field study found that males showed more courtship and agonistic behaviour in a high male density, male-biased situation, while females showed more courtship and agonistic behaviour in a low male density, female-biased situation (Forsgren et al. 2004). Therefore, depending on which of the cues they react to, we expected males to compete more in either high male density or male-biased treatments or both, and females to compete more in low male density or female-biased treatments or both. Additionally, we tested for a lag in the behavioural response to a subsequent change in sex ratio. To our knowledge, this is the first study to explore the effects of OSR on competitive behaviour in a laboratory setting in a species where a change in behaviour with OSR has been found in the field.

METHODS

Model Species

The two-spotted goby is a small marine fish, common along the coast of Europe from northwest Spain to northern Norway. It typically lives only a year. During its breeding season (May–July in our study area) it inhabits shallow waters along rocky shores, where nonbreeding individuals often form semipelagic foraging shoals (Miller 1986; Svensson et al. 2000). Breeding males defend a nest in empty blue mussel, *Mytilus edulis*, shells, in crevices, or in brown algae (*Saccharina latissima* and *Laminaria digitata*). Females visit males to spawn, and the male provides all parental care, defending the nest and cleaning and aerating the eggs until they hatch (Skolbekken & Utne-Palm 2001). Males care for clutches of up to six different females simultaneously (Mobley et al. 2009). Females generally lay all eggs of one clutch in the same nest (Skolbekken & Utne-Palm 2001). Both males and females have ornaments, which they emphasize in courtship. Males have coloured fins and an iridescent blue pattern on their body, whereas females develop bright orange bellies during egg development (Amundsen & Forsgren 2001; Svensson et al. 2006).

Experimental Design

Our experiment ran from May until July 2006 at the The Sven Lovén Centre for Marine Sciences at Kristineberg, situated on the west coast of Sweden (58.15°N, 11.27°E). Treatments were designed to investigate OSR and density effects on mating behaviour. We created two different sex ratios at two different densities, resulting in four treatments: 8:4, 4:2, 4:8 and 2:4 males:females adult sex ratio (Table 1). Treatments lasted for 4 days. After 4 days, we exposed two males and two females from each treatment to an equal sex ratio treatment (2:2) for another day, to test for a potential lag in the response to the social environment, that is, whether the response to the previous treatment was preserved.

Fish Collection and Husbandry

All fish were caught by snorkellers with hand-held dip nets around islands up to 3 km from the research station. We stored the

Table 1
Experimental treatments and the resulting densities and sex ratios

Treatment	8:4	4:2	4:8	2:4
Number of males	8	4	4	2
Number of females	4	2	8	4
Total density	High	Low	High	Low
Male density	High	Medium	Medium	Low
Female density	Medium	Low	High	Medium
Sex ratio	M-biased	M-biased	F-biased	F-biased
M/(M+F)	0.66	0.66	0.33	0.33

M: male; F: female.

sexes separately in aquaria of approximately 60 litres. Individual aquaria held between 10 and 40 fish. Males went through a screening procedure (see Selection of experimental fish) after a maximum of 12 h in the storage aquaria, while females stayed in the storage aquaria until the start of the experiment (maximum 3 days). We measured total length of all fish on a millimetre grid to the nearest 0.5 mm and weighed them on digital Mettler scales to the nearest 1 mg. To remove excess water before weighing, we held each fish on a moist paper before releasing it gently into a pre-weighed cup containing sea water. All aquaria had a layer of 1–2 cm of gravel on the bottom and artificial plants to provide cover. We provided an uninterrupted flow of surface water (7 m depth). Temperature therefore reflected ambient sea water temperature (range 10 °C in May to 23 °C in July). We measured the temperature in the experimental aquaria once a day. Light conditions followed a typical Swedish summer with 16 h light and 8 h dark. In the experimental room there was natural light from windows, assisted by some spotlights in darker areas of the room. After completion of the experiment, we used the focal fish for a separate study and released all others back into the fjord.

Selection of Experimental Fish

To make sure we used only ready-to-mate individuals, we selected ready-to-spawn females by the roundness of their belly, an indicator of gonad maturity, and screened males for willingness to court. Screening aquaria (25 × 30 cm and 30 cm deep) were equipped with a PVC tube (length 80 mm, diameter 14 mm) attached to a plastic plant, as nest substrate. Males were allowed to acclimatize for 12–24 h, after which we introduced a free-swimming, round female into the screening aquarium. We used all males that showed any courtship in the first 5 min after they spotted the female. If a male did not court in the first trial, he was retested a maximum of three times.

To ensure a selection of sizes that mimics a natural distribution, we selected sets of fish to fit four size classes, matching quartiles of the frequency distribution in the field. Within each size class we then divided the fish randomly over the different treatments. For the treatments with eight same-sex individuals we used two fish per size class. For the treatments with four individuals we used one fish per size class and for the treatments with only two individuals, we selected fish from size classes 2 and 3. Because it proved difficult to find individuals of the exact right size for every replicate, the size classes slightly overlapped (females 1: 36.5–40.0 mm; 2: 40.0–43.0 mm; 3: 42.0–45.5 mm; 4: 45.0–50.0 mm; males 1: 35.0–40.5 mm; 2: 39.5–42.5 mm; 3: 42.0–45.5 mm; 4: 45.0–52.0 mm). However, the size difference between fish of adjoining classes was never less than 0.5 mm within a replicate. For the equal sex ratio period, we used the two focal fish of the medium size classes (2 and 3) from each treatment for each sex. The two focal males stayed in their compartment, while we removed all other males and their nests, but not the plants and stones. We replaced the females in the

adjoining compartment by two new females. The two focal females were moved to aquaria with two new males.

To recognize fish individually, we marked each fish with visible implant elastomer (Northwest Marine Technology TM, www.nmt.us). We used four colours, red, green, yellow and pink, and injected it subcutaneously at one of two positions, to obtain eight unique combinations. We anaesthetized the fish with 60 µl of 2-phenoxy-ethanol diluted in 500 ml of surface sea water and, after the elastomer injection, each fish was transferred to fresh sea water. All fish regained normal swimming behaviour within 10 min after this procedure. This method of tagging has been shown not to influence mortality or behaviour in other gobies (Malone et al. 1999; Griffiths 2002; Whiteman & Côté 2004).

Experimental Aquaria

Experimental aquaria had a water volume of 60 × 38 × 30 cm. To keep the OSR constant during the treatments we prevented the fish from spawning by dividing the aquaria into male (40 × 38 cm and 30 cm deep) and female (20 × 38 cm and 30 cm deep) compartments with a transparent divider, perforated to allow water flow (and thus chemical communication). Male compartments had as many nests as males to minimize nest competition. The nests were attached either just above the gravel to stones or to artificial plants at approximately 10 cm height (four plants and four stones per compartment). Thus, we created eight potential nest sites at two different heights. We distributed the available nests evenly over these sites, to maximize the distance to the next nest and the aquarium wall, thus minimizing competition over space. This resulted in a minimum distance of about 15 cm between nests in our highest density treatment, which is not unusual in the field (personal observations), although the overall density in the treatment was relatively high. Female compartments contained two artificial plants to provide shelter. We allowed the fish to acclimatize to both the treatment situation and the equal sex ratio situation for 12 h while a blind covered the transparent divider (allowing neither water flow nor visual contact between the sexes), plus a further 6 h with only the transparent divider (as per experimental conditions) before observation.

Behavioural Observations

Two observers observed courtship and agonistic behaviour in both sexes simultaneously, on the first day of treatment, the fourth day of treatment and on the fifth day in the equal sex ratio. We observed four focal fish of each sex sequentially for 10 min each, adding up to 40 min total observation time per sex in each aquarium. In treatments with eight fish of one sex, we randomly chose one fish in each of the four size classes to observe. In treatments with only two fish of one sex, we observed these two individuals twice to standardize the total observation time per aquarium.

We recorded five types of courtship behaviour and three types of agonistic behaviour for males. A male typically starts courtship by erecting his dorsal fins (fin display). This can be followed by vibrating his body perpendicular to the female (quiver), and swimming to the nest waving his tail in a very characteristic manner (lead swim) often entering it (nest entry) before he returns to the female (Amundsen & Forsgren 2001; Pélabon et al. 2003). This sequence of behaviours can be (partially) repeated several times. In some cases it is preceded by a fast swim towards the female (approach). Male agonistic behaviour most often starts with a fin display while the bodies of the two males involved are aligned; after this, one of the males may either chase or attack the other male.

We recorded two types of courtship and response behaviours and three types of agonistic behaviours for females. Female courtship typically consists of following the male (along the divider in our set-up) followed by bending her body in an S-shape, presenting her orange belly to the male (sigmoid; Amundsen & Forsgren 2001; Borg et al. 2006). Female agonistic behaviour is much more subtle than male agonistic behaviour (Forsgren et al. 2004). A sigmoid display to another female is relatively clear, but a chase is often very slow and an attack resembles a push. For females, we also noted the number of male courtship bouts (sequences of male courtship behaviours) she received.

We scan-sampled the location of the focal fish every 10 s as in one of three zones. Males could be in (1) the female association area less then 5 cm from the female compartment, (2) less than a fish length from his nest, or (3) somewhere else. Females could be (1) moving up and down along the divider to the male compartment, which they often did continuously before, during and after courtship, (2) in the male association area less then 5 cm from the male compartment without moving up and down or (3) somewhere else. Any male that entered a nest during an observation or was scan-sampled at the same nest more than nine times, which was the observed median number of scans at any nest per male, was assigned nest-holder status for that day.

Actual OSR in Treatments

Since our experiment was designed to ensure that all individuals would be both qualified and ready to mate, males were provided with a nest site each. Moreover, we prevented the fish from mating and fed them in excess to avoid time out. However, it could be argued that males that did not take up nests were not qualified to mate and should not be considered when calculating OSR. We did not take this rigid course, because the number of nest holders could be higher than detected in our 10 min observations ($\overline{X} \pm SD = 53.5 \pm 3.1\%$), and because in the field males often do not establish a nest before a spawning event (personal observations). Rather, we tested whether the percentage of nest-holding males differed significantly between treatments (Kruskal–Wallis test: day 1: $P = 0.41$; day 4: $P = 0.34$; day 5: $P = 0.55$). Thus, a higher density did not lead to a decrease in the proportion of nest holders, indicating that competition for space was not a major reason for males to forgo establishing nests. We are therefore convinced that the

differences in our experimental sex ratios reflected differences in OSR in the same direction and order of magnitude.

Data Analyses

We recorded data for seven replicates of each treatment. An eighth set of replicates was started but not finished and we included only data from the first day of observation in this last set. We replaced diseased or deceased females immediately with females from the same size class. We did not observe replacement fish. Over the 4-day treatment, only four of 144 females needed replacement; one died and three developed fin rot, a decay of the fins caused by a fungus, which commonly occurs in the wild. We discarded two female observations on day 1 and nine on day 4 because the focal fish showed impaired movement from fin rot or because they buried themselves in the gravel. None of these females were transferred to the equal sex ratio treatment afterwards. In the equal sex ratio period, we discarded six of 56 female observations for the same reasons. We did not replace any males, so as not to disturb any territorial relations, but we avoided using them for focal observations if they developed fin rot. This happened very rarely; out of 144 males, one developed fin rot on day 1, one on day 4 and one on day 5. None of the males died during the experiment.

Because the occurrence of most behaviours was significantly correlated, we used principal components analyses (PCA) to produce a reduced number of uncorrelated variables (principal components) using SPSS 16.0 (SPSS Inc., Chicago, IL, U.S.A.). We entered log-transformed frequencies of all behaviours, to reduce the effect of outliers, and arcsine-square-root transformed proportions of the time spent in different areas in the PCA. We applied a varimax rotation and thereafter used all components with an eigenvalue larger than one. We tested for the effects of treatment on behaviour on each day separately, because the loadings for the different variables and the relative order of the components differed between days. For our tests on the differences in courtship and agonistic behaviour between days we added those behaviours that had the highest loadings on the components concerned, and were thus most representative of either courtship or agonistic behaviour, to calculate total courtship and total agonistic frequency.

We used linear mixed models (Bates et al. 2008) in R version 2.7.2 (R Development Core Team 2008) to test for the effects of sex

Table 2
Loadings of male two-spotted goby behaviours on three PCA components

Components	Day 1			Day 4			Day 5 (2:2)		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Courtship behaviour									
Courtship fin display	**0.902**		−0.201	**0.911**			**0.897**	0.242	
Quiver	**0.948**			**0.928**	0.242		**0.942**		
Lead swim	**0.922**			**0.856**	0.304		**0.897**		
Enter nest	**0.753**	0.438	0.232	**0.711**	0.346	0.308	0.311		**0.854**
Approach	0.438	0.303		**0.528**			0.234	**0.760**	
Agonistic behaviour									
Agonistic fin display		**0.778**	−0.215		**0.604**			0.400	**0.533**
Chase	0.219	**0.690**		0.371	**0.704**		0.446	**0.512**	
Attack	0.224	**0.776**			**0.806**			**0.849**	
Time spent in area									
Time in association area	0.314		−0.770	0.383		−0.774	**0.610**	0.346	−0.331
Time at nest			**0.873**	0.256		**0.838**			**0.804**
% Total variance explained	36	21	16	36	18	15	32	20	17

Loadings for eight male behaviours and time in two scan-sampling areas on three components for each of the 3 observation days. Loadings range from 0 to 1. Loadings lower than 0.2 are omitted for the sake of clarity. Some behaviours load on more than one component; the highest loading for each behaviour is given in bold.

ratio and density on mean courtship and agonistic behaviour. We used the scores on each PCA component per individual as the dependent variables. We could not test for the effect of male and female density separately, because they are inherently correlated with sex ratio. Therefore we made a full factorial model with total density and sex ratio as fixed factors, controlling for the effect of relative length (individual total length minus mean total length of same-sex individuals in the aquarium), adding individual, aquarium and observer as random factors. In such a model, a significant interaction between sex ratio and total density would indicate an effect of either male or female density. To test for changes in individual behaviour between days 4 and 5, we only used individuals that we observed on both days and calculated the difference in total courtship frequency and total agonistic frequency between those days for each individual. We then tested whether the mean overall difference in total frequency between days 4 and 5 differed significantly from zero with a t test and we used a mixed model with group as a random factor to test for an effect of treatment sex ratio, density or the interaction on the differences in total frequency. In the mixed models we tested for significance of the effects with a Markov Chain Monte Carlo resampling method and we report the confidence interval (CI), but only if t values were higher than 1.5, below which significance is not to be expected. For all other tests we used SPSS 16.0. All tests were two tailed with a 95% confidence interval.

Ethical Note

All procedures were carried out with permission from the ethical committee in Gothenburg. We did not detect any physical injuries resulting from agonistic behaviour during our experiment. Only one of 152 fish died during our experiment, which is an extremely low mortality rate given the high natural mortality during the mating season in this short-lived species.

RESULTS

Males and females showed all aspects of courtship and aggressive behaviours that have been observed in the field (Forsgren et al. 2004; K. de Jong, unpublished data). Both sexes courted actively with a mean $\pm$ SE of 12.7 ± 1.4 behaviours in 10 min for males and 6.1 ± 0.7 behaviours in 10 min for females. Agonistic behaviours also occurred frequently with an average rate of 4.0 ± 0.3 behaviours in 10 min for males and 0.8 ± 0.2 behaviours in 10 min for females. There were no significant differences between treatments in body length, wet weight at the start of the experiment, or water temperature on any of the days (Kruskal–Wallis test: $P > 0.86$ in all cases).

Male Competitive Behaviour

For males, behavioural patterns for the 3 sampling days were similar. The PCA resulted in three components (Table 2) for all 3 days after varimax rotation. The first component (PC1) contained the highest loadings for courtship behaviours and we call this the courtship component. The second component (PC2) contained the highest loadings for agonistic behaviours and we call this the agonistic component. The third component (PC3) was mainly based on the time spent at the nest and we therefore call this the nest component. Although component loadings for days 1 and 4 (treatment sex ratio) were near identical, the pattern in the PCA for day 5 (equal sex ratio) differed somewhat from the other days. Time in association on day 5 was more closely correlated with the courtship component (PC1) than on other days, suggesting that most of the time in the association area was actually spent courting.

Furthermore, agonistic fin display was more related to the nest component on day 5 than on the previous days, suggesting that more of the aggression took place around the nest. This would also result in more activity around the nest, which could explain the higher loading for nest entries.

Male courtship score (PC1; Fig. 1a, b, c) was not affected by sex ratio ($t = -1.10$, $N = 126$), total density ($t = -1.17$, $N = 126$) or their interaction ($t = 0.55$, $N = 126$) on day 1. On the fourth day of treatment, sex ratio did have a significant effect on average male courtship score (PC1; $t = -3.47$, $N = 111$, CI $= -1.70$ to -0.62), but total density did not ($t = -1.03$, $N = 111$), while the interaction was borderline significant ($t = 1.62$, $N = 111$; CI $= 0.003$ to 1.52). The effect of sex ratio was opposite of expected: males courted more in a female-biased sex ratio than in a male-biased sex ratio. The interaction appeared to be mainly caused by an effect of male density, because only in the highest male density treatment (8:4) did the response differ substantially from what is expected from the model estimates for sex ratio and density alone (expected means for the male courtship score in the four treatments calculated from the model estimates for the intercept and the effects of sex ratio and density: 8:4 $= -0.89$; 4:2 $= -0.56$; 4:8 $= 0.24$; 2:4 $= 0.58$). Thus, high male density tended to have a positive effect on male courtship score. In the equal sex ratio (day 5), no lasting effects of former treatments were found. The effect of sex ratio on male courtship (PC1) disappeared ($t = -0.48$, $N = 55$) and the interaction was not close to significance any more ($t = 0.83$, $N = 55$). There was no effect of previous total density ($t = -0.81$, $N = 55$). Fin displays, quivers and lead swims had the highest loadings on the courtship component on all days and were therefore added to calculate total courtship frequency on days 4 and 5. Although the individuals from the male-biased treatments did seem to increase total courtship frequency when they entered the equal sex ratio, while those from the female-biased treatments did not (Fig. 2a), we found no effect of sex ratio ($t = 0.86$, $N = 53$), density ($t = -0.65$, $N = 53$) or the interaction ($t = 0.32$, $N = 53$) on the differences. This could suggest that the disappearance of the effect of sex ratio on day 5 was caused by a change in group composition, rather than a change in individual behaviour. However, the effect of sex ratio on day 4 remained significant when we tested only those individuals that later went on to the equal sex ratio on day 5 ($t = -2.71$, $N = 53$, CI $= -1.98$ to -0.37). The overall within-individual difference between days 4 and 5 regardless of treatment was not significantly different from zero ($t_{52} = 0.88$, $P = 0.38$).

Mean male agonistic score (PC2; Fig. 1d, e, f) was not affected by sex ratio ($t = 0.81$, $N = 126$), total density ($t = 0.19$, $N = 126$) or the interaction ($t = 0.22$, $N = 126$) on day 1. On day 4, male agonistic behaviour (PC2) was not affected by sex ratio ($t = 0.71$, $N = 111$), total density ($t = -0.26$, $N = 111$) or the interaction ($t = 0.02$, $N = 111$) either. We also found no effect of previous treatments on agonistic behaviour on day 5 (PC2; sex ratio: $t = 1.13$; total density: $t = 0.27$; interaction: $t = -0.25$, $N = 55$). We could not test for differences between days 4 and 5 in individual male agonistic behaviour, because the behaviours with the highest loadings on the agonistic component on all days, attacks and chases, seldom occurred.

The courtship and agonistic frequency of nest holders was not correlated with the distance from their nest to the divider on any of the observation days (day 1: $P > 0.32$ in all treatments; day 4: $P > 0.10$ for all; day 5: $P > 0.15$ for all).

Female Competitive Behaviour

For females, the principal component analysis resulted in two components (Table 3). PC1 had the highest loadings for courtship and response behaviours and time spent in the association area,

Figure 1. (a, b, c) Male courtship and (d, e, f) male agonistic behaviour in the two-spotted goby under different sex ratios on 3 observation days, day 1 ($N = 8$) and day 4 ($N = 7$) in the different sex ratio treatments and day 5 ($N = 7$) in an equal sex ratio. Courtship is expressed as the average score on PC1 per aquarium. Graphs are box plots representing the median and 25% quartiles, lines represent minima and maxima and circles denote outliers. The dashed line is at zero. The PCA is calculated separately for each day and therefore cannot be compared between days.
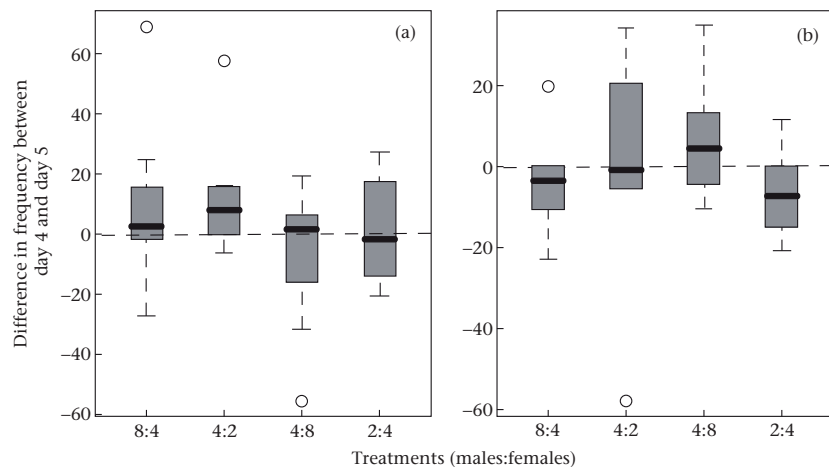
Figure 2. Within-individual difference from day 4 (treatment sex ratio) to day 5 (equal sex ratio) in (a) male total courtship frequency (fin displays + quivers + lead swims) and (b) female total courtship frequency (follows + sigmoids) in the two-spotted goby. Graphs are box plots representing the median and 25% quartiles, lines represent minima and maxima and circles denote outliers. The dashed line is at zero.

Table 3
Loadings of female two-spotted goby behaviours on three PCA components

Components	Day 1		Day 4		Day 5 (2:2)	
	PC1	PC2	PC1	PC2	PC1	PC2
Courtship behaviour						
Follow	**0.728**		**0.706**		**0.759**	
Courtship sigmoid	**0.886**		**0.855**		**0.884**	
Agonistic behaviour						
Agonistic sigmoid	0.375	**0.536**	0.332		**0.652**	
Chase		**0.834**		**0.819**		**0.988**
Attack	−0.207	**0.562**		**0.787**	—[*]	—[*]
Time spent in area						
Time in association area	**0.773**		**0.852**		**0.794**	
Time up and down	**0.873**		**0.860**		**0.905**	
% Total variance explained	41	19	40	19	53	16

Loadings for five female behaviours and time in two scan-sampling areas on 3 components for each of the 3 observation days. Loadings range from 0 to 1. Loadings lower than 0.2 are omitted for the sake of clarity. Some behaviours load on more than one component; the highest loading for each behaviour is given in bold.

[*] Attack could not be entered in the PCA of day 5, because it did not occur in any replicates.

both while moving up and down the divider and otherwise (we call this the courtship component). PC2 had the highest loadings for the more escalated agonistic behaviours and we this the agonistic component. Agonistic sigmoids had the highest loading on PC2 (agonistic behaviour) on day 1, while the highest loadings were on PC1 (courtship) on days 4 and 5.

We found no significant effects of treatment on female courtship score (PC1; Fig. 3a, b, c), on day 1 (sex ratio: $t = -0.69$; total density: $t = -0.94$; interaction: $t = 0.52$, $N = 126$) or day 4 (sex ratio: $t = -0.61$, total density: $t = -0.61$; interaction: $t = 0.69$; $N = 103$). We also did not find any effect of previous treatments on female courtship score (PC1) on day 5 in the equal sex ratio (sex ratio: $t = 0.97$; total density: $t = 1.09$; interaction: $t = -0.51$, $N = 50$). Follows and sigmoids had the highest loadings on the courtship component on all days and were therefore added to calculate total courtship frequency on days 4 and 5. There was no effect of sex ratio ($t = 0.64$, $N = 46$), density ($t = 1.15$, $N = 46$) or the interaction ($t = -1.13$, $N = 46$) on the differences in total courtship frequency between days 4 and 5 within individuals (Fig. 2b). The mean within-individual difference between days 4 and 5 regardless of treatment was not significantly different from zero ($t_{45} = -0.35$, $P = 0.72$).

Mean female agonistic score (PC2; Fig. 3d, e, f) was not significantly affected by treatment on day 1 (sex ratio: $t = -0.68$; total density: $t = 1.09$; interaction: $t = -0.64$, $N = 126$), day 4 (sex ratio: $t = -1.39$; total density: $t = -1.66$, CI $= -1.02$ to 0.08; interaction: $t = 1.15$, $N = 103$) or day 5 (sex ratio: $t = -0.58$; total density: $t = -0.59$; interaction: $t = 0.32$, $N = 50$). Chases had the highest loadings on the agonistic component on all days, but these occurred so rarely that we were not able to test for differences in frequency within individuals between days.

Correlation of Courtship Behaviour between the Sexes

Average male and female courtship scores (PC1) within an aquarium were significantly correlated on day 1 (Spearman rank correlation: $r_S = 0.57$, $N = 32$, $P = 0.001$), but not significantly so on day 4 (Spearman rank correlation: $r_S = 0.33$, $N = 27$, $P = 0.09$). On day 5 (equal sex ratio), male courtship score (PC1) was correlated with the number of received female courtship bouts (Spearman rank correlation: $r_S = 0.43$, $N = 28$, $P = 0.02$). Female courtship score (PC1) was also strongly correlated with the number of received male courtship bouts on all days (Spearman rank

correlation: day 1: $r_S = 0.77$, $N = 32$, $P < 0.001$; day 4: $r_S = 0.77$, $N = 27$, $P < 0.001$; day 5: $r_S = 0.83$, $N = 27$, $P < 0.001$).

DISCUSSION

We predicted that a change in either OSR or same-sex density would lead to an increase in competitive behaviour in the more abundant sex. However, contrary to our predictions, males courted more after prolonged exposure to a female-biased sex ratio, although we also found a tendency for a positive effect of male density on male courtship frequency. Apart from this we did not find any effects of treatment on either male or female behaviour. The effects of sex ratio and male density on male courtship behaviour were not maintained after a 6 h exposure to an equal sex ratio.

Male Competitive Behaviour

Seemingly contrary to predictions from theory (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996), we found that males courted more in female-biased treatments. A potential explanation for the high male courtship frequency in female-biased treatments is that males may have been more stimulated by the sight of many females. However, we did not see an effect of absolute female density on male courtship behaviour, only of the actual adult sex ratio. In a female-biased sex ratio, both the encounter rate and the potential for matings are very high. Males should therefore have much to gain, and may be highly motivated to compete for females, which could potentially explain the higher courtship frequency per individual, although in the field both courtship frequency and propensity to court decrease when females are abundant (Forsgren et al. 2004). High courtship motivation in our experiment may be partly explained by the fact that the sexes were not allowed to mate. However, this restriction would only explain a higher overall frequency, not a difference in frequency between treatments. Other laboratory studies that did allow free interaction between the sexes have also found higher frequencies of certain male courtship behaviours in female-biased sex ratios (Farr 1976; Grant et al. 1995, 2000; Jirotkul 1999; Mills & Reynolds 2003; Shine et al. 2003; Spence & Smith 2005). The authors proposed different valid explanations for their findings, but the overall pattern seems to indicate that, in a laboratory setting, males can increase the frequency of certain courtship behaviours when they encounter more mates. However, courtship frequency may not be directly linked to the level of competition. Theory predicts an increase in competition for mating opportunities with OSR and density, although there are some recent examples to the contrary (Fitze & Le Galliard 2008; Head et al. 2008). Even if competition increases, however, it is questionable whether we should expect an increase in the frequency of all behaviours. In retrospect, we realize that a male's courtship frequency could increase with an increasing number of encountered females, even if his propensity to court each female decreases, simply because there are so many females to court. While the propensity to court each encountered female may reflect his innate tendency to court and thus the strength of competition, frequencies could thus reflect the strength of the stimulus as well as the level of competition. The total courtship frequency would then be modified by both propensity and the number of encounters, rendering it plausible that an increase in competition could result in both an increase in courtship frequency, as in the field (Forsgren et al. 2004), or a decrease in courtship frequency, as in this study. However, we found an increase in courtship frequency not only with sex ratio, but also with male density (as revealed by the borderline significant interaction between sex ratio and total density). This density effect is most easily interpreted as a result of increased competition.
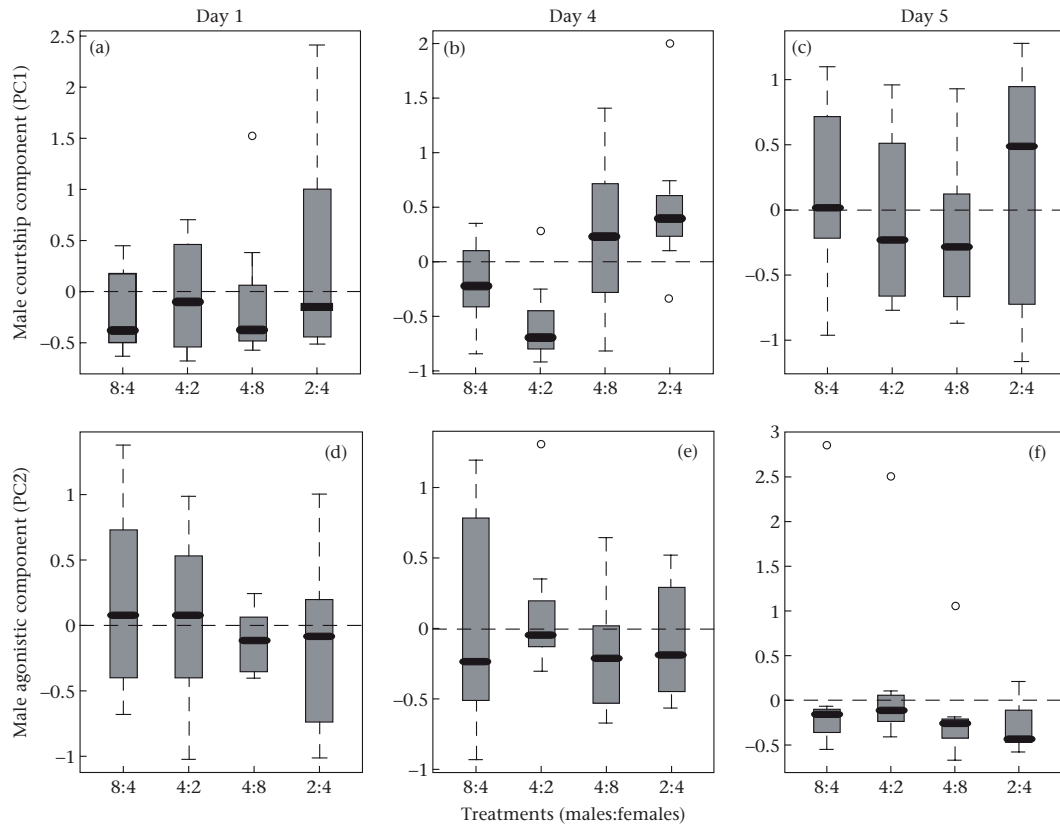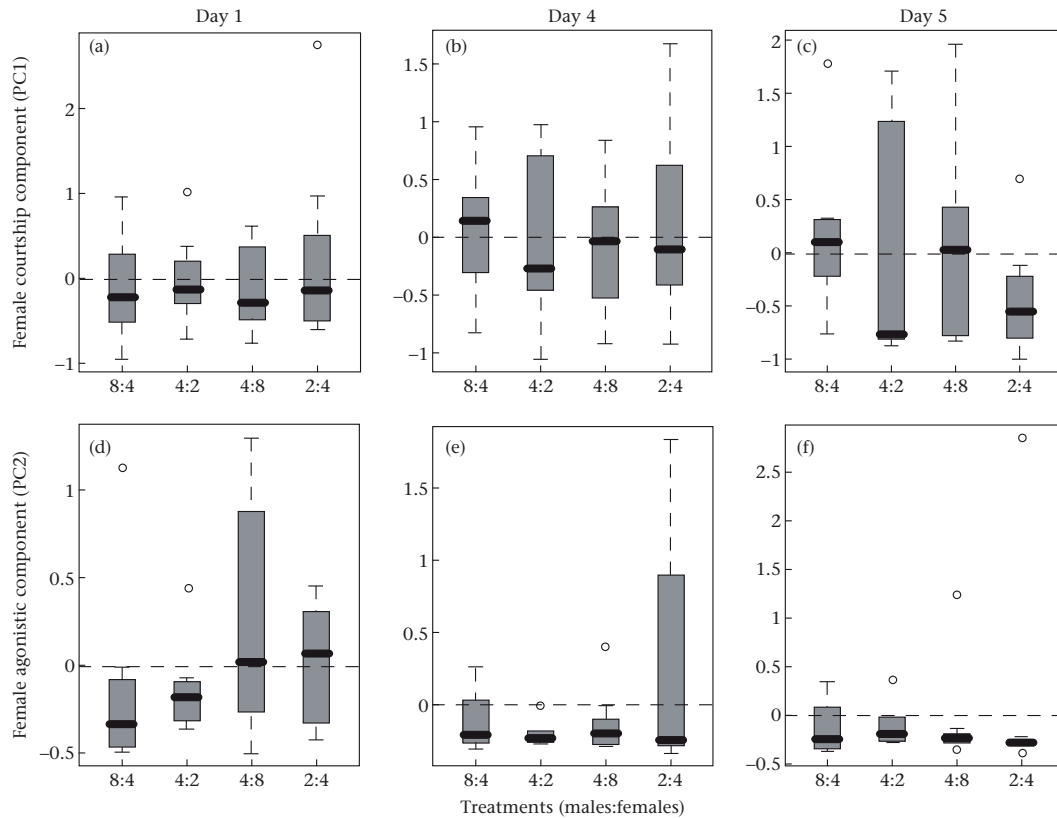
Figure 3. (a, b, c) Female courtship and (d, e, f) female agonistic behaviour in the two-spotted goby under different sex ratios on 3 observation days, day 1 ($N = 8$) and day 4 ($N = 7$) in the different sex ratio treatments and day 5 ($N = 7$) in an equal sex ratio. Courtship is expressed as the average score on PC1 per aquarium. Graphs are box plots representing the median and 25% quartiles, lines represent minima and maxima and circles denote outliers. The dashed line is at zero. The PCA is calculated separately for each day and therefore cannot be compared between days.

We found no effect of OSR or density on male agonistic behaviour. For agonistic behaviours, one would expect total frequencies of agonistic acts per male to show the same pattern as the propensity of each male to behave agonistically at a given male–male encounter. This is because, unlike the case for courtship, an increase in the relative number of male competitors should theoretically lead to an increased propensity to behave agonistically at each encounter, as well as an increase in the frequency of male–male encounters. Male agonistic behaviour can be caused by mating competition, but also by competition for other resources such as nest sites. However, because there were as many nests as males in our experiment and no difference between treatments in the percentage of nest holders, there does not appear to have been any large difference in the level of resource competition between treatments. Moreover, we found no evidence for competition for particular nest positions, as courtship and agonistic behaviours by males were not correlated with the distance of a male's nest from the female compartment.

Female Competitive Behaviour

Female courtship did not differ between treatments in our study. Since the courtship score of each individual female was directly correlated with the number of received male courtship bouts on all days, it is possible that females reacted more to male courtship than to the treatment. However, female courtship has been observed to change with OSR independently of male courtship in a field study on the same species (Forsgren et al. 2004).

There was no effect of treatment on agonistic behaviours among females. However, female aggression in two-spotted gobies may not always be so easy to detect and quantify, since it is far more subtle than male aggression. Agonistic sigmoid displays had the highest loading on the agonistic component on day 1, while the highest loadings were on the courtship component on days 4 and 5. This may indicate a switch from aggression towards unknown females to direct competition for courtship opportunities. Females were often seen to push each other away during courtship (K. de Jong & S. Wacker, personal observations).

Lag in the Behavioural Response

The absence of treatment effects on anything but male courtship behaviour precluded most planned tests of a lag in behavioural response to changes in OSR and density. However, the effect of sex ratio on male courtship frequency we found on day 4 did not appear on day 1. This may suggest that extended exposure to the

treatments was required to elicit the behavioural response. On the other hand, it may also be caused by insufficient acclimatization to experimental conditions on day 1 rather than a lag in the response to a change in sex ratio. The transfer from the field via storage and marking is likely to have a larger effect on the time needed for acclimatization (day 1) than the switch from one sex ratio to another (day 5), especially because males remained in the same aquarium during this last switch. The effect of sex ratio on male courtship on day 4 was not retained on day 5 (equal sex ratio), suggesting that there was no major lag in adjusting to the equal sex ratio. On the other hand, the disappearance of this effect could be caused by a change in group composition, rather than a change in individual behaviour. We did not find a significant change in total courtship frequency within individuals between days 4 and 5, although individuals from the male-biased treatments did seem to increase courtship when they entered the equal sex ratio and those from the female-biased treatments did not (Fig. 2a). However, because the effect of sex ratio on day 4 was significant also when we used only those individuals that would later be used in the equal sex ratio on day 5, we conclude that the disappearance of this effect on day 5 has to be attributed to a change in individual behaviour.

Conclusions

This study adds to a growing body of evidence that the frequencies of different competitive behaviours do not react uniformly to a change in OSR or density. Field studies have generally found a decrease in courtship propensity when the opposite sex is abundant (e.g. Almada et al. 1995; Borg et al. 2002; Forsgren et al. 2004), as predicted by theory. In contrast, laboratory studies, which most often quantify behaviour as frequencies, have produced surprisingly inconsistent results with respect to the effects of OSR and density on competitive behaviour. Results vary from an increase (Farr 1976; Grant et al. 1995, 2000; Jirotkul 1999; Mills & Reynolds 2003; Shine et al. 2003; Spence & Smith 2005) to a decrease (Gwynne 1993) in courtship frequency with relative abundance of the opposite sex and some studies found no effect (Kvarnemo 1995; Magellan & Magurran 2007; Head et al. 2008). This poses the question whether frequencies per individual are the best measure to use when estimating the effects of OSR and density on mating competition. In this study we found a tendency towards increased male courtship frequency with male density, which could indicate increased competition. However, we also found that courtship frequency increased in female-biased treatments, where one would expect the level of competition to be lower (but see Fitze & Le Galliard 2008). Because we do not know whether the increase in courtship frequency is caused by an increase in the propensity of a male to court each female, or by an increase in the number of female encounters per male, we cannot conclude whether OSR affected competition in this set-up. For female courtship, we did not find an effect of treatment. Although the propensity to court may well have increased in the female-biased treatments, any positive effect of the increased propensity on total courtship frequency may have been masked by a negative effect of a decrease in the number of encountered males. Behaviour is generally quantified as frequencies per individual in laboratory studies for reasons of practicality. In most cases the sexes are in constant visual contact and it is therefore impossible to separate individual encounters. However, since there is now some evidence that an excess of potential mates can increase mating competition (Fitze & Le Galliard 2008), it becomes increasingly important to relate the effects of OSR and density on behaviour to their effects on competition. We suggest that propensities may reflect the innate motivation to act of an individual, while frequencies may reflect both the motivation and the strength of the stimulus. Thus,

propensities may be more informative of the strength of competition. Clearly, there is a need for more studies on the effect of OSR and density on mating competition, estimating frequencies and propensities of behaviours in both field and laboratory set-ups for the same species.

References

Ahnesjö, I., Kvarnemo, C. & Merilaita, S. 2001. Using potential reproductive rates to predict mating competition among individuals qualified to mate. *Behavioral Ecology*, **12**, 397–401.

Almada, V. C., Goncalves, E. J., Oliveira, R. F. & Santos, A. J. 1995. Courting females: ecological constraints affect sex roles in a natural population of the blenniid fish *Salaria pavo*. *Animal Behaviour*, **49**, 1125–1127.

Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. *Proceedings of the National Academy of Sciences, U.S.A*, **98**, 13155–13160.

Andersson, M. 1994. *Sexual Selection*. Princeton, New Jersey: Princeton University Press.

Bates, D., Maechler, M. & Dai, B. 2008. *Lme4: Linearb Mixed-effects Models using s4 Classes. R Package Version 0.999375-24.* http://r-forge.r-project.org/projects/lme4.

Borg, Å. A., Forsgren, E. & Magnhagen, C. 2002. Plastic sex-roles in the common goby: the effect of nest availability. *Oikos*, **98**, 105–115.

Borg, Å. A., Forsgren, E. & Amundsen, T. 2006. Seasonal change in female choice for male size in the two-spotted goby. *Animal Behaviour*, **72**, 763–771.

Clutton-Brock, T. H. & Vincent, A. C. J. 1991. Sexual selection and the potential reproductive rates of males and females. *Nature*, **351**, 58–60.

Clutton-Brock, T. H. & Parker, G. A. 1992. Potential reproductive rates and the operation of sexual selection. *Quarterly Review of Biology*, **67**, 437–456.

Colwell, M. A. & Oring, L. W. 1988. Sex-ratios and intrasexual competition for mates in a sex-role reversed shorebird, Wilson phalarope (*Phalaropus tricolor*). *Behavioral Ecology and Sociobiology*, **22**, 165–173.

Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. New York: Appleton.

Davis, L. S. & Murie, J. O. 1985. Male territoriality and the mating system of Richardson ground-squirrels (*Spermophilus richardsonii*). *Journal of Mammalogy*, **66**, 268–279.

Denoël, M., Hector, M. P. & Poncin, P. 2005. Courtship behavior in the alpine newt *Triturus alpestris* at two different densities of males. *Herpetologica*, **61**, 373–379.

Emlen, S. T. 1976. Lek organization and mating strategies in bullfrog. *Behavioral Ecology and Sociobiology*, **1**, 283–313.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215–223.

Farr, J. A. 1976. Social facilitation of male sexual behavior, intra sexual competition, and sexual selection in the guppy, *Poecilia reticulata* (Pisces Poeciliidae). *Evolution*, **30**, 707–717.

Fitze, P. S. & Le Galliard, J. F. 2008. Operational sex ratio, sexual conflict and the intensity of sexual selection. *Ecology Letters*, **11**, 432–439.

Forsgren, E., Amundsen, T., Borg, Å. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles in a fish. *Nature*, **429**, 551–554.

Goldman, B. D., Gwinner, E., Karsch, F. J., Saunders, D., Zucker, I. & Ball, G. F. 2004. Circannual rhythms and photoperiodism. In: *Chronobiology: Biological Timekeeping* (Ed. by J. C. Dunlap, J. J. Loros & P. J. DeCoursey), pp. 107–142. Sunderland, Massachusetts: Sinauer.

Grant, J. W. A., Bryant, M. J. & Soos, C. E. 1995. Operative sex ratio, mediated by synchrony of female arrival, alters the variance of male mating success in Japanese medaka. *Animal Behaviour*, **49**, 367–375.

Grant, J. W. A., Gaboury, C. L. & Levitt, H. L. 2000. Competitor-to-resource ratio, a general formulation of operational sex ratio, as a predictor of competitive aggression in Japanese medaka (Pisces: Oryziidae). *Behavioral Ecology*, **11**, 670–675.

Griffiths, S. P. 2002. Retention of visible implant tags in small rockpool fishes. *Marine Ecology Progress Series*, **236**, 307–309.

Gwynne, D. T. 1984. Sexual selection and sexual differences in mormon crickets (Orthoptera: Tettigoniidae *Anabrus simplex*). *Evolution*, **38**, 1011–1022.

Gwynne, D. T. 1985. Role-reversal in katydids: habitat influences reproductive behavior (Orthoptera: Tettigoniidae *Metaballus sp*). *Behavioral Ecology and Sociobiology*, **16**, 355–362.

Gwynne, D. T. 1993. Food quality controls sexual selection in mormon crickets by altering male mating investment. *Ecology*, **74**, 1406–1413.

Head, M. L., Lindholm, A. K. & Brooks, R. 2008. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. *Evolution*, **62**, 135–144.

Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour*, **58**, 287–294, doi:10.1111/j.1558-5646.2007.00277.x.

Kim, T. W. & Choe, J. C. 2003. The effect of food availability on the semilunar courtship rhythm in the fiddler crab *Uca lactea* (de Haan) (Brachyura: Ocypodidae). *Behavioral Ecology and Sociobiology*, **54**, 210–217.

Kokko, H. & Monaghan, P. 2001. Predicting the direction of sexual selection. *Ecology Letters*, **4**, 159–165.

Kokko, H. & Rankin, D. J. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society*, **361**, 319–334.

Kolluru, G. R., Grether, G. F. & Contreras, H. 2007. Environmental and genetic influences on mating strategies along a replicated food availability gradient in guppies (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology*, **61**, 689–701.

Kvarnemo, C. 1995. Size-assortative nest choice in the absence of competition in males of the sand goby, *Pomatoschistus minutus*. *Environmental Biology of Fishes*, **43**, 233–239.

Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution*, **11**, 404–408.

Kvarnemo, C., Forsgren, E. & Magnhagen, C. 1995. Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. *Animal Behaviour*, **50**, 1455–1461.

McNamara, J. & Houston, A. 1980. The application of statistical decision theory to animal behavior. *Journal of Theoretical Biology*, **85**, 673–690.

Magellan, K. & Magurran, A. E. 2007. Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. *Animal Behaviour*, **74**, 1545–1550.

Malone, J. C., Forrester, G. E. & Steele, M. A. 1999. Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. *Journal of Experimental Marine Biology and Ecology*, **237**, 243–253.

Michener, G. R. & McLean, I. G. 1996. Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. *Animal Behaviour*, **52**, 743–758.

Miller, P. J. 1986. Gobiidae. In: *Fishes of the North-eastern Atlantic and the Mediterranean* (Ed. by P. J. P. Whitehead, M. L. Bauchot, J. C. Hureau, J. Nielsen & E. Tortonese), pp. 1019–1085. Paris: UNESCO.

Mills, S. C. & Reynolds, J. D. 2003. Operational sex ratio and alternative reproductive behaviours in the European bitterling, *Rhodeus sericeus*. *Behavioral Ecology and Sociobiology*, **54**, 98–104.

Mobley, K. B., Amundsen, T., Forsgren, E., Svensson, P. A. & Jones, A. G. 2009. Multiple mating and a low incidence of cuckoldry for nest-holding males in the two-spotted goby, *Gobiusculus flavescens*. *BMC Evolutionary Biology*, **9**, 6.

Pélabon, C., Borg, Å. A., Bjelvenmark, J., Forsgren, E., Barber, I. & Amundsen, T. 2003. Do male two-spotted gobies prefer large fecund females? *Behavioral Ecology*, **14**, 787–792.

R Development Core Team 2008. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Schwagmeyer, P. L. & Brown, C. H. 1983. Factors affecting male-male competition in thirteen-lined ground squirrels. *Behavioral Ecology and Sociology*, **13**, 1–6.

Shibata, J. & Kohda, M. 2006. Seasonal sex role changes in the Blenniid *Petroscirtes breviceps*, a nest brooder with paternal care. *Journal of Fish Biology*, **69**, 203–214.

Shine, R., Langkilde, T. & Mason, R. T. 2003. The opportunistic serpent: male garter snakes adjust courtship tactics to mating opportunities. *Behaviour*, **140**, 1509–1526.

Silva, K., Vieira, M. N., Almada, V. C. & Monteiro, N. M. 2007. The effect of temperature on mate preferences and female-female interactions in *Syngnathus abaster*. *Animal Behaviour*, **74**, 1525–1533.

Skolbekken, R. & Utne-Palm, A. C. 2001. Parental investment of male two-spotted goby, *Gobiusculus flavescens* (Fabricius). *Journal of Experimental Marine Biology and Ecology*, **261**, 137–157.

Smith, C. C. 2007. Independent effects of male and female density on sexual harassment, female fitness, and male competition for mates in the western mosquitofish *Gambusia affinis*. *Behavioral Ecology and Sociobiology*, **61**, 1349–1358, doi:10.1007/s00265-007-0365-7.

Sogabe, A. & Yanagisawa, Y. 2007. Sex-role reversal of a monogamous pipefish without higher potential reproductive rate in females. *Proceedings of the Royal Society B*, **274**, 2959–2963.

Spence, R. & Smith, C. 2005. Male territoriality mediates density and sex ratio effects on oviposition in the zebrafish, *Danio rerio*. *Animal Behaviour*, **69**, 1317–1323.

Stephens, D. W. & Krebs, J. R. 1986. *Foraging Theory*. Princeton, New Jersey: Princeton University Press.

Svensson, P. A., Barber, I. & Forsgren, E. 2000. Shoaling behaviour of the two-spotted goby. *Journal of Fish Biology*, **56**, 1477–1487.

Svensson, P. A., Pélabon, C., Blount, J. D., Surai, P. F. & Amundsen, T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens*, Gobiidae)? *Functional Ecology*, **20**, 689–698.

Svensson, P. A., Pélabon, C., Blount, J. D., Forsgren, E., Bjerkeng, B. & Amundsen, T. 2009. Temporal variability in a multicomponent trait: nuptial coloration of female two-spotted gobies. *Behavioral Ecology*, **20**, 346–353, doi:10.1093/beheco/arn154.

Thomas, L. K. & Manica, A. 2005. Intrasexual competition and mate choice in assassin bugs with uniparental male and female care. *Animal Behaviour*, **69**, 275–281.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871–1971* (Ed. by B. Campbell), pp. 136–179. London: Heinemann.

Vincent, A., Ahnesjö, I. & Berglund, A. 1994. Operational sex ratios and behavioural sex differences in a pipefish population. *Behavioral Ecology and Sociobiology*, **34**, 435–442.

Whiteman, E. A. & Côté, I. M. 2004. Dominance hierarchies in group-living cleaning gobies: causes and foraging consequences. *Animal Behaviour*, **67**, 239–247.

Paper II

Are we measuring mating competition correctly?

Karen de Jong[1], Elisabet Forsgren[2], Hanno Sandvik[1] & Trond Amundsen[1]

[1]Department of Biology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway; [2]The Norwegian Institute for Nature Research (NINA), 7485 Trondheim, Norway

ABSTRACT: Central to sexual selection theory is the question why one sex usually competes more for mates than the other sex. Theory predicts that the abundant sex in the sex ratio of ready-to-mate individuals (OSR) will compete most. In accordance, the OSR matches the strength and direction of mating competition in field populations of several species. However, experimental tests have provided inconclusive results, possibly because experiments generally measure the frequency of courtship and agonistic behaviour. Frequency is affected by the number of potential mates or opponents encountered, as well as by the level of mating competition. The propensity to behave at each encounter, generally used in field studies, should reflect a behavioural response, but is used in very few experiments. In two simple models, we show that courtship frequency can respond differently from courtship propensity to a change in OSR. Agonistic frequency and propensity respond similarly, but not identically, and an increase in frequency could merely reflect an increase in encounters with competitors. In a meta-analysis we show that studies measuring courtship frequency indeed produce different results from studies measuring courtship propensities. We do not claim originality for the idea that encounter rate affects frequencies of behaviour, but stress its importance in the design of future experiments. We provide suggestions how to conclusively test OSR theory.

Introduction

Competition for mates is a major driving force behind sexual selection. The number of mates obtained is generally correlated to reproductive success, particularly in males (Bateman 1948; Arnold 1994), but sometimes also in females (e.g. Jennions & Petrie 2000). Thus, mating success has a direct effect on fitness. Although several models have been proposed to predict the strength of mating competition (reviewed in Ahnesjö et al. 2008, Jennions and Kokko 2010), empirical data are relatively scarce. Behavioral sex roles can provide insight in which sex invests most during the process of competition and suggest which traits are favored. In most species, males compete more for matings than females. This is described as conventional sex roles, compared to reversed sex roles when females compete most (Vincent et al. 1992; Andersson 1994). However, the strength of mating competition can also vary within a sex, both temporally and spatially (e.g. birds: Colwell and Oring 1988; mammals: Michener and McLean 1996; insects: Gwynne 1985; fishes: reviewed in Ahnesjö et al. 2008). This variation can even lead to a switch in sex roles (e.g. Gwynne 1985; Almada et al. 1995; Forsgren et al. 2004; Shibata and Kohda 2006).

A main theory to explain variation in mating competition is that the operational sex ratio (OSR; sex ratio of ready-to-mate individuals; box 1) drives mating competition (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). OSR theory predicts the strength of intra-sexual competition for matings to increase when potential mates become scarce relative to the number of competitors. However, this straightforward and intuitive theory has proven hard to test and empirical results are surprisingly inconsistent (de Jong et al. 2009; table 1). De Jong et al. (2009) pointed out that there is striking difference in findings between field and laboratory studies. In field studies, variations in OSR have largely been found to match variations in the strength of mating competition (Colwell and Oring 1988; Cratsley and Lewis 2005; Lengkeek and Didderen 2006), and to match which sex is the most competitive (Gwynne 1985; Almada et al. 1995; Forsgren et al. 2004; Shibata and Kohda 2006) although a switch in sex roles does not have to occur at an OSR of 0.5 (Okuda 1999; Kokko and Monaghan 2001). The majority of laboratory studies on the other hand, have failed to find a similar match between OSR and mating competition (table 1). If we accept these tests as robust tests of the theory, the data should lead us to question OSR theory, in particular in light of some recent evidence that OSR does not necessarily affect the opportunity for sexual selection (Fitze and le Gaillard 2008; Head et al. 2008). However, the apparent inconsistency between results from the field and from the laboratory could result from a mismatch between theory and empirical tests (de Jong et al. 2009). In this study we will explore this mismatch in two models and test

whether it has affected empirical results with a meta-analysis.

Mismatch between Theory and Practice

Theory predicts increased mating competition when OSR becomes increasingly biased towards the own sex, and mates are therefore scarce (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996). Such an underlying resource conflict (competition) can be resolved in various ways, and theory does naturally not specify exactly how an increase in competition is reflected in specific behaviors. The most common and also most well studied way to resolve competition for matings is through courtship and agonistic behavior (Andersson 1994). Thus, most authors of mating competition experiments to date have taken an increase in competition to mean "more acts of aggression" or "more courtship displays" when potential mates are scarce. Contrary to such a -seemingly-predicted increase, the majority of these experiments find a decrease in courtship frequency, agonistic frequency, or both, when potential mates become scarce. There are several biological reasons why the frequency of agonistic and courtship behavior could decrease, rather than increase, in highly competitive situations. For example, increased risk of injury may lead to a breakdown of agonistic behavior or there may be a trade-off between agonistic and courtship behavior (e.g. Grant et al. 2000, Grant and Foam 2002) or individuals may switch from courtship to alternative tactics, such as sneaking, to obtain mates (e.g. Evans and Magurran 1999; Jirotkul 1999; Shine et al. 2003).

However, the most obvious reason why courtship frequency per individual may decrease when potential mates are scarce does not require a behavioral response and is often overlooked. The frequency of any behavior is affected by the number of opportunities to display the behavior (box 1). This is problematic because a change in OSR will lead to a numerical change in stimuli. For example, a change towards a more male-biased OSR leads to more male-male encounters and fewer male-female encounters. This low male-female encounter rate could result in a lower male courtship frequency, even if a male would be more likely to court each encountered female. In contrast, a change in courtship propensity, the likelihood to court at each encounter, would reflect a change in the behavioral response to a given stimulus (de Jong et al. 2009; box 1).

If a behavioral response is indeed what theory predicts, we expect each individual to be more likely to court at each opportunity if competition is high. Thus, courtship propensity will increase when potential mates become scarce, unless individuals switch to alternative mating behaviors. Courtship frequency on the other hand, could easily decrease when potential mates become scarce, simply because of a decrease in the number of potential mates encountered. Thus, while frequency

and propensity have been used as equally valid measures of mating competition, they can produce opposite results. Using frequency as a measure of the strength of competition is in fact no less problematic for agonistic behavior. Although both the propensity and frequency of agonistic acts should increase in a male-biased OSR, one could find false positives when recording frequencies. Because the encounter rate with competitors will increase as well as the level of competition, the frequency of agonistic behaviors can increase purely due to competitor abundance. Therefore it is impossible to judge if an increase in agonistic frequency is caused by a change in the behavioral response or simply by an increase in the number of encounters with competitors. The logic of the argument why propensity and frequency measures could produce opposite patterns is in its essence rather trivial. Moreover, we do not claim originality for the idea that encounter rates affect frequencies of behavior. However, the fact that the literature in the field has been muddled with confusion on whether empirical data actually support or go against OSR theory shows that the issue is not trivial in practice. At a more general level, our work illustrates the importance of translating model predictions accurately to relevant parameters for empirical tests.

To clarify the difference between frequency and propensity measures, we explored the effect of OSR on both frequency and propensity of courtship and agonistic behavior in two simple graphical models. Furthermore, to test if quantifying behavior as frequency versus propensity affects empirical results, we compared studies that report frequencies and studies that report propensities in a meta-analysis. In the meta-analysis we focused on courtship behavior, because the different measures produce opposite predictions in this case: a scarcity of potential mates, i.e. an OSR that is biased towards the own sex, should lead to an increase in courtship propensity, but a decrease in courtship frequency.

Box 1. Definitions of the terms used

Mate competition: The competition for mates, expected to occur if mates are a limiting resource. Changes in the strength of competition are expected to be expressed in changes in competitive behavior, such as courtship and agonistic behavior.

Operational sex ratio (OSR): The sex ratio of ready-to-mate individuals in a population (males/(males+females)). OSR is expected to affect mate competition, because a change in OSR leads to a change in the availability of potential mates, and competition is expected to increase when potential mates are scarce (Emlen and Oring 1977; Kvarnemo and Ahnesjö 1996)

Competitor-to-resource ratio (CRR): A sex-neutral expression of OSR that allows us to compare studies on male and female behavior (competitors/(potential mates + competitors; sensu Grant et al. 2000). For example, an CRR of 0.9 for females would correspond to an OSR of 0.1 (female bias), whereas an CRR of 0.9 for males would correspond to an OSR of 0.9 (male bias).

Mate encounter rate: The number of potential mates encountered per period of time.

Courtship propensity: The probability that an individual will court any potential mate, which should reflect the motivation to compete for a mate. This can be measured as, for example, the percentage of encountered potential mates courted, or the proportion of courtship events initiated by the individual.

Courtship frequency: The number of courtship displays per individual, generally measured as displays per time or total time spent displaying. This is a compound measure of courtship propensity and mate encounter rate, therefore it will not accurately reflect levels of competition.

Methods

Because a change in OSR affects the ratio of potential mates to competitors in opposite directions for the two sexes, using this variable would complicate a comparison between studies on male and female behavior. We therefore used competitor-to-resource ratio (CRR; competitors/(potential mates + competitors); box 1; sensu Grant et al. 2000), in both our model and our meta-analysis.

Model

We developed two simple graphical models. In the first model we explored the relation between CRR and the frequency and propensity of courtship behavior. We assumed encounter rate to decrease with CCR, because, at a given population density, the density of potential mates will

decrease with CRR. For simplicity, we set the decrease to be fixed and linear. The propensity to court we assumed to increase with CRR, because there will be more competition for mates, increasing the need to invest in courtship. However, we also explored the situation when the propensity to court did not change. For simplicity, we assumed this relation to be linear as well, but allowed slopes and intercepts to vary. We then calculated the frequency of courtship behavior as: frequency = encounter rate * propensity. In the second model we explored the relation between CRR and the frequency and propensity of agonistic behavior. We assumed the encounter rate with competitors to increase with CRR, because, at a given population density, the density of competitors will increase with CRR. We assumed the propensity to behave agonistically towards each encountered competitor to increase with CRR. However, we also explored the situation when this propensity did not change. Similar to the first model, we set the encounter rate to a fixed linear relation with CRR. We assumed the relation between CRR and propensity to behave agonistically to be linear as well, although we varied the slope and the intercept. We calculated the frequency of agonistic behavior as frequency = encounter rate * propensity.

Meta-analysis

In a meta-analysis, we tested if the used method (propensity versus frequency) significantly affected empirical results with a meta-analysis. We performed a topical literature search for ("sex ratio" AND ("sexual compet*" OR court*)) and an extensive, though not exhaustive, search for references in these articles. We found that the majority of studies either record individual frequencies of courtship (most common, particularly in laboratory experiments) or propensities of courtship (more common in field studies; table 1). We included all studies that tested for an effect of OSR on courtship behavior as a frequency or propensity if they reported a measure of OSR that could be transformed to CRR.

Because some of the studies did not report effect sizes, and the sample size was already low, we used the reported P-values to compare the results. We treated each test within each study as separate data points, but weighed them by the inverse of the number of tests that reported a measure from the same class (frequency or propensity) per study. For each P-value, we calculated a variable z_p based on the quantile function of the normal distribution (qnorm; R Development Core team 2008). Given random P-values, the qnorm function returns normally distributed z-values, with a mean of zero and a variance of one. To take the direction of the effects into account, we transformed two-sided P-values into one-sided P-values, such that no effect ($P = 1$) became $P = 0.5$, $P > 0.5$ meant that a particular measure of courtship behavior increased with CRR ($P > 0.975$

for significant effect), and $P < 0.5$ meant that particular measure decreased significantly with CRR ($P < 0.025$ for a significant effect). This resulted in a positive sign to z_p for results that were in accordance with theoretical expectations, and a negative sign to z_p in the opposite case. If exact P-values were not given, we calculated them from the reported test statistic and degrees of freedom.

We then used a linear model (lm; R Development Core team 2008) to test for the effects of the measure used (propensity versus frequency) on z_p. We controlled for the type of study (field versus laboratory) and sex (female versus male), by including them in the model as explanatory variables. Additionally, to test for an overall effect of CRR on propensity and frequency measures, we tested whether the average z_p for propensity measures differed from zero with a one sample t-test, and similarly for frequency measures.

Results

Model

In figure 1, we show the relation between courtship frequency, courtship propensity and CRR that follows from our model. We set encounter rate with potential mates to decrease from 100 to zero encounters per unit time when CRR increases from zero to one. In figure 1A we set courtship propensity (the percentage of encountered potential mates courted) to increase from 50 % to 100 % when CRR increases from zero to one. As a result, there is a negative correlation between courtship frequency and CRR, seemingly opposite of what is predicted by theory. In figure 1B we can see that an overall decrease in courtship propensity can lead to the courtship frequency is being at a CRR of 0.5 (equal OSR). In figures 1B to 1D we explore the effect of a change in the slope of the increase in the propensity to court with CRR. The courtship frequency decreases in all cases after CRR becomes higher than 0.5, but before that point the frequency response is dependent on the slope of the response of the propensity to court. Note that the frequency to court will decrease even if the propensity to court does not change at all (fig. 1D), simply because the encounter rate changes with CRR.
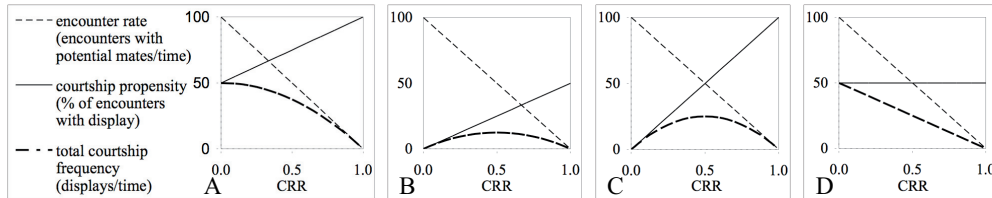
Figure 1. Courtship behavior: A graphical representation of the relation between encounter rate, the propensity to court and the resulting frequency of courtship behaviors (propensity * encounter rate) as a function of CRR: competitors/(potential mates + competitors). We assume a linear decrease in the encounter rate with potential mates with CRR. The difference between A and B illustrates the effect of an overall decrease in the propensity to court, whereas comparing A, C and D illustrates the effect of a change in the slope of the propensity to court. Note that D illustrates that there will be a decrease in the courtship frequency with CRR, even if the propensity to court does not change.
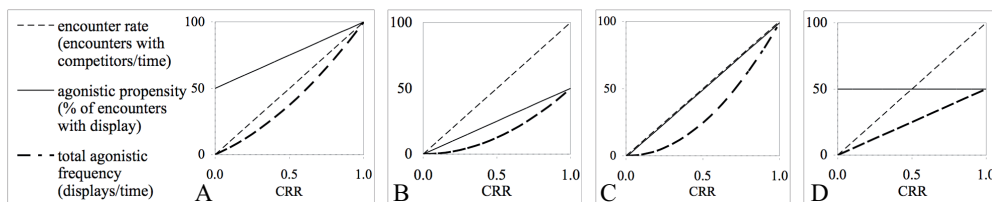
Figure 2. Agonistic behavior: A graphical representation of the relation between encounter rate, propensity to behave agonistically and the resulting frequency of agonistic behavior (propensity * encounter rate) as a function of CRR: competitors/(potential mates + competitors). We assume a linear increase in the encounter rate with competitors with CRR. The difference between A and B illustrates the effect of an overall decrease in propensity to behave agonistically, whereas comparing A, C and D illustrates the effect of a change in the slope of the propensity. Note that D illustrates that there will be an increase in the frequency of agonistic behaviors with CRR, even if the propensity to behave agonistically does not change.

In figure 2, we show the relation between agonistic frequency, agonistic propensity and CRR that follows from our second model. We set encounter rate with potential mates to increase from zero to 100 encounters per unit time when CRR increases from zero to one. In figure 2A agonistic propensity (percentage of encountered competitors attacked or displayed to) increases from 50 % to 100 % when CRR increases from zero to one. As a result, the agonistic frequency is highest at high CRR, when there are many competitors relative to the number of potential mates. Comparing figures 2A and 2B we can see that an overall decrease in agonistic propensity could lead to a very small effect of CRR on courtship frequency in the lower ranges of CRR. In figures 2B to 2D we explore the effect of a change in the slope of the increase in the propensity to behave agonistically with CRR. The frequency of agonistic behaviours increases in all cases, but the curvature of the increase depends on the slope of the increase in propensity. Notably, a change in encounter rate

produces an increase in agonistic frequency, even in the absence of a change in the propensity to behave agonistically (fig. 2D).

Meta-analysis

In our literature search, we found far more studies on male than on female behavior (table 1). We also found far more studies using frequency measures than studies using propensity measures (table 1). For males, propensity measures generally showed a positive effect of CRR on courtship, as predicted from theory, while frequency measures in general showed a negative effect (fig. 3A). That the distribution of the P-values of the studies in testing frequencies closely resembles a normal distribution (fig 3A), suggests that there is no strong publication bias. Thus, males generally court a higher proportion of encountered potential mates, when potential mates are scarce, but this does not result in a higher courtship frequency. On the contrary, males generally court less frequently when potential mates are scarce. Unfortunately, very few studies tested the effect of OSR on female behavior. Nevertheless, these few studies show the same pattern as the studies on male behavior. Three out of four studies that measured propensity to court found a positive effect of CRR on female courtship, while two out of three studies that measured courtship frequency found no effect (fig. 3B). The direction of the effect of the used measure on the findings was thus similar for the two sexes. In the full model however, we found a significant interaction between sex and measure. Therefore we did not pool tests of male and female behavior in our analyses. Because there were too few studies on female behavior to warrant meaningful analyses, we only present analyses on male behavior. We found a significant positive effect of propensity measures vs. frequency measures on the z_p (estimate $\pm$ SE = 4.81 $\pm$ 1.19, t_{23} = 4.04, P = 0.0005), controlling for type of study (field vs. lab: estimate $\pm$ SE = 2.54 $\pm$ 1.02, t_{23} = 2.50, P = 0.02) in a linear model (R^2 = 0.6, $F_{2,23}$ = 15.39, P = 0.00006, intercept $\pm$ SE = -2.75 $\pm$ 0.46). These results are robust to the removal of outliers (N = 3). Thus, the used measure significantly affects the results, even if we control for the difference between field and laboratory studies. Furthermore, the average z_p for frequency measures was negative ($\mu \pm$ SE: -2.17 $\pm$ 0.48) and significantly different from zero (t-test: t_{21} = -4.48, P = 0.0002). The average z_p for propensity measures on the other hand was positive ($\mu \pm$ SE: 3.13 $\pm$ 1.13) and three out of four z_p were positive and one zero. However, this did not result in a significant difference from zero (t_3 = 2.76, P = 0.07). Thus, in males, courtship frequency decreases with CRR, while courtship propensity tends to increase with CRR.

A

P < 0.0001	P < 0.001	P < 0.05	N. S.	N. S.	P < 0.05	P < 0.001	P < 0.0001
		Ward & Fitzgerald 1988					
		Soukouris & Cade 1993: *1*					
		Soukouris & Cade 1993: *2*					
		Mills & Reynolds 2003					
		Kodric-Brown 1988: *1*	Faleiro et al. 2008				
		Jirotkul 1999: *1*	Berglund 1994: *1*				
		Jirotkul 1999: *2*	Berglund 1994: *2*				
Shine et al. 2003		Grant et al. 2000	Kvarnemo et al. 1995				
Magellan & Magurran 2007	Spence & Smith 2005	Grant et al. 1995	Kodric-Brown 1988: *2*		Forsgren et al. 2004: *1*		Borg et al. 2002
Evans & Magurran 1999	de Jong et al. 2009	Grant & Foam 2002	Head et al. 2008	Takahashi 2000	Forsgren et al. 2004: *2*		Berglund 1994: *3*

Number of studies (y-axis); 5 marked on axis.

Decrease with CRR   Increase with CRR

B.

P < 0.0001	P < 0.001	P < 0.05	N. S.	N. S.	P < 0.05	P < 0.001	P < 0.0001
		Faleiro et al. 2008		Cratsley & Lewis 2005			Forsgren et al. 2004
		Kvarnemo et al. 1995	de Jong et al. 2009	Grant & Foam 2002			Borg et al. 2002

Number of studies (y-axis)

Decrease with CRR   Increase with CRR

Figure 3. All studies on the effects of OSR (converted to CRR) on male (A) and female (B) courtship used in our meta-analyses: Studies that report frequencies are marked grey, studies reporting propensities are marked white. The x-axis represents *P*-values in increasing order of significance, to the left of the black line for a negative effect of CRR on that particular measure of courtship behavior (as predicted by theory), to the right of the black line for a positive effect of CRR on the measure (seemingly opposing theory). Each measure of behavior is represented as one box with the reference inside. Thus, studies reporting several measures will appear more than once, the italic numbers correspond to the numbers of the measures for that study in table 1. This figure categorizes studies according to significance levels. However, our statistical tests are based on a continuous measure of significance and correct for the fact that some studies report multiple tests.

Discussion

In a meta-analysis of 20 experimental studies on the effect of OSR on male mating behavior, we found that the method used to quantify courtship behavior has significantly affected results. Male courtship frequency decreases when potential mates become scarce (increasing CRR: a male-biased OSR; box 1), whereas male courtship propensity tends to increase when potential mates become scarce. This illustrates the importance of using the right method to measure mating competition. The two main methodologies that to date have been adopted as equally valid tests of theory have led to opposite findings, and they are indeed expected to, as shown in our model.

The model

In the model, we used a sex-neutral conversion of OSR, CRR (competitor to resource ratio; box 1). Our simple graphical model for the relation between CRR, encounter rate, courtship frequency and courtship propensity clarifies why measuring courtship frequency leads to inconsistent results (fig. 1). It shows that, depending on the overall level of courtship (fig. 1A vs. 1B) and the slope of the increase in courtship propensity with CRR (fig. 1A-D), courtship frequency can show a variety of relationships with CRR, and with courtship propensity. With such non-linear relationships, predictions for empirical outcomes would depend entirely on which levels of OSR are established in an experiment. Furthermore, it shows that a decrease in the courtship frequency in competitive situations could be caused by an effect of the encounter rate with potential mates in the absence of a behavioral response (Fig. 1D).

For agonistic behavior (fig. 2), recording agonistic frequency should qualitatively give the same results as recording agonistic propensity. However, the effect of CRR on agonistic behavior can be strongly over or underestimated when frequencies are measured, depending on the curve of the relation and on which levels of CRR are established (fig. 2A-D). Moreover, an increase in encounter rate can produce an effect of CRR on frequency, even in the absence of a change in the behavioral response (the propensity; fig. 2D). It is important to realize that this could produce false positives in studies testing for an effect of OSR on the frequency of agonistic behaviors. Such studies may find a significant increase in frequency with increasing competition, as expected from theory, while the only thing that is actually increasing is the number of encounters between competitors.

It is important to note that population density will also affect encounter rates. In our model an

increase in overall density could lead to an increase of the slope of the relation between encounter rate and CRR. Thus, when comparing populations, both an increase in CRR and an increase in density would lead to an increase in encounter rate. In addition, encounter rates do not necessarily change linearly with OSR, because mate search behavior may change with mate availability (e. g. Kokko and Wong 2007). Future studies should assess how OSR and density affect both encounter rates and the propensity to show competitive behavior.

The meta-analyses

Although much is written about the theoretical effects of OSR on mating competition, empirical tests are still surprisingly scarce (table 1). In table 1 we list only those studies that report observations of courtship behavior, because for courtship behavior, the predictions from our model for propensity vs. frequency measures are qualitatively different, in contrast to the predictions for agonistic behavior. Within this subset, 25 of the 30 studies we found are on teleost fishes (table 1). Thus, there is an obvious and urgent need for a broadening of the taxonomic range of model organisms used to test predictions for how OSR affects mating competition. However, the five studies done on other taxa than fish show a similar pattern as the fish studies (Souroukis and Cade 1993; Jivoff and Hines 1998; Debuse et al. 1999; Shine et al. 2003; Cratsley and Lewis 2005; table1). Four of these five studies test for an effect of OSR on courtship frequency, and one recorded female propensity to court (table 1). Two of the studies that report frequencies corrected for the proportion of individuals of the opposite sex (table 1). Only the study that reports courtship propensity, and one of the studies that report corrected frequencies, find the expected decrease of courtship behavior with increasing mate availability (table 1). Apart from this taxonomic bias, there is also a bias towards species with male parental care (15 studies, of which 12 on nest brooding males; table 1). Since the cost of care can affect mating competition independent of OSR (Kokko and Monaghan 2001), it would be preferable to study the effects of OSR in a more balanced sample of care systems. In general, field studies report frequencies and laboratory experiments report propensities (but see Ward and Fitzgerald 1988; Berglund 1994; Takahashi 2000; Forsgren et al. 2004; Faleiro et al. 2008; table 1). Two studies use an even different measure and report the occurrence of behaviors as a proportion of all behaviors (Vincent et al. 1994; Kvarnemo 1996; table 1), these were not used in our meta-analyses. Only in one species (the two-spotted goby) has the effect of OSR on behavior of a sex been tested both in the laboratory and in the field (table 1). In the laboratory only frequencies of behavior were tested, which did not change with OSR, except for male courtship frequency, that was higher in a female-biased OSR (de Jong et al. 2009). In a field study on the other hand, both frequency and propensity of courtship and agonistic behavior

increased with CRR (OSR-bias towards the own sex; box 1) in both sexes (Forsgren et al. 2004; table 1). If a combination of field and laboratory studies on the same species would have been more common, the striking difference in results between field and laboratory studies might have been noted earlier. Apart from the previously mentioned field study, only one more study has tested both frequency and propensity measures simultaneously, in the pipefish *Syngnathus typhle* the latency to court (a propensity measure) increased with CRR, while CRR did not significantly affect the two frequency measures reported (Berglund 1994; table 1). Please note that there is only one experimental field study among the studies included in table 1 (Borg et al. 2002). Experiments in the field may be a fruitful future direction, because it combines the possibility to draw strong inferences about causality with a natural environment and manner of encountering potential mates.

For courtship behavior, our meta-analysis shows that the two methods used to quantify behavior, propensities versus frequencies, produce different results. While the propensity to court tends to increase with CRR, the courtship frequency decreases on average (fig. 3). Additionally, there is a large variation in the results when frequency measures are used to test the effects of OSR on both courtship and agonistic behavior, even between closely related species and within species (table 1). This could very well be caused by variation in encounter rates and chosen OSR-levels in different set-ups, as predicted by our models (figs. 1C and 2C).

Another reason why courtship frequency could vary, which we did not include in our model, is variation in courtship intensity. Courtship frequency is affected by the number of encounters and at how many of these encounters an individual decides to court (the propensity), as we have shown. However, although courtship intensity may often be expressed as a change in the nature or the duration of the behavior, courtship intensity could also affect the number of displays per encounter, and thus courtship frequency if scored as the total number of a certain behavior per individual per time. One would expect courtship intensity to increase when competition is high, thus increasing courtship frequency when mates are scarce and decreasing frequency when mates are readily available. This would effectively have the same effect as an increased slope of the propensity in our model.

Both for agonistic and for courtship behavior there are several biological reasons why these could decrease, rather than increase, in highly competitive situations. Agonistic behavior has been found to break down at high levels of competition or when resources are hard to monopolize (e.g. Grant et al. 1995). Courtship could also decrease at high levels of competition. Instead of displaying

courtship, individuals may switch to alternative tactics to obtain matings, such as sneaking (e.g. Evans and Magurran 1999; Jirotkul 1999; Shine et al. 2003), or competitors could prevent each other from courting (Grant et al. 1995; Jirotkul 1999). In addition, there could be a trade-off between courtship and agonistic behavior (e. g. Grant et al. 2000), reducing either the one or the other. However, our model shows that the effect of encounter rates could produce such a pattern, in the absence of a real trade-off.

In some cases, agonistic behavior may not reflect mating competition, but competition for resources. Even though competition for resources such as nest sites may often happen before individuals become ready-to-mate (Ahnesjö et al. 2008), in several species these processes overlap in time and individuals also have to defend these resources from competitors during mating competition (Almada et al. 1995; Borg et al. 2002; Saraiva et al. 2009). Finally, other factors, such as the cost of reproduction or how much you benefit from an extra mating, could be more important to explain changes in mating behavior than OSR (Kokko and Monaghan 2001; Jennions and Kokko 2010; Klug et al. 2010). These issues only increase the urgency of conclusively testing OSR theory, using relevant measures.

Suggestions for Experiments

Our findings do not imply that courtship frequency is not a worthwhile measure to record. On the contrary, it should reflect both the strength of the stimulus and the actual energy spent on courtship. Also, the variation in courtship frequency between individuals could give us an idea of whether certain individuals are excluded from courtship, through competition or mate choice by the other sex. However, if the aim of the study is to estimate the strength of mating competition, we think the propensity to court is the better measure.

To accurately measure courtship propensity, it is essential to distinguish separate encounters. Exactly how an overall population OSR translates to how animals experience a competitive situation is unclear, but it is likely that important clues are given by the encounter rates of same-sex and opposite-sex individuals. There are several problems to be solved if one wants to record encounter rates and the propensity to perform certain behaviors in the laboratory. In most of the species studied in the field, encounters appear to be clear cut, which may be why most of those field studies report courtship per encounter (table 1). In the laboratory however, encounter rates are not always easily recorded, because individuals often are in constant visual contact. In the typical design used to date, a certain number of animals of both sexes are put together in an open enclosure,

be it an aquarium or a cage, normally so small that any individual can travel the whole distance in minutes or less. This makes it impossible to separate individual encounters and to record propensities, which may explain why frequencies are so often used to reflect courtship motivation in the laboratory (table 1). Another issue to consider is that visual encounters could be less important for certain behaviors. For example, courtship calls could broadcast the availability to mate of an individual to a larger audience than just its immediate surroundings, therefore it should be carefully assessed what kind of encounter is relevant for the behavior under study.

One approach to solve the problem of encounter rates has been to correct individual frequencies for the number of available opposite sex individuals in a standard laboratory setting (Farr 1976; Kodric-Brown 1988; Grant et al. 1995, 2000; Jivoff and Hines 1998; Debuse et al. 1999; Silva et al. 2010). The core problem with such an approach is that it is probably impossible to tell how numbers of individuals relate to stimulus strength or encounter rate when individuals are in constant visual contact. Thus, it is rather arbitrary how to correct the frequencies exactly and overcompensation could lead to false positives. The inherent difference between the treatments in relative number of potential mates would, if corrected for, create a difference between the treatments, even if, for example, in each of the treatments one female was courting one male with similar intensity.

A first step towards testing the effect of competition on behavior, is to test the effect of an audience of one or more added competitors on the dyadic interaction between two individuals of the opposite sex. Such tests can provide information on the behavioral repertoire of a certain species, i.e. whether individuals are likely to increase courtship or rather switch to other mating tactics in the presence of competitors. How individuals respond to increased competition can differ substantially between species. For example, in the presence of competitors, male guppies (*Poecilia reticulata*) court more frequently (Farr 1976), male haddock (*Melanogrammus aeglefinus*) increases sound production (Bremner et al. 2002) and male sailfin mollies (*Poecilia latipinna*) show a higher number of sexual behaviors, although courtship duration does not change (Makowicz et al. 2010). On the other hand, male alpine newts (*Ichthyosaura alpestris*) do not change their courtship behavior in the presence of another male (Denoël et al. 2005). When competition is very high male red-spotted newts (*Notophthalmus viridescens*) abandon courtship in favor of amplexus (Verrell 1983) and male mole salamanders (*Ambystoma talpoideum*) even abandon any attempt to court and mate (Verrell & Krenz 1998). Although these studies do not actually test the effect of a gradual change in OSR, they are important sources for predictions on possible behavioral responses to OSR.

However, behavior may change differently in more complex social settings. For example, several studies have shown that guppies can switch to sneaking in a male-biased OSR (Evans & Magurran 1999; Jirotkul 1999; Magellan & Magurran 2007; but see: Head and Brooks 2006).

Thus, we suggest that a way to test behavioral responses in the laboratory is to develop set-ups that allow quantification of encounter rates, for example by presenting potential mates asynchronously (Grant et al.1995), or by creating structure in the experimental arena, so that individuals have to actively visit each other. Alternatively, experiments changing OSR in the field (Borg et al. 2002) would provide a natural environment and encounter rates. With such designs it would be possible to record encounter rates, the propensity to behave (e.g. the propensity to court at an encounter) and the frequencies of behaviors, and test how these relate to mate availability, all in one set-up.

Conclusion

Our data show that the empirical basis for generalizations on how OSR affects mating competition is surprisingly limited. This highlights the need for more studies, including controlled experiments both in the laboratory and in the field, measuring the effect of OSR on propensities to behave competitively to obtain mates. The majority of extant tests use teleost fishes, future tests need to include model organisms from a broader range of taxa. Additionally, although controlled laboratory experiments studies are a stronger approach to detect causality than observational field studies, the current study illustrates that field studies are still an important tool to investigate what is really going on. Ideally, any laboratory experiment should be firmly based on knowledge from the field as to what are relevant situations and parameters.

Furthermore, this study reveals a fundamental problem in how the effect of ORS on mating competition has been measured. The core of the problem is that OSR-based theory for mating competition predicts a behavioral response to an altered social environment, while the majority of studies have quantified purely numerical responses to a change in the number of opportunities to display the behavior (frequencies of behavior). Our model shows that these two measures can produce opposite results when studying the effects of OSR on courtship behavior, and our meta-analysis shows that this is indeed what has happened. This highlights the importance of translating model predictions accurately to relevant parameters for empirical tests.

References

Ahnesjö I., E. Forsgren, and C. Kvarnemo. 2008. Variation in sexual selection in fishes. Pages 303-335 *in* C. Magnhagen, V. A. Braithwaite, E. Forsgren, and B. G. Kapoor, eds. Fish behaviour. Science Publishers, Enfield, NH.

Almada, V. C., E. J. Concalves, R. F. Oliveira, and A. J. Santos. 1995. Courting females - Ecological constraints affect sex-roles in a natural-population of the Blenniid fish *Salaria pavo*. Animal Behaviour 49:1125-1127.

Arnold, S. J. 1994. Bateman principles and the measurement of sexual selection in plants and animals. American Naturalist 144:126-149.

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton, N. J.

Bateman, A. J. 1948. Intra-sexual selection in drosophila. Heredity 2:349-368.

Berglund, A. 1994. The operational sex-ratio influences choosiness in a pipefish. Behavioral Ecology 5:254-258.

Borg, Å. A., E. Forsgren, and C. Magnhagen. 2002. Plastic sex-roles in the common goby - the effect of nest availability. Oikos 98:105-115.

Bremner, A. A., E. A. Trippel, and J. M. Terrhune. 2002. Sound production by adult haddock, *Melanogrammus aeglefinus*, in isolation, pairs and trios. Environmental Biology of Fishes 65:359-362.

Colwell, M. A., and L. W. Oring. 1988. Sex-ratios and intrasexual competition for mates in a sex-role reversed shorebird, Wilson`s phalarope (*Phalaropus tricolor*). Behavioral Ecology and Sociobiology 22:165-173.

Cratsley, C. K., and S. M. Lewis. 2005. Seasonal variation in mate choice of *Photinus ignitus* fireflies. Ethology 111:89-100.

Debuse, V. J., J. T. Addison, and J. D. Reynolds. 1999. The effects of sex ratio on sexual competition in the European lobster. Animal Behaviour 58:973-981.

de Jong, K., S. Wacker, T. Amundsen, and E. Forsgren. 2009. Do operational sex ratio and density affect mating behaviour? An experiment on the two-spotted goby. Animal Behaviour 78:1229-1238.

Denoël, M., M. Mathieu, and P. Poncin. 2005. Effect of water temperature on the courtship behavior of the Alpine newt *Triturus alpestris*. Behavioral Ecology and Sociobiology 58:121-127.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and evolution of mating systems. Science 197:215-223.

Evans, J. P., and A. E. Magurran. 1999. Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. Animal Behaviour 58:1001-1006.

Faleiro, F., L. Narciso, and L. Vincente. 2008. Seahorse behavior and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? Aquaculture 282:33-40.

Farr, J. A. 1976. Social facilitation of male sexual-behavior, intra-sexual competition, and sexual selection in guppy, *Poecilia reticulata* (Pisces - Poeciliidae). Evolution 30:707-717.

Fitze, P. S., and J. F. le Galliard. 2008. Operational sex ratio, sexual conflict and the intensity of sexual selection. Ecology Letters 11:432–439.

Forsgren, E., T. Amundsen, Å. A. Borg, and J. Bjelvenmark. 2004. Unusually dynamic sex roles in a fish. Nature 429:551-554.

Grant, J. W. A., and P. E. Foam. 2002. Effect of operational sex ratio on female-female versus male-male competitive aggression. Canadian Journal of Zoology 80:2242-2246.

Grant, J. W. A., M. J. Bryant, and C. E. Soos. 1995. Operational sex-ratio, mediated by

synchrony of female arrival, alters the variance of male mating success in Japanese medaka. Animal Behaviour 49:367-375.

Grant J. W. A., C. L. Gaboury, and H. L. Levitt. 2000. Competitor-to-resource ratio, a general formulation of operational sex ratio, as a predictor of competitive aggression in Japanese medaka (Pisces : Oryziidae). Behavioral Ecology 11:670-675.

Gwynne, D. 1985. Role-reversal in katydids: habitat influences reproductive behavior (Orthoptera: Tettigoniidae, *Metaballus sp.*). Behavioral Ecology and Sociobiology. 16:355-361.

Head, M. L., and R. Brooks. 2006. Sexual coercion and the opportunity for sexual selection in guppies. Animal Behaviour 71, 515-522.

Head, M. L., A. K. Lindholm, and R. Brooks. 2008. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. Evolution 62:135-144.

Jennions, M. D., and H. Kokko. 2010. Sexual selection. Pages 343-364 *in* D. F. Westneat, and C. W. Fox, eds. Evolutionary Behavioral Ecology. Oxford University Press, Oxford.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews 75:21-64.

Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. Animal Behaviour 58:287-294.

Jivoff, P., and A. H. Hines. 1998. Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. Animal Behaviour 55:589-603.

Klug, H., J. Heuschele, M. D. Jennions, and H. Kokko. 2010. The mismeasurement of sexual selection. Journal of Evolutionary Biology 23:447-462.

Kodric-Brown, A. 1988. Effects of sex-ratio manipulation on territoriality and spawning success of male pupfish, *Cyprinodon pecosensis*. Animal Behaviour 36:1136-1144.

Kokko, H. and P. Monaghan. 2001. Predicting the direction of sexual selection. Ecology Letters 4:159-165.

Kokko, H., and D. J. Rankin. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philosophical Transactions of the Royal Society B 361:319-334.

Kokko, H., and B. B. M. Wong. 2007. What determines sex roles in mate searching? Evolution 61:1162-1175.

Kvarnemo, C. 1996. Temperature affects operational sex ratio and intensity of male-male competition: Experimental study of sand gobies, *Pomatoschistus minutus*. Behavioral Ecology 7:208-212.

Kvarnemo, C., and I. Ahnesjö. 1996. The dynamics of operational sex ratios and competition for mates. Trends in Ecology and Evolution 11:404-408.

Kvarnemo, C, E. Forsgren, and C. Magnhagen. 1995. Effects of sex ratio on intra- and inter-sexual behaviour in sand gobies. Animal Behaviour 50:1455-1461.

Lengkeek, W., and K. Didderen. 2006. Breeding cycles and reproductive behaviour in the river blenny *Salaria fluviatilis*. Journal of Fish Biology 69:1837-1844.

Magellan, K., and A. E. Magurran. 2007. Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. Animal Behaviour 74:1545-1550.

Makowicz, A., M. Plath, and I. Schlupp. 2010. Using video playback to study the effect of an audience on male mating behaviour in the Sailfin molly (*Poecilia latipinna*). Behavioural Processes 85:36-41.

Michener, G. R., and I. G. McLean. 1996. Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. Animal Behaviour 52:743-758.

Mills, S. C., and J. D. Reynolds. 2003. Operational sex ratio and alternative reproductive behaviors in the European bitterling, *Rhodeus sericeus*. Behavioral Ecology and Sociobiology 54:98-104.

Okuda, N. 1999. Sex roles are not always reversed when the potential reproductive rate is higher in females. American Naturalist 153:540-548.

R Development Core Team. 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Reichard, M., P. Jurajda, and C. Smith. 2004. Male-male interference competition decreases spawning rate in the European bitterling (*Rhodeus sericeus*). Behavioral Ecology and Sociobiology 56:34-41.

Saraiva, J., E. Barata, A. Canário, and R. Oliveira. 2009. The effect of nest aggregation on the reproductive behaviour of the peacock blenny *Salaria pavo*. Journal of Fish Biology 74:754-762.

Shibata, J., and M. Kohda. 2006. Seasonal sex role changes in the Blenniid *Petroscirtes breviceps*, a nest brooder with paternal care. Journal of Fish Biology 69:203-214.

Shine, R., T. Langkilde, and R. T. Mason. 2003. The opportunistic serpent: Male garter snakes adjust courtship tactics to mating opportunities. Behaviour 140:1509-1526.

Silva K, M. N. Vieira, V. C. Almada, and N. M. Monteiro. 2010. Reversing sex role reversal: compete only when you must. Animal Behaviour 79:885-893.

Souroukis, K., and W. H. Cade. 1993. Reproductive competition and selection on male traits at varying sex-ratios in the field cricket, *Gryllus pennsylvanicus*. Behaviour 126:45-62.

Spence, R., and C. Smith. 2005. Male territoriality mediates density and sex ratio effects on oviposition in the zebrafish, *Danio rerio*. Animal Behaviour 69:1317-1323.

Takahashi, D. 2000. Conventional sex roles in an amphidromous *Rhinogobius* goby in which females exhibit nuptial coloration. Ichthyology Research 47:303-306.

Verrell, P. A. 1983. The influence of the ambient sex-ratio and intermale competition on the sexual behavior of the red-spotted newt, *Notophthalmus viridescens* (Amphibia, urodela, salamandridae). Behavioral Ecology and Sociobiology 13:307-313.

Verrell, P. A., and J. D. Krenz. 1998. Competition for mates in the mole salamander, *Ambystoma talpoideum*: Tactics that may maximize male mating success. Behaviour 135:121-138.

Vincent, A. C. J., I. Ahnesjö, A. Berglund, and G. Rosenqvist. 1992. Pipefishes and seahorses - are they all sex-role reversed. Trends in Ecology and Evolution 7:237-241.

Vincent, A. C. J., I. Ahnesjö, and A. Berglund. 1994. Operational sex-ratios and behavioral sex-differences in a pipefish population. Behavioral Ecology and Sociobiology 34:435-442.

Ward, G., and G. J. Fitzgerald. 1988. Effects of sex-ratio on male-behaviour and reproductive success in a field population of threespine sticklebacks (*Gasterosteus aculeatus*) (Pisces, gasterosteidae). Journal of Zoology (London) 215:597-610.

Table 1: An overview of all studies we found that reported the effect of a variation in OSR on courtship behavior in taxonomical order. We also show results for agonistic behavior, but we did not include studies that only report agonistic behavior. The English and the scientific name of each species are given in the first column. Under system we give a description of the mating situation and parental care if this affects the mating situation. Studies are described as laboratory (lab) if study subjects were artificially constrained, including field enclosures, and as field studies otherwise. Under OSR estimate, we describe the method for estimating OSR and under response variable we describe how behavior was quantified. In the column used in test, we state if each response variable was used as a frequency measure (freq) or a propensity measure (prop) or not at all (no) in our meta-analyses. We did not use response variables that: 1. did not reported a measure of courtship behavior that was either a frequency or a propensity measure, 2. corrected in some way for the number of (encounters with) available opponents or 3. reported an OSR estimate that could not be converted to the competitor-to-resource rate (CRR). The OSR-range used in each study is given in the 7th column. In columns 10-13 we give the results for each measure of courtship and agonistic behavior for males and females with "+" denoting a significant increase with CRR (as expected from theory), "-" denoting a significant decrease with CRR (seemingly opposite to predictions from theory) and "0" denoting no significant effect of CRR.

Species	System	Reference	Type study	OSR estimate	OSR range M/(M+F)	Response variable	In test	Male courtship	Female courtship	Male agonistic	Female agonistic
European lobster (*Homarus gammarus*)	Polygamous, female carries eggs	Debuse et al. 1999	Lab, exp	Adult sex ratio	0.33-0.66	1: Total duration per individual[1]	No[1]	0		0	+
						2: Intensity: proportion of displays with claw contact	No			-	0
Blue crab (*Callinectes sapidus*)	Polygamous, female carries eggs	Jivoff and Hines 1998	Lab, exp	Adult sex ratio	0.16-0.83	Frequency of displays[1]	No[1]	+	+		
Firefly (*Photinus ignitus*)	Polygamous, male spermatophore	Cratsley and Lewis 2005	Field, corr	Number of males courting a female	0.5-0.83	Proportion of male flashes to which females responded	Prop		+		
Field cricket (*Gryllus pennsylvanicus*)	Female visits male	Souroukis and Cade 1993	Lab, exp	Adult sex ratio	0.33-1	1: Frequency of displays per individual	Freq	-		+	
						2: Time spent calling per individual	Freq	+			
Long-snouted seahorse (*Hippocampus guttulatus*)	Polygamous, pouch brooding males	Faleiro et al. 2008	Lab, exp	Adult sex ratio	0.25-0.75	Proportion of courtship initiated by the male	Prop	0	0		
Pipefish (*Syngnathus abaster*)	Polygamous, pouch brooding males	Silva et al. 2010	Lab, exp	Adult sex ratio	0.25-0.75	Frequency of four different behaviors per individual[1]	No[1]	+	0/+[2]	-/0/+[2]	0/+[2]
Pipefish (*Syngnathus typhle*)	Polygamous, pouch brooding males	1: Berglund 1994	Lab, exp	Adult sex ratio	0.33-0.66	1: Frequency of displays per individual	Freq	0			
						2: Time spent courting per individual	Freq	0			
						3: Latency to court	Prop	+			

Species	System	Reference	Type study	OSR estimate	OSR range M/(M+F)	Response variable	In test	Male courtship	Female courtship	Male agonistic	Female agonistic
Pipefish (Syngnathus typhle)	Polygamous, pouch brooding males	2: Vincent et al. 1994	Field, corr	Ratio of females to males with pouch space	0–0.65	Interactions as proportion of all interactions observed	No		+[3]	+	+
Japanese medaka (Oryzias latipes)	Polygamous, synchronous mating, no care	1: Grant and Foam 2002	Lab, exp	Adult sex ratio	0.16–0.5	Frequency of displays per individual	Freq	-	+	+	+
		2: Grant et al. 1995	Lab, exp	Sex ratio (receptive)	0.33–0.75	Frequency of displays per individual	Freq	-		+	
		3: Grant et al. 2000	Lab, exp	Adult sex ratio	0.33–0.88	Frequency of displays per individual	Freq	-		+/-	
Zebra fish (Danio rerio)	Polygamous, spawning grounds[4]	Spence and Smith 2005	Lab, exp	Adult sex ratio	0.33–0.66	Frequency of displays per territorial male	Freq	-		0	
European bitterling (Rhodeus sericeus)	Polygamous, nest brooding males	1: Mills and Reynolds 2003	Lab, exp	Adult sex ratio	0.5–0.85	Frequency of displays per size class	Freq	-		-	
		2: Reichard et al. 2004	Lab, exp	Adult sex ratio	0.15–0.27	Frequency of displays per individual	No	?[5]		?[5]	
Pupfish (Cyprinodon pecosensis)	Polygamous, male territories, no care	Kodric-Brown 1988	Lab, exp	Adult sex ratio	0.33–0.66	1: Frequency of displays per individual	Freq	-		+	
						2: Total time spent	Freq	0			
Guppy (Poecilia reticulata)	Polygamous, live-bearing females	1: Evans and Magurran 1999	Lab, exp	Adult sex ratio	0.31–0.68	Frequency of displays per individual	Freq	-			
		2: Head et al. 2008	Lab, exp	Adult sex ratio	0.42–0.6	Frequency of displays per individual	Freq	0			
		3: Jirotkul 1999	Lab, exp	Adult sex ratio	0.16–0.83	1: Frequency of displays per individual	Freq	-		+	
						2: Total time per individual	Freq				
		4: Magellan and Magurran 2007	Lab, exp	Adult sex ratio	0.3–0.7	Frequency of displays per individual	Freq	-[6]			
Threespine sticklebacks (Gasterosteus aculeatus)	Polygamous, nest brooding males	Ward and Fitzgerald 1988	Field, exp	Adult sex ratio	0.33–0.66	1: Percentage of time spent displays	No[7]	+		0	
						2: Frequency of male displays	Freq	-			
Blenny (Petroscirtes breviceps)	Polygamous, nest brooding males	Shibata and Kohda 2006	Field, corr	Nest surface covered with eggs		1: Proportion of courtship events initiated by females	No[8]		+		
						2: Proportion of visiting males	No[8]			0	
						3: Frequency of displays per individual	No[8]	-		-	+

Species	System	Reference	Type study	OSR estimate	OSR range M/(M+F)	Response variable	In test	Male courtship	Female courtship	Male agonistic	Female agonistic
River blenny (*Salaria fluviatilis*)	Polygamous, nest brooding males	Lengkeek and Didderen 2006	Field, corr	Proportion of females gravid	...	Proportion of potential mates courted	No[8]	0	+		
Blenny (*Salaria pavo*)	Polygamous, nest brooding males	Saraiva et al. 2009	Lab, exp	Aggregated versus dispersed nest sites; nr. Of nest holding males	~0.35-0.4	1: Frequency of displays per individual	No[8]	0	+	0	0
						2: Proportion of individuals displaying	No[8]	0	+		
Common goby (*Pomatoschistus microps*)	Polygamous, nest brooding males	Borg et al. 2002	Field, exp	Ratio of gravid females to males with nests	0.17-0.27	Proportion of events	Prop	+	+	0	0
Sand goby (*Pomatoschistus minutus*)	Polygamous, nest brooding males	1: Kvarnemo 1996	Lab, exp	Sex ratio of sexually active individuals	0.4-0.8	Interactions as proportion of all observed interactions	No	0	0	+	+
		2: Kvarnemo et al. 1995	Lab, exp	Adult sex ratio	0.33-0.66	Frequency of interactions per individual	Freq	0	0	-	-
Two-spotted goby (*Gobiusculus flavescens*)	Polygamous, nest brooding males	1: Forsgren et al. 2004	Field, corr	Ratio of gravid females to estimated male nest space	0.15-0.8	1: number of interactions per focal male	Freq	+	+	+	+
						2: visits with display as proportion of all female visits	Prop	+	+	+	+
						3: proportion of focal males with interaction	No	+	+	+	+
		2: de Jong et al. 2009	Lab, exp	Adult sex ratio	0.33-0.66	Frequency of displays per individual	Freq	-	0	0	0
Rhinogobius sp.	Polygamous, nest brooding males	Takahashi 2000	Field, corr	Sex ratio of receptive individuals	0.7-1.0	Frequency of displays per individual	Freq	0	0	+	
Red-sided gartner snake (*Thamnophis sirtalis parietalis*)	Polygynous, aggregations, life bearing females	Shine et al. 2003	Lab, exp	Adult sex ratio	0.5-0.96	1: Frequency of displays per individual	Freq	-			
						2: Mean wave-rates	No	-			

Footnotes: [1]corrected for the proportion of active individuals of the relevant sex, [2]results differed between behaviors, [3]dome shaped in one year, [4]territorial males in this experiment, [5]a significant interaction effect between total and local OSR, [6]only a significant difference between male-bias and equal sex ratio, [7]only recorded for agonistic behavior, [8]no measure of OSR that could be converted to CRR.

Paper III

Operational sex ratio affects male courtship propensity, but not courtship frequency, in the two-spotted goby

Karen de Jong[1], Trond Amundsen[1] & Elisabet Forsgren[2]

[1]Department of Biology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, Norway; [2]The Norwegian Institute for Nature Research (NINA), 7485 Trondheim, Norway

ABSTRACT: The operational sex ratio (OSR; the sex ratio of ready-to-mate individuals) was proposed to affect the strength of mating competition in 1976. The suggestion has received correlational support from field studies, but results from controlled experiments have not matched patterns found in the field. To date, most experiments measure frequencies of competitive behaviour. However, frequencies could be affected by the number of encounters with mates and competitors, as well as by the level of competition. The propensity to compete at each encounter should better reflect the behavioural response. Here, we adopt an experimental design that allows for recording encounter rates and propensities to compete. In a laboratory experiment we changed the OSR via the number of ready-to-mate females, without changing the adult sex ratio. We provided structured aquaria with male compartments at each end that were partly closed off from the central compartment, where we entered the females. This allowed us to record all visits of males and females to focal males. As predicted, we found that males in a male-biased OSR were more likely to court visiting ready-to-mate females, while total courtship frequency was not affected by OSR. These results corroborate a recent model that predicts that if OSR affects courtship propensity, this does not necessarily result in a similar effect on courtship frequency. Moreover, males spent less time in their nests and interacted more in a male-biased OSR. Our results provide the first conclusive experimental evidence that a difference in OSR alone affects competitive mating behaviour.

Introduction

Mating success can directly affect the reproductive success and thus fitness of an individual (Darwin 1871, Bateman 1948). Competition for mates is therefore an important episode in sexual selection. The operational sex ratio (OSR; the sex ratio of ready-to-mate individuals) has been proposed to affect the strength of mating competition and related behaviours more than three decades ago (Emlen 1976, Emlen & Oring 1977). Mating competition can be expressed as agonistic behaviour and courtship behaviour (Andersson 1994). Both agonistic and courtship behaviour is thus expected to be affected by OSR (Emlen & Oring 1977, Kvarnemo & Ahnesjö 1996). Controlled experiments have generally found that agonistic behaviour increases when OSR becomes biased towards the own sex (e. g. Kodric-Brown 1988, Gwynne & Simmons 1990, Souroukis and Murray 1995, Debuse et al. 1999, Jirotkul 1999), but often only up to a point where competition becomes too costly (e. g. Sih and Krupa 1995, Jirotkul 1999, Grant et al. 2000, Clark & Grant 2010). Male courtship behaviour, on the other hand, generally decreases in a male biased OSR in laboratory experiments (reviewed in Paper II). This is surprising, because courtship behaviour is expected to increase with an OSR bias towards the own sex, and has been found to do so in correlational field studies (e. g. Gwynne 1984, Vincent et al 1994, Almada et al. 1995, Forsgren et al. 2004, Cratsley & Lewis 2005, Lengkeek and Didderen 2006, but see Okuda 1999). Several explanations have been proposed for the decrease of courtship behaviour with OSR in laboratory experiments. First, OSR has been suggested not to affect mating competition as expected (Head et al. 2008, Fitze & le Gaillard et al. 2008). Second, there could be a trade-off between courtship and agonistic behaviour and some individuals could switch to alternative mating tactics when competition is high (e.g. Jirotkul 1999, Grant et al. 2000, Shine et al. 2003, Magellan & Magurran 2007). Third, we proposed recently that the lack of consistency between field data and laboratory data could be the result of not using appropriate methods when estimating mating competition (Paper II). Most experiments to date have measured frequencies of competitive behaviour. However, frequencies are affected by the number of encounters with mates and competitors, as well as by the level of competition (Paper II).

Thus, courtship frequency could decrease simply because any individual will encounter fewer potential mates when the OSR is biased towards the own sex. This could also call into question the increase in agonistic frequency found in most laboratory experiments when the OSR is biased towards the own sex. This increase could have been caused merely by an increase in encounters between same-sex individuals, and is hence not an evidence for a behavioural response to the level

of mating competition (Paper II). By contrast, the propensity of an individual to behave at each encounter with a potential mate or competitor, should express behavioural motivation, and is thus expected to increase with the strength of mating competition (Paper II).

In the two-spotted goby (*Gobiusculus flavescens*), a small marine fish, a change in OSR over the course of a breeding season has been shown to coincide with a switch in sex roles from conventional (male competing for females) to reversed (females competing for males; Forsgren et al. 2004). In a laboratory experiment on the same species however, frequencies of courtship and agonistic behaviours were not affected in the expected direction in either sex (de Jong et al. 2009). In this experiment, we tested the effect of OSR on the propensity to behave in the two-spotted goby. Propensities to behave can be approximated by the latency to behave (Berglund 1994) or by the proportion of times courtship is initiated by a certain sex (Borg et al. 2002, Faleiro et al. 2008). Ideally however, competitive behaviours should be recorded on a per encounter basis. This has been done in a number of correlative field studies (Forsgren et al. 2004, Cratsley & Lewis 2005, Lengkeek & Didderen 2006, Shibata & Kohda 2006), but to our knowledge never in any experimental test of OSR theory. A main reason why laboratory studies have generally not recorded behaviours per encounter could be that individuals are typically in constant visual contact in traditional laboratory designs. This makes it impossible to distinguish separate encounters.

In this experiment we therefore applied a novel design. We partly shielded male nest sites from the rest of the aquarium, so that females had to actively visit males to spawn. This allowed us to record encounter rates between males and females and the propensity of each male to behave competitively at any encounter, in addition to overall frequencies of behaviours. We expected male propensity to court to be higher in a male-biased OSR, while courtship frequency should be more affected by how many female visits a male received. In addition, we tested whether male movement patterns differed between treatments, which could affect encounter rates.

Methods

The experiment was conducted during 17 June - 12 July 2008 and 15 May - 9 June 2009 at the Sven Lovén Centre for Marine Sciences at Kristineberg, Sweden (58.15° N, 11.27° E).

Model Species

The two-spotted goby (*Gobiusculus flavescens*) is a small marine fish, which typically lives only one year (Collins 1981). The species is common along the coast of Europe from northwest Spain to northern Norway. During its breeding season (May-July in our study area), the two-spotted goby inhabits shallow waters along rocky shores, where females and non-breeding males often form semi-pelagic foraging shoals (Miller 1986; Svensson et al. 2000). Breeding males defend a nest in empty mussel shells (e.g. *Mytilus edulis*) in crevices, or in brown algae (typically *Saccharina latissima* and *Laminaria digitata*) (Amundsen & Forsgren 2001, Mobley et al. 2009). Females visit these nests to spawn, whereafter the male provides all parental care, which consists of nest defence and cleaning and aerating (fanning) the eggs until they hatch (Skolbekken & Utne-Palm 2001). Males can care for eggs of up to six different females simultaneously (Mobley et al. 2009). Both males and females have colourful ornaments, which they emphasize in courtship (Amundsen & Forsgren 2001). Males have iridescent blue spots along their body and blue stripes on their fins, whereas females develop bright orange bellies during egg development (Amundsen & Forsgren 2001; Svensson 2006).

Fish Collection and Husbandry

All fish were caught by snorkelers using hand-held dip nets around islands up to three kilometres from the research station. Upon arrival at the research station, we stored the sexes separately in aquaria of approximately 60 litres. Individual aquaria held between 10 and 40 fish. All aquaria had a layer of 1 - 2 cm of gravel on the bottom and artificial plants to provide cover. We provided an uninterrupted flow of surface water (7 m depth). Temperature therefore reflected ambient seawater temperature. Artificial light followed a typical Swedish summer with 16 hours light and 8 hours dark, and there was additional light from windows in the experimental room. We measured total length of each fish on a grid (to the nearest 0.5 mm) and weighed them on digital Mettler scales (to the nearest mg), just before transfer to the experimental aquaria. To remove excess water before weighing, we held each fish on a moist paper before releasing it gently into a cup of pre-weighed seawater. Females were classified according to roundness class from 1 (slim) to 3 (very round). In order to recognize the two males individually, we marked them by subcutaneously injecting Visible Implant Elastomer (VIE; Northwest Marine Technology TM, www.nmt.us). We injected each male dorsally on both sides of his body with one of four colours: green, yellow, orange or pink.

Experimental Design

We created two treatments, a male biased and a female biased OSR, by varying the number of ready-to-mate females, in a constant adult sex ratio of 2 males to 10 females (Figure 1). This way we could test the effect of OSR without confounding OSR with adult sex ratio. Male compartments, one on each side, were partly closed off from the rest of the aquarium, to allow us to record separate visits from females and males (Figure 1). Females were introduced in the middle, and actively had to visit males to interact and spawn. Males were also able to swim freely through the aquarium, but most males spent most of their time in their own compartment. The treatments lasted for two days.

Experimental aquaria had a water volume of about 60 l (60 x 38 x 30 cm). Two males of similar size (< 1 mm difference in total length) were allowed to take up nests in PVC-tubes (length 80 mm, inner diameter 14 mm) attached to stones at approximately 3 cm height, at opposite sides of the tank. The distance between male nests was thus approximately 50 cm. When males had taken up nests, we partly shielded the area around each male's nest with an opaque divider (30 x 20 cm). A divider was placed on the gravel 15 cm from the side of the aquarium, at an angle of approximately 30 degrees towards the nest, so that fish could pass above and on the sides (Figure 1). Two to twelve days after, we introduced females in the middle of the tank. This time did not differ between the treatments (MWU: 104, Z =-0.12, P = 0.91, N = 92).
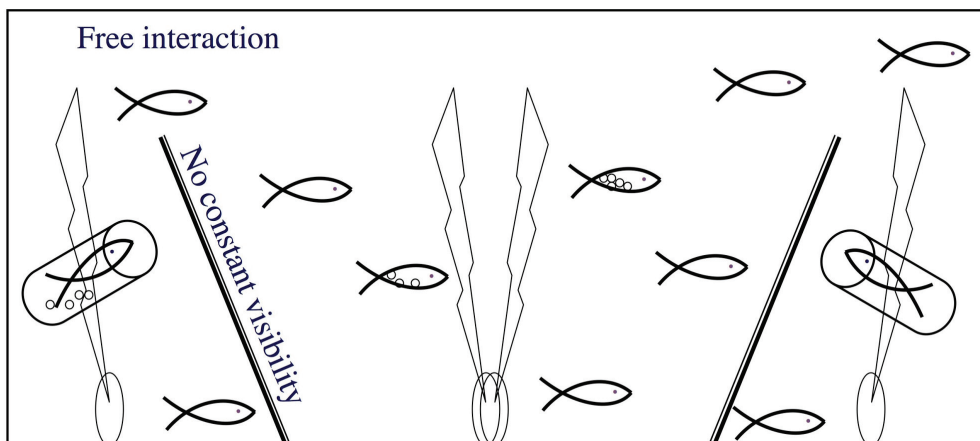
Figure 1. A novel experimental set up to test effects of OSR on mating competition in the two-spotted goby. Males are shown in their nest. Both males and females could move freely between compartments (on the side of or above the divider) and could interact. In this example, the male on the left has received some eggs in his nest, whereas the male to the right has not. OSR was manipulated solely through a difference in the number of round ready-to-spawn females (depicted as females with eggs in their belly), while the adult sex ratio was kept constant at two males to ten females.

We judged if a female was ready to mate by the roundness of her belly. Round females have mature eggs in their belly and are thus ready to spawn (Svensson 2006, Svensson et al. 2009). In the male-biased OSR treatment we introduced two ready-to-mate (round) females and eight other (slim) females. In the female-biased treatment we introduced ten ready-to-mate females. OSR in the treatments (males/(males + ready-to-mate females)) was thus 0.50 and 0.17 respectively. This is within the natural range (0.18 - 0.79 over the breeding season; Forsgren et al. 2004).

OSR during observations

We calculated OSR at the moment of observation (current OSR) in two different ways (Table 1). The first (OSR1) is the sex ratio of ready-to-mate individuals, while the second (OSR2) takes into account that each male can accommodate eggs from several females. Because of this, a larger number of mating-ready females than males need not imply that competition is stronger in females (Forsgren et al. 2004). We calculated OSR2 as: available nest space/(available nest space + number of round females), with available nest space = empty nest space/average female clutch size in this experiment. The median egg area per female that spawned in the experiment was 7.26 cm^2 in 2008 and 7.32 cm^2 in 2009. We therefore used 7.3 cm^2 in the calculation of OSR2 in both years. This was within the range of what was found in a previous lab study on the same population (Bjelvenmark & Forsgren 2003), but larger than in an experiment on a different population of two-spotted gobies (Skolbekken & Utne-Palm 2001). At the day of observation both OSR1 and OSR2 were female biased (< 0.5) in the treatment where we added 10 ready-to-mate females at the start (Table 1), hereafter called female biased treatment. In the other treatment where we added two ready-to-mate and eight other females, both measurements of OSR were male biased (> 0.5) on average at the time of observation (Table 2). The latter treatment will from here on be called male biased treatment.

Table 1. Two calculations of the OSR (mean and 95% confidence interval) at the time of observation in two treatments in an experiment on the two-spotted goby. OSR1 = males/(males + round females), OSR2 = available nest space/(available nest space + (round females * mean egg area per female)).

Treatment	OSR1 mean	95% CI	OSR2 mean	95% CI
Female bias	0.31	0.26 - 0.35	0.30	0.26 - 0.33
Male bias	0.56	0.52 - 0.60	0.71	0.68 - 0.74

Observations and video recordings

One observer (K.d.J.) observed the two males in one aquarium sequentially for ten minutes each, while both males were simultaneously video recorded for the whole twenty minutes. The order of observation, both between males within an aquarium, and between aquaria within a set of replicates, was decided by flipping a coin. From the video recordings, the same observer (K.d.J.) recorded the number and duration of visits by ready-to-mate females and the other male. At each encounter we recorded if the focal male approached the visitor, and if and when courtship or agonistic behaviour was initiated by the focal male. For visits by other (not ready-to-mate) females, we recorded the number of visits, and if the male approached or courted the female and if he showed agonistic behaviour during each visit. Courtship behaviour consisted of fin displays, quiver displays and lead swims (Amundsen & Frosgren 2001, Pélabon 2003, Forsgren et al. 2004). Agonistic behaviour included only male-male fin displays (Pélabon 2003, Forsgren et al. 2004) in this set-up. Because the video cameras recorded only what happened in and around each male's compartment, the recording did not give information on the position of the male and male behaviour in the rest of the aquarium. Therefore the same observer recorded by direct observation how much time a male spent in his nest and in his own compartment, and any male-male interactions anywhere in the aquarium.

We aimed to observe at a stage when some but not all females had spawned in both of the aquaria in a replicate. We did not observe before any spawning had taken place, because this might imply a lack of acclimatization to the experimental (captive) situation and the treatment. As a result, all observations took place the day after introduction of the females. In four cases, we found post observation that all females had spawned; these cases were excluded from analyses. In total we conducted 23 successful replicates of the female biased treatment (13 in 2008, 10 in 2009) and 24 replicates of the male biased treatment (13 in 2008, 11 in 2009). We excluded seven males from analyses (one died, one disappeared, and five showed aberrant behaviour indicative of lack of acclimatisation to the experimental setting; three males sat motionless and two males did not visit their own compartment during the observation; all 2008). In these replicates we did use the other male. There was no significant difference in male standard length ($t = 0.38$, df $= 85$, $P = 0.71$) or weight ($t = 0.14$, df $= 85$, $P = 0.89$) between the treatments (including only males used in analyses).

Post-treatment test

With free interaction between males and females, our set-up could not entirely exclude the possibility that male responses may have been affected by potential differences in female behaviour between treatments. We therefore wanted to test if males of the two treatments would respond

differently to a standardized visit by a ready-to-mate female. Directly after we had observed both males (see above), we closed one male compartment off from the rest of the aquarium with an opaque divider to prevent further visits. We then introduced a naive, ready-to-mate, female in a transparent cylinder into the compartment. We recorded on video for ten minutes. We then did the same for the other male. From video, we noted if and when the male approached and if and when he started courting. The time of approach was closely correlated to the time when the test female first moved after introduction. We therefore calculated approach and courtship latencies from this moment. Only one male approached the female before she moved, in this case latency was set to zero. After the test, we removed both nests and recorded the area covered with eggs in each nest (to the nearest 0.25 cm^2).

Measures of behaviour

We used the 20 min video recordings to calculate encounter rate with potential mates (the number of visits by ready-to-mate females), courtship frequency (the number of these visits with male courtship) and courtship propensity (the proportion of these visits with male courtship). We only used males that received visits from ready-to-mate females during the video recording for these calculations (52 males in 36 replicates). We did not include fin displays as courtship events, because they may be used in both courtship and agonistic behaviour and the difference between those was difficult to assess on some of the videos. Latency for both courtship and agonistic behaviour was calculated as the time in seconds from the start of the visit to the start of the behaviour, as seen in the video recordings. Six males seemed to court slim females once during the 20 minutes video observation, five in the male biased treatment, one in the female biased treatment. This courtship was never more than one fin display. Therefore, we did not consider courtship to slim females. Only eleven males in ten replicates received a visit from the other male, of which only two occurred in the female biased treatment. Therefore, we did not analyse the propensity to behave agonistically to a visiting male. We calculated the frequency of male-male fin displays per aquarium as a measure of frequency of agonistic behaviour. Furthermore, we calculated the proportion of time during the ten minutes direct observation a male spent in his nest and in his own compartment.

Statistical analyses

We tested for treatment effects on all our measures of behaviour in a linear mixed model (lmer; R) with male and replicate as a random factor. We used models assuming a binomial error distribution

for propensities, and a Poisson error distribution for frequencies. We first tested if there was an overall effect of treatment on each of these measures of behaviour. Secondly, because we expected the OSR at the moment of observation (OSR1; Table 1), the number of visits received and nest fullness to differ between the treatments, we tested if these factors were associated with the various behaviours using a path analyses (Figure 2). In the path analyses, we first tested if treatment indeed differed in these three factors (vector 1-3 in Figure 2) with a Mann-Whitney U test on the means per aquarium. For the second part of the path analyses we used the same models as for testing the overall treatment effects on behaviour, but entered the area of eggs, number of visits and current OSR (OSR1; Table 1) as additional fixed factors (vector a-c in Figure 2). We did not include OSR2 (OSR corrected for available nest space; Table 1) in the model, because this variable is inherently correlated with both OSR1 and nest fullness. Using treatment as a factor in the full model was done to test wether treatment affected behaviour in some other way than through OSR, nest fullness or the encounter rate (vector d in Figure 2). To allow for comparisons between effect sizes in the model, all fixed factors were standardized. In addition, we included the mean per aquarium in the model for all fixed factors, to test if within aquaria effects were similar to between aquaria effects (van de Pol & Wright 2009). A between aquaria effect is an effect of differences between aquaria in the variable, while a within aquarium effect is an effect of the difference in the variable between the males in an aquarium. For all models, we present both the full model, including all effects, and the reduced model, where we removed fixed effects one by one on the basis of z-values and P-values, to obtain the model with the lowest AIC (Aikaike information criterium). Linear mixed models were done in R (version 2.7.2 R development core team). All other statistics were done in SPSS 16.0.

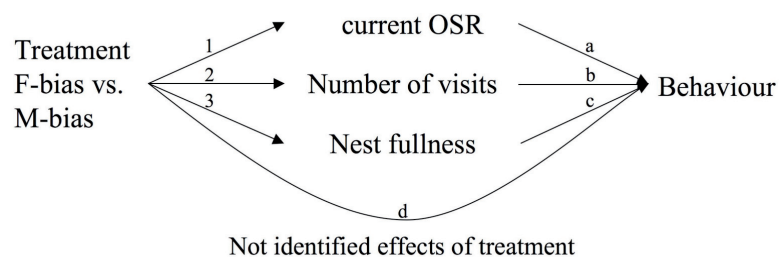**Figure 2.** Path analysis to test how the OSR treatment could affect behaviour of two-spotted gobies. We first tested if treatment affected OSR, encounter rate (number of visits) and nest fullness (1, 2 and 3). In the second model, we tested if these factors were associated with behaviour, and if there were any additional effects of treatment on behaviour when we controlled for these factors (a, b, c and d).

Ethical Note

All procedures in this experiment followed Swedish law and were carried out with permission from the ethical committee in Göteborg (Göteborgs djurförsöksetiska nämnd). We did not detect any physical injuries resulting from agonistic behaviour during our experiment. Before marking, we anaesthetized the fish with 60μl 2-phenoxyethanol diluted in 500ml of surface seawater. Following elastomer injection, the fish was immediately released in a container with fresh seawater. All fish regained normal swimming behaviour within 10 minutes after this procedure. VIE tagging has been shown not to influence mortality or behaviour in other gobies (Malone et al. 1999; Griffiths 2002; Whiteman & Côté 2004). Only one of 152 fish died during our experiment, which is an extremely low mortality rate given the high natural mortality during the breeding season in this short-lived species. After completion of the experiment, all fish were released back into the fjord.

Results

OSR, encounter rates and nest fullness

The treatments significantly differed in OSR at the moment of observation (current OSR) and nest fullness (vector 1 & 2 in Figure 2), as expected. Current OSR was higher in the male biased treatment (OSR1: $U = 28$, $P < 0.0001$, $N = 47$; Table 1). Nest fullness was higher in the female biased treatment ($U = 55.5$, $P < 0.0001$, $N = 47$; Figure 3A). Surprisingly, the number of visits by ready-to-mate females (vector 2 in figure 2) did not significantly differ between the treatments ($U = 216$, $P = 0.20$, $N = 47$; Figure 3B).

Courtship propensity

Overall, males in the male biased treatment were significantly more likely to court visiting ready-to-mate females than males in the female biased treatment (estimate $\pm$ SE: 2.16 $\pm$ 1.06, $z = 2.03$, $P = 0.043$, $N_{obs} = 320$, $N_{ind} = 52$, $N_{repl} = 36$; Figure 4A). Males also tended to approach a higher proportion of the round females that visited (estimate $\pm$ SE: 1.49 $\pm$ 0.80, $z = 1.84$, $P = 0.07$, $N_{ind} = 54$, $N_{repl} = 37$) and courted a higher proportion of the females they approached (estimate $\pm$ SE: 1.61 $\pm$ 0.79, $z = 2.04$, $P = 0.04$, $N_{obs} = 318$, $N_{ind} = 36$, $N_{repl} = 28$) in the male biased treatment. Only a minority of the males (nine in each treatment) showed one or more of the more advanced courtship behaviours (quiver and lead swim). Median courtship latency per male tended to be shorter in the male biased treatment (estimate (log-transformed) $\pm$ SE: -0.96 $\pm$ 0.59, t = -1.63, CI = -2.2 - 0.2).
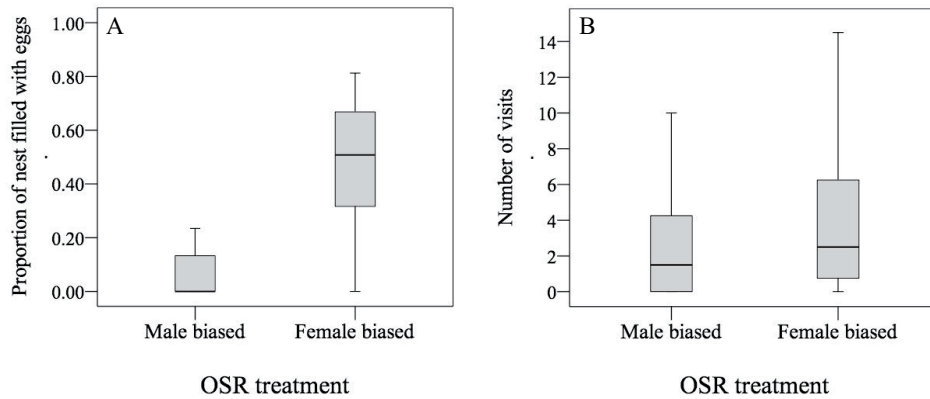
Figure 3. Differences between the male biased treatment and the female biased OSR-treatment in an experiment on male two-spotted gobies. A. Nest fullness as the proportion of the area in the nest covered with eggs. B. Encounter rate as the number of visits from ready-to-mate females each male received. Graphs are box plots representing the median and 25th-75th percentiles and lines represent minima and maxima. Data used are means per aquarium. N = 24 for male bias and N = 23 for female bias.

Figure 4. Differences in male behaviour between the male biased treatment and the female biased OSR-treatment in an experiment on two-spotted gobies. A. Courtship propensity as the proportion of visits by ready-to-mate females with male courtship. B. Courtship frequency as the number of these visits with male courtship. Graphs are box plots representing the median and 25th-75th percentiles and lines represent minima and maxima. The circles and stars denote outliers. Data shown are means per aquarium, while the tests are done on binomial data per visit for the propensity and counts per male for the frequency.

Courtship frequency

The total number of visits with male courtship (only including quiver and/or lead swim) did not differ significantly between treatments (estimate $\pm$ SE: 0.67 ± 0.58, $z = 1.15$, $P = 0.25$, $N_{ind} = 52$, $N_{repl} = 36$; Figure 4B), although among the males that courted, males in the male biased treatment tended to court more frequently (estimate $\pm$ SE: 0.66 ± 0.40, $z = 1.66$, $P = 0.096$, $N_{ind} = 18$, $N_{repl} = 17$). The number of round female visitors approached did not differ between treatments (estimate $\pm$ SE: 0.35 ± 0.31, $z = 1.13$, $P = 0.26$, $N_{ind} = 52$, $N_{repl} = 36$).

Male spatial and agonistic behaviour

Males in the male biased treatment spent on average a lower proportion of the time in their nest (estimate $\pm$ SE: -1.34 ± 0.46, $z = -2.95$, $P = 0.0032$, $N_{ind} = 87$, $N_{aq} = 47$; Figure 5A) and tended to spend less time in their own compartment (estimate $\pm$ SE: -1.21 ± 0.63, $z = -1.94$, $P = 0.053$, $N_{ind} = 87$, $N_{aq} = 47$; Figure 5B) than males in the female biased treatment. There were too few encounters between males in their own compartment to calculate the propensity to display. In the female biased treatment, two males in two replicates received visits of the other male (three visits in total); no agonistic behaviour was noted during these visits. In the male biased treatment, nine males in eight replicates received visits of the other male; at four of these thirteen visits, males displayed to each other. We did not observe any escalated agonistic behaviour between males during the observations. Males did meet in the neutral compartment and displayed to each other there. We observed 11 male agonistic interactions (in seven replicates), all in the male biased treatment.

Post treatment test

Males from the male biased treatment did not approach the contained stimulus female more often ($X^2 = 0.55$, df $= 1$, $P = 0.50$, $N = 87$) nor did they court her more often ($X^2 = 0.73$, df $= 1$, $P = 0.39$, $N = 87$), than males from the female biased treatment. However, out of 20 males from the male biased treatment that approached the female, 19 initiated courtship, compared to 14 out of 22 males from the female biased treatment ($X^2 = 6.12$, df $= 1$, $P = 0.013$, $N = 42$). Hence, among males showing interest in the stimulus female, the propensity to court was higher in the male biased treatment. Courtship latency (s) also tended to be shorter in the male biased treatment (estimate $\pm$ SE $= -0.71 \pm 0.41$, $z = -1.75$, $P = 0.08$, $N_{ind} = 33$, $N_{aq} = 28$), while the latency to approach the test female did not differ between treatments (male bias: estimate $\pm$ SE $= -0.51 \pm 0.45$, $z = -1.16$, $P = 0.25$, $N_{ind} = 42$, $N_{aq} = 32$). Time to first movement of the female did not differ between treatments (male bias: estimate $\pm$ SE $= -0.23 \pm 0.74$, $z = -0.30$, $P = 0.76$, $N_{ind} = 77$, $N_{aq} = 43$).
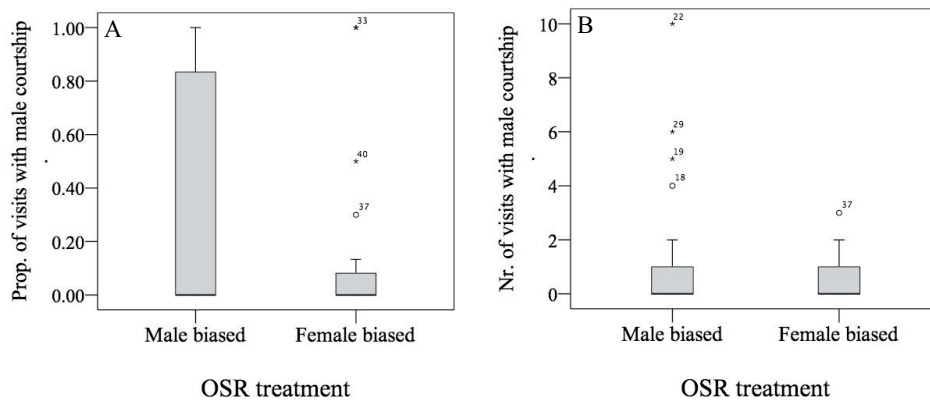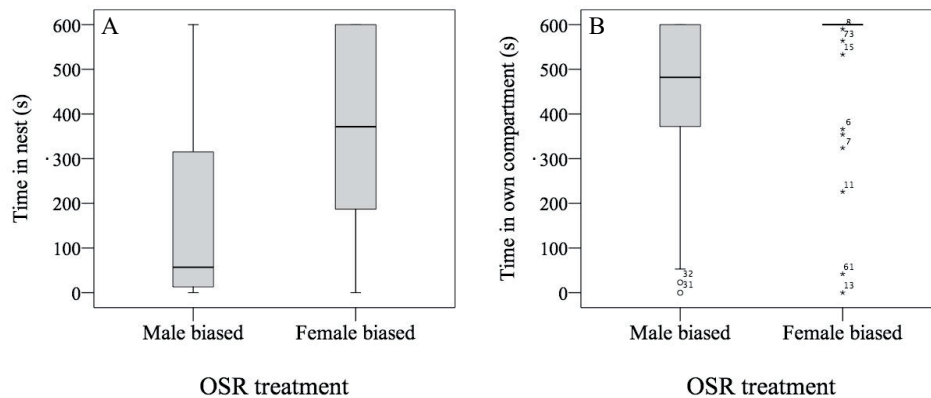
Figure 5. Differences in male movement patterns between the male biased and the female biased OSR-treatment in an experiment on two-spotted gobies. A. The total time (s) a male spent in his nest. B. The total time (s) a male spent in his own compartment. Graphs are box plots representing the median and 25th-75th percentiles and lines represent minima and maxima. The circles and stars denote outliers. Data shown are means per aquarium during ten minutes direct observation, while the tests are done on proportions of the total time per male. N = 24 for male bias and N = 23 for female bias.

OSR, nest fullness, encounter rate and male behaviour

In the path analyses we tested if OSR, encounter rate or nest fullness were associated with each of the recorded behaviours (Figure 2). The propensity to court was most strongly associated with current OSR and the number of visits in the full model, but was only significantly associated with current OSR in the reduced model (Table 2A). Males in a more male-biased OSR were significantly more likely to court ready-to-mate females (Table 2A). The frequency of male courtship on the other hand, which did not differ between treatments, was significantly associated with the number of visits by ready-to-mate females, but not with nest fullness or OSR. Within aquaria, the male that received most visits courted most often, as expected (Table 2A). However, between aquaria, the association was the opposite of expected, courtship frequency was higher in aquaria where males received less visits (Table 2A). A non-significant association with the average area of eggs in an aquarium also remained in the reduced model (Table 2A).

The path analyses for approaches to ready-to-mate females showed a similar pattern as the one for courtship behaviour, but there were some differences. The propensity to approach was mainly associated with current OSR, as for the propensity to court (Table 2B), although the association was

only borderline significant. However, in the reduced model, there was also a non-significant negative association with the mean number of female visits per aquarium (Table 2B). Approach frequency was significantly associated with within aquaria differences in the number of female visits, as for courtship frequency. In contrast to courtship frequency, the frequency of approaches was not significantly associated with the between aquarium differences in the number of visits, and a non-significant additional effect of treatment remained in the reduced model (Table 2B).

The proportion of time a male spent in his nest was positively associated with nest fullness (Table 2C), while the proportion of time a male spent in his own compartment was not significantly associated with any of the variables in the model (Table 2C).

Table 2. Results from a path analyses exploring how current OSR, number of visits and nest fullness are associated with behaviour in male two-spotted gobies. A. Courtship behaviour, B. Approaches to ready-to-mate females, C. Male spatial behaviour. We tested for any additional effects of OSR-treatment by including treatment in the model. For each measure of behaviour both the full model and the maximum reduced model (based on AIC selection) are given. Effects are split into within and between-aquaria effects. We present estimates (logit) ± SD and the values in brackets are z-values and P values, respectively. Significant effects (P < 0.05) are given in bold. Below the line are AIC values and sample size for each model.

Table 2A	Courtship propensity		Courtship frequency	
	Full	Reduced	Full	Reduced
Intercept	-3.65 ± 1.15	-3.70 ± 0.60	-1.52 ± 0.75	-1.26 ± 0.35
Current OSR	0.79 ± 1.02 (0.78, 0.44)	**1.06 ± 0.49 (2.17, 0.03)**	-0.11 ± 0.68 (-0.16, 0.88)	
Eggs-within	-0.04 ± 1.02 (-0.04, 0.97)		0.03 ± 0.44 (0.06, 0.96)	
Eggs-between	-0.42 ± 1.38 (-0.31, 0.76)		-0.41 ± 0.67 (-0.61, 0.54)	-0.58 ± 0.35 (-1.64, 0.10)
Visits-within	2.02 ± 1.86 (1.09, 0.28)		**1.91 ± 0.62 (3.11, 0.002)**	**1.92 ± 0.60 (3.20, 0.0014)**
Visits-between	-2.06 ± 1.83 (-1.13, 0.26)		**-1.48 ± 0.65 (-2.27, 0.02)**	**-1.48 ± 0.62 (-2.38, 0.018)**
Treatment	0.27 ± 2.58 (0.11, 0.92)		0.64 ± 1.57 (0.41, 0.68)	
AIC	190.9	183.0	89.6	85.4
N obs/aq (male)	320/36 (52)	320/36 (52)	52/36	52/36

Table 2B	Approach propensity		Approach frequency	
	Full	Reduced	Full	Reduced
Intercept	-1.24 ± 0.80	-1.53 ± 0.42	0.04 ± 0.38	0.24 ± 0.16
Current OSR	0.89 ± 0.67 (1.33, 0.18)	0.69 ± 0.36 (1.95, 0.05)	0.06 ± 0.33 (0.19, 0.85)	
Eggs-within	0.17 ± 0.58 (0.29, 0.77)		-0.01 ± 0.26 (0.04, 0.97)	
Eggs-between	-0.38 ± 0.80 (-0.48, 0.63)		0.01 ± 0.35 (0.03, =0.98)	
Visits-within	0.07 ± 0.84 (0.09, 0.93)		**0.84 ± 0.29 (2.85, 0.004)**	**0.84 ± 0.28 (3.00, 0.003)**
Visits-between	-0.93 ± 0.94 (-0.99, 0.32)	-0.93 ± 0.49 (-1.92, 0.06)	-0.50 ± 0.32 (-1.56, 0.12)	-0.52 ± 0.30 (-1.73, 0.08)
Treatment	-0.74 ± 1.78 (-0.42, 0.68)		0.45 ± 0.80 (0.57, 0.57)	0.55 ± 0.31 (1.75, 0.08)
AIC	321.1	313.4	97.6	91.7
N obs/aq (male)	318/36 (52)	318/36 (52)	52/36	52/36

Table 2C	Proportion of time in nest		Proportion of time in own compartment	
	Full	Reduced	Full	Reduced
Intercept	-0.96 ± 0.57	-0.89 ± 0.17	-0.41 ± 0.40	-0.21±0.12
current OSR	-0.13 ± 0.36 (-0.36, 0.72)		-0.08 ± 0.26 (-0.32, 0.75)	
Eggs-within	0.01 ± 0.28 (0.04, 0.97)		0.06 ± 0.22 (0.25, 0.80)	
Eggs-between	0.41 ± 0.40 (1.01, 0.31)	**0.41 ± 0.15 (2.65, 0.0081)**	0.22 ± 0.30 (0.73, 0.47)	0.16 ± 0.12 (1.38, 0.17)
Visits-within	-0.19 ± 0.32 (-0.58, 0.56)		-0.00 ± 0.24 (-0.00, 1.00)	
Visits-between	0.06 ± 0.33 (0.18, 0.86)		0.01 ± 0.25 (0.04, 0.97)	
Treatment	0.12 ± 1.02 (0.11, 0.91)		0.37 ± 0.73 (0.51, 0.61)	
AIC	43.2	34.0	26.6	16.9
N obs/aq	87/47	87/47	87/47	87/47

Discussion

We found that OSR as such, regardless of adult sex ratio, affects competitive mating behaviour in male two-spotted gobies. Moreover, we found a difference in results between courtship propensity and courtship frequency. Males were more likely to court encountered ready-to-mate females in the male biased treatment, but courtship frequency did not differ between treatments. In the post-treatment test, more of the males from the male biased treatment that approached the female initiated courtship. Male-male interactions only occurred in the male biased treatment, and were overall uncommon.

OSR and courtship propensity
Both our calculations of OSR were higher in the male biased treatment (Table 1). Hence, we succeeded in changing the operational sex ratio in this experiment, without changing the sex ratio or density. To our knowledge, there are very few other studies that changed the OSR without changing adult sex ratio. These few studies have typically changed OSR by changing potential reproductive rates through food availability (Gwynne & Simmons 1990), food quality (Gwynne 1993), temperature (Kvarnemo 1996) or nest availability (Borg et al. 2002, Saraiva et al. 2009). In this experiment, we did not change anything but the number of ready-to-mate females.

As expected, male courtship propensity was significantly higher in a male biased OSR, and was significantly associated with current OSR, not with nest fullness or the encounter rate with ready-to-mate females. Only eighteen males showed advanced courtship behaviours within our observation time, it is therefore reassuring that the propensity to approach ready-to-mate females was similarly, though not significantly, associated with OSR. In the post-treatment test, males from the male biased treatment were more likely to court than males from the female biased treatment, but only among males that approached the stimulus female. It is possible that the lack of an overall treatment effect on courtship propensity in the post-treatment test was caused by some unavoidable disturbance during the removal and introduction of females and the introduction of a divider. Therefore, it is likely that the results from the sub-set of males that approached the stimulus female are more informative.

To our knowledge, very few other laboratory experiments have looked at an effect of OSR on male propensity to court individual females, even though courtship propensity should reflect the motivation of an individual to court better than courtship frequencies (Paper II). Berglund (1994)

reported that, as expected, male latency to court females was higher in a male biased treatment in the pipefish *Syngnathus typhle*. On the other hand, Faleiro and co-authors (2008) found that males of the long-snouted seahorse (*Hippocampus guttulatus*) initiated all courtship events, regardless of the sex ratio treatments. In field studies, using propensities is more common (Almada et al. 1995, Borg et al. 2002, Forsgren et al. 2004, Cratsley & Lewis 2005, Lengkeek & Didderen 2006, Shibata & Kohda 2006) and most of these studies have found an increase in courtship propensity in situations where the OSR was likely biased towards the own sex. In a field study on the two-spotted goby, male propensity to court was found to decrease over the season, which coincided with a change in OSR from male to female biased (Forsgren et al. 2004). Our data suggest that the change in courtship behaviour in the field may be caused by the change in OSR, although other factors such as a cost of breeding may also be important (Kokko & Monaghan 2001, Kokko & Johnstone 2002).

Courtship frequency

Courtship frequency did not differ between treatments, and was associated with the encounter rate with potential mates, not with the OSR. The number of visits by ready-to-mate females was significantly and positively associated with courtship frequency within aquaria; males that received more visits courted more, as expected. Between aquaria, however, there was a negative association between the mean number of visits and mean male courtship frequency. Because both the courtship frequency and the mean number of visits did not significantly differ between treatments, this is likely to be a within treatment effect and not an effect related to OSR. An alternative explanation would be that individual variation in male eagerness may have caused a lower average courtship frequency in some aquaria, and as a result, females had to visit males more often to spawn.

In the two-spotted goby, courtship frequency was lower in a male biased OSR in a set-up were individuals were in constant visual contact (de Jong et al. 2009). In the present study courtship frequency did not differ between treatments. This suggests that effects of OSR on courtship frequency can vary between study designs or levels of OSR. Courtship frequency has been shown to increase in competitive situations (e.g. Farr 1976, Bremner et al. 2002, Makowicz et al. 2010), but may decrease when competition becomes too high (e.g. Verrell 1983, Verrell & Krenz 1998). Studies that test the effect of OSR on courtship frequency report variable results, but most studies have found a decrease in the number of courtship displays in a male-biased OSR (reviewed in Paper II). For example, in the Japanese medaka, males are reported to switch from courtship to agonistic behaviour to scramble competition in increasingly male biased OSR (Grant et al. 1995, Grant & Foam 2002, Grant et al. 2000, Clarck & Grant 2010). In guppies, males switch to coersion tactics in

stead of courtship displays, when the OSR becomes male biased (Jirotkull 1999, Magellan & Maguran 2007, Evans & Magurran 1999, but see Head et al. 2008), However, in guppies, the courtship each female received did not decrease in a male-biased OSR (Jirotkull 1999), suggesting the decreased courtship frequency in males may be caused by a decrease in encounter rates with females. If courtship frequency is mainly affected by encounter rates, as our data suggest, these patterns could be explained without inferring a trade-off. Thus, encounter rates should be carefully considered in future experiments.

Encounter rates

Surprisingly, the encounter rate with potential mates, as the number of visits from ready-to-mate females in twenty minutes, was not significantly higher in the female biased treatment (Figure 3B). This may suggest that females adjust their visiting behaviour to the operational sex ratio. Females may be choosier in a male biased OSR (e.g. Emlen & Oring 1977, Balshine-Earn 1996, Owens & Thompson 1994), and therefore visit males more often before they decide to spawn. On the other hand, females in the female biased treatment could restrain from visiting to avoid female-female competition, which should be higher in female biased OSR (Emlen & Oring 1977, Kvarnemo & Ahnesjö 1996, 2002) or females could show restricted mate search in high competition (Dale et al. 1992). This experiment cannot distinguish between these alternative explanations. Nevertheless, these data show that inter-sexual encounter rate is not necessarily linearly related to the density of potential mates in the population. Therefore, correcting courtship and agonistic frequencies for the number of other individuals in an aquarium, as some studies have done (Jivoff & Hines 1998, Debuse et al. 1999, Silva et al. 2010), may not be as straightforward as previously thought.

Agonistic behaviour

Male-male interactions were only observed in the male biased treatment, even though male density did not differ between treatments. Male mate search behaviour could also to change in relation to female density (e. g. Kokko & Wong 2007). In the present study, males spent less time in their nest and tended to spend less time in their own compartment in the male biased treatment. Time in nest was significantly affected by the mean area of eggs in the nest in an aquarium, suggesting that males Thus, males were probably more likely to encounter each other in the male biased treatment. An increase in the frequency of male agonistic behaviours in a male biased OSR has been found repeatedly (e. g. Kodric-Brown 1988, Grant et al. 1995, Kvarnemo et al. 1995, Gwynne & Simmons 1990, Jirotkul 1999, Forsgren et al. 2004), although a decrease has also been found, especially in

high male densities (e. g. Grant et al. 2000, Michener & Mclean 1996, Smith et al. 2007) suggesting a dome-shaped response (Grant & Foam 2002, Clark & Grant 2010). In this experiment, males did not encounter each other regularly, and were not able to see each other from their nest, thus males may have experienced the set-up as a rather low male density, leading to very few agonistic acts overall. Thus, our results corroborate the general pattern that male agonistic frequency increases with OSR in low male density. However, we cannot conclude whether males were more likely to behave agonistically (higher agonistic propensity) or if the agonistic behaviour we observed was simply caused by an increase in male-male encounter rates.

Conclusions

Our data show that, all other factors being equal, OSR can affect the strength of mating competition. We show that male propensity to court was affected by OSR, while male courtship frequency was affected by the encounter rate with potential mates. This is to our knowledge the first laboratory experiment that quantified encounter rates, courtship propensity and courtship frequency in relation to OSR. We show that encounter rates are not necessarily linearly related to OSR. Moreover, our data suggest that males increase movement outside the nest in a male-biased OSR, while females visit males less often in a female biased OSR. In addition, we show that male courtship frequency is affected by inter-sexual encounter rate, while agonistic frequency may be affected by intra-sexual encounter rate. More studies on the effect of OSR on the propensity to behave competitively are urgently needed. In particular, future studies should aim to disentangle the effects of propensities to behave and encounter rates on the reported effects of OSR on frequencies of courtship and agonistic behaviour.

Acknowledgements

References

Almada, V. C., Gonçalves, E. J., Oliveira, R. F. & Santos, A. J. 1995. Courting females - ecological constraints affect sex-roles in a natural-population of the blenniid fish *Salaria pavo*. Animal Behaviour 49, pp. 1125-1127.

Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. Proceedings Of The National Academy Of Sciences Of The United States Of America 98, pp. 13155-13160.

Andersson, 1994. Sexual Selection. Princeton University Press, Princeton, NJ.

Balshine-Earn, S. 1996. Reproductive rates, operational sex ratios and mate choice in St. Peter's fish. Behavioral Ecology and Sociobiology 39, pp. 107-116.

Bateman, A. J. 1948. Intra-sexual selection in Drosophila. Heredity 2, 349-368.

Berglund, A. 1994. The operational sex-ratio influences choosiness in a pipefish. Behavioral Ecology 5, pp. 254-258.

Bjelvenmark, J. & Forsgren, E. 2003. Effects of mate attraction and male-male competition on paternal care in a goby. Behaviour 140, pp. 55-69.

Borg, Å. A., Forsgren, E. & Magnhagen, C. 2002. Plastic sex-roles in the common goby - the effect of nest availability. Oikos 98, pp. 105-115.

Bremner, A. A., Trippel, E. A. & Terrhune, J. M. 2002. Sound production by adult haddock, *Melanogrammus aeglefinus*, in isolation, pairs and trios. Environmental Biology of Fishes 65, pp. 359-362.

Collins, S. P. 1981. Littoral and benthic investigations on the west-coast of ireland. XII. The biology of *Gobiusculus flavescens* (Fabricius) on the Connemara coast. Proceedings Of The Royal Irish Academy Section B. 81, pp. 63-87.

Clark, L. & Grant, J. W. A. 2010. Intrasexual competition and courtship in female and male Japanese medaka, Oryzias latipes: effects of operational sex ratio and density. Animal Behaviour 80, pp. 707-712.

Cratsley, C. K. & Lewis, S. M. 2005. Seasonal variation in mate choice of *Photinus ignitus* fireflies. Ethology 111, pp. 89-100.

Dale, S., Rinden, H. & Slagsvold, T. 1992. Competition for a mate restricts mate search of female pied flycatchers. Behavioral Ecology and Sociobiology 30, pp. 165-176.

Darwin, C. 1871. The descent of Man, and Selection in Relation to Sex. Appleton, New York.

Debuse, V. J., J. T. Addison, and J. D. Reynolds. 1999. The effects of sex ratio on sexual competition in the European lobster. Animal Behaviour 58, pp. 973-981.

de Jong, K., Wacker, S., Amundsen, T. & Forsgren, E. 2009. Do operational sex ratio and density affect mating behaviour? An experiment on the two-spotted goby. Animal Behaviour 78, pp. 1229-1238.

Emlen, S. T. 1976. Lek organization and mating strategies in the bullfrog. Behavioral Ecology and Sociobiology 1, pp. 283-313.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. Science 197, pp. 215-223.

Evans, J. P. & A. E. Magurran. 1999. Male mating behaviour and sperm production characteristics under varying sperm competition risk in guppies. Animal Behaviour 58, pp. 1001-1006.

Faleiro, F., Narciso, L. & Vicente, L. 2008. Seahorse behaviour and aquaculture: How to improve *Hippocampus guttulatus* husbandry and reproduction? Aquaculture 282, pp. 33-40.

Farr, J. A. 1976. Social facilitation of male sexual-behavior, intra-sexual competition, and sexual selection in guppy, *Poecilia reticulata* (Pisces - Poeciliidae). Evolution 30, pp. 707-717.

Fitze, P. S. & Le Galliard, J. 2008. Operational sex ratio, sexual conflict and the intensity of sexual selection. Ecology Letters 11, pp. 432-439.

Forsgren, E., Amundsen, T., Borg, Å. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles in a fish. Nature 429, pp. 551-554.

Grant, J. W. A. & Foam, P. E. 2002. Effect of operational sex ratio on female-female versus male-male competitive aggression. Canadian Journal Of Zoology 80, pp. 2242-2246.

Grant, J W. A., Bryant, M. J. & Soos, C. E. 1995. Operational sex-ratio, mediated by synchrony of female arrival, alters the variance of male mating success in Japanese medaka. Animal Behaviour 49, pp. 367-375.

Grant, J. W. A., Gaboury, C. L. & Levitt, H. L. 2000. Competitor-to-resource ratio, a general formulation of operational sex ratio, as a predictor of competitive aggression in

Japanese medaka (Pisces: Oryziidae). Behavioral Ecology 11, pp. 670-675.

Griffiths, S. P. 2002. Retention of visible implant tags in small rockpool fishes. Marine Ecology Progress Series 236, pp. 307-309.

Gwynne, D. T. 1984. Sexual selection and sexual differences in Mormon crickets (Orthoptera: Tettigoniidae, Anabrus simplex). Evolution 38, pp. 1011-1022.

Gwynne, D. T. 1993. Food quality controls sexual selection in Mormon crickets by altering male mating investment. Ecology 74, pp. 1406-1413.

Gwynne, D. T. & Simmons, L. W. 1990. Experimental reversal of courtship roles in an insect. Nature 346, pp. 172 - 174.

Head, M. L., Lindholm, A. K. & Brooks, R. 2008. Operational sex ratio and density do not affect directional selection on male sexual ornaments and behavior. Evolution 62, pp. 135-144.

Jirotkul, M. 1999. Operational sex ratio influences female preference and male-male competition in guppies. Animal Behaviour 58, pp. 287-294.

Jivoff, P., & Hines. A. H. 1998. Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. Animal Behaviour 55, pp. 589-603.

Kodric-Brown, A. 1988. Effects of sex-ratio manipulation on territoriality and spawning success of male pupfish, *Cyprinodon pecosensis*. Animal Behaviour 36, pp. 1136-1144.

Kokko, H. & Johnstone, R. 2002. Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. Philosophical Transactions of the Royal Society B. 357, pp. 319-330.

Kokko, H. & Monaghan, P. 2001. Predicting the direction of sexual selection. Ecology Letters 4, pp. 159-165.

Kokko, H. & Wong, B. B. M. 2007. What determines sex roles in mate searching?. Evolution 61, pp. 1162-1175.

Kvarnemo, C. 1996. Temperature affects operational sex ratio and intensity of male-male competition: Experimental study of sand gobies, *Pomatoschistus minutus*. Behavioral Ecology 7, pp. 208-212.

Kvarnemo, C. & Ahnesjö, I. 1996. The dynamics of operational sex ratios and competition for mates. Trends In Ecology & Evolution 11, pp. 404-408.

Kvarnemo, C. & Ahnesjö, I. 2002. Operational sex ratios and mating competition. In: Sex ratios: concepts and research methods (ed.

Hardy, I. C. W.), Cambridge University Press, Cambridge, pp 366-382.

Kvarnemo, C., Forsgren, E. & Magnhagen, C. 1995. Effects of sex ratio on intra-and inter-sexual behaviour in sand gobies. Animal Behaviour 50, pp. 1455-1461.

Magellan, K. & Magurran, A. E. 2007. Behavioural profiles: individual consistency in male mating behaviour under varying sex ratios. Animal Behaviour 74, pp. 1545-1550.

Makowicz, A., Plath, M. & Schlupp, I. 2010. Using video playback to study the effect of an audience on male mating behaviour in the Sailfin molly (*Poecilia latipinna*). Behavioural Processes 85, pp. 36-41.

Malone, J. C., Forrester, G. E. & Steele, M. A. 1999. Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. Journal of Experimental Marine Biology and Ecology 237, pp. 243-253.

Michener, G. R. & McLean, I. G. 1996. Reproductive behaviour and operational sex ratio in Richardson's ground squirrels. Animal Behaviour 52, pp. 743-758.

Miller, P. J. 1986. Gobiidae. In: Fishes of the North-eastern Atlantic and the Mediterranean (ed. P. J. P. Whitehead, M. L. Bauchot, J. C. Hureau, J. Nielsen & E. Tortonese), UNESCO, Paris, pp. 1019-1085.

Mobley, K. B., Amundsen, T., Forsgren, E., Svensson, P. A. & Jones, A. G. 2009. Multiple mating and a low incidence of cuckoldry for nest-holding males in the two-spotted goby, *Gobiusculus flavescens*. BMC Evolutionary Biology 9:6.

Lengkeek, W. & Didderen, K. 2006. Breeding cycles and reproductive behaviour in the river blenny *Salaria fluviatilis*. Journal of Fish Biology 69, pp. 1837-1844.

Okuda, N. 1999. Sex roles are not always reversed when the potential reproductive rate is higher in females. American Naturalist 153, pp. 540-548.

Owens, I. P. F. & Thompson, D. B. A. 1994. Sex differences, sex ratios and sex roles. Proceedings of the Royal Society B. 258, pp. 93-99.

Pélabon, C., Borg, Å. A., Bjelvenmark, J., Forsgren, E., Barber, I. & Amundsen, T. 2003. Do male two-spotted gobies prefer large fecund females? Behavioral Ecology 14, pp. 787-792.

Pol, M. vd & Wright, J. 2009. A simple method for distinguishing within- versus between-

subject effects using mixed models. Animal Behaviour 77, pp. 753-758.

R Development Core Team. 2008. R: A Language and Environment for Statistical Computing, Vienna, Austria.

Saraiva, J., Barata, E., Canário, A. & Oliveira, R. 2009. The effect of nest aggregation on the reproductive behaviour of the peacock blenny *Salaria pavo*. Journal of Fish Biology 74, pp. 754-762.

Shibata, J. & Kohda, M. 2006. Seasonal sex role changes in the blenniid *Petroscirtes breviceps*, a nest brooder with paternal care. Journal of Fish Biology 69, pp. 203-214.

Shine, R., Langkilde, T. & Mason, R. T. 2003. The opportunistic serpent: Male garter snakes adjust courtship tactics to mating opportunities. Behaviour 140, pp. 1509-1526.

Sih, A., Krupa, J. J. 1995. Interacting effects of predation risk and male and female density on male–female conflicts and mating dynamics of stream water striders. Behavioral Ecology 6, pp. 316-325.

Silva, K., Vieira, M. N., Almada, V. C. &. Monteiro, N. M. 2010. Reversing sex role reversal: compete only when you must. Animal Behaviour 79, pp. 885-893.

Skolbekken, R. & Utne-Palm, A. C. 2001. Parental investment of male two-spotted goby, *Gobiusculus flavescens* (Fabricius). Journal Of Experimental Marine Biology And Ecology 261, pp. 137-157.

Smith, C. C. 2007. Independent effects of male and female density on sexual harassment, female fitness, and male competition for mates in the western mosquitofish *Gambusia affinis*. Behavioral Ecology and Sociobiology 61, pp. 1349-1358.

Souroukis, K. & Murray, A. M. 1995. Female mating-behavior in the field cricket, *Gryllus pennsylvanicus* (Orthoptera, Gryllidae) at different operational sex-ratios. Journal Of Insect Behavior 8, pp. 269-279.

Svensson, P. A. 2006. Female coloration, egg carotenoids and reproductive success: gobies as a model system. Thesis for the degree of Philosophiae Doctor, NTNU, Trondheim.

Svensson P. A., Barber, I. & Forsgren, E. 2000. Shoaling behaviour of the two-spotted goby. Journal of Fish Biology 56, pp. 1477-1487.

Svensson, P. A., Pélabon, C., Blount, J. D., Forsgren, E., Bjerkeng, B. & Amundsen, T. 2009. Temporal variability in a multicomponent trait: nuptial coloration of female two-spotted gobies. Behavioral Ecology 20, pp. 346-353.

Verrell, P. A. 1983. The influence of the ambient sex-ratio and intermale competition on the sexual-behavior of the red-spotted newt, *Notophthalmus viridescens* (Amphibia, urodela, salamandridae). Behavioral Ecology and Sociobiology 13, pp. 307-313.

Vincent, A. C. J., Ahnesjö, I., Berglund, A. and Rosenqvist, G. 1992. Pipefishes and seahorses - are they all sex-role reversed. Trends in Ecology and Evolution 7, pp. 237-241.

Whiteman, E. A. & Côté, I. M. 2004. Dominance hierarchies in group-living cleaning gobies: causes and foraging consequences. Animal Behaviour 67, pp. 239-247.

Paper IV

Seasonal variations in reproductive behaviour of the two-spotted goby, a fish with dynamic sex roles

Karen de Jong[1], Lise Cats Myhre[1], Sebastian Wacker[1], Elisabet Forsgren[2] & Trond Amundsen[1]

[1]Department of Biology, Norwegian University of Science and Technology (NTNU), 7491 Trondheim, [2]The Norwegian Institute for Nature Research (NINA), Trondheim

ABSTRACT: To interpret results of experimental studies, we need to understand the ecology of our model organism. The two-spotted goby has proven useful as a model system in a variety of topics, but knowledge about the reproductive biology in natural populations is still relatively scant. In this study we describe the reproductive behaviour of male two-spotted gobies breeding in artificial nests in the Gullmar fjord on the west coast of Sweden. We placed 10 artificial nests at one location in 2007 and 45 artificial nests at each of two locations in 2008. We inspected nests regularly over the season and marked, measured and weighed males that attended the nests. Our results indicate that males cared for relatively synchronous broods and did not generally attend the same nest longer than the developmental period of one brood. Our results also indicate that males competed for breeding opportunities early in the season, when male density is high. Early in the season, more of the nests were occupied and males attending a nest early were larger (in 2008) or in better condition (in 2007) than males attending a nest late in the season. In addition, males attending a nest were in better condition than males in feeding shoals (only data for 2007). Late in the season, males received more visits from ready-to-mate females in one of the years (2008), but they did not have fuller nests (both years). Brood size was smaller late than early in the season and was associated with nest size. Early in the season brood size was also associated with male size. Brood survival was associated with brood size and lower late than early in the season.

Introduction

Theory is typically designed to be general. However, model systems used to test theory have different ecological backgrounds. To interpret experimental results and to compare across model species it is therefore important to be able to relate experimental results to the biology of the species in its natural habitat. In addition, there is a danger that laboratory studies without sufficient knowledge on a species' natural biology become unrealistic, with limited scope for inferences. Fish are becoming more and more important as model species for testing theory in a variety of areas (e.g. Avise et al. 2002, Hart & Reynolds 2002, Amundsen 2003, Hendry & Stearns 2004, Ahnesjö et al. 2008). However, there is often a lack of biological background knowledge, such as the reproductive biology from field populations (Amundsen 2003, Spence et al. 2008).

Here, we investigate the reproductive biology of a temperate marine fish, the two-spotted goby, *Gobiusculus flavescens*, in the wild. The two-spotted goby has been very useful as a model species to test theory in a wide range of topics. These include sex roles and mating competition (Forsgren et al. 2004, de Jong et al. 2009, Paper III), male and female mate choice (Amundsen & Forsgren 2001, 2003, Pélabon et al. 2003, Borg et al. 2006), sexual ornamentation (Sköld et al. 2008, Svensson et al. 2005, 2006, 2009a, 2009b), parental care (Skolbekken and Utne-Palm 2001, Bjelvenmark & Forsgren 2003), habitat use (Utne et al. 1993, Folkestad 2005), parasitology (Zander et al. 1993, Pelabon et al. 2005, Zander 2005a, 2005b, Barber et al. 2009) and predator-prey interactions (Steingrund & Ferno 1997, Utne & Bacchi 1997, Utne-Palm 2001). The species inhabits the shallow algal zone (ca 0-10 m depth) during the breeding season. It is probably the most abundant fish species in kelp forests along rocky shores of Norway and Sweden, and a key species in the ecosystem (e.g. Fosså 1991). Interestingly, the two-spotted goby also seems to be among the first fish species to colonise new man-made structures like the foundations of wind turbines (Anderson et al. 2009, Anderson & Öhman 2010).

The two-spotted goby has remarkably dynamic sex roles (Forsgren et al. 2004). Early in the season males compete for females, while late in the season females compete for mates. This switch in sex roles coincides with a change in operational sex ratio (OSR; Emlen & Oring 1977) over the season, from male to female biased (Forsgren et al. 2004). A change in OSR has subsequently been shown to affect competitive mating behaviour in this species (Paper III). This raises several questions about the reproductive behaviour of both males and females. A recent field study has investigated female mate sampling behaviour (L. C. Myhre, K. de Jong, E. Forsgren & T.

Amundsen, unpublished data), but little is known about male breeding behaviour in the wild. A study on the genetic mating system in the two-spotted goby has shown that successful sneaking is very limited in this species, and that parental males mate with multiple females (Mobley et al. 2009). The study also shows that nest size affects mating and reproductive success, while mating success was not associated with male size late in the season (Mobley et al. 2009), when females are abundant (Forsgren et al. 2004). In the present study we placed artificial nests (PVC-tubes) in the field to follow males over multiple breeding events and describe male reproductive behaviour over the breeding season. In addition, we tested whether brood size and brood survival was associated with time of season, male size and condition, and nest size. Below we will elaborate on these questions.

In animals with a restricted breeding season, it should be advantageous to breed as early as possible, to increase the time the offspring can spend feeding and growing before winter, enhancing winter survival (Fairbairn 1977, Hendersson et al. 1988, but see Schultz 1993). However, the reproductive success of a male depends both on brood survival and mating success. For male two-spotted gobies that might have a low chance to gain a nest and mate early in the season, it may be advantageous to postpone breeding to later in the season, when mating competition is relaxed, even if conditions for egg survival and offspring fitness may be worse. Thus, we expected more of the artificial nests to be occupied early in the season and more competition for nests and mates. On the other hand, we expected brood size to be larger late in the season, when females compete for males (Forsgren et al. 2004).

A laboratory experiment on the two-spotted goby showed that larger males display more competitive mating behaviour than smaller males (S. Wacker, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). In addition, early in the season, females have been shown to prefer large males (Borg et al. 2006). Thus, size is a clear candidate for a trait that may be associated with mating success. Moreover, nest size affects mating success in this species (Mobley et al. 2009). Thus, there may be competition for larger nests, leading to larger males attending larger nests (Lindström 1988, Wong et al. 2008), at least early in the season. We also tested whether high quality (in size and / or condition) males bred early in the season, while low quality males bred late.

Methods

This study was conducted in the summers of 2007 and 2008, at three sites in the Gullmar fjord at the west coast of Sweden. We divided the breeding season (late April to July) into three parts; early (29 April - 2 June), mid (3 - 24 June) and late (25 June -17 July) and focussed on early and late for seasonal comparisons.

Model Species

The two-spotted goby belongs to the group of sand gobies (Huyse et al. 2004). Year-old gobies become mature when they are between 30 and 45 mm long in the Danish waters, the Baltic and along the coast of South Norway, and they do typically not survive to the next breeding season (Johnson 1944, Collins 1981). Further north however, the size-distribution that suggests they live longer, due to a delay in reaching maturity (Johnson 1944). Their main food source consists of copepods (Berg 1979). During its breeding season (April–July in our study area) it inhabits shallow waters near rocky shores of the coast of Europe from northwest Spain to northern Norway (Miller 1986). Breeding males defend a nest in empty mussel shells, e.g. *Mytilus edulis* (Breder & Rosen 1966, Mobley et al. 2009), in crevices, or in brown algae (typically *Saccharina latissima* and *Laminaria digitata*; Zostera & Laminaria Sacherina; Petersen 1891). The male provides all parental care, defending the nest and cleaning and aerating the eggs until they hatch (Petersen 1891; Bjelvenmark and Forsgren 2003; Skolbekken & Utne-Palm 2001). A male can care for clutches from several different females simultaneously (Gordon 1983, Mobley et al. 2009). Both males and females are ornamented (Guitel 1895, Amundsen & Forsgren 2001). Males have colourful fins and iridescent spots along the body (Guitel 1895, Amundsen & Forsgren 2001) Females develop round orange bellies with egg development (Amundsen & Forsgren 2001, Svensson et al. 2006), with round females being ready-to-spawn. Ready-to-spawn females visit several males before they spawn (L. C. Myhre, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). Females generally lay all eggs of one brood in the same nest (Skolbekken & Utne-Palm 2001), but can spawn several times over the breeding season (Guitel 1895, Rodrigues-Graña & de Jong unpublished data). Non-breeding individuals often form semi-pelagic foraging shoals (Collins 1981; Svensson et al. 2000).

Study sites

Three study sites were chosen to be suitable habitat, but different in the level of shelter from

prevailing winds. In 2007, in what was originally planned as a pilot study, we placed ten artificial nests at one site (Location 1: Råttholmen, 58° 15' 3" N, 11° 26' 55" E). In 2008 we placed 45 artificial nests at two locations (Location 2: Pittleskär, 58° 14' 45" N, 11° 24' 23" E) and Location 3: Pittlehuvud, 58° 14' 31" N, 11° 24' 59" E). At Location 1, nests were divided along the shore of an island, seven were placed relatively sheltered in a bay and three were placed on the outside of the island. In 2008, location 2 was relatively sheltered from prevailing winds while location 3 was not. At location 2, we distributed the nests in the shallow area around a small island that was sheltered by land tongs at three sides. At Location 3, nests were positioned along a stretch of coast in a main canal of the Gullmar fjord. Artificial nests were made of a PVC-tube, which contained a sheet of acetate to facilitate inspection of the eggs. Nests were distributed with 3-5 meter intervals in between brown algae with the nest opening circa on level with the top of the algae.

Nests and males 2007

In 2007, we placed ten nests of similar size at Location 1 (Table 1) the 15th of May. Breeding had started before that moment (personal observations). That year we caught, measured and marked 14 attendant males in the artificial nests (7 early, 2 mid and 5 late in the breeding season), 82 were caught attending natural nests (41 early, 29 mid and 12 late in the breeding season) and 31 were caught in shoals (30 early, 1 mid season). Males were judged to attend an artificial nest if they were seen to enter the nest, or if they were clearly defending the nest. Of the 14 males caught in artificial nests, 11 males were observed to attend an artificial nest at at least one more nest inspection. Males were judged to attend natural nests if they were stationary and relatively solitary, while they were judged to be floaters if they were caught in a mixed-sex feeding shoal. Males that did not clearly belong to one of these categories were not used. Four of the 82 attendant males of natural nests and two of the 31 shoaling males were later found to attend artificial nests. Condition was calculated as the standardized residual of the regression between size and weight on a log-log scale.

Nests and males 2008

In 2008, at Location 2 and 3, we placed 40 nests of two different sizes, plus five very small nests to test if our smaller size was not the lower limit of what was physically possible (Table 1). We placed the artificial nests in mid April (15th and 16th), well before the start of the breeding season. We then inspected the nests for eggs and presence of an attendant male regularly between April 29th and July 17th. Early in the breeding season (until June 2nd) we performed seven nest inspections at Location 2 and eight at Location 3 (at four to seven day intervals) and during late season (from June 25th) we

performed seven nest inspections at both sites (at three to four day intervals). The intervals were shorter late in the season, because egg development is faster in higher temperatures (Skolbekken & Utne-Palm 2001). Additionally, we inspected both sites once during mid season (June 12th). We marked attendant males progressively during the season, at eight (Location 2) and six (Location 3) occasions. In total we marked 41 attendant males at Location 2, 39 early and two late. We marked 35 attendant males at Location 3, 32 early, and three late. We also marked 31 and 12 males mid season at Location 2 and 3 respectively, but of those, only two were still attending their nest at the following nest inspection. Because the period between nest inspections in mid season in 2008 was too long to judge brood survival or male nest attendance period, we excluded all males that were only seen at the mid season nest inspection from the analyses.

Marking protocol

We caught attendant males at their nests and marked them on shore nearby. We measured them on a measuring board to the nearest 0.5 mm, and weighed them on portable digital field scales (Accuracy 0.01 g). Before weighing, we blotted each fish carefully with a moist tissue to remove excess water and released him into a pre-weighed cup with seawater. We marked males with visible implant elastomer (VIE; Northwest Marine Technology TM, www.nmt.us). We used red, orange, yellow and green and injected it subcutaneously at two out of five dorsal positions. We anaesthetized the fish with 60 µl of 2-phenoxyethanol diluted in 500 ml of surface seawater and, after the elastomer injection, each fish was transferred to fresh seawater. All fish regained normal swimming behaviour within two minutes after this procedure. VIE tagging has been shown not to influence mortality or behaviour in other gobies (Malone et al. 1999; Griffiths 2002; Whiteman & Côté 2004). Marks did not fade under laboratory circumstances for three months (personal observations). After marking, we released the male close to his nest and waited till he entered. All males were returned to their nest within ten minutes.

We found no evidence of an effect of marking in this study. In total, we marked 90 males attending artificial nests in 2008 (excluding the mid season males), of these, 37 (41%) were not observed at the following nest inspection. In comparison, of 18 males that were marked before they arrived at a nest, 11 (61%) were observed at only one nest inspection, suggesting that we did not increase the turn-over rate of attendant males by marking. Including only those males that had eggs in the nest, 10 (21%) of 48 males abandoned their brood, compared to five of ten (50%) males that had been marked before they received the brood. Overall, 24 % of the broods were abandoned before hatching. The likelihood that a brood potentially hatched (see below) was not significantly

different between marked males (marked during the current brood or previously) and unmarked males (intercept: estimate ± SD = 1.69 ± 0.40, newly marked: estimate ± SD = 0.33 ± 0.41, z = 0.80, P = 0.43, previously marked: estimate ± SD = 0.65 ± 0.40, z = 1.62, P = 0.10, N_{obs} = 154, N_{nests} = 76).

Nest inspection protocol

In 2007, we inspected how long we should observe each nest to get some visits for each nest inspection, and thus observed for different periods of time. In 2008, we observed each nest for 2 minutes, but occasionally waited longer if this was necessary to establish if a present male was attending the nest (i. e. entered the nest). During these observations, we noted the number and sex of visitors that approached the nest within a 30 cm radius, and for females the roundness on a scale from one (slim) to three (very round; Forsgren et al. 2004). After the observation, we assessed brood size as the area covered with eggs, by carefully taking the acetate partly out of the nest to check the outline of the brood, and if there were any areas without eggs within the brood. The area of eggs was correlated with the number of eggs in a sample of freshly caught females that spawned in the laboratory within 48 hours (on log-log scale: r = 0.92, P < 0.001, N = 11), although there was variation in egg density (mean ± SD = 159 ± 20 eggs/cm^2, range 134 - 192). The acetate was lined with a centimetre grid, which we used to copy the outline of the brood onto a paper sheet with a similar grid. If eggs were hatching we recorded this as eggs present, but without drawing the outline. If there was a female in the nest with the male we did not interfere, but returned after we inspected all other nests. If the female had not left when we returned, we regarded this as a missing data point for the area of eggs, but recorded a possible spawning event. We did not nest inspection all nests at all nest inspections in 2008, because they were sometimes hard to find. Additionally, some nests disappeared during the season. Therefore, nest occupation and nests with eggs are always given as the proportion of the nests inspected. There was no significant difference in the number of nests inspected between early and late season (table 2).

Paternity assignment

Attendant males were assigned paternity for a brood if they were seen attending the brood at more than one nest inspection, because we found that in some cases of nest take-overs by a new male, the old eggs remained till the following nest inspection (see results), but never longer. Males were also assigned paternity if they had attended the same nest before and they were seen at least once during the current brood. Males were marked as soon as possible after they took up a nest, but males that

were marked after the first nest inspection where they were attending a brood, only were assigned paternity if the brood survived to the next nest inspection.

Not all VIE tags were equally distinguishable in all circumstances, which together with (likely) human error caused some mismatches in tagging identity of males attending a certain nest. Therefore, we allowed for one mistake in the reading of the mark to still be counted as the same attendant male. However, we carefully ascertained that similar marks had not been used at the time of reading, and in case of doubt did not use the observation. There were ten cases were the identity of an attending male could not be established with these rules.

Brood size and brood survival

Males can take care of eggs from several females at the same time, and in most cases, all eggs in the nest hatch within 24 hours, although they were spawned by several females (Mobley et al. 2009). Thus, males likely receive all clutches they care for within a short period. We termed such a group of clutches in the nest a brood, and assumed that all clutches were relatively synchronous, but tested this assumption by recording if all eggs within a brood were in the same developmental stage (see below). Because brood size often decreased over the developmental period, we estimated maximum brood size as the maximum area of eggs recorded at any inspection during the development of a brood. The total area of eggs that potentially hatched (see below) from each brood was used to calculate the proportional brood success (area of likely hatched eggs / maximum brood size). Reproductive success for each male was estimated as the sum of the areas of eggs that potentially hatched in all broods he fathered.

Egg development time until hatching depends strongly on temperature (Dannevig 1895, Pauly & Pullin 1988). Two-spotted goby eggs hatch at around 24 days at 8.5 ºC, at 10-15 days in the interval 10 - 16 ºC (163 daydegrees; Skolbekken and Utne-Palm 2001, Svensson 2006, own observations) and at eight days at 18ºC (J. Bjelvenmark, unpublished data). In this study, no breeding was observed in water below 10 ºC. Two-spotted goby eggs develop pigmented eyes roughly half way through the developmental period (unpublished data K. de Jong, L. Rodrigues-Graña). Therefore, we could use the presence of pigmented eyes as a cue to the developmental stage of the eggs in a nest. This allowed us to assess if a brood potentially hatched from the time between the last nest inspection before it appeared and the first nest inspection after it disappeared. The temperature during our study period ranged from 12 to 20 degrees in 2007 (at 1m depth) and from 10 to 20 degrees in 2008 (measured with a continuous logger at 1.5m depth at Location 2; Hobo (R)

Datalogger, Onset Computer Corporation). We inspected nests every four to seven days early in the breeding season, and every three to four days late in the season. This meant that successful broods would be inspected at least twice during the developmental period. Due to missed nest inspections for some nests, we inspected five potentially successful broods only once, three early and two late in the season, these were excluded from the analyses. We also excluded all broods that did not have time to develop pigmented eyes at the last nest inspection before the mid season and the last nest inspection before the end of the season. For all other broods that disappeared from a nest we estimated if they would have been expected to hatch before. We assumed that all 'eyed' eggs present at the last inspection before estimated hatching actually hatched. To test if these estimates were repeatable, the number of broods, the fate of the broods and paternity were assessed independently by two of the authors (KdJ and SW) and compared. There were only five cases where the assessments did not agree; in all these cases a new male had taken over the nest before a previous clutch could have developed pigmented eyes. In two of these cases it remained unclear after discussion if eggs belonged to the evicted male or to the new male, these cases were therefore excluded from analyses.

Table 1. Artificial nests (PVC-tubes) placed at three localities in two breeding seasons for a study on reproductive behaviour of male two-spotted gobies in the wild.

	length (cm)	circumference (cm)	area for eggs (cm^2)	number of nests per site
2007				
All	7.5 - 8.5	4.0	30-34	10
2008				
Small	5.0	3.5	18	5
Medium	8.0	4.0	32	20
Large	9.0	5.0	45	20

Statistical analyses

Because years differed significantly in encounter rates and nest occupancy, we analysed all data separately for 2007 and 2008. In all of the following models, we included locality and the interaction with locality as additional fixed factors, but excluded the interaction if removal decreased AIC (Akaike information criterion) of the model. Mid season nest inspections were excluded from the analyses. For comparison between early and late in the season of the likelihood that a nest was attended or contained eggs, we used each check of each nest as a data point. We used mixed models with binomial error distributions (lmer; R development core team) and entered nest as a random factor. For analyses of encounter rate we used the mean number of visits to all

Paper IV: Reproductive biology

attended nests, and used a linear model with time of season as fixed effect. For the effects of season and brood size on brood reduction and the likelihood that a brood hatched (only data for 2008), we used linear mixed models with binomial error distributions, and included time of season and brood size as fixed effects, and nest as random effect. We also checked whether male size or condition affected potential brood success in a model including male id as a random effect, only including marked males. We tested whether length, condition or time of season affected the length of the period a male attended any artificial nest, mating and reproductive success, in linear models assuming Gaussian error distributions. Because there was only one male observed to breed both early and late in the season, we could not check for within-male effects of time of season. In all analyses that tested the effect of nest size we excluded the smallest nests, because very few males attended these nests and a few extreme values may cause significant correlations to appear in a linear model where there is no true effect. In all models, the effect of time of season and the interaction with time of season were included.

Results

General description of reproductive behaviour

Combining both years, only 11 males (8 early, 4 late, of 90 males in total) were observed at an empty nest. Of these, four received eggs before the following nest inspection and remained. No male attended a nest without eggs at two consecutive nest inspections. Correspondingly, in both years, the proportion of nests with eggs closely resembled the proportion of nests attended (Figure 1). Thus, males did not frequently attend a nest for more than a few days before they received eggs.

The period between the first and the last sighting of a marked male at any artificial nest was median 12 days (1-63, $N = 77$) in 2008 and 9 days (1-38, $N = 17$) in 2007. These males were seen at a median of two nest inspections in both 2008 (1-8, $N = 77$) and 2007 (1-7, $N = 17$). Males were usually found at their nest at all consecutive nest inspections until permanently leaving the nest, no male was not observed at the nest for more than one nest inspection during a period of attendance. During the observation periods early and late in the season of 2008, marked males initiated a median of two broods (range 0-4, $N = 45$) in any artificial nest of which a median of one brood (range 0-3, $N = 45$) potentially hatched. The number of initiated broods was significantly lower in the observation period of 2007 (median = 1.0, range 0 - 4, $N = 16$; -0.57 ± 0.23, $t = 2.53$, $P = 0.014$). Maximum brood size was a median of 25.66 cm^2 (range: 4.5 – 45, $N = 233$) and 24.3 ± 8.4

cm^2 potentially hatched per successful brood in 2008.

As a general pattern, we saw first an increase in the area of the nest covered with eggs to a certain maximum at the second inspection, where after the area often decreased slightly. The majority of the eggs in a brood developed pigmented eyes at the same time, and all eggs disappeared before the same nest inspection, sometimes being replaced by a fresh clutch without pigmented eyes. In cases where hatching was observed, all of the non-hatched eggs either had pigmented eyes or had turned opaque (a sign of egg death; personal observations). This suggests that the clutches in a brood were spawned within a few days.

Differences in nest attendance over the season

As expected, the occupation of artificial nests decreased over the season in 2008, and the likelihood a nest was attended was significantly lower late in the season (Figure 1B & C; Table 2). In accordance, attending males received fewer visits from other males late than early in the season (Figure 2 B & C; Table 2). In 2007, however, six to eight of the ten nests were typically occupied during the whole observation period and this did not significantly differ between early and late in the breeding season (Figure 1A; Table 2). In contrast, attending males received very few visits from attending males during the whole observation period (Figure 2C; Table 2).

The median male attendance period (per nest) was shorter late than early in the season, in both years. During early season, median attendance time was seven days (1-33, $N = 75$) and two nest inspections (1-8, $N = 75$) in 2008, and ten days (1-10, $N = 7$) and three nest inspections (1-3, $N = 7$) in 2007. During late season, median attendance time was one day (1-26, $N = 10$) and one nest inspection (1-5, $N = 10$) in 2008, and one day (1-16, $N = 9$) and one nest inspection (1-3, $N = 9$) in 2007. Only one male attended a nest for a continuous period from early to late in the season (for 38 days, 7 nest inspections; 2007).

Five males sequentially attended more than one artificial nest, four early in the season of 2008 and one late in the season of 2007. All of the five males in 2008 left their initial nests before their brood hatched and in all cases another male was attending the nest at the nest inspection following the last sighting of the male at his initial nest. In one of these cases we saw agonistic behaviour between the previous and the new male, suggesting nest take-overs do happen. In 2008, there were 25 cases where we found a new male attending a nest that was attended by a marked male at the previous nest inspection, all early in the season. Seventeen of these replacements took place when

there were eggs in the nest. In five of these cases, the brood of the previous male had been replaced with a new brood, and in another five all eggs disappeared. In two cases we could not conclude if the brood was from the previous or the new male, and in five cases the new male was attending the brood of the previous male.

Differences in mating success over the season

Attending males received more visits from ready-to-mate (round) females late than early in the season (Figure 2 B & C; Table 2), as expected. In 2007 on the other hand, attendant males received more visits from ready-to-mate females than from males during the whole study period. The mean number of all female visits (including slim and round females) did not differ between the early and the late part of the season in 2007 (Figure 2C; Table 2). In 2008, males initiated significantly fewer broods during the period they attended a nest late than early in the season (Table 2).

However, contrary to our predictions, in both years, the proportion of attendant males with an empty nest did not differ between early and late in the season (Table 2). Maximum brood size was significantly lower late than early in the season of 2008 (Table 2). Mean fullness of attended nests did not differ between early and late in the season in 2007 and tended to be lower late than early in the season of 2008 (Table 2).

Differences in reproductive success over the season

We observed 146 broods early and 69 late in the breeding season of 2008. Early in the season broods were more likely to hatch than late in the season (Table 2). The median area of potentially hatched eggs in successful broods was also significantly lower late in the season (Table 2). However, the proportion of the maximum brood area that potentially hatched was not significantly lower late in the season (Table 2). The likelihood that a brood containing live eggs was attended by a male was significantly lower late in the season of 2008 (Figure 1A & C; Table 2). In 2007, this did not differ between early and late in the season (Figure 1A, Table 2). None of the marked males were seen to breed successfully both early and late in the season (only data for 2008). Although five of the marked males that had attended eggs early in the season were observed to attend a nest again late in the season, none of these males were assigned paternity according to our rules and none of the broods they attended ($N = 2$) potentially hatched.
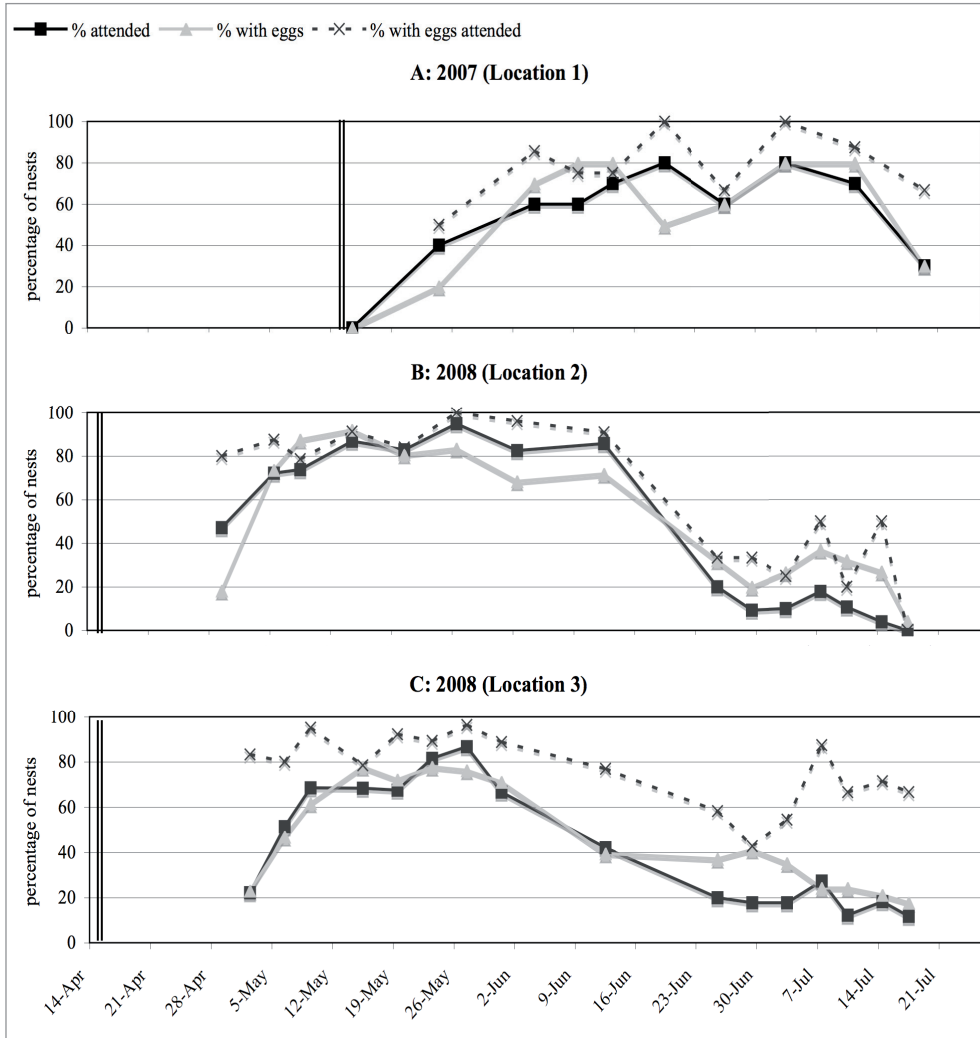
Figure 1. Percentage of artificial nests that was attended by a two-spotted goby male (solid black line), or that contained eggs (solid grey line) at each nest inspection during the breeding season of 2007 (A) and 2008 (B-C). The dashed line indicates the percentage of nests with live eggs that was attended by a male at each nest inspection. The double lines indicate the time of introduction of artificial nests to each study site.
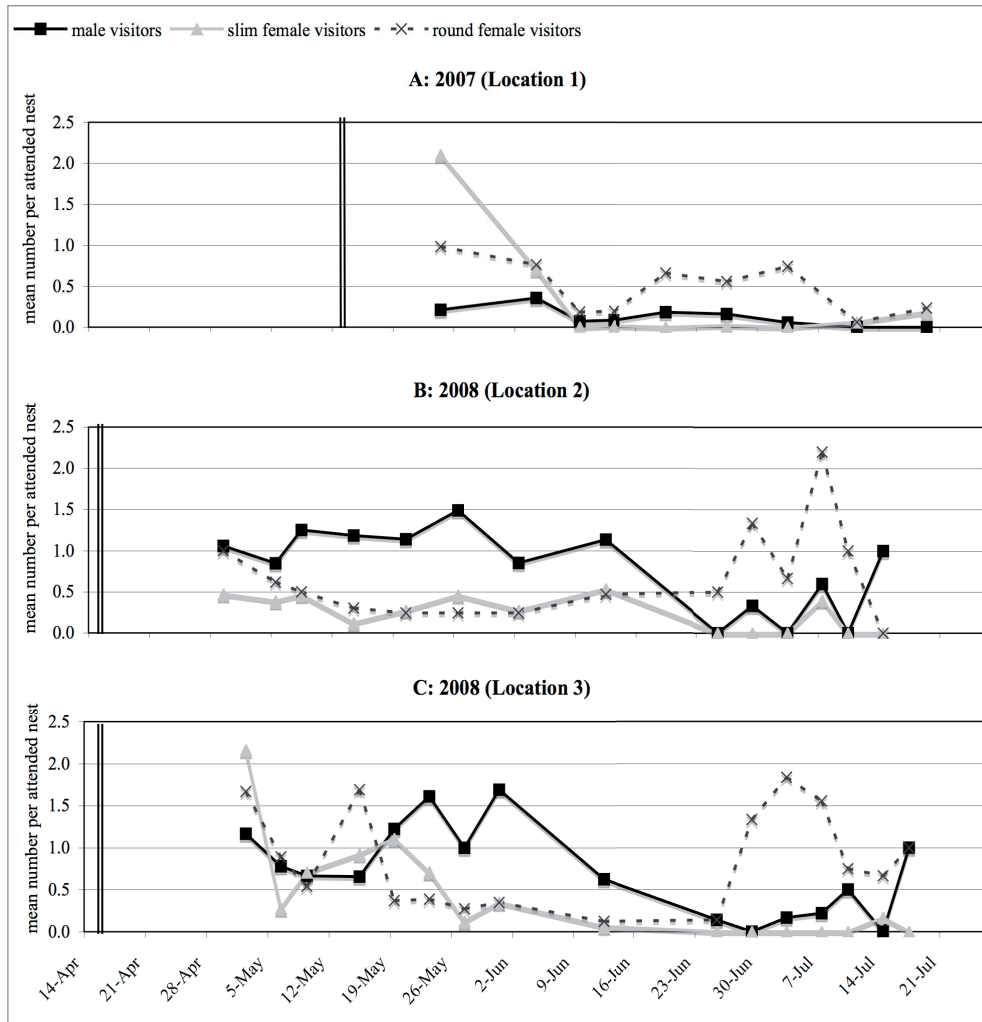
Figure 2. Mean number of visitors in two minutes observation of artificial nests attended by a two-spotted goby male for each nest inspection in the breeding season of 2007 (A) and 2008 (B & C).

Differences in male traits over the season

Early in the season, attendant males were significantly larger than late in the season of 2008, and there was a tendency for a similar effect in 2007 (Table 2; Figure 3). In addition, attendant males marked early in the season were larger than attendant males marked late in the season in 2008 (int: estimate ± SD = 4.87 ± 0.05, mid: estimate ± SD = 0.06 ± 0.08, t = 0.69 P = 0.49, late: estimate ± SD = -0.59 ± 0.19, t = -3.14, P = 0.002, N = 118) and there was a tendency for a similar effect in
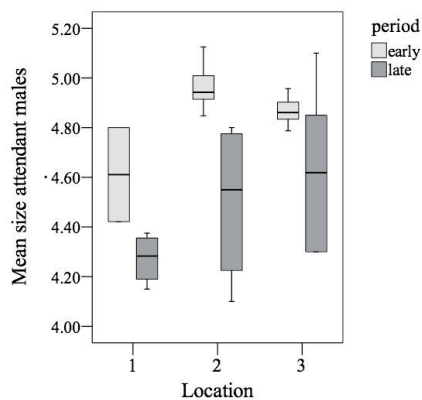

Figure 3. Mean size (total length) of attendant males over the season. Graphs are box plots with the median and quartiles in the boxes. Lines denote minima and maxima.

2007 (int: estimate ± SD = 4.58 ± 0.05, mid: estimate ± SD = 0.02 ± 0.20, t = 0.08, P = 0.94, late: estimate ± SD = -0.03 ± 0.02, t = -1.94, P = 0.055, N = 100). In 2007, when we also marked shoaling males, there was no significant difference over the season in total length of all males (int: estimate ± SD = 4.58 ± 0.07, mid: estimate ± SD = 0.03 ± 0.23, t = 0.11, P = 0.91, late: estimate ± SD = -0.11 ± 0.07, t = -1.60, P = 0.11, N = 131). There was a large significant difference in total length of males between the years (int: estimate ± SD = 4.53 ± 0.04, 2008: estimate ± SD = 0.34 ± 0.05, t = 6.60, P < 0.0001, N = 249).

In 2008, attendant males marked in early season were in significantly better condition than males that were first seen at the mid season nest inspection and they tended to be in better condition than attendant males marked late in the season (int: 0.36 ± 0.11, mid: -0.91 ± 0.17, t = -5.24, P < 0.0001, late: -0.68 ± 0.41, t = -1.65, P = 0.10, N = 118). This effect was similar in 2007, although here, the difference between early and late season was significant, while in mid season, males tended to be in slightly better condition (int: estimate ± SD = 0.36 ± 0.15, mid: estimate ± SD = 0.05 ± 0.58, t = 0.09, P = 0.93, late: estimate ± SD = -0.44 ± 0.20, t = -2.20, P = 0.03, N = 100). Overall condition of all males did not differ significantly over the season (int: estimate ± SD = 0.03 ± 0.12, late: estimate ± SD = -0.093 ± 0.18, t = -0.52, P = 0.60, mid: estimate ± SD = 0.38 ± 0.59, t = 0.65, P = 0.52, N = 131).

Male traits and nest attendance

There was no difference in total length between stationary and shoaling males (int: estimate ± SD =

4.56 ± 0.07, Stationary: estimate ± SD = 0.02 ± 0.10, t = 0.218, P = 0.83, N = 71; only data for 2007). However, stationary males were in significantly better condition than shoaling males (int: -0.42 ± 0.18, stationary males: 0.78 ± 0.24, t = 3.25, P = 0.002, N = 71; only data for 2007). The time a male attended a nests on the other hand, was not significantly associated to nest size (estimate ± SD = 0.02 ± 0.02, t = 0.29) male total length (estimate ± SD = -0.24 ± 0.83, t = -0.29) or male condition (estimate ± SD = 0.12 ± 0.43, t = 0.29, int: 2.71 ± 0.37, N_{males} = 44, N_{nests} = 41; model assuming quasipoisson error distributions; only data for 2008).

The smallest nests were only attended when the majority (< 80 %) of other nests were attended. Attendant males were on average 4.59 ± 0.36 (range: 4.05-4.90, N = 5) cm long, which was smaller than average in 2008 (Table 2). This suggests that males in general avoid these smallest nests, but small males accept them at times of high competition. There was no overall significant difference between medium and large nests in the likelihood that a nest was attended by a male (int: -0.89 ± 0.26, large nests: -0.11 ± 0.36, z = -0.32, P = 0.75, N_{obs} = 903, N_{nests} = 75). However, there was a significant three-way interaction between size, season and location (-2.30 ± 0.81, z = -2.82, P = 0.005; same model), which was caused by larger nests being more likely to be attended late in the season at Location 2 (int: -2.49 ± 0.41, large nests: 1.39 ± 0.50, z = 2.82, P = 0.005, N_{obs} = 221, N_{nests} = 33). Males that attended large nests tended to be larger than marked males that attended medium nests (Location 2: t = -1.77, df = 55, P = 0.083, Location 3: t = -1.97, df = 42, P = 0.055). If we only included males that arrived early in the season, this difference was significant for Location 3 (t = -2.37, df = 27, P = 0.025), and a strong tendency for Location 2 (t = -1.97, df = 28, P = 0.059; Figure 4). There were too few males arriving in the late part of the breeding season for a similar analysis, but there was no difference in total length between males attending large nests and males attending small nests at the mid season nest inspection (Location 2: t = 0.25, df = 24, P = 0.98, Location 3: t = 0.17, df = 9, P = 0.87).
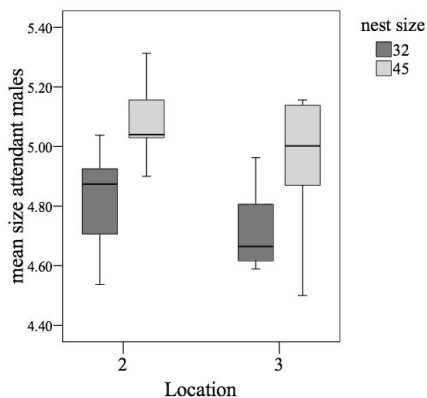

Figure 4. Mean size (total length) of attendant males in the medium (32 cm^2) and large (45 cm^2) nests early in the season of 2008. Graphs are box plots with the median and quartiles in the boxes. Lines denote minima and maxima.

Male traits, nest size and brood size

Overall, maximum brood size was positively associated with nest size (int: estimate ± SD = 3.94 ±

2.66, nest size: estimate ± SD = 0.55 ± 0.07, t = 1.48, CI = 0.42 – 0.69, N_{obs} = 232, N_{nest} = 78). Within broods attended by marked males only nest size was significantly associated with maximum brood size (int: estimate ± SD = -1.61 ± 6.54, nest size: 0.62 ± 0.09, t = 7.08, CI = 0.50 – 0.81, N_{obs} = 102, N_{nest} = 53, N_{males} = 62), not standard length (estimate ± SD = 1.07 ± 1.51, t = 0.71) or condition (estimate ± SD = 0.34 ± 0.71, t = 0.50), and these were removed from the model.

Early in the season however, maximum brood size of marked males was not only significantly associated with nest size (int: estimate ± SD = -104.4 ± 45.4, nest size: estimate ± SD = 3.5 ± 1.23, t = 2.87, CI = 1.07 – 6.02, N_{obs} = 91, N_{nests} = 50, N_{males} = 55), but also with male size (estimate ± SD = 21.36 ± 9.23, t = 2.32, CI = 2.85 – 39.58) and the interaction (estimate ± SD = -0.57 ± 0.25, t = -2.31, CI = -1.06 - -0.07). The interaction was caused by a difference in the association of male size with maximum brood size between large and medium nests. Male size was associated with maximum brood size in medium nests (int: estimate ± SD = 9.15 ± 6.32, male size: estimate ± SD = 3.01 ± 1.33, t = 2.27, CI = 0.23 – 5.70, N_{obs} = 46, N_{nest} = 24, N_{males} = 28) but not in large nests (int: 55.28 ± 16.33, male size: -4.45 ± 3.21, t = -1.39, CI = -10.9 – 2.1, N_{obs} = 45, N_{nest} = 26, N_{males} = 29).

Male traits and reproductive success

The likelihood that any brood potentially hatched was significantly and positively associated with maximum brood size (estimate ± SD = 0.09 ± 0.03, z = 3.17, P = 0.002, N_{broods} = 194, N_{nests} = 78), but not with the number of inspections no male was attending (estimate ± SD = 0.13 ± 0.32, z = 0.42, P = 0.68; with the previously mentioned effect of late season included in the model: estimate ± SD = -1.48 ± 0.42, z = -3.45, P = 0.0006). If we only consider broods that were sired by a male that bred repeatedly, the best model (lowest AIC) to explain the differences within males, included the number of previous broods a male had sired (estimate ± SD = -20.7 ± 38.6, z = -0.54, P = 0.59, N_{broods} = 51, N_{males} = 30). In this model, there was no association between brood size and the likelihood a brood potentially hatched (estimate ± SD = 0.10 ± 5.87, z = 0.02, P = 0.99). The total number of broods that was in the nest before, regardless of paternal male, was not significantly associated with the likelihood that a brood potentially hatched (estimate ± SD =12.9 ± 105.9, z = 0.12, P = 0.90), and was removed from the model. All first broods of males that bred repeatedly potentially hatched (N = 30). Brood size decreased with the number of broods a male had sired before (estimate ± SD =12.9 ± -3.30 ± 0.88, t = -3.7, CI = -5.06 - -1.52, N_{broods} = 64, N_{males} = 32).

Table 2. Differences in some general variables (A) and variable related to male reproductive success (B) early and late in the breeding season of a two-spotted goby population. For each variable, we show median (range) or mean ± SD for early and late season of 2007 and 2008. Test results are for the difference between early and late season. Location and the interaction between location and time of season are included in all models for 2008. For the tests, we give estimates ± SD for the intercept and late vs. early season, all estimates are on logit or log scale. We give the total number of observations (obs) and the number of groups in the random factors for each model. Significance is given as a *t* or *z*-value and a *P*-value, significant values are given in bold. P-values for models with binomial error distributions (z-values) are computed by the lmer function in R, for models with a Gaussian error distribution (t-values), we log-transformed the data and used MCMC-sampling to compute 95 % confidence intervals. The median and range of proportions of nests attended, nests with eggs, males with eggs and live broods attended are calculated per nest inspection, the tests are done on the binomial scores for each nest at each nest inspection.

Table 2A	Year	Early	Late	Test: estimates	N obs/nests	Significance
Nr. of male visits to attended nests	2007	1.33 ± 0.24	0.35 ± 0.55	int: -0.53 ± 0.43 late: -1.28 ± 0.68	31/10	z = -1.88 P = 0.061
	2008	1.11 ± 0.32	0.31 ± 0.37	int: 0.12 ± 0.05 late: -1.56 ± 0.26	455/80	**z = -5.93 P < 0.0001**
Nr. of ready-to-mate female visits to attended nest	2007	4.75 ± 3.18	2.27 ± 1.64	int: -0.33 ± 0.45 late: -0.21 ± 0.39	31/10	z = -0.55 P = 0.58
	2008	0.62 ± 0.49	1.00 ± 0.64	int: -0.64 ± 0.13 late: 0.74 ± 0.16	455/80	**z = 4.78 P < 0.0001+**
Proportion of nests attended by a male	2007	0.40 (0.0 - 0.6)	0.65 (0.3 - 0.8)	int: -0.71 ± 0.40 late: 1.12 ± 0.51	70/10	**z = 2.22 P = 0.027**
	2008	0.72 (0.22 – 0.95)	0.15 (0.0 – 0.27)	int: 0.72 ± 0.19 late: -2.53 ± 0.23	986/83	**z = -10.96 P < 0.0001+**
Proportion of all nests that contained eggs	2007	0.40 (0.0 – 0.70)	0.70 (0.30 – 0.80)	int: -0.90 ± 0.44 late: 1.56 ± 0.53	70/10	**z = 2.96 P = 0.003**
	2008	0.74 (0.18 – 0.92)	0.27 (0.04 – 0.41)	int: 0.73 ± 0.20 late = -1.62 ± 0.20	963/83	**z = -7.99 P < 0.0001**
Mean nest fullness of attended nests	2007	0.49 ± 0.39	0.44 ± 0.12	int: 1.27 ± 0.91 late: -1.25 ± 1.01	29/10	z = -1.24 P = 0.21
	2008	0.58 ± 0.12	0.45 ± 0.13	int: 0.70 ± 0.12 late: -0.53 ± 0.30	366/77	z = -1.76 P = 0.078
Proportion of attending males with eggs	2007	0.63 (0.25 – 1.0)	0.83 (0.67 – 1.0)	int: 0.85 ± 0.69 late: 1.55 ± 1.01	34/10	z = 1.53 P = 0.13
	2008	0.88 (0.29 – 1.0)	0.83 (0.33 – 1.0)	int: 2.05 ± 0.32 late: 0.51 ± 0.64	436/80	z = 0.80 P = 0.42+
Proportion of all live broods that were attended	2007	0.68 (0.50 – 0.86)	0.81 (0.40 – 1.0)	int: 1.25 ± 0.80 late: 0.45 ± 0.97	35/10	z = 1.56 P = 0.64
	2008	0.88 (0.79 – 1.0)	0.47 (0.0 – 0.71)	int: 2.00 ± 0.23 late: -1.95 ± 0.32	502/81	**z = -6.04 P < 0.0001+**
Mean size attending males per nest inspection	2007	4.61 ± 0.27	4.27 ± 0.10	int: 4.61 ± 0.11 late: -0.34 ± 0.14	5 N. ins.[1]	t = -2.43 (P = 0.072)
	2008	4.91 ± 0.09	4.57 ± 0.33	int: 4.92 ± 0.05 late: -0.34 ± 0.09	24 N. ins.[1]	**t = -3.79 P = 0.001**
Number of nests inspected	2007	10	10	-	7 N. ins.[1]	t = 0 (P = 1.0)
	2008	37 ± 3.2	31 ± 3.4	int: 36.37 ± 0.97 late: -2.66 ± 1.41	28 N. ins.[1]	t = -1.88 P = 0.07+

+Indicates a significant effect of location or a significant interaction with location in the model.

[1]Calculated per nest inspection in the test as well as for the median and range.

Paper IV: Reproductive biology

Table 2B	Year	Early	Late	Test: estimates	N obs/nests	Significance
Number of attempted broods per male	2008	1 (0 - 3)	1 (0 - 2)	int: 1.95 ± 0.13 late: -0.9 ± 0.19	obs: 148 nests: 82	**z = -4.89** **P < 0.0001**
Proportion of successful broods	2008	0.70	0.54	int: 0.48 ± 0.24 late: -0.98 ± 0.41	obs: 123 nests: 80	**z = -2.37** **P = 0.018**
Proportion of unsuccessful broods	2008	0.09	0.41	int: -2.29 ± 0.40 late: 2.02 ± 0.52	obs: 123 nests: 80	**z = 3.90** **P < 0.0001**
Proportion of broods without known fate	2008	0.22	0.06	int: -0.96 ± 0.26 late: -0.21 ± 0.49	obs: 123 nests: 80	z = -1.00 P = 0.32
Brood size (all broods)	2008	28.3 (4.5 - 45.0)	21.0 (4.8 - 35.3)	int: 3.25 ± 0.03 late: -0.32 ± 0.05	obs: 233 nests: 79	**t = 96.99** **(-0.42 - -0.21)**
Area of potentially hatched eggs in successful broods	2008	26.9 (4.5 - 41.6)	20.5 (2.0 - 32.5)	int: 3.24 ± 0.04 late: -0.34 ± 0.08	obs: 152 nests: 77	**t = -4.47** **(-0.49 - -0.19)**
Proportion of the brood that potentially hatched	2008	1.0 (0.3 - 1.0)	0.93 (0.2 - 1.0)	int: 2.44 ± 0.24 late: -0.86 ± 0.55	obs: 152 nests: 77	z = -1.55 P = 0.12

Reproductive success per male tended to be similarly associated with male standard length and nest size as mating success. (int: 1.41 ± 0.73; nest size: estimate ± SD = 0.02 ± 0.01, $t = 1.9$, 0.065; male size: estimate ± SD = 0.28 ± 0.16, $t = 1.80$, $P = 0.08$, $N = 41$), but not with condition (estimate ± SD = 0.02 ± 0.08, $t = 0.27$, $P = 0.79$). Within successful broods, maximum brood size and the area of potentially hatched eggs were correlated, both in early season (r = 0.84, $t = 15.77$, df = 108, $P < 0.001$), and in late season (r = 0.70, $t = 5.84$, df = 35, $P < 0.0001$). The proportion of the mating success resulting in potentially hatched eggs was not associated with male size (estimate ± SD = 0.08 ± 1.10, $z = 0.07$, $P = 0.94$), nest size (estimate ± SD = -0.03 ± 0.06, $z = -0.50$, $P = 0.61$) or condition (estimate ± SD = 0.35 ± 0.55, $z = 0.53$, $N = 41$).

Discussion

Most marked males in this study attended an artificial nest for the time it took for one brood to successfully hatch (7 - 12 days), but some remained longer (one male stayed for 63 days). Attendance periods were shorter and males initiated fewer broods late than early in the breeding season.

In 2008, the proportion of nests attended and the number of visits from other males decreased over the season. Early in the season, we observed ten cases suggestive of nest take-overs. In addition, attendant males were larger early than late in the season and nest size was associated with male size early in the season, but not late in the season, suggesting competition for nests. Maximum

brood size was associated with nest size, and early in the season also with male size. Males received more visits from ready-to-mate females late than early in the season, as expected. However, neither the proportion of males with empty nests or nest fullness differed between early and late in the season. Maximum brood size was even smaller late than early in the season. Broods were less likely to hatch and were less often attended by males late than early in the season. The probability of a brood to hatch was also associated with brood size. Reproductive success (the total area of eggs that potentially hatched in all broods of a male) nor the proportion of eggs that potentially hatched was associated with male size or nest size.

In 2007 however, during the whole observation period, males received fewer male visits than visits from ready-to-mate females, while the proportion of nests attended was relatively high. In 2007, attendant males were in better condition than shoaling males early in the season, and attendant males were in better condition early than late in the season. There were no significant size differences between these groups. Similar to 2008, neither the proportion of males with empty nests or nest fullness differed between early and late in the season

Reproductive behaviour

All eggs in a broods were generally in the same developmental stage and potentially hatched within a few days, even if a nest was not fully filled. This indicates that broods are relatively synchronous, although there were three to seven days between nest inspections. If males would have taken on eggs continuously, we should have seen more cases where only a part of the brood had developed pigmented eyes and cases where broods increased in size over the developmental period. The synchrony of broods varies widely among substrate brooding fish from complete synchrony (Van Iersel 1953, Hunter 1963) to continuous acceptance (Kraak 1996). In the two-spotted goby, males have been found to decrease courtship activity with brood age, although courtship did not stop completely (Bjelvenmark & Forsgren 2003). It would thus be interesting to test how synchronous broods exactly are in the field.

Very few males cared for more than one brood in the same nest. This is unexpected, because in the laboratory, males can breed repeatedly in the same nest (Breder & Rosen 1966, personal observations). Very little is known about reproductive biology in the field of other goby species, but among nest brooders, patterns seem to vary. For example in the Mediterranean blenny, males defended the same nest during the whole breeding season (Kraak 1996) and in damselfish, some individuals remain in the same territory, while turn over is very high for most individuals (Itzkowitz

1985). We do not know how many of the males that left an artificial nest continued breeding in natural nests. Two of ninety males cared for broods in two different artificial nests, indicating that nest switching is not uncommon. A study on female mate sampling that shared one location observed that many males were breeding in natural nests at Location 3 (L. C. Myhre, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). Thus, our estimate of the length of a breeding period for the males is likely to be an underestimate. The long periods between nest inspections in mid season of 2008 may have caused us to miss one clutch for each male that remained until or just before the mid season nest inspection. However, only three of the males that were breeding at the last nest inspection before the gap remained until mid season, so this underestimation is unlikely to be large.

Seasonal differences in reproductive behaviour

In temperate animals with a restricted breeding season, it may be very important to breed as early as possible to enhance offspring fitness (Fairbairn 1977, Hendersson et al. 1988). In addition, temperature during the development is negatively correlated with larval size at hatching (Kamler 1992). We found that brood hatching probability was lower during late season (Table 2). Our estimate of hatching probability was not exact, we estimated if a brood disappeared before it could have hatched based on water temperature. However, because the intervals between nest inspections were based on the estimated development time of a brood, it is not likely that this introduced a bias towards one part of the season.

We also found evidence for nest competition. Attendant males were smaller late than early in the season of 2008, and in worse condition in 2007, suggesting that high quality males disappeared and were replaced by lower quality males, similar to what was suggested in common gobies (Pampoulie et al. 2001). Early in the season, there was also a significant different in size between males occupying large nests and males attending small nests. Overall, this suggests there may be competition for nests in the season.

Both size and condition may be important traits in competition for nests (Parker 1974, Andersson 1994). Because two-spotted gobies have been seen to breed on many different substrates (Breder & Rosen 1966, personal observations), nest competition may be mainly associated with nest quality. Nest size has been shown to limit brood size in species that lay eggs in a single layer on the substrate (Hastings 1988, Marconato et al. 1989, Lindström 1992) and nest size was positively associated with mating success in this study. Large males are superior in competition for

nests (S. Wacker, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). Thus, the correlation between nests size and male size could be caused by larger males being able to defend large nests better than small males (e. g. Lindström 1988, Wong et al. 2008). On the other hand, costs related to defending large nests could make smaller males prefer smaller nests (Kvarnemo 1995, Takegaki et al. 2008). Late in the season, few nests were attended and males should have been able to express their preference. A higher proportion of large nests was attended in one of the localities, but not in the other, indicating that not all males prefer the large nests. The total time a male attended an artificial nest was not associated with male size or male condition, suggesting that there may be other traits involved as well.

Two-spotted goby males do not only show more agonistic behaviour early than late in the season, but also more courtship behaviour (Forsgren et al. 2004). Thus, there is competition for mates as well as nests early in the season. Two-spotted goby females have been found to prefer large males in the laboratory, but only early in the season (Borg et al. 2006). We show that this preference is realized in the field, with large males receiving larger broods early in the season. However, nest size was the main correlate of mating success, and male size was not associated with brood size within the largest nests. Territory quality could be more important than male traits in mating success (Alatalo et al. 1986). On the other hand, male size was associated with nest size. Overall, this suggests that size may be an important trait in mating success. However, reproductive success was not associated with male size or nest size, although reproductive output per brood was correlated to brood size.

We observed very few males without eggs in the nest, suggesting that unsuccessful males may postpone breeding (Warner 1984, Berglund 1991). The occupancy of our artificial nests decreased dramatically over the season in 2008 (Figure 2B & C), corresponding with a previously found significant decrease in male density over the season (Forsgren et al. 2004). In contrast to male density, female density has not been found to decrease significantly over the season (Forsgren et al. 2004), suggesting that males incur higher costs than females early in the breeding season. In the laboratory a decrease in condition was found for breeding males of the two-spotted goby (Skolbekken & Utne-Palm 2001, but see Eriksen 2007). In addition, egg development is slower in colder temperatures and the period of parental care correspondingly longer (Breder & Rosen 1966, Pauly & Pullin 1988), although the proportion of time spent on parental duties is higher in warmer water (Skolbekken & Utne-Palm 2001). Thus, both costs of competition and costs of parental care could be high for males, increasing a potential gain for less successful males to postpone breeding.

Unexpectedly, the occupancy of artificial nests remained high in late season 2007, while the pattern of visitors to the nest suggested that male density was already low from the start of the observation period. Artificial nests have been shown to be preferred over natural nests both in birds (reviewed in Møller 1992, Lambrechts et al. 2010) and in fish (Itkowitz & Makie 1986). On the other hand, in 2008, many artificial nests were empty during late season, while males were still breeding in natural nests (L. C. Myhre, K. de Jong, E. Forsgren & T. Amundsen, unpublished data). In 2007, filamentous algae growth seemed to be more abundant than in other years, including 2008 (personal observations). Artificial nests may then have been easier to clean and less overgrown with filamentous algae than the surrounding natural nest sites. Sample size was also very low in 2007 (N = 10) compared to 2008 (N = 90), suggesting that the decrease in nest occupancy over the season in 2008 was the more robust pattern.

If lower quality males breed later in the season, the low reproductive success late in the season may also be associated with decreasing quality of paternal care. Marked males were more often absent from their brood late than early in the season and overall, a higher proportion of clutches were unattended late in the season. On the other hand, brood hatching probability was not associated with the number of inspections without attending male, but the power of this test may be rather low, because we inspected most clutches only twice. Nest fullness has been shown to positively affect parental expenditure (Skolbekken & Utne-Palm 2001). Smaller clutches may also be more prone to filial cannibalism (e.g. Sargent 1988, Stott & Poulin 1996, Forsgren et al. 1996, Lindstrom & Sargent 1997, Kvarnemo et al. 1998, Svensson et al. 2010). However, within males, we did not find a significant association between brood size and the likelihood for a brood to potentially hatch, when we controlled for the number of broods a male had before. Brood size was negatively correlated with the number of broods a male had before, suggesting that male mating success decreased over time. An explanation for this pattern could be that male condition decreases over a breeding period (Skolbekken & Utne-Palm 2001), and that females prefer better fathers, which would also result in a positive association between maximum brood size and brood survival (e. g. Ridley and Rechten 1981, Forsgren 1997, Lindström & st Mary 2006).

Late in the season, females have been shown to compete for males (Forsgren et al 2004). Thus, we expected all males that were breeding late in the season to receive full nests, as was reported in the same study (Forgren et al. 2004). In the present study, we found no significant difference in nest fullness between early and late in the season. Maximum brood size was lower late than early in the

season. In particular, we found many very small broods late in the season, while early in the season nests were generally at least half full. These small broods may have been breeding attempts of males that disappeared, but also attended broods were smaller late than early in the season. The differences between these studies may be caused by variation in years or by differences in sample sizes. A potential explanation for our results would be that, late in the season, males are relatively spread out, and females sample fewer males and are less choosy (Myhre, de Jong, Forsgren & Amundsen, unpublished data). Thus, there may be relatively more of the males that get some eggs, while in early season only high quality males get nests that will be filled with eggs, due to female preference or female copying, while other males do not receive eggs and consequently leave the nest again.

Conclusions

Our data suggest that there is competition for early breeding opportunities among two-spotted goby males and low quality males breed later in the season. Reproductive success is lower late than early in the season, although we cannot conclusively state that this is due to seasonality or the quality of parental males. There is likely competition for nests as well as mates and nest size is the most important factor associated with mating success. In addition, the preference for male size in the laboratory is likely realized in mating success in the field, although only early in the season. Contrary to predictions, mating success was higher early in the season when male mating competition was found to be high and males receive few female visits. Thus, males breeding early may enjoy both a higher mating success and a higher reproductive success.

References

Ahnesjö, I., Forsgren, E. & Kvarnemo C. 2008. Variation in sexual selection in fishes. In: Fish behaviour (ed. Magnhagen, C., Braithwaite, V. A., Forsgren, E & Kapoor, B. G.). Science Publishers, Enfield, NH, pp. 303-335.

Alatalo, R., Lundberg, A. & Glynn, C. 1986. Female pied flycatchers choose territory quality and not male characteristics. Nature 323, pp. 152-153.

Amundsen, T. 2003. Fishes as models in studies of sexual selection and parental care. Journal of Fish Biology 63, pp. 17-52.

Amundsen, T. & Forsgren, E. 2001. Male mate choice selects for female coloration in a fish. Proceedings Of The National Academy Of Sciences Of The United States Of America 98, pp. 13155-13160.

Andersson, M. 1994. Sexual Selection. Princeton University Press, Princeton, NJ.

Andersson, M. H. & Öhman, M. C. 2010. Fish and sessile assemblages associated with wind-turbine constructions in the Baltic Sea. Marine And Freshwater Research 61, pp. 642-650.

Andersson, M. H., Berggren, M., Wilhelmsson, D. & Öhman, M. C. 2009. Epibenthic colonization of concrete and steel pilings in a cold-temperate embayment: a field experiment. Helgoland Marine Research 63, pp. 249-260.

Avise, J. C., Jones, A. G., Walker, D., DeWoody, J. A. et al. 2002. Genetic mating systems and reproductive natural histories of fishes: Lessons for Ecology and Evolution. Annual Review of Genetics 36, pp. 19-45.

Barber, I., Davies, A., Inronside, J., Forsgren, E. & Amundsen, T. 2009. First record of a *Kabatana sp microsporidium* infecting fish in the Atlantic Ocean. Diseases Of Aquatic Organisms 83, pp. 145-152.

Berg, J. 1979. Discussion of methods of investigating the food of fishes, with reference to a preliminary study of the prey of *Gobiusculus flavescens* (gobiidae). Marine Biology 50, pp. 263-273.

Berglund, A. 1991. Egg competition in a sex-role reversed pipefish: subdominant females trade reproduction for growth. Evolution 45, pp. 770-774.

Bjelvenmark, J. 2004. Mating decisions and parental care in the two-spotted goby, *Gobiusculus flavescens,* Thesis (PhD), Gothenburg University, Sweden.

Bjelvenmark, J. & Forsgren, E. 2003. Effects of mate attraction and male-male competition on paternal care in a goby. Behaviour 140, pp. 55-69.

Borg, Å. A., Forsgren, E. & Amundsen, T. 2006. Seasonal change in female choice for male size in the two-spotted goby. Animal Behaviour 72, pp. 763-771.

Breder, C. M., Jr. & Rosen, D. E. 1966. Modes of reproduction in fishes. T. F. H. Publications, Jersey City.

Collins, S. P. 1981. Littoral and benthic investigations on the west-coast of Ireland.13. The biology of Gobiusculus flavescens (Fabricius) on the Connemara coast. Proceedings of the Royal Irish Academy Section B: Biological, Geological and Chemical Science 81, pp. 63-87.

Dannevig, H. 1895. The influence of temperature on the development of the eggs of fishes. Republican Fisheries Board Scotland 1894, pp. 147-152.

de Jong, K., Wacker, S., Amundsen, T. & Forsgren, E. 2009. Do operational sex ratio and density affect mating behaviour? An experiment on the two-spotted goby. Animal Behaviour 78, pp. 1229-1238.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. Science 197, pp. 215-223.

Eriksen, J. M. 2007. Parental care behaviour and costs in male two-spotted gobies (*Gobiusculus flavescens*). Thesis (MSc), NTNU, Trondheim, Norway.

Fairbairn, D. J. 1977. Why breed early? A study of reproductive tactics in *Peromyscus*. Canadian Journal of Zoology 55, pp. 862-871.

Folkestad, H. 2005. Stage dependent habitat use under conflicting predation pressure: An experimental test with larval and juvenile two-spotted gobies, *Gobiusculus flavescens* Fabricius. Journal Of Experimental Marine Biology And Ecology 323, pp. 160-171.

Forsgren, E., Amundsen, T., Borg, Å. A. & Bjelvenmark, J. 2004. Unusually dynamic sex roles in a fish. Nature 429, pp. 551-554.

Forsgren, E., Karlsson, A., Kvarnemo, C., 1996. Female sand gobies gain direct benefit by choosing males with eggs in the nests. Behav. Ecology and Sociobiology 39, 91-96.

Fosså, J. H. 1991. The ecology of the 2-spot goby (*Gobiusculus flavescens* Fabricius) - the

potential for cod enhancement. Ecology and Management Aspects of Extensive Mariculture 192, pp. 147.

Gordon, J. C. D. 1983. Some notes on small kelp forest fish collected from *Saccorhiza polyschides* bulbs on the Isle of Cumbrae Scotland. Ophelia 22, pp. 173-183.

Griffiths, S. P. 2002. Retention of visible implant tags in small rockpool fishes. Marine Ecology Progress Series 236, pp. 307-309.

Guitel, F. 1895. Observations sur les mïurs du *Gobius ruthensparri.* Archives de Zoologie Experimentale et Génerale 3, pp. 63-288.

Hart, P. J. B. & Reynolds, J. D. 2002. Handbook of Fish Biology and Fisheries. Blackwell Science Ltd., Oxford.

Hastings, P. A. 1988. Female choice and male reproductive success in the angel blenny, *Coralliozetus angelica* (Teleostei: Chaenopsidae). Animal Behaviour 36, 115-124.

Henderson, P. A., Holmes, R. H. A. & Bamber, R. N. 1988. Size-selective overwintering mortality in the sand smelt, *Atherina boyeri* Risso, and its role in population regulation. Journal of Fish Biology 33, pp. 221-233.

Hendry, A. P. & Stearns, S. C. 2004. Evolution illuminated: Salmon and their relatives. Oxford University Press, Oxford.

Hosken, D. J. & Stockley, P. 2003. Benefits of polyandry: A life history perspective. Evolutionary Biology 33, pp. 173-194.

Hunter. J.R. 1963. The reproductive behaviour of the green sun-fish. *Lepornis cyanellus.* Zoologica 48, pp. 13-24.

Huyse, T., Van Houdt, J. & Volckaert, F. A. M. 2004. Paleoclimatic history and vicariant speciation in the "sand goby" group (Gobiidae, Teleostei). Molecular Phylogenetics and Evolution 32, pp. 324-336.

Iersel. J.J.A. van. 1953. Analysis of parental behaviour of the male three-spined stickleback (Gasterosteus aculeatus L.). Behaviour 3, suppl., pp. 1-159.

Itzkowitz, M. 1985. Aspects of the population dynamics and reproductive success in the permanently territorial beaugregory damselfish. Marine Behavior and Physiology 12, pp. 57–69.

Johnsen, S. 1944. Studies on variation in fish in north-european waters. I. Variation in Size. Bergen Museums Årbok, Vol. 4. Bergen: Naturvitenskapelig Rekke, pp. 9-129.

Kamler, E. 1992. 5. Mixed Feeding Period and 6. Early exogenous feeding period. Early life history of fish - and energetics approach.

Chapman & Hall, London, pp. 177-210.

Kvarnemo, C. 1995. Size-assortative nest choice in the absence of competition in males of the sand goby, *Pomatoschistus minutus.* Environmental Biology of Fishes 43, 233-239.

Kvarnemo, C., Svensson, O. & Forsgren, E. 1998. Parental behaviour in relation to food availability in the common goby. Animal Behaviour 56, 1285-1290.

Kraak, S. B. M. 1996. A quantitative description of the reproductive biology of the Mediterranean blenny *Aidablennius sphynx* (Teleostei, Blenniidae) in its natural habitat. Environmental Biology Of Fishes (1996) vol. 46 (4) pp. 329-342.

Lambrechts, M. M. et al. 2010. The design of artificial nestboxes for the study of secondary hole-nesting birds: a review of methodological inconsistencies and potential biases. Acta Ornithologica 45, pp. 1-26.

Lindström, K. 1988. Male–male competition for nest sites in the sand goby, *Pomatoschistus minutus.* Oikos 53, 67-73.

Lindström, K. 1992. Female spawning patterns and male mating success in the sand goby, Pomatoschistus minutus. Marine Biology 113, 475–480.

Lindstrom, K. & Sargent, R.C., 1997. Food access, brood size and filial cannibalism in the fantail darter, *Etheostoma flabellare.* Behavioral Ecology and Sociobiology 40, 107-110.

Malone, J. C., Forrester, G. E. & Steele, M. A. 1999. Effects of subcutaneous microtags on the growth, survival, and vulnerability to predation of small reef fishes. Journal of Experimental Marine Biology and Ecology 237, pp. 243-253.

Marconato, A. & Bisazza, A. & Marin, G. 1989. Correlates of male reproductive success in Padogobius martensi (Gobiidae). Journal of Fish Biology 34, pp. 889-899.

Miller, P. J. 1986. Gobiidae. In: Fishes of the North-eastern Atlantic and the Mediterranean Volume 3 (ed. Whitehead, P. J. P., Bauchot, M.-L., Hureau, J.-C., Nielsen, J. & Tortonese, E.). UNESCO, Paris, pp. 1019-1085.

Mobley, K. B., Amundsen, T., Forsgren, E., Svensson, P. A. & Jones, A. G. 2009. Multiple mating and a low incidence of cuckoldry for nest-holding males in the two-spotted goby, *Gobiusculus* flavescens. BMC Evolutionary Biology 9:6.

Møller A.P. 1992. Nest boxes and the scientific rigour of experimental studies. Oikos 63: 309–311.

Pampoulie, C., Sasal, P., Rosecchi, E., Auby, I., Bouchereau, J. -L., Lindström, K. & Crivelli, A. 2001. Nest use by the common goby Pomatoschistus microps in Camargue (France). Ethology Ecology & Evolution 13, pp. 181-192.

Pauly, D. & Pullin, R. S. V. 1988. Hatching time in spherical, pelagic, marine fish eggs in response to temperature and egg size. Environmental Biology Of Fishes 22, pp. 261-271.

Pélabon, C., Borg, Å. A., Bjelvenmark, J., Forsgren, E., Barber, I. & Amundsen, T. 2003. Do male two-spotted gobies prefer large fecund females? Behavioral Ecology 14, pp. 787-792.

Pélabon, C., Borg, Å. A., Bjelvenmark, J., Barber, I., Forsgren, E. & Amundsen, T. 2005. Do microsporidian parasites affect courtship in two-spotted gobies?. Marine Biology 148, pp. 189-196.

Petersen, C. G. J. 1891. On the eggs and breeding of our Gobiidae. Rep. Dan. biol. Stn, 2, pp. 1-9.

Sargent, R.C. 1988. Paternal care and egg survival both increase with clutch size in the fathead minnow, Pimephales promelas. Behav. Ecol. Sociobiol. 23: 33–37.

Schultz, E. T. 1993. The effect of birth date on fitness of female dwarf perch, *Micrometrus minimus* (Perciformes: Embiotocidae). Evolution 47, pp. 520-539.

Skolbekken, R. & Utne-Palm, A. C. 2001. Parental investment of male two-spotted goby, *Gobiusculus flavescens* (Fabricius). Journal Of Experimental Marine Biology And Ecology 261, pp. 137-157.

Sköld, H. N., Amundsen, T., Svensson, P. A., Mayer, I., Bjelvenmark, J., & Forsgren, E. 2008. Hormonal regulation of female nuptial coloration in a fish. Hormones and Behavior 54, pp. 549-556

Spence, R., Gerlach, G., Lawrence, C. & Smith, C. 2008. The behaviour and ecology of the zebrafish, *Danio rerio*. Biological Reviews 83, pp. 13-34.

Steingrund, P. & Fernö, A. 1997. Feeding behaviour of reared and wild cod and the effect of learning: Two strategics of feeding on the two-spotted goby. Journal of Fish Biology 51, pp. 334-348.

Stott, M.K. & Poulin, R., 1996. Parasites and parental care in male upland bullies Eleotridae. Journal of Fish Biology 48, 283-291.

Svensson, O., Lissaker, M. & Mobley, K. B. 2010. Offspring recognition and the influence of clutch size on nest fostering among male sand gobies, *Pomatoschistus minutus*. Behavioral Ecology and Sociobiology 64, pp. 1325-1331.

Svensson, P. A. 2006. Female coloration, egg carotenoids and reproductive success: gobies as a model system. Thesis (PhD), NTNU, Trondheim, Norway.

Svensson P. A., Barber, I. & Forsgren, E. 2000. Shoaling behaviour of the two-spotted goby. Journal of Fish Biology 56, pp. 1477-1487.

Svensson, P. A,, Forsgren, E., Amundsen, T. & Sköld, H. N. 2005. Chromatic interaction between egg pigmentation and skin chromatophores in the nuptial coloration of female two-spotted gobies. Journal Of Experimental Biology 208, pp. 4391-4397.

Svensson, P. A., Pélabon, C., Blount, J. D., Surai, P. F. & Amundsen, T. 2006. Does female nuptial coloration reflect egg carotenoids and clutch quality in the two-spotted goby (*Gobiusculus flavescens, Gobiidae*)? Functional Ecology 20, pp. 689-698.

Svensson, P. A., Blount, J. D., Forsgren, E., & Amundsen, T. 2009a. Female ornamentation and egg carotenoids of six sympatric gobies. Journal of Fish Biology 75, pp. 2777-2787.

Svensson, P. A., Pélabon, C., Blount, J. D., Forsgren, E., Bjerkeng, B. & Amundsen, T. 2009b. Temporal variability in a multicomponent trait: nuptial coloration of female two-spotted gobies. Behavioral Ecology 20, pp. 346-353.

Tagegaki, T., Matsumoto, Y., Tawa, A., Miyano, T. & Natsukari, Y. 2008. Size-assortative nest preference in a paternal brooding blenny *Rhabdoblennius ellipes* (Jordan & Starks). Journal of Fish Biology 72, pp. 93-102.

Utne, A. C. W. & Bacchi, B. 1997. The influence of visual and chemical stimuli from cod *Gadus morhua* on the distribution of two-spotted goby *Gobiusculus flavescens* (Fabricius). Sarsia 82, pp. 129-135.

Utne, A. C. W., Aksnes, D. L. & Giske, J. 1993. Food, predation risk and shelter - an experimental study on the distribution of adult 2-spotted goby *Gobiusculus-flavescens* (Fabricius). Journal Of Experimental Marine Biology And Ecology 166, pp. 203-216.

Utne-Palm, A. C. W. 2001. Response of naïve two-spotted gobies Gobiusculus flavescens to visual and chemical stimuli of their natural predator, cod *Gadus morhua*. Marine Ecology-Progress Series 218, pp. 267-274.

Warner, R. R. 1984. Deferred reproduction as a response to sexual selection in a coral reef fish:

a test of the life historical consequences. Evolution 38, pp. 148-162.

Whiteman, E. A. & Côté, I. M. 2004. Dominance hierarchies in group-living cleaning gobies: causes and foraging consequences. Animal Behaviour 67, pp. 239-247.

Wong B. B. M., Lehtonen, T. & Lindström, K. 2008. Male nest choice in sand gobies, *Pomatoschistus minutus*. Ethology 114, pp. 575-581.

Wootton, R. J. 1999. Ecology of Teleost Fishes, 2nd edn. Dordrecht: Kluwer Academic Publishers.

Zander, C. 2005a. Comparative studies on goby (Teleostei) parasite communities from the North and Baltic Sea. Parasitology Research 96, pp. 62-68.

Zander, C. 2005b. Four-year monitoring of parasite communities in gobiid fishes of the southwest Baltic - III. Parasite species diversity and applicability of monitoring. Parasitology Research 95, pp. 136-144.

Zander, C., Strohbach, U. & Groenewold, S. 1993. The importance of gobies (gobiidae, teleostei) as hosts and transmitters of parasites in the sw baltic. Helgolander Meeresuntersuchungen 47, pp. 81-111.

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 sympar[tic] 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, I	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