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Effects of Nest Spacing on Nest Occupation, Mating Success and Mating Behaviour in the Two-spotted Goby (*Gobiusculus flavescens*)

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Abstract

Male-male competition over access to essential resources necessary for reproductive success, is common in many animal systems. The intensity of intra-sexual competition is hereby not only influenced by the quality and availability of such resources, but also by its distribution. The distribution of breeding resources can have pronounced impacts on a population, by directly affecting the number of breeding individuals and the reproductive success of a population. Clumped resources can easily be monopolized by only a few individuals, preventing other members of the population access to these resources, leading to higher competition for the restricted resource. In this study I tested how different nest spacing (clumped-nest-sites vs. dispersed-nest-sites) affects nest occupation and mating success, nest fullness, male mating behaviour (agonistic and courtship behaviour) and the operational sex ratio (OSR) in the two-spotted goby (*Gobiusculus flavescens*), a small marine fish. Results show fewer nest occupations and lower mating success in the clumped treatment and lower nest fullness per mated male. Agonistic interaction among nest holders was significantly higher in the clumped than in the dispersed treatment. The development of the OSR was different between the treatments, with a consistently more female-biased OSR in the clumped treatment. Based on the results of lower nest occupations and higher agonistic behaviour among nest holder in the clumped than in the dispersed treatment, I suggest that males not just compete for nests but that they defend territories. I propose that the competition for nests and territories induced fewer performed matings, which was recorded as lower nest fullness in the clumped than in the dispersed treatment. I conclude that two-spotted gobies have a resource-based-polygyny where providing a nest site/territory has priority and influences the access to females and therefore also mating and reproductive success.

Introduction

Darwin (1871) recognised two distinct processes of sexual selection: intra-sexual selection, usually male-male competition and inter-sexual selection, usually female-choice. Intra-sexual competition for access to mates and resources that are essential for reproduction is often strongest among males (Andersson 1994). Male-male competition commonly includes occupation and defense of resources required for reproduction, to which females are attracted (Emlen & Oring 1977; Kodric-Brown 1983). Females often select males on the basis of their ownership of resources, which lets females' choice act in combination with male competition (Thornhill 1980; Andersson 1994). The strength of intra-sexual competition can be strongly affected by population density (Emlen & Oring 1977; Kokko & Rankin 2006), due to a higher contact rate between competitors.

Males often establish territories around resources, and thereby enjoy superior reproductive success by attracting females to mate in their territories. The term territory is here defined as "any defended area" (Noble 1939). Species vary to which degree they are territorial. For example many birds and fish defend large contiguous territories (van Balen 1973; Larson 1980; Norman & Jones 1984; Both & Visser 2000), but also the defense of small non-contiguous territories with non-defended space between the territories is widely spread (Stenning et al. 1988; Genner et al. 1999). The quality of a territory is related to mating success in many vertebrate-classes, e.g. in fish (Jones 1983; Petersen 1988; Hoelzer 1990), birds (Verner 1964; Askenmo 1984; Davies & Lundberg 1984) and anurans (Welles 1978; Howard 1983). According to the theory of economic defendability (Brown 1964), animals should only defend a territory if the fitness benefits of the defense exceed the costs. Or, if the net benefit of the defense exceed the net benefits of not defending. The costs of defending and agonistic behaviour may be energetic (Haller 1991; Ros et al. 2006), an increased risk of injury (Huntingford & Turner 1987), loss of mating opportunities (Huntingford & Turner 1987; Reichard et al. 2004b; Spence & Smith 2005) or a higher risk of predation (Brick 1998; Kelly & Godin 2001). Benefits of defense and agonistic behaviour in the context of mating competition can be access to territories or breeding resources, access to mates directly or preference in female choice (reviewed in Andersson 1994). Males with a higher *resource holding potential* win competitions e.g. over territories and are therefore often favored by females, resulting in higher reproductive success (Parker 1974). Many studies have shown that size differences can predict outcomes of such competitions (Bisazza et al. 1989; Magnhagen & Kvarnemo 1989; McPeck 1992; reviewed in Andersson 1994).

A low availability of breeding resources or differences in quality often induces intensive intra-sexual competition in order to gain reproductive success (Darwin 1871; Turner & Huntingford 1986). When reproduction is limited to a nest site (resource-based mating systems), not only the availability, but also the distribution of resources is expected to affect intra-sexual competition. Resource scarcity may be described by its low abundance or high clumping, both of which can independently result in a small number of e.g. territories or nest sites available for reproduction and therefore increase intra-sexual competition. A low abundance or high clumping of resources may hence have similar effects on the success of individual males. Different behavioural interactions arising due to resource clumping can have strong consequences not only on the individual level, but also on the population level. Resource distribution can for example directly affect the proportion of individuals that breed and the relative reproductive success (Reichard et al. 2004a) in a population, due to higher male-male competition e.g. for nest sites (Lindström 1988). Highly clumped resources are expected to increase male-male competition.

Spatial clumping of resources is one of the six ecological-key-variables that are believed to influence economic defendability (Grant 1997). The spatial-clumping-effect of breeding sites might be the reason why e.g. fish defend them more often than for example food (Grant 1997). Clumping of essential resources such as food, shelter or breeding sites, increases the opportunity for a small percentage of a population to control a high proportion of the given resources (Emlen & Oring 1977). Mating success among males may differ, because some males are more successful than others in obtaining and defending breeding resources essential for attracting females. A high degree of control or monopolization, results in a higher variance in mating success. A higher variance in reproductive success leads to a greater opportunity for sexual selection (Emlen & Oring 1977).

Another important aspect influencing competition over mates is the operational sex ratio (OSR) of a population (Forsgren et al. 2004). The OSR is the ratio of ready-to-mate males and females in a population at a given time (Emlen & Oring 1977; Kvarnemo & Ahnesjö 1996). Among the ready-to-mate individuals, mating competition takes place. OSR is known to affect male behaviour (Kvarnemo & Ahnesjö 1996), and it is commonly suggested that under male-biased OSR, male-male interference competition for mates increases (Emlen 1976; Enders 1993; Clutton-Brock et al. 1997).

For vertebrates, and especially birds, many studies dealing with the influences of nest limitation on reproductive success exist (Nweton 1994; Pöysä & Pöysä 2002; Salinas-Melgoza et al. 2009). However, research on how nest spacing, independent of quantity-changes of nest sites or individuals, affects mating behaviour and reproductive success is rare, especially in fish (Reichard et al. 2008;

Konečná et al. 2010). Lindström (1988) found that male sand gobies' (*Pomatoschistus minutus*) nest-occupation-behaviour was affected by the spacing of the nests. Two nests tiles placed 50 cm apart from each other were both frequently occupied independent of their size, whereas for nest tiles placed only 10 cm apart very few such cases occurred. To my knowledge there is no laboratory study and very few field studies on fish, which directly address how nest spacing influences the number of individuals that breed in a population (Lindström 1988). Furthermore, many nest-spacing-studies done on fish, do not record behaviour (Lindström 1988; Reichard et al. 2008).

The aim of this study was to investigate how the spatial distribution of nest sites, critical to reproduction, affects: (1) nest occupation and mating success, (2) nest fullness, (3) mating behaviour (agonistic and courtship behaviour) and (4) the operational sex ratio in the two-spotted goby (*Gobiusculus flavescens*), a small marine fish. I compared effects of nest spacing by creating two different treatments: (1) clumped-nest-site and (2) dispersed-nest-site.

The two-spotted goby is a suitable and particularly interesting model species to test how the questions raised above, would influence its biology. It has a resource-based mating system where nest occupation is essential for reproduction (Ahnesjö et al. 2001) and competition for nest sites is therefore expected. There are to my knowledge no studies on *G. flavescens* available, which directly investigate male nest-occupation-behaviour, nest-competition-behaviour and territoriality. The basis of this study was therefore to address these topics. A recent study on two-spotted gobies suggested the occurrence of nest competition (de Jong 2010) and it has been shown to occur also in the closely related sand goby (*Pomatoschistus minutus*) and the common goby (*Pomatoschistus microps*) (Lindström 1988; Magnhagen & Kvarnemo 1989; Magnhagen 1994, 2006; Borg et al. 2002; Lethonen & Lindström 2004). The two-spotted goby breeds on many different substrates (Breder & Rosen 1966, personal observations) and faces therefore basically no nest limitations. Nest competition may be mainly associated with the quality of nests. This is supported by a study on female mate sampling where females inspected nests before they spawned and often reject males after nest inspections (Myhre 2009). An obvious question to ask was therefor: does the distribution of high-quality nest sites affect mating- and nest-competition? And if so: might this induce territoriality in the two-spotted goby?

For the questions raised above, the following predictions were made: (1) clumped nest spacing leads to fewer nest occupations due to higher male-male competition over possession of nests and (2) therefore also to fewer mated males (mating success); (3) nest holders in the clumped treatment have lower nest fullness than nest holders in the dispersed treatment, indicating fewer matings, due to

more agonistic behaviour and competition for nests and females; (4) male agonistic behaviour is higher in the clumped treatment due to close nest neighbours; (5) more agonistic interactions reduce male courtship behaviour; (6) due to monopolizing resources essential for reproduction, a higher opportunity for selection occurs in the clumped treatment than in the dispersed treatment; (7) fewer nest occupations means fewer males are ready-to-mate, leading to a more female-biased operational sex ratio (OSR) in the clumped than in the dispersed treatment.

Methods

The study was conducted from the 19th June 2010 to the 12th of July 2010 at the The Sven Lovén Centre of Marine Sciences at Kristineberg, on the west coast of Sweden (58.15°N, 11.27°E).

Model species

The two-spotted goby (*Gobiusculus flavescens*) occurs in high density along the European west coast, from NW-Spain to N-Norway including the Baltic Sea. Along the rocky shores of Norway and Sweden it is probably one of the most common fish species in kelp forests and a key species in the ecosystem (Fosså 1991). During the breeding season this small marine, semipelagic fish (TL 3-6cm) inhabits the shallow algal zone (ca. 0-5 m depth) (Amundsen & Forsgren 2003), where non-breeding individuals often forage in shoals (Svensson et al. 2000). Shoals of between two and several hundred adult *G. flavescens* were observed commonly in my study area, as well as single individuals. The reproductive season lasts from May-July in the study area.

The two-spotted goby has a resource-based mating system and breeding males defend nest sites (Forsgren et al. 2004). Agonistic behaviour in the two-spotted goby is more pronounced in males than in females. *Fin displays*, the erection of the colorful dorsal fin, shown by males while courting a female (see below) also occur in an agonistic context to conspecific males. Males usually start agonistic interactions with *fin displays* while the involved males align their bodies (de Jong et al. 2009). Male agonistic behaviour also includes *chases*, fast approaches towards another male, which also can end in physical contact and biting (Amundsen & Forsgren 2001; Forsgren et al. 2004; de Jong et al. 2009). Males involved in agonistic interactions often change their color and become darker than usual (personal observations).

The two-spotted goby is sexually dimorphic, and the visual ornamentation plays an important role during courtship of both sexes. Typically the male starts courtship with a *fin display*, often followed by vibrating his body perpendicular to the female (*quiver*) before swimming to his nest, moving the tail in a very characteristic manner (*lead swim*) (Amundsen & Forsgren 2001; Pélabon et al. 2003). Females are characterized by orange bellies (egg development) during the reproductive season (Svensson et al. 2006), which they bend in an s-shape (*sigmoid display*) during courtship to the maximal extent towards the male (Amundsen & Forsgren 2001).

Nest sites for spawning are essential in order to assure reproductive success. Males take up nests during the breeding season in shallow waters in empty blue mussels, (*Mytilus edulis*), in brown algae (*Saccharina latissima* and *Laminaria digitata*) or in crevices e.g. between small rocks (Amundsen & Forsgren 2001; Mobley et al. 2009). Males inspect several nests (*nest inspections*), whereby they swim headfirst very close (less than 5 cm) to a potential breeding site to inspect it, before they occupy a suitable nest (personal observations). Females are courted by males and led to their nest, where eggs are laid in a single layer and fertilized by the males. The clutch size of a wild caught female placed with a single male in laboratory settings is on average between 1000-1500 (Pélabon et al. 2003; Svensson et al. 2006). Males can take care of several clutches from up to six different females at the same time (Mobley et al. 2009). Uniparental care including fanning, cleaning and defense is provided to the eggs by the male until they hatch, normally for a period of 1-3 weeks, depending on the water temperature (Skolbekken & Utne-Palm 2001; Svensson et al. 2006).

Experimental design

In order to investigate how nest spacing affects nest occupation, reproductive success and male mating behaviour, two different treatments were created (1) dispersed nest site (Fig. 1a) and (2) clumped nest site (Fig. 1b). Both treatments were arranged in 2x2m mesocosm tanks (fiberglass) which had a water depth of ca. 40 cm and a continuous flow of surface sea water (7 m depth). The water temperature in the mesocosm tanks reflected the current water temperature in the fjord (range 15°C mid-June to 19°C mid-July). Considering the limitations a laboratory study always brings along, I tried nevertheless to have a fairly natural density of fish. Each treatment contained the same number of males (eight) and ready-to-mate females (sixteen). A recent study done on two-spotted gobies using a similar experimental set up and density of fish, achieved a high proportion of reproducing males (S Wacker, K Mobely, LC Myhre, E Forsgren, T Amundsen, unpublished data). Eight artificial nests were placed in different spacing in the two treatments to assure that theoretically each male had the possibility to occupy a nest. The artificial nests were 80 mm long PVC tubes with a 13 mm inner Ø diameter, attached to a stone with a rubber band and individually marked with a number from 1-8. Previous studies have shown that males occupy these artificial nests even in the wild (Forsgren et al. 2004; de Jong 2010). One artificial nest of the given size can accommodate eggs from about four females (Forsgren et al. 2004). In the dispersed treatment the eight nests were spaced out in the mesocosm tanks with the nest openings (directed to the center of the tank) 60 cm apart from each other (Fig. 1a). In the clumped treatment the nests were placed close together in the center of the mesocosm tanks with the nest openings (directed to the center of the tank) 10 cm apart from

each other (Fig. 1b). One set of replicates consisted of: one mesocosm tank with the clumped treatment and one mesocosm tank with the dispersed treatment which always ran simultaneously. All 14 replicates ran for 3 days (d0, d1, d2), d0 functioned for acclimatisation (Table 1).

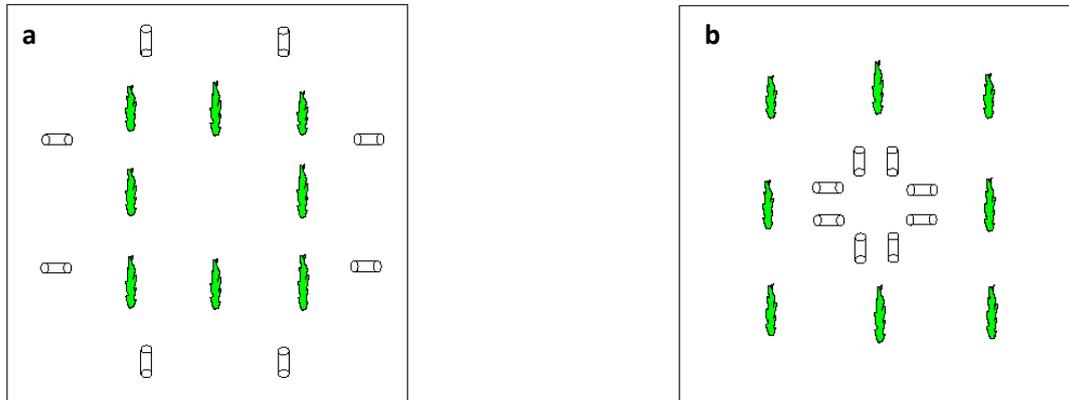


Figure 1: Experimental set up to test how nest spacing affects nest occupation, reproductive success and male mating behaviour in the two-spotted goby (*Gobiusculus flavescens*). Each 2mx2m mesocosm tank (water depth ca. 40 cm) contained eight artificial nests and plants, eight males and sixteen females, whereby nests were distributed with different spacing in the two treatments: **(a)** dispersed treatment with the nest entrances facing the tank-center 60 cm apart and **(b)** clumped treatment with the nest entrances facing the tank-center 10 cm apart.

Fish collection and husbandry

All fish used for the experiments were caught by hand-held dip nets (for details see Svensson 2006), while snorkeling around islands and shores around the research station (0 - 3 km). Fish were stored separated by sex and location of collection in aquaria of approximately 60 liters. Only females with obviously round bellies were kept for use in the experiment, slim females were released. With one exception (acclimatisation only 24 h), the fish were always kept in storage aquaria for acclimatisation for a minimum of 48h. All storage aquaria were equipped with a 1-2 cm layer of gravel, 2-3 artificial plants and had a continuous flow of surface sea water (7 m depth). The water temperature in the aquaria reflected the current water temperature in the fjord (range 15°C mid-June to 19°C mid-July). Fish were fed twice a day *ad libitum* with *Artemia* nauplii. All fish were released into their natural habitat after a replicate ended.

Selection of experimental fish

I selected ready-to-mate females for the experiments based on the roundness of their bellies. I assessed female-roundness on a scale from 1 to 3; 1 for slim females, 2 for round females and 3 for very round females (Forsgren et al. 2004). I used twice as many (sixteen) females as males (eight), and only very round females (3) to assure, that half of the males were able to fill the nest completely

with eggs (cf. Experimental design). With one exception in replicate seven, where I used also females with roundness (2), due to a shortage of very round females (3). All fish of both sexes were measured using a measuring board, to the nearest 0.5 mm. Male mean total length TL \pm SE was 42.5 mm \pm 0.24 mm (range 35.5- 53.0 mm; N = 224). Females mean total length TL \pm SE was 42.5 mm \pm 0.12 mm (range 35.0-50.5 mm; N = 448). To assure a distribution of male sizes as similar as possible in each of the two treatments, I measured and weighed all 16 males from a set of replicates and made eight size classes. A size class represented respectively the two males that were closest in their body length. For each of the eight size classes I flipped a coin to randomly decide which of the two males was meant for which treatment. In the end all eight size classes were represented once in both treatments. The wet body mass (WM) of all fish was measured on an electronic scale to the nearest 0.01 g by blotting the fish gently with tissue paper before putting it in a plastic cup with seawater of known weight and recording the total weight of the fish. Males had a mean WM \pm SE of 0.629 g \pm 0.01g (range 0.353 - 1.215 g; N = 224). Females' mean WM \pm SE was 0.726 g \pm 0.006 g (range 0.397 - 1.253 g; N = 448). Males' condition was calculated as the unstandardized residuals from the regression of mass on length. During the experiment we had to replace one male and three females that died. Males were marked in order to be able to recognize them individually in behavioural observations. I used four colors (red, green, yellow and orange) of visible implant elastomer (Northwest Marine Technology TM, www.nmt.us). Studies on comparably-sized gobies did not detect that internal elastomer tags increase mortality or influence behaviour (Griffiths 2002; Whiteman & Côté 2004). No indications of any negative impacts caused by the internal elastomer tags were found in previous studies using marked two-spotted gobies (de Jong et al. 2009; Myhre 2009). I anesthetized the males with 60 μ l of 2-phenoxyethanol diluted in 500 ml of surface sea water before I injected the internal elastomer tags. The colors were then injected subcutaneously at one of two positions (front and back) to obtain eight unique combinations. After the elastomer-injection, males were transferred separately to fresh surface sea water. After a few minutes they all regained normal swimming behaviour. After all fish were measured, weighed and the males marked, males and females were released simultaneously to the experimental mesocosm tanks.

Observational protocol

After the fish had been in the mesocosm tanks for 24 hours, I checked all nests for eggs. To disturb the fish as little as possible, I used sticks with a small mirror attached to the end to inspect the nests. I noted for each nest the presence or absence of a nest holder and the males' color-code. A male was considered a nest holder if he stayed inside a nest the moment I checked the nest. If a male was close

(< 15 cm) to a nest and I had found him inside that nest at an earlier nest-check, or he had eggs in the nest, he was also determined a nest holder. If I found a nest with eggs but without a male the moment I checked the nest, I could determine the owner of the nest often during the later followed behavioural observations (see Table 1). Moreover, I assessed the percentage of the nest area covered with eggs (“nest fullness”) by visual inspection to the nearest 10 %. Nest-checks were done twice a day (Table 1), after 24 h (d1), 36 h (d1), 48 h (d2) and 60 h (d2). At each check I also noted the roundness of each of the 16 females in a tank, to assess how many females were ready-to-mate.

Behavioural observations were made on day one (d1) and day two (d2) (Table 1). Each marked male was observed for 10 minutes both on d1 and d2. I randomized the order of observation with respect to the colour codes. I recorded behaviours of the focal male (given/received) including agonistic behaviour and courtship behaviour; for male agonistic behaviour I counted *fin displays* either given, received or both and *chasing* given, received or both. Courtship behaviour of males is compounded of different single behaviours: *fin display*, *quiver display* and *lead swim* (Amundsen & Forsgren 2001; Pélabon et al. 2003). Additional behaviours recorded during 10 min. observation time: the time a male spent shoaling, swimming solitary or remaining stationary at his nest, the number of nest-inspections a male made, time a male spent spawning and time a male spent in the nest. I also noted female behaviour towards the focal male including: *sigmoid displays* shown to the focal male (i.e. courtship-display by females), and whether females followed the focal male to his nest after courtship and if she entered his nest. After the last nest- and female-roundness-check (60 h), a replicate was terminated and the fish were released into the fjord in front of the research station.

Table 1: Time table of the recording scheme including: **(1)** weighing, measuring and marking experimental fish, **(2)** time period of acclimatisation of fish in mesocosm tanks, **(3)** nest-checks of all eight nests per mesocosm tank, **(4)** female-roundness-checks, categorising females’ fecundity from 1-3, and **(5)** 10 min. behavioural observation of each male. One exception: **(3)** and **(4)** between 12.30–13.00 and 21.00–22.30.

	d0	d1	d2	Time
(1) weighing, measuring, marking males	X			08:00 – 10:30
(2) acclimatisation	X	X		10:00 – 08:30
(3) nest - check		X	X	08:30 – 09:00 18:00 – 20:30
(4) female - roundness - check		X	X	08:30 – 09:00 18:00 – 20:30
(5) behavioural observations		X	X	09:00 – 15:00

The study was approved by The Swedish National Board for Laboratory Animals. One out of 224 males and six out of 448 females died in the laboratory. This happened in end of June/early July, a time when it is common for the fish to die or become sick also in their natural environment. This is an extremely low mortality rate for such a small, short lived fish. None of the marked males seemed to be negatively affected by the VIE tags.

Data analysis

Data were analysed using PASW Statistics 18.0 for Windows. Most analyses were made on a per tank basis in order to avoid pseudoreplication, as the behaviour and reproduction of individuals within a tank were not independent. Standard transformation of the non-parametric data failed in achieving normal distribution, and non-parametric statistics were therefore used. To test for differences between the treatments a non-parametric Man-Whitney-U test was used. I applied the term “tendency” for P-values between 0.10 and 0.05.

For analyses of behavioural data I distinguished between d1 and d2. A longer habituation to the experimental setting and a higher nest occupation rate for example, could have possibly affected the males’ behaviour and made it therefore important to distinguish between the two observation days. When proportions of behaving males (nest holders/none nest holders) were calculated, I used data on nest holding males from nest check one (after 24 hours) for d1. For d2 I used data on nest holding males from nest check three (after 48 hours), since these data on nest holding males are closest to the behavioural observations. For agonistic behaviour I pooled the data for *fin displays* given, *fin displays* given/received, *chases* given and *chases* given/received by the focal male, to achieve a bigger sample size. I did the same for courtship behaviour and pooled data of *fin displays*, *quivers* and *lead swims*. In both cases I created a new variable that included all courtship behaviours, respectively all agonistic behaviours. These new variables were used to calculate the proportion of males showing agonistic or courtship behaviour in each set of replicates.

I calculated the opportunity for selection of both treatments as the variance nest fullness divided by the square of mean nest fullness (Shuster & Wade 2003). Selection differentials for male body length and condition were calculated as difference between average body length/condition of all males and the average body length/condition of mated males. Negative values indicate a positive selection. The normal distribution of males’ body length and condition allowed using parametric statistics to test whether the mean differed from zero within a treatment (one-sample t-test) or between the two treatments (two-sample independent t-test).

The sample size was always 14 (replicates) for both treatments (clumped = N_c and dispersed = N_d). However, testing for behaviour shown by nest holders on d2, the sample size was always $N_c = 11$ and $N_d = 13$ (three replicates in the clumped, one replicate in the dispersed treatment without any nest holders). I did not test for differences of nest holders behaviour between the treatments on d1, due to the low sample size, $N_c = 3$.

Results

Nest occupation

At all nest checks a significantly higher proportion of males occupied nests in the dispersed than in the clumped treatment (Fig. 2a; Mann-Whitney U tests: 24 h: $Z = - 2.87$, $P = 0.007$; 36 h: $Z = - 2.93$, $P = 0.003$; 48 h: $Z = - 2.55$, $P = 0.011$; 60 h: $Z = - 2.40$, $P = 0.016$). Out of a total of 112 males in each treatment, 63 (56.3%) males occupied nests in the dispersed treatment, whereas only 40 (35.7 %) males occupied nests in the clumped treatment after 60 hours.

Mating success

A significantly higher proportion of mated males was found after the first three nest checks in the dispersed than in the clumped treatment (Fig. 2b; Mann-Whitney U tests: 24 h: $Z = - 2.94$, $P = 0.009$; 36 h: $Z = - 2.89$, $P = 0.004$; 48 h: $Z = - 2.52$, $P = 0.012$), while this pattern was only marginally significant at the last nest check (Fig. 2b; 60 h: $Z = - 1.99$, $P = 0.050$). In the dispersed treatment 47 out of 112 (42.0 %) males mated and in the clumped treatment 30 out of 112 (26.8%) males mated. In the dispersed treatment 47 out of 63 (74.6 %) nest holders mated and in the clumped treatment 30 out of 40 (75.0 %) nest holders mated.

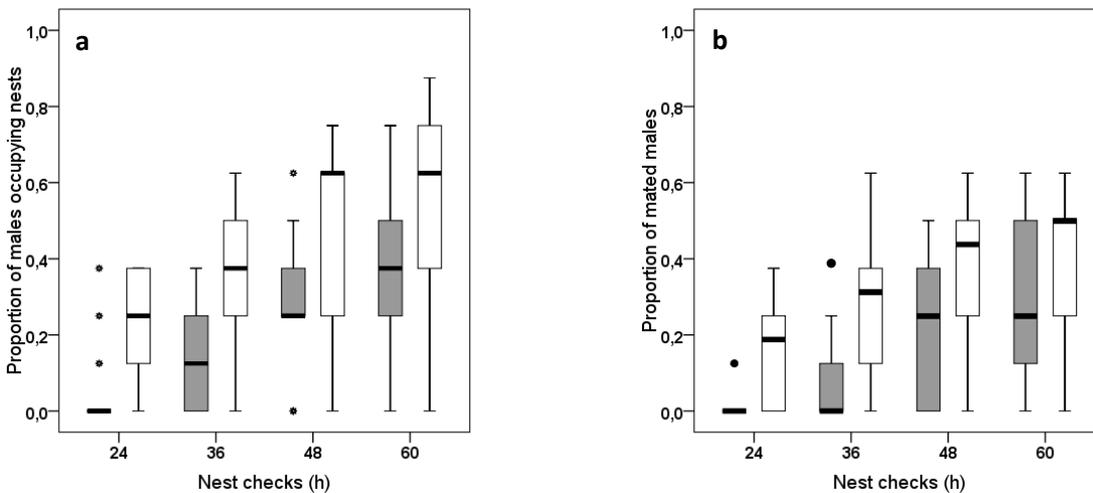


Figure 2: Proportion of two-spotted goby males **(a)** occupying nests and **(b)** received mating, after 24, 36, 48 and 60 hours in two different nest distribution treatments: the clumped treatment (shaded boxes) and the dispersed treatment (open boxes). Graphs are box plots representing the median and 25% quartiles, lines represent minima and maxima and dots/asterisks denote outliers.

Nest fullness

I found a significant difference in the nest fullness between the two treatments, with a higher nest fullness for mated males in the dispersed treatment than for mated males in the clumped treatment after 60 hours (Fig. 3; Mann-Whitney U test: $Z = - 2.38$, $P = 0.015$). A significant difference was also found, including all males per tank, with a higher nest fullness per male in the dispersed than in the clumped treatment (Fig. 3; Mann-Whitney U test: $Z = - 2.78$, $P = 0.004$).

Operational sex ratio (OSR)

Nest spacing had a strong effect on numbers of males and females ready-to-mate in both treatments. Table 2 shows how numbers of males and females ready-to-mate changed in both treatments, over the course of the experiment. The number of females' ready-to-mate decreased constantly in both treatments, and differed significantly between the two treatments after 36h, 48h and 60h (Table 2). The number of males' ready-to-mate increased constantly in both treatments, and differed significantly between the treatments after 24h, 36h and 48h (Table 2). The OSR differed already significantly between the two treatments after 24h (Fig. 4; Mann-Whitney U test: $Z = - 2.80$, $P = 0.008$), this was also the case after 36 h ($Z = - 3.18$, $P = 0.001$), 48 h ($Z = - 2.34$, $P = 0.015$), and 60 h ($Z = - 2.15$, $P = 0.030$).

Table 2: Differences between the two treatments (clumped and dispersed) in the number of **(a)** ready-to-mate males and **(b)** ready-to-mate females at all nest checks (24h, 36h, 48h and 60h). Presented are results of the Mann-Whitney U-test: median (M) of ready-to-mate individuals, minimum and maximum number of ready-to-mate individuals (range), the interquartile range (IQR) and the significance of the difference between the treatments (P).

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

The OSR in the clumped treatment stayed female-biased at all nest checks (Fig. 4; Wilcoxon Rank test: 24h: $Z = -3.49$, $P = 0.000$; 36h: $Z = -3.33$, $P = 0.001$; 48h: $Z = -3.11$, $P = 0.002$; 60h: $Z = -2.59$, $P = 0.010$). In the dispersed treatment the OSR stayed significantly female-biased after 24h and 36h (Fig. 4; Wilcoxon Rank test: 24h: $Z = -3.30$, $P = 0.001$; 36h: $Z = -3.30$, $P = 0.001$) but did not significantly differ from 0.5 after 48h and 60h (Fig. 4; Wilcoxon Rank test: 48 h: $Z = -0.97$, $P = 0.33$; 60 h: $Z = -0.43$, $P = 0.67$).

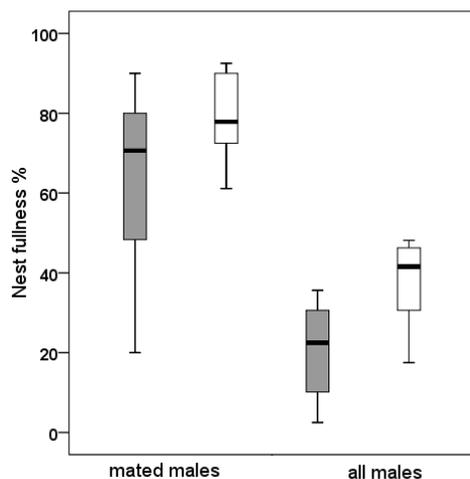


Figure 3: Nest fullness in % after 60 hours, for mated males and for all males in a tank. Shaded boxes represent the clumped treatment, open boxes the dispersed treatment. Box plot details: see Fig. 2.

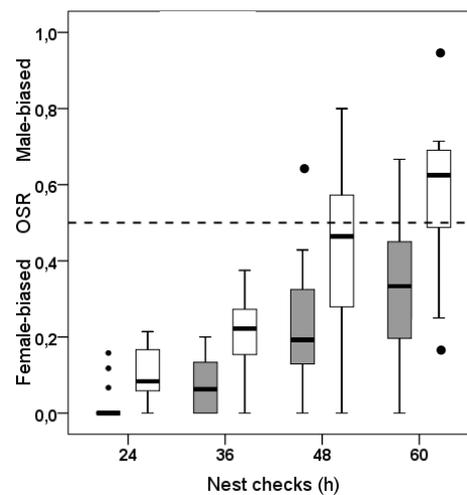


Figure 4: Operational sex ratio (OSR) of ready-to-mate females and males after 24, 36, 48 and 60 hours in the clumped (shaded boxes) and in the dispersed treatment (open boxes). Box plot details: see Fig. 2.

Selection differentials and opportunity for selection

Selection differentials were calculated to test whether male body length or condition had an effect on mating success. The body length of mated males did not significantly differ from the average body length of a male in the clumped treatment (one sample t-test: $t = -0.92$, $P = 0.38$, $N_c = 11$). There was a tendency that mated males were larger in the dispersed treatment, than an average male in this treatment (one sample t-test: $t = -2.16$, $P = 0.052$, $N_d = 13$). No significant difference was found in the selection differential for male body length between the two treatments (Fig. 5a; two-sample t-test: $t = 0.42$, $P = 0.68$). Mated males of the clumped treatment did not differ significantly in condition to an average male of the clumped treatment (one sample t-test: $t = 1.07$, $P = 0.31$, $N_c =$

11). There was a tendency for mated males to be in better condition in the dispersed treatment than an average male of that treatment (one sample t-test: $t = -1.87$, $P = 0.086$, $N_d = 13$). I found a tendency for a difference between the treatments in the selection differential on males' condition (Fig. 5b; two-sample t-test: $t = 1.99$ $P = 0.059$).

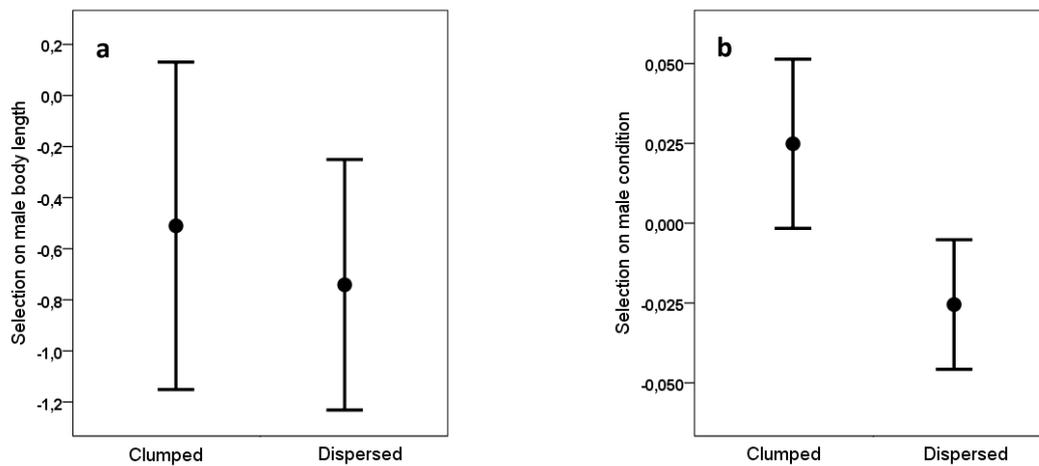


Figure 5: Selection differential (mean \pm SE 1) for **(a)** male body length (mm) and for **(b)** male condition (mg/mm) in the clumped treatment and the dispersed treatment. Negative values indicate positive selection.

There was a significantly higher opportunity for selection in the clumped than in the dispersed treatment (Fig. 6; Mann-Whitney U test: $Z = -2.17$, $P = 0.030$). In the clumped treatment nest fullness ranged from 10.5 % to 90.0 %, in the dispersed treatment from 61.0 % to 100 %.

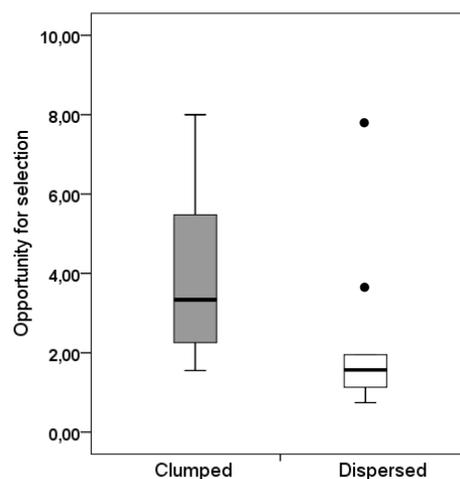


Figure 6: The opportunity for selection of mated males in the clumped (shaded boxes) and dispersed treatment (open boxes). Box plot details: see Fig. 2.

Agonistic behaviour

I found no significant difference in the proportion of none-nest holders performing agonistic behaviour between the two treatments on d1 (Fig 7a; Mann-Whitney U test: $Z = -0.36$, $P = 0.73$) or on d2 ($Z = -0.92$, $P = 0.48$). A significantly higher proportion of nest holders behaved agonistically in the clumped than in the dispersed treatment on d2 (Fig. 7b; Mann-Whitney U test: $Z = -2.26$, $P = 0.026$, $N_c = 11$, $N_d = 13$; not tested for d1, see Methods).

For those males that behaved agonistically on d2, I tested for a difference between treatments in the frequency of agonistic behaviour. I found no significant differences between the frequency of agonistic behaviour shown, by agonistically behaving nest holder between the two treatments (Mann-Whitney U test: $Z = -0.68$, $P = 0.56$, $N_c = 10$, $N_d = 11$) with a median frequency of 1.75 in the clumped treatment (range 1 – 5.5, IQR 2.18) and a median frequency of 1.00 in the dispersed treatment (range 1 – 3.75, IQR 1.00).

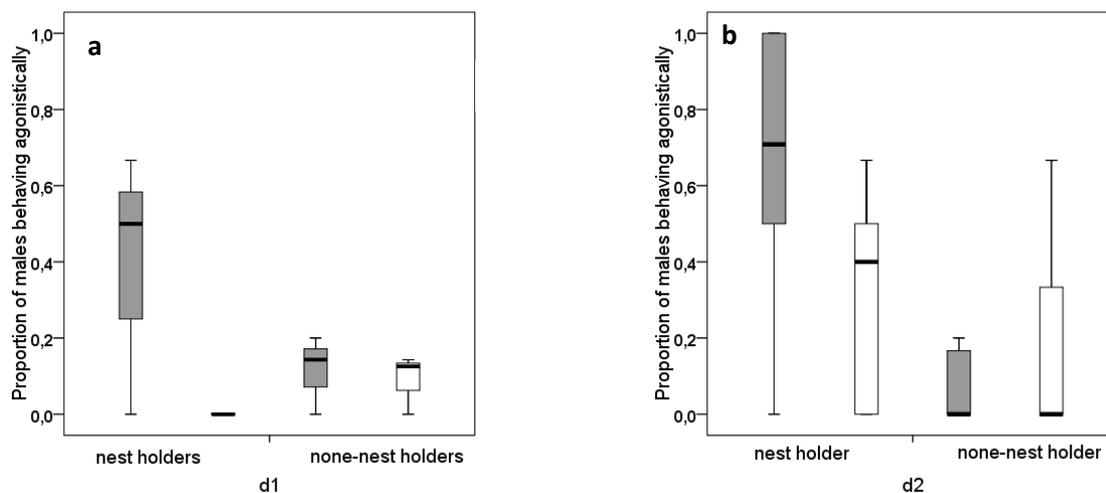


Figure 7: Proportion of nest holders and none-nest holders behaving agonistically in the clumped (shaded boxes) and the dispersed treatment (open boxes) on **(a)** d1 and on **(b)** d2. Box plot details: see Fig. 2.

Male courtship

None-nest holders showed almost no courtship behaviour and the proportion of none-nest holders showing courtship did not differ between the treatments on d1 or on d2 (Fig. 8a,b; Mann-Whitney U test: d1: $Z = -1.80$, $P = 0.35$; d2: $Z = -0.51$, $P = 0.80$). The proportion of nest holders showing courtship behaviour did not significantly differ between the two treatments on d2 (Fig. 8b; Mann-Whitney U test: $Z = -2.10$, $P = 0.72$, $N_c = 11$, $N_d = 13$; not tested for d1, see Methods).

Female courtship

The proportion of none-nest holding males receiving courtship from females showed only a tendency to be higher in the clumped than in the dispersed treatment on d1 (Fig. 9a; Mann-Whitney U test: $Z = -2.87$, $P = 0.050$). On d2 the proportion of none-nest holders receiving female courtship did not significantly differ between the two treatments (Fig. 9b; Mann-Whitney U test: $Z = -2.31$, $P = 0.069$). A significantly higher proportion of nest holders was courted by females in the clumped than in the dispersed treatment on d2 (Fig. 9b; Mann-Whitney U test: $Z = -3.01$, $P = 0.011$, $N_c = 11$, $N_d = 13$; not tested for d1, see Methods).

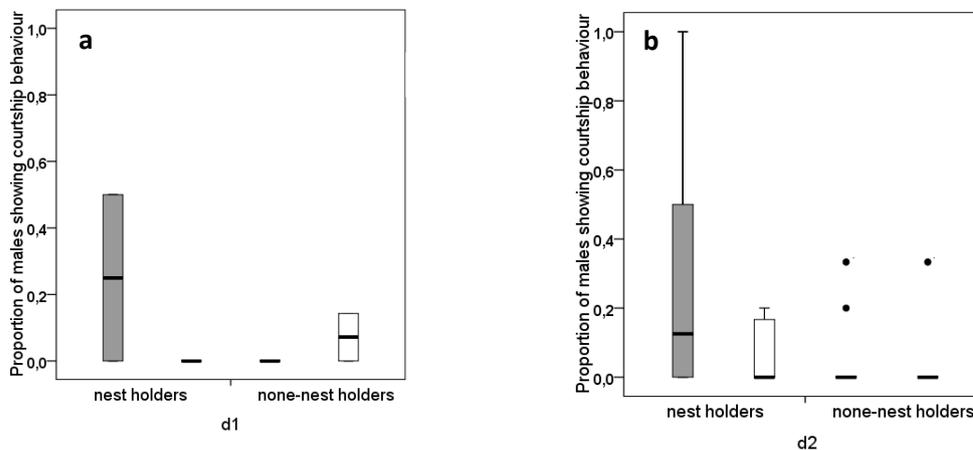


Figure 8: Proportion of nest holders and none-nest holders showing courtship behaviour in the clumped (shaded boxes) and in the dispersed treatment (open boxes) on (a) d1 and on (b) d2. Box plot details: see Fig. 2.

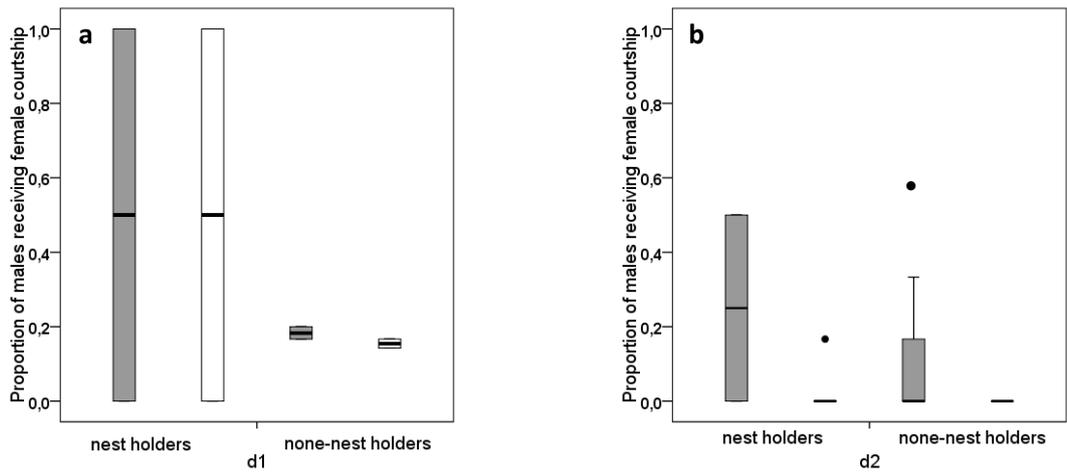


Figure 9: Proportion of nest holders and non-nest holders receiving female courtship in the clumped treatment (shaded boxes) and in the dispersed treatment (open boxes) on **(a)** d1 and on **(b)** d2. Box plot details: see Fig. 2.

Discussion

My results show that a significantly lower proportion of males occupied nests (Fig. 2a) and mated (Fig. 2b) in the clumped than in the dispersed treatment. Evidence was also found that nest clumping induces lower nest fullness, which in turn implies that fewer matings occurred in the clumped than in the dispersed treatment (Fig. 3). Nest spacing also affected the behaviour of males differently in the two treatments. A significantly higher proportion of nest holders showing agonistic behaviour was found on d2 in the clumped treatment, than in the dispersed treatment (Fig. 7b). The proportion of nest holders receiving female courtship was also significantly higher in the clumped treatment than in the dispersed treatment on d2 (Fig. 9b). These results show that nest clumping leads to a decrease in nest occupation and mating success in the two-spotted goby suggesting nest competition and territoriality in the two-spotted goby males during the breeding season. Based on my results, I suggest that these main findings are mainly a result of a higher proportion agonistically behaving nest holders in the clumped than the dispersed treatment.

Does nest-clumping prevent males from nest occupation?

Different studies have shown that the spatial distribution of breeding resources affects demographic parameters such as recruitment, juvenile distribution, competition (Einum et al. 2008) and effective population size (Twiss et al. 2007) in fish and other taxa. However, not much evidence can be found how the actual proportion of breeding individuals in a population is affected by the spacing of nest sites. I predicted that fewer males succeed in nest occupation when nests are clumped, and therefore inducing fewer mated males. This prediction was supported with a generally higher proportion of both nest holding males (Fig. 2a) and mated males (Fig. 2b), in the dispersed treatment. The proportion of mated nest holders was similar in both treatments (~ 75 %) indicating that once a male occupied a nest, nest-clumping did not affect mating success (see discussion below).

The lower proportion of males occupying nests in the clumped treatment was most likely a result of more agonistic behaviour among males in the clumped than in the dispersed treatment (Fig. 7b). Agonistic interaction among males could have been facilitated and promoted by the short distance between nests or male territories. A high encounter rate can lead to an increase in the intensity of intra-sexual competition (Kokko & Rankin 2006). The two-spotted goby has a resource-based mating system where reproduction is limited to a nest site. The higher proportion of males failing to occupy a nest in the clumped treatment is therefore also inducing the higher proportion of unmated

males in that treatment. That spacing of nest sites affects the number of males occupying nests, and hence also the number of mated individuals in a population, has previously also been shown for the closely related sand goby (*Pomatoschistus minutus*) (Lindström 1988).

Replicates lasted only for 60 hours, therefore it is uncertain to say if clumped nest spacing prevents males completely from occupying nests or if it just delays it. It is conceivable that in such a dense clumping of nests, a full occupation of all eight nests would almost never occur. A total prevention of some individuals from breeding due to clumped nest spacing could affect the effective size of a population after a certain time (Twiss et al. 2007). However, it is possible that after a longer period of time, male-male competition over unsolved dominance decreases and more nest occupations and matings occur. But even just a delay in nest occupation and mating, can have pronounced effects on such a short-lived species with a high mortality rate, where reproduction is limited to a short period of time. Two-spotted goby males are reproducing repeatedly during a single breeding season and a delay in nest occupation could strongly affect the reproductive success of an individual male. Furthermore, in animals with a restricted reproductive season, it is advantageous to breed as early as possible, to increase the winter-survival-rate of the offspring, by increasing the time-span in which it can feed and grow (Fairbairn 1977; Hendersson et al. 1988). Even though clumped spacing of nests could affect over a longer period of time the effective population size and the productivity of a population, one need to consider in this species, the basically unlimited supply of natural nest sites (Breder & Rosen 1966, personal observation). However, we do not know if suitable nest sites or high-quality nest sites are unlimited available. Males have been seen to breed very close together in small mussel banks, which are considered high quality nests (Amundsen, personal observations; Brevik 2007), which shows that clumped-breeding occurs naturally. Males may often face a trade-off in high-quality nest sites being clumped and poorer-quality nest sites being dispersed inducing competition for high-quality clumped-nest-sites.

Lower nest fullness induced by nest-clumping?

Restricted access to breeding sites often reduces the reproductive rate of a population (Oliveira et al. 1999; Ekman et al. 2001). I predicted that males of the clumped treatment have lower nest fullness, indicating fewer matings, than males in the dispersed treatment. The prediction was supported by my results that showed higher nest fullness for males of the dispersed treatment than for males of the clumped treatment (Fig. 3). Even though nest-clumping seemed not to affect mating success once a male successfully occupied a nest, these results show that nest-clumping affected the nest fullness,

which in turn indicates that nest holders of clumped nests received fewer matings. Even though nest holders of dispersed nests had to share females with more ready-to-mate males, the nest fullness was still higher here than in the clumped treatment.

I therefore suggest that the difference in the proportion of nest holders showing agonistic behaviour (Fig. 7b) between the treatments is the reason for less received matings in the clumped treatment, recorded as lower nest fullness (Fig. 3). Studies have shown that costs of agonistic behaviour can be loss of mating opportunities (Huntingford & Turner 1987; Reichard et al. 2004b; Spence & Smith 2005). To receive matings males of many substrate-breeding species have to offer two important requirements: (1) a breeding resource and (2) courtship. Males of the clumped treatment were confronted with a trade-off, once successfully occupying a nest: (1) either to focus on defending the nest/territory and be able to acquire a breeding site essential for reproduction, or (2) to focus on courting females to achieve matings, but risk losing the nest/territory to another conspecific-male seeking a nest. Much evidence can be found in fish that defending a nest or territory has priority over courting females (Candolin 1997; Santangelo et al. 2002; Reichard et al. 2004b; Spence & Smith 2005; Smith et al. 2006) inducing reduction of the total spawning rate (Reichard et al. 2004b). Focusing on the defense of a nest/territory, inducing high intra-sexual competition, could have led the focus away from attracting females in the clumped treatment. I suggest that the reproductive system of the two-spotted goby is resource-based-polygyny (Orians 1969), in which providing a nest has priority and influences the access to females. However, providing a nest does not necessarily lead to a high number of matings, as the results show. Factors like nest competition, nest spacing and possibly female choice could determine the number of matings a male receives, and therefore his nest fullness (Fig. 3).

Does nest spacing influence male-male competition?

Males often experience intense competition for access to resources, which is not surprising, knowing that resource quality can have a strong impact on the fitness of a male (Bisazza et al. 1989; Lindström 1992). The intensity of agonistic behaviour an individual shows should depend on the respective costs and benefits (Maynard Smith 1982; Huntingford & Turner 1987). I predicted to see more agonistic behaviour in the clumped than in the dispersed treatment as a consequence of higher competition over nests. The benefits of achieving a resource that is essential for reproduction should exceed the costs of competition (see Introduction). The prediction was supported with a higher proportion of nest holders showing agonistic behaviour in the clumped treatment than in the

dispersed treatment on d2 (Fig. 7b). I suggest that the most likely reason for more agonistic events among nest holders in the clumped treatment was competition for nests and territories. Agonistic interaction among males was almost exclusively observed near nests, which coincides with personal observations in the field and supports my suggestion. Occupying a nest and defending a territory in the clumped treatment might have required a higher resource holding potential and a greater ability to defend. Under natural conditions the species faces basically no nest limitations (Breder & Rosen 1966) and competition for nests and territories should only occur if differences in quality occur. The clumped treatment, which had a limited number of nests and a dense spacing, could have induced higher competition for resources that are essential for reproduction, a behaviour which is common in many substrate-breeding fish species (Lindström 1988; Almada et al. 1995). It is reasonable to think that by creating a very dense nest area, I induced a higher encounter rate of males, which additionally may have led to more agonistic interaction (Kokko & Rankin 2006). For future research it would be interesting not only to measure the encounter rate but also the propensity of a male to behave agonistically (de Jong 2010).

Intra-sexual competition among nest holders in the clumped treatment may have been caused by competition for nests and territories, but also by mating competition. In the dispersed treatment, the approach of a female towards a nest-holding male would probably in most cases remain undetected by other nest holders. However, females approaching the dense nest area in the clumped treatment were simultaneously visible to many, maybe all males. One female could easily attract the attention of several nest holders at the same time, inducing competition among these males. Close nest sites/territories often lead to intrusion from neighbouring rivals (Balsby & Dabelsteen 2003). For example in the variegated pupfish (*Cyprinodon variegatus*) the entrance of a female into a male's territory stimulated neighbours to intrude and disrupt ongoing courtship (Itzkowitz 1974). I observed a similar behaviour in two-spotted goby males, where courting nest holders were interrupted by fast approaches of conspecifics (*chases*). Another reason to assume that mating competition induced the high proportion of agonistic nest holders in the clumped treatment is the low proportion of agonistic none-nest holders (Fig. 7b). Suggesting that two-spotted goby males occupy nests before they start courting females (see Fig. 8b: almost no courtship of none-nest holders) this would explain the higher agonistic behaviour among nest holders as a consequence of competition for females. It is crucial to understand the consequences of resource competition on mating competition without confounding them. However, cases where e.g. interruption of courtship leads to male-male disputes show that it is often difficult to strictly distinguish between competition for nests and competition for mates.

Does nest-clumping increase the opportunity for selection?

Assuming that clumped nest sites lead to a high degree of monopolization by only a few males (Emlen & Oring 1977), I predicted to see a higher opportunity for selection in the clumped than in the dispersed treatment. The results show a significantly higher opportunity for selection in the clumped treatment (Fig. 6). This is based on the fact that there was a significant difference in the variation of the nest fullness between the two treatments (Fig. 3). In the clumped treatment only a small percentage of males was able to monopolize nests and constrained others completely from reproducing (Fig. 2a). This, most likely, induced the high variation in recorded nest fullness. Assuming territoriality, the higher variation in nest fullness in the clumped treatment could also be explained by a variation in the quality of territories in the clumped treatment. For example direct nest neighbours/no direct nest neighbours could lead to different preferences in female choice. Warner and Hoffman (1980) showed that territorial male mating success and mating pattern varied with the proportion of territorial and non-territorial males in the population. Another reason for the higher variation in nest fullness of clumped nests could have been differences between replicates. Ongoing male-male competition in some replicates and low agonistic interactions in others might have induced instability in nest fullness. The argument that the variation of nest fullness is a result of a low proportion of ready-to-mate females because of some highly successful males can be dismissed for the clumped treatment (see Fig. 4).

Selection on body length or condition of mated males did not show significant differences in and between the treatments (Fig. 5a, b). However, I found a tendency for a difference between the treatments in the selection differential on male body length (Fig. 5a). On the other hand there was also a tendency for a difference between the treatments in the selection differential on males' condition (Fig. 5b). It has previously been found (Wacker 2007) that body size matters for nest ownership in the two-spotted goby and that the condition of a male for mating success (S Wacker, K Mobely, LC Myhre, E Forsgren, TAmundsen, unpublished data). Different studies have shown that large males often are superior in male-male competition (Bisazza et al. 1989; Magnhagen & Kvarnemo 1989; McPeck 1992; reviewed in Andersson 1994). However, high aggressiveness and intense competition pressure can lead to a dome-shaped rather than a linear relationship between resource scarcity and fitness variation (Grant et al. 2000; Dubois & Giraldeau 2005). Further research is however needed to fully understand how male size and condition affects nest ownership, mating competition and mating success in the two-spotted goby (especially under high intra-sexual competition).

Are two-spotted goby males territorial?

Many substrate-breeding fish are territorial (Genner et al. 1999; Reichard et al. 2004b; Spence & Smith 2005; Amorim & Neves 2008). Low nest occupation rate (Fig. 2a) and mating success (Fig. 2b), in addition to high agonistic interaction between males (Fig. 7b), suggest not only the occurrence of intra-sexual competition for nest sites, but also the occurrence of territoriality in two-spotted goby males during the reproductive season (Amundsen & Forsgren 2003). Of all the results, the lower nest occupation rate found in the clumped than in the dispersed treatment is the strongest indicator to assume territoriality in two-spotted goby males (Fig. 2a). That nest-occupation-behaviour is influenced by nest spacing has been shown also in other territorial fish, including the closely related sand goby (*Pomatoschistus minutus*) (Lindström 1988; Reichard et al. 2004b). The behavioural observations of this study and also personal observations in the field suggest that territorial-aggressiveness increases near nests. The results of low nest occupations and high intra-sexual competition in the clumped treatment imply that already established nest holders restricted access of other males to nearby nest sites; a fact which can be interpreted as territorial behaviour. Another result that speaks in favor for the occurrence of territoriality is the low proportion of agonistic none-nest holder (Fig. 7a, b). This result directly relates agonistic behaviour to the defense of territories. However, more research, directly addressing territoriality, is needed for determining if this widely common behaviour in fish (Genner et al. 1999; Amorim & Neves 2008; Smith 2011) also occurs in the two-spotted goby.

Is the OSR influenced by nest spacing?

A bias in the OSR can often predict which sex will compete most for access to mates (Emlen & Oring 1977). Predicting fewer nest occupations in the clumped treatment, causing fewer males ready-to-mate, it was expected to find a more female-biased OSR in the clumped than in the dispersed treatment. This prediction was supported by the results, which showed a constantly female-biased OSR in the clumped treatment (Fig. 4). In the dispersed treatment the OSR was initially significantly female biased (after 24h and 36h) but after 48h it did not significantly differ from 0.5 anymore (Fig. 4). At the start of the experiment, all females were ready-to-mate whereas males first had to occupy a nest to become ready-to-mate (only ready-to-mate individuals are included when calculating the OSR). The clumped spacing of the nests led to high competition for nests among males (Fig. 7b), and nests became a limiting factor. Males precluded other males from nest occupation in the clumped treatment (Fig. 2a), which induced that the OSR stayed female-biased. I

found a significant difference in the OSR between the treatments at all nest checks (Fig. 4). This shows that nest spacing strongly affected the number of individuals' ready-to-mate in a population (Table 2). However, the OSR was created artificially and it remains to test if clumping of nests shows the same effect on OSR under natural conditions.

Is courtship behaviour affected by nest spacing?

Much evidence can be found on fish, of how increased male intra-sexual competition induces interruption of courtship events. This can lead to females leaving the spawning site, which can lower the total spawning rate in a population (Candolin 1997; Spence & Smith 2005; Reichard et al. 2004b). I predicted to see a negative impact on male courtship behaviour in the clumped treatment, caused by higher male-male competition. Other studies on fish have found that high male-male competition can directly affect the courtship frequency (Kangas & Lindström 2001; Spence & Smith 2005; Price & Rodd 2005). Based on the clumped nest area I expected more interruptions of courtship events to take place among competitive conspecifics. However, the results show no significant difference between the treatments, in fact overall only very few courtship events were observed in both treatments (Fig. 8a, b). It is possible that the observation scheme simply did not capture male courtship well as a consequence of too short observation time per male. My results do not show much about male courtship behaviour, except that in 10 min observation time more agonistic (Fig. 7a, b) than courtship behaviours were observed.

However, female courtship behaviour seemed to be strongly affected by nest spacing. A significantly higher proportion of males in the clumped treatment were courted by females than in the dispersed treatment (Fig. 9b). Forsgren et al. (2004) showed that for a female-biased OSR female courtship behaviour, and the competition between females for breeding opportunities increased. A constantly female-biased OSR in the clumped treatment (Fig. 4) could therefore explain the significant higher proportion of female courtships towards nest holders in this treatment (Fig. 9b). It might simply be the result of having more unmated males in the clumped than in the dispersed treatment (Table 2). However, even though nest holders of the clumped treatment received more female courtship, they ended up with lower nest fullness than nest holders in the dispersed treatment (Fig. 3). This could be another indicator that high male-male competition (Fig. 7b) prevents nest holders in the clumped treatment from focusing on mating success (Fig. 2b). For future studies it would be interesting to determine the female propensity to court, this might provide a better basis for interpreting results like this.

Conclusion

This study showed that clumped nest spacing induced fewer nest occupations and lower mating success. I found that nest holders in the clumped treatment had lower nest fullness, inducing fewer performed matings, than nest holders in the dispersed treatment. I suggest that these findings are a result of more agonistic behaviour among males in the clumped than in the dispersed treatment. Moreover, the low nest-occupation-rate of males in connection with high proportion of nest holders showing agonistic behaviour in the clumped treatment, suggests that two-spotted goby males defend not just nests but also territories. The nest fullness of nest holders in the clumped treatment showed a higher variation than for nest holders in the dispersed treatment leading to a higher opportunity for selection in the clumped treatment. A significantly higher proportion of males received female courtship in the clumped than the dispersed treatment, a possible effect of a constantly stable female-biased OSR in this treatment. To sum the most important findings up: clumped nest spacing led to fewer nest occupations and therefore lower mating success, to lower nest fullness per nest holder indicating fewer performed matings and to higher agonistic interaction among nest holders suggesting competition for nests and territories.

Spatial distribution of breeding resources can affect the strength of sexual selection on traits, as a consequence of variation in reproductive success among individuals of the same sex (Andersson 1994). It would be very interesting to test in further studies on the two-spotted goby, how selection on traits associated with intra-sexual competition is affected by nest spacing. In addition, further research is necessary directly addressing territoriality in *G. flavescens* to support my assumption of territorial two-spotted goby males. Field studies are needed to test if my results coincide with results of complementary experiments in the field.

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