Spatial and temporal patterns of nest distribution influences sexual selection in a marine fish

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Summary

In many species, the natural distribution of material resources important for reproduction can profoundly impact reproductive success among individuals and, hence, the opportunity and intensity of sexual selection. Here, we report on a field-based experiment investigating the effects of nest aggregation on sexual selection in a fish, the sand goby *Pomatoschistus minutus*. We found that the distribution of potential nests (sparse versus aggregated nest treatments) affected patterns of nest colonization and reproductive success. Specifically, in the treatment with aggregated nesting resources, a greater proportion of nests remained unoccupied by sand goby males. Although the size of nesting males did not differ between treatments, eggs accumulated more rapidly when nests were sparsely distributed. We found that the opportunity for selection decreased over time with the accumulation of eggs in the nests in both the aggregated and sparse treatments. Moreover, the effect of male size on reproductive success was influenced by an interaction between nest distribution and time, with the selection gradient being highest right after nest colonization when nests were aggregated, while the opposite pattern was observed in the sparse nest treatment. Such findings highlight the vital role that environmental and social factors can play in determining the importance of male phenotypic traits (in this case, male size). More broadly, our results also underscore how the natural distribution of resources, both in space and time, can impact the strength of sexual selection acting on wild animal populations.

Keywords: selection gradient, mating system, density dependence

Introduction

Sexual selection theory predicts greater variance in reproductive success and, therefore, a higher opportunity for sexual selection, with increasing mating competition (Andersson 1994). Such competition can influence the distribution of reproductive success in multiple ways, especially in so called 'resource defense' mating systems, where resources needed for reproduction (e.g. nest sites) are monopolized by a subset of individuals. First, mating competition can determine the phenotypes that manage to successfully obtain and defend a breeding resource (Parker 1974) and, hence, qualify to mate (reviewed in Hardy and Briffa 2013). For instance, male red collared widowbirds (*Euplectes ardens*) that possess larger red collars are more successful in contests over territories even though females prefer males with longer tails, a trait that is negatively correlated with the size of the male's collar (Andersson et al. 2002). Second, competition can determine the quality of the resource that an individual is able to acquire. For example, in many species of nest-guarding fish, the size of a male's nest can directly affect the number of clutches he is able to receive, thus acting as a physical limit to the number of mating opportunities a male can obtain (Hastings 1988, Marconato et al. 1989). Finally, the distribution of reproductive success can be affected by competitive interactions that result in courtship/mating interference (Casalini et al. 2010). An example of this is seen in the rose bitterling (*Rhodeus ocellatus*), with density affecting male courtship rate due to a trade off between male competition and female attraction (Casalini et al. 2010).

Because successful resource monopolization can give individuals direct or indirect access to mates and determine how reproductive success is distributed among individuals, it has a direct bearing on the opportunity and intensity of sexual selection (Emlen and Oring 1977, Shuster and Wade 2003). For example, when reproduction is limited to nesting sites or breeding territories, the availability and distribution (e.g. sparse versus aggregated) of these resources can influence the degree of multiple mating enjoyed by the resource (Reichard et al. 2009, Mück et al. 2013). Variation in reproductive success is predicted to increase with population density (Kokko and Rankin 2006), as has been shown in species as diverse as seed bugs (McLain 1992) and red deer (Clutton-Brock et al. 1997). However, in other taxa, higher density has been shown to have little or no effect on sexual selection, as in two-spotted gobies (*Gobiusculus flavescens*) (Wacker et al. 2013), or may even decrease the strength of selection, as in a number of beetle (Coleoptera) species (Conner 1989, Pomfret and Knell 2008), broad-nosed pipefish (*Syngnathus typhle*) (Aronsen et al. 2013) and common yellowthroats (*Geothlypis trichas*) (Taff et al. 2013). The reasons for these seemingly conflicting results are yet to be resolved.

A number of factors can result in temporal and spatial variation in the distribution of reproductively active individuals and the availability of resources they require for mating. For instance, sexual selection estimates can change markedly between (Madsen and Shine 1993, Gosden and Svensson 2008, Lehtonen et al. 2010), or even within (Forsgren et al. 2004), breeding seasons. Due to varying degrees of seasonal synchrony in female breeding cycles, temporal variation in selection can also occur without changes in resource availability or sex ratio (Lindström 2001). Similarly, sexual selection can vary spatially (Lehtonen and Lindström 2004, Gosden and Svensson 2008), which could be an important driver of population divergence and speciation (Lande 1982), especially when coupled with limited gene flow (Hendry et al. 2002).

Resource aggregations, combined with the presence of many resource-holding males, could also favor the ability of females to compare different male phenotypes and, therefore, facilitate sexual selection through female choice. In contrast, when breeding resources and, hence, resource-holding males, are spread out, sampling of potential suitors may become more cognitively demanding (Janetos 1980, Real 1990), energetically costly and time consuming (Janetos 1980, Real 1990, Milinski and Bakker 1992), and perilous in terms of heightened predation risk (Kasumovic et al. 2007). Therefore, we might expect a tighter relationship between reproductive success and key male traits when males are more readily available for simultaneous comparison.

Manipulative studies related to population density and resource availability have usually taken two different approaches. Specifically, studies typically manipulate either the number of prospecting individuals relative to resource availability, in which case they have directly manipulated population density (e.g. Casalini et al. 2010, Wacker et al. 2013), or the availability of resources, including how these are distributed (e.g. Borg et al. 2002, Lehtonen and Lindström 2008, Mück et al. 2013). In the vast majority of cases, studies have been performed on laboratory populations, whereas manipulation of resource aggregation in free-living, wild populations, have been far less common (but see Borg et al. 2002, Lehtonen and Lindström 2008).

Here, we report the results of a field-based experiment testing the effect of nest aggregation on sexual selection in the sand goby (*Pomatoschistus minutus*), a small marine fish with a resource defense mating system. By manipulating nest aggregation, we created spatial heterogeneity in the prerequisites for phenotypic selection, while allowing population density, and colonizer and intruder pressure to follow natural conditions. The sand goby is a benthic species native to sandy coastal habitats across Europe (Miller 1986). During their single breeding season (Healey 1971, Fonds 1973), male sand gobies construct their nests under empty mussel shells or rocks by piling sand on top of—and excavating under—the substrate, leaving a single narrow opening. Males attract females using vigorous courtship displays and, within the limits of the size of the nest, are capable of receiving eggs from multiple females (Jones et al. 2001). Depending on the population, nesting resources (i.e. shells and rocks) can be in short supply, with males competing vehemently for access to nests (Forsgren et al. 1996), with the intensity of nesting resource competition significantly increasing the variance in male reproductive success (Lindström 2001, Lehtonen and Lindström 2004).

In this study, we examined how nest site aggregation in the field affected patterns of male settlement and reproductive success over time. Male-male competition is expected to be stronger in more aggregated sites because males would have more opportunities for competitive interactions when in close physical proximity (*sensu* Kangas and Lindström 2001). Since previous work has shown that male size in sand gobies is relevant for resource holding potential (Lindström and Pampoulie 2005), we predicted that aggregated nest sites would favor larger individuals due to heightened male-male competition. There is also evidence that differences in male-male competitive interactions could, in turn, affect both the ability of additional males to settle in neighboring nests (see Lehtonen and Lindström 2008), as well as the ability of males to accumulate egg clutches from females (Lindström and Seppä 1996). Accordingly, we expected that a bigger proportion of nests would remain unoccupied in aggregated sites. Lastly, we predicted that males should receive fewer eggs when nesting resources are aggregated because courtship would be interrupted more often due to competitive interference (Kangas and Lindström 2001). We also expected that when nesting resources are aggregated, a male's reproductive success should be more strongly dependent on his competitive ability relative to the other males nesting in his vicinity than when nesting resources are sparsely distributed.

Material and methods

General experimental design
This study was carried out on the south coast of Finland near the Tvärminne Zoological Station in June 2005 during the sand goby breeding season, which typically lasts from ~ mid May to mid July. The area where we carried out our experiment was situated underwater (<1.5 m depth) on the sandy, landward side of Vargskär Island. Here, as is typical for this population (Lindström 1988, Forsgren et al. 1996), natural nesting resources (i.e. suitable shells and rocks) were scarce, providing us with an excellent opportunity to experimentally manipulate nest aggregation locally. This was achieved by introducing ceramic tiles into the study area as potential nesting resources. The size of the tiles chosen (length × width =10 cm × 10 cm) fell within the natural range of nesting resources exploited by male sand gobies (Wong et al. 2008) and are readily accepted as nests (Forsgren et al. 1996, Lehtonen and Lindström 2004). For each replicate, four tiles were carefully placed onto the sandy substrate in an unoccupied area (i.e. an area where there were no nesting sand gobies or potential nesting resources within a min 2 meter radius from a tile). The four tiles were arranged on the substrate in a 2 × 2 square configuration, with the tiles spaced either 50 or 200 cm apart to simulate sparse and aggregated nest sites, respectively. Adjacent replicates were separated by at least 20 m to ensure independence. A 1 m long iron pole was then hammered into the substrate, with a length of tape fastened on top of the pole to mark the location of each replicate. The two different nest aggregations used in our study are reflective of those encountered naturally in the population (Forsgren et al. 1996). In the aggregated nest set-up, nesting males would be able to very easily see and interact with each other. It is also expected that females venturing into an area where nests are more aggregated would be able to see and interact with multiple nesting males at a time. By contrast, the distances between males in the sparse nest set-up was expected to reduce the ability of nesting males to interact, as nesting males rarely move further than 50 cm from their nest (Lindström and Hellström 1993). Further, females venturing into a replicate where nests were sparsely distributed would be unable to see or interact with more than one nesting male at a time.

To investigate any temporal changes in egg accumulation and size distribution of the nest occupants, we allowed replicates to remain in the field for 1, 2, or 5 days before the nests were checked for the presence of nest-holding males and eggs. Both egg accumulation and nest occupants may change over time: new females can deposit additional eggs in the nest (Lindström 1992), and the size distribution of nest holders may change due to predation on males (Lindström and Ranta 1992) and nest take-overs (Lindström 1992).

In total, we carried out 88 replicates (with $88 \times 4 = 352$ tiles), arranged in a 2×3 factorial design (aggregation $\times$ time). In each treatment combination, we had 15 replicates, except in the sparse treatment that was checked after one day, which had 13 replicates. The experiment was run during the four weeks of June, with approximately similar numbers of replicates starting each week (first week = 27, second week = 21, third week =22 and fourth week = 18 replicates).

Nest occupancy, size of nesting males, and distribution and size of clutches

We checked each replicate (n = 88) by using a mask and snorkel to firstly identify the number of tiles that had been colonized. We attempted to catch each nesting male with a hand net. Captured males were individually photographed in a shallow dish next to a small ruler for scale before being removed from the study site and brought back to the research station for use in unrelated experiments. The underside of each occupied tile was also carefully retrieved and the clutch photographed. Any remaining (i.e. unoccupied) tiles and the marking post were then removed.

Opportunity for sexual selection and selection gradients

A number of previous studies have shown that male size is an important trait explaining reproductive success in sand gobies (Forsgren et al. 1996, Lindström and Seppä 1996). Since females lay their eggs in a single layer, the area of the egg mass corresponds to the number of eggs and is therefore a good measure of a male's reproductive success (see also Jones et al. 2001). As a consequence, egg mass area was our fitness measure and male length was the selected trait. Our main objective was to compare selection in the different nest treatments (i.e. sparse vs aggregated). This was accomplished in two ways. First, we calculated the opportunity for selection, I, for each replicate using egg area as our measure of reproductive success. The opportunity for selection, which is the variance in reproductive success divided by the squared mean reproductive success (Arnold and Wade 1984, Krakauer et al. 2011), gives the potential for selection, and is therefore a useful description of how the mating system depends on resource distribution (Krakauer et al. 2011). We were able to calculate the opportunity for selection for all replicates with at least 2 occupied nests. Hence, 86 replicates were used in calculating the selection opportunity.

Second, we calculated the standardized linear selection gradient using a regression with standardized fitness on standardized trait (Arnold and Wade 1984). We standardized the reproductive success within each replicate of four nests by dividing the egg mass area by the corresponding replicate mean egg mass area for each male. Similarly, male size was standardized to have a mean of zero and a variance of one for each replicate of four nests. This was done by subtracting the mean of all males within a replicate and dividing this value by the standard deviation of the lengths of these males. Because we did not have direct information on the reproductive success of each male, which would have required genotyping, and possible survival estimates of offspring, it was not possible to apply other measures of selection (sensu Henshaw et al. 2016), such as the Jones index (Jones 2009). In some instances, the nesting males (n = 58 of 323 occupied nest) could not be captured due to inclement weather conditions or poor visibility, or because fish simply alluded capture. As a result, we calculated the selection gradient only for replicates in which male length and egg mass were known for at least two nests. There were a total of 80 such replicates.

Statistical analyses

We analyzed the proportion of unoccupied tiles using a generalized linear model with a multinomial distribution and a cumulative logit link function. The two treatments of our experiment, time and nest aggregation, were used as the explanatory variables in all analyses. Egg mass area was analyzed with a generalized mixed model using a gamma error distribution and a log link function to account for the data being biased towards larger values. Replicate (four tiles in each replicate) was included as a random

effect. The opportunity of selection was biased towards larger values and therefore also analyzed using a generalized linear model with a gamma error distribution and a log link function. The selection gradient was analyzed using a generalized linear model using a normal error distribution with an identity link function. Male length fulfilled the requirements of parametric general linear model. Likewise, the coefficient of variation in male length fulfilled the requirements of parametric general linear model after arc sine transformation. All models always included all main factors and their interactions. All analyses were done in SPSS v 23.

Results

Nest occupancy
Of the 352 tiles made available, 323 were occupied within the observation period. The proportion of unoccupied tiles was higher in the aggregated treatment but was not affected by time (aggregation effect, Wald X^2 = 7.125, df = 1, p = 0.008, Fig. 1).

Distribution and size of clutches
In total, 307 out of the 323 occupied nests contained eggs. The empty nests were equally distributed among the treatments (time, Fisher's exact p = 0.217; nest aggregation, Fisher's exact p = 0.781) and were therefore excluded. Time had the most pronounced effect as the egg area increased strongly with the length of time the nests had remained in the field (generalized mixed model, $F_{2,298}$ = 111.432, p < 0.001, Fig. 2a). Nests in the sparse treatment contained, on average, slightly more eggs than nests in the aggregated treatment ($F_{1,298}$ = 4.683, p = 0.045, Fig. 2a). Eggs accumulated more slowly in the aggregated than sparse treatment (interaction, $F_{2,298}$ = 3.779, p = 0.024, Fig. 2a).

Size of nesting males
We found that the size of nesting males increased with time (nested two factor ANOVA, time effect; $F_{2,98.75}$ = 6.958, p = 0.001, Fig. 2b) but was not affected by nest aggregation ($F_{1,99.05}$ = 2.034, p = 0.131). Replicates also did not differ significantly in the mean size of males occupying the nests (nested factor, $F_{79,177}$ = 1.043, p = 0.402). Lastly, there was no effect of treatment on the variation in male size, as measured by the CV in male length, within each replicate (arcsine square root transformed CV values, total df = 82, all p ≥ 0.146).

In order to test if the size of resident males affected the probability of all nests in a replicate being inhabited, we compared mean male size between replicates where all nests had been colonized with replicates that contained one or more empty nests. Male size was slightly larger, but not significantly so, in incompletely colonized replicates (t-test comparing mean male length standardized for treatment effects, t = 1.195, df = 83, p = 0.235).

Opportunity for sexual selection and selection gradients
The opportunity for selection was similar in the sparse and aggregated treatments (aggregation treatment effect, Wald X^2 = 0.189, df = 1, p = 0.664). Specifically, the opportunity for selection was highest after one day but, as nests started to accumulate eggs, the opportunity for selection decreased with time (time effect Wald X^2 = 11.288, df = 2, p < 0.004, Fig. 3).

The main factors did not affect the selection gradient (nest aggregation effect, Wald X^2 = 0.279, df = 1, p = 0.598; time effect, Wald X^2 = 5.179, df = 2, p = 0.075) but there was a significant interaction between nest aggregation and time on the selection gradient (Wald X^2 = 13.561, df = 2, p = 0.001). In the aggregated nest treatment, the gradient was initially strong and positive, with larger males enjoying a higher reproductive success (Fig. 4). In the sparse treatment, the selection gradient remained at very low values and showed no change over time indicating that there was no relationship between male size and reproductive success (Fig. 4).

Discussion

Our field-based experimental study shows that the distribution of nesting resources can have a profound impact on patterns of nest colonization and reproductive success in sand gobies. First, we found that a greater proportion of nesting resources (i.e. tiles) remained unoccupied when nesting resources were aggregated. Second, although there was no difference between treatments in the size of the males occupying the tiles, we did find a significant interaction between nest distribution and reproductive success, with eggs accumulating more rapidly in the sparse nest treatment. Third, the opportunity for selection decreased over time, and this was true in both the sparse and aggregated treatments. Lastly, we found an interaction effect of nest aggregation and time on the selection gradient on male body size. Specifically, following nest colonization, we found a significant, positive selection gradient in the aggregated treatment, with larger males enjoying a higher reproductive success, which disappeared with time. In the sparse nest treatment, the gradient remained weak.

Nest distribution influenced patterns of nest settlement, with a greater proportion of nests remaining unsettled in the aggregated nest treatment. One possible explanation for this is that individuals already occupying a nest may prevent others from settling nearby. This has been shown, for example, in the fiddler crab (*Uca mjobergi*), where the behavior of resident males can prevent others from settling into neighboring burrows (Backwell and Jennions 2004). In this respect, the phenotype of both the resident and the prospective settler can be important, with size often being a mediating factor. This is because, in many species, including the sand goby, larger males often have a physical advantage over smaller rivals (e.g. Rosenberg and Enquist 1991, Lindström 1992, Jennions and Backwell 1996, Hack et al. 1997). However, it is important to point out that the outcome of agonistic encounters is not always resolved by straightforward asymmetries in the size of the contestants alone (Olsson and Shine 2000). For example, a territory owner may value the resource more than an intruder and, as a result, is more motivated to fight (Enquist and Leimar 1987, Elwood et al. 1998). Thus, factors other than body size can also be important in explaining settlement patterns and the lack of any apparent difference in the size of males that managed to settle in the aggregated treatment compared to those that settled in the sparse.

In regard to male-male interactions, males may also be disinclined to settle into areas that already have a high concentration of nesting competitors since nesting in close physical proximity is known to increase the frequency of encounters between neighboring individuals (Lehtonen and Lindström 2008, Mück et al. 2013). Apart from the risk of serious injury, such encounters can also impact male reproductive success. In the variegated pupfish (*Cyprinodon variegatus*), for example, males nesting nearby have been shown to intrude upon, and disrupt, the courtship of the territory holder (Itzkowitz 1974). This can result in missed mating opportunities, especially as males, of many species, often prioritize the defense of breeding resources over mate attraction (Santangelo et al. 2002, Reichard et al. 2004, Wong 2004, Mück et al. 2013). In this respect, heightened interference competition at higher male densities has been shown to reduce spawning rate, as in the European bitterling (*Rhodeus sericeus*) (Reichard et al. 2004). In sand gobies, neighboring males are known to interfere with one another's courtship displays when in close physical proximity (≤ 50 cm, *sensu* Kangas and Lindström 2001). Hence, heightened interference and potential impacts on male reproductive success could potentially explain not only the lower levels of nest occupancy in the aggregated nest treatment, but also why males nesting in the aggregated treatment accumulated eggs more slowly compared to those nesting in the sparse treatment. We found no evidence that the size of resident males influenced the probability that all four nests in a replicate were colonized. However, caution should be exercised in the interpretation of this result given that this measure may not have been sensitive enough to detect an effect, and the fact that we would only have expected to see a size effect in the aggregated treatment where interactions among neighboring males were assumed to be more important.

Although there was no difference between treatments in the size of nesting males, large males may nevertheless end up enjoying a mating advantage as a result of direct male-male aggression or interference of the courtship of smaller rivals, especially when nesting resources are aggregated (Lindström 1988, Reichard et al. 2004). In this regard, the success of large males is likely to be bolstered particularly when more space within the nests becomes available due to seasonal effects (Kvarnemo 1994, Lindström 2001), predation events (Lindström and Ranta 1992), nest take-overs (Lindström 1992, Lindström and Pampoulie 2005) or hatching of older egg clutches (Kvarnemo 1994). This pattern was supported by the strong positive selection gradient in the aggregated treatment. However, when successful males are no longer able to physically accommodate additional egg clutches to their nests, their reproductive success becomes constrained by the size of the nest (Lindström 1992). When this happens,

neighboring, smaller males may begin to enjoy higher levels of reproductive success (Lindström 2001), thereby explaining patterns of male reproductive success observed in the aggregated nest treatment over time.

By contrast, direct interference by nesting neighbors is less likely when nesting resources are dispersed. This is especially true in the sand goby, as nesting males stay in close proximity to their nests (Lindström and Hellström 1993). Indeed, when nesting resources are more sparsely distributed, other factors, such as resource holding potential, are likely to become more important. This is because, in contrast to the aggregated nest treatment, most of the males encountered by residents would be potential nest intruders, rather than established neighbors. Previous work has shown that competition for nests in this population of sand gobies is heightened due to a shortage of suitable nesting resources, such as shells and rocks (Forsgren et al. 1996, Lehtonen and Lindström 2004). As a result, nest-holding males not only have to invest time and resources into attracting mating opportunities, but also defending their nests from potential take-overs. The increase in the size of nesting males over time, in both treatments of the current study, is consistent with the displacement of nest holders by larger individuals over time. In this respect, smaller nesting males often face a greater trade-off between nest defense and mate attraction, as seen, for example, in common gobies (*Pomatoschistus microps*) (Borg et al. 2002). By contrast, in common with many other species (reviewed in Hardy and Briffa 2013), larger nest holding sand goby males typically enjoy a higher resource holding potential compared to their smaller counterparts in the aggregated treatment (Lindström and Pampoulie 2005).

It is important to point out that patterns of male reproductive success can also be influenced by factors that were not directly measured in the current study. For example, the reproductive success of nesting sand goby males can be affected by the presence of sneaker males (Jones et al. 2001). In the context of the current study, patterns of sneak fertilizations could be relevant if rates of sneaking differ between aggregated and sparse treatments. Unfortunately, it was not logistically possible to genotype the broods in our current experiment. However, in a previous field study, Jones et al. (2001) compared the sneaking frequencies in two populations differing in nest availability (nest site density) and found no differences in sneaking rates. Further, in a subsequent experimental study under controlled laboratory conditions, Singer et al. (2006) manipulated the number of nests available and similarly found no effect of nest density on sneaking rates. Hence, previous work suggests that different nest aggregation levels may not have a profound impact on sneaking rates, although we cannot completely exclude this possibility. Similarly, in the absence of paternity analyses, we also cannot definitively rule out the possibility that, due to nest take-overs, at least some of the eggs within a male's nest may belong to a previous owner if those eggs were not immediately consumed by the usurping male (e.g. Jones et al. 2001). Lastly, filial cannibalism and egg predators can also be influential, although these have specifically been taken into account through the use of reproductive success in our estimates (as opposed to mating success), which considers the actual number of eggs present.

In conclusion, the results of our study provide insights into how the distribution of nesting resources may influence patterns of nest occupation and reproductive success in wild, free-living populations. Specifically, our findings underscore how both spatial and temporal patterns of nest distribution, by influencing the nature and extent of behavioral interactions among individuals, have the potential to affect reproductive success and, in so doing, impact the strength and direction of sexual selection. As our findings suggest, the spatial distribution of breeding resources can influence their availability to individuals for reproduction and the relationship between resource availability and the number of breeding individuals is not necessarily linear. Our results also suggest that the estimates of selection opportunity and gradients can vary over time, thus emphasizing the need to move beyond cross-sectional studies, which, although informative, may lead to a distorted view of the evolutionary potential of sexual selection. Finally, our study highlights how the importance of a male's phenotype can be dependent upon the environmental and social setting.

References

Andersson, M. 1994. Sexual selection. - Princeton University Press.

Andersson, S., et al. 2002. Multiple receivers, multiple ornaments, and a trade-off between agonistic and epigamic signaling in a widowbird. - Am. Nat. 160: 683-691.

Arnold, S. J. and Wade, M. J. 1984. On the measurement of natural and sexual selection: theory. - Evolution 38: 709-719.

Aronsen, T., et al. 2013. Sex ratio and density affect sexual selection in a sex-role reversed fish. - Evolution 67: 3243-3257.

Backwell, P. R. Y. and Jennions, M. D. 2004. Coalition among male fiddler crabs. - Nature 430: 417.

Borg, Å. A., et al. 2002. Plastic sex-roles in the common goby - the effect of nest availability. - Oikos 98: 105-115.

Casalini, M., et al. 2010. The effect of crowding and density on male mating behaviour in the rose bitterling (*Rhodeus ocellatus*). - Behaviour 147: 1035-1050.

Clutton-Brock, T. H., et al. 1997. Density-related changes in sexual selection in red deer. - Proc. R. Soc. B. 264: 1509-1516.

Conner, J. K. 1989. Density-dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. - Evolution 43: 1378-1386.

Elwood, R. W., et al. 1998. Probing motivational state during agonistic encounters in animals. - Nature 393: 66-68.

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

Enquist, M. and Leimar, O. 1987. Evolution of fighting behaviour: the effect of variation in resource value. - J. Theor. Biol. 127: 187-205.

Fonds, M. 1973. Sand gobies of the Dutch Wadden Sea (Pomatoschistus, Gobiidae, Pisces). - Neth. J. Sea. Res. 6: 417-478.

Forsgren, E., et al. 2004. Unusually dynamic sex roles in a fish. - Nature 429: 551-554.

Forsgren, E., et al. 1996. Mode of sexual selection determined by resource abundance in two sand goby populations. - Evolution 50: 646-654.

Gosden, T. P. and Svensson, E. I. 2008. Spatial and temporal dynamics in a sexual selection mosaic. - Evolution 62: 845-856.

Hack, M. A., et al. 1997. Fighting in males of the autumn spider, *Metellina segmentata*: Effects of relative body size, prior residency and female value on contest outcome and duration. - Ethology 103: 488-498.

Hardy, I. C. W. and Briffa, M. 2013. Animal contests. - Cambridge University Press.

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

Healey, M. C. 1971. Gonad development and fecundity of the sand goby, *Gobius minutus* Pallas. - Trans. Amer. Fish. Soc. 100: 520-526.

Hendry, A. P., et al. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the misty system. - Evolution 56: 1199-1216.

Henshaw, J. M., et al. 2016. A rigorous comparison of sexual selection indexes via simulations of diverse mating systems. - Proc. Natl. Acad. Sci. 113: E300-E308.

Itzkowitz, M. 1974. The effects of other fish on the reproductive behaviour of the male variegated pupfish, *Cyprinodon variegatus* (Pisces: Cyprinodontidae). - Behaviour 48: 1-22.

Janetos, A. C. 1980. Strategies of female mate choice: a theoretical analysis. - Behav. Ecol. Sociobiol. 7: 107-112.

Jennions, M. D. and Backwell, P. R. Y. 1996. Residency and size affect fight duration and outcome in the fiddler crab *Uca annulipes*. - Biol. J. Linn. Soc. 57: 293-306.

Jones, A. G. 2009. On the opportunity for sexual selection, the Bateman gradient and the maximum intensity of sexual selection. - Evolution 63: 1673-1684.

Jones, A. G., et al. 2001. Surprising similarity of sneaking rates and genetic mating patterns in two populations of the sand goby experiencing disparate sexual selection regimes. - Mol. Ecol. 10: 461-470.

Kangas, N. and Lindström, K. 2001. Male interactions and female mate choice in the sand goby, *Pomatoschistus minutus*. - Anim. Behav. 61: 425-430.

Kasumovic, M. M., et al. 2007. Risky mate search and mate preference in the golden orb-web spider (*Nephila plumipes*). - Behav. Ecol. 18: 189-195.

Kokko, H. and Rankin, D. J. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating systems. - Proc. R. Soc. B. 361: 319-334.

Krakauer, A. H., et al. 2011. The opportunity for sexual selection: not mismeasured, just misunderstood. - J. Evol. Biol. 24: 2064-2071.

Kvarnemo, C. 1994. Temperature differentially affects male and female reproductive rates in the sand goby: consequences for operational sex ratio. - Proc. R. Soc. B. 256: 151-156.

Lande, R. 1982. Rapid origin of sexual isolation and character divergence in a cline. - Evolution 36: 213-223.

Lehtonen, T. and Lindström, K. 2004. Changes in sexual selection resulting from novel habitat use in the sand goby. - Oikos 104: 327-335.

Lehtonen, T. K. and Lindström, K. 2008. Density-dependent sexual selection in the monogamous fish *Archocentrus nigrofasciatus*. - Oikos 117: 867-874.

Lehtonen, T. K., et al. 2010. Fluctuating mate preferences in a marine fish. - Biol. Lett. 6: 21-23.

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

Lindström, K. 1992. The effect of resource holding potential, nest size, and information about resource quality on the outcome of intruder- owner conflicts in the sand goby. - Behav. Ecol. Sociobiol. 30: 53-58.

Lindström, K. 1992. Female spawning patterns and male mating success in the sand goby *Pomatoschistus minutus.* - Mar. Biol. 113: 475-480.

Lindström, K. 2001. Effects of resource distribution on sexual selection and the cost of reproduction in sandgobies. - Am. Nat. 158: 64-74.

Lindström, K. and Hellström, M. 1993. Male size and parental care in the sand goby, *Pomatoschistus minutus.* - Eth. Ecol. Evol. 5: 97-106.

Lindström, K. and Pampoulie, C. 2005. Effects of resource holding potential and resource value on tenure at nest sites in sand gobies. - Behav. Ecol. 16: 70-74.

Lindström, K. and Ranta, E. 1992. Predation by birds affects structure of breeding population in male sand gobies, *Pomatoschistus minutus.* - Oikos 64: 527-532.

Lindström, K. and Seppä, T. 1996. The environmental potential for polygyny and sexual selection in the sand goby, *Pomatoschistus minutus*. - Proc. R. Soc. B. 263: 1319-1323.

Madsen, T. and Shine, R. 1993. Temporal variability in sexual selection acting on reproductive tactics and body size in male snakes. - Am. Nat. 141: 167-171.

Marconato, A., et al. 1989. Correlates of male reproductive success in *Padogobius martensi* (Gobiidae). - J. Fish Biol. 34: 889-899.

McLain, D. K. 1992. Population density and the intensity of sexual selection on body length in spatially or temporally restricted natural populations of a seed bug. - Behav. Ecol. Sociobiol. 30: 347-356.

Milinski, M. and Bakker, T. C. M. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. - Proc. R. Soc. B. 250: 229-233.

Miller, P. J. 1986. The tokology of gobioid fishes. - In: Potts, G. W. and Wootton, R. J. (eds.), Fish reproduction: strategies and tactics. Academic Press, pp. 119-153.

Mück, I., et al. 2013. Nest distribution affects behaviour and mating success in a marine fish. - Behav. Ecol. Sociobiol. 67: 609-619.

Olsson, M. and Shine, R. 2000. Ownership influences the outcome of male-male contests in the scincid lizard, *Niveoscincus microlepidotus*. - Behav. Ecol. 11: 587-590.

Parker, G. A. 1974. Assessment strategy and the evolution of fighting behaviour. - J. Theor. Biol. 47: 223-243.

Pomfret, J. C. and Knell, R. J. 2008. Crowding, sex ratio and horn evolution in a South African beetle community. - Proc. R. Soc. B. 275: 315-321.

Real, L. 1990. Search theory and mate choice. I. models of single-sex discrimination. - Am. Nat. 136: 376-404.

Reichard, M., et al. 2004. Male-male interference competition decreases spawning rate in the European bitterling (*Rhodeus sericeus*). - Behav. Ecol. Sociobiol. 56: 34-41.

Reichard, M., et al. 2009. Breeding resource distribution affect slection gradients on male phenotypic traits: experimental study on lifetime reproductive success in the bitterling fish (*Rhodeus amarus*). - Evolution 63: 377-390.

Rosenberg, R. H. and Enquist, M. 1991. Contest behaviour in Weidemeyer's admiral butterfly *Limenitis weidemeyerii* (Nymphalidae): the effect of size and residency. - Anim. Behav. 42: 805-811.

Santangelo, N., et al. 2002. Resource attractiveness of the male beaugregory damselfish and his decision to court or defend. - Behav. Ecol. 13: 676-681.

Shuster, S. M. and Wade, M. J. 2003. Mating systems and strategies. - Princeton University Press.

Singer, A., et al. 2006. Sneaking in sand gobies: a genetic study with manipulated nest site availability in two different populations. - J. Evol. Biol. 19: 1641-1650.

Taff, C. C., et al. 2013. Spatial distribution of nests constrains the strength of sexual selection in a warbler. - J. Evol. Biol. 26: 1392-1405.

Wacker, S., et al. 2013. Operational sex ratio but not density affects sexual selection in a fish. - Evolution 67: 1937-1949.

Wong, B. B. M. 2004. Male competition is disruptive to courtship in the Pacific blue-eye. - J. Fish Biol. 65: 333-341.

Wong, B. B. M., et al. 2008. Male nest choice in sand gobies, *Pomatoschistus minutus*. - Ethology 114: 575-581.

FIGURE LEGENDS

Figure 1: Percentage of tiles that remained uncolonised. The percentage of empty (i.e. uncolonised) tiles is higher in the aggregated (grey bars) than sparse treatment (white bars) but independent of time. Numbers above the bars indicate number of replicates (with each replicate comprising a set of 4 tiles) in each treatment group. The error bars indicate 1 standard error.
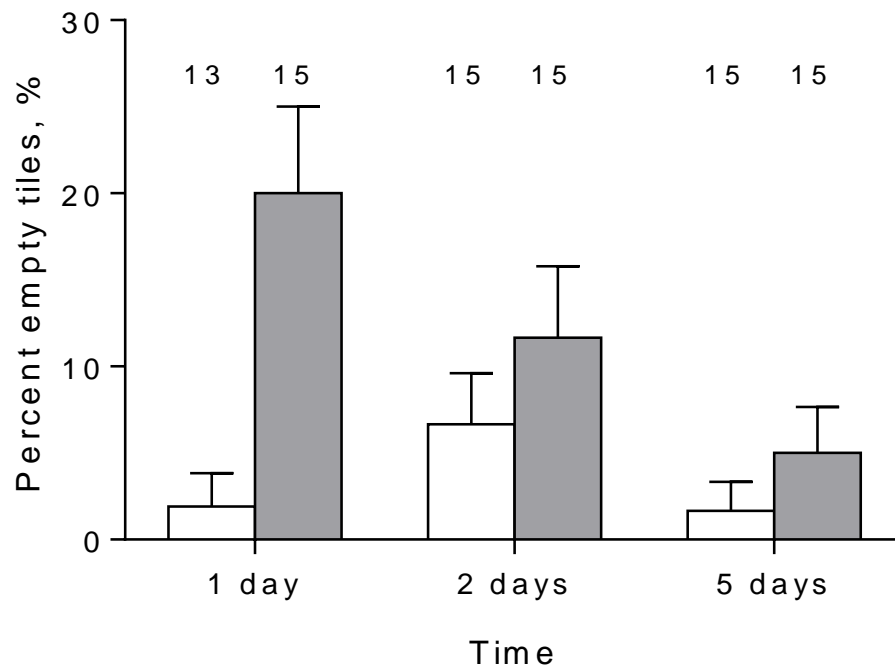
Figure 2: Distribution of (A) reproductive success measured as egg mass area as a box and whiskers plot and (B) male size measured as total body length. There was an interaction effect of nest distribution and time the nest were in the field, such that eggs accumulated more slowly in the aggregated (grey) treatment but still reached the same egg mass size on day 5 than eggs in the sparse (white) treatment. Later caught males were bigger than earlier caught males, while nest distribution had no effect on size distribution. Numbers above the bars indicate the number of (A) individual nests and (B) males in each treatment group. Whiskers in (A) indicate the 10 to 90 % interval. The error bars in (B) indicate 1 standard error.
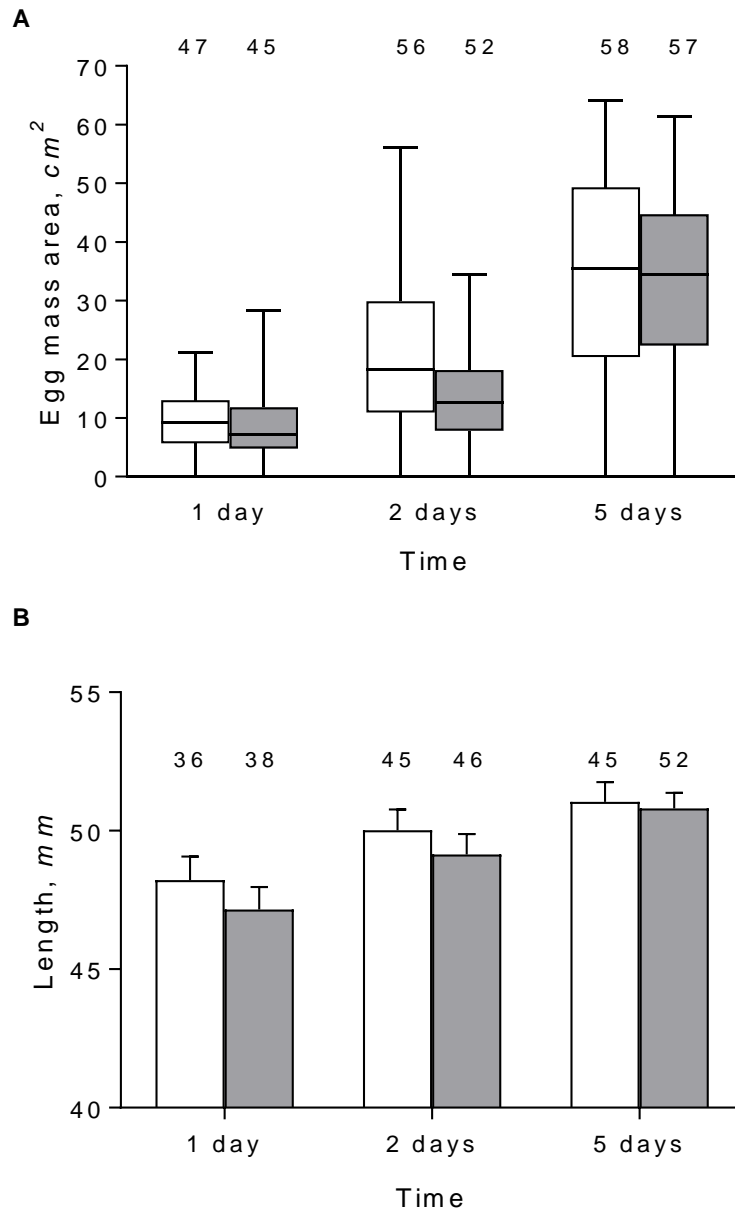
Figure 3: Box and whiskers plots of the opportunity for sexual selection, *I*. The opportunity decreased over time in the aggregated (grey bars) and the sparse treatments (white bars). Numbers above the boxes indicate the number of replicates (with each replicate comprising a set of 4 tiles) for which *I* could be calculated in each treatment group. Whiskers indicate the 10 to 90 % interval with observations outside of that indicated as circles.
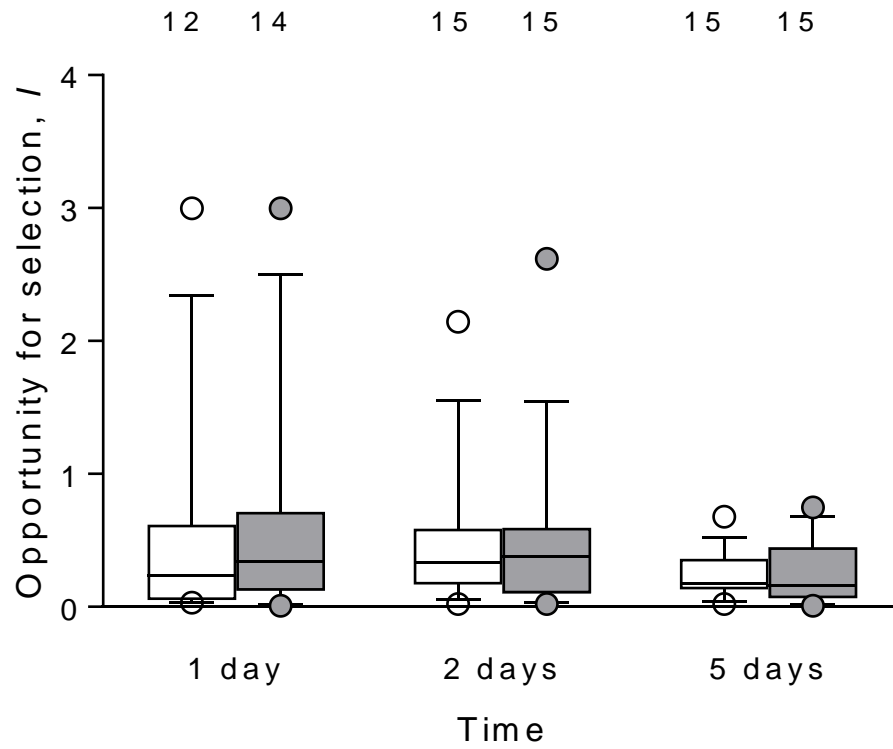
Figure 4: Box and whiskers plots showing the selection gradient on male body length for the different treatment combinations. In the aggregated treatment (grey bars) there is initially a strong positive gradient that decrease with time. In the sparse (white bars) treatment the relationship between reproductive success and male body length was weak throughout. Numbers above the boxes indicate the number of replicates (with each replicate comprising a set of 4 tiles) for which the gradient could be calculated in each treatment group. Whiskers indicate the 10 to 90 % interval with observations outside of that indicated as circles.