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Effect of sand texture on nest quality and mating success in a fish with parental care

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Abstract:	Nest quality is an important aspect of courtship and mate choice, offering females direct benefits through offspring survival and, if it reflects male genetic quality, also indirect ones. Nest characteristics may thus affect both male mating success and reproductive success. Using the sand goby, where males build nests by covering mussel shells or stones in sand, we tested the role of nest material in male nest site choice, nest construction, and female mate choice. We examined the effect of sand texture (coarse or fine, depending on grain size) in two different settings: A. when the male was free to choose between nest sites in different sand textures and other males were absent, and B. when the male was denied a choice of sand texture and another male was present behind a partition. In B, we also examined the effects of sand texture on female preference. In A, males took up nest sites equally often in coarse and fine sand, but nests built in fine sand had greater sand cover. In B, there was no difference in nest sand cover, but a greater number of males, and in particular males that weighed less and had been assigned coarse sand, refrained from building a nest at all. This suggests that sand texture does affect nest building in sand gobies, manifesting itself directly through nest sand cover, or indirectly through failure to build a nest. Moreover, we found that females preferred to spawn in well covered nests regardless of sand texture.
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Title: Effect of sand texture on nest quality and mating success in a fish with parental care

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Abstract

Nest quality is an important aspect of courtship and mate choice, offering females direct benefits through offspring survival and, if it reflects male genetic quality, also indirect ones. Nest characteristics may thus affect both male mating success and reproductive success. Using the sand goby, where males build nests by covering mussel shells or stones in sand, we tested the role of nest material in male nest site choice, nest construction, and female mate choice. We examined the effect of sand texture (coarse or fine, depending on grain size) in two different settings: A. when the male was free to choose between nest sites in different sand textures and other males were absent, and B. when the male was denied a choice of sand texture and another male was present behind a partition. In B, we also examined the effects of sand texture on female preference. In A, males took up nest sites equally often in coarse and fine sand, but nests built in fine sand had greater sand cover. In B, there was no difference in nest sand cover, but a greater number of males, and in particular males that weighed less and had been assigned coarse sand, refrained from building a nest at all. This suggests that sand texture does affect nest building in sand gobies, manifesting itself directly through nest sand cover, or indirectly through failure to build a nest. Moreover, we found that females preferred to spawn in well covered nests regardless of sand texture.

Significance statement

Nests offer eggs and offspring protection from predators and inclement weather, but building material may affect both the properties of the nest and the quality of the construction. Here, we presented male sand gobies with nest sites in either fine-grained or coarse-grained sand, assessed the sand cover of the nest and allowed females to spawn. We found that grain size influenced the amount of sand cover on the nest and affected the fraction of males that refrained from building a nest. Female spawning decision depended on the amount of sand cover, but neither males nor females expressed a preference for sand texture. Our results show that nest material is an important but indirect aspect of mating success, which may influence habitat utilization in the wild.

Keywords: Gobiidae, mate choice, nest building, reproduction, sand goby

Introduction

To animals that rely on a nest to house eggs or offspring, aspects of the nest can be expected to affect offspring survival. If so, these aspects reflect nest quality and may play a part not only in reproductive success but also in mating success. While much attention has been devoted to the effect of male competition on nest site occupancy, male traits on nest building, and the role of nest quality on female mate choice, less attention has been given to how nest materials affect nest quality, and how this in turn affects mate choice.

If the male builds a nest, female assessment of available nests may benefit the female both directly in terms of ensuring offspring protection and indirectly if it is linked to the genetic quality of the male. For example, both nest site and quality may affect how well offspring are protected from predators and adverse environmental conditions. Thus, female baya weaverbirds (*Ploceus philippinus*) prefer nests on high and slender branches which may protect against predators (Quader 2005). Similarly, nests sheltered from the waves increases the nesting success, in terms of successful nest building and the eventual production of fry, in five-spotted wrasse (*Symphodus roissali*) (Raventos 2006). Nest quality may also be indicative of the quality of the nest-building male, and even act as an extended phenotype. For example, in extreme cases where females do not use the nest for egg laying, such as the satin bowerbird (*Ptilonorhynchus violaceus*), nest quality is nonetheless a key aspect of mate choice (Borgia 1985). Furthermore, if nest quality contributes to reproductive success and there is variation in nest building ability in one sex, nest building itself may come under sexual selection through a preference by the opposite sex for high-quality nests. In both three-spined (*Gasterosteus aculeatus*) and fifteen-spined (*Spinachia spinachia*) sticklebacks, males build nests from plant matter held together with secretional threads of glycoproteins, such that the quality of the nest reflects the condition and stress level of the male (Barber et al. 2001; Östlund-Nilsson 2001), likewise in black wheatears (*Oenanthe leucura*), males with larger wing area can carry heavier stones to the nest, which results in earlier and higher frequency of egg laying (Møller et al. 1995). On the other hand, in barn swallow (*Hirundo rustica*), attractive males contributed less to the nest building, suggesting that in situations where female reproductive success is affected both by the quality of the male and the quality of the nest, nest-building can also represent a compensatory tactic for less attractive males (Soler et al. 1998).

Even though nest quality may be an important factor in female mate choice, the link between nest material and nest quality has only occasionally been examined, and then often with a focus on parasite load. For example, nest parasite load was reduced by the inclusion of aromatic plants in spotless starlings (*Sturnus unicolor*; Soler et al. 2017) and old nest material in pied flycatchers (*Ficedula hypoleuca*; Mappes et al. 1994). It was also reduced in the presence of smoked cigarette butts in urban house finches (*Carpodacus mexicanus*) and house sparrows (*Passer domesticus*; Suárez-Rodríguez et al. 2013), although at the price of elevated genotoxicity in the blood cells of chicks (Suárez-Rodríguez and Macías Garcia 2014). The scarcity of studies examining how nest material influences the physical properties of nests is notable, but the few existing studies have revealed important effects of material on nest architecture. Generally, both the size and the lining material can affect the thermal properties of a nest (Hilton et al. 2004). For example, heat loss and water absorption in nests of the thorn-tailed rayadito (*Aphrastura spinicauda*) were influenced by the surface-to-volume ratio as well as the inclusion of plant materials and feathers (Botero-Delgadillo et al. 2017).

In this study, we address the link between nest material, nest appearance and their effect on male and female nest preferences in the sand goby (*Pomatoschistus minutus*). Male sand gobies build nests by excavating a burrow underneath a mussel shell or stone and covering it in sand, leaving only a small opening. Sand gobies inhabit shallow bays with a range of sandy substrates, and we focus on the importance of sand texture as defined by grain size. Previous work on nest-building in sand gobies has shown that nest properties, such as degree of sand cover and nest opening size, vary between males and that nest appearance (among other cues) affects female spawning decision with females preferring nests that are well covered by sand (Svensson and Kvarnemo 2005; Lehtonen et al. 2007; Lehtonen and Wong 2009). Furthermore, males adjust nest appearance in response to the environment. In the presence of potential sneaker males (Svensson and Kvarnemo 2003, 2005) and egg-predators (Lissåker and Kvarnemo 2006; Olsson et al. 2016) the opening is made smaller, while it is enlarged under lower levels of dissolved oxygen (Lissåker et al. 2003; Lissåker and Kvarnemo 2006; Olsson et al. 2016). Finally, nest coverage has also been shown to be important in avoiding nest predation (Lindström and Ranta 1992; Jones and Reynolds 1999; Lissåker and Kvarnemo 2006).

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Previous studies have shown that nest building is a costly investment to male sand gobies (Olsson et al. 2009) and that males choose nests in sandy habitats over rocky habitats in the field, unless the rock nest is larger (Lehtonen and Lindström 2004). This suggests that choice of nest site is an important decision and that males may express preferences based on sand texture. We thus hypothesize that sand grain size may affect nest appearance and properties, and that this in turn may influence male nest material preferences and female nest choice. Using two experimental settings, with either a solitary male given a free choice of sand texture, or a male being assigned sand texture in the presence of another male (behind a partition) and a female being allowed to choose a mate and spawn, we address the following questions: 1) Which sand texture (fine vs. coarse) do males prefer? 2) Does sand texture affect nest appearance? 3) Are there differences in nest building and nest appearance in the different settings, e.g. if the male does or does not have a choice of sand texture? 4) Do females show a preference for nests built from fine or coarse sand?

Material and methods

Study species

The sand goby inhabits near-shore marine and brackish waters in northern Europe (Miller 1986) and during the breeding season, which typically lasts from April to June, adult fish migrate to shallow, sandy bays (Hesthagen 1977). Males build nests by excavating a burrow underneath a mussel shell or stone and covering it with sand. Both males and females are polygamous and spawn repeatedly, with territorial nest-holding males courting females by fin displays and “lead swims” towards the nest (reviewed in Forsgren 1999). In addition to nest characteristics, females have been found to use male size, coloration, courtship display, presence of eggs in the nest, and fanning rates as cues in mate choice (Forsgren 1992, 1997a; Forsgren et al. 1996; Pampoulie et al. 2004). The male guards and ventilates the clutch until hatching, which happens up to three weeks after spawning, depending on temperature (Kvarnemo 1994). Sand gobies are common in a range of sandy habitats, while on muddier substrates it is often replaced by the phenotypically similar common goby (*Pomatoschistus microps*; Tallmark and Evans 1986).

Experimental design

The study was carried out at the Swedish west coast (The Sven Lovén Centre Kristineberg, University of Gothenburg; lat 58.24, long 11.44), in May and June 2007. Sand gobies were caught in a nearby bay (Bökevik) using a hand trawl. The fish were brought to the lab, separated by sex and placed in 115-L storage aquaria furnished with approximately 2 cm of sand to burrow in. Fish numbers in storage tanks varied due to field collections and use in experiments but did not exceed 40 fish. All tanks (storage and experimental tanks) were continuously supplied with seawater delivered by the laboratory surface water pumps. Consequently, experiments were run at natural seawater temperature and we obtained recordings of sea surface temperature, logged each hour at Väderöarna WR buoy (lat 58.48, long 10.93), from the open database provided by the Swedish Meteorological and Hydrological Institute (SMHI 2017).A large window together with timer-controlled lamps ensured that natural light conditions were maintained. Fish in storage tanks were fed daily with chopped mussel meat (*Mytilus edulis*).

Data availability

The datasets generated during the current study are available in the Open Science Framework repository [https://osf.io/uetjz].

Sand texture

We defined two classes of sand texture, coarse and fine, depending on grain size. We obtained these by taking sand from a beach where sand gobies build nests, and sifting it through sieves (mesh sizes of 0.5 mm and 1 mm) such that coarse sand was composed of grains with diameters between 0.5-1.0 mm and fine sand of grains with a diameter < 0.5 mm (mostly > 0.25 mm but also some fraction smaller than that). Sand in the field comprises a mixture of grain sizes, and this method produced sand consistent with finer and coarser sand of local sand goby habitats.

Experiment A: One male, choice of nest site

In the first experiment, individual males were introduced to tanks measuring 50 x 36 cm and 30 cm deep (50 L); eight tanks were used simultaneously. Each tank was partially divided by an opaque partition that created two nesting compartments, both of which connected to an open foreground area (Fig. 1a). Each nesting compartment was furnished with a layer (about 3 cm deep) of either fine or coarse sand and an empty nest site (a halved clay flower pot). In the foreground area, where inflow and outflow of water were located, sand was a 50:50 mixture of fine and coarse sand. The relative position (left/right) of the coarse and fine sand compartments was randomized for each tank, but once a tank was furnished the sand texture in the compartments was not changed. To stimulate nest building, two ripe females assigned at random to each tank, were confined inside a plastic container placed in the

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foreground area, visible from both nest compartments. The male was released into the tank in the middle of the foreground area and allowed to freely choose a nest site. The male was given a maximum of three days to initiate nest building, and another 24 hours to complete it once it had started (i.e. cover the pot with sand and excavate underneath). At this point, nests were photographed (as described below), the chosen sand texture was noted, the male was captured and his total length was measured. If no nest building activity was detected within three days, the replicate was excluded from analyses. After the trial ended, the sand was smoothed and the pots replaced, before a new replicate was started.

A total of 31 trials were successfully conducted and only three males did not build, however, in one case the male built nests in both compartments. This trial was retained for nest quality analysis but excluded from the male preference analysis.

Experiment B: Two males, no nest site choice, female choice

In the same tanks used in experiment A, a female compartment was created by adding a clear Plexiglas partition that separated the foreground area from the two nesting compartments (Fig. 1b). The tank was also replumbed to have an inflow of water in each nest compartment and outflow in the female area; small perforations in the clear partition allowed for water flow. In the first phase of the experiment, two males were size-matched to within 1 mm and weighed before they were assigned to the two nest compartments of the aquarium. The opaque divider prevented males from visual interaction, but did not necessarily prevent knowledge of a second male via auditory, vibrational, or olfactory means. To stimulate male behavior, two ripe females, again chosen at random and confined inside a plastic container, were placed in the foreground compartment, visible to both male compartments. Any male that failed to build a nest within two days was replaced with another size-matched male. On the morning that both nests had been built, the stimulus females were removed and the nests were photographed, as described below. In the second phase of the experiment, a ripe female was introduced to the female compartment and allowed to move freely inside it. The position and behavior of the males and the female were recorded every 15-20 minutes until 15 observations had been made. Males were recorded as being inside the nest, displaying by the nest (including any display behavior such as fin flaring, tail-lifting, or leading display; i.e., approaching the female and then swimming towards the nest), showing other behavior by the nest (lying still, swimming around, or burrowed in sand), displaying at the partition or showing other behavior at the partition (also as detailed above). Female display of dark eyes, indicating readiness to spawn, was also recorded. All fish were observed on 15 occasions, all behavior and position information was recorded for each male at each observation point. In some cases more than one behavior or position would be observed (e.g. if a male was moving to interact with a female and then back to his nest). In case the fish had completely burrowed in the sand and could not be sighted, no behavior was recorded at that observation point. After the final observation, the transparent partition separating the foreground from the nest compartments was removed and the fish were observed for 15 minutes to determine if the female would immediately spawn. At this point all fish could freely interact. The female was allowed two nights to spawn, although most had spawned after the first night. Spawning latency was categorized as 'immediate' if it occurred within the observed 15 minutes, else 'overnight' or 'second night', depending on when eggs were discovered in a nest. After spawning, the sand texture of the chosen nest was noted. After the trial ended the sand was smoothed and the pots and fish were replaced. If the female did not spawn, the second phase of the experiment was repeated with another female. The males were not reused if the female spawned or if two successive females failed to spawn.

Of the 47 trials conducted, females spawned in 32, although in one trial one of the males died and in another the female spawned in both nests. These replicates were excluded from the female preference analyses.

Quantification of nest appearance

Halved clay flowerpots with an outer diameter of 7 cm were used as standardized nest sites. All completed nests were photographed from above, from the front, and from an angle facing the nest opening, to allow measurement of three aspects of nest appearance: sand height on top of the nest, area of the nest opening, and exposed area of the pot. The rim of each pot was marked at 10 mm intervals to provide a scale in the images. ImageJ (Schindelin et al. 2012; Schneider et al. 2012) was used to quantify the height of the nest cover, nest opening area and exposed pot area. In some cases (54 images) the scale was obscured and other aspects of those nests were used to set a scale, usually the thickness of the pot. In one case, the pot was so completely covered that the rim was obscured and the sand height could not be accurately estimated. In this case, sand height was set to 10 mm, which was judged to be the lowest possible value when compared to other nests. The relationship between the three nest appearance measurements was examined by performing a principal component analysis (rda, package vegan, Oksanen et al.

2017). The first component of the PCA explained 67.13% of the total variance (loadings: sand height = -0.36, nest opening area = 0.43, pot exposure = 0.43) and was used to create a single nest score parameter. The second and third components had eigenvalues < 1 and were thus not considered further. It should be noted from the signs of the loadings that a higher nest score means that the nest had a larger opening, less sand on top and a more exposed pot, i.e., less sand cover. Therefore, to make the nest score parameter more intuitive, it was multiplied with -1 so that a higher score denotes a nest with more sand cover and a smaller opening.

Quantification of male size

Male total length was measured to the nearest mm on a measuring board. We measured male weight by carefully wiping excess water off the fish before gently placing it in a tared cup of water. Male weight was recorded on a digital balance (Mettler PM600) to the nearest 0.01 g. We calculated a male condition index as 100 * male weight / $(\text{male length})^3$.

Quantification of behavior

In the second phase of experiment B, we calculated apparent female sand texture preference while the transparent partition was in place as the difference between the number of times she was observed on the coarse sand side and the fine sand side. We calculated a dark eye score for females as the sum of the number of instances she was recorded displaying dark eyes. We summarized male behavior based on the frequency of a given behavior relative to the total behavioral observations from that male, e.g., display score was the total number of display behaviors noted divided by the total number of behaviors observed for that male (typically 15 but on occasion slightly more than 15 or slightly less, as detailed above). Approximately 48% of all observations consisted of males being in the nest, while courtship display at the nest or at the partition was observed only on 8.7% and 10.1% of observations, respectively. These patterns of behavior is not atypical for this species, especially for observations made in person rather than via video (Kvarnemo et al. 1995).

Statistical analyses

Which sand texture (fine vs. coarse) do males prefer?

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Male choice of sand texture (experiment A) was tested using a binomial test with an assumed null-hypothesis probability of 0.5. We examined factors affecting male preference by fitting a logistic regression with sand texture at the chosen nest site as the response variable and male length and temperature as predictors (model specification: sand texture of the chosen nest site ~ male length + temperature, fine sand arbitrarily assigned a value of 0 and coarse sand a value of 1) and obtained the minimal adequate model by stepwise removal of terms (beginning with the least significant term) as long as the difference between the full and reduced model was not significant ($p > 0.05$, assessed by likelihood ratio test), and checked it for overdispersion. Since model coefficients are affected by other variables included in the model specification, a stepwise selection process allows us to examine whether terms close to significance remain non-significant during model reduction.

Does sand texture affect nest appearance?

In both experiment A and B, we examined how sand, temperature and male length affected nest score. In experiment A, we fitted a linear model with nest score as response variable and sand texture, temperature and male length as predictors (model specification: nest score ~ sand texture + temperature + male length). In experiment B, we fitted a mixed-effects model, with nest score as response variables, sand texture, temperature and male length as fixed effects and replicate as random effect, to account for the two nest builders per replicate (model specification: nest score ~ sand texture + temperature + male length + (1|replicate)). Again, we obtained the minimal adequate model through stepwise removal of non-significant terms and inspected the residuals of the minimal model for deviance from normality. We used restricted likelihood ratio test (RLRT; exactLRT, package RLRsim, Scheipl et al. 2008), to determine the significance of the random factor (RLRT = 5.41, $p = 0.008$).

Are there differences in nest building and nest appearance in the different settings?

To compare nest building performance between the two experimental setups, we performed a mixed effects ANOVA with nest score as response variable, experiment as fixed effect and replicate as random effect (model specification: nest score ~ experiment + (1|replicate)), as there were two nests per replicate in experiment B. Again, we used restricted likelihood ratio test to determine the significance of the random factor (RLRT = 5.60, $p = 0.0085$).

Males that did not build a nest within the allowed time were replaced. We tested the fraction of males replaced in experiment A, compared to experiment B, using Fisher's Exact test. We examined the effect of sand texture on the fraction of males that were replaced in experiment B using a binomial test with a null hypothesis of 0.5. The effect of male size, measured as total length, weight and condition index, was analyzed in separate Mann-Whitney tests, after Shapiro-Wilk tests showed that the size variables deviated from normality. We investigated the relationship between the display score of individual males to their nest score using Spearman's rank correlation.

Do females show a preference for nests built in fine or coarse sand?

We tested apparent female preference for sand texture (partition down) using a t-test against $\mu = 0$. We tested female choice of sand texture (based on where females spawned) using binomial tests with an assumed null-hypothesis probability of 0.5. Because each female in experiment B was offered a choice between two males and nests, and to allow us to analyze the effect of nest score on female choice, we created a variable to reflect nest score difference - the difference between the nest scores of the nest in coarse sand and the nest in fine sand. We did the same with male weight difference, and display score difference. A similar variable for the difference in length would have been redundant, since the males were matched for body length. We examined factors affecting preference by fitting a logistic regression with sand texture of the chosen nest site as dependent variable and male length and temperature as predictors (model specification: sand texture of the chosen nest site ~ nest score difference + display score difference + weight difference + temperature, sand texture scored as described above), and again obtained the minimal adequate model by stepwise removal of non-significant terms, and checked it for overdispersion. The frequency of dark eyes relative to spawning latency was tested using a Conover-Iman test, which performs a Kruskal-Wallis test and, if this is significant, post-hoc pairwise comparisons with Bonferroni correction between the three spawning groups (immediately, overnight and second night; conover.test, package conover.test, Dinno 2017).

It was not possible to record data blind because our study involved focal animals in the laboratory. All statistical tests were performed in R version 3.5.0 (R Core Team 2018).

Data availability

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The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Results

Which sand texture (fine vs. coarse) do males prefer?

Males showed no preference for either fine or coarse sand in experiment A (binomial test: $n_{coarse\ sand} = 14$, $n_{fine\ sand} = 16$, $p = 0.86$), and sand texture choice was also unaffected by male length and temperature (Table 1).

Does sand texture affect nest appearance?

In experiment A, nests built in fine sand had higher nest scores, i.e. more sand cover, than nests in coarse sand, but there was no effect of temperature or male length (Table 2; Fig. 2). In experiment B, there was no effect of sand texture, temperature or male length on nest score (Table 3).

Are there differences in nest building and nest appearance in the different settings?

There was a non-significant trend towards higher nest scores, i.e. more sand cover, in experiment A (mean ± SE: 0.28 ± 0.13) compared to experiment B (mean ± SE: -0.10 ± 0.11; mixed effects ANOVA, $F_{1,95.96} = 3.26$, $p = 0.074$). There was no difference between the fraction of males that were replaced (i.e., did not build a nest) in experiment A compared to experiment B (A: 3 males replaced, 32 males retained, B: 21 males replaced, 94 males retained; Fisher's Exact test: p = 0.20). However, of the replaced males in experiment B, most (n = 16) had been assigned coarse sand (binomial test: p = 0.027). Comparing all the males in experiment B that built nests to those that were replaced, the replaced males weighed less, and while the difference in length was close to being significant, there was no difference in condition index (Mann-Whitney test: weight: W = 707.5, p = 0.043; length: W = 740.5, p = 0.074; condition index: W = 793.5 p = 0.16; Fig. 3). Males with higher display scores had higher nest scores, although the correlation was weak (Spearman's test: n = 94, adj.$rho^2 = 0.06$, p = 0.01).

Do females show a preference for nests built in fine or coarse sand?

Prior to the removal of the partition in experiment B, females did not differ in the amount of time spent near the coarse and fine sand compartments, thus, females showed no apparent preference for either sand texture (mean ± SE number of times -0.06 ± 1.26, t-test against $\mu = 0$: $t_{46} = -0.05$, $p = 0.96$). Of the 30 replicates in which females spawned in only one nest and both males survived, 8 resulted in immediate (i.e. within the observed 15 minutes) spawning, 17 in overnight spawning and 5 in spawning the second night. Females that spawned immediately had higher dark eye scores than females that spawned overnight or second night (Conover-Imam test: n = 30, Kruskal-Wallis $\chi^2_{df=2} = 7.70$, $p = 0.02$, pairwise comparisons: immediate-overnight z = 3.10, p = 0.007, immediate-second night z = 1.89, p = 0.11, overnight-second night z = -0.50, p = 0.93; Fig. 4). Female spawning decision was not affected by sand texture (coarse sand spawning: n = 13, fine sand spawning: n = 17; binomial test: p = 0.58), but was influenced by of the difference in nest scores (Table 4). For identical nest scores (i.e. nest score difference = 0), the minimum adequate model thus predicted that the female were equally likely to spawn in either fine sand or coarse sand (predicted probability (95% CI) = 0.5 (0.38 – 0.62); Fig. 5).

Discussion

We found that female spawning decision was affected by nest appearance, with females preferentially choosing nests with more sand cover Indeed, we found no preference for sand texture *per se* amongst either males or females. This seems somewhat surprising since males that were offered a choice between sand textures built nests with higher nest score, i.e. more sand cover, in fine sand than in coarse sand, and males that were only offered coarse sand were more likely to refrain from building a nest at all.

We found that nests in fine sand had higher nest scores than nests in coarse sand, but this difference was only significant when males were given a choice between nest sites and no other male was present. Conversely, when males were denied a choice and another male was present, a significant number of males that had been assigned a nest site in coarse sand, and especially males of lower weight, did not build a nest at all. These results suggest that coarse sand is more difficult to build in, especially for lighter males. Furthermore, if male-male competition extends to nest building and the perceived presence of another male is interpreted as greater competition, small males in coarse sand may be at a prohibitive disadvantage and therefore refrain from nest building. In other animals, type and availability of nest material can affect both the structure of the nest and the number of nesting individuals. For example, the ability of laboratory mice to build complex nests, similar to nests found in the wild, depended on available nest material (Hess et al. 2008). Moreover, depletion of nest material reduced the total number of nests but not average nest quality in rooks (*Corvus frugilegus*), suggesting that the abundance of material constituted a threshold for building rather than a predictor of quality (Rutnagur 1990, as cited in Hansell 2000). Another possibility is that choosing a nest site itself affects nest score. When male sand gobies in another study were allowed to choose between nests of different sizes, successive nests had consistent degrees of sand cover, while males that were denied a choice built nests of variable appearance (Japoshvili et al. 2012).

Despite the effect of sand texture on nest score and nest building, we did not find a male preference for either sand texture. Sand goby nests are built by swirling up sand at the nest site, which may explain the male preference for a sandy habitat (Lehtonen and Lindström 2004), but the difference between sand textures in our experiment was much smaller than the difference between the sand and cobbles found in the natural habitats studied by Lehtonen and Lindström. Our study was also limited to the initial building of the nest, whereas a male that acquires a clutch must guard it until hatching, which requires nest maintenance. If different sand textures carry different maintenance costs, for instance because smaller sand grains are more easily transported by wave action or currents (McLaren and Bowles 1985), this may affect the successful rearing of offspring and the total cost of the brood cycle to the male.

Surprisingly, there was no effect of temperature on nest score, even though water temperature rose as the season progressed. Metabolic rates increase as temperature rises (Clarke and Johnston 1999), which may leave less energy for nest building. In addition, since warmer water holds less dissolved oxygen, males could have responded by increasing the nest opening to ensure adequate oxygenation (Lissåker et al. 2003; Lissåker and Kvarnemo 2006; Olsson et al. 2016). Nevertheless, no effect of temperature on nest building was found in this study.

We also found no effect of the time the female spent on the coarse and fine sand sides, prior to removing the partition, on female spawning decision, but females displaying dark eyes spawned more quickly. That dark eyes indicate readiness to spawn is consistent with previous work (Olsson et al 2017), but previous studies carried out under laboratory conditions similar to ours suggest that the time allowed should be sufficient for females to arrive at a spawning decision (Forsgren 1997b), which is often made even more quickly in the field (Forsgren 1997a).

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Female spawning decision was influenced by nest score, but not by sand texture, male weight or courtship display. If the purpose of the nest is to protect offspring from predation or harsh conditions, choosiness may produce direct benefits. In penduline tits (*Remiz pendulinus*), nest quality affects sheltering capacity during brooding (Hoi et al. 1994). Similarly, both sand gobies and common gobies have been observed to increase nest sand cover in the presence of a predator (Jones and Reynolds 1999; Lehtonen et al. 2013), and small nest entrances offer better protection against egg predators (Olsson et al. 2016). If females also gain indirect benefits, a link between some aspect of male genetic quality and nest quality is expected. For example, it has been suggested that male three-spined sticklebacks may advertise their paternal skills through decorated nests openings, which would explain why females prefer to spawn in such nests (Östlund-Nilsson and Holmlund 2003). It is therefore not uncommon that female mate choice is influenced by multiple signals (e.g. Wagner and Reiser 2000; Candolin 2003; Berson and Simmons 2018; Mowles et al. 2018). Previous work has shown that female sand gobies prefer larger (Forsgren 1992) and intensely courting males (Forsgren 1997a; but see Lehtonen 2012), and also that there is a link between males preferred by females and hatching success (Forsgren 1997b). However, previous evidence on whether nest appearance is associated with male attractiveness or offers less attractive males an alternative means to attract females has been ambiguous (Svensson and Kvarnemo 2005; Lehtonen and Wong 2009). In our study, we found no effect of male length on nest score and only a weak correlation between male courtship display and nest score, and also no effect of male courtship or weight on female spawning choice, although our observations did not capture all occasions when males may have engaged in courtship, while the size-matching of males may have obscured the effect of size. We therefore suggest that female preference for nest appearance is consistent with seeking direct benefits.

In conclusion, we found that in the sand goby, females preferentially spawn in nests with substantial sand cover, making nest appearance a key factor in mate choice. Moreover, nest appearance is influenced by sand texture, and it appears that coarser sand hampers nest building. Finally, our results imply that the decision on whether to build a nest or not is complex, and affected by sand texture, male size, freedom of choice and perhaps also the presence of other males.

Compliance with ethical standards

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Ethical permission for the experimental procedures was obtained from the Swedish Animal Welfare Agency (dnr 211-2007) and University of Florida (UF IACUC #E644).

Conflict of Interest

The authors declare that they have no conflict of interest.

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Captions

Fig. 1 Experimental setup in experiment A (a) and experiment B (b) investigating sand texture choice in the sand goby. The aquarium was divided into two adjacent nest compartments with either coarse or fine sand (dark and light grey, respectively) which both bordered a female area with a 50:50% mix of coarse and fine sand (medium grey). The nest compartments were separated by an opaque partition, while the female area was accessible in experiment A but closed off during the first phase of experiment B by a transparent partition (dashed line)

Fig. 2 Mean nest score (bars: ± SE) in experiment A and B in the sand goby for nests in fine (grey) and coarse (black) sand. A high nest score indicates a nest with more sand cover

Fig. 3 Male characteristics and nest building in different sand textures in the sand goby. Boxplots (horizontal line: median, box hinges: first and third quartiles, whiskers: largest value maximum 1.5*IQR from the hinge, dots: outliers, N: sample size) of condition factor, length and weight of males that built a nest (dark grey) and males that did not (light grey), and were thus replaced, for coarse and fine sand

Fig. 4 Dark eye score (i.e. the number of instances dark eyes were observed) in female sand gobies relative to latency of spawning. The size of the point indicates the number of females while N is the sample size

Fig. 5 The effect of nest score difference on female spawning decision in the sand goby. Higher nest scores indicate nests with more sand cover and a nest score difference > 0 shows that the chosen nest had a higher score than the rejected nest. The black line shows the predicted probability of spawning occurring in coarse sand nests (according to the minimum adequate model; Table 4), while black and grey points show the nest score difference of the coarse and fine sand nests in which females spawned

Table 1 Full and minimal adequate models for the logistic regression of male sand goby nest choice (experiment A). Nests built in fine sand were arbitrarily scored as 0, while nests built in coarse sand were scored as 1

Table 2 Full and minimal adequate models for the linear regression of nest score in the sand goby (experiment A)

Table 3 Full and minimal adequate models for the mixed effects linear regression of male sand goby nest score (experiment B); with p-values calculated using the Satterthwaite approximation for degrees of freedom (package lmerTest, Kuznetsova et al. 2017)

Table 4 Full and minimal adequate models for the logistic regression of female sand goby spawning choice (experiment B). Spawning in fine sand was arbitrarily scored as 0 and spawning in coarse sand as 1

Table 1

Dependent variable:	Male nest choice				
Full model	Independent variables	Coefficient	SE	z	P
	Intercept	8.06	12.05	0.67	0.50
	Length	-0.01	0.08	-0.08	0.94
	Temp	-0.73	1.19	-0.61	0.54
Min. ad. model	Independent variables	Coefficient	SE	z	p
	-0.1335	0.366	-0.365	0.715	0.72

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Table 2

Dependent variable:	Nest score				
Full model	Independent variables	Coefficient	SE	z	P
	Intercept	-4.16	4.08	-1.02	0.32
	Sand	-0.26	0.12	-2.12	0.04
	Temp	0.27	0.40	0.66	0.51
	Length	0.03	0.03	1.09	0.28
	$F_{3,28}$=2.55, p=0.08, adj r^2=0.13				
Min. ad. model	Independent variables	Coefficient	SE	z	p
	Intercept	0.27	0.12	2.18	0.04
	Sand	-0.28	0.12	-2.27	0.03
	$F_{1,30}$=5.14, p=0.03, adj r^2=0.12				

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Table 3

Dependent variable:	Nest score				
Full model	Fixed effects	Coefficient	SE	t	p
	Intercept	-0.84	1.36	-0.62	0.54
	Sand	-0.13	0.09	-1.45	0.15
	Temp	0.02	0.04	0.41	0.69
	Length	0.01	0.02	0.43	0.67
	Random effects variance	Replicate = 0.42, residual = 0.85			
Min. ad. model	Fixed effects	Coefficient	SE	t	p
	Intercept	-0.10	0.13	-0.76	0.45
	Random effects variance	Replicate = 0.38, residual = 0.86			

Table 4

Dependent variable:	Female spawning choice				
Full model	Independent variables	Coefficient	SE	z	P
	Intercept	-3.48	2.18	-1.60	0.11
	Nest score diff	1.40	0.63	2.21	0.03
	Display score diff	-0.18	1.47	-0.13	0.90
	Weight diff	0.91	2.85	0.32	0.75
	Temp	0.23	0.15	1.50	0.13
Min. ad. model	Independent variables	Coefficient	SE	z	p
	Intercept	-0.30	0.42	-0.71	0.48
	Nest score diff	1.15	0.54	2.11	0.04

Figure 1

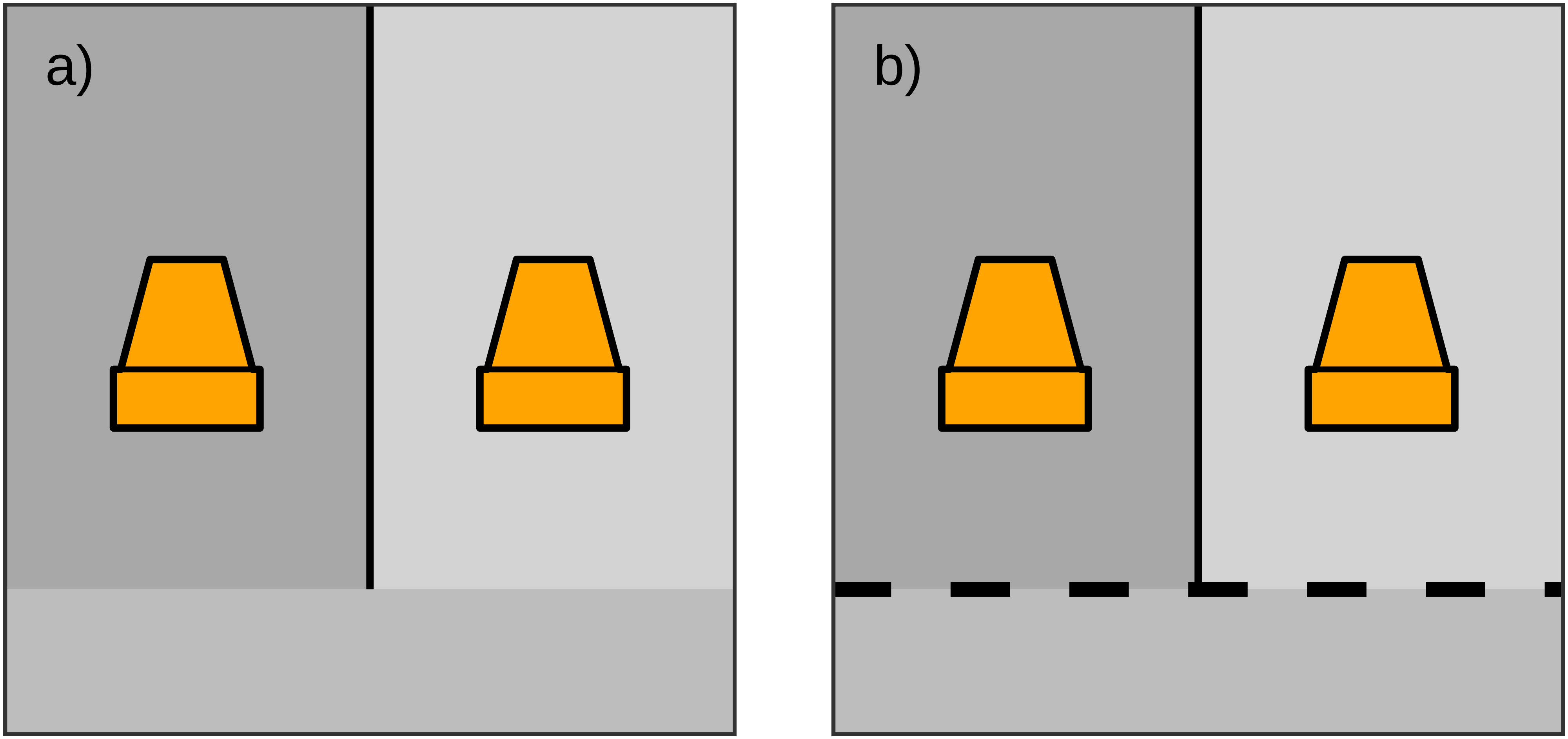

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Figure 2

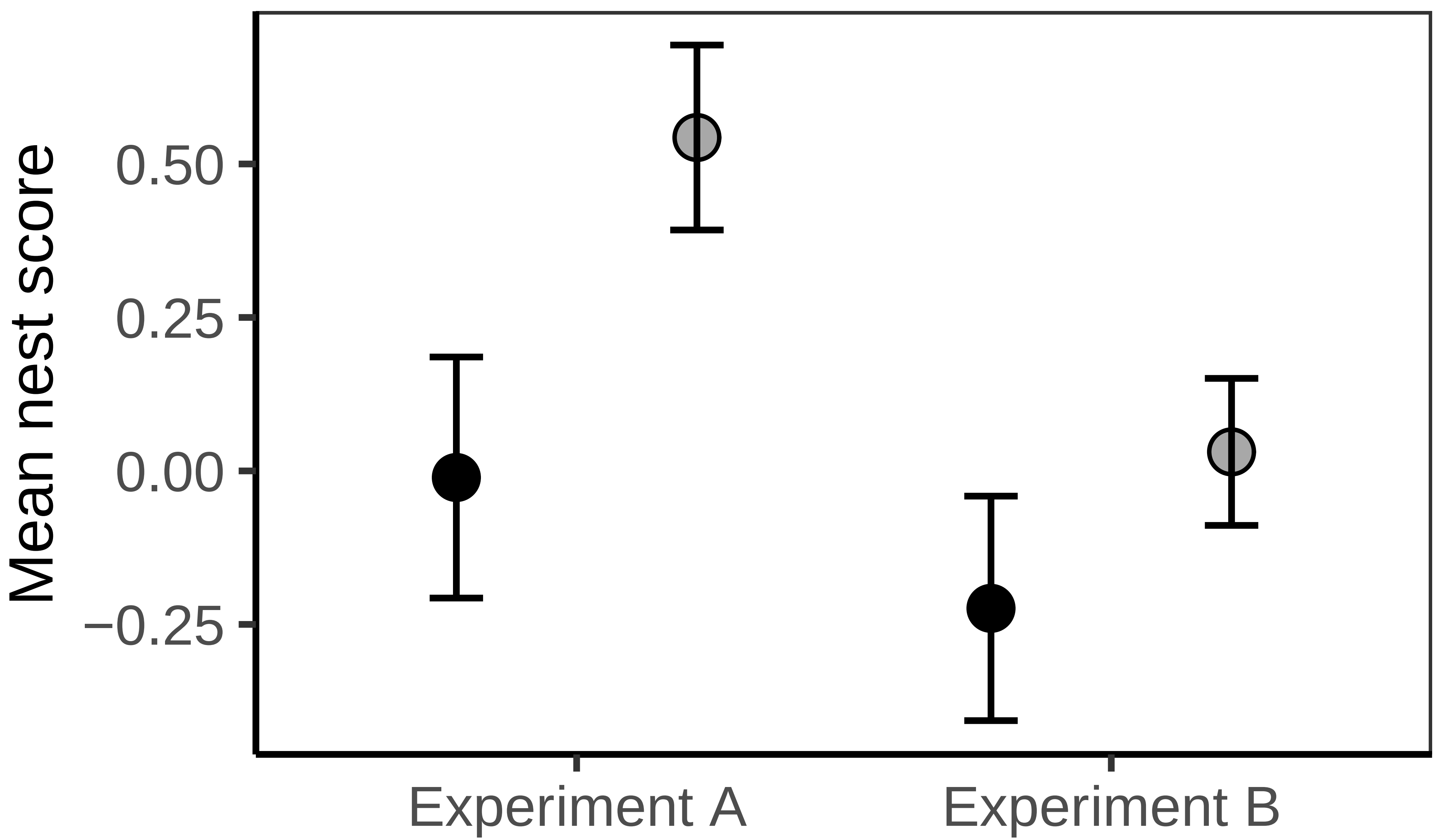

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Figure 3

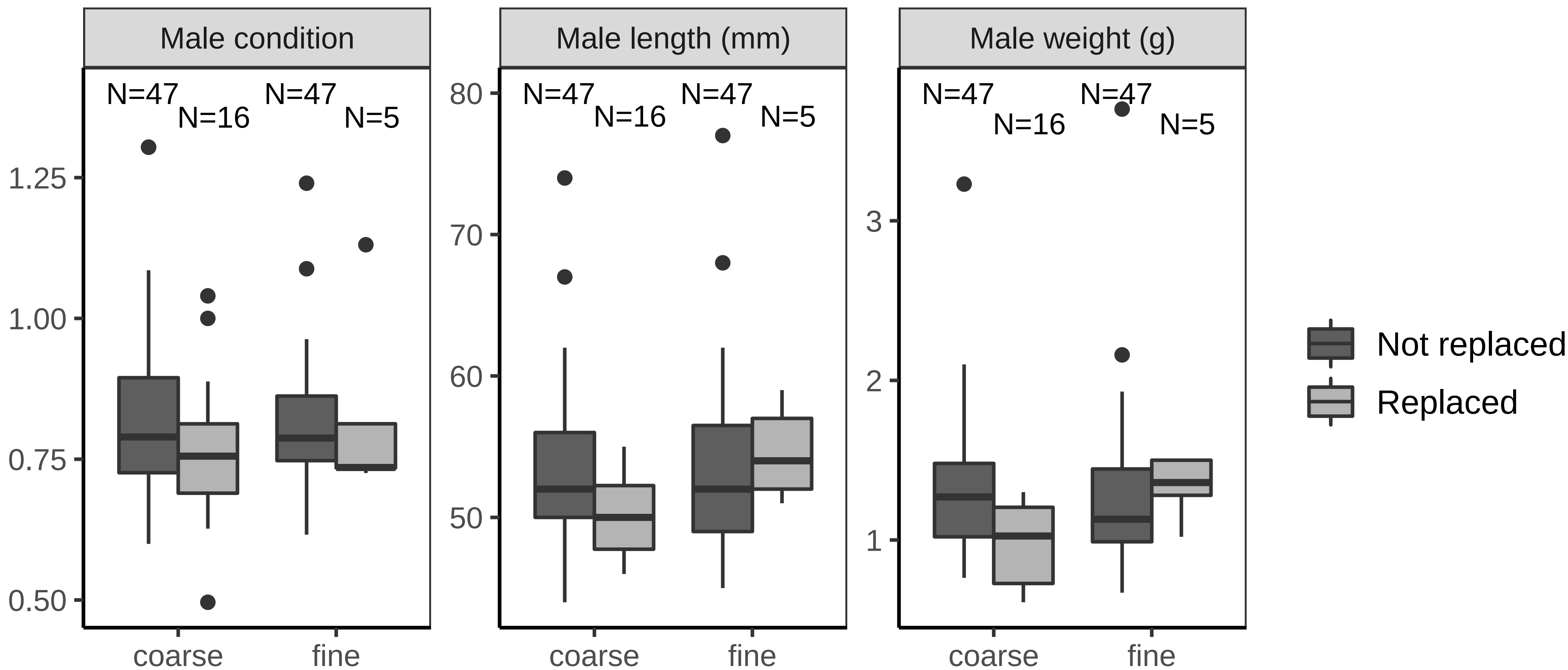

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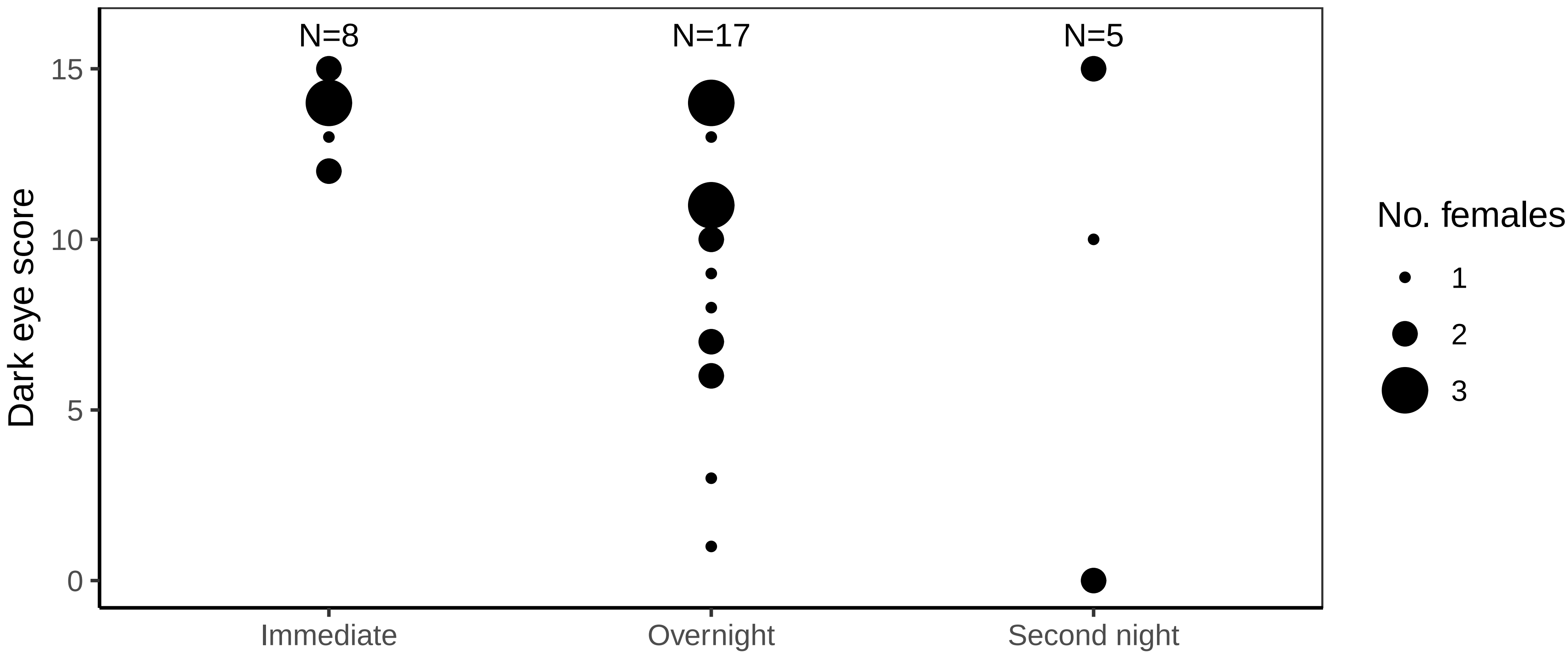
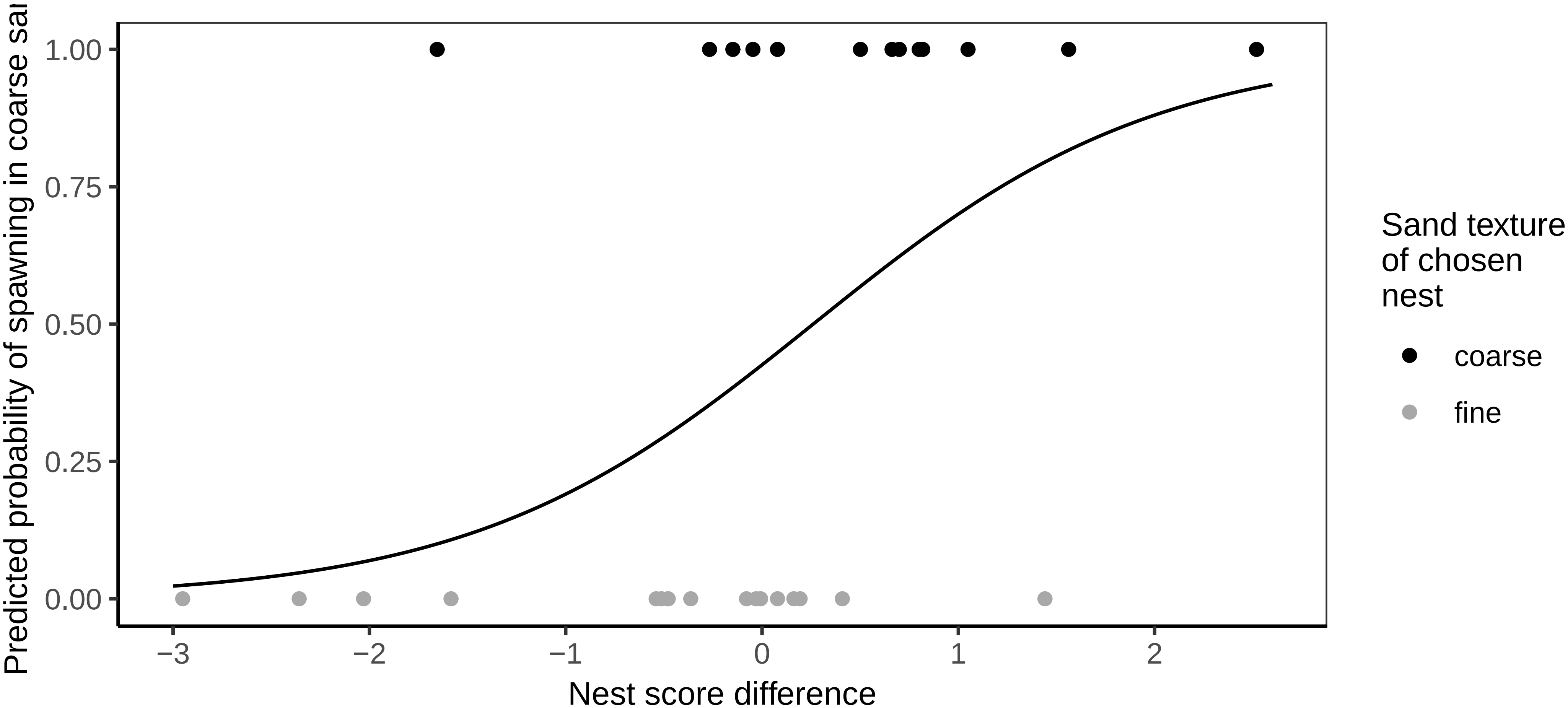
Figure 4



N=8
N=17
N=5
Dark eye score
15
10
5
0
Immediate
Overnight
Second night
No. females
1
2
3

Figure 5



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Response table

#	Comments for the author	Response	Location in (marked) revised MS
	Reviewer #1:		
	In the study "Effect of sand texture on nest quality and mating success in a fish with parental care", Olsson et al. examined the effect of different sand grain size on nest construction and mating in the sand goby. This is an interesting study, targeting a relatively unexplored area - most studies have measured nest quality, whereas few have examined how nest materials directly affect nest quality and, ultimately, mating success.	n/a	n/a
	Olsson et al. make use of two experimental designs. First, they give males the choice between two sand grain sizes, testing where they prefer to build their nests. In this part of the study, they find that males build equally in small vs. large grain sand and that nests built in fine grain sand had greater cover. In a follow-up experiment, males were given no choice in grain size but were given the opportunity to mate with a free ranging female. Grain size had little to no effect on female mate choice but females did prefer to mate with males that had built nests with higher sand cover. Finally, there was some effect of grain size on male nest building - it seems males assigned to the larger grain size more often failed to build nests.	n/a	n/a
	This study provides evidence that choice of nest material can contribute to male mating success and that nest appearance may act as a female choice criterion. These results are likely to be appealing to a wide audience. However, I feel there are a few issues to address:	n/a	n/a
1	1) The authors note direct vs. indirect benefits females may derive from male nest construction but I feel the delineation between these factors is not clear, particularly as it relates to this study. The authors discuss that sand texture may "manifest itself directly through next cover or indirectly through failure to build a nest" but this does not speak to the benefits to a female from the nest. Thus, I feel the study would benefit from more clear discussion of this in the introduction and, in particular, in the discussion, as it relates to the results. One relevant study that comes to mind: https://doi.org/10.1007/s00265-002-0574-z. The sand gobies may not be decorating their nests, but perhaps ability to cover the nest in sand acts in a similar manner?	Parts in the Introduction and Discussion which relate to direct and indirect have been rewritten, with the suggested reference included, to clarify this distinction.	L31-34, L65-87, L484-508
2	2) The use of two experimental designs is helpful to address specific questions relevant to the study. However, it can be confusing to the reader, when the results and interpretations of the two overlap. Rearrangement of the text and/or the use of subheadings to direct the reader may be helpful. I found that I was often having to go back to re-read methods descriptions to follow which experiment addressed which question. The authors list 4 questions at the end of the introduction section - these might be useful guidelines for the reader throughout.	We have rearranged Method and Results sections, as advised, and agree it improves the presentation.	Methods and Results sections

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3	3) I have some concern over the use of the differential scores in the analyses. I understand the logical, given the experimental design, but for female preference, this only makes sense if females have adequately interacted with each male. Was this the case? Was there a minimum amount of interactions per male for the trial to be counted as successful? Likewise, are analyses with absolute nest or courtship scores quantitatively similar?	Each female had 4-5 hours to inspect both males, which is substantially longer than would occur in the field. The tank set up is such the females can easily see both males and both nests, and given the confined space of the setup, we think it would have been practically impossible for the female to avoid noticing both males. Indeed from the point of introduction into the tank, females could see the two males and have the opportunity to examine each. Thus we are confident the female is aware of her options and how much time she spent on either side is part of her decsion making process; we are only looking at the outcome of that process. Since there are two nests in each trial in experiment B, using absolute nest score would not make sense as the female is limited to a choice between those two nests only, irrespective of their absolute scores. Finally, the focus of the study was nest appearance as quantified by sand cover, and while we made note of other factors that previous work has indicated may influence female mate choice, these were not the central to the question and we do not make strong interpretations of their impact. Text revised to clarify this	L329-334, L499-507
	Other comments:		
	Introduction		
4	Lines 104-108: Prior work suggest males prefer to build nests in sand vs. rocks, but the authors interpret this as "males prefer finer-grained sand". This may or may not be true. Thus, it seems that this study follows up on prior work to explore if males differentiate sand grain size in similar ways.	Wording has been clarified.	L122-125
5	Line 106: delete "because".	Word deleted.	L121
6	Line 107: in finer-grained sand, THUS we hypothesize…	Rewritten.	L122-125
	Material and methods		
7	Line 136: I understand that the fish were supplied with fresh seawater and that water temperature can vary (thus the need for temperature recordings). However, this may not be immediately clear to the reader - I had to reread to remember why temperature was included as a variable. Best to clearly state this to the reader (also in the stats section).	Wording has been clarified.	L150-154
8	Line 146: What defines 'fine' vs. 'coarse' sand? I know this information comes later in the text but as the reader, I was left asking this for a long time. Best to move this information up and present it with the 'experimental design' section.	Section has been moved as advised.	L167-173
9	Line 149: Were the females size matched or at least similar?	Males were provided a pair of gravid females selected at random. There was no attempt to size match the male and females provided. The intent here was to suggest to the male that multiple gradvid females are present and ready to mate. This stimulus female approach is widely used with studies	L183

		of this and similar species. The text has been updated to reflect this point.	
10	Line 151: Males were given one day to complete nest building. Is this enough or do males continue to build? I ask because of the impact on next scores - where all males given an equal amount of time to build following the onset of building? Or did some males potentially build longer, thus impacting nest scores?	Males are able to build a nest in very little time; from personal experience this can happen over a lunch break. We considered that giving them a day, especially during the quiet period of the night, was ample time.	(L185-187)
11	Line 156: Any indication of carry over effects between trials? In other words, were males in later trials less likely to build because of prior male activity? Given that the sand was smoothed and not changed between trials, I wonder if this could have had an effect.	This species naturally builds in sand alongside and in competition with other fish, and we have no reason to expect, either from nature or other studies, that this is an issue. Checking our data using binomial tests, we found no significant left/right side bias for any tank, and the number of times a nest was followed by a nest on the same side of the tank, compared to a change of sides, for the whole experiment was also not significant.	n/a
12	Lines 174-175: Vague description of behaviour. Earlier, the authors cite work highlighting the importance of courtship behaviour (lines 122-123).	Description and details added to the text	L211-222
13	Lines 177-178: Again, vague. So, some fish had multiple behaviours recorded, while others had none? Is this why courtship behaviour was corrected by the total behaviours recorded (lines 230-231)?	Yes, male behavior are recorded as a fraction of what was observed for the male. This provides an estimate of what the female could have observed. We have now clarified this in the analysis section.	L215-219, L274-280
14	Line 188: It is not clear to me if male-male interactions were possible. I assume so? If this is the case, were they scored and did they have an effect (is this why one male died)?	Males could interact, but such interactions were not frequently observed, partly because the divider between the male compartments was opaque and partly because they rarely left their own compartment. Such interactions when they happened were not scored. We have now clarified when fish could interact vs. not. Males of this species rarely if ever compete to the death. The observed death was not related to competition.	L203-205, L222
15	Lines 191-196: As noted above, this should come earlier. Details on nest photographs can remain here.	Text has been rearranged as advised.	L167-173, L238-254
16	Line 206: What about other PCs - informative?	The additional PCs were not significant by conventional criteria; information of the non-informative 2nd and 3rd PC added.	L250-251
17	Lines 219-221: If I understand correctly, the authors use a condition factor score, incorporating both SL and weight. This should be detailed here.	The formula for calculating the condition factor has been moved.	L265-266
18	Lines 224-225: Can be removed, begin with "we calculated…."	Sentence removed.	L269-270

19	Lines 230-231: Males courted VERY little. Courtship was then corrected by total behaviour and then calculated as a difference score. Was male courtship so low because of female disinterest? Did the female interact to some minimal amount with each male? As above, do absolute courtship scores give similar results?	The behavior described is fairly typical for this species, as we now mention in the text. This is particularly the case for in person observations, which are more likely to show reduced activity overall. This should not impede our ability to make comparisons among males. Furthermore, the focus of the study was nest appearance and while we made note of other aspects of the male which earlier work has shown may influence female mate choice, these are not the core question we address here.	L279-280
	Statistical analyses		
20	In general, I think this section may benefit by organization and subheadings according to the question at hand. It is rather long and complex for the reader to take in. Additionally, I find it helpful to explicitly state the models in the text as they were tested. For example: nest score ~ experimental + (1| replicate) to accompany your text description. I found myself writing this in the margin to make sure I understood the models.	Section rearranged, along with Results, as advised. We have also stated the formulas explicitly, along with a verbal description of the model, to keep the method text accessible to non-R users.	Method and Results sections
21	Relatedly: did you models include interaction terms? From the tables, it seems not. What effect would this have? Could the combination of nest score and male courtship behaviour be a better predictor than any single variable alone?	No, they did not. A preliminary data exploration did not indicate significant interactions, but more importantly there is the number of parameters per observation: our sample sizes are not very large and we judged it outside the commonly recommended rules of thumb to also include interactions.	n/a
	Results		
22	Similar to the stats section, organization according to question, with appropriate subheadings (instead of experiment A vs B), would help the reader.	Section rearranged, along with Method, as advised.	Method and Results sections
23	Lines 278-281: remind the reader what nest scores mean. What is a low vs. high nest score?	Higher nest score meant more sand cover. Now clarified.	L393, L398
24	Line 289: I thought males were size matched, yet the difference in size was significant? Also, how was condition index calculated?	The male pairs were size-matched to within 1 mm but the reported (non-significant, p=0.07) difference is for all males that were replaced compared to all males that built nests in Experiment B. text clarified. The formula for calculatinng condition index has been moved up.	L408-410
25	Line 308: 'Nest' instead of 'Thest"	Sentence removed in the rearrangement process.	L433-434
	Discussion		
26	Lines 320-323: The authors state males in coarse sand more often failed to build a nest when another male was present, potentially related to male competition. How was male competition a factor in this experiment? Could males interact? From my understanding, they could not during the building phase and the water flow was from males to females, so they theoretically had little opportunity of chemical interaction. How then was competition a factor? Likewise, males built nests equally well when in coarse sand when given a choice. The contradictory nature of these results is very interesting and deserves more clarification and discussion. Do the	We have clarified the description of the experimental setup to describe when fish could and could not interact. We do not believe that individuals in the female choice experiment (exp B) were unaware of each other. The knowledge of another male in close proximity may serve as a signal of potential competition. Thus, we hyopthsize in the Discussion that knowledge may affect behavior which explains our results. We have clarified the text in the Methods and Discussion on this point.	L203-222, L444-452

	analyses with condition factor provide any insight - less fit males are less likely to build in coarse sand, only when another male is present?		
27	Line 331: Here and throughout the discussion, there are extra spaces following citations; likely due to the citation manager.	Yes, done.	Throughout Discussion
28	Line 335-336: So, the differences in grain size were not extreme enough? Would more extreme differences change the results?	It probably would, if the difference was extreme enough. As mentioned in the introduction sand gobies occur over a range of sandy substrates, but are usually rare where substrates are very muddy or gravelly. Here we set out to examine substrates that appeared to be consistent with the habitats where we encountered the local sand gobies. This is now clarified in the text. Although it would be interesting to examine the limits of 'buildable' sand textures, it is not within the scope of this study	L167-173
28	Lines 359-372: I mentioned this above, but I wonder about the combined affect (interaction) of nest score and courtship. The authors state that courtship has little function outside of initially attracting the females and that nest characteristics are important to mating success. Is this an artifact of the experiment (courtship was extremely low) or is this a boarder strategy in sand gobies?	We do not state it as such, only that we are unable to find a link. We also mention a previous study which put forward this hypothesis (Lehtonen 2012), as well as a study where the results were the reverse (Forsgren 1997b). Clarification of the low levels of courtship added.	L279-280, L497-507
30	Line 376: Why is it that coarse sand hampers male nest building, but only when there is another male is present? I find this result extremely interesting, but also very hard to reconcile if male-male competition is not a factor (as I've already mentioned above).	We agree that this is an interesting result! (Also, see our response to comment #26). However, although it is tempting to speculate on the effect of porosity caused by grain size, on both the stability of the piled sand as well as its permeability (which would affect oxygenation during incubation), our study was not designed to reveal the proximate cause and we feel that elaborating on this speculation would suffer from a lack of baseline data.	n/a
	Reviewer #2:		

	This study examines the role of sand texture on nest site choice and appearance in nest-building sand goby males. Two experimental conditions tested 1) male preference for sand texture and resulting nest appearance, and 2) nest appearance when males could not choose sand texture and were in the presence of another male, and female preferences on sand texture and nest appearance. Males did not show a preference between sand textures, but sand texture impacted nest appearance. Females preferred nests with more cover. This study adds to our broader understanding nest building and sexual selection. I hope the authors find my comments helpful.	n/a	n/a
	Introduction:		
31	Considering the question regarding male nest building and nest appearance when males are given a choice or not, include information about individual variation in male nest building. Difference in material/texture preferences may stem from intrinsic characteristics of sand type in conjunction with natural variation in males (e.g. boldness, cognition, etc.).	We have now included information about variation in nest appearance among males. While we do not exclude the possibility that intrinsic variability in other male traits, such as boldness or cognition, may affect sand texture preference, this question is beyond the scope of this study.	L109-110
32	Line 66: nesting success measured as what?	Measured as nests having progressed through building-sexual-fanning stages and produced fry. Clarified in text.	L71
33	Lines 94-102: I was looking for some information about parental care and nest use during parental care in this section, though it appears in the methods. Is there any reason to think that sand texture might affect parental care?	Sand texture may affect nest quality, which in turn may influence the quality of care, but we do not expect texture to have direct effects on parental care. In the introduction, we include some benefits associated with well built nests, and mentioned the benefits related to reduced nest predation, but this is a bit beyond the scope of the study, as we did not measure parental care, thus we did not expand its discussion at this point in the intro.	L115-117
34	Line 108: Male preferences for what? Female preferences for what?	For sand texture and nest; now clarified.	L123-124
35	Line 112: In the third question, expand on "different settings" (e.g. 'when males do or do not have a choice of sand')	Sentence expanded upon, however, choice is not the only difference between the experimental settings.	L129
36	Some more parallelism between the introduction questions, methods, and results would help with flow. It was not always clear which section in the methods, and especially the results, was addressing which of the major questions in the introduction. For example, in the results, what question is being addressed by "Experiment A and B: Nest score"? Some more descriptive subsection headings and reorganization/merging of subsections might help.	We have rearranged the methods and results sections, along with subheadings according to this comment and those of referee nr 1.	Methods and Results sections
	Methods:		
37	It is not clear when or if sand texture was randomized between tank sizes among testing tanks. If sand texture was always the same between sides and among tanks, sand and side may be confounded. Please provide some more detail.	The relative position (left/right) of the coarse and fine sand compartments was randomized for each tank, but once a tank was furnished the sand texture in the compartments was not changed. This information has now been added to the text.	L181-182
38	Line 131: At what densities were fish housed? Number of fish per tank?	Fish numbers in storage tanks varied due to field collections and use in experiments but did not exceed 40 fish; clarified in text	L149-150

39	Lines 134-137: How was temperature maintained? Ambient conditions?	Text rewritten to clarify this.	L150-154
40	Line 165-166: So males could not see each other but could smell each other? Is this biologically sufficient to detect male presence and influence behavior in these fish?	The aim was not to induce male-male competition, but our design does not exclude that it happened. We have now clarified in several place that males were likely to be, or cannot be assumed not to have been, aware of each other, even if they could not interact directly.	L203-222, L444-452
41	Given that the experimental design is not fully factorial, it's difficult to make conclusions about freedom of sand choice and male presence between experiments A and B. The experimental conditions between A and B are very different. Take into consideration the presence of the other male and female in your interpretations/discussion.	We have taken this into consideration when revising the discussion.	Throughout Discussion
42	Line 178: Why was burrowing/not sighted considered differently than no behavior/position? By increasing/decreasing the total number of observations and dividing numbers of displays/behaviors by that total, is that not then influencing the display score?	A male that was out of sight for us was also out of sight for the female; we chose to focus on the behavioral signal that male sent while visible. However it's important to note that males rarely burrowed in this way. Most of the time they were visible even when partially borrowed in the sand.	L274-280
43	It is not clear what the behavioral measurements taken over the course of 15-minutes after removing the partition were used for vs the "every 15-20 minutes" observations that were used to produce the male display score.	Clarified in the text	L221-222
44	Line 192: Why were the size ranges for sand texture chosen? Is this a biological relevant and significant range?	Yes, it is biologically relevant, as the used sand represents the grain size distribution in the breeding area. Clarified in text.	L171-173
45	Were other sand characteristics consistent between textures (e.g. color)?	All sand was sourced from the same beach and only afterwards sieved to produce the different grain size distributions used in the experiment. Therefore, we expect only particle size to differ between the fine and coarse sand.	n/a
46	Lines 240-242: Please justify your reasoning for conducting a stepwise regression (many papers in the ecology literature discuss alternatives to reducing full models). It looks like the significant effects in the minimum adjusted models are also significant in the full model.	We acknowledge that the question of model selection is subject to debate. The interpretation of any variable in a model depends on which other variables are included in the model. Our stepwisely reduced model selection showed that no close-to-significant terms became signficant in the final (minimum adequate) model. Clarified in text.	L292-294
	Results:		
47	Line 280: Remind readers what a higher nest score indicates for nest appearance here.	Higher nest score meant more sand cover. Now clarified.	L393, L398
48	Line 289: There was a significant difference in length? Were males not size matched using length?	See comment and response to #24; the male pairs were size-matched but this test was carried out for all males in experiment B.	L408-410

49	Line 294: In experiment A, fine sand nests had higher scores, and in B, males with higher displays had higher nest scores. Is there a relationship between sand texture and male display (interaction) on nest scores?	The models were specified with the same variables to make the model outcome comparable between experiments and since display data was not collected for experiment A we did not include this parameter. As stated above, we also did not include interactions in light of the small sample sizes. We used a Spearman correlation to analyse the effect of display rate on nest score. Re-running the model for experiment B only with main factors and interactions, and proceeding with likelihood ratio tests to reduce the model in the manner described in the manuscript did not retain the sand:display interaction, or any of the main effects.	n/a
50	Line 308: Typo-"Thest"	See response #25	L433-434
51	Female decisions were not affected by sand texture but were affected by nest score. If nests with higher scores tend to be in fine sand, is there an interaction between sand texture and nest score on female decisions?	No, because female choice in the logistic regression is modelled in terms of sand texture. We know from the binomial test that females did not favour either sand type; in the logistic regression sand type is the reponse variable, hence there is no statistical interaction. As stated, for identical nest scores, females may spawn in either sand type.	n/a
	Discussion:		
52	If female preference varies with nest appearance, what are some possible implications for sexual selection?	The paragraph on female choice discusses direct and indirect benefits, and this discussion has now been expanded.	L484-508
53	Conclusions about male choice of sand texture in the presence of other males are only speculative, since that was not properly tested here.	The presence of another male in the same tank, and the implications thereof, have been clarified.	Throughout
54	Figure 5: There are several things a reader has to keep track of to interpret this figure. It would be helpful to add, either to the x-axis or caption, descriptions of what kind of nest appearance would occur with the nest score differences: positive numbers indicating greater nest cover in the chosen nest and vice versa.	Explanation provided in the legend has been expanded to provide this information.	L666-668

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