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LETTER

Who to include in measures of sexual selection is no trivial matter

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Abstract

In many animals acquiring limited reproductive opportunities involves competition for resources, mates and opposite-sex gametes. There is ambiguity in which competitive steps are included in measures of sexual selection: individuals who fail to obtain resources necessary for reproduction are often excluded. We illustrate the implications of variation in who is included in measures of selection. We quantified selection on male length and the opportunity for selection associated with nest acquisition, mate acquisition, and fertility of mates at two levels of density and two levels of nest availability in the sand goby. Both measures varied significantly across the three episodes of selection. Nest and mate acquisition contributed substantially to the overall opportunity for selection and selection on male size. Focusing only on males with nests led to lower estimates of selection. The effects of density and nest availability depended on the selective episodes considered. While there is nothing wrong with focusing on particular episodes of interest, inconsistency in who is included in measures of sexual selection across studies will make it difficult to answer broad research questions.

Keywords

Density-dependent sexual selection, mate competition, mating system, natural selection, resource competition, sexual selection.

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INTRODUCTION

Sexual selection is defined as the process by which limited access to mates or opposite-sex gametes leads to variation in mating and ultimately reproductive success (Darwin 1871). Sexual selection can occur when members of one sex compete directly for access to mates and/or gametes (intrasexual selection) or to be chosen by members of the opposite sex (intersexual selection) (Darwin 1871). In many animals, competing for and acquiring limited reproductive opportunities involves multiple sequential steps (Arnold & Wade 1984a,b; Ahnesjö *et al.* 2001; Shuster & Wade 2003). First, individuals of a given sex must compete for and acquire general resources, such as nesting sites, territories or food (Fig. 1). Then, after acquiring such resources males or females engage in direct mate competition or attraction (Fig. 1). After mating, gametes of one sex potentially compete directly for access to opposite-sex gametes (Birkhead & Pizzari 2002). Each step can influence sexual selection (Forsgren *et al.* 1996; Ahnesjö *et al.* 2001; Shuster & Wade 2003). In practice though, there is disparity in which of these competitive steps are accounted for in measures used to predict, explain and quantify sexual selection. In particular, selection associated with intrasexual resource competition is frequently not accounted for in such measures (Shuster & Wade 2003; Shuster 2009).

Shuster & Wade (2003) and Wade & Shuster (2004) suggest that excluding non-mating males, who are typically inconspicuous or absent from breeding aggregations, is common and will underestimate the opportunity for sexual selection. Wade & Shuster (2004) advocate accounting for non-mating individuals in calculations of sexual selection and provide a way to do so even when non-mating individuals cannot be counted directly – their approach thus accounts for variation in mating success that stems from both resource and mate acquisition. Ahnesjö *et al.* (2001) instead place greater emphasis on distinguishing between different episodes of selection, which allows them to

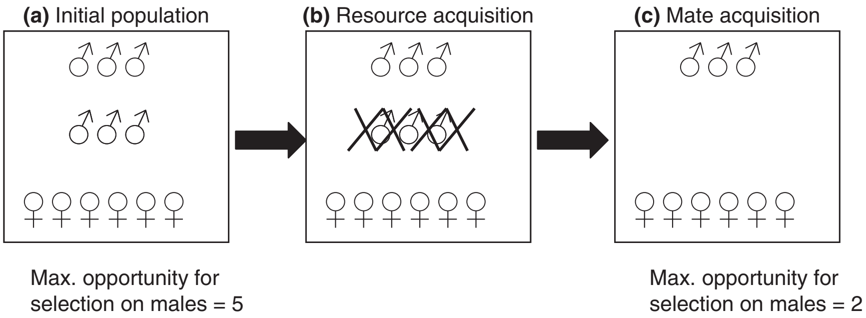

Figure 1 An illustration of the relationship between resource and mate competition. (a) We consider a population that consists of six reproductive males and six reproductive females. The opportunity for selection is greatest when a single male monopolizes all possible mating opportunities. If we consider all males, the maximum possible opportunity for selection in this initial population is 5. (b) Now, let us assume that males must compete for and acquire some sort of limited resource, such as a nest, before they can mate. If only three nests are available, three males will be left without a nest. (c) Thus, only three males will be qualified to mate and capable of engaging in direct mate competition. If we only consider males who are currently qualified to mate, the maximum opportunity for selection is 2. In this example, nest competition decreases the potential intrasexual variance in mating success associated with direct mate competition, which in turn decreases the maximum opportunity for sexual selection associated with direct mate competition. There is inconsistency in whether both competitive steps, i.e. acquisition of resources required for mating and direct mate acquisition, are accounted for in empirical measures of sexual selection. Including or excluding certain individuals affects that maximum selection that can be quantified and will potentially affect the conclusions we draw about sexual selection. This example is modified directly from fig. 1 of Ahnesjö *et al.* (2001). The opportunity for selection is defined as the coefficient of variation in mating success squared (following Shuster & Wade 2003 and eqns 1 and 2 in the text; note: the maximum opportunities of selection are 6 and 3 if the statistical formula for variance is used).

specifically focus on the stage of sexual selection most directly associated with mating competition (Kvarnemo & Ahnesjö 1996, 2002).

It is clear that sexual selection measures should include unsuccessful individuals (Wade 1979; Arnold & Wade 1984a,b; Shuster & Wade 2003; Wade & Shuster 2004; Shuster 2009). However, the issue of *which* non-mating individuals to include has not been widely addressed or resolved. Should all non-mating individuals who are alive at a given time and place be included in calculations of selection? Or should only those who are actively seeking reproductive opportunities or who have the resources required to mate be included? There is tremendous variation in who is included in measures of sexual selection, and this variation creates inconsistency in the selective episodes that are accounted for in measures of sexual selection. For example, numerous studies include nesting and non-nesting males (Singer *et al.* 2006; Mobley & Jones 2007; Duval & Kempenaers 2008), whereas several other studies exclude non-nesting males (Westneat 2006; Dolan *et al.* 2007; Perlut *et al.* 2008). As an example of the magnitude of variation that exists, Dolan *et al.* (2007) consider only males who have a nest and potential mate in calculations of the opportunity for sexual selection. In contrast, Vanpé *et al.* (2008) include males who have died in previous years in some (but not all) calculations of the opportunity for sexual selection.

Disparity across studies in who is included in measures of sexual selection might stem from logistical constraints (Wade & Shuster 2004). Alternatively, authors might exclude certain individuals because they aim to address questions related to a particular episode of selection (e.g. Ahnesjö *et al.* 2001). Few studies explicitly discuss why certain individuals are excluded from measures of sexual selection, though, making it difficult to discern an author's intent. Regardless, variation in who is included in measures of sexual selection is expected to affect our general understanding of sexual selection (Shuster & Wade 2003; Wade & Shuster 2004).

To explore the implications of excluding certain individuals in measures of sexual selection, we examined the effect of nest limitation and density on the opportunity for sexual selection and selection on male size in the sand goby (*Pomatoschistus minutus*). Sand goby males compete for and guard limited nest sites. Acquiring mates in the sand goby is a two-step process: first, males compete for and acquire nests; then, after attaining a nest, males directly compete for females. We calculated the opportunities for selection and selection differentials associated with male nest acquisition (which accounts for all males), mate acquisition (which accounts for mated and non-mated males who are 'qualified to mate', Ahnesjö *et al.* 2001), and fertility of mates (which accounts for males who have mated). We also quantified the overall opportunity for selection and selection differential (which accounts for all males). Our goals were to (1) determine whether there were differences among the selective episodes, (2) identify the effects of nest limitation and density on distinct episodes of selection and (3) illustrate the potential implications of excluding certain individuals in measures of sexual selection.

MATERIALS AND METHODS

Experimental set-up

The study was conducted at Tvärminne Zoological Station (University of Helsinki) during the summers of 2006 and 2007. Fish were caught just prior to use, and only reproductive individuals were used. The experiment was performed in outdoor aquaria (80 cm × 80 cm) experiencing semi-natural conditions (natural lighting, flow-through seawater, sandy bottom). In the wild males build nests under shells and rocks, which they cover with sand. We used halved clay flowerpots (6 cm diameter), which are accepted by sand gobies as nesting sites in the laboratory and field. Prior to the experiment, we measured and weighed all males. During 2007, males were tagged with elastomer-like paint that helped us indentify individual males. Four and two blocks of the experiment were completed in 2006 and 2007, with one to three replicates per block, depending on fish availability.

To evaluate the effect of density and nest limitation on sexual selection, we crossed two levels of density (high density = six males and six females per aquarium; low density = four males and four females) with two levels of nest availability (limited = one nest per every two males in the aquarium; unlimited = one nest per every one male). Thus, there were four treatments: (1) high density, limited nest availability (six males, six female, three nests per tank; $n = 9$), (2) high density, unlimited nest availability (six males, six females, six nests; $n = 12$), (3) low density, limited nest availability (four males, four females, two nests; $n = 10$), (4) low density, unlimited nest availability (four males, four females, four nests; $n = 8$). The nests could accommodate eggs from all females present within a tank, making it possible for a single male to monopolize all matings. Males were thus mate-limited, whereas females were not. Sexual selection is expected to act most strongly on the mate-limited sex (Darwin 1871), and thus, we focused on male sexual selection.

Each replicate began by adding the appropriate number of nests and males to the aquarium. The corresponding number of females was then added to a transparent container in the centre of the aquarium that allowed for chemical and visual, but not physical, contact with the males. Males were given 24 h to establish nests; after 24 h one female was released from the holding container into the main compartment of the tank every 2 h until all females were added. Adding females sequentially is likely most representative of natural conditions, as it is unlikely that numerous females will simultaneously appear at a male's nest.

Nests were checked for eggs 24 h after the last female had been added. Preliminary trials revealed that filial cannibalism is minimal during this period. In 2007, when males were tagged, we determined which specific males had nests. Digital images were taken and used to determine how many eggs each male received. We estimated the number of females each male spawned with by comparing female gravidity (distension of the belly) before and after the 24 h period (to determine which females spawned) and noting between-clutch variation in egg coloration (to estimate how many distinct clutches, and hence mates, each male had).

Opportunity for selection and selection differential calculations

For each experimental replicate (each aquarium), we calculated the total opportunity for selection (I) for males. The opportunity for selection is a measure of variation in reproductive success that reflects the upper limit of the strength of sexual selection (Wade 1979; Arnold & Wade 1984a; Shuster & Wade 2003). The opportunity for selection is measured as the variance in mating success (V_{m}) divided by the mean mating success (M) squared (Arnold & Wade 1984a; Shuster & Wade 2003), following:

$$M = \sum_{i=1}^{n} (k_i m_i) / \sum_{i=1}^{n} m_i \qquad (1)$$

and

$$V_{\mathrm{m}} = \sum_{i=1}^{n} (k_i^2 m_i) / \sum_{i=1}^{n} m_i - \left[\sum_{i=1}^{n} (k_i m_i) / \sum_{i=1}^{n} m_i \right]^2, \qquad (2)$$

where k_i represents the number of females mated by the ith male and m_i is the number of males in the ith mating class.

When calculating I, we used the total number of eggs a male received as our measure of reproductive success and simply divided the variance in reproductive success by the mean reproductive success squared for each replicate (eqns 1 and 2 above; eqn. 13 of Arnold & Wade 1984a). Thus, the total opportunity for selection in this study accounts for potential sexual selection associated with nest and mate acquisition and fertility of mates (number of eggs received). We then calculated the opportunity for selection associated with each of these competitive steps separately (see, e.g. eqns 13–18 of Arnold & Wade 1984a). The opportunity for selection associated with nest acquisition (I_{nest}) was calculated as the variance in nesting success (the number of nests each male received, 0 or 1) divided by the mean nesting success squared. All males had the opportunity to compete for nests and were included in these calculations. The opportunity for selection associated with mate acquisition ($I_{\mathrm{mates|\,nest}}$) was calculated as the variance in mating success, i.e. the number of mates each male received (which equals the number of nests received × mates per nest) divided by mean mating success squared. Only nesting males were able to acquire mates, and thus, only nesting males were included in $I_{\mathrm{mates|\,nest}}$. The opportunity for selection associated with fertility of mates ($I_{\mathrm{eggs|\,mate}}$) was calculated as the variance in reproductive success, i.e. the number of eggs each male received (or the total number of nests received × mates per nest × eggs per mate) divided by mean reproductive success squared. Only males who had at least one mate were included in these calculations. It is important to note that our measures of fitness for each selective episode (i.e. number of nests, mates, or eggs) are multiplicative, which is consistent with the methods proposed by Arnold & Wade (1984a) (eqns 15 and 16).

For each replicate in 2007 (when males were marked) we calculated the selection differential with respect to male length: the difference in mean length between successful males and all males (Arnold & Wade 1984a,b). Given a constant level of additive genetic variation in the direction of selection, the selection differential is proportional to the evolutionary change in a trait. Specifically, we calculated the total selection differential (S_{total}), i.e. the difference in mean length between presumed sires (weighted by number of eggs received) and all males. This measure accounts for selection associated with nest acquisition, mate acquisition and fertility of mates. Additionally, we calculated the selection differential associated with nest acquisition, S_{nest} (the difference in mean length between males who got nests and all males), mate acquisition, $S_{\text{mates}|\text{nest}}$ (the difference in mean length between mated males, weighted by the number of female mates, and all males who had nests) and fertility of mates $S_{\text{eggs}|\text{mate}}$ (the difference in mean length between presumed sires, weighted by the number of eggs received, and all males who mated). Standardized selection differentials were also computed (following eqn 2 of Jones 2009). Results based on standardized selection differentials were qualitatively the same as those based on the un-standardized selection differentials; thus, we present only results using un-standardized selection differentials. We could only determine all of the specific males who got nests or mated in three to four of the aquaria in each experimental treatment. Thus, we had reduced sample sizes for the selection differential calculations. While we had sufficient power to identify differences among the selective episodes considered (see Results), we were lacking power for some analyses examining the effect of density and nest limitation on selection differentials (see Results and Discussion).

Five fish died during the experiment (one to three fish per treatment). Because death altered density, these replicates were excluded from analyses. No reproduction occurred in one to two replicates of each treatment group. Because variances in mating and reproductive success were response variables, we excluded these cases from our analyses. These excluded replicates are not included in the sample sizes listed above.

Nest-holding and non-nest holding sand goby males spawn parasitically (Singer *et al.* 2006). Previous work has found no clear predictor of parasitic spawning (Singer *et al.* 2006; M. Järvi-Laturi and K. Lindström, unpublished data), and Singer *et al.* 2006 found no effect of nest limitation on the rate of parasitic spawning. While parasitic spawning likely lowers the opportunity for selection in all treatment groups, it is unlikely to have differential effects on any treatment groups (Singer *et al.* 2006; M. Järvi-Laturi and K. Lindström, unpublished data) and thus, it is unlikely to affect our general conclusions. Nonetheless, it is important to note that we did not measure parasitic spawning in this experiment, and thus, we clearly do not consider all potential episodes of sexual selection. Selection associated with fertilization success is an additional selective episode that warrants explicit consideration in future work.

Data analysis

We used stepwise ANOVAs to evaluate the effect of density and nest availability on the total opportunity for selection (I) and the total selection differential (S_{total}). Density, nest availability, block, density × nest availability and all block × treatment interactions were included in the initial model. We then simplified the model in a stepwise manner (remove if $P > 0.15$, higher-order terms removed first in a sequential, stepwise manner based on P-value; see e.g. Crawley 2002; Quinn & Keough 2002).

We used repeated measures ANOVA to examine differences between the selective episodes. For the opportunity for selection data, I_{nest}, $I_{\text{mates}|\text{nest}}$ and $I_{\text{eggs}|\text{mate}}$ were treated as response variables. In the analysis of selection on male size, S_{nest}, $S_{\text{mates}|\text{nest}}$ and $S_{\text{eggs}|\text{mate}}$ were treated as response variables. In the initial models, we considered the effects of episode, density, nest availability, block, density × nest availability, all episode × treatment interactions, and the interaction between nest availability, density and episode. We then simplified the model in a stepwise manner, as described above. Covariance matrices were not homogenous in the repeated measures analysis; however, this did not reduce the power to detect treatment effects in the opportunity for selection analyses, and we report the multivariate statistic Pillai's Trace, which is robust against violations of model assumptions (Olson 1974). Greenhouse-Geisser correction was used when the repeated measures ANOVA assumption of sphericity was violated.

RESULTS

Considering all males in a tank, males received an average of 371 ± 36 eggs ($\overline{X} \pm \text{SE}$, $N = 39$) and mated with 0.5 ± 0.05 females. There was no significant effect of density or nest availability on the mean number of eggs males received or the number of female mates within a tank (eggs: density, $F_{1,31} = 0.1$, $P = 0.81$; nest availability, $F_{1,32} = 2.7$, $P = 0.11$; mates: density, $F_{1,13} = 1.0$, $P = 0.31$;

nest availability, $F_{1,26} = 1.6$, $P = 0.22$). Block affected the mean number of eggs received ($F_{5,32} = 2.7$, $P = 0.04$), but had no effect on the mean number of female mates ($F_{5,5} = 1.3$, $P = 0.4$). Specifically, females laid fewer eggs in the beginning of each breeding season. There were no significant interactions in these analyses ($P > 0.07$ for all block × treatment and treatment × treatment interactions). Only one available nest ever went unoccupied and a single male never occupied more than one nest. Further, mate monopolization was high: a single male monopolized all matings within a tank 63% of the time and only in one case did more than two males mate.

Effects of nest availability and density on overall selection

Nest availability did not affect the total opportunity for selection using variance in number of eggs for all males in the experiment (thereby encompassing all episodes considered) (ANOVA: $F_{1,35} = 3.7$, $P = 0.10$; Fig. 2a). Increasing density increased the opportunity for selection ($F_{1,35} = 16.4$, $P < 0.001$; Fig. 2a). Specifically, on average, a 50% increase in density increased the opportunity for selection by 56% (Fig. 2a). However, there was also a significant interaction between density and nest availability ($F_{1,35} = 5.8$, $P = 0.02$): the magnitude of the density effect was much greater when nests were unlimited vs. limited (Fig. 2a). There was no effect of block ($F_{5,30} = 0.3$, $P = 0.91$) and no block × treatment interactions were significant ($P > 0.45$).

There was no effect of density, nest availability, block, or interaction between density and nest availability on the overall selection differential (density: $F_{1,11} = 2.0$, $P = 0.18$; nest availability: $F_{1,12} = 2.9$, $P = 0.11$; block: $F_{1,10} = 0.6$, $P = 0.47$; density × nest availability: $F_{1,9} = 1.1$, $P = 0.32$; Fig. 3a). The lack of significance in this analysis was potentially due to low statistical power; thus, we cannot draw firm conclusions using these data.

Sequential selection: not all episodes are the same

The opportunity for selection and the selection differential varied significantly across the three episodes of selection considered (repeated measures ANOVA, episode effect: opportunity for selection, Pillai's Trace = 0.92, $F_{1,35} = 121.9$, $P < 0.001$; selection differential, Pillai's Trace = 0.61, $F_{1,24} = 6.5$, $P = 0.02$; Fig. 4a,b). Of the three selective episodes considered, mate acquisition was associated with the largest opportunity for selection and selection differential (Fig. 4a,b). Variability in nest acquisition among males also created substantial opportunity for selection (Fig. 4a) and

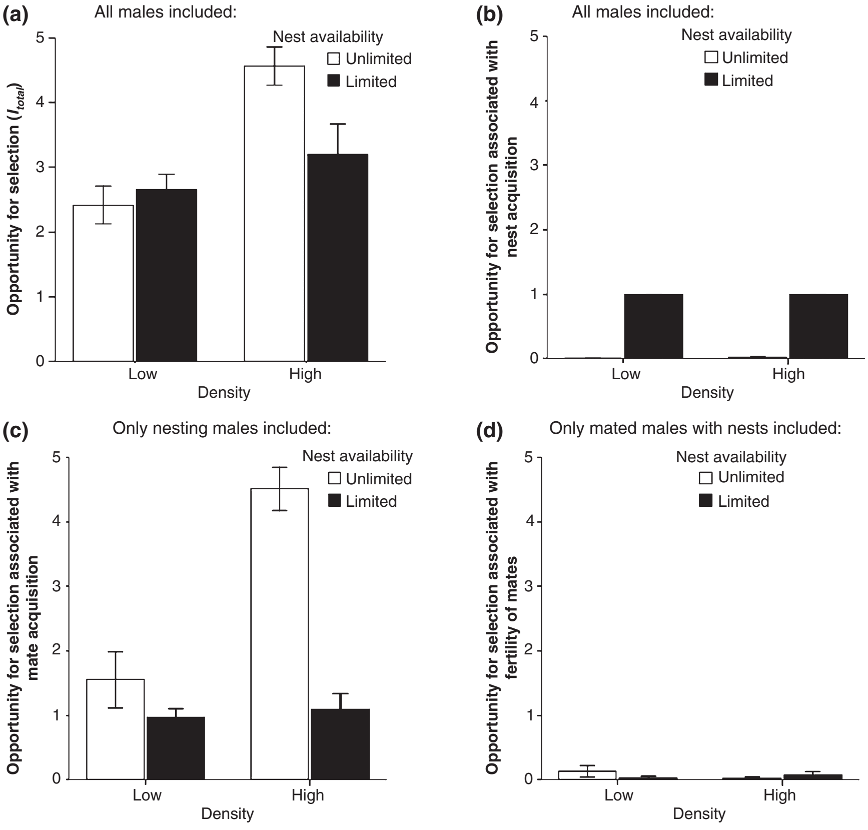

Figure 2 Effect of density and nest availability on the (a) total opportunity for selection I associated with nest and mate acquisition and fertility of female mates, (b) opportunity for selection associated with nest acquisition I_{nest}, (c) opportunity for selection associated with mate acquisition $I_{mates|\,nest}$ and (d) opportunity for selection-associated with fertility of female mates $I_{eggs|\,mate}$. All bars represent means and error bars are standard error.

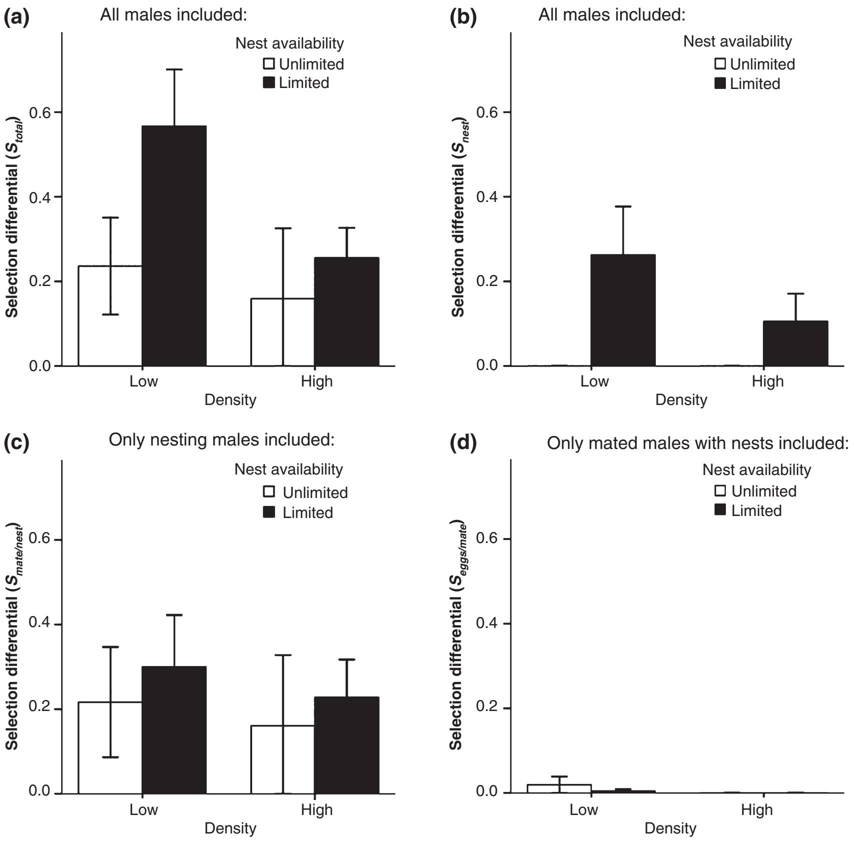

Figure 3 Effect of density and nest availability on the (a) total selection differential with respect to male length S_{total} (cm) associated with nest and mate acquisition and fertility of female mates, (b) selection differential with respect to male length associated with nest acquisition S_{nest} (cm), (c) selection differential with respect to male length associated with mate acquisition $S_{\mathrm{mates|\,nest}}$ (cm) and (d) selection differential with respect to male length associated with the fertility of female mates $S_{\mathrm{eggs|\,mate}}$ (cm). All bars represent means and error bars are standard error.

positive selection on male size (Fig. 4b). Overall, competition among males for both nests and mates is expected to select for larger males (Fig. 4b). In contrast, there was relatively less opportunity for selection or selection on male size associated with mate fertility (Fig. 4a,b).

Density, nest availability and the interaction between density and nest availability explained significant amounts of variation in the average opportunity for selection across the three selective episodes (repeated measures ANOVA: density, $F_{1,34} = 25.4$, $P < 0.001$; nest availability, $F_{1,34} = 14.1$, $P = 0.001$; density × nest availability, $F_{1,34} = 23.5$, $P < 0.001$; Fig. 2b–d). However, the effects of density and nest availability and the interaction between density and nest availability varied significantly across the episodes of selection (repeated measures ANOVA: density × episode, Pillai's Trace = 0.40, $F_{1,35} = 21.9$, $P < 0.001$; nest availability × episode, Pillai's Trace = 0.95, $F_{1,35} = 70.5$, $P < 0.001$; density × nest availability × episode, Pillai's Trace = 0.40, $F_{1,35} = 22.3$, $P < 0.001$; Fig. 2b–d). Block did not account for significant amounts of variation in the opportunity for selection across episodes ($F_{5,29} = 0.7$, $P = 0.73$) and there was no significant interaction between block and episode ($F_{5,29} = 0.5$, $P = 0.60$).

Density, nest availability, block and the interaction between density and nest availability did not explain significant amounts of variation in the average selection differential across episodes (repeated measures ANOVA: density, $F_{1,11} = 1.1$, $P = 0.32$; nest availability, $F_{1,12} = 3.6$, $P = 0.08$; block, $F_{1,9} = 1.0$, $P = 0.35$; density × nest availability, $F_{1,10} = 0.2$, $P = 0.64$). Likewise, there were no significant interactions between episode and any factors, and there was no significant density × nest availability × episode interaction (repeated measures ANOVA: density × episode, Pillai's Trace = 0.09, $F_{1,22} = 0.2$, $P = 0.77$; nest availability × episode, Pillai's Trace = 0.42, $F_{1,24} = 1.4$, $P = 0.26$; block × episode, Pillai's Trace = 0.08, $F_{1,18} = 0.4$, $P = 0.57$; density × nest availability × episode, Pillai's Trace = 0.15, $F_{1,20} = 0.3$, $P = 0.66$; Fig. 3b–d). Again, the lack of significance in these analyses is potentially due to low statistical power.

DISCUSSION

Density and nest availability impact the sequential process of mate acquisition in the sand goby, but the effects differ between the stages considered (Figs 2–4). Increasing density increased the opportunity for sexual selection when all selective episodes (nest acquisition, mate acquisition, fertility of mates) were considered. However, the magnitude of this effect was dependent on the level of nest availability

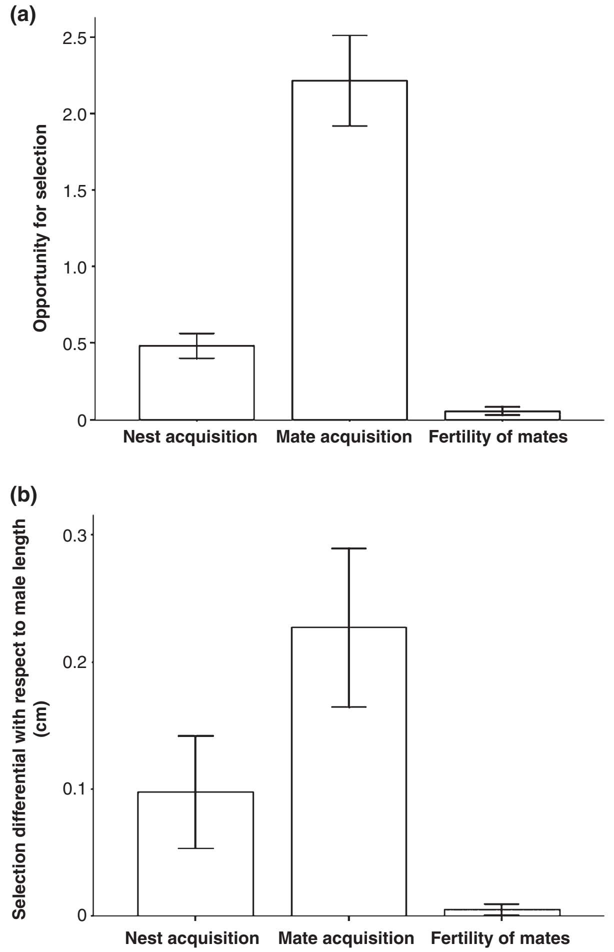

Figure 4 Sequential selection in the sand goby. (a) The opportunity for selection and (b) the selection differential with respect to male size (cm) associated with nest acquisition, mate acquisition and fertility of female mates. Bars represent means and error bars are standard error.

experienced. Specifically, the effect of density was much greater when nests were unlimited. The finding that there is greater variation in male reproductive success at higher densities is consistent with the prediction that male–male interference might increase at higher densities, leaving more males unmated (Kokko & Rankin 2006). Furthermore, our findings suggest that the effect of density on mating success can interact with other ecological factors, such as nest limitation. Such interactions might explain why empirical studies have found mixed results regarding the effect of density on sexual selection (McLain 1992; Jirotkul 1999; Reichard *et al.* 2004; Head *et al.* 2007).

Ideally, results based on opportunity of selection should be corroborated with data on selection on phenotypic traits (Klug *et al.* 2010). Using male size as a likely trait under sexual selection, we found no effect of density on overall selection associated with male size. This lack of significance is potentially due to low statistical power across replicates. Small within-replicate sample sizes (four or six males) might also cause chance to play a large role in mate selection. Alternatively, the opportunity for selection is not always expected to reflect realized selection on traits (Sutherland 1985, 1987; Koenig & Albano 1986; Ruzzante *et al.* 1996; Westneat 2006; Klug *et al.* 2010), and it is possible that the potential for selection (unequal male mating success) is simply not realized with respect to male size in this system.

While our results thus call for caution in evaluating the effects of density, the role of nest and mate acquisition is clearer. The opportunity for selection and selection differential varied across the episodes of selection considered. Nest and mate acquisition contributed most to the overall opportunity for selection and selection on male size, whereas mate fertility contributed relatively less. Increasing nest limitation increased the opportunity for selection associated with nest acquisition. When nests were limited, nest and mate acquisition contributed similarly to the overall opportunity for selection. In contrast, when nests were unlimited, mate acquisition contributed most strongly to the overall opportunity for selection. These findings are consistent with previous work (Forsgren *et al.* 1996; Lindström 2001; see also Ahnesjö *et al.* 2001), which suggests that competition for nesting sites affects sexual selection in gobies. The finding that fertility of mates contributed relatively less to the overall opportunity for selection is also consistent with previous work. Sand goby females tend to spawn all of their eggs with a single male during a given breeding episode (Lindström 1992; Singer *et al.* 2006), and male reproductive success is largely determined by mating success (Lindström 1992).

It is noteworthy that nest limitation continues to impact the opportunity for selection when focusing on males who have already passed that stage of competition (i.e. when considering mate acquisition). Any effect of nest limitation on the opportunity for selection associated with mate acquisition reflects the fact that the maximum the opportunity for selection can reach depends on the number of males considered (Fig. 1; Wade & Shuster 2004). The maximum opportunity for selection is realized when one male monopolizes all females. In our case, the maximum opportunity for sexual selection is 1 and 2 at low and high densities when only nesting males are considered, whereas when all males are included the maxima are 3 and 5 (eqns 1 and 2). Nest limitation therefore decreases the potential intrasexual variance in mating success, which in turn decreases the maximum level of selection that can potentially operate during mate acquisition.

Our experimental study highlights the implications of excluding certain individuals from measures of sexual selection. Our general conclusions depended heavily on whether we included or excluded certain males from our measures. In particular, the effects of density and nest limitation differed between the episodes of selection. Further, we found that in some cases nest acquisition can contribute as strongly to the opportunity for selection and the strength of selection on male size as mate acquisition. Focusing only on males who secured nests (i.e. ignoring resource competition) can lead to much lower estimates of selection and might result in one ignoring a potentially significant source of sexual selection (assuming that nest acquisition is considered sexual selection). Likewise, if one were to only consider selection among mated individuals, the opportunity for selection and selection differential with respect to male size would appear minimal. This finding is consistent with the theoretical findings of Wade (1979), Shuster & Wade (2003) and Wade & Shuster (2004).

There is variation in who is included in measures of sexual selection (e.g. Singer *et al.* 2006; Westneat 2006; Dolan *et al.* 2007; Mobley & Jones 2007; Duval & Kempenaers 2008; Perlut *et al.* 2008). In particular, competition associated with resources required for mating is often excluded from measures of sexual selection and in some cases unsuccessful males are excluded entirely (see Shuster & Wade 2003). Our findings, in combination with those of others (Shuster & Wade 2003; Wade & Shuster 2004) suggest that this is not a trivial matter. Variation in who is included in measures of sexual selection will affect the general conclusions that we draw about sexual selection.

Is there a correct answer to the 'who to include' question? It is important to note that an answer to this question essentially defines the boundary of sexual and natural selection: one includes individuals that participate in those selective episodes that are considered sexual selection. There is no unique clear answer to where this boundary should lie (see Clutton-Brock 2007 and Jennions & Kokko 2010 for contrasting views). We suspect that in many cases the question goes away because it is not necessary to predict, quantify or explain 'sexual selection' *per se*. For example, if one is interested in the effect of size on one or more components of reproductive success, there is no need to label size as a sexually selected trait: the sequential process comprising several selection episodes can be considered without paying attention to definition problems.

However, this simple solution is unavailable when aiming to reach general conclusions about sexual selection. Idiosyncrasy in the measurement of sexual selection for each case or species will make it impossible to answer broad questions, such as whether sexual selection impacts speciation (Ritchie 2007), population viability and extinction (Doherty *et al.* 2003; Kokko & Brooks 2003), and adaptation to novel environments (Rundle *et al.* 2006) including anthropogenic change (Candolin & Heuschele 2008), whether sexually selected traits show more heritable variation than naturally selected traits (Pomiankowski & Møller 1995) or different patterns of allometry (Bonduriansky 2007), and whether there is a positive or negative association between sexually and naturally selected species properties (Tobias & Seddon 2009).

It is not our aim in this study to state where natural selection ends and sexual selection begins. With regard to who should be included in measures of sexual selection, we suggest that it is critical to be explicit about and justify which aspects of sexual selection are being considered and/or quantified (e.g. selection associated with resources acquisition, mate acquisition, fertility of mates, fertilization success), and to address whether the selective episodes accounted for are likely to be sexually selected, and how these episodes relate to the overall sexual selection that is likely to occur in a system based on biological knowledge.

We additionally suggest two future avenues of research: (1) an explicit examination of the importance of general resource competition in mating behaviour, as this competitive step has received relatively less attention; and (2) more explicit discussion of who is included in empirical measures of sexual selection and what these measures reflect (see Jones 2009). We argue that, when possible, it is worthwhile to consider sequential episodes of selection separately, and in particular, to explicitly consider the role of specific traits in various steps associated with mate acquisition (see Arnold & Wade 1984a,b; Ahnesjö *et al.* 2001; Shuster & Wade 2003). Doing so provides a more complete view of selection, and allows a reader to make their own decision about what traits they consider sexually selected.

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