Original Article

Effects of habitat complexity on mating behavior and mating success in a marine fish

Lise Cats Myhre,[a] Elisabet Forsgren,[b] and Trond Amundsen[a]
[a]Department of Biology, Norwegian University of Science and Technology (NTNU), NO-7491 Trondheim, Norway and [b]Norwegian Institute for Nature Research (NINA), PO Box 5685, Sluppen, NO-7485 Trondheim, Norway

The environments animals inhabit vary greatly in structural complexity, both naturally and as a consequence of human disturbance. Structural complexity might affect communication by visual and other means, impair detection of potential partners, and affect sexual selection processes. Previous studies on shallow water fishes suggest that sexual selection can be relaxed when visibility is reduced. Here, we test whether habitat complexity affects mate search, mate choice, and the opportunity for sexual selection in the two-spotted goby, *Gobiusculus flavescens*, a marine fish with paternal care. In 2×2 m tanks, we established environments with low or high habitat complexity and introduced a mixed-sex group of fish (8 males, 8 females), which were allowed to breed. Two days later, we released additional (focal) ready-to-mate females in the tanks and observed female mate search and mating behaviors of both sexes. For females, habitat complexity negatively affected rate of movement, encounter rate with males, courtship rate, and time until mating. For males, habitat complexity resulted in fewer cases of multiple-male simultaneous courtships. Additionally, fewer courtship interactions were interrupted by male–male aggression in the complex habitat. However, these clear behavioral effects did not appear to affect the mating skew among males. Despite the absence of a difference in the opportunity for selection between treatments, we detected positive selection for male length in the open but not in the structurally complex environment. The results indicate that habitat complexity affects mating behaviors of both females and males and that a more structurally complex habitat might relax sexual selection. *Key words:* environmental change, female choice, *Gobiusculus flavescens*, habitat structure, mate sampling, sexual selection, two-spotted goby. *[Behav Ecol]*

INTRODUCTION

In nature, habitats vary greatly in complexity. Within the same species, populations may sometimes inhabit widely different habitats. The same population may also experience dramatic temporal changes in its habitat, both between and within years. Finally, within populations, individuals may inhabit different habitat types. Such differences in habitat complexity could have profound effects on animal communication and behavior. For example, the expression and transmissibility of signals could be affected by environmental factors (e.g., Moyaho et al. 2004; Brumm and Slater 2006; van der Sluijs et al. 2011). A simple habitat (e.g., open fields, clear waters) might permit the transmission of visual signals over long distances, whereas a physically and structurally complex habitat (e.g., dense vegetation, rocky environments) will impede visibility and decrease visual range. The complexity of a habitat can affect a range of behaviors, such as movement patterns (e.g., Longepierre et al. 2001; Orpwood et al. 2008), aggressiveness (e.g., Carfagnini et al. 2009; Kadry and Barreto 2010; Danley 2011), and foraging behavior (e.g., Ryer et al. 2004; Andruskiw et al. 2008; Michel and Adams 2009). The physical complexity of a habitat might also influence behavioral processes important for sexual selection, such as

species recognition, mate choice, and intrasexual competition. For instance, a structurally complex habitat might relax intrasexual competition by making it difficult for animals to detect competitors (e.g., Hibler and Houde 2006). Structural complexity might also relax mate choice by making it harder for animals to locate or assess potential mates. Thus, animals might adapt their behavior to the structure of the environment they inhabit. For instance, calls used for mate attraction often differ between open and densely vegetated habitats (birds: Morton 1975; Boncoraglio and Saino 2007; Smith et al. 2008; mammals: Peters and Peters 2010). Additionally, male mate-locating behavior in an insect has been found to vary between different forest structures (Bonte and Van Dyck 2009). If an increased habitat complexity causes females (or males) to sample fewer potential mates before mating, then this lesser sampling should lead to weaker sexual selection (Jennions and Petrie 1997; Benton and Evans 1998). Among shallow water fishes, more complex habitats are associated with reduced competitive interactions among males (Hibler and Houde 2006), increased male courtship intensity (Dzieweczynski and Rowland 2004; Candolin et al. 2007), increased female inspection of males (Hibler and Houde 2006; Candolin et al. 2007), and a decreased mating skew (Candolin 2004).

The marine coastal environment contains habitats ranging from very simple (pure sand/gravel bottom) to very complex (rugged rocky shores, complex algal vegetation, etc.). Both natural and anthropogenic influences could have effects on habitat structural complexity. Along Scandinavian coasts, for instance, habitat complexity can be affected by the increased growth of filamentous algae as a result of natural seasonal growth, increased nutrient concentrations (Rosenberg et al.

1996), and the removal of top predators, which can decrease the abundance of grazers through cascading trophic effects (Eriksson et al. 2009). It has been documented that the increased growth of fast-growing seasonal filamentous algae reduces visibility and alters habitat complexity (Larsson et al. 1985; Pihl et al. 1995; Rosenberg et al. 1996). In addition to clusters of filamentous algae on existing macroalgae, the invasive macroalgae *Sargassum muticum* (Thomsen et al. 2006) contributes to increased structural complexity. In the more extreme cases, the growth of filamentous algae has "taken over" and suppressed the original kelp forest, thus creating carpets of filamentous algae (Moy et al. 2008) and habitats with almost no structure. Thus, environmental changes currently observed in marine coastal environments of Scandinavia (Karlsson 2007; Moy et al. 2008) might both increase and decrease the complexity of a habitat. Similar changes seem to occur in coastal waters around the World. Likewise, the structural complexity of global marine environments is highly variable, from uniform sandy bays with little vegetation to coral reefs of sometimes extreme structural complexity.

Visibility in aquatic environments might be similarly affected by increased turbidity, which can be caused by natural or human-induced phytoplankton blooms or pollution. Increased turbidity has been found to reduce male mating skew (Järvenpää and Lindström 2004), impair male mate choice (Sundin et al. 2010; Lindqvist et al. 2011), and might, in a worst-case scenario, lead to the breakdown of reproductive isolating mechanisms between species (e.g., Seehausen et al. 1997).

In order to understand the operation of sexual selection and other important processes in nature, it is crucial to know how these processes are affected by environmental variability. This study aims to determine if a more structurally complex environment affects female and male mating behaviors and sexual selection. We used a small marine fish, the two-spotted goby (*Gobiusculus flavescens*), as a model species. Two-spotted gobies inhabit and breed in a range of coastal habitats. They are abundant in moderately exposed environments in fjords, bays, and around islands and skerries of all sizes, but seem to be less common in the most exposed outer localities, and at very sheltered, inshore localities. Typically, their habitat is dominated by large macroalgae, usually dominated by *Laminaria* spp. and *Saccharina latissima* (Wheeler 1980; Svensson 2006), which by themselves create a highly structured environment, but also with a range of other algae growing either on the substrate or on the kelp. The species might also seem to prefer some natural structure, as one often find shoals and individuals in crevices, depressions, and other somewhat less exposed locations. However, the species is also common in and near gravel-dominated bays, where the environment might be more open, often only with various algae (e.g., *Fucus* spp.) creating some structure. The light environment is variable depending on weather conditions and physical structure (darker in crevices and in the shadows of rocks and algae). Water transparency varies naturally in accordance to time of season, algal blooms, weather, and other conditions (Myhre LC, Forsgren E, Amundsen T, personal observations). The two-spotted goby is a keystone species in the coastal ecosystem and constitutes an important food source for juvenile cod (*Gadus morhua*) (Fosså 1991; Giske et al. 1991; Salvanes and Nordeide 1993). Changes in the coastal environment are therefore likely to influence this species.

To test how habitat complexity affects mating behavior and sexual selection in two-spotted gobies, we conducted an experiment in which the habitat complexity (spatial structure) was manipulated as either low (open habitat) or high (spatially structured habitat) in large indoor tanks. Individually marked, mixed-sex groups of gobies were released into the tanks and were allowed to interact freely. We allowed the initial group of fish 2 days in the tank before additional females were released for focal observations. Along with male reproductive success (monitoring of egg batches), both female and male mating behaviors were observed.

We predicted that a structured environment (complex habitat) would affect behaviors in the following 2 ways: 1) because of difficulty in detecting males, females would sample fewer males before mating and 2) because of a reduction in the visual contact between males, male–male competition would be relaxed, which would lead to less male courtship interference. As a result of these predicted responses, we expected eggs to become more evenly distributed among males (lower mating skew and lower potential for sexual selection) in the complex habitat.

METHODS

Model species

The two-spotted goby (*G. flavescens*) is a small (adult mostly 35–55 mm), sexually dimorphic, marine fish. This species is quite common along the rocky shores of western Europe (Collins 1981). Two-spotted gobies are semipelagic (Wheeler 1969) and often form large shoals near the algal vegetation (Svensson et al. 2000). In nature, fish density varies between localities and with time of season (Forsgren et al. 2004). On a local scale, there is often large variation within localities as fish can occur from single individuals up to shoals including several hundred individuals (Svensson et al. 2000). During the breeding season, many males are relatively sedentary, which is likely to reflect them having and defending a nearby nest or nest site (Forsgren et al. 2004; de Jong 2011). Females, by contrast, mostly live in shoals. Such shoals are relatively unstable (Svensson et al. 2000), both in terms of size and composition of individuals, and seem not to have any strong spatial association. Breeding-ready females often encounter sedentary males in multiple-female groups (Myhre et al. 2012). Both males and females might occur in sometimes very large (many hundred or more individuals) feeding shoals, typically in the open water just outside the algal vegetation. Two-spotted gobies often seek shelter among the algae when threatened by predators (Utne et al. 1993; Utne and Aksnes 1994). The species typically live for only 1 year (Johnsen 1945), but have a polygamous mating system where both sexes can reproduce repeatedly during a breeding season (Collins 1981; Mobley et al. 2009; de Jong K, Rodrigues-Graña L, unpublished data). The species is a substrate brooder with paternal care. Breeding males take up a nest in mussel shells, on kelp leaves, or in crevices (Gordon 1983; Amundsen and Forsgren 2001; Svensson 2006). Females typically deposit clutches of 1000–1500 eggs (Pélabon et al. 2003; Svensson et al. 2006) in a male's nest and can successively lay several batches of eggs in the nest of different males over the breeding season, at intervals likely ranging from 1 to several weeks. Males might simultaneously care for clutches from several (2–6) females (Gordon 1983; Mobley et al. 2009) and they tend the eggs by guarding, fanning, and cleaning them until hatching (Skolbekken and Utne-Palm 2001; Bjelvenmark and Forsgren 2003). In the laboratory, a male may court and attract females and care for their eggs just one or a few days after hatching of earlier clutches (Eriksen 2007).

Two-spotted gobies exhibit dynamic sex roles (Forsgren et al. 2004; Myhre et al. 2012). At the start of the breeding season, mating competition is strongest among males (conventional sex roles), whereas later in the season, mating competition is stronger among females (reversed sex roles,

Forsgren et al. 2004). Both sexes display visual ornamentation and distinct courtship behaviors during the breeding season (Amundsen and Forsgren 2001; Pélabon et al. 2003; Forsgren et al. 2004; for definitions see Table 1). During their mate search, ready-to-mate females actively search for males and typically visit several males before mating (Myhre et al. 2012). The males, if interested, begin courting the female and attempt to lead the female to the nest; the female might then respond with courtship displays and follow the male to his nest (Myhre et al. 2012). Both sexes may initiate courtship, and mate choice has been demonstrated in both sexes (Amundsen and Forsgren 2001; Borg et al. 2006; Myhre et al. 2012). Males prefer females with more orange belly coloration (Amundsen and Forsgren 2001), and females prefer large males early in the breeding season (Borg et al. 2006).

General procedures

The experiment was conducted between 11 May and 21 June 2010 at the Sven Lovén Centre for Marine Sciences, Kristineberg (58°15′N, 11°27′E), on the west coast of Sweden.

The fish were collected around islands up to 2 km from the research station by snorkelers, using hand-held dip nets. The fish were separated by sex in the field and brought back to the laboratory by boat. Females and males were collected from different localities to ensure that they had no familiarity with each other. In the laboratory, the males were placed individually into aquaria ($25 \times 30 \times 30$ cm, $L \times W \times H$) and acclimatized for approximately 40 h. The females were kept together for 2 days (up to 35 females in $60 \times 40 \times 35$ cm aquaria) before being used in the experiment. Females used for focal observations were held in an aquarium ($35 \times 35 \times 35$ cm) for 2 additional days, with 5 fish in each aquarium. All of the fish were fed ad libitum twice daily (morning and evening) with *Artemia* sp. nauplii. Aiming to include only individuals that were ready to mate, we selected healthy-looking males and females with a high "belly roundness", which is an indicator of gonad maturity (Svensson et al. 2006).

Table 1

Behaviors recorded during observations of focal female two-spotted gobies *(Gobiusculus flavescens)*

Behavior	Definition
Courtship	
Sigmoid display	Female bending to a sigmoid shape, displaying distended orange belly
Fin display	Male erecting dorsal and anal fins
Quiver display	Male quivering his body
Lead display	Male swimming with undulating body movements toward nest
Agonistic behavior	
Male–male fin display	Males lining up side-by-side, erecting dorsal, and anal fins
Chase	Darting toward another individual, often with extended fins
Other definitions	
Male encounter	Focal female <2 body lengths from a male
Mating	Focal female staying >10 min in a male's nest
Search time	Time from commencement of normal swimming until entering nest for mating. If no mating occurred within 30 min, the search time was set at 30 min
Courtship event	Courtship by the male, the female, or both
Nest inspection	Female entering a male's nest

All of the fish used in the experiment were marked individually with visible implant elastomer tags (North-West Marine Technology, Shawn Island, Washington), as described by de Jong et al. (2009). Using 4 colors (blue, red, green, and orange), each male was marked in 1 of 2 possible locations. The females were given 2 marks, in 2 of 4 possible locations, using 3 colors (blue, red, and green; 54 combinations). Both before and after the trials, we measured the total body length (TL) for each fish to the nearest 0.5 mm. The TL measurements were conducted using a measuring board, and we determined the wet body mass (BM, to an accuracy of 0.01 g) using a Mettler digital balance. These measurements allowed us to calculate a rough estimate of how many females had laid their eggs during the experiment. The females (introduced on day 2) that had lost weight (>0.03 g) by the termination of the experiment were judged likely to have spawned. The females that likely spawned lost a median of 12.2% (range 4–29%) of their BM.

All of the males were tested in a "personality assay" before they were used in the experiment. These results will be published elsewhere.

Experimental design

The treatments were designed to test whether visual obstructions induced by a spatially structured (complex) environment affected mating behaviors and sexual selection. The experiment was conducted indoors under controlled light and water flow conditions. We created 2 treatments that consisted of an open and a structured (spatially complex) environment in 200×200 cm gray tanks with a water depth of circa 35 cm. Two sets of replicates of each treatment were run in parallel (4 tanks), which added up to a total of 16 replications of each treatment. Each trial was run for 3 days, and we let the fish interact freely during that time. All of the fish were randomly assigned (by flipping a coin) to a treatment. Neither females nor males differed in TL or BM between treatments (Table 2), and the sizes represented the natural range of sizes in the field. The size of the males ranged from 37.8 ± 0.3 mm to 47.8 ± 0.5 mm (smallest and largest fish, respectively, in each trial), the within-trial size range did not differ between treatments ($t_{29.21} = -0.06$, $P = 0.95$), and the coefficient of variation (CV) was very similar between the treatments (structured: CV = 0.091 and open: CV = 0.085).

For both treatments, we used white tape to mark the bottom of the tanks in sections ($\sim 50 \times 50$ cm). The tape facilitated the recordings of female movements during the observations. We provided each tank with 8 artificial nests and 20 plastic plants. The nests were placed next to 1 plant, approximately 20 cm from the wall, and 12 plants were placed in the 4 central sections of the tank, approximately 10 cm apart (Figure 1). The open environment had only the artificial nests and plants as structural elements in the tank (Figure 1A). Thus, the fishes in this treatment could potentially see most of the other fish in the tank the majority of the time. The structurally complex environment treatment (hereafter termed structured environment) had the same basic setup with respect to the artificial nests and plants, but also included 6 opaque (white) plastic dividers across the length and width of the tank (Figure 1B). These dividers created partly separated sections ($\sim 50 \times 50$ cm, which matched the division of the marked sections) around the artificial nests, but they did not preclude movement between the sections. Around the nest sections the dividers were 40 cm high with a "V" section removed. The bottom of the "V" was 20 cm above the bottom of the tank so the fish could swim through. We also cut an approximately 2 cm high "arch" under each "V" in the bottom of the dividers. Hence, the fish could swim both below and above the dividers and

Table 2

The characteristics of female and male two-spotted gobies *(Gobiusculus flavescens)* **used to test the effect of habitat complexity on mating behavior**

	Open	Structured	*t*	df	*P*
Initial females					
N	128	128			
Length	41.83 ± 0.25 (35.0–48.5)	41.51 ± 0.25 (36.0–49.0)	0.89	253.68	0.37
Body mass	0.666 ± 0.011 (0.387–0.979)	0.652 ± 0.011 (0.408–1.033)	0.92	253.74	0.36
Males					
N	128	128			
Length	42.01 ± 0.31 (35.0–52.0)	42.42 ± 0.34 (35.5–53.5)	−0.87	252.17	0.38
Body mass	0.614 ± 0.014 (0.349–1.091)	0.635 ± 0.015 (0.321–1.193)	−0.99	252.37	0.32
Nonfocal females					
N	32	32			
Length	42.56 ± 0.48 (36.0–48.5)	43.19 ± 0.59 (36.5–49)	−0.91	61.94	0.36
Body mass	0.728 ± 0.022 (0.414–0.979)	0.749 ± 0.025 (0.456–0.980)	−0.62	60.92	0.55
Focal females					
N	48	48			
Length	43.18 ± 0.45 (35.0–50.5)	43.90 ± 0.42 (36.5–48.5)	−1.18	93.29	0.24
Body mass	0.761 ± 0.023 (0.423–1.205)	0.793 ± 0.021 (0.459–1.089)	−1.01	93.02	0.32

Total length (mm) and body mass (g) are given as the mean ± 1 SE (range). Differences between the treatments, open, and structurally complex (structured) environment are tested with a 2-sample *t*-test. Males and initial females are those introduced to the tank on day 0, the nonfocal females were introduced on day 2 but were not observed, and the focal females are those fish that were released and observed on day 2. See METHODS for further explanations.
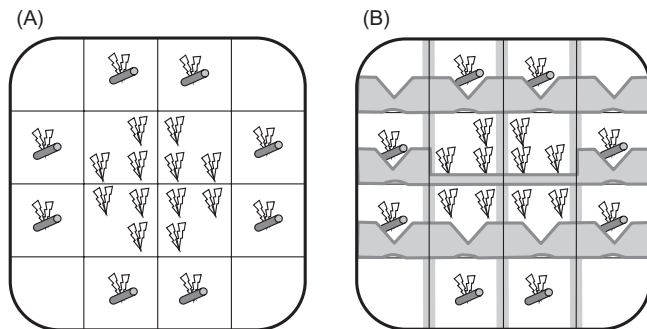

Figure 1
Design of the experiment to test for effects of habitat complexity on mating behavior and mating success in two-spotted gobies (*Gobiusculus flavescens*). (A) The open environment treatment with only plastic plants and 8 artificial nests. (B) The structurally complex environment treatment, where opaque plastic dividers across the tank (gray area) reduce the visual range and contact among the fish. The dividers had the same shape for both dimensions. The thin lines illustrated where we marked the tank in sections. The dark gray tubes are the artificial nests. See METHODS for further details.

slip past the edges. To create a more open area where the fish could shoal, the dividers were only 10 cm high in the central sections (see Figure 1B). The dividers hampered visual contact between the fish in the tank and are thus likely to have made the detection of both potential mates and competitors more difficult.

We used a 2-phase design for the experiment. First, we established a breeding population in each tank and allowed the males and females time to acclimatize to the laboratory conditions and interact freely and spawn (no behavioral observations at this stage). Two days later, we released additional (focal) females and observed the mating behavior of these females and any males they interacted with during their mate search. Apart from the data on male mating success over the course of the experiment, all data in the study are based on these behavioral observations.

For the first phase (the establishment of breeding populations), individually marked males and females were released in the middle of each tank (sex ratio males:females 8:8) at circa 14:00 h on day 0 (hereafter called "initial" females and males). An even sex ratio was chosen so that males would take up nests and mating could take place. Additionally, this ratio was chosen so that all or most males would have room for additional clutches in their nests at the time of focal female introduction (second phase). We provided PVC nest tubes (80 mm long and 13 mm inner diameter, lined with an acetate sheet) capable of holding clutches from approximately 4 females (Bjelvenmark and Forsgren 2003; Forsgren et al. 2004). The second phase began on day 2 between 10:00 and 14:00 h (approximately 44 h after the initial males and females were introduced to the tank). We released 5 marked ready-to-mate females (see details below) and observed behaviors (performed and received) for 3 of these females (focal females). We released 2 nonfocal females just before the release of the first focal female to avoid a situation where the first of these focal females would be the only ready-to-mate female in the tank at that time. The primary reason for observing females introduced at a later stage than the initial females was to simulate a situation where the females would have no prior knowledge of the males and would sample mating options in a natural environment of already breeding males and females. The fact that the focal females were introduced to an already acclimatized group of fish that performed natural breeding behaviors also seemed to minimize stress in focal females, with the result that most of them display natural mate search behavior almost immediately on release. The trials were terminated on day 3 (after 15:00 h), by which time most of the females had spawned. All of the fish and nests were collected at the end of the trial (approximately 73 h after the trial initiation). The fish were measured (as described above) and fin clipped before being released back into the sea. The egg content of each nest was photographed to allow for the counting of the number of eggs. The eggs were counted as an estimate of male reproductive success at the end of the experiment.

The tanks had a continuous flow of sea water (from 7 m depth). The water transparency was high and did not restrict

visual range in any of the treatments. The water temperature followed the natural sea temperatures and ranged from 10.5 to 16.8 °C during the experiment (the temperature was not recorded for the first 2 replications). The light schedule followed a natural summer light regime for that latitude, with a light:dark ratio of 17:7.

Observational protocol

Because females are the sex that actively searches for mates (Myhre et al. 2012), females were chosen for the focal observations. On the morning before the release of the focal females, typically 4–5 of the males held a nest and approximately 3 of these males had eggs in their nest (Figure 5). In 7 (open: 3, structured: 4) out of 32 trials, one of the males in a tank had a full nest prior to the release of focal females, which left 7 out of 8 males available for mating. Most (6–7 out of 8 in most trials) of the initial females had already spawned at the time of the focal female introduction. This figure was calculated from the number of nest holders with eggs and the mean nest fullness (Figure 5), and we assumed 1 spawning to typically fill up 25% of a nest (Bjelvenmark and Forsgren 2003). We first released 2 nonfocal and then 3 focal females in each trial. The focal females were released one at a time, and they were observed until mating (see Table 1) or for 30 min if mating did not occur. The mean observation time was 18.4 min (range 0.3–30.0 min). In addition to female movement between sections, all of the behaviors (Table 1) performed and received within a radius of 10 cm from the focal female were recorded. We flipped a coin to randomly determine in which treatment, out of a pair of treatments run in parallel, we would first release the focal females. Thus, we randomly determined where the observations began. The observations were alternated between the treatments. All of the females were released in the middle of the tank. At the time of their release, the females sometimes immediately laid down on the bottom, but began swimming normally within <3 min (mean 45±12 s, 1 female stayed 15 min on the bottom). The behavioral recording did not begin until normal swimming commenced. In total, we observed 96 focal females (48 for each treatment). The focal females did not differ significantly in either TL or BM between the treatments (Table 2).

As a measure of male success, every morning (around 8:00 h) and evening (around 19:00 h) we recorded which males were holding a nest, the position of the nest, and the percentage of nest area covered with eggs (nest fullness, in 10% increments).

Statistical analyses

The data analyses were performed using R v. 2.13.1 (R Development Core Team 2011). We used generalized linear mixed models (GLMM) to analyze the data with the appropriate error distributions (proportion data: binomial with a logit link; count data: Poisson with a log link). When analyzing the female mate search, we included "tank" as a random effect and "treatment," "release order," and the interaction between these effects as fixed effects. We checked for over-dispersion. If the model was over-dispersed, we then fitted the model again by adding a random effect at the individual level. We tested the fit of the models using a likelihood ratio test (LRT) and removed nonsignificant variables. For the analysis of the position of events in the tank, we entered "male identity," "female identity," and "tank" as random factors. Estimates are given as a contrast to the intercept and on a log or logit scale depending on the error distribution used, ±1 SE. The release order affected the observation time of the focal females (the mean

observation time, first focal: 14±2 min, second focal: 23±2 min, and third focal: 18±2 min; GLMM: intercept [first] 6.05±0.22, estimate [second] 0.77±0.25, z = 3.07, P = 0.002, estimate [third] 0.29±0.25, z = 1.14, P = 0.25), regardless of the treatment (z = 1.56, P = 0.12). Thus, to make analyses more comparable among the females, most of the analyses are focused on how many times an event occurred per minute. The time until mating during the 30 min observations was analyzed using a survival analysis with a constant hazard assumed.

To quantify variation in egg acquisition among males, we used the square centimeter of the nest that was covered with eggs, and we calculated the opportunity for selection (I) as the variance in reproductive success divided by the square of mean reproductive success (Wade 1979; Wade and Arnold 1980) over time for each treatment and replicate. At termination of each replicate, the number of eggs was counted (from photos) and used to calculate I. To test whether male traits (TL and condition) affected reproductive success, we analyzed the relationships between these traits and reproductive success and then tested if the male selection differentials differed from zero. The means are presented ±1 SE; medians are presented with the range.

RESULTS

Space use

After having been released in the center of the tank, the focal females in the open environment typically swam to the peripheral sections where the males had their nests. On average, it took the females less than a minute (mean time 40±10 s, N = 47) to reach the peripheral sections. By contrast, females in the structured environment usually remained in the center of the tank for an average of approximately 5 min (the mean time until reaching the peripheral sections was 292±53 s, N = 46; GLMM: intercept [open] = 2.19±0.31, estimate [structured] = 2.73±0.44, z = 6.25, P < 0.001). In the central sections, the focal females typically formed loose shoals together with the initial females. The positions of the initial females were not systematically recorded, but these females (which had mostly spawned) often appeared to shoal in the central (more open) part of the tank in the structured treatment. In the open environment, these females would often shoal across the full tank.

Both release order and treatment had an effect on the movement of focal females (number of section boundaries crossed per minute; LRT: treatment P = 0.003, release order P < 0.001). Thus, we performed separate tests in relation to the release order of the females. The focal females in the structured environment moved around in the tank less actively (crossed fewer section boundaries per minute) than did the females in the open environment (2-sample t-test, first female: $t_{26.98}$ = 2.20, P = 0.037; second female: $t_{26.21}$ = 2.97, P = 0.006; and third female: $t_{29.86}$ = 1.82, P = 0.08, Figure 2A). Compared with the focal females of the open environment, the focal females in the structured environment visited a lower number of unique male nest sections per minute (open: median 0.37, range 0.13–3.33; structured: median 0.17, range 0–1.53; GLMM: intercept [open] = 3.73±0.12, estimate [structured] = −0.87±0.18, z = −4.91, P < 0.001).

The likelihood that a courtship event (i.e., courtship by the male, the female, or both) took place in 1 of the 4 central sections of the tank (see Figure 1) was much higher in the structured environment (150 of 235 [64%] courtship events) than in the open environment (96 of 368 [26%] courtship events; GLMM: intercept [open] = −1.20±0.31, estimate [structured] = 1.61±0.45, z = 3.62, P < 0.001, N = 597).
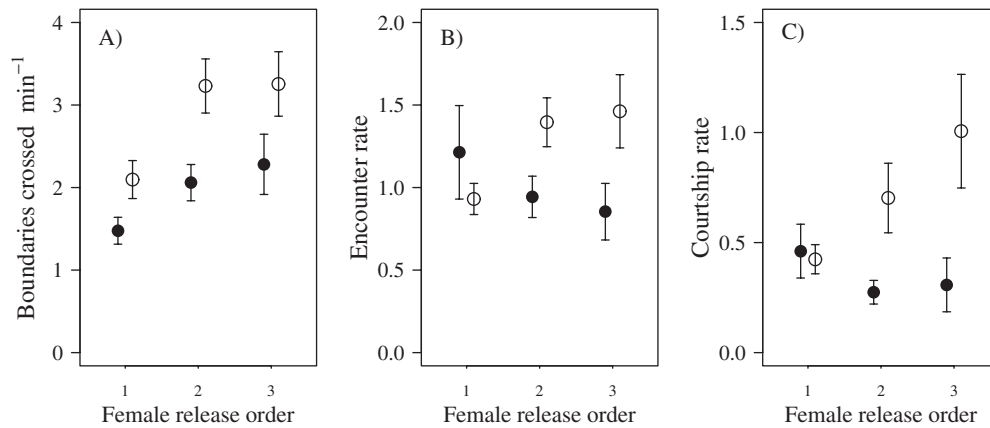
Figure 2
The effects of habitat complexity (open environment: open dots; structured environment: filled dots) and the release order of the focal female two-spotted gobies (*Gobiusculus flavescens*) on the number of (A) section boundaries crossed per minute, (B) male encounters per minute, and (C) courtship events per minute. Each point represents the mean ± 1 SE (*N* = 16, for each point).

Female mate search

The focal females (*N* = 48 in both treatments) were frequently in contact with males during their mate search. Because the release order affected the female search time, our analyses are based on the rates of events per time unit. The release order and treatment had a significant interaction effect on the encounter rate (number of males encountered per minute; LRT: *P* = 0.010). Thus, we conducted separate tests in relation to the release order of females. The male encounter rate for the first-released focal female did not differ between the open and structured environment ($t_{18.29}$ = −0.95, *P* = 0.36, Figure 2B). However, the second and third focal females had lower encounter rates with males in the structured environment than in the open environment (second focal female: $t_{29.20}$ = 2.33, *P* = 0.027; third focal female: $t_{28.21}$ = 2.17, *P* = 0.039, Figure 2B).

Compared with the focal females that mated in the open environment, the focal females that mated during observations in the structured environment experienced approximately half as many courtship events (i.e., encounters that included courtship) before mating (open: mean 5.69±1.15, *N* = 32; structured: mean 2.70±0.49, *N* = 23; GLMM: intercept [open] = 1.39±0.16, estimate [structured] = −0.63±0.26, *z* = −2.41, *P* = 0.016). Of all of the courtship events recorded, only 12 out of 597 such events were by a focal female courting an unresponsive male. We recorded 585 courtship events that included male courtship (open: 359, structured: 226) and only 65 courtship events including female courtship (open: 34, structured: 31). When both females that mated and those that did not mate during the observations were included, the proportion of encounters that included courtship tended to be lower in the structured environment (median 0.32) than in the open environment (median 0.55; GLMM: intercept [open] = −0.21±0.30, estimate [structured] = −0.73±0.42, *z* = −1.73, *P* = 0.08). Accordingly, focal females experienced courtship events with fewer males in the structured (median 2 males, range 0–5) environment than they did in the open environment (median 3 males, range 0–7; GLMM: intercept [open] = 1.09±0.09, estimate [structured] = −0.31±0.13, *z* = −2.28, *P* = 0.023). The release order and treatment had a significant interaction effect on the courtship rate (number of courtship events per minute; LRT: *P* = 0.014). We therefore conducted separate tests to account for the release order of the females. The courtship rate for the first-released focal female did not differ between the open and the structured

environment ($t_{23.11}$ = −0.27, *P* = 0.79, Figure 2C). However, when compared with the females in the open environment, the second and third focal females in the structured environment experienced a lower male courtship rate ($t_{18.42}$ = 2.56, *P* = 0.019 and $t_{21.40}$ = 2.45, *P* = 0.023, respectively, Figure 2C).

During their mate search, the focal females inspected the nests of males without staying to spawn in 42 out of 98 (43%) cases. The median nest inspection lasted for 16 s (range 1–304 s, interquartile range 8–54, *N* = 40). The number of nest inspections per focal female did not differ between the treatments (open: median 1, range 0–4, and structured: median 1, range 0–3; GLMM: *z* = −1.40, *P* = 0.16, *N* = 42). However, if a female made a nest inspection, the likelihood for her to mate in that nest was higher if there were eggs in the nest (GLMM: slope [egg] = 1.72±0.46, *z* = 3.73, *P* < 0.001). The treatment had no effect on this result (GLMM: *z* = 0.57, *P* = 0.57).

Compared with the open environment, focal females searched for a longer time before mating during observations in the structured environment (survival analysis: intercept [open] ± SE = 7.29±0.17, estimate [structured] ± SE = 0.56±0.27, *z* = 2.04, df = 1, *P* = 0.042, *N* = 96, Figure 3). Only 23 of 48 (48%) females mated during observations in the structured environment (mean time until mating ± SE = 10.2±1.8 min, range 1.0–28.0 min) compared with 32 of 48 (67%) in the open environment (mean time until mating ± SE = 9.5±1.6 min, range 0.3–29.5 s; GLMM: *z* = 0.43, *P* = 0.67). Almost all of the females released on day 2 (153 of 160, 96%) mated during the 1.5 days between the release and termination of the experiment.

Male–male competition

Compared with the focal females in the open environment, fewer of the focal females in the structured environment were simultaneously courted by several males (GLMM: intercept [open] = 0.19±0.35, estimate [structured] = −1.82±0.55, *z* = −3.31, *P* < 0.001, Figure 4A). In line with this result, when compared with the focal females in the open environment, fewer focal females in the structured environment experienced male–male aggression during courtship (GLMM: intercept [open] = −0.99±0.32, estimate [structured] = −1.16±0.57, *z* = −2.03, *P* = 0.043, Figure 4B).

Male reproductive success

Except for fewer nest holders the evening after the introduction of focal females in the structured environment, we found
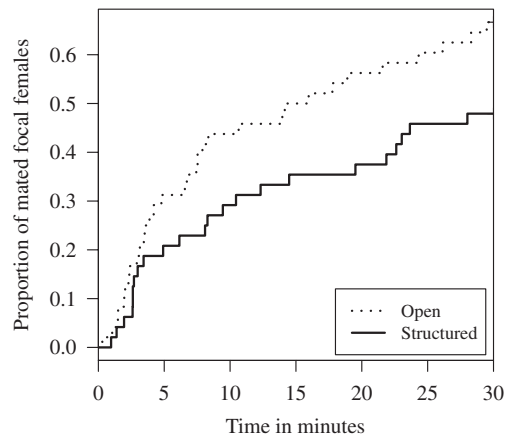
Figure 3
The effects of habitat complexity on the proportion of focal female two-spotted gobies (*Gobiusculus flavescens*) that mated over time (*N* = 48 for both open and structured environment) during 30 min observations (0 = commencement of normal swimming).
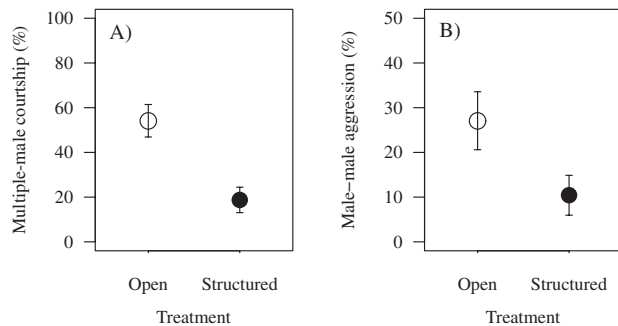

Figure 4
The effect of habitat complexity on multiple-male courtship and male–male aggression during courtship events. The percentage (mean ± 1 SE, *N* = 48 for both treatments) of focal female two-spotted gobies (*Gobiusculus flavescens*) that experienced courtship events in the open (open dots) and structured environment (filled dots) in which (A) several males courted them simultaneously and (B) a courting male showed aggressive behavior toward other males.

no differences between the treatments for any measures of male success or opportunity for selection (Figure 5).

The selection differentials (i.e., the mean trait values for breeding males compared with the mean trait of all the males in the population, here: tank) indicated no selection for male length (1 sample *t*-test: $t_{15} = 0.69$, $P = 0.50$) in the structured environment. However, in the open environment, we found a nearly significant positive selection for length (Wilcoxon signed rank test: $V = 106$, $P = 0.052$, Figure 6A), and a statistically significant selection for length after the removal of an extreme outlier ($V = 104$, $P = 0.013$, $N = 15$, Figure 6A). We did not detect any selection on male body condition in either the open ($t_{15} = 0.44$, $P = 0.67$) or the structured ($t_{15} = -0.45$, $P = 0.66$) environment (Figure 6).

DISCUSSION

Habitat complexity had significant impacts on the mating behavior of both males and females. When compared with females in the open environment, the females in the structured environment were slower to begin exploring, moved around less, had lower male encounter rates, experienced

courtship interactions with fewer males, and experienced a longer search time before mating. Fewer females also experienced multiple-male courtship and male–male aggression in the structured environment. The opportunity for selection, which was estimated from the variation in reproductive success, was unaffected by the treatment. Despite this finding, we found evidence of selection on male length in the open, but not in the structured, environment. Thus, habitat complexity might relax sexual selection by affecting the mating behaviors of both males and females.

The effects of habitat complexity on female mating behavior

We found that the order of release for the focal females significantly affected what they experienced during their mate search (see Figure 2). We typically detected a greater interest by the males for the second and third focal females compared with the first. An inevitable consequence of the chosen design was that density changed during the course of the experiment. However, the changes in density were modest (range 4–5.25 fish/m²) and are unlikely to have affected the observed behaviors. The changes in density of the 2 sexes also affected the operational sex ratio, but again to a modest degree that cannot explain the major behavioral effects seen. Also, the differences between treatments were minimal (see Figure 5). The greater interest by males for the second and third focal female might rather be due to a lag in the male's recognition of the presence of mating-ready females, which might cause the sexual activity of the males to increase as time elapsed and as more females were released for observations. Such a scenario is in line with a recent study on guppies (*Poecilia reticulata*) in which males responded to their recent social environment rather than to immediate stimuli (Jordan and Brooks 2012). Because there was little or no difference between the 2 environments for the first-released female, but clear patterns for the 2 later-released females, we base our general inference on the patterns revealed by the later-released females.

Compared with females in the open environment, the females in the structurally complex environment were slower to move out of the central parts of the tank and start exploring the environment. The females in the complex environment also moved around less, crossed fewer section boundaries, and visited fewer unique male nest sections per minute. These findings suggest that habitat complexity constrains female movement and the detection of males, both of which could affect sexual selection.

Compared with the focal females in the open environment, the second- and third-released focal females in the structured environment had a lower male encounter rate and experienced a lower rate of courtship events (number of courtship events per minute observed). A lower courtship rate is inevitable if the females experience a lower encounter rate. However, the proportion of encountered males that courted the focal females also tended to be lower in the structured environment compared with the open environment. This indicates that the lowered courtship rate is not only a direct result of a lower encounter rate. Likewise, in three-spined sticklebacks (*Gasterosteus aculeatus*), it has been found that female encounter rate was lower for males in less open territories (Candolin and Voigt 2001). If lower male encounter rates lead to overall fewer males visited (as is likely in the wild), our results suggest that sexual selection by mate choice would be weakened by increasing habitat complexity.

Fewer of the females mated within the 30 min observation time in the structured environment, but most (96%) of the females had mated by the end of the experiment.
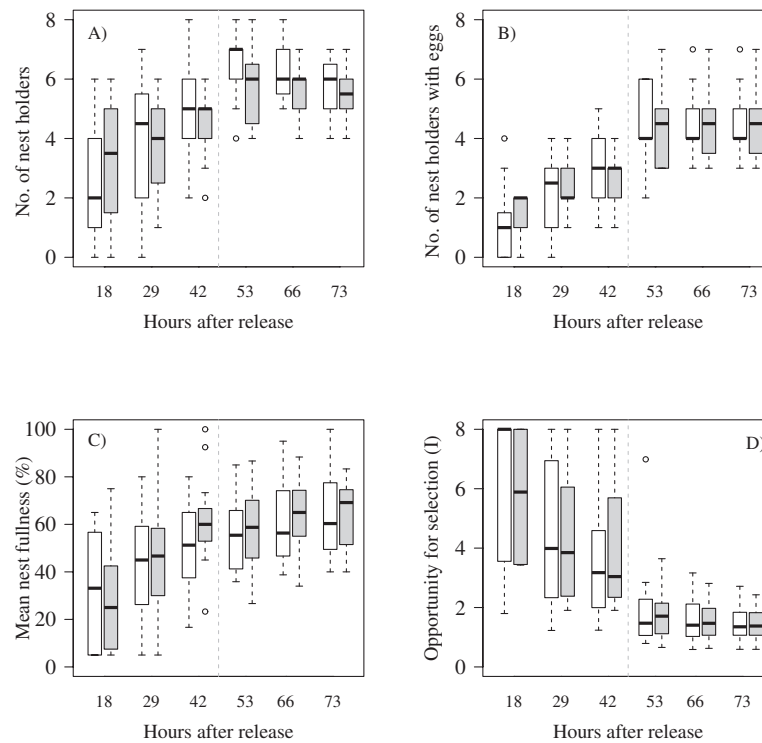
Figure 5

The effects of habitat complexity on nest ownership, mating success, and mating skew in male two-spotted gobies (*Gobiusculus flavescens*) over time for an open environment (open bars, $N = 16$) and a structurally complex environment (shaded bars, $N = 16$). (A) The mean number of nest holders, (B) the mean number of nest holders with eggs, (C) the mean nest fullness (%) for males with eggs (after 18 h: open $N = 10$, structured $N = 13$, for the other observation times $N_{open} = N_{structured} = 16$), and (D) the opportunity for selection (*I*) in egg cover (cm^2) for all males. Except for the number of nest holders after 53 h ($t_{28.10} = 2.37$, $P = 0.025$), there were no significant difference between open and structured environments at any time interval (Welch 2-sample *t*-tests or Wilcoxon tests: all $P > 0.15$). The dashed gray line represents the time where we introduced focal females for observations in the tanks. The thick lines in the boxes represent the median for each distribution, whereas the top and bottom of the boxes represent the 75th and 25th percentiles. The dashed error bars extend to the most extreme data point ≤1.5 times the interquartile range from the box. Outliers are shown as separate data points.
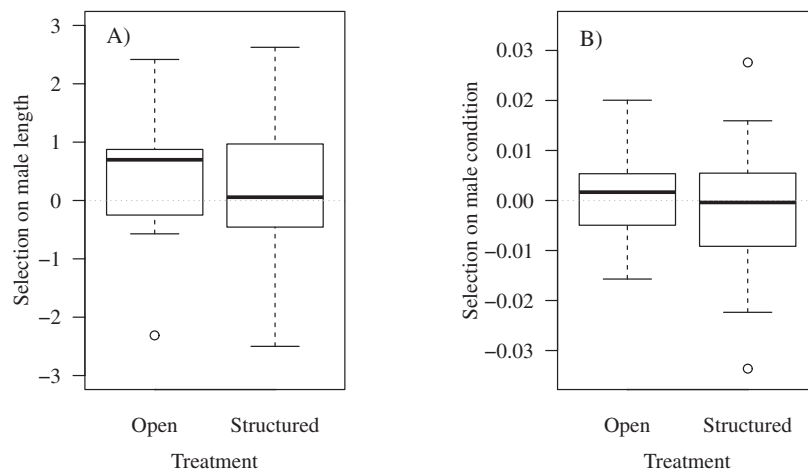

Figure 6

The effect of habitat complexity on the selection for male total length and male body condition in two-spotted gobies (*Gobiusculus flavescens*). The selection differentials (the mean trait for breeding males compared with the mean trait of all the males in the tank, $N = 16$ for each environment) for (A) male total length (mm) and (B) male condition (the residuals from the regression between male length and body mass). For boxplot details, see Figure 5.

A structurally complex environment might make it harder to detect males (and for the males to detect females). Thus, it might take longer to find a suitable mate. Some previous studies have found that females spend more time evaluating a male when the visibility is poor (guppy: Hibler and Houde 2006; sticklebacks: Candolin et al. 2007; Engström-Öst and

Candolin 2007). In order to discriminate between males of different sizes, female two-spotted gobies seemingly need to see both males at the same time (Borg ÅA, Forsgren E, Amundsen T, unpublished data). Thus, a structurally complex environment could hamper female choice by making it difficult to compare males. Regardless of the treatment, females were more likely to stay and spawn in nests if there were eggs present. This suggests that females prefer males with eggs in their nest, as found in many other fishes (e.g., Jamieson 1995; Forsgren et al. 1996; Reynolds and Jones 1999). To summarize, when compared with the females in the open environment, the focal females in the structured environment swam around less, encountered interested males less frequently, had courtship events with fewer of the males, and experienced fewer opportunities for directly comparing males. These findings suggest that it was harder for the females to choose between males in the structured than in the open environment and that their mate search process was slower.

The effects of habitat complexity on male mating behavior

Two-spotted goby males compete over nests, a resource necessary for breeding (i.e., to be qualified to mate; Ahnesjö et al. 2001), and for access to mates (Forsgren et al. 2004; de Jong et al. 2009; Wacker et al. 2012). Compared with the open environment, fewer females in the structured environment experienced simultaneous courtship by several males, and there were fewer male–male aggressive interactions during courtship. It might have been more difficult for the males in the structured environment not only to detect females but also to detect and join ongoing courtship events. Thus, a more structurally complex environment appears to reduce male–male competition by means of courtship interference. Reduced courtship interference as a consequence of visual obstructions has also been found in guppies (Hibler and Houde 2006). Male–male interactions could help females assess male qualities, but male dominance may also constrain females from choosing freely between potential mates (Qvarnström and Forsgren 1998; Kangas and Lindström 2001, reviewed in Wong and Candolin 2005).

In the open environment, males did not have to leave their nest sections to attract females because the females frequently visited these sections. However, in the structurally complex environment, a higher proportion of courtship events took place in the central part of the tank, away from the males' nests, where the females appeared to be primarily shoaling. The results suggest that when males detect where females are gathered, the males go there to seek out a potential mate. This finding is consistent with another study which indicated that male two-spotted gobies spend less time in their nests when the encounter rate with ready-to-mate females is low (de Jong 2011).

The effects of habitat complexity on the scope for sexual selection

We found no difference among the treatments in the number of males that received eggs or in the opportunity for selection. Previous studies have found that the mating skew decreases under increased algal cover (three-spined sticklebacks: Candolin 2004) and with more turbid conditions (sand gobies, *Pomatoschistus minutus*: Järvenpää and Lindström 2004). In fishes, the time window from when a female has ovulated until she needs to spawn is, at most, only a few days (Mollah and Tan 1983; Kjørsvik et al. 1990; Legendre et al. 2000). We introduced 2 nonfocal and 3 focal females during a relatively short time period (<3 h). In the related sand goby, the duration of a spawning event is approximately 1–2 h and our impression is of

a similar duration for two-spotted gobies (Myhre LC, Forsgren E, Amundsen T, personal observations). Thus, if superior males were all engaged in spawning, females would face a choice between either postponing spawning or mating with an inferior male. Hence, the rather synchronous release of females might explain why we did not detect any difference in the opportunity for selection between treatments. Synchronous arrival of females is generally considered to reduce the potential for males to monopolize females (e.g., Emlen and Oring 1977; Grant et al. 1995; Lindström and Seppä 1996). Unlike the situations in the wild, the females of this study were confined to the tank and had no other mating options.

Comparing the males that mated (i.e., had eggs in their nest) to all of the males in each tank, we detected a positive selection for male length in the open environment (after removing an outlier). However, we did not detect such selection in the structured environment. A positive selection for male length could have resulted from female choice or from male competition (e.g., Censky 1997; Howard et al. 1998; Hagelin 2002; Schütz and Taborsky 2011), both of which are important in two-spotted gobies (Borg et al. 2006; Wacker et al. 2012; Amundsen T, Bjelvenmark J, unpublished data). There is evidence that habitat complexity affected both mate choice and male competition processes (discussed above). These effects seem to a large extent to occur because habitat structure affects the social structuring of individuals, their behavior, and the rates of male–female and male–male encounters (e.g., Oh and Badyaev 2010). Hence, when investigating potential effects of habitat structure on animal behavior, these must be analyzed in a social rather than in an individual perspective.

CONCLUSION

Habitat complexity strongly affected the mating behaviors of both females and males. In the complex habitat, females generally moved around less, experienced fewer male encounters and less courtship, and took longer to mate. For males, a complex habitat appeared to hamper their detection of searching females and also of other males engaged in courtship, which resulted in less frequent multiple-male courtship events and less frequent male–male courtship interference. From a female point of view, it appears that habitat complexity makes it harder to detect potential mates. It might also be more difficult to visually compare alternative mates, partly because females cannot simultaneously see neighboring nest holding males, but also because the females more rarely experience 2 or more males courting at the same time. From a male perspective, the structure appears to reduce male–male competition. Thus, a structurally complex habitat might weaken sexual selection through effects on both male and female behavior. Consistent with this finding, and despite the treatments' lack of effect on the opportunity for sexual selection, we found a positive selection for male size in the open, but not in the structured, environment.

In conclusion, our results suggest that variation in habitat complexity, which occurs naturally in most species but can also result from human impacts, significantly affects central processes of sexual selection. This is an insight of wide-ranging implication and relevance, given that sexual selection is a major force in shaping animal behavior and morphology, and important for the reproductive potential of populations. Knowing how habitat structure affects sexual selection will be important in predicting animal responses to environmental change. Also, habitat structure should be taken into account when interpreting results from studies on sexual selection in the wild (or in the laboratory). More research is needed to reveal the importance of habitat complexity for the processes of mate choice and intrasexual mating competition across animal taxa.

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