Territorial male gobies respond aggressively to sneakers but do not adjust their sperm expenditure

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We investigated whether mating behavior (sperm expenditure, courtship rate, and nest guarding) varied according to different levels of sperm competition in territorial males of two goby species, the grass and the black gobies. We measured sperm expenditure (sperm released after 30 min from the beginning of the spawning), male courtship rate, and nest-guarding behavior in territorial males of both species during simulated spawnings, in which we varied the number of attending sneakers. Our results showed that, in both species, territorial males adjusted their effort in nest guarding to the presence of rival sneakers by increasing the time spent patrolling the territory and attacking the sneakers. In contrast, sperm expenditure and male courtship rate were not influenced by the number of attending sneakers. These results are in agreement with those reported for other fish with alternative mating tactics and help to interpret previous inconsistencies between theoretical predictions and measured levels of sperm released at different levels of sperm competition by sneakers of the two gobiids studied here. *Key words:* alternative male mating tactics, fish, sperm expenditure. *[Behav Ecol 16:1001–1007 (2005)]*

Sperm competition, defined as the competition between sperm from two or more males for the fertilization of a given set of ova (Parker, 1970), is a widespread and powerful evolutionary force affecting many animal taxa and inducing a broad variety of adaptations at behavioral, anatomical, and physiological levels (Birkhead and Møller, 1998; Smith, 1984). For example, theory predicts that high levels of sperm competition favor a greater investment in gonads and a greater sperm expenditure (Parker, 1990a,b, 1998; Parker et al., 1996). Indeed, in several animal taxa, species exposed to high levels of sperm competition possess relatively large testes and increase sperm investment to raise their chances to fertilize more eggs (Gage, 1994; Harcourt et al., 1981; Hosken, 1997; Møller, 1988; Parker, 1998; Stockley et al., 1997). Data from several studies suggest that sperm have nontrivial energetic costs to produce (Nakatsuru and Kramer, 1982; Olsson et al., 1997; Shapiro et al., 1994). As a consequence, individual sperm expenditure is expected to be strategically adjusted, if the sperm competition level varies among matings, and males can assess this variation (Parker, 1990a,b). In particular, the number of sperm allocated by a male to a given mating should be greatest when there is another male competitor (the so-called "risk" model, Parker et al., 1997) and then decrease when the number of competitors is above two (the so-called "intensity" model, Ball and Parker, 1996; Parker et al., 1996).

Instead of adjusting sperm expenditure (Evans et al., 2003; Pilastro et al., 2002; Simmons and Kvarnemo, 1997), males could alternatively respond to the presence of competing males by guarding the mate or the nest (Alonzo and Warner, 2000a; Birkhead and Møller, 1992; Komdeur, 2001; Zamudio and Sinervo, 2000) or making the nest less accessible to sneakers (Svensson and Kvarnemo, 2003). Evidence of a trade-off between mate guarding and sperm expenditure has been found in some fish species, suggesting that both strategies are expensive in terms of time and/or energy (Candolin and Reynolds, 2002; Henson and Warner, 1997; Marconato et al., 1996; Warner et al., 1995).

We investigated the mating allocation pattern (sperm allocation, courtship, and mate guarding) of territorial males in two goby species: the grass goby (*Zosterisessor ophiocephalus*) and the black goby (*Gobius niger*). In both species, smaller males adopt alternative mating tactics, and the level of sperm competition is high (Immler et al., 2004; Mazzoldi and Rasotto, 2002; Mazzoldi et al., 2000). Large territorial males monopolize females and invest energy primarily in building and defending a nest and in providing egg parental care. By contrast, smaller and younger males adopt a parasitic behavior, sneaking inside the nests of territorial males to fertilize eggs during pair-spawns. In addition to behavior, territorial and sneaker males differ in body size, age, testes investment, and seminal vesicle and ejaculate characteristics (Mazzoldi and Rasotto, 2002; Mazzoldi et al., 2000; Rasotto and Mazzoldi, 2002; Scaggiante et al., 1999). When exposed to different levels of sperm competition intensity, sneaker males respond by tailoring ejaculate expenditure, first increasing and then decreasing their sperm output as the number of competing males increases (Pilastro et al., 2002). Although qualitatively in agreement with the intensity model of sperm competition (Ball and Parker, 1997; Parker et al., 1996), the sperm expenditure of sneakers peaked when three males were competing during the spawning (i.e., the territorial male and two sneakers, Pilastro et al., 2002) and not when two males (i.e., one territorial male and one sneaker) were competing, as predicted by these models (Ball and Parker, 1996; Parker et al., 1996). This result, however, could be reconciled with the theory if territorial males have a "fixed" sperm expenditure strategy that depends on the average sperm competition level (Parker, 1998). If this is the case, sneakers are expected to adjust their ejaculate expenditure at each mating according to the number of competing sneakers and not to the total number of males (Parker et al., 1996).

To test how territorial males respond to the presence of sneakers, we measured the behavior and sperm output of territorial males facing different levels of sperm competition in the grass and black gobies. Our aim was to investigate whether territorial males adjust their sperm output to the number of sneaker males attending the spawn and to test whether they respond to the presence of sneakers by increasing their mate guarding and courtship rate.

METHODS

Study species

The grass goby is a large coastal fish inhabiting sea grass (*Zostera* sp.) meadows in shallow brackish water. In the Venetian Lagoon (North Adriatic Sea, Italy), it achieves sexual maturity at 1 year of age and lives up to 3 years. During the breeding season, lasting from March to June, sexually mature males range in size from 7.4 to 25 cm (Mazzoldi et al., 2000). The largest males in the population (usually >16-cm total length, TL) dig and defend a burrow under the sea grass rhizomes. Females lay their eggs on the nest ceiling, while the nest-owner males release sperm trails by rubbing their urogenital papilla on the nest surface. The ejaculate (sperm trail) is a mucous trail with embedded sperm that slowly dissolve and release active sperm in the water (Marconato et al., 1996; Scaggiante et al., 1999). Egg deposition lasts on average 7–8 h (Scaggiante et al., 1999), and territorial males perform parental care (Gandolfi et al., 1991). Smaller males (usually <12.5-cm TL) mate by employing an alternative mating behavior, that is, approaching spawning pairs and releasing a large ejaculate in the nest or in its vicinity. Males with intermediate size can be either sneakers or territorial, according to social conditions (Mazzoldi et al., 2000). In this field survey, sneakers were found in 20 out of 98 nests and their number ranged between one and five. Preliminary observations based on molecular paternity analyses suggest that in natural nests the number of putative fathers is usually greater than two, suggesting a high level of sperm competition (Gallini A, Scaggiante M, Bernardinelli F, Zane L, unpublished data).

The black goby is a coastal fish inhabiting sandy environments and frequently entering brackish lagoons and estuaries (Joyeux et al., 1991, 1992; Whitehead et al., 1986). In the Venetian Lagoon, it reaches a maximum TL of 16 cm, 4 years of age, and sexual maturity at the first year of life with a polygynous mating system (Mazzoldi and Rasotto, 2002). During the breeding season, from May to August, males longer than 9-cm TL and 3–4 years old build nests under different types of hard substratum, and females lay eggs on the nest surface, while males release sperm trails similar to that of grass goby; egg deposition lasts on average 5–6 h, and males perform parental care (Gandolfi et al., 1991; Marconato et al., 1996; Mazzoldi and Rasotto, 2002). Several sneaker males have been observed to congregate around and inside nests when territorial males were spawning. In particular, in seven out of eight spawnings observed in the field from one to five sneakers were present around the nest, and some of them were observed to sneak inside the nest for a few seconds before being chased away by the resident male (Mazzoldi and Rasotto, 2002).

Fish collection

Fish used for this study were either collected in the Venetian Lagoon, under a fishing scientific permit from the Regione Veneto, or provided by local professional fishers. Animals were collected during the breeding season of 2002 and were sexed based on the sexually dimorphic genital papilla (Gandolfi et al., 1991). TL was measured to the nearest millimeter with a ruler, and male sexual maturity was assessed by
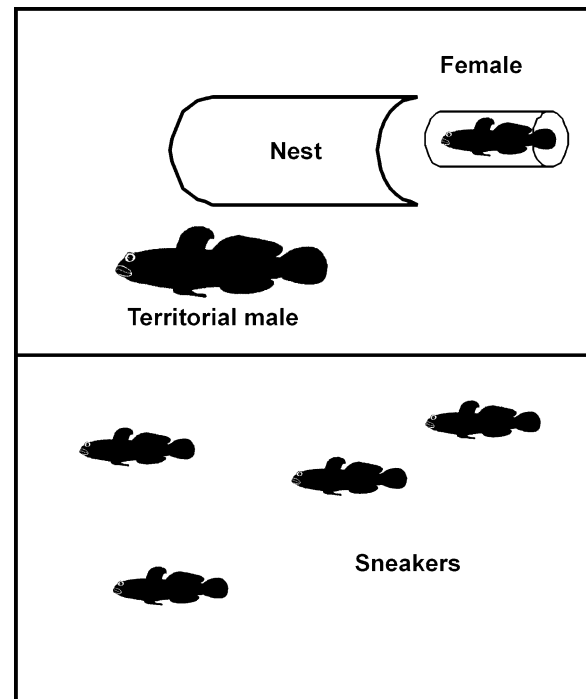
Figure 1
Plan view of the experimental tank (60 × 36 × 40–cm height). The tank was divided in two equal-sized compartments: the compartment of the territorial male contained a plastic nest (20-cm long and with a diameter of 15 cm) and a ripe female confined into a transparent Plexiglas tube. The sneaker compartment contained zero, one, or four competitor sneakers, according to the treatment.

checking for seminal fluid release after a gentle abdominal pressure (Scaggiante et al., 1999). Territorial and sneaker males were separated on the basis of body size, presence or absence of sexual secondary traits, and ejaculate characteristics. In particular, territorial males produce a viscous ejaculate, rich in mucins, that is released in the form of sperm trails laid on the nest surface. In contrast, sneakers release sperm trails that are less viscous, poorer in mucins, and have a higher sperm concentration (Marconato et al., 1996; Rasotto and Mazzoldi, 2002; Scaggiante et al., 1999).

Fish were housed at Chioggia Hydrobiological Station of the University of Padova. At the end of the experiments they were released back into the wild. Housing and experimental procedures have been conducted in accordance with Italian national law (DL116/92).

Experimental trials

Experiments were performed from 24 April to 23 May 2002 for the grass goby and from 4 July to 7 August for the black goby. Aquaria used for the experiments (60 × 36 × 40 cm high, 70 l) were divided in two equal sections by a glass partition, attached to the bottom and sides with silicone, to prevent water and sperm flow across sections (Figure 1). Every aquarium was provided with a sandy bottom, water temperature was kept at 16°C for grass goby and 22°C for black goby, and water was renewed daily. The artificial light regimen followed natural conditions, and fish were fed once a day with fresh black mussel meat.

The experimental design was similar to the one used in a previous experiment (Pilastro et al., 2002). Territorial males (grass goby: $N = 12$, TL = 17.92 ± 0.50 cm, range = 15–21.6 cm; black goby: $N = 21$, TL = 10.37 ± 0.12 cm, range = 9.2–11.2 cm) were allowed to settle for 2 days in one section of the aquarium

containing a polyvinyl chloride (PVC) half pipe as artificial nest. After this acclimation period, territorial males were exposed to the three sneaker density treatments during three consecutive days, always at the same hour of the day, randomizing the order of treatment to minimize the possible effect of sperm expenditure. Grass goby sneakers ($N = 60$, TL = 10.02 ± 0.99 cm, range = 8.5–11.3 cm) and black goby sneakers ($N = 105$, TL = 6.60 ± 0.04 cm, range = 5.5–7 cm) were chosen randomly for each replicate from a large stock of animals, renewed weekly with new catches.

On the day of the experiment, we introduced zero, one, or four sneakers in the other aquarium section and allowed them to acclimate for 5 min. In trials with zero sneakers, we mimed the act of releasing sneakers with a hand net and we ran the observations on territorial male as for trials with sneakers. We recorded for 20 min two types of territorial male behaviors associated with nest defense: (1) the time spent by the territorial outside the nest and (2) the time spent in attacks toward the glass partition. We measured the behavior of 12 individual territorial males in each condition for the grass goby (total = 36 trials) and 21 individual territorial males in each condition for the black goby (total = 63 trials). After each trial, the water in the tank was renewed with seawater and the PVC nest substituted with a new one. Because of the glass partition, territorial males had visual but not olfactory access to sneakers. The lack of olfactory and chemical cues was not a likely restriction for our experiment because it has been demonstrated in the black goby that sneakers are pheromonally silent (Locatello et al., 2002).

At the end of the first observation period, a female ready to spawn (i.e., showing a round belly and a swollen genital papilla) was placed in the compartment of the territorial male. She was confined in a transparent Plexiglas tube that was placed in front of the nest (Pilastro et al., 2002). Grass goby females ($N = 36$, TL = 11.04 ± 0.23 cm, range = 8.0–13.5 cm) and black goby females ($N = 63$, TL = 7.87 ± 0.06 cm, range = 6.7–8.5 cm) were chosen randomly from a large stock renewed daily with new catches. As soon as the territorial male saw the female, he started alternatively courting her outside the nest and rubbing the genital papilla on the ceiling of the nest, a behavior associated to sperm trail release (Marconato et al., 1996). Five minutes after female introduction, we gently moved the female inside the territorial male nest to simulate her readiness to spawn and continued to record the territorial male behavior for a further 15 min (for a total of 20 min of observation). We recorded behaviors associated with nest defense, as for the previous observation period (namely, time out of the nest and number of attacks), and with mating (duration of courtship displays). Male courtship display includes extending the dorsal fin, pursuing, and eventually biting the female (Mazzoldi et al., 2000). As for a previous study (Pilastro et al., 2002), we simulated the behavior of a female ready to spawn by introducing, when the female was moved into the nest, an aliquot of 500 μl of female ovarian fluid into the nest compartment. Ovarian fluid is released by females during egg laying and acts as a spawning cue for males (our unpublished data, Pilastro et al., 2002). Ovarian fluid was collected from the genital papilla of five ripe females that were previously anaesthetized, by exerting gentle pressure on their abdomen. Aliquots of ovarian fluid were prepared from the pool of the ovarian fluids of five females, stored at −20°C, and used within 1 week. After 1 week, a new ovarian fluid stock was prepared from another five females. Stripped females were then released back to the point of capture. Simulated spawnings were used because natural spawnings usually last 4–12 h, and females can enter and exit the nest several times during spawning (Mazzoldi and Rasotto, 2002; Scaggiante et al., 1999). This would have introduced a large variance in the characteristics

of the spawns that may have influenced the behavior of territorial males. In contrast, our approach allowed to control for such differences and to refer any difference in the behavior of the territorial male to the presence and the number of sneakers in the vicinity of his nest.

Thirty minutes after the introduction of the female under the nest, 80 ml water sample was collected via a small plastic tube placed under the nest. One of us (M.S.), blind of the experimental group, determined the sperm concentration in the nest according to the method described in previous experiments (Pilastro et al., 2002; Scaggiante et al., 1999). In both species, spermatozoa remain viable in water up to 2 h, without changing their concentration over time (Mazzoldi and Rasotto, 2002; Scaggiante et al., 1999). The sperm concentration recorded is therefore representative of the total number of sperm released over the 30-min period. In preliminary trials conducted on the black goby with the same experimental design, we found that the concentrations of sperm measured after 30 and 60 min did not differ significantly (sperm concentration: 30 min, 158 sperm ml^{-1} $\pm$ 155 SD; 60 min, 153 sperm ml^{-1} $\pm$ 140, $n = 28$; paired t test, $t_{27} = 0.37$, $p = .72$). For a smaller data set based on natural spawnings in which the females were free to move in the tank and did lay the eggs in the nest of the territorial male, we measured the concentration of the sperm released by the territorial male three times, at 30 min, 1 h, and 1.5 h after the beginning of the spawn. Again, we found no evidence that the concentration of sperm increased or decreased significantly during the spawning (black goby—30 min: 643 sperm ml^{-1} $\pm$ 484 SD, 60 min: 661 sperm ml^{-1} $\pm$ 406 SD, 90 min: 569 sperm ml^{-1} $\pm$ 353 SD; $F_{2,22} = 0.65$, $p = .53$; grass goby, 30 min: 5263 sperm ml^{-1} $\pm$ 8595 SD, 60 min: 7165 sperm ml^{-1} $\pm$ 10,678 SD, 90 min: 6234 sperm ml^{-1} $\pm$ 7978 SD; $F_{2,14} = 0.98$, $p = .40$). TLs of territorial males, females, and sneakers did not differ among treatments group (ANOVA, all $p > .05$ for the two species).

Statistical analyses

We tested all distributions for normality using Shapiro-Wilks test. Some behavioral observations were not normally distributed, and we therefore used log transformation when appropriate. We compared territorial male behavior and sperm expenditure under three conditions, that is, with zero, one, and four sneakers, using a Generalized Linear Model (GLM) model in which territorial male behavior and sperm expenditure were the dependent variables, number of attending sneakers was the fixed factor, and identity of territorial male was the random factor (to control for repeated observations). The test was repeated for each of the two phases of the experiment (before and after female introduction). Probabilities are two tailed. Where not otherwise stated, mean $\pm$ SE is reported. Statistical analyses were performed using SPSS 12.

RESULTS

Territorial behavior

Territorial males spent more time patrolling the territory (time out of the nest) when one and four sneakers were present rather than in the absence of sneakers (Table 1, Figure 2a). Attacks (rapid movements toward the sneaker compartment) were observed only when sneakers were present (Table 1, Figure 3a). Considering only the trials in which sneakers were present, territorial males performed more attacks when there were four sneakers compared to a single sneaker in the black goby ($F_{1,20} = 6.12$, $p = .022$) but not in the grass goby ($F_{1,11} = 0.21$, $p = .66$). After the female was introduced, territorial males decreased the time spent patrolling the

Table 1

Defense behavior of territorial males according to the number of attending sneakers during two 20-min observation periods (sneakers = before female introduction; female = after a female introduction)

	Source	df	mean square	F^a	p
Time out of nest[b]					
With sneakers					
Black goby	Intercept	1,20	180.52	65.24	<.0001
	Number of sneakers[c]	2,40	3.77	3.10	.055
	Male identity[d]	20,40	2.77	2.28	.013
Grass goby	Intercept	1,11	66.25	47.37	<.0001
	Number of sneakers[c]	2,22	11.98	14.58	<.0001
	Male identity[d]	11,22	1.40	1.70	.139
With sneakers and female					
Black goby	Intercept	1,20	52.29	31.40	<.0001
	Number of sneakers[c]	2,40	5.42	6.61	.003
	Male identity[d]	20,40	1.67	2.03	.027
Grass goby	Intercept	1,11	10.20	7.23	.021
	Number of sneakers[c]	2,22	0.92	1.01	.379
	Male identity[d]	11,22	1.41	1.56	.181
Number of attacks[b]					
With sneakers					
Black goby	Intercept	1,20	11.46	53.72	<.0001
	Number of sneakers[c]	2,40	3.75	20.01	<.0001
	Male identity[d]	20,40	0.21	1.14	.352
Grass goby	Intercept	1,11	11.05	55.18	<.0001
	Number of sneakers[c]	2,22	2.76	26.83	<.0001
	Male identity[d]	11,22	0.20	1.94	.089
With sneakers and female					
Black goby	Intercept	1,20	1.99	17.83	<.0001
	Number of sneakers[c]	2,40	0.68	6.81	.003
	Male identity[d]	20,40	0.11	1.11	.376
Grass goby	Intercept	1,11	0.63	4.48	.058
	Number of sneakers[c]	2,22	0.24	3.98	.033
	Male identity[d]	11,22	0.14	2.32	.045

The results of GLM in which territorial male behavior was the dependent variable, the number of attending sneakers the fixed factor, and territorial male identity the random factor are shown. Interactions were nonsignificant and were removed from the model.

[a] ANOVA F test.

[b] After log transformation.

[c] Fixed factor.

[d] Random factor.

territory and attacking the sneakers, and male territorial behavior did not differ according to the number of sneakers (Table 1, Figures 2b and 3b).

Mating behavior and sperm expenditure

Territorial males did not vary their courtship rate according to the number of attending sneakers (Figure 4a). In both goby species, the sperm expenditure (log sperm per milliliter) of the territorial males did not vary significantly with the number of attending sneakers (Tables 2 and 3, Figure 4b). In absolute numbers, grass gobies shed more sperm than black gobies. This difference was probably due to grass goby territorial males being larger than the black goby counterparts (see Methods). An analysis of the effect sizes (partial eta square) showed that 55.1% and 49.8% of the variance in sperm expenditure was explained by male identity and only 2.5% and 1.5% was related to the number of attending sneakers (black and grass goby, respectively). In five trials, black goby territorial males did not release sperm during the simulated spawning. Removing these trials did not change the results (number of sneakers: $F_{2,33} = 0.35$, $p = .71$, all others $p > .15$). The sperm expenditure when no sneakers were attending the

mating was not significantly different from that observed when sneakers (either one or four) were present (GLM—grass goby, number of sneakers: $F_{1,21} = 0.00$, $p = .99$; male identity: $F_{11,21} = 1.87$, $p = .11$; order: $F_{2,21} = 0.01$, $p = .99$; black goby, number of sneakers: $F_{1,39} = 0.01$, $p = .98$; male identity: $F_{20,39} = 2.39$, $p = .01$; order: $F_{1,39} = 0.46$, $p = .64$; all interactions were nonsignificant and were removed from the model). To test if body size influenced the sperm expenditure under the three experimental conditions, we also run a repeated measure ANOVA, in which sperm expenditure measures with zero, one, and four sneakers were the dependent variables and territorial male body length was the covariate. No significant effect of body size and number of sneakers was found (repeated measure analysis of covariance—grass goby, between subjects: $F_{1,20} = 1.15$, $p = .34$, male TL: $F_{2,20} = 1.22$, $p = .32$; black goby, between subjects: $F_{1,38} = 0.35$, $p = .71$, male TL: $F_{2,38} = 0.36$, $p = .70$).

DISCUSSION

The presence of sneakers triggered a violent reaction of the territorial male toward them. Field observations confirm that sneakers usually stay around the nests, and when they try to
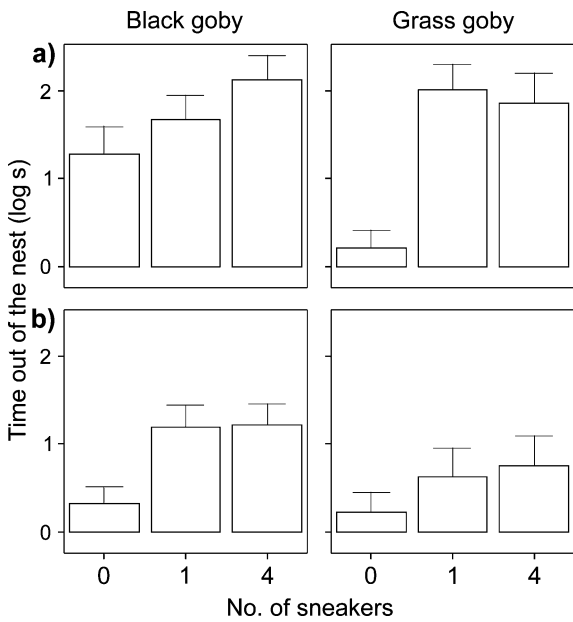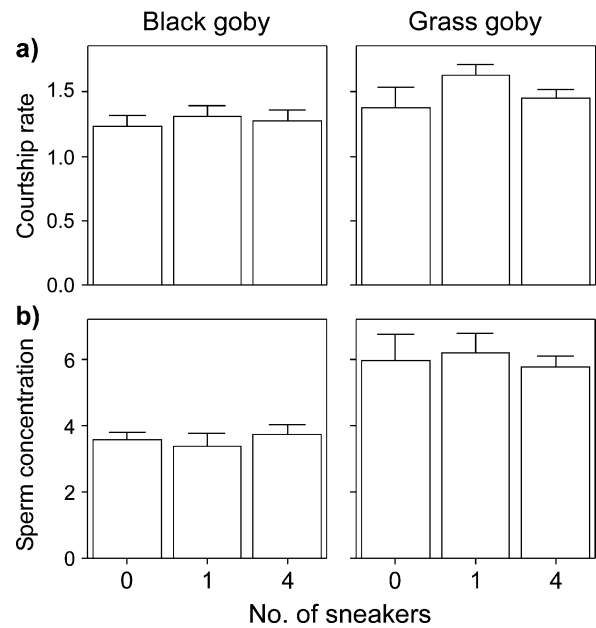
Figure 2
Time spent patrolling the territory (time out of the nest, s) by the
territorial male according to the number of sneakers (zero, one, or
four) and the phase of the experiment. (a) After the introduction of
the sneakers and before the introduction of the female (s out of nest
20 min^{-1}); (b) after the introduction of the female (s out of nest
20 min^{-1}). Data were log transformed.

approach or to enter into the nest, they are immediately
chased away by the territorial male (Mazzoldi and Rasotto,
2002; Mazzoldi et al., 2000). An ongoing study on fertilization
success in natural nests of grass gobies suggests that territorial
male fertilization success can be substantially reduced by
sneakers (Gallini A, Scaggiante M, Bernardinelli E, Zane L,
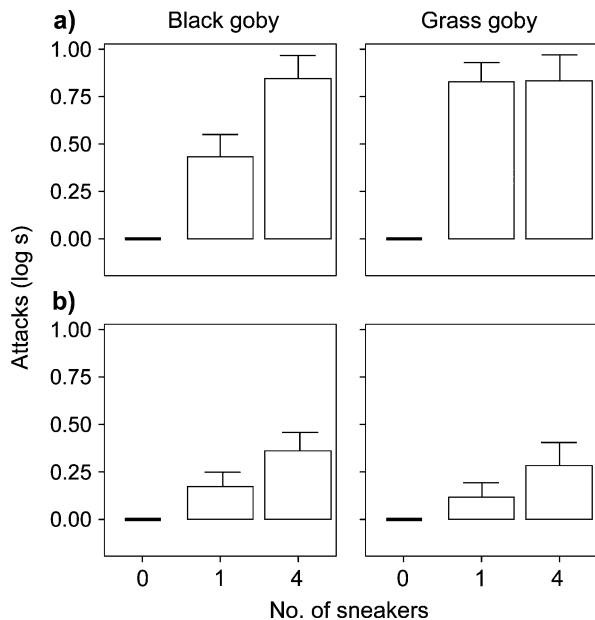
Figure 3
Time spent attacking the sneakers (i.e., hitting the transparent di-
vider, s) by the territorial male according to the number of sneakers
(zero, one, or four) and the phase of the experiment. (a) After
the introduction of the sneakers and before the introduction of
the female (s of attack 20 min^{-1}); (b) after the introduction of the
female (s of attack 20 min^{-1}). Data were log transformed.

Figure 4
Courtship behavior and sperm released of territorial male according
to the number of sneakers (zero, one, or four). (a) Courtship rate of
territorial male (seconds of display 20 min^{-1}); (b) sperm release
(log of sperm concentration, sperm per milliliter) by the territorial
male. Data were log transformed.

unpublished data; see also Jones et al., 2001, for a compari-
son), and the aggressive reaction of territorial males is there-
fore not surprising. Indeed, as soon as sneakers were
introduced in their compartment, territorial males, which
were usually within the nest, immediately exit the nest to pa-
trol their compartment and to perform attacks in the direc-
tion of the transparent partition. On the contrary, when
sneakers were not present, territorial males never displayed
aggressively, and patrolling behavior around the nest was re-
duced. This suggests that territorial males perceived the pres-
ence of the sneakers even if they were only in visual contact. In
the black goby, territorial males spent increasing time in at-
tacks toward sneakers as the number of sneakers attending the
spawning increased from one to four, suggesting that territo-
rial males invest more in mate and nest guarding as the in-
tensity of sperm competition increases. The same pattern was
found also in the grass goby, although in this species the
difference in territorial behavior between the two conditions

Table 2

**Sperm expenditure (sperm per milliliter) of territorial males
according to the number of sneakers attending the simulated
spawning**

Species	Number of sneakers	Mean	Median	SD	n
Black goby	0	52	32	47	21
	1	78	39	111	21
	4	98	40	207	21
	Total	76	37	137	63
Grass goby	0	3712	165	6308	12
	1	2649	351	5322	12
	4	691	245	1101	12
	Total	2351	241	4837	36

Each territorial male was tested in the three conditions (with zero,
one, and four attending sneakers).

Table 3

Courtship behavior and sperm release of territorial males according to the number of attending sneakers

	Source	df	mean square	F[a]	P
Courtship rate[b]					
Black goby	Intercept	1,20	17.00	144.40	<.0001
	Number of sneakers[c]	2,40	0.006	0.13	.878
	Male identity[d]	20,40	0.118	2.58	.005
Grass goby	Intercept	1,11	14.519	168.19	<.0001
	Number of sneakers[c]	2,22	0.069	2.20	.135
	Male identity[d]	11,22	0.086	2.75	.021
Sperm released[b]					
Black goby	Intercept	1,20	805.31	253.19	<.0001
	Number of sneakers[c]	2,40	0.67	0.52	.599
	Male identity[d]	20,40	3.18	2.45	.008
Grass goby	Intercept	1,11	1292.53	210.77	<.0001
	Number of sneakers[c]	2,22	0.52	0.17	.850
	Male identity[d]	11,22	6.13	1.99	.098

The results of GLM in which territorial male behavior was the dependent variable, the number of attending sneakers the fixed factor, and territorial male identity the random factor are shown. Interactions were nonsignificant and were removed from the model.

[a] ANOVA F test.

[b] After log transformation.

[c] Fixed factor.

[d] Random factor.

was not significant. In both species, the aggressive reaction of the territorial males toward the sneakers decreased when the female was introduced in the tank and the male started to court the female and to lay sperm trails. This reduction of nest guarding may be determined by a trade-off between courting the female and releasing the sperm trails on one hand and territory patrolling on the other hand.

Our results also showed that territorial males of both species did not vary their sperm expenditure according to the number of sneakers attending the mating, although there was a large variation in the sperm concentration we measured in the nest. Pooling the observations with one and four sneakers did not change the results, confirming that the sperm expenditure of territorial males was not influenced by the presence of sneaker rivals. Territorial males also courted the female at the same rate, regardless of whether sneakers were present. Altogether, these results suggest that, in these two species, the response of territorial males to different levels of sperm competition mainly consists of mate and nest guarding and does not include an adjustment of the sperm expenditure. A similar strategy has been found in the cricket *Gryllodes sigillatus* (Bateman et al., 2001), a species in which males are constrained in their ejaculate production and respond to the sperm competition risk (associated with female fecundity in this species) by investing more in mate guarding.

It has to be noted, however, that even if it pays for territorial males to allocate more energy to mate guarding than to ejaculate (Alonzo and Warner, 2000b), one may expect them to strategically tune the allocation in both functions in response to varying levels of sperm competition. In the bitterling (*Rhodeus sericeus*), for example, territorial males have been shown to adjust both their mating effort and their sperm output to the level of sperm competition (Candolin and Reynolds, 2002; Reichard et al., 2004; Smith et al., 2002). Why then do territorial gobies of the two species studied here not adjust their sperm output, considering that they are able to do it when, before becoming territorial, they sneak matings (Pilastro et al., 2002)? The observed lack of response in terms of ejaculation rate may be because it is constrained by the

particular fertilization mode adopted by territorial males: in these species the ejaculate is a very viscous fluid, containing high concentration of mucins, in which sperm are embedded (Marconato et al., 1996; Scaggiante et al., 1999). It is therefore possible that the semen of territorial males is designed to ensure the fertilization of all the eggs during the prolonged spawning, which can last several hours, but does not allow a fine adjustment of ejaculation rate according to the level of sperm competition. Alternatively, it is possible that in nature territorial males cannot always detect the presence of sneakers; therefore, a fixed-response strategy in terms of sperm allocation has been selected (i.e., territorial males assume a constant level of sperm competition).

In this study, we only measured the number of sperm released. It is clear, however, that sperm competition can lead to qualitative changes of the ejaculate (Snook, 2005). It would be therefore interesting to verify if territorial males change other traits of their ejaculate (e.g., the proportion of mucins) in the presence of sneakers males. A different composition of the ejaculate as a response to varying level of sperm competition has been found, for example, among butterflies (Karlsson, 1995), but the instantaneous adjustment of the ejaculate quality (other than sperm concentration) to varying levels of sperm competition has never been reported, as far as we know. An alternative, non–mutually exclusive explanation is that nest guarding is energetically less expensive and/or more efficient in reducing cuckoldry than a response based on numerical sperm competition, as suggested by the fact that usually territorial males invest less in gonads than sneakers (Candolin and Reynolds, 2002; Henson and Warner, 1997; Marconato et al., 1996; Warner et al., 1995). On the other hand, the deposition of sperm trails allows the territorial male to temporarily leave the nest, attack, and chase away sneakers without risking that some eggs are not fertilized.

In grass and black gobies, the sperm expenditure of sneaker males peaks when the territorial male and two sneakers are attending the spawning (Pilastro et al., 2002), whereas theory predicts a peak when two males are competing (Ball and Parker, 1996; Parker et al., 1996). This discrepancy may be

reconciled considering that sperm expenditure of territorial males is invariant with respect to the number of sneakers, as suggested by the results of the present study. Whatever the reason for the fixed strategy of sperm allocation in territorial males, our results indicate that alternative mating strategies in these two goby species do not only differ in the total investment allocated to the different reproductive functions by males (e.g., mate-guarding allocation versus gametic allocation, Alonzo and Warner, 2000a) but also in the way males modulate their sperm investment among matings.

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