

Socially induced tactic change in 2 types of sand goby sneaker males

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Abstract

Male alternative reproductive tactics, like satellite or sneaking tactics, typically parasitize reproductively on a larger resource-holding tactic. In the sand goby, *Pomatoschistus minutus*, 2 types of sneaker males are known. Sneaker males with melanization, a typical male breeding coloration, have small testes and large sperm-duct glands, and sneaker males without melanization have large testes and small sperm-duct glands. We tested their potential to change into the nest-holding tactic experimentally by keeping them with or without a large nest-holding male. With nest-holding males, neither sneaker male type built nests. However, without nest-holding males, a large proportion of both types of sneaker males built nests and became nest-holders, and all the nest-building nonmelanized sneaker males developed melanization. Furthermore, nest-building nonmelanized sneaker males had larger sperm-duct glands (used to produce a sperm-containing mucus) than nonnest-building nonmelanized sneaker males. However, contrary to our expectation, treatment did not affect testes size. Compared with melanized sneaker males nonmelanized sneaker males tended to have a lower proportion of nest-building males and showed significantly less reproductive activity, especially in the early experimental period. Finally, in a separate experiment, we confirmed that nonmelanized sneaker males that build nests can spawn and tend eggs normally. Taken together, our results suggest that these tactics are not genetically or ontogenetically fixed but condition dependent. However, this does not exclude an underlying genetic variation in phenotype expression.

Key words: alternative reproductive strategies bourgeois genetic polymorphism Gobiidae seminal vesicles sperm competition

INTRODUCTION

Sexual selection sometimes produces sexually dimorphic traits, often with males having the more conspicuous ones, such as large body size, bright colors, and elaborate ornaments and weapons (Andersson 1994). These traits contribute to enhance male mating success through female mate choice and male–male competition over females. However, in some species, even males with no or small such competitive traits participate in reproduction within the same population by adopting different reproductive styles: that is, alternative reproductive phenotypes (Waltz 1982; Dominey 1984; Gross 1996). Theory predicts the existence of different reproductive phenotypes within a species to be maintained because they maximize the fitness of each individual (Koprowski 1993; Gross 1996; Shuster and Wade 2003). The difference in reproductive phenotype is generally based on differences in reproductive traits, such as body size, color, morphology, and behavior.

Alternative reproductive phenotypes can be divided into different types based on flexibility: several alternative reproductive tactics (ARTs) that are used by an individual during its lifetime (plastic ART) and a fixed phenotype that is used by an individual throughout its life. Although the latter can reflect an alternative reproductive strategy, in which the phenotypes are genetically determined with Mendelian inheritance (e.g., Shuster and Wade 1991; Ryan et al. 1992; Lank et al. 1995), more often it is due to an ART. ARTs are often condition dependent, sometimes expressed as a threshold response to the status of the individual or an environmental cue (Roff 1996; Tomkins and Hazel 2007). In a number of species, conditions early in life determine which tactic is adopted and the tactic becomes fixed due to the ecological or physiological constraints (fixed ART) (Gross 1996). In comparison with species having fixed ARTs, species with plastic ARTs generally have an advantage if the physical or social environment of the animal or its own physical condition is unpredictable (West-Eberhard 2003). A change in tactics of a species with fixed ARTs may be prevented by physiological constraints or other costs of the change or limited by environmental conditions (Tomkins and Hazel 2007). In particular, tactic-specific morphological and physiological sexual traits are generally less plastic than behavioral traits (West-Eberhard 1989). To understand the life-history strategy of a species, it is necessary to examine the potential for change in tactics (e.g., in bluegill sunfish, 2 tactics are plastic and another one is genetically fixed; Gross 1984, 1991).

ARTs are common in fishes, and the most widespread pattern is the one in which large males monopolize reproductive resources and females through male–male competition, whereas small males adopt surreptitious reproductive tactics (Taborsky 2008). In many species (e.g., *Salarias pavo*: Gonçalves et al. 1996; *Bathygobius fuscus*: Taru et al. 2002), small males that adopt parasitic spawning tactics (e.g., sneaking, streaking, and satellite tactics) change their tactics to a resource-holding bourgeois tactic at some stage in their life because the benefits of bourgeois tactics generally increase with body size. However, little is known about size-related reproductive success of males that adopt parasitic and bourgeois tactics. In the common goby *Pomatoschistus microps* and black goby *Gobius niger*, male ARTs have been suggested to follow an ontogenetic gradient because small sneaker males do not try to use nest sites even when they are available and large males do

not try to adopt sneaking tactics (Magnhagen 1992; Rasotto and Mazzoldi 2002). On the other hand, Immler et al. (2004) showed that sneaker black goby males change their tactics to nest-holding tactics when they are placed in a tank in the absence of other males.

Parasitic spawning also produces intense sperm competition in fish (Taborsky 1994; Petersen and Warner 1998). Dominant males prevent parasitic males from intruding into nests during spawning to maximize their fertilization success, making opportunities for fertilization by parasitic males severely limited. Males that adopt parasitic tactics invest much energy in producing large testes and ejaculating larger numbers of sperms compared with large dominant males (Parker 1998; Taborsky 1998) to increase their fertilization success. Moreover, they often mimic the body color of females to enable them to intrude into the nest without being attacked by dominant males (Dominey 1980; Gonçalves et al. 1996). In contrast, in some gobies and blennies, dominant nest-holding males develop larger reproductive accessory organs (see below) than sneaker males that produce a mucus that contains sperm and antimicrobial substances. These tactic-specific investments into primary and secondary reproductive traits are expected to be reversed with change in tactics, but very few studies have ever tried to investigate these changes experimentally (Immler et al. 2004; Scaggiante et al. 2004).

The sand goby *P. minutus* is a small marine fish that is distributed along the coasts of Europe and that breeds in shallow sandy areas during spring and early summer (Miller 1986). In the study area, they usually have only one reproductive season, but both sexes breed several times during this season (Forsgren 1999). Males occupy empty mussel shells and build nests by excavating sand from beneath the shells and by covering them with the sand. Nest-holding males court females by fanning, tail beats, and erect fins (Kvarnemo et al. 1995). Females deposit eggs on the inner surface of the nest. After spawning, only the male tends the eggs until they hatch (1–3 weeks). Each nest usually contains eggs deposited by several females, and one-tenth of the eggs are fertilized by males other than the nest-holder due to parasitic fertilizations (Jones et al. 2001). Parasitic fertilization is performed by small subordinate males, as well as by large nest-holding males (Singer et al. 2006; Svensson and Kvarnemo 2007). There are 2 different types of males in this population; males with or without breeding coloration (Svensson and Kvarnemo 2007; Kvarnemo et al. 2010). Most males show distinct breeding coloration, with a melanized black-colored edge along the anal and tail fins, a blue band inside the black edge of the anal fin, and a blue and black spot on the first dorsal fin. In the field, only colored males breed as nest-holding males. The smallest colored males do not occupy nests and are hence forced to breed as sneaker males (“melanized sneaker males”). However, morphologically there is no difference between colored males of different sizes (Kvarnemo et al. 2010). Males without breeding coloration have extremely large testes compared with colored males, and they breed as sneaker males (“nonmelanized sneaker males”) (Svensson and Kvarnemo 2007; Kvarnemo et al. 2010). Nonmelanized sneaker males are slightly smaller and relatively rare compared with melanized sneaker males (Kvarnemo et al. 2010).

As in some other gobiid fishes (Marconato et al. 1996), sand goby males attach a sperm-containing mucus to the surface of the nest by rubbing their genital papilla against

the substrate (Svensson and Kvarnemo 2005). The eggs that are attached afterward can be fertilized by the sperm that is released from the mucus (Ota et al. 1996). Prespawning sperm-depositing behavior is considered a tactic of nest-holding males to ensure their paternity in the face of sperm competition from other males, and they increase the frequency of mucus attachment behavior in the presence of sneaker males (Svensson and Kvarnemo 2005). However, both types of sneaker males also enter the nests “before” spawning, behaving as if they attach mucus to the surface of the nests (Svensson and Kvarnemo 2007). The amount of mucus produced is related to the size of the sperm-duct glands (to use the vocabulary recommended by Miller 1984: also referred to as seminal vesicles, e.g., Fishelson 1991) in 2 gobies (Scaggiante et al. 1999; Rasotto and Mazzoldi 2002). In addition, the mucus contains antimicrobial substances to protect eggs from infections (Giacomello et al. 2008) and sexual pheromones to attract females (Locatello et al. 2002). These glands are specialized accessory organs near the testes. In the sand goby, males with melanization (i.e., nest-holding and melanized sneaker males) have large sperm-duct glands, whereas males without melanization (i.e., nonmelanized sneaker males) have only rudimentary or small sperm-duct glands (Svensson and Kvarnemo 2007; Kvarnemo et al. 2010).

Our objective in this study was to experimentally test whether both nonmelanized and melanized sneaker sand goby males have the potential to change their tactics to nest-holding tactics. In a preliminary observation, melanized sneaker males built nests when they were kept in a tank without competition from other males (Svensson O, unpublished data). However, whether nonmelanized sneaker males have the potential to become nest-holding males has never been investigated before. Because nonmelanized sneaker males were not caught on nests in the wild (Kvarnemo et al. 2010), there is a possibility that their phenotype represents a genetic polymorphism. A second objective was to examine the differences between the 2 types of sneaker males in the process of changing tactics. For nonmelanized sneaker males in particular, we expected that they would develop melanization, their huge testes would be reduced, and their small sperm-duct glands would increase in size with a change in tactics.

MATERIALS AND METHODS

General experimental procedure

The experiment was conducted between 30 April and 15 June 2009 at the Sven Lovén Centre for Marine Sciences, Kristineberg (lat 58°150' N, long 112°80' E) on the west coast of Sweden. The fish used in this experiment were caught in a nearby bay using a hand trawl. They were sexed, and then the males were sorted into melanized and nonmelanized males. Males that showed extremely faint black color were classified as having no melanization because even such males have large testes and small sperm-duct glands as observed in males with no melanization (Kvarnemo et al. 2010): The melanization is a continuous variable but it shows a distinct bimodal distribution (Kvarnemo et al. 2010). Moreover, the melanized males were divided into large (>50 mm in total length) and small

males as potential nest-holding males and melanized sneaker males, respectively. Because there is no difference in anything else other than mating behavior and relative body size between nest-holding males and melanized sneaker males, in this study, they were divided on the basis of the body size of nest-holding males reported in the previous study (Kvarnemo et al. 2010). These fish groups were kept separately in 130-l storage tanks with a layer of sand at an approximate depth of 3 cm. All tanks including experimental tanks were continuously supplied with running natural seawater of ambient salinity and temperature (9.5–14 °C, the ambient temperature increased as the study progressed). The fish were fed chopped mussels (*Mytilus edulis*), shrimps (*Crangon crangon*), and Alaska pollock (*Theragra chalcogramma*) every day during storage and every fourth day during the experimental period. Experiments were performed indoors, and an ambient photoperiod was maintained during the experiments by supplementing natural light from windows. All tanks were screened off with plastic sheets to prevent fish from seeing each other.

Potential for change in tactics

To examine the potential of the 2 types of sneaker sand goby males to change their tactics to nest-holding, whether the presence of nest-holding males constrains their change in tactics, and whether there is a difference in the tactic-changing process between nonmelanized and melanized sneaker males, we used a 2 × 2 design, thus with 4 treatments in our experiment. Each 20-l experimental tank (38 × 21 × 25 cm) used for observation had a 3-cm layer of sand and one half of a clay flowerpot, 6 cm in diameter, was used as a nest site. In each experimental tank, we placed 1) one nonmelanized sneaker male alone (mean initial TL ± standard deviation [SD] = 42.4 ± 3.3 mm, range = 37.0–49.0 mm, N = 15); 2) one nonmelanized sneaker male (42.9 ± 2.4 mm, 39.5–47.0 mm, N = 15) and one large melanized male (51.3 ± 0.8 mm, 50.0–53.0 mm); 3) one small melanized (sneaker) male alone (45.7 ± 1.6 mm, 43.0–49.0 mm, N = 16); or 4) one small melanized (sneaker) male (45.5 ± 2.3 mm, 40.5–48.0 mm, N = 16) and one large melanized male (53.2 ± 1.4 mm, 51.0–56.0 mm). These fish were taken out from storage tanks, their body size was measured (nearest 0.5 mm), and then randomly transferred into each experimental tank. The initial body sizes of melanized sneaker males were significantly larger than those of nonmelanized sneaker males in both solitary treatment (t-test, $t = -3.48$, $P < 0.01$) and treatment with a large male ($t = -2.99$, $P < 0.01$), however, the initial body sizes of both types of sneaker males did not differ between the treatments used (t-test, $t = -0.44$ and 0.27 , $P = 0.66$ and 0.79 , respectively). In all 4 treatments, a female in a transparent plastic cup was introduced once every fourth day (ca. 7 h/day) to encourage nest building. The cups (0.5 l) were provided with a net over the top and holes in the sides to enable water circulation. The experiments started on 30 April for 50 tanks and on 6 May for the remaining 12 tanks and lasted until 10 (41 days) and 15 (40 days) June, respectively.

Every day during the experimental period, we checked whether males built nests, and for tanks with a large male (i.e., treatments 2 and 4), we determined which male built and occupied the nest. Because fish of this species build nests only as spawning sites,

nest-building behavior is a good indicator of change to the nest-holding tactic. If nests had been built, nest volume and nest-opening size were measured as indices of nest quality because these traits may affect the female mate choice in this species (Svensson and Kvarnemo 2003, 2005). The volume of the sand covering the nest was estimated by comparing it with a photographic scale prepared by pouring sand onto a flowerpot in steps of 50 ml of sand (Svensson and Kvarnemo 2005). We measured the arc length of the nest opening using a scale marked along the rim of the flowerpot (diameter, $d = 65$ mm), and the size of the opening was approximated from the arch area.

At the end of the experiment, melanization of all males was checked, and total length was measured to the nearest 0.5 mm for growth rate analysis. They were then sacrificed using a lethal 2-phenoxyethanol solution (2 ml/l) before storing the fish in a freezer at -20 °C for later analysis by dissection. Some fish that died before the end of the experiments were also stored at -20 °C. We dissected all males under a stereomicroscope (Leica Wild M3Z) to compare the investment in testes and sperm-duct glands between treatments. The gutted bodies, testes, and sperm-duct glands were separated and dried at 60 °C for at least 24 h before being weighed on a microbalance (Sartorius, LE26P) to the nearest 0.001 mg. All measurements of mass were conducted by the T.T.

To examine the change in male behavior and difference between nonmelanized and melanized sneaker males, behaviors of sneaker males were recorded in the presence of a female in a plastic cup in the early (12 and 13 May) and late (1 June) experimental periods using digital video cameras (Sony, HDR-HC7, and DCR-HC62). Behaviors in each tank were recorded for 20 min, and the last 15 min was used for analysis. Male behaviors analyzed were the amount of time the males spent 1) inside and outside the nest; 2) displaying courtship toward the female (outside the nest); 3) fanning (with head visible in the nest opening); 4) nest building and the frequency of 5) mucus preparation (male turns upside down and rubs the anal-urogenital area toward the ceiling inside the nest); and 6) aggressive interaction between sneaker male and large male in treatments 2 and 4. All behavioral analyses were conducted by the T.T.

During the experimental period, some fish died from jumping out of the tanks and from an unknown cause. Because time frames for behavioral and morphological changes are likely to differ and because nest building was established by daily observations, whereas color change, growth rate, and gonadal investment required the fish to be found and in good shape, the sample size differs between analyses. Specifically, we omitted experimental fish that died ($N = 11$) within 2 weeks after the start of the experiments from the analysis of change in body color. In addition, 2 males that died on day 16 and 18 were omitted because they were severely damaged. Fish that died without building nests within 2 weeks after the start of the experiment ($N = 3$) were omitted from the analysis of nest building, but fish that built nests within the initial 2 weeks and then died ($N = 7$) were included the analysis. Fish that died ($N = 20$) within 4 weeks after the start of the experiment were omitted from the analyses of behavior, testes, sperm-duct glands, and of growth rates. This left us with a total sample size of 49 for color change (no initial size difference of nonmelanized and melanized sneaker males between the treatments, t -test, $t = -0.94$ and 0.55 , $P = 0.36$ and

0.59, respectively), 59 for nest building ($t = -0.67$ and 0.42 , $P = 0.51$ and 0.68 , respectively), and 42 concerning behavior, gonadal investment, and growth rate ($t = -0.72$ and 0.28 , $P = 0.48$ and 0.78 , respectively).

Potential for spawning and parental care

To confirm whether nonmelanized sneaker males that built nests can spawn and tend eggs normally, a spawning experiment was performed with an additional set of 11 nonmelanized sneaker males between 15 May and 15 June 2009. We used the same experimental design as for treatment (1), except that a female was placed in a cup in the tank throughout the experiment and released into the tank after nest building to spawn with the nest-building nonmelanized sneaker male. After the release of the female, we checked for spawning, and the female was immediately removed from the tank if spawning had occurred. We then observed whether the males tended the eggs in their nests. To confirm egg fertilization and survival, we removed the nest from the tank and checked the eggs 3 days after spawning.

Statistical analyses

Proportions of color change and nest-building males were compared using a chi-square test. Body sizes were compared between nest-building and nonnest-building sneaker males by t-test. Differences in nest volume and nest-opening area between the treatments used were examined in two-factor analyses of variance (ANOVAs), with sneaker type (melanized/nonmelanized) and presence of large male (yes/no) as factors. Time before the start of nest building was examined using the Kruskal–Wallis test because the data did not show a normal distribution. Relationship between body size of nest-building sneaker males and the period until start of nest building was analyzed using Spearman's rank correlation coefficient. Differences in testes size, sperm-duct gland size, and growth rate between the treatments were examined in two-factor ANOVAs and between nest-building and nonnest-building males were analyzed using analysis of covariance (ANCOVA) with the body mass as covariate and treatment groups as factor. To fulfill the assumptions of the parametric analyses, all data on body, testes, and sperm-duct gland masses were log 10-transformed. The effects of sneaker male types and month on male behaviors in the absence of large males were analyzed using generalized linear model (GLM) because a large proportion of the data are zeros. For Poisson-distributed data (confirmed by a chi-square goodness-of-fit test), we used a log link function and assessed the significance of the explanatory terms with the chi-square Wald statistic. Differences in the frequency of being attacked by large males between sneaker male types were tested using the Mann–Whitney U test and differences between months were tested using the Wilcoxon matched pairs test to account for repeated observations of the same males. All statistical analyses were performed with Statview (version 5.0, SAS Institute Inc.) and SPSS (version 16.0, SPSS Inc.).

RESULTS

Nonmelanized sneaker males under the solitary treatment developed melanization at a significantly higher proportion than those under the treatment with a large male (χ^2 -test, $\chi^2 = 9.1$, degree of freedom [df] = 1, $P < 0.01$; Table 1). In contrast, most melanized males did not change, with the exception of one male in each treatment that lost color. Thus, the breeding color of most melanized sneaker males was not lost whether they were placed with a large male or not (Table 1). In the presence of a large male, all nests were built by the large male and not by cohoused sneaker male, whereas 6 of 14 nonmelanized sneaker males and 11 of 14 melanized sneaker males built nests in the absence of a large male (Table 2). The proportion of nest-building solitary melanized sneaker males was about 36% higher than that of nonmelanized sneaker males (χ^2 -test, $\chi^2 = 3.7$, df = 1, $P = 0.053$). All nonmelanized sneaker males that built a nest had developed melanization. These nest-building nonmelanized sneaker males (mean initial TL \pm SD = 44.1 \pm 3.2 mm, range = 41.0–49.0 mm, N = 6) tended to be slightly larger than the nonnest-building nonmelanized sneaker males (40.8 \pm 2.7 mm, 37.0–45.0 mm, N = 8; t-test, $t = 2.11$, df = 12, $P = 0.057$), whereas no size difference was observed between nest-building (45.9 \pm 1.8 mm, 43.0–49.0 mm, N = 11) and nonnest-building melanized sneaker males (45.3 \pm 1.2 mm, 44.0–46.0 mm, N = 3; $t = 0.52$, df = 12, $P = 0.61$). The median time before initiation of nest building was more than 3 times longer in nonmelanized than in melanized sneaker males (Table 2), but, due to large variation, the period did not differ statistically between the 2 types of sneaker males or the nest-holding males that built the nests in treatments 2 and 4 (Kruskal–Wallis test, $H = 0.96$, df = 3, $P = 0.79$; Table 2). For sneaker males that built nests, larger individuals tended to start nest building faster than smaller ones among melanized males, but not among nonmelanized males (melanized male: Spearman's rank correlation coefficient, $r_s = -0.62$, N = 11, $P = 0.052$; nonmelanized male: $r = -0.49$, N = 6, $P = 0.28$). There were no significant differences in nest volume (one-factor ANOVA, $F_{3,43} = 1.57$, $P = 0.21$; Table 2) or nest-opening area ($F_{3,43} = 0.82$, $P = 0.49$; Table 2) between the 4 treatments. Nonmelanized sneaker males had larger testes than melanized sneaker males, but there was no effect of presence of nest-holding males or interaction between sneaker type and presence of nest-holding male on testes size (two-factor ANOVA, sneaker type: $F_{1,39} = 20.2$, $P < 0.001$; presence of nest-holding male: $F_{1,39} = 1.04$, $P = 0.31$; interaction: $F_{1,39} < 0.01$, $P = 0.99$; Table 3). In contrast, sperm-duct glands were larger in melanized than nonmelanized sneaker males (two-factor ANOVA, sneaker type: $F_{1,39} = 5.74$, $P < 0.05$; Table 3) and smaller when they were housed with nest-holding males (presence of nest-holding male: $F_{1,39} = 9.22$, $P < 0.01$; Table 3). There was no interaction between sneaker type and presence of nest-holding male on sperm-duct glands size (interaction: $F_{1,39} = 0.02$, $P = 0.90$; Table 3). Across the 4 treatments, no significant differences in testes size were observed between nest-building (N = 6) and nonnest-building nonmelanized sneaker males (N = 13: 4 nonnest-building males kept alone and 9 nonnest-building males kept with a large male) (ANCOVA, $F_{1,17} = 0.004$, $P = 0.95$; Figure 1) or between nest-building (N = 11) and nonnest-building melanized sneaker males (N = 9: 2 nonnest-building males kept alone and 7 nonnest-building males kept with a large male) (F

$F_{1,18} = 1.68$, $P = 0.21$; Figure 1), whereas both nest-building nonmelanized and melanized sneaker males had larger sperm-duct glands compared with nonnest-building nonmelanized (ANCOVA, $F_{1,17} = 11.3$, $P < 0.01$; Figure 2) and melanized sneaker males ($F_{1,18} = 13.2$, $P < 0.01$; Figure 2), respectively. Daily growth rates of sneaker males did not differ among the 4 treatments (two-factor ANOVA, sneaker type: $F_{1,39} = 0.82$, $P = 0.37$; presence of nest-holding male: $F_{1,39} = 1.77$, $P = 0.19$; interaction: $F_{1,39} = 1.31$, $P = 0.26$; Table 3). In ANCOVA with body size as a covariate, there was no effect of nest-building on growth rate of sneaker males irrespective of sneaker type (nest-building: $F_{1,34} = 0.18$, $P = 0.68$; sneaker type: $F_{1,34} = 0.05$, $P = 0.82$; interaction: $F_{1,34} = 0.03$, $P = 0.87$; Figure 3).

In the presence of large males in the tank, most sneaker males did not enter the nests, and all their reproductive activities were severely limited (Table 4). They were sometimes attacked by the large males on approaching the nests. The number of attacks by large males differed between male types neither in May nor in June (Mann–Whitney U test: May, $Z = -1.1$, $P = 0.28$; June, $Z = -1.4$, $P = 0.16$) nor between months (Wilcoxon matched pairs test: nonmelanized sneaker males, $Z = -1.2$, $P = 0.23$; melanized sneaker males, $Z = -1.4$, $P = 0.17$). In comparison with sneaker males that were kept with nest-holding males, both types of sneaker males without large males spent more time inside the nests and in displaying courtship and fanning behavior, though the time was relatively short in nonmelanized sneaker males in May (Table 4). Mucus preparation and nest-building behaviors by sneaker males were observed only under the treatments without large males, except one nonmelanized sneaker male with a nest-holding male (Table 4). In the absence of large males in the tanks, the melanized sneaker males showed higher reproductive activities compared with the nonmelanized sneaker males, especially in May (GLM: stay inside nest: sneaker type, Wald $\chi^2 = 376.0$, $df = 1$, $P < 0.0001$; month, Wald $\chi^2 = 80.6$, $df = 1$, $P < 0.0001$; courtship display: sneaker type, Wald $\chi^2 = 572.2$, $df = 1$, $P < 0.0001$; month, Wald $\chi^2 = 153.6$, $df = 1$, $P < 0.0001$; fanning: sneaker type, Wald $\chi^2 = 839.4$, $df = 1$, $P < 0.0001$; month, Wald $\chi^2 = 4.98$, $df = 1$, $P < 0.05$; mucus: sneaker type, Wald $\chi^2 = 5.93$, $df = 1$, $P < 0.05$; month, Wald $\chi^2 = 7.16$, $df = 1$, $P < 0.01$; nest-building: sneaker type, Wald $\chi^2 = 49.5$, $df = 1$, $P < 0.0001$; month, Wald $\chi^2 = 43.4$, $df = 1$, $P < 0.0001$; Table 4).

In the spawning experiment, 9 of 11 nonmelanized sneaker males changed body color and 8 of them built nests (mean period until the initiation of nest building \pm SD = 4.6 ± 1.3 days, range = 1–15 days, $N = 8$), spawned, and then tended eggs. Three days after spawning, all eggs had developed normally and were alive.

DISCUSSION

The present study demonstrates that in the sand goby *P. minutus* not only melanized sneaker males but also nonmelanized sneaker males have the potential to change their tactics from sneaking to nest-holding when nests and females are available without competition from other males. The tactic-changed nonmelanized sneaker males showed

melanization typical of melanized sneaker and nest-holding males and their sperm-duct glands, which are essential for reproduction as nest-holding males, increased in size. They also built nests that were equivalent in quality to the nests built by large nest-holding males, exhibited courtship, and they spawned and tended eggs normally if allowed to spawn with a female. These results suggest that these reproductive tactics are not genetically or ontogenetically fixed, but condition dependent.

In some fishes that adopt ARTs, male mating tactics may follow an ontogenetic gradient, that is, tactics change at a particular age or size (Magnhagen 1992; Rasotto and Mazzoldi 2002). In case of *P. microps*, small sneaker males do not try to hold nests even when they are available, and large males do not try to sneak fertilization even when they have the chance (Magnhagen 1992). At the Swedish west coast, nonmelanized sneaker sand goby males tended to be less common in the late breeding season, and it was therefore suggested that they might change their tactics to nest-holding males later in the season as age or size-dependent tactics (Kvarnemo et al. 2010). Although larger nonmelanized sneaker males tended to change their tactic to nest-holder relatively more often in this study, extremely small nonmelanized sneaker males (38 and 41 mm TL) also became nest-holders when given a chance. We have never observed such small nest-holders in the field (Kvarnemo et al. 2010, personal observations). These results suggest that the tactic of nonmelanized sneaker males is not primarily size dependent but instead depend on the social context (also see below). Social context giving access to nests and females may be the important factors inducing the change in tactics in nonmelanized sneaker males as well as melanized sneaker males.

Although both nonmelanized and melanized sneaker males had the potential to change to nest-holder males, nonmelanized sneaker males appeared to change their tactics less readily than melanized sneaker males. This is based primarily on the fact that a lower proportion of nonmelanized sneaker males tended to build a nest but also that these males spent significantly less time fanning, courting, and being inside the nest especially in the early part of the experimental period. These differences might not be attributable to a physiological constraint on nonmelanized sneaker males because nonmelanized sneaker males used in the spawning experiment built nests at a similar proportion and within a similar time period as the melanized sneaker males did in the solitary treatment. In the spawning experiment, however, a female was present in the tank throughout the experiment to stimulate male mating behavior, whereas a female was introduced only at 4-day intervals under the solitary condition. Therefore, uncertainty about female availability might have caused the delay in change in tactics among nonmelanized sneaker males. Operational sex ratio and population density may affect change in tactics in relation to mate and resource availability (e.g., Forchhammer and Boomsma 1998; Zamudio and Chan 2008). The lack of change in tactics in the small common goby males that were mentioned above might be partly caused by the short experimental period (48 h; Magnhagen 1992). Then why were the nonmelanized sneaker males conservative in their decision to change tactic compared with the melanized sneaker males? Flexibility of change in phenotypes may be affected by the costs of change from one form to the other (DeWitt et al. 1998). For nonmelanized

sneaker sand goby males, the costs involved in developing melanization and large sperm-duct glands seem to be associated with the delayed change in tactics. In addition, since smaller sneaker males spent more time until start of nest building and nonmelanized sneaker males were slightly smaller than melanized sneaker males, we suggest the following 2 possible reasons. The first is a low mating success of smaller nest-holding males because female sand gobies prefer larger males as their mates (Kvarnemo and Forsgren 2000) and have a low resource-holding potential (Lindström and Pampoulie 2005). The second reason is relatively higher maintenance costs of nests and eggs incurred by smaller nest-holding males. These costs may result in low parental success of small males as known in many fishes (e.g., Downhower and Brown 1980; Kuwamura et al. 1993). Such size-dependent benefits and costs may make it unbeneficial for very small males to change tactic, which thus may explain why the nonmelanized sneaker males in our study tended to be less likely to build nests.

Although we expected a reduction in testes size of nonmelanized sneaker males with a change in tactics from sneaking to nest-holding, contrary to our expectation, their testes size did not differ between males that changed tactic and males that did not (Figure 1). This may be partly because the tactic-changed nonmelanized sneaker males had no chance to use their sperms during the experiment. In similar experiments, but allowing focal males to spawn with a female, males of the black goby, *G. niger*, showed a reduction in testes size with a change in tactics from sneaking to nest-holding (Immler et al. 2004), whereas significant testis size reduction was not detected in the male grass goby, *Zosterisessor ophiocephalus* (Scaggiante et al. 2004). On the other hand, nest-building nonmelanized and melanized sneaker males had larger sperm-duct glands than nonnest-building nonmelanized and melanized sneaker males, respectively (Figure 2). Because sperm-duct glands play an important role in producing mucus, these nest-building nonmelanized and melanized sneaker males should be able to attach sperm-containing mucus to the inner surface of the nests, and more so than the males that did not build a nest. An increased mucus attachment may enable nest-holding males to devote more time to nest guarding against intruders, because they do not have to rely exclusively on continuously releasing sperm during the time consuming spawning act, while the female attaches each egg one-by-one to the nest ceiling. Increased mucus attachment may also reduce the risk of losing paternity even after an intrusion by sneaker males (Marconato et al. 1996; Kvarnemo et al. 2010), as suggested by the increased frequency of mucus attachment behavior in nest-holding males in the presence of sneaker males (Svensson and Kvarnemo 2005). Moreover, the mucus may contribute to other aspects of reproductive behavior of nest-holding males. In *Z. ophiocephalus*, the mucus contains antimicrobial substances that protect eggs from infections (Giacomello et al. 2008), and the mucus of *G. niger* nest-holding males contains sexual pheromone that attracts females (Locatello et al. 2002). In addition to the increased investment into sperm-duct glands and maintained investment into testes, nest-building sneaker males must have allocated energy into nest building and courtship behaviors. These facts predict lower growth rate of nest-building males compared with nonnest-building males, however, neither nonmelanized and melanized sneaker males showed any difference in growth rate between nest-builders and nonnest-builders. Thus, the increased male

investment into sperm-duct glands production that was detected in this study appears not to have affected investment in growth or sperm production.

This study suggests that the reproductive phenotypes of sand goby males are condition dependent and not genetically or ontogenetically fixed, while not excluding the possibility of existence of genetically fixed nest-holding males. Before the breeding season, immature males probably get divided into nonmelanized and melanized males depending on their situation, such as social status and resource availability. Although there is a considerable overlap in size range, nonmelanized sneaker males are smaller than melanized sneaker males in the breeding season (Kvarnemo et al. 2010). This suggests that the decision rule is influenced by size, that is, at an early stage of life, absolutely or relatively small males in a population may become nonmelanized. The choice of reproductive tactics may depend on the size difference at a particular life stage, which may be determined by the time available to grow (birth date effect: Alonzo et al. 2000; Oliveira et al. 2001) or nutritional condition (Emlen 1994; Moczek and Nijhout 2002). Because nest site and female availability generally are size dependent in male sand gobies, small males, which have a low probability of changing to nest-holding tactic within the breeding season, may not invest their energy in growth to become nest-holders but may invest it in testes to breed as sneaker males (i.e., as nonmelanized sneaker males). On the other hand, melanized sneaker males may have been expected to invest more energy in growth compared with nonmelanized sneaker males to change their tactics into nest-holding. However, there was no difference in growth rate between 2 types of sneaker males. Furthermore, with the exception of one individual, most melanized sneaker males that were kept with a large nest-holder did not lose breeding color and change into nonmelanized sneaker males, and we do not know if the melanized males are able to develop the huge testes and small sperm-duct glands that are typical of the nonmelanized sneaker males. Hence, although both melanized and nonmelanized males are able to behaviorally act as both parasitic spawners and nest-holders (present study; Singer et al. 2006; Svensson and Kvarnemo 2007), there may still be genetic variation underlying phenotype expression.

To conclude, this study demonstrates that the melanized as well as nonmelanized sneaker males have a potential to change their tactics to nest-holding males. Which alternative tactic the males use depends on the social context. However, while melanized males are able to switch between tactics immediately by only changing behavior when the context changes, nonmelanized males also produce color pigment and mucus when changing from sneaker to nest-holding male tactic. Hence, we think that melanized sneaker males adopt a sneaking tactic simply because they are not socially dominant males. In contrast, nonmelanized males are likely to sneak as a consequence of lacking the breeding coloration needed to attract females and because they lack ability to produce a sufficiently large quantity of mucus. Probably, even if they make a decision to change tactic, they are not able to reproduce as nest-holding males until they have completed the body color change and development of sperm-duct glands. We hypothesize that before the breeding season, both body size and social context contribute to the decision to be a melanized or nonmelanized male. In practice, nonmelanized sneaker males may have little or no chance of changing

tactics during a single breeding season because they are relatively few in number and smaller in body size compared with melanized sneaker and nest-holding males and therefore likely to be at a disadvantage socially. Moreover, if nonmelanized sneaker males do change tactics, their reproductive success is probably not as great as that of large nest-holding males because of low mating and parental success. Because some individuals of this species may live for more than a year (Healey 1971), it is also conceivable that nonmelanized sneaker males continue to grow after their first breeding season and reproduce as nest-holders in their second year.

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Table 1. Change in body color shown by 2 types of sneaker (nonmelanized and melanized) males with and without large (nest-holding) males. If body color of sneaker males changed, nonmelanized sneaker males showed melanization and melanized sneaker males lost melanization. ^aMales that died within 2 weeks after the start of the experiment were omitted from the analysis (details in the text).

Treatment conditions	N ^a	Change in the body colour of sneaker males	
		Change	No change
Non-melanized sneaker male	12	10	2
Non-melanized sneaker male + large male	13	3	10
Melanized sneaker male	14	1	13
Melanized sneaker male + large male	10	1	9

Table 2. Nest building by the 2 types of sneaker (nonmelanized and melanized) males with and without large (nest-holding) males. ^aMales that died without building nests within 2 weeks after the start of the experiment were omitted from the analysis (details in the text).

Treatment conditions	N ^a	Nest building		Days until building		Nest volume (max. ml)		Nest-opening area (avg. cm ²)		
		Built	Non-built	Median	Range	Mean (SD)	Range	Mean (SD)	Range	
Non-melanized sneaker male	14	6	8	9.5	1-26	442 (49)	400-500	0.63 (0.34)	0.15-1.04	
Non-melanized sneaker male + large male	15	Sneaker male	0	15	-	-	-	-	-	-
		Large male	14	1	2.0	1-21	464 (66)	250-500	0.69 (0.62)	0.06-2.13
Melanized sneaker male	14	11	3	3.0	1-21	477 (34)	400-500	0.38 (0.28)	0.15-1.04	
Melanized sneaker male + large male	16	Sneaker male	0	16	-	-	-	-	-	-
		Large male	15	1	2.0	1-26	487 (23)	450-500	0.54 (0.52)	0.10-2.13

Table 3. Testes mass, sperm-duct gland mass, and growth rate of the 2 types of sneaker (nonmelanized and melanized) males with and without large (nest-holding) males. ^aMales that died within 4 weeks after the start of the experiment were omitted from the analysis (details in the text).

Treatment	N ^a	Testes mass (mg)		Sperm-duct gland mass (mg)		Growth rate (mm/day)	
		Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Non-melanized sneaker male	10	2.19 (0.94)	0.70-3.68	0.86 (0.75)	0.20-2.77	0.087 (0.072)	0.00-0.250
Non-melanized sneaker male + large male	11	1.94 (1.19)	0.39-4.20	0.34 (0.24)	0.09-0.76	0.043 (0.059)	- 0.08-0.122
Melanized sneaker male	13	1.06 (0.38)	0.60-1.87	1.31 (0.59)	0.57-2.44	0.050 (0.052)	- 0.04-0.175
Melanized sneaker male + large male	8	0.80 (0.29)	0.61-1.22	0.74 (0.58)	0.14-1.69	0.047 (0.042)	0.00-0.113

Table 4. Behaviors of the 2 types of sneaker (nonmelanized and melanized) males with and without large (nest-holding) males in early (May) and late (June) experimental periods. Numbers in parenthesis indicate the number of males performing the behavior. *Some males died before the observation (details in the text).

Treatment conditions	Month	N ^a	Stay inside nest (sec)		Courtship display (sec)		Displacement fanning (sec)		Mucus preparation (freq)		Nest building (sec)		Attacked by large male (freq)	
			Mean ± SD	(n)	Mean ± SD	(n)	Mean ± SD	(n)	Mean ± SD	(n)	Mean ± SD	(n)	Mean ± SD	(n)
Non-melanized sneaker male	May	10	196.9 ± 243.4	(5)	66.5 ± 148.4	(2)	5.3 ± 16.8	(1)	0.1 ± 0.3	(1)	0.0 ± 0.0	(0)	-	-
	June		326.7 ± 336.1	(3)	145.9 ± 211.4	(5)	72.2 ± 136.3	(3)	0.1 ± 0.3	(1)	0.2 ± 0.6	(1)	-	-
Non-melanized sneaker male + large male	May	11	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	2.3 ± 2.7	(6)
	June		36.8 ± 122.1	(1)	22.6 ± 75.1	(1)	27.8 ± 92.3	(1)	0.3 ± 0.9	(1)	0.0 ± 0.0	(0)	0.8 ± 1.7	(3)
Melanized sneaker male	May	12	360.8 ± 339.5	(8)	188.3 ± 232.9	(7)	146.8 ± 167.4	(7)	0.1 ± 0.3	(1)	9.5 ± 21.8	(5)	-	-
	June		340.5 ± 247.7	(9)	208.2 ± 218.0	(8)	102.4 ± 127.5	(6)	1.2 ± 1.9	(6)	19.9 ± 45.6	(5)	-	-
Melanized sneaker male + large male	May	9	5.0 ± 15.0	(1)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.7 ± 0.7	(5)
	June		2.0 ± 6.0	(1)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	0.0 ± 0.0	(0)	2.7 ± 3.3	(5)

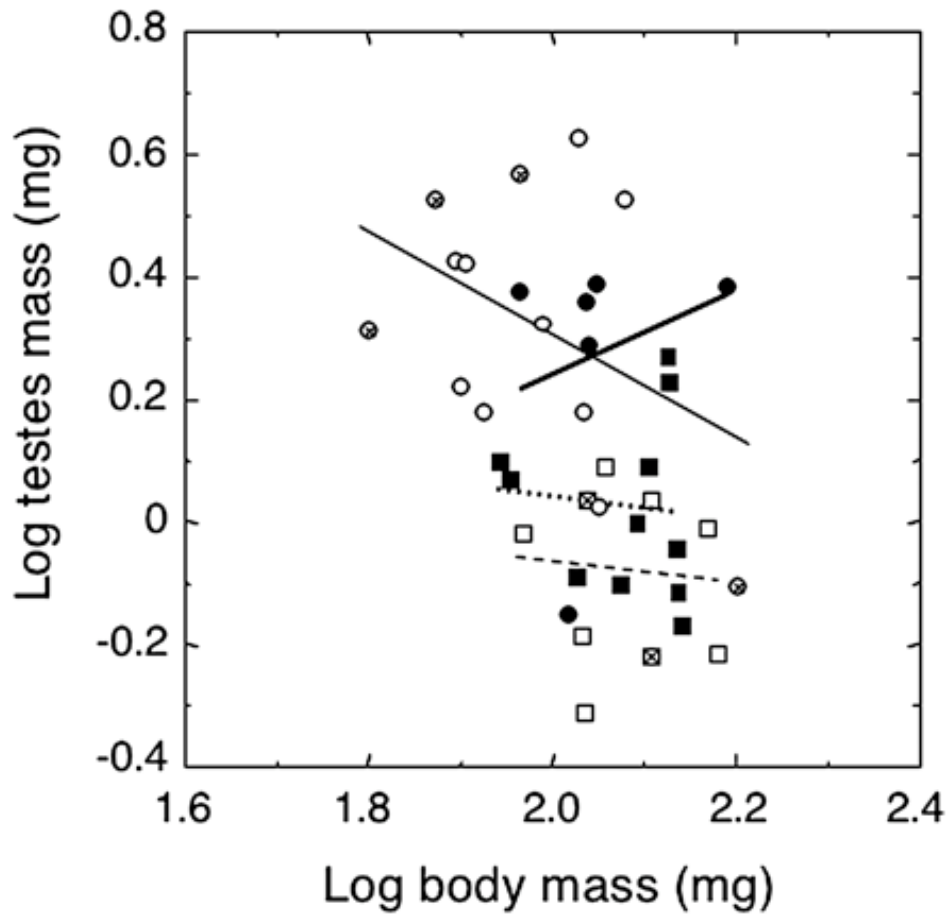


Figure 1

Relationship between body size (log dry mass, mg) and testes size (log dry mass, mg) in melanized (square symbols and dotted regression lines) and nonmelanized (circle symbols and solid regression lines) sneaker males of the sand goby, *Pomatoschistus minutus*. Closed and open symbols indicate nest-building and nonnest-building males, respectively, that were housed alone. Square and circle symbols with cross indicate sneaker males that were kept with a large male (none of which built nests). Thick and thin lines indicate nest-building and nonnest-building males, respectively. Males that died within 4 weeks after the start of the experiment were omitted from the analysis (details in the text).

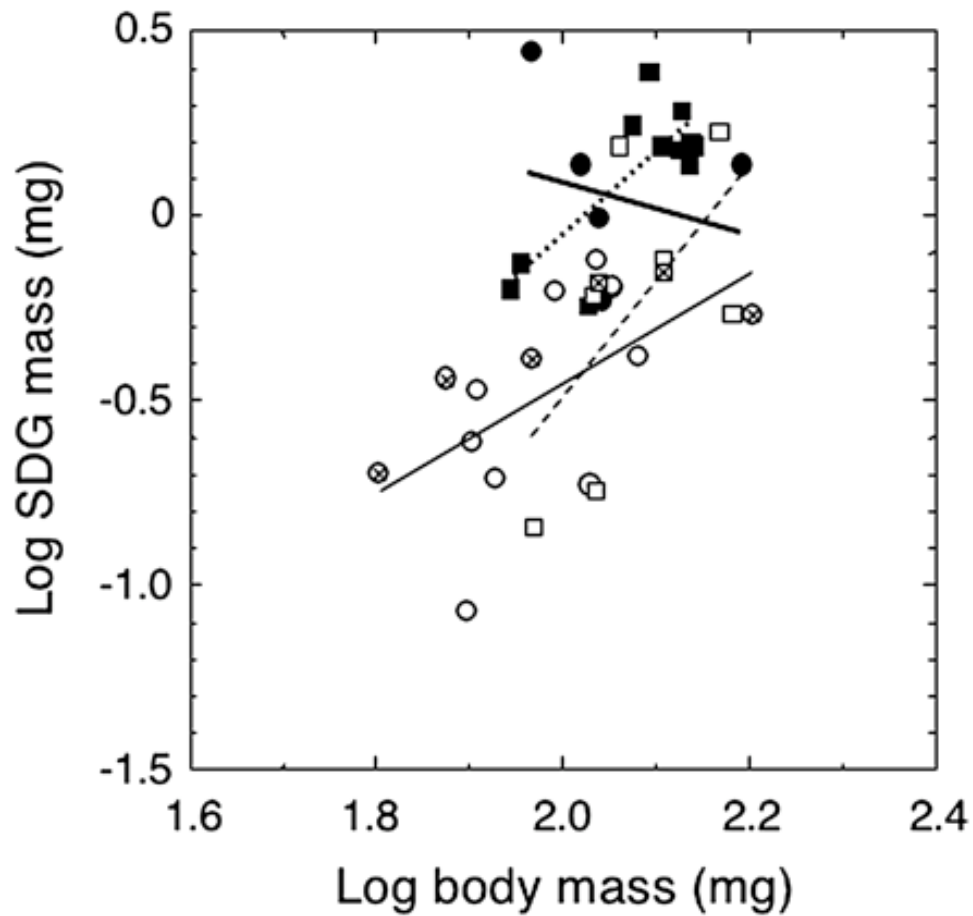


Figure 2

Relationship between body size (log dry mass, mg) and sperm-duct gland size (log dry mass, mg) in melanized (square symbols and dotted regression lines) and nonmelanized (circle symbols and solid regression lines) sneaker males of the sand goby, *Pomatoschistus minutus*. Closed and open symbols indicate nest-building and nonnest-building males, respectively, that were housed alone. Square and circle symbols with cross indicate sneaker males that were kept with a large male (none of which built nests). Thick and thin lines indicate nest-building and nonnest-building males, respectively. Males that died within 4 weeks after the start of the experiment were omitted from the analysis (details in the text).

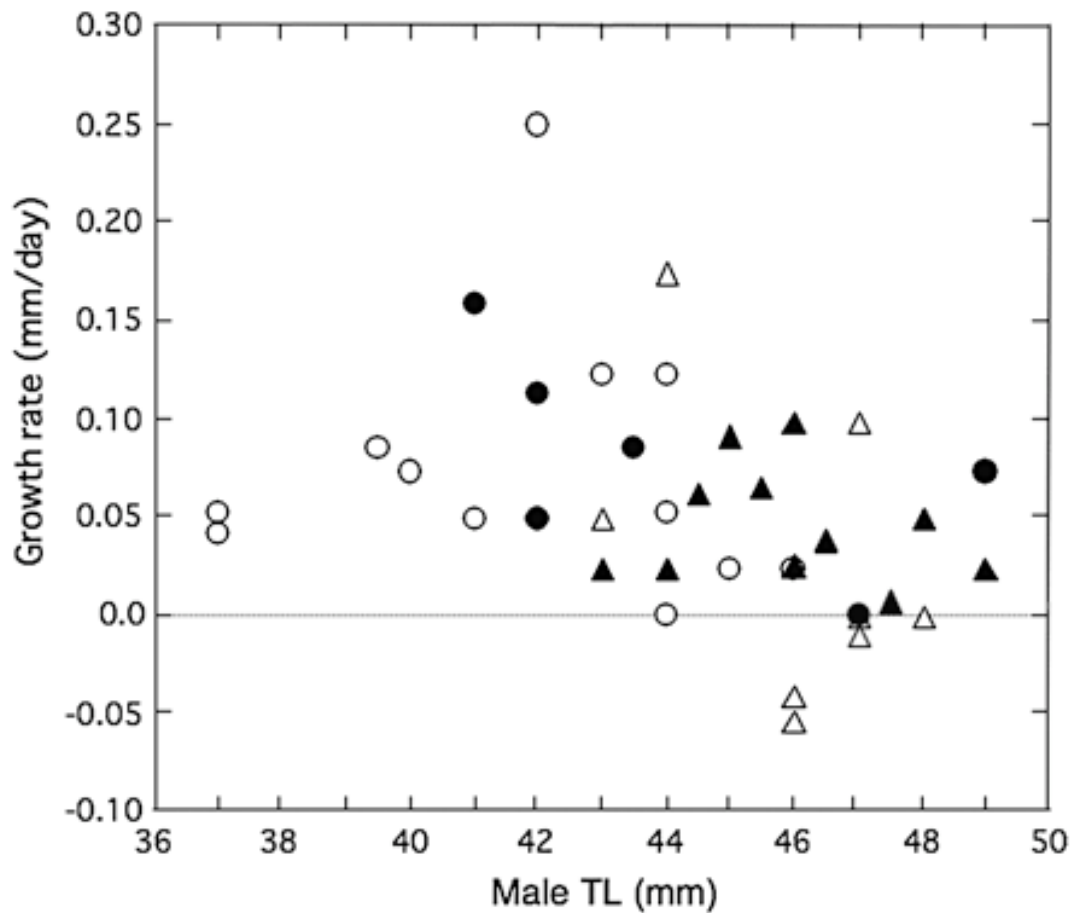


Figure 3

Relationship between body size (total length, mm) and growth rate (mm per day) in sneaker males of the sand goby, *Pomatoschistus minutus*. Closed and open circles indicate nest-building and nonnest-building nonmelanized sneaker males, respectively, and closed and open triangles indicate nest-building and nonnest-building melanized sneaker males, respectively. Males that died within 4 weeks after the start of the experiment were omitted from the analysis (details in the text).