

**Large- and small-size advantages in sneaking behaviour in the dusky
frillgoby *Bathygobius fuscus***

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Abstract

Sneaking tactic, a male alternative reproductive tactic involving sperm competition, is generally adopted by small individuals because of its inconspicuousness. However, large size has an advantage when competition occurs between sneakers for fertilization of eggs. Here, we suggest that both large- and small-size advantages of sneaker males are present within the same species. Large sneaker males of the dusky frillgoby *Bathygobius fuscus* showed a high success rate in intruding into spawning nests because of their advantage in competition among sneaker males in keeping a suitable position to sneak, whereas small sneakers had few chances to sneak. However, small sneaker males were able to stay in the nests longer than large sneaker males when they succeeded in sneak intrusion. This suggests the possibility of an increase in their paternity. The findings of these size-specific behavioural advantages may be important in considering the evolution of size-related reproductive traits.

Keywords Alternative reproductive tactics Size advantage Sperm competition

Introduction

Sneak mating as an alternative reproductive tactic occurs in various animal taxa with internal and external fertilization; especially, in the latter case, such as fishes and amphibians, sneak fertilization of eggs is the most widespread style of male alternative reproductive tactics (Oliveira et al. 2008). Larger, dominant males try to prevent sneaker males from intruding in spawning to maximize their own fertilization success. In such a disadvantageous condition for sneaker males in terms of fertilization opportunity, to enhance the fertilization success as much as possible, sneaker males usually invest much energy in producing testes, which are relatively larger than size compared to those of larger-bodied dominant males (Taborsky 1998). In addition, sneaking behaviour is generally favoured by inconspicuousness, such as small body size and female-mimicking traits (Gross 1985; Pilastro et al. 1997; Stoltz and Neff 2006). In some salmonid fishes, however, male–male competition for spawning occurs not only between large dominant migratory males and small mature male parr, but also between small mature parr and relatively larger parr that have a size advantage in competition for sneaking opportunities (Myers and Hutchings 1987; Thomaz et al. 1997; Koseki and Maekawa 2000). These studies suggest the possibility that body size of sneaker males is affected potentially by conflicting selection pressures, i.e. small-size advantage in competition with dominant males and large-size advantage in competition among sneaker males (Koseki and Maekawa 2000). Such selection pressures have potential of producing size-related reproductive and life history traits of sneaker males, for example, disruptive testis investment between large and small sneaker males or fixed sneaking tactic of small males. However, evidence for within-species large- and small-size advantages in sneaking behaviour is lacking.

Here, we report the large- and small-size advantages of sneaker males of the dusky frillgoby *Bathygobius fuscus*, a small marine fish with a life-span of approximately 3–4 years (Dotu 1955). Relatively large males occupy small rock holes as spawning nests. Spawning occurs in pairs, and eggs are tended only by the nest-holding (NH) males until the eggs hatch (4–5 days). On the other hand, relatively small males adopt sneaking tactics without occupying nests; they

intrude into a spawning nest where spawning is occurring and quickly fertilize the eggs (Taru et al. 2002). Testis size of sexually mature small males is absolutely equivalent to or larger than those of large males, including NH males (Yamasaki 2002). Taru et al. (2002) reported that some but not all small males in the population change their tactics to an NH tactic within a single breeding season and suggested that males of this species adopt a conditional strategy. However, they also reported that not only small males but also relatively large males spawn as sneaker males (Taru et al. 2002); this species is therefore a good model for investigating the effects of body size on sneaking behaviour.

Materials and methods

This study was conducted in rocky intertidal pools on the Mieasaki coast, Nagasaki, Japan, throughout the 2009 breeding season (mid June to late August) of *Bathygobius fuscus*. Male sneaking behaviour was observed for 5–7 days during every neap and spring tide. Almost all adult males found in the study pools were captured for individual identification ($n = 158$). They were anaesthetized, the standard length (SL) was measured (± 0.1 mm), and sexes were determined by the shape of the genital papillae (Dotu 1955). The males were marked by hypodermically injecting visible implant elastomer tag into the body sides (Northwest Marine Technology, Inc., Washington, USA), which are widely used in experimental studies and have proven harmless (e.g. Weston and Johnson 2008), and then returned to the respective collection sites.

Reproductive behaviour in some of the nests where spawning occurred was automatically recorded using several underwater video cameras at the same time (Sanyo, DMX-WH1). Each video camera was mounted on a flexible tripod stand (Joby, Gorillapod) with a 1-kg weight and fixed on the sea floor about 80 cm apart from a focal spawning nest. The recording covered about 30 cm around the nest and basically lasted from male courtship behaviour until the end of spawning; however, some long spawnings (>3 h) were not recorded until the end because of camera memory shortage. A total of 42 courtship and spawning bouts were recorded, and sneaking was observed 54 times in 20 of them. Each NH male had only one spawning female in his nest at a time.

Analyses of agonistic interaction and social status of sneaker males were performed for only 11 of 20 spawnings with sneak intrusions because, in the remaining nine cases, it was hard to identify some sneaker males individually because of the turbidity of the water. However, identifiable sneaker males were included in the analysis of the duration of sneak intrusion for all 20 spawnings (45 of 54 sneak intrusions were available). In three cases of the sneakings repeated twice by the same male in a single spawning, mean duration was used for the analysis. As a result, the analysis of the duration of sneak intrusion was performed for 42 sneaker males. Body sizes of unmarked males observed in the videos were estimated on the screen displays using a vinyl tape of known length fixed visibly near each nest.

In addition to several non-nesting marked and unmarked males, females other than paired females were observed rarely around the nests where spawning was occurring. The unpaired females frequently approached paired nest-holding males and displayed their abdomen, whereas sneaker males showed dark body colour around the spawning nests, enabling us to discriminate females from sneaker males. Agonistic interactions were observed not only between sneaker males and NH males but also between sneaker males; we therefore examined the relationships among within-tactic social status of sneaker males, body size and sneak intrusion success. For analysis, we used the video data of the first sneak intrusion (5-min duration before and after) in each spawning event because the composition of sneaker males around the focal spawning nests usually varied during spawning. On the basis of the video recordings, we measured (1) the frequency and outcome of agonistic interactions between sneaker males, (2) the frequency and outcome of agonistic interactions between sneaker males and NH males, (3) the frequency of sneaking attempts (not all attempts succeeded in intrusion), (4) the frequency of sneak intrusions into the nests, and (5) the duration of each sneak intrusion. Among the non-nesting males observed around the nests, males that attempted to sneak were regarded as sneaker males in this study. The social status of sneaker males within each spawning group was determined by assembling a win–loss matrix based on the outcome of agonistic interactions among them.

Sneaking nest intrusions of longer duration may increase the chance of fertilizing eggs, as demonstrated in the sand goby sneaker males (Svensson and Kvarnemo 2007). In some gobies including sand goby, males attach a sperm-containing mucus to the surface of the nest by rubbing the urogenital papilla before and during female egg deposition, and the eggs attached afterwards can be fertilized by the sperm released from the mucus (Ota et al. 1996). Therefore, sneaker males are able to increase their paternity by attaching a sperm-containing mucus in the nests without synchronizing to the timing of female egg deposition (e.g. Yeates et al. 2007). The mucus is produced in sperm-duct glands (also referred to as seminal vesicles), which are reproductive accessory organs near the testes (Mazzoldi et al. 2011). Since dominant bourgeois males generally have larger sperm-duct glands than sneaker males, pre-spawning sperm-depositing behaviour is considered as a fertilization tactic of bourgeois males to defend their paternity against other males (Svensson and Kvarnemo 2005). Since *B. fuscus* males have sperm-duct glands (Takegaki et al., unpublished data), they are supposed to have similar sperm release and fertilization mechanisms, though such behaviours have not been observed. In this study, we regarded the duration of sneak intrusion as an indicator of sneaking success and examined the effect of male body size on the duration of sneak intrusion.

To examine the relationship between male body size and testis size in this study site, non-nesting *B. fuscus* males observed around the nests and egg-tending NH males in the nests were collected far from the observation area. Since this species spawns synchronously with semilunar periods (Taru et al. 2002), the non-nesting males were collected 5 to 7 days before spring tide to avoid the influence of sperm release on testis size. In this study, the non-nesting males were regarded as sneaker males because NH males usually don't leave their own nests just before spawning. Then, all males (62 non-nesting males and 9 egg-tending NH males) were sacrificed using MS 222, and SL, body weight and testes weight were measured.

The frequency of agonistic interactions between sneaker males was compared with that between sneaker males and NH males in each spawning event with the paired *t* test. The effect of body size of sneaker males on the

frequency of being chased by NH males was tested with Pearson's correlation coefficient. Relationships between social rank and body size of sneaker males, frequency of sneaking trials and frequency of sneak intrusions were tested by ANOVA. Because the number of sneaker males differed among spawning events (6–14 males), data on sneaker males of social rank below 6 were pooled for analysis. The effect of male body size on time retained in nests when they succeeded in intruding into the nests was analysed using generalized linear model (GLM). For gamma-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. The difference in testis size between large sneaker males (≥ 39 mm SL, see "Results") and NH males was analysed using ANCOVA, with the body mass as covariate and male status as factor. However, the testis size between large and small sneaker males (< 39 mm SL) was compared with the paired *t* test because a significant interaction between body mass and male status was detected. To fulfil the assumptions of the parametric analyses, all data on body and testis masses were log 10-transformed. All statistical analyses were conducted using Statview (version 5.0, SAS Institute Inc.) and SPSS (version 16.0, SPSS Inc.).

Results

Sneaking behaviour was observed 54 times in 20 spawning events (mean number of sneak intrusions \pm SD = 2.7 ± 1.6 , range = 1–6). The number of sneaker males observed around the nests where spawning was occurring was 6–14 (mean \pm SD = 10.5 ± 2.6 ; mean SL \pm SD = 39.2 ± 6.4 mm, range = 22.5–58.5 mm, $n = 11$ spawning events). The frequency of agonistic interactions between sneaker males (mean \pm SD = 0.036 ± 0.019 /min/male, range = 0.013–0.071/min/male) was significantly higher than that of chasing of sneaker males by NH males (0.015 ± 0.009 /min/male, 0.005–0.032/min/male; paired *t* test, $t = 4.1$, $df = 10$, $p < 0.003$, $n = 11$). As the result of competition among sneaker males, a size-dependent rank order was detected (ANOVA, $F_{5,106} = 8.5$, $p < 0.0001$; Fig. 1a). Higher ranked sneaker males tried to sneak more frequently than lower ranked ones ($F_{5,106} = 4.6$, $p = 0.0008$; Fig. 1b) and

showed a higher success rate of sneaker intrusion into the nests ($F_{5,106} = 7.0$, $p < 0.0001$; Fig. 1c), although larger sneaker males tended to be chased by NH males more frequently than smaller ones (Pearson's correlation coefficient, $r = 0.16$, $p = 0.095$, $n = 112$ males). On the other hand, among the sneaker males that succeeded in intruding into the nests, smaller males stayed longer in the nests than larger males ($n = 42$, GLM: Wald $\chi^2 = 10.7$, $df = 1$, $p < 0.01$, Fig. 2).

Discussion

This study is the first to suggest advantages of both large and small sizes in sneaking behaviour within a single species. Large *B. fuscus* sneaker males with small testes had an advantage in competition among sneaker males for opportunities to sneak into nests, whereas small sneaker males with large testes stayed in the nests longer than large sneaker males when they succeeded in sneak intrusion. The findings of these size-specific behavioural advantages may be important when considering the evolution of testis size and associated energy allocation strategy in this species.

Sneaking usually occurred when NH males were chasing other sneaker males or just after they had moved from the nest entrance into the nest. To avoid missing these brief moments of opportunity to intrude into the nests, it may be necessary for sneaker males to obtain a position as close as possible to the nest entrance. The similar importance of waiting position to intrude into spawning was suggested in the bluegill sunfish sneaker males (Stoltz and Neff 2006). The high intrusion success of large sneaker males may depend on their greater ability to obtain suitable positions at nest sites compared with competing sneaker males. Nest intrusion by small sneaker males was prevented by competition not only with NH males but also with larger sneaker males. Sneaker males competed more frequently with other sneaker males than with NH males. Their low intrusion success seems to be a severe disadvantage with regard to the reproductive success of sneaker males because fertilization success cannot be expected without intruding into nests. As theory predicts (Birkhead and Møller 1992), sneaker males in this species had larger testes than NH males, suggesting the presence of sperm competition between sneaker and NH males.

Moreover, small sneaker males had the largest testes relative to their body weight probably because a relatively large amount of ejaculate per sneaking and/or relatively high frequency of participation in spawning throughout the breeding season compensates for their small number of chances of sneak intrusion. Thus, it seems possible that the large investment into testes of small sneaker males is affected not only by sperm competition with NH males for fertilization success but also by competition with large sneaker males for sneak intrusion. Similar testis size dimorphism among sneaker males was reported in the sand goby (Takegaki et al. 2012).

Most *B. fuscus* sneaker males that had succeeded in intruding into a nest were finally chased out of the nest by NH males. Among the successfully intruding sneaker males, smaller males stayed in the nests longer than larger males. The longer stay of the sneaker males in the nests may be expected to increase the possibility of releasing sperm and the amount of sperm released, and consequently increase their fertilizing eggs. This has been reported previously for the sand goby (Svensson and Kvarnemo 2007); the specific fertilization mechanism using sperm-containing mucus reported in the sand goby is considered to occur in *B. fuscus*. In fishes, a smaller body size is generally considered to be favourable in parasitic spawning tactics because small fish are less conspicuous and more mobile and therefore have less risk of being detected by dominant males. For example, in coho salmon (Gross 1985) and bluegill sunfish (Stoltz and Neff 2006), smaller parasitic males obtain a position closer to females than larger parasitic males during spawning. Smaller *B. fuscus* sneaker males may be harder to detect in the nests by NH males. Although small sneaker males have few chances to intrude into the nests, if they succeed in intrusion, they may have higher fertilization success compared to larger sneaker males because they can stay longer in the nests and have larger testis.

The existence of both small- and large-size advantages in sneaking behaviour suggests that sneaker males face conflicting selection pressures, i.e. whether they should become large by investing in growth or remain small. For example, in a shell-brooding cichlid fish *Lamprologus callipterus*, dwarf males remain small in body size to perform sneak fertilization hiding in a small shell; however, a comparative study across population revealed that selection

pressure favouring large bodies may work in accordance with inner space of the shell (Ota et al. 2010). These conflicting pressures may produce variation in their reproductive and life history traits. The large size of the testes observed only in small sneaker *B. fuscus* males may be one such example, which is generally considered to evolve in company with the sneaking tactic. On the other hand, the relatively small testes of large sneaker males suggest that they invest more energy into growth instead of testis development to become NH males at an early date, or physical competition with other sneaker males and NH males. A further skewed distribution of traits between two types of sneaker males may cause disruptive selection within a sneaking tactic, that is, possibility of fixed sneaking tactic of small males. However, both large and small sneaker males have at least the potential to change their tactic to the NH tactic when nests and females are available in the tanks (Takegaki et al., unpublished data), though it is not clear whether or not small sneaker males have such conditions in the wild. In the present study, the size-related fertilization success of sneaker and NH males was not demonstrated; however, the results of this study did highlight the importance of variation in traits within a tactic for understanding the evolution of alternative reproductive tactics.

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Fig. 1

Relationships between social rank of sneaker males and (a) body size (SL), (b) frequency of sneaking trials and (c) frequency of sneak intrusions. The letters 'a' and 'b' above the *error bars* indicate a significant difference from first- and second-rank males, respectively. *Error bars* indicate standard error

Fig. 2

Relationship between time (s) retained in nest and body size of sneaker males (SL, mm) that succeeded in sneak intrusion into the nest ($n = 42$)

The body size of NH males ranged 39.0–69.2 mm (standard length, SL) and overlapped highly with that of sneaker males observed in this study (Fig. 3). The relatively small sneaker males (<39 mm SL, $n = 30$) had large testes equivalent to large sneaker males (≥ 39 mm SL, $n = 32$) (t test, $t = 1.2$, $p > 0.05$), and large sneaker males had larger testes compared to the NH males ($n = 9$; ANCOVA, $F_{1,38} = 4.6$, $p < 0.05$; Fig. 3).

Fig. 3

Relationship between somatic mass (g) and testis mass (g) in males collected at the study site from June to August in 2009. *Closed* and *open circles* indicate small ($n = 30$) and large ($n = 32$) sneaker males, respectively. *Crosses* indicate NH males ($n = 9$) with eggs in the nests. *Solid thick and thin lines*, and *dotted line* indicate the slopes of regressions fit for small and large sneaker males, and NH males, respectively

Fig. 1

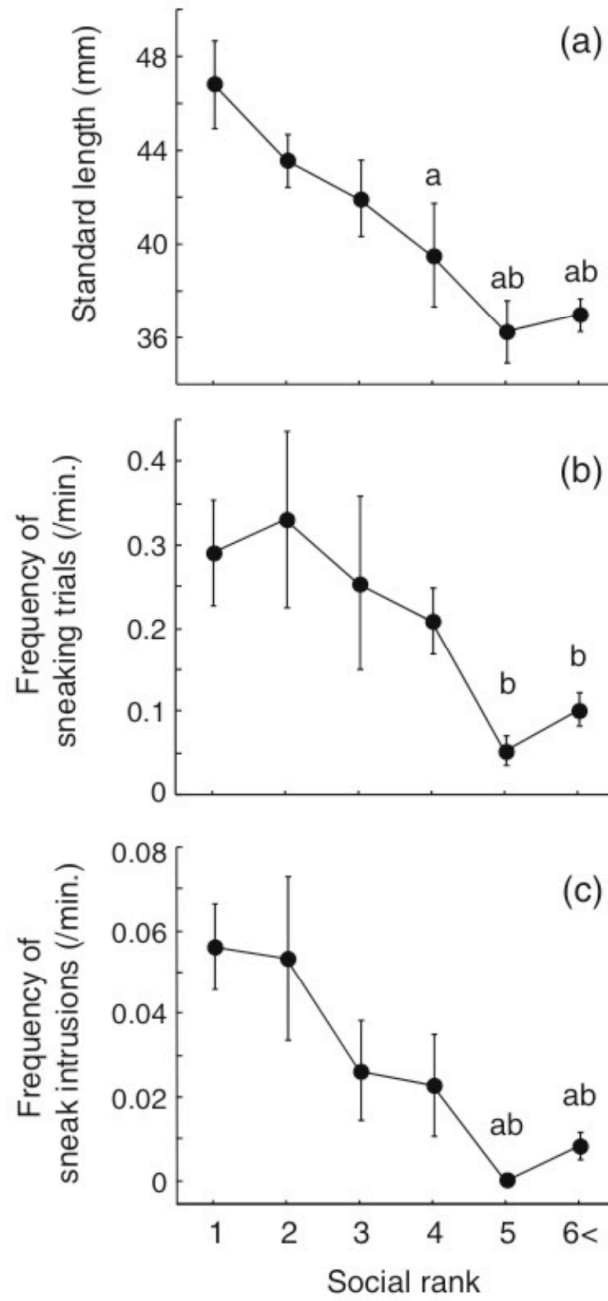


Fig. 2

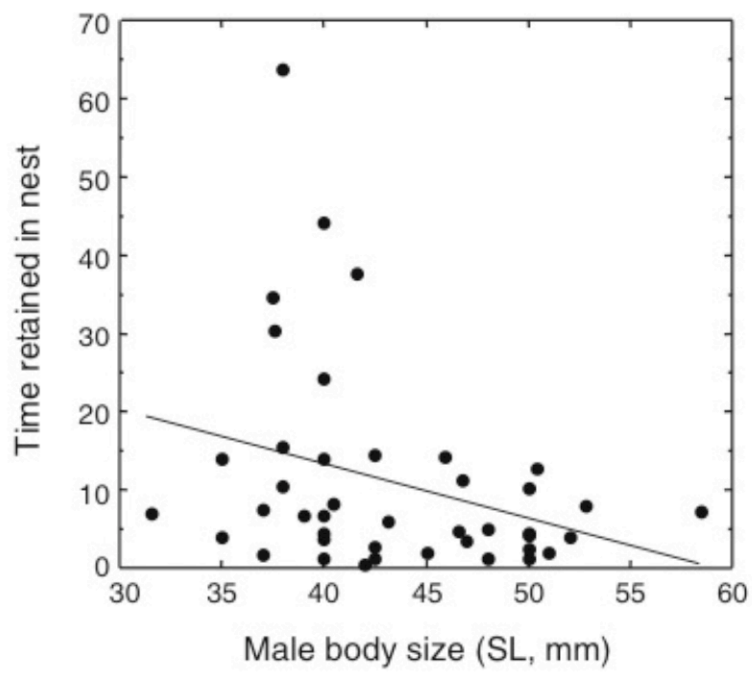


Fig. 3

