Behavioral Ecology
doi:10.1093/beheco/arp058

Female reproductive tactics in a sex-role reversed pipefish: scanning for male quality and number

K. Silva,[a,b] V.C. Almada,[c] M.N. Vieira,[a,b] and N.M. Monteiro[b,d]
[a]Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade do Porto, Praça Gomes Teixeira 4099-002, Portugal, [b]Laboratório de Ecologia, Centro Interdisciplinar de Investigação Marinha e Ambiental, Rua dos Bragas 177, 4050-123 Porto, Portugal, [c]Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal, and [d]Centro de Estudos Biomédicos, Faculdade de Ciências da Saúde da Universidade Fernando Pessoa, Rua Carlos da Maia 296, 4200-150 Porto, Portugal

Theoretical studies predict that females should invest in current reproduction according to both the expected payoffs from mating with different-quality males and their future mating prospects. The Syngnathidae family, with its male pregnancy together with the occurrence of varying degrees of sex-role reversal, constitutes an exceptional model to study female allocation strategies. The present work tests for the influence of male availability and quality (translated into body size) on the egg allocation pattern of different-sized females of the sex role–reversed pipefish *Syngnathus abaster.* Besides revealing a multiple mating strategy and showing that females do not produce enough eggs to fully occupy a male's brood pouch during the extent of a pregnancy, results reveal a complex pattern with different-sized females adopting different investment tactics. In contrast to small, less attractive females, who show a much more constant reproductive effort through the tested mating contexts, large females seem able to monitor the number and quality of available males responding accordingly by 1) laying more eggs in the presence of several large males or saving efforts for future breeding and 2) laying larger eggs in larger males while depositing smaller ones in lower quality individuals as a consequence of a serial mating process (large males first, small males later). *Key words:* egg allocation, female mate choice, mate quality, pipefish, serial mating. *[Behav Ecol]*

Reproductive effort is a central topic in life-history theory (Roff 1992). In iteroparous species, a high investment in current reproduction might reduce the capacity to invest in future reproductive events (Williams 1966; Trivers 1972). According to the differential allocation hypothesis, individuals facing trade-offs between investment in current and future reproduction should strategically modulate their reproductive effort depending on the attractiveness of their mate and the likelihood of finding a better one in the future (Burley 1986, 1988; Sheldon 2000). As proxies for differential allocation, many studies have examined variation in clutch characteristics (e.g., egg number and size) or parental behavior, revealing that females across a vast range of taxa invested more resources when paired with attractive rather than with nonattractive males (e.g., insects, Simmons 1987; amphibians, Reyer et al. 1999; birds, Gorman et al. 2005; or mammals, Drickamer et al. 2000). Surprisingly, given the diversity and amount of work conducted on parental care in fishes, female differential allocation has not been reported until very recently. Females of the Banggai cardinalfish (*Pterapogon kauderni*), for instance, were found to produce heavier eggs when paired with larger, preferred males (Kolm 2001). Also, females of the Mediterranean blenniid (*Aidablennius sphinx)* were observed spawning more eggs per time unit when paired with large, preferred males (Locatello and Neat 2005). A similar pattern has been described by Skinner and Watt (2007) for the zebra fish (*Danio rerio*).

The family Syngnathidae, comprising pipefishes, seahorses, and seadragons, is a particularly interesting group to study reproductive investment and differential allocation patterns in fish. In this family, females lay eggs in a specialized incubating structure of the males that undergo a more or less prolonged "pregnancy" providing protection, aeration, osmoregulation (Carcupino et al. 2002), and nutrients to the developing brood (Haresign and Schumway 1981).

Berglund et al. (1986) argued that, in *Syngnathus typhle*, larger males carried more eggs and provided more energy per offspring than smaller individuals. If males do vary in their investment in offspring, females would benefit from differentially allocating their eggs toward large or small mating partners. Nevertheless, due to several factors, such as male choosiness, brood pouch limitations or female–female competition, females might not always be able to lay their eggs in the preferred partner. In this scenario, only preferred, dominant females would gain from an increased opportunity to access high quality males. Thus, it seems plausible that differential egg allocation according to the expected pay-offs from mating with different-quality males together with the likelihood of finding a better partner (or any other partner at all) might have evolved, in females, as an adaptive strategy.

Despite the growing interest in syngnathid reproductive biology, particularly on sex-role reversed species usually seen as mirror-like models specially useful to highlight factors affecting the intensity of sexual selection, there is still no experimental data supporting the hypothesis of female differential egg allocation. To this extent, the present work focuses on female

---

Address correspondence to N.M. Monteiro. E-mail: nmonteir@fc.up.pt.

Received 6 May 2008; revised 17 December 2008; accepted 1 March 2009.

reproductive strategies in the sex role–reversed pipefish, *Syngnathus abaster*. Although no molecular study has been conducted on this species, previously reported observations refer to a polygynandrous mating system, with females mating with different males and males receiving eggs from distinct females (Tomasini et al. 1991; Silva et al. 2006b).

Five major questions were addressed in this study: 1) Do females indeed scatter the eggs over multiple partners when given the opportunity or, instead, do they concentrate their eggs into 1 preferred male? 2) Do females differentially allocate their reproductive effort (here quantified as the total number of spawned eggs and its mean size) according to mate quality? 3) Does mate availability (1 vs. several potential partners) influence female egg allocation? 4) Do females spawn more eggs than a male can care for over the extent of a pregnancy? 5) Do different-quality females exhibit distinct responses to the questions raised above?

METHODS

Syngnathus abaster is a euryhaline species that inhabits the Mediterranean, Black Sea, and the Atlantic coast of Southwest Europe up to southern Biscay (Dawson 1986). This black-striped pipefish occurs either in coastal areas or in brackish and fresh waters (Cakic et al. 2002) and can be found mainly among sand, mud, or eelgrass meadows, at depths between 0.5 and 5 m, within a temperature range of 8–24 °C. Males have a brood pouch located ventrally on the tail (subfamily Urophori), which consists of 2 skin folds that contact medially along their free edges. Females are larger and more competitive than males under even sex-ratio conditions (Silva et al. 2006b).

Fish were collected with a hand net, in a salt pond storage tank at the Ria de Aveiro estuarine lagoon (40°45′N, 8°40′W), in Portugal, and transported to the laboratory where they were maintained in a closed system of 250-l aquaria illuminated by natural light supplemented with 18-W fluorescent lamps. Tank substrata consisted mainly of sand and plastic seagrass laid in order to mimic the original habitat where the fish were caught. The continuously running natural seawater was physically and biologically filtered and temperature was maintained at 18–19 °C. Aeration was performed before entering the experimental aquaria in order to prevent the "gas bubble disease," common in pipefishes (Monteiro et al. 2002). Fishes were fed daily with fresh *Artemia franciscana* nauplii.

The experiments were designed in order to assess the females' number of reproductive partners and the characteristics of the transferred clutches within the time span of a male pregnancy (30 days, according to Silva et al. 2006a). Apart from the estimation of the time required until the first mating event, all the other variables were measured on 30 days. Large or small females were individually placed in 40-l aquaria together with one large ($1♂_{\mathrm{L}}$) or 1 small male ($1♂_{\mathrm{S}}$) or 4 identically large ($4♂_{\mathrm{L}}$) or small males ($4♂_{\mathrm{S}}$). In order to assess female responses to a heterogeneous group of potential mating partners, females (large or small) were also placed in aquaria containing a group of 4 males, comprising 2 large and 2 small individuals ($2 + 2♂$). In this group, $2 + 2♂$, females were able to assess males of different sizes. Because it could be expected that some variables, such as the size of the egg clutch, could vary according to male size, these measurements could not be registered for large and small males within the same treatment replicate. Thus, 2 independent trials of the $2 + 2♂$ treatment were conducted and results recorded solely for large ($2 + 2♂_{\mathrm{L}}$) or small males ($2 + 2♂_{\mathrm{S}}$). Nevertheless, variables such as the number of pregnant males within the $2 + 2♂$ treatment were registered regardless of male size (data were pooled from the $2 + 2♂_{\mathrm{L}}$ and $2 + 2♂_{\mathrm{S}}$). Sexually mature males and females, initially kept in separate tanks, were randomly assigned to each treatment according to their size.

Five replicates were conducted for each experimental unit {[$1♂_{\mathrm{L}}$, $1♂_{\mathrm{S}}$, $4♂_{\mathrm{L}}$, $4♂_{\mathrm{S}}$, $2 + 2♂_{\mathrm{L}}$ and $2 + 2♂_{\mathrm{S}}$] × 2 female size classes}. A total of 240 pipefish were used (30 large and 30 small females, 90 large and 90 small males). No fish was used more than once, and all individuals were released into the wild at the end of the experiments.

All males presented well-developed brood pouches and carried no offspring at the beginning of the experiment. Size cutoffs for "large" and "small" individuals were defined according to Silva et al. (2007), as ½ standard deviation (SD) below and above the mean size (L_{T}) of each sex (♀: mean = 9.4 cm, SD = 1.38 cm; ♂: mean = 8.5 cm, SD = 1.26 cm). Large males and females were longer than 9.1 and 10.1 cm, respectively. Small males and females were shorter than 7.9 and 8.7 cm, respectively.

Males were inspected for eggs 3 times per day (early morning, noon, and late afternoon). Both time required for the first mating and time elapsed between mating events were recorded. Good quality digital photos of the brood pouch (Nikon Coolpix 995), together with an external ruler for calibration, were imported into UTHSCA© Image Tool, and only perfectly visible eggs were measured in order to calculate the average egg diameter per clutch. Due to brood pouch opacity, good photographs of the eggs were not possible for all pregnant males.

In order to see if females spawn more eggs than a male can care for, an estimation of the female's potential reproductive rate (the number of males that each tested female could fill up to capacity) was calculated for each treatment ($4♂_{\mathrm{L}}$, $4♂_{\mathrm{S}}$, $2 + 2♂_{\mathrm{L}}$, $2 + 2♂_{\mathrm{S}}$, $1♂_{\mathrm{L}}$, and $1♂_{\mathrm{S}}$). To this purpose, the area occupied by the eggs laid by each female (A_{E}) {total number of eggs × π [egg diameter/2]^2} was divided by the approximately rectangular brood pouch area (A_{M}) of an average large or average small male according to treatment (e.g., in the $4♂_{\mathrm{L}}$ the area of the eggs laid was divided by the A_{M} of an average large male). A value smaller than 1 indicates that the total number of eggs deposited during 1 month was unable to fully occupy a male's brood pouch, whereas, as an example, a value of 2 would indicate that the number of eggs was enough to occupy 2 males. Brood pouch area was estimated according to the formula $A_{\mathrm{M}} =$ brood pouch height (H_{M}) × brood pouch width (W_{M}) where H_{M} and W_{M} were inferred from 2 regressions: $H_{\mathrm{M}} = 83.606 \times \log(L_{\mathrm{T}}) - 134.36$, $r = 0.938$, $P < 0.001$ and $\log(W_{\mathrm{M}}) = 1.609 \times \log(\mathrm{L_T}) - 2.649$, $r = 0.966$, $P < 0.001$. The estimation of the presented regressions was obtained from 95 wild males coming from the same population (see Silva et al. 2008 for additional details).

All statistical analyses were performed using STATISTICA 7.0 (Statsoft). Data were tested for deviance from normality and homoscedasticity when parametric tests were used. Assumptions were not met solely for the analysis of variance (ANOVA) conducted on the size of the eggs (Cochran's test; $P < 0.05$). However, because according to Lindman (1974), the F statistic is quite robust when there is no significant correlation between means and variances across the cells of the design (Spearman correlation; $R = 0.258$, $P > 0.05$), parametric statistics were used. Post hoc comparisons were conducted using Student–Newman–Keuls test (SNK) in all analyses. Probabilities were 2-tailed, and a significance level of 0.05 was used.

RESULTS

When considering the choice on depositing all eggs into 1 male or scattering clutches among partners, we observed that females opted to distribute their eggs among several males (Figure 1). Indeed, a T-test of means against a reference constant (1 mating

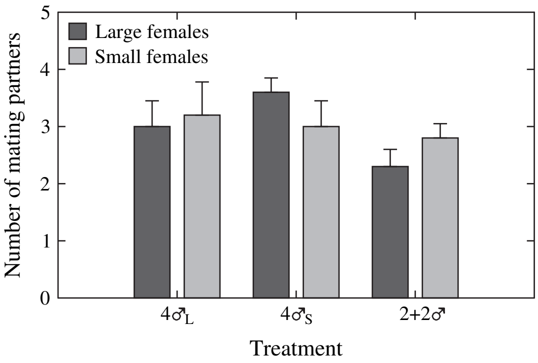

Figure 1
Mean number of mating partners for large and small females in each treatment. Error bars represent SDs.

partner) showed that all females, independently of size, consistently chose to mate with more than 1 partner when given the opportunity (Large females: 4♂$_{\mathrm{L}}$, df = 4, $P = 0.01$; 4♂$_{\mathrm{s}}$, df = 4, $P < 0.01$; 2 + 2♂, df = 9, $P = 0.02$ and Small females: 4♂$_{\mathrm{L}}$, df = 4, $P = 0.02$; 4♂$_{\mathrm{s}}$, df = 4, $P = 0.01$; 2 + 2♂, df = 9, $P < 0.01$). Additionally, a 2-way ANOVA with 2 factors (treatment [3 levels = 4♂$_{\mathrm{L}}$, 4♂$_{\mathrm{S}}$ and 2 + 2♂] and female size [2 levels = large and small]) revealed that the number of mating partners was neither affected by female size [$F(1,34) = 0.0114$; $P > 0.05$] nor by treatment [$F(2,34) = 25.328$; $P > 0.05$].

When analyzing the total number of spawned eggs (2-way ANOVA with 3 factors: male number [2 levels = 4 and 2], male size [2 levels = large and small] and female size [2 levels = large and small]), we observed only a significant interaction between female size and male number [$F(1,32) = 7.802$; $P < 0.01$] (Figure 2A). Contrarily to small females that laid the same number of eggs irrespective of the number of available partners, large females laid significantly more eggs when presented with more than 1 male (SNK post hoc test).

When analyzing the number of eggs laid by different-sized females in small or large males within the heterogeneous groups (2 + 2♂) (2-way ANOVA with 2 factors: male size [2 levels = large and small] and female size [2 levels = large and small]), we found a significant interaction between female and male size [$F(1,16) = 12.132$; $P < 0.01$]. Contrastingly to small females that laid the same number of eggs independently of male size, large females deposited bigger clutches in large males while laying fewer eggs in small mating partners (SNK post hoc test; Figure 2B).

When considering the mean size of the eggs (2-way ANOVA with 3 factors: male number [2 levels = 4 and 2], male size [2 levels = large and small] and female size [2 levels = large and small]), we observed a significant interaction between male number and female size [$F(1,32) = 6.31$; $P = 0.02$] (Figure 3A). Although small females laid eggs of similar dimensions in all mating contexts, large females laid larger eggs in the presence of more than 1 male (SNK post hoc test). Additionally, we found that large females laid larger eggs than small ones [$F(1,32) = 31.44$; $P < 0.001$].

When analyzing the size of eggs laid by different-sized females in small or large males within the heterogeneous groups (2-way ANOVA with 2 factors: male size (2 levels = large and small) and female size (2 levels = large and small)], we observed a significant interaction between female and male size. Although both large and small females laid larger eggs in larger males, the size increase was considerably notable in large female's eggs (SNK post hoc test; Figure 3B).

Because we observed that larger males tended to receive larger eggs when small males were present, we tried to detect if these differences were due to the female ability to control the size of the laid eggs according to male size or, on the other hand, a result of a much simpler mechanism, mainly explained by mating precedence. To this extent, the mean egg size of the clutches laid by 13 females from which we were able to monitor 2 consecutive breeding episodes (occurring within less than 9 days into males of similar size) were recorded. Egg size significantly varied over matings, with the eggs from the first clutch being larger than the second (*T*-test for dependent samples, 1st = 1.428 mm, 2nd = 1.389 mm, df = 12, $P < 0.05$). This reduction in size occurred 12 times over the 13 measured events ($\chi^2 = 9.308$, df = 1, $P < 0.05$).

Independently of male number (1 or 4) and size (large or small) females started depositing eggs beginning on the first day of observations. Nevertheless, if different-sized males were available in the aquaria, both large and small females consistently mated with large males first (2-way ANOVA on the time elapsed until the first breeding episode with a specific large or small male, with 2 factors: male size [2 levels = large and small] and female size [2 levels = large and small]). As such, a significant effect on male size was observed [$F(1,16) = 7.98$; $P = 0.01$]. In fact, it took on average more than 4 days for small males to receive an egg clutch.

Finally, the estimated male brood pouch fullness was always smaller than 1 in every treatment, indicating that the total number of eggs laid during the extent of a male pregnancy could not fill a male pouch up to full capacity (Average male brood pouch fullness due to large female egg laying: 4♂$_{\mathrm{L}}$ = 0.67; 4♂$_{\mathrm{S}}$ = 0.90; 2 + 2♂$_{\mathrm{L}}$ = 0.74; 2 + 2♂$_{\mathrm{S}}$ = 0.10; 1♂$_{\mathrm{L}}$ = 0.27; 1♂$_{\mathrm{S}}$ = 0.63; Average male brood pouch fullness due to small female egg laying: 4♂$_{\mathrm{L}}$ = 0.27; 4♂$_{\mathrm{S}}$ = 0.38; 2 + 2♂$_{\mathrm{L}}$ = 0.32; 2 + 2♂$_{\mathrm{S}}$ = 0.44; 1♂$_{\mathrm{L}}$ = 0.30; 1♂$_{\mathrm{S}}$ = 0.54).

DISCUSSION

The impact of female decisions on a species reproductive strategy remains largely unknown, thus being 1 of the most challenging areas of debate in biology (Lee and Hays 2004). Mating with 1 or many partners is a critical decision that may have a significant impact on a female's reproductive fitness (Fedorka and Mousseau 2002; Fox and Rauter 2003).

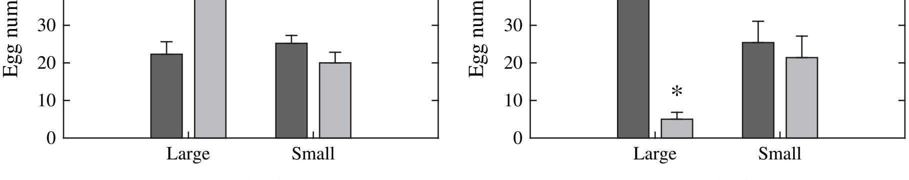

Figure 2
Mean number of eggs laid by large and small females in 1 and 4 males (A); Mean number of eggs laid by different-sized females in large or small males in the heterogeneous group (B); Error bars represent SDs and asterisks indicate significantly different values.

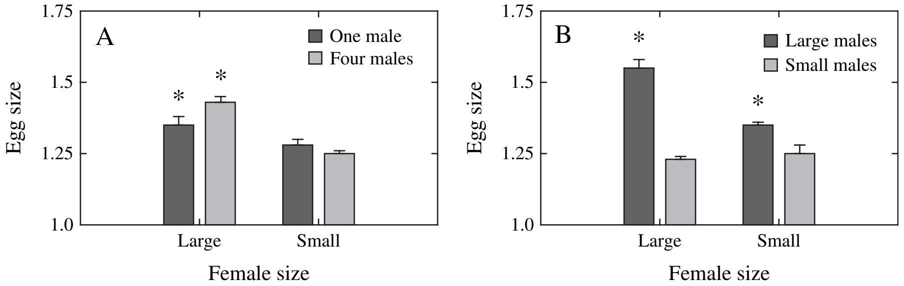

Figure 3
Mean size of the eggs laid by large and small females in 1 and 4 males (A); Mean size of the eggs laid by different-sized females in large or small males in the heterogeneous group (B). Error bars represent SDs and asterisks indicate significantly different values.

Confirming preliminary observations (Silva et al. 2006b), the present study showed that *S. abaster* females clearly opted for a multiple mating strategy, with both large and small females distributing their eggs among more than 1 male. Similar observations have been made in other pipefish species such as *S. typhle*, *S. scovelli*, and *S. floridae* (Jones and Avise 2001) where the female's multiple mating strategy can be viewed as a consequence of a genetically predisposed "bet-hedging" strategy, reducing both variance in the number of produced offspring as well as competition among embryos in the brood pouch (Berglund et al. 1988; Jones et al. 2000).

Together with the decision of dividing their egg clutches through multiple partners, *S. abaster* females revealed a particularly interesting egg allocation pattern, with different-sized females showing distinct investment tactics. Despite the previously described preference for large males, measured as the time spent by large and small females near a particular male (Silva et al. 2007), females did not avoid mating with small individuals. Instead, the preference for large partners, assessed in the heterogeneous treatment (containing large and small males), was translated into different mating timings. Females, large and small, opted to mate sooner with large partners, only then depositing eggs in smaller males. A nonexclusive hypothesis, helping to explain the observed mating timings, deals with male–male competition in a skewed sex-ratio setting (4♂:1♀), where large males may have access to females not only due to female preference but also because of a hypothetically higher hierarchical status.

Within large females, the preference for larger individuals was reinforced through differential egg allocation according to male size, with larger males receiving more and larger eggs. Interestingly, this differential egg allocation was especially visible when large females were allowed to simultaneously evaluate both large and small males. These results are in accordance with theoretical studies addressing female mate choice behavior, which argue that, in the absence of some internal standard, females may assess male quality through a comparison process (Halliday 1983; Wiegmann et al. 1999; Bateson and Healy 2005). A differential egg number allocation strategy seems to be restricted to large females because smaller ones showed no significant differences.

According to Kolm and Olsson (2003), differential allocation in egg size according to male quality can be facilitated either through an extended pair bond or by an ability to rapidly adjust egg size prior to mating. Thus, decreasing an already defined high egg investment seems highly improbable. Females of the Banggai cardinalfish, for example, are capable of rapidly increasing their egg size investment in response to a new, more attractive, male even after the onset of egg maturation. Contrastingly, they are unable to adjust egg size in response to a decrease in mate attractiveness (Kolm and Olsson 2003).

In *S. abaster*, when males of different sizes were available, females deposited smaller eggs in smaller partners while transferring larger eggs into high quality males. These results might suggest that females are adjusting egg size according to male quality. Nevertheless, the observation that egg size diminished over 2 sequential matings indicates that the observed egg allocation pattern might result mainly from a process of serial matings: larger males first, smaller males later. This hypothesis would help justify not only the wide difference in egg size measured in the heterogeneous group but also why the other "homogeneous" groups revealed similar values.

Interestingly, besides being differentially allocated according to size, eggs of larger females were also laid in greater number in treatments involving more than one available large male. The reduced egg number in treatments involving only 1 male could be thought to result from brood pouch space constraints. Nevertheless, the estimations of females' potential reproductive rate clearly revealed that, during the extent of a male pregnancy, females did not spawn enough eggs to fully occupy a male's pouch, thus corroborating the in situ observations of Silva et al. (2008). Furthermore, the fact that the number of eggs laid by large females in the presence of 1 male is significantly smaller than that recorded when females interact with 4 males strongly suggests that large females can have a fine-tuned control over their reproductive investment, being able to "deliberately" save a share of their resources for future breeding opportunities.

It is unclear why investing more and larger eggs in larger males is not a common strategy to both large and small females of *S. abaster* when considering the direct benefits they could accrue from mating with high quality partners (e.g., Berglund et al. 1986). Even considering that smaller females, due to physiological constraints, might not be able to produce as much nor as large eggs as those laid by large females, a similar pattern of egg number allocation could be expected. The recorded constant reproductive effort suggests that, contrary to large females, small females seem to invest heavily in each breeding attempt. Maximizing investment in current reproduction might well be an adaptive tactic for smaller females to compensate their lower attractiveness (see Silva et al. 2007). Contrarily to large preferred females who have increased opportunities to mate with high quality males, small females might not have access to a better partner, or any other partner in the future. This adaptive tactic might be especially relevant in populations located in areas where the extent of the breeding season raises the opportunity of small females to reproduce. In areas where the extent of breeding season is reduced to a few months, small females could opt to allocate resources into growth rather than reproduction. Large, preferred females, on the other hand, seem able to simultaneously monitor the number of available mates and their intrinsic quality, responding accordingly through the deposition of eggs that vary in number and size. Assuming that the size of the fish reflects age (Billing et al. 2007) this strongly suggests the existence of a "switch point' in females" reproductive tactics through lifetime.

Globally, results show not only that females seem to continuously scan their social environment, making decisions based on mate number and quality, but also that they become able to differentially allocate resources as they grow. Interestingly, if the increased size recorded in a female first egg batches correlates into higher nutritional quality, then we might be unravelling the importance of mating order effect on male reproductive success. The male's active role in courtship reported for *S. abaster* (Silva et al. 2006b; Silva K, Vieira MN, Almada VC, Monteiro NM, unpublished data) might be interpreted as a selected trait aiming at securing the larger eggs through mating precedence.

FUNDING

Programa Plurianual de Apoio às Unidades de Investigação (partial funding to V.A.); Fundação para a Ciência e a Tecnologia (FCT-SFRH/BPD/14992/2004 to N.M.) and Programa Plurianual de Apoio às Unidades de Investigação (to N.M.); Fundação para a Ciência e a Tecnologia (FCT-SFRH/BD/13171/2003 to K.S.); and FCT (POCI/MAR/60895/2004, this work).

We would like to thank Dr Candy Rowe and the anonymous referees for their criticism and suggestions, which greatly improved the manuscript. We also would like to thank everybody who helped during the laboratorial work, especially Pedro Correia. **Ethical note:** All experiments were conducted in agreement with the pertinent European and Portuguese legislation on animal welfare.

REFERENCES

Bateson M, Healy SD. 2005. Comparative evaluation and its implications for mate choice. Trends Ecol Evol. 20:659–664.

Berglund A, Rosenqvist G, Svensson I. 1986. Reversed sex roles and parental energy investment in zygotes of two pipefish (Syngnathidae) species. Mar Ecol Prog Ser. 29:209–215.

Berglund A, Rosenqvist G, Svensson I. 1988. Multiple matings and paternal brood care in the pipefish *Syngnathus typhle*. Oikos. 51: 184–188.

Billing AM, Rosenqvist G, Berglund A. 2007. No terminal investment in pipefish males: only young males exhibit risk-prone courtship behavior. Behav Ecol. 18:535–540.

Burley N. 1986. Sexual selection for aesthetic traits in species with biparental care. Am Nat. 127:415–445.

Burley N. 1988. The differential-allocation hypothesis: an experimental test. Am Nat. 132:611–628.

Cakic P, Lenhardt M, Mickovic D, Sekulic N, Budakov LJ. 2002. Biometric analysis of *Syngnathus abaster* populations. J Fish Biol. 60: 1562–1569.

Carcupino M, Baldacci A, Mazzini M, Franzoi P. 2002. Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. J Fish Biol. 61:1465–1480.

Dawson CE. 1986. Syngnathidae. In: Whitehead PJP, Bauchot ML, Hureau JC, Nielsen J, Tortonese E, editors. Fishes of the north-eastern Atlantic and the Mediterranean. Paris: UNESCO. p. 628–639.

Drickamer LC, Gowaty PA, Holmes CM. 2000. Free female mate choice in house mice affects reproductive success and offspring viability and performance. Anim Behav. 59:371–378.

Fedorka KM, Mousseau TA. 2002. Tibial spur feeding in ground crickets: larger males contribute larger gifts (Orthoptera: gryllidae). Fla Entomol. 85:317–323.

Fox CW, Rauter CM. 2003. Bet-hedging and the evolution of multiple mating. Evol Ecol Res. 5:273–286.

Gorman HE, Orr KJ, Adam A, Nager RG. 2005. Effects of incubation conditions and offspring sex on embryonic development and survival in the zebra finch (*Taeniopygia guttata*). Auk. 122:1239–1248.

Halliday TR. 1983. The study of mate choice. In: Bateson P, editor. Mate choice. Cambridge (MA): Cambridge University Press. p. 3–32.

Haresign TW, Schumway SE. 1981. Permeability of the marsupium of the pipefish *Syngnathus fuscus* to [14C]-alpha animo-isobutyric acid. Comp Biochem Physiol. 69:603–604.

Jones AG, Avise JC. 2001. Mating systems and sexual selection in male-pregnant pipefishes and seahorses: insights from microsatellite-based studies of maternity. J Hered. 92:150–158.

Jones AG, Rosenqvist G, Berglund A, Avise CJ. 2000. Mate quality influences multiple maternity in the sex-role-reversed pipefish *Syngnathus typhle*. Oikos. 90:321–326.

Kolm N. 2001. Females produce larger eggs for larger males in a paternal mouthbrooding fish. Proc R Soc Lond B Biol Sci. 268: 2229–2234.

Kolm N, Olsson J. 2003. Differential investment in the Banggai cardinalfish: can females adjust egg size close to egg maturation to match the attractiveness of a new partner? J Fish Biol. 63:144–151.

Lee PLM, Hays GC. 2004. Polyandry in a marine turtle: females make the best of a bad job. Proc Natl Acad Sci USA. 101:6530–6535.

Lindman HR. 1974. Analysis of variance in complex experimental designs. San Francisco (CA): Freeman, W.H. & Co.

Locatello L, Neat FC. 2005. Reproductive allocation in *Aidablennius sphinx* (Teleostei, Blenniidae): females lay more eggs faster when paired with larger males. J Exp Zool. 303:922–926.

Monteiro NM, Vieira NM, Almada VC. 2002. The courtship behaviour of the pipefish *Nerophis lumbriciformis*: reflections of and adaptation to intertidal life. Acta Ethol. 4:109–111.

Reyer HU, Frei G, Som C. 1999. Cryptic female choice: frogs reduce their clutches when amplexed by undesired male. Proc R Soc Lond B Biol Sci. 266:2101–2107.

Roff DA. 1992. The evolution of life histories. London: Chapman & Hall.

Sheldon BC. 2000. Differential allocation: tests, mechanisms and implications. Trends Ecol Evol. 15:397–402.

Silva K, Monteiro NM, Almada VC, Vieira MN. 2006a. Early life history of *Syngnathus abaster* (Pisces: syngnathidae). J Fish Biol. 68: 80–86.

Silva K, Monteiro NM, Vieira MN, Almada VC. 2006b. Reproductive behaviour of the black-striped pipefish, *Syngnathus abaster* (Pisces; Syngnathidae). J Fish Biol. 69:1860–1869.

Silva K, Vieira MN, Almada VC, Monteiro NM. 2007. The effect of temperature on mate preferences and female-female interactions in *Syngnathus abaster*. Anim Behav. 74:1525–1533.

Silva K, Vieira MN, Almada VC, Monteiro NM. 2008. Can the limited marsupium space be a limiting factor for *Syngnathus abaster* females: insights from a population with size assortative mating. Anim Ecol. 77:390–394.

Simmons LW. 1987. Female choice contributes to offspring fitness in the field cricket *Gryllus bimaculatus* (De Geer). Behav Ecol Sociobiol. 21:313–321.

Skinner AJ, Watt PJ. 2007. Strategic egg allocation in the zebra fish, *Danio rerio*. Behav Ecol. 18:905–909.

Tomasini JA, Quignard JP, Canapé C, Bouchereau JL. 1991. Facteurs du succés reproductif de *Syngnathus abaster* Risso, 1826 (Pisces, Teleostei, Syngnathidae) en milieu lagunaire méditerranéen (lagune de Mauguio, France). Acta Oecol. 12:331–355.

Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man. Chicago (IL): Aldine Press. p. 136–179.

Wiegmann DD, Mukhopadhyay K, Real LA. 1999. Sequential search and the influence of male quality on female mating decisions. J Math Biol. 39:193–216.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat. 100:687–690.