

# Can the limited marsupium space be a limiting factor for *Syngnathus abaster* females? Insights from a population with size-assortative mating

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## Summary

1. Some syngnathid species show varying degrees of sex role reversal aside from male pregnancy, with females competing for access to mates and sometimes presenting conspicuous secondary sexual characters. Among other variables, brooding space constraints are usually considered a key element in female reproductive success, contributing strongly to the observed morphological and behavioural sexual differences. Nevertheless, a close relationship between sex role reversal and male brooding space limitation has not yet been accurately demonstrated in field studies.

2. The present work, conducted over two consecutive breeding seasons in a wild population of the sex role-reversed pipefish *Syngnathus abaster*, simultaneously analysed egg number and occupied space, as well as the free area in the male's marsupium. The number of eggs that would fit in the observed unoccupied space was estimated.

3. Contrary to what would be expected, given the marked sexual dimorphism observed in the population studied, where females were larger and more colourful, male brooding space did not appear to limit female reproduction as neither large nor small individuals presented a fully occupied pouch. Interestingly, the largest unoccupied areas of marsupium were found in the larger individuals, although they received more and larger eggs. Laboratory data also showed that larger females lay larger eggs.

4. Together, these results suggest the existence of assortative mating, which may result from: (i) the reluctance of larger males (which tend not to receive small eggs usually laid by small females) to mate with lower quality females, even at the expense of a smaller number of offspring; or (ii) female–female competition, which might strongly reduce the hypothesis of a small female mating with a large male.

The potential impact of temperature on reproduction and population dynamics is also discussed in the light of ongoing climatic changes.

**Key-words:** assortative mating, egg allocation, reproductive success, sex role reversal, Syngnathidae

## Introduction

In many sexually reproducing animals, one sex clearly competes more intensely for mates than the other. Differences in the relative intensity of mating competition are usually attributed to sex differences in potential reproductive rates that determine which sex limits the reproductive success of the other (Clutton-Brock & Parker 1992; Andersson 1994; Ahnesjö, Kvarnemo & Merilaita 2001). Sex roles are termed 'conventional'

when males compete among themselves for female access. In contrast, when females compete over mates sex roles are usually described as reversed (Vincent *et al.* 1992).

Although uncommon in nature (Colwell & Oring 1988; Gwynne & Simmons 1990; Balshine-Earn & McAndrew 1995), sex role reversal can be observed in some species of the Syngnathidae family, a fish group characterized by a highly specialized male pregnancy, the costs of which are believed to exceed those of most vertebrates (Clutton-Brock & Vincent 1991). Syngnathid females deposit unfertilized eggs into a male's specialized ventral incubating surface. The anatomical complexity of this varies among species, from a simple incubating ventral surface to a sealed pouch (Herald 1959). In

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some pipefish species, females compete for male access, sometimes presenting conspicuous secondary sexual characters (Monteiro, Vieira & Almada 2002; Silva *et al.* 2006). Energy investment, brooding space constraints and pregnancy length, operating alone or in combination, may limit female reproductive success, thus providing an explanation for such sexual differences (Berglund & Rosenqvist 2003). Despite many attempts to identify clearly the mechanisms behind the evolution of sex role reversal in syngnathids, such as whether the male's limited brooding space can contribute effectively to sex role reversal, the causes have yet to be fully demonstrated, especially in wild populations where a species' behavioural repertoire is modulated by far more variables than in aquaria.

Laboratory studies showed that brooding space constraints and pregnancy length, rather than energy investment, might limit female reproduction in two northern European populations of sex role-reversed pipefish, *Nerophis ophidion* (Linnaeus, 1758) and *Syngnathus typhle* (Linnaeus, 1758) (Berglund, Rosenqvist & Svensson 1989). In both species, females were found to produce more eggs than a male can brood during a pregnancy episode (Berglund *et al.* 1989). The need to contextualize these findings with field observations has encouraged the present study on a wild population of the sex role-reversed *Syngnathus abaster* (Risso, 1827), morphologically similar to *S. typhle*. Both the space occupied by eggs and the remaining free area in the male's pouch were analysed to test whether the male's limited brooding space could constrain female reproductive success. The importance of male and female size was also considered in order to investigate potential differences in egg allocation and their consequences for mating patterns.

## Methods

*Syngnathus abaster* is a euryhaline species that inhabits the Mediterranean, Black Sea and Atlantic coast of south-west Europe up to southern Biscay (Dawson 1986). The 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, in a temperature range of 8–24 °C. Males have a brood pouch located ventrally on the tail (Urophori), which consists of two skin folds that contact medially with their free edges. Sex roles seem to be reversed, as females are larger and apparently more competitive than males, at least under even sex ratio conditions (Silva *et al.* 2006).

Bimonthly samples were conducted over two consecutive breeding seasons in the Ria de Aveiro estuarine lagoon (40°45' N, 8°40' W) in northern Portugal. A total of 295 pregnant males were captured and measured for total length (LT). For each pregnant male, eggs were counted and macrophotographs, including an external ruler for size reference, were taken in the most transparent area of the marsupium. A subsample of 95 individuals (≈32%) was brought to the laboratory for additional marsupium width and height measurements. All other captured males were immediately released in the same area where they were initially collected. Good quality photographs, available for 121 individuals, were imported into UTHSCA IMAGE TOOL and perfectly visible eggs were measured in order to calculate the average egg diameter per male ( $E_D$ ). The total number of fish captured, including non-pregnant males and females, was also recorded in each sampling event for sex-ratio calculation.

The approximately rectangular marsupium area ( $A_M$ ) was calculated according to the formula:

$$A_M = \text{marsupium height } (H_M) \times \text{marsupium width } (W_M)$$

$H_M$  and  $W_M$  (measured in the 95 individuals that were transported to the laboratory specifically for this goal) were inferred for all males according to the regressions presented in the Results section. The number of missing eggs,  $M_E$  [the number of eggs, with the average diameter observed for each male, that would fill the unoccupied marsupium space ( $U_M$ )], was calculated for each individual according to the formula:

$$M_E = U_M / \pi(\text{egg diameter}/2)^2$$

where  $U_M = A_M - \text{egg number} \times \pi(\text{egg diameter}/2)^2$ .

Other approaches could have been taken to calculating the number of eggs that could fit the available marsupium area: those that would consider space between the theoretically spherical eggs as unusable. Alternatively, the degree of compression sometimes alters egg sphericity into a cube-like form. It could be expected that the most accurate of these two approaches [ $U_M = A_M - \text{egg number} \times \pi(\text{egg diameter}/2)^2$  or  $U_M = A_M - \text{egg number} \times \text{egg diameter}^2$ ], when subtracted from the direct measurement of the actual area occupied by eggs (determined using photographs of the marsupium of aquarium-kept males using UTHSCA IMAGE TOOL), would tend to zero. A *t*-test of means against a reference constant (0) showed that the selected approach was accurate ( $N = 4$ , d.f. = 3,  $P = 0.64$ ) when compared with the hypothesis that eggs have a cube-like form or that the space between spherical eggs cannot be accounted as free space ( $N = 4$ , d.f. = 3,  $P = 0.03$ ).

Regressions were conducted to test for possible relationships between male and egg size. Additionally, data gathered in the aquarium, using female size and the diameter of the laid eggs, were used to test for a possible correlation between these two variables.

Taking into consideration that mate size is an important variable in syngnathid mate choice (Berglund & Rosenqvist 2003; Silva *et al.* 2007), two male groups were considered, with the size cut-offs for 'large' and 'small' pregnant males defined according to Silva *et al.* 2007, as 0.5 SD below and above the mean male size ( $L_T$ ) (mean = 8.5 cm, SD = 1.26 cm, values obtained from prior measurements in the same population,  $N = 214$  males). Large males were longer than 9.1 cm, while small individuals were shorter than 7.9 cm. Accordingly, two one-way ANOVAs were conducted to test for differences between male size classes (large and small; untransformed data) in the number of eggs carried and the number of 'missing' eggs [transformed data: square root ( $x + \text{constant}$ )]. The homogeneity of variances assumption was met for all analyses. All probabilities are two-tailed and a significance level of 0.05 was used.

## Results

The number of males and females captured was not significantly different during the 29 sampling visits (*t*-test for dependent samples, female = 13.24, male = 15.03, d.f. = 28,  $P = 0.239$ ).

The regression equations used to estimate marsupium height and width were: (a) marsupium height:  $H_M = 83.606 \times \log(L_T) - 134.36$ ,  $r = 0.938$ ,  $P < 0.001$ ,  $N = 95$  males; (b) marsupium width:  $\log(W_M) = 1.609 \times \log(L_T) - 2.649$ ,  $r = 0.966$ ,  $P < 0.001$ ,  $N = 95$  males (Fig. 1a,b).

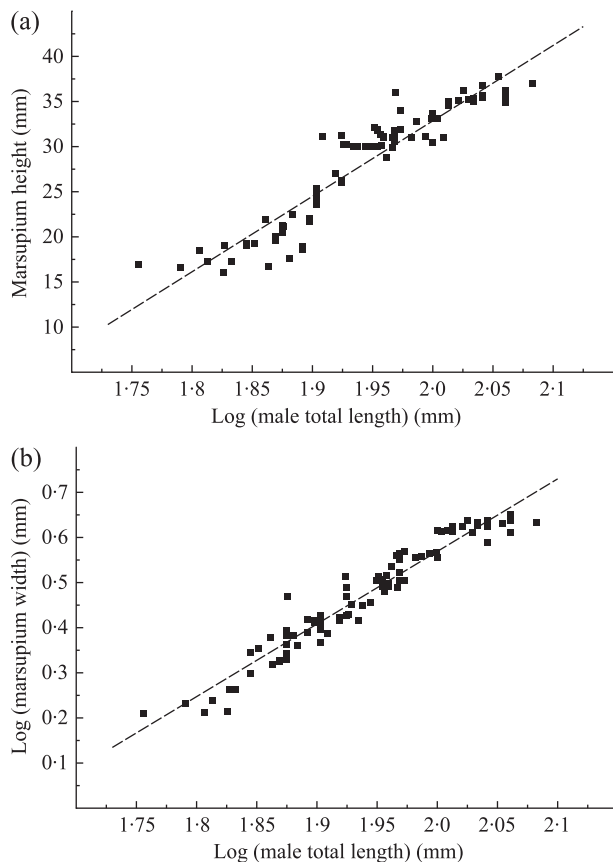


Fig. 1. Relationship between (a) male size and male marsupium height; (b) male size and male marsupium width.

A significant correlation was found between male size and egg size (egg size =  $0.0089 \times \text{male size} + 0.5307$ ;  $r = 0.5762$ ;  $P < 0.001$ ,  $N = 121$  males), with larger males accommodating larger eggs (Fig. 2a). Also, a significant correlation was found between female size and egg size [female  $L_T = 45.215 \times \text{egg size} + 34.574$  [ $r = 0.582$ ,  $P < 0.001$ ,  $N = 92$  females (Fig. 2b)]. Larger males presented more eggs than smaller individuals [ANOVA: d.f. (1,88),  $F = 87.64$ ,  $P < 0.001$ ; Fig. 2c]. Neither large nor small males presented a fully occupied pouch (percentage occupation for large males = 64.36,  $N = 51$ ; percentage occupation for small males = 70.02,  $N = 39$ ). In contrast to what would be expected, the number of missing eggs, specifically calculated taking into consideration the average egg size per male, was found to be significantly higher in large than in small individuals [ANOVA: d.f. (1,88),  $F = 19.79$ ,  $P < 0.001$ ; Fig. 2d].

## Discussion

It is not always clear how to demonstrate that one sex actually limits the reproductive success of the other. For example, even if a male makes a substantial investment of time and energy in offspring, either this may not exceed female investment, or time and energy may not be limiting factors during the breeding period (Berglund *et al.* 1989).

The present study showed that neither large nor small *S. abaster* males presented fully occupied pouches over the breeding season, at least in the population studied, where the number of males and females was similar. Thus it seems that the limited marsupium space may not to be a limiting factor for *S. abaster* females. The fact that only brooding males were considered in this work reinforces the results obtained, as otherwise the areas available for females to lay their eggs on would be even greater. Also, it could be argued that, even though space is available, males could refrain from accepting eggs for physiological or allocation trade-off reasons. Nevertheless, it is important to stress that several fully occupied pouches were observed during the 2-year sampling period, showing that males can be filled up to maximum capacity.

Berglund *et al.* (1989) showed that, in aquaria, *S. typhle* females, captured from a population under an even sex ratio, produced far more eggs than a male could bear during the extent of a pregnancy event. If these results could be transposed to the field, than a marsupium space limitation would be expected. These differences between *S. abaster* and *S. typhle* could be interpreted from distinct viewpoints: (i) even considering that *S. typhle* is a larger species, with males presenting a bigger marsupium, the far greater number of eggs produced by *S. typhle* females (105; Monteiro, Almada & Vieira 2005) may still fully occupy the increased brooding area. Alternatively, (ii) the results obtained in aquaria during a male pregnancy, and those observed in the wild during the full extent of a breeding season, might not be fully comparable. For instance, in preliminary aquarium observations, an *S. abaster* female was able to lay 78 eggs during the extent of a male's pregnancy. No male captured in the wild showed more than 62 eggs.

The observed differences in egg number and size among *S. abaster* male length classes, with larger individuals receiving more and larger eggs, seem to be the result of mating with larger females, as inferred from laboratory data on female size and eggs laid. As large body size proved to be a sexually selected trait (Silva *et al.* in press), it would be expected that large males would have more mating opportunities and thus more occupied pouches than smaller individuals. The largest unoccupied marsupium areas were found in the group of larger individuals. Apparently discordant, this observation might be the result of male choosiness, as larger males were observed also to prefer large partners (Silva *et al.* in press). It should be stressed that the difference in marsupium occupation observed in large and small males could also be the result of different trade-offs between egg size and egg numbers, with a larger empty space in the brood pouch being more beneficial for large males. Furthermore, it seems that larger males may also be reluctant to mate with lower-quality females, even at the expense of a smaller number of offspring. By mating with large females, which produce larger eggs, males could gain in offspring quality. Offspring hatching from larger eggs have been reported to have higher survival, higher resistance to starvation, and increased swimming performance (Kolm & Ahnesjö 2005). A non-exclusive hypothesis explaining the apparent absence of small female eggs in large male's marsupium deals with female–female competition. Large, dominant females

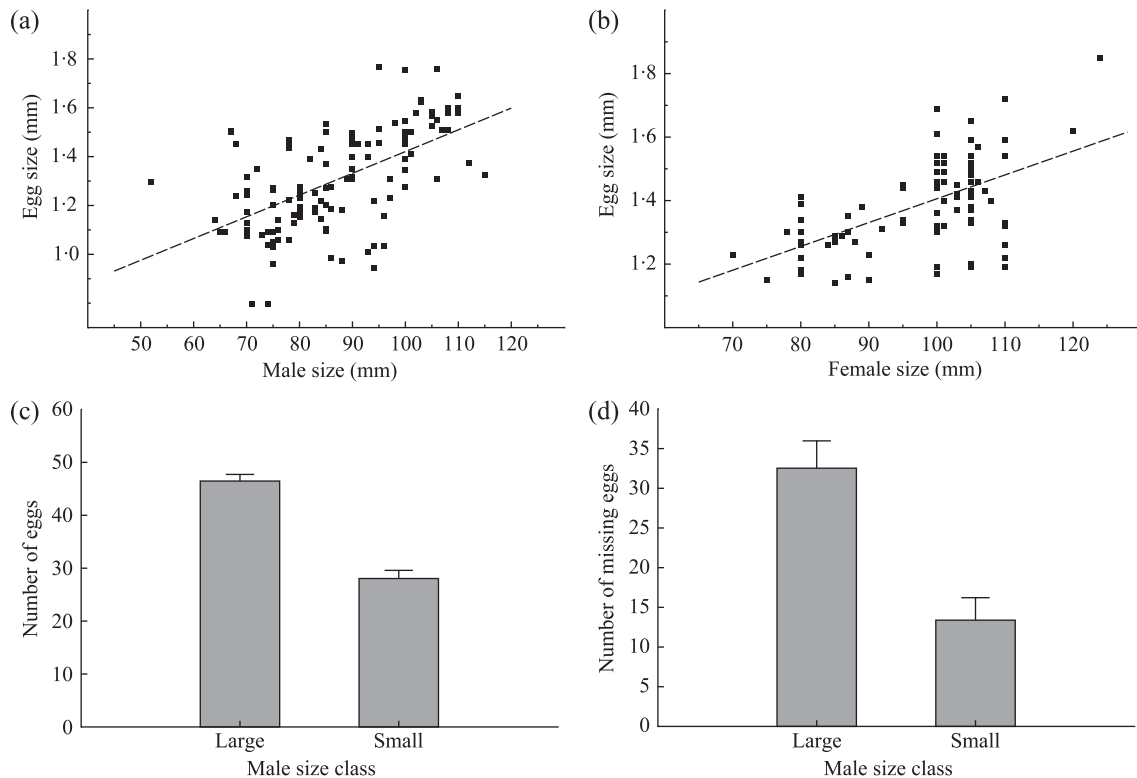


Fig. 2. Relationship between (a) male size and egg size; (b) female size and egg size; (c) number of eggs carried in large and small males; (d) number of missing eggs estimated for large and small males. Error bars represent standard errors.

may strongly reduce the opportunity of small females to mate with large males, as suggested by Silva *et al.* 2007). Small females of *S. typhle* were also found to be reproductively constrained by large, dominant females (Berglund & Rosenqvist 2003).

Male mate choice for larger females was observed not only in sex role-reversed syngnathids (e.g. *S. abaster*, Silva *et al.* 2007; *S. typhle*, Berglund & Rosenqvist 2003), but also in 'conventional' sex role species outside this family, such as *Gasterosteus aculeatus* (Kraak & Bakker 1998), *Poecilia reticulata* (Herdman, Kelly & Godin 2004) and *Pseudomugil signifer* (Wong, Jennions & Keogh 2004). Thus it could be expected that small males, that also prefer large mates, might be able to carry eggs laid by large females. Nevertheless, the significant correlation obtained between male and female size suggests that larger females may avoid reproducing with small males.

Together, these results suggest the occurrence of assortative mating, as proposed by Silva *et al.* (2006), where *S. abaster* courting pairs with more asymmetrical body sizes were less successful in achieving mating. Size-assortative mating has been observed in many fish species [e.g. *Sarotherodon galilaeus* (Ros, Zeilstra & Oliveira 2003); *Gasterosteus aculeatus* (Olafsdottir, Ritchie & Snorrason 2006); *Cichlasoma nigrofasciatum* (Beeching & Hopp 1999)]. Assortative mating is believed to have played a significant role in the seahorse speciation process (Jones *et al.* 2003). Size-assortative mating might also contribute to a female reproductive limitation, as the number of preferred males (that are also choosy) tends to be smaller, reinforcing sex role reversal.

It would be interesting to see if a similar pattern of results may be seen in other *S. abaster* populations, especially those inhabiting different latitudes, where the extent of the breeding season differs markedly. It could be predicted that the limited marsupium space could indeed be a limiting factor for females, particularly those of small size, in areas where the extent of the breeding period would not allow for time-scattered spawnings. Moreover, given the current climatic changes, the local expression of a species mating system will surely vary, with consequences that cannot yet be foreseen given the small amount of information available on estuarine and coastal fish population dynamics.

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