*Journal of Fish Biology* (2006) **69,** 1860–1869
doi:10.1111/j.1095-8649.2006.01229.x, available online at http://www.blackwell-synergy.com

# Reproductive behaviour of the black-striped pipefish *Syngnathus abaster* (Pisces; Syngnathidae)

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*(Received 25 July 2005, Accepted 3 July 2006)*

The reproductive behaviour of *Syngnathus abaster* is described and compared with those of other syngnathids. The need for standardized behavioural data is discussed in light of the actual theories of evolution of mating patterns and sex-role reversal within this family.

Key words: courtship; reproduction; sex-role reversal; Syngnathidae; *Syngnathus abaster*.

The Syngnathidae (comprising pipefishes, seahorses and seadragons) is well known for its unique form of parental care, where females deposit unfertilized eggs into the male's specialized ventral incubating surface (Herald, 1959). Together with male pregnancy, some syngnathid species also exhibit varying degrees of sex-role reversal (Monteiro *et al.*, 2002; Berglund & Rosenqvist, 2003), with females competing for access to mates and sometimes presenting conspicuous secondary sexual characters. These specializations have promoted the use of syngnathids as models in which to study not only the evolution of parental care (Trivers, 1972; Ridley, 1978), but also to test theoretical assumptions of sexual selection (Johnstone, 1995).

The phylogenetic relationships among syngnathids have been studied both by Herald (1959), based on the morphological organization of the male's incubating area, and Wilson *et al.* (2003), using mitochondrial DNA, producing similar results. Attempts to determine the evolution of mating patterns or the degree of sex-role reversal, superimposing information from behavioural and ecological data are, nevertheless, still far from producing definitive results. Despite the

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ubiquity of male pregnancy in syngnathids, mating patterns and sexual dimorphism differ greatly among species. Thus, comparative studies must be preceded and supported by objective and reasonably standardized behavioural observations, able to highlight not only differences between species but also variation among populations.

In order to further extend knowledge of syngnathid reproduction, a study area where information on the most basic life-history parameters is scarce, data were collected on the reproductive biology of the black-striped pipefish *Syngnathus abaster* Risso, based on aquarium observations of individuals captured in the wild. The reproductive behaviour of this species is poorly understood and information on its reproductive biology is still insufficient. Tomasini *et al.* (1991) described factors that influence the reproductive success of a population of *S. abaster* in a Mediterranean lagoon (Mauguio, France), Carcupino *et al.* (1997) described the ultrastructural organization of the male brood pouch epithelium and Silva *et al.* (2006) presented a description of the embryonic development and juvenile behaviour. Furthermore, comparisons are presented and discussed, highlighting similarities and differences in the reproductive biology of the black-striped pipefish when compared to other Western European pipefish species.

*Syngnathus abaster*, a euryhaline species with a restricted distribution that includes the Mediterranean and Black Sea northward to southern Biscay (Dawson, 1986), occurs either in coastal areas or in brackish and fresh waters (Cakic *et al.*, 2002). The black-striped pipefish is a small brown-green pipefish, with dark or pale spots on the trunk and tail. It can be found mainly among sand, mud or eelgrass meadows, between depths of 0·5 and 5 m, within a temperature range of 8 and 24° C. Males of the Urophori have a brood pouch located ventrally on the tail, which consists of two skin folds that contact medially with their free edges.

Fish were collected with a hand-net, in a salt pond reservoir, at the Ria de Aveiro estuarine lagoon (40°45′ N; 8°40′ W), Portugal. One hundred and seventy-six mature individuals, including males and females, were captured during a 2 year period (March 2003 to June 2005), measured for total length ($L_T$) and the number of eggs (visible through the marsupium folds) was counted in each pregnant male. These individuals were transported to the laboratory and maintained in several 80 l aquaria, illuminated with natural light and supplemented with 18 W fluorescent lamps. The tank substrata consisted mainly of sand and plastic seagrass laid in order to mimic the original habitat where the fish were caught. Due to the 'gas bubble disease', common in pipefishes, aeration was performed outside the fish tanks (Monteiro *et al.*, 2002). The continuously running sea water was physically and biologically filtered and its temperature and salinity (33) maintained constant. Fish were fed daily with fresh *Artemia franciscana* nauplii. Adult males and females were initially kept separate in order to synchronize the 'disposition to mate' and simultaneously to allow the fish to be accustomed to the presence of an observer. An equal number of females and non-brooding males (not exceeding six individuals) were then placed together to mate. Since no successful mating rituals were observed in the first courtship trials, with the water temperature ranging from 14 to 15° C, and considering that temperature is one of the strongest factors in the control of marine fish reproduction (Monteiro *et al.*, 2001), water temperature was gradually increased to 18–19° C. This is in agreement with *in situ* observations (during 2005, adult *S. abaster* migrated to salt ponds, in order to reproduce, when temperature reached values >18° C; K. Silva, pers. obs.). This adjustment in water temperature resulted in the observation of several successful matings. More than 230 h of *ad libitum* observations were conducted (Martin & Bateson, 1993), at random intervals, and the main stages of the courtship rituals were defined, measured and described. More than 15 h of video-tape recordings were also used in order to further describe the behavioural patterns of courtship and mating.

Differences between male and female initiative, flickering movements and leads were determined using ADERSIML (Almada & Oliveira, 1997). This computer programme implements a procedure to access the significance of goodness of fit tests that would usually be addressed using the $\chi^2$ distribution. This procedure was chosen because it allows the analysis of data where expected frequencies are very low (<4 or even 0) in several classes, a condition in which the $\chi^2$ distribution must not be used (Zar, 1984).

*Syngnathus abaster* males and females differed significantly in $L_T$ [*t*-test, $n = 176$ (79 females and 97 males), $P < 0{\cdot}001$] with females being larger than males (female: mean = 9·19 cm, range: 6·7–12·7 cm; male: mean = 8·46 cm, range: 5·2–13·5 cm).

Pregnant males caught in salt ponds carried an average of 37 eggs ($n = 58$, mean ± S.D. = 37 ± 11 eggs, range: 10–64 eggs) and a significant correlation was found between male size and the number of eggs in the marsupium ($n = 58$, $r = 0{\cdot}554$, $P < 0{\cdot}01$) (data from males that were already pregnant when collected) (Fig. 1).

The courtship and mating ritual, unlike the otherwise cryptic nature of the species, was marked by rather conspicuous behaviours, namely several movements in the water column, above the vegetation canopy. Mating occurred

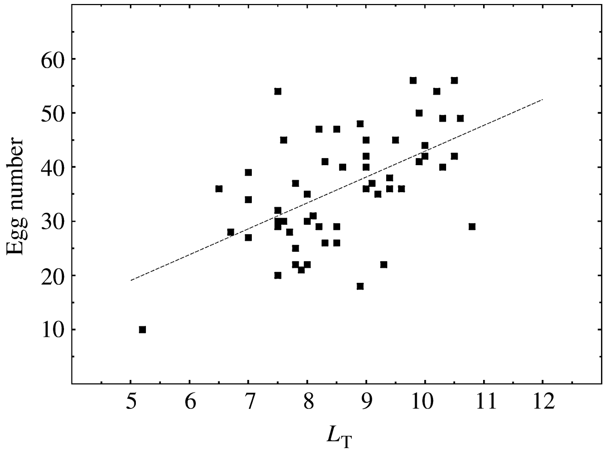

FIG. 1. Relationship between male total length and the number of eggs carried. The curve was fitted by $y = 4{\cdot}7742x - 4{\cdot}8064$.

throughout the day and was preceded by an increase in basal activity levels together with numerous interactions among the fish. This increase was specially pronounced in females that usually initiated displays, mainly consisting of vertical swimming movements in the water column. The onset of courtship was also marked by easily perceivable morphological alterations on both sexes. Females showed an expanded genital papilla (a small, fleshy area near the anus, from which the eggs were released) and a more contrasted colouration, especially in the trunk (Fig. 2). Males presented swollen pouch folds, particularly in the vicinity of the genital area.

The reproductive ritual of *S. abaster* consisted of three distinct phases, marked by prominent behavioural changes that can be summarized as follows. Initial courtship [Fig. 3(a), (b), (c)]: the first stage of the courtship ritual was characterized by mutual flickering movements that affected the entire body rather than just the anterior section of the fish, as observed in *Nerophis lumbriciformis* (Jenyns) (Monteiro *et al.*, 2002). It consisted mainly of rapid and vigorous bends moving along the main axis of the body. Both females and males approached the opposite sex, without either of the sexes showing greater initiative ($n = 17$ courtship trials, male: nine initiatives, female: eight initiatives, $P > 0{\cdot}05$). Females tended to flicker first ($\chi^2$, $n = 17$ courtship trials, male: 3, female: 14, $P < 0{\cdot}05$), but not more often ($\chi^2$, $n = 142$ flickering movements observed in 17 courtship trials, male: 69, female: 73, $P > 0{\cdot}05$). After the first flicker, if the opposite sex flickered in response, both individuals performed rapid side-by-side vibrations while swimming through the aquarium in a more or less parallel position (73% of trials). Females tended to lead these short movements ($\chi^2$, $n = 34$ leads in 17 courtship trials; male: nine leads, female: 25 leads; $P < 0{\cdot}01$) that spanned over mean ± S.D. 50·2 ± 21·1 cm, and generally occurred only a few cm above the substratum (mean ± S.D. 8·8 ± 3·8 cm) but were sometimes ($n = 5$ courtship trials) interrupted by more pronounced elevations in the water column (up to 35 cm). During this stage, both males and females approached its partner, both from below (35%) and above (65%), and touched either with the tails (57%) or flanks (43%) [Fig. 3(b), (c)]. The flickering behaviour continued, with increased frequency, and the female's genital papilla became clearly visible. The relative length of time of this phase, from the first flicker to the beginning of spawning, was highly variable among observed pairs ($n = 10$, range: 1–18 min, mean ± S.D. = 8 ± 7 min), due to a possible variability in the predisposition to mate, but mainly due to disruptions caused by other individuals (especially females; 15 out of 16 observed disruptions) that tended to place their bodies between the courting

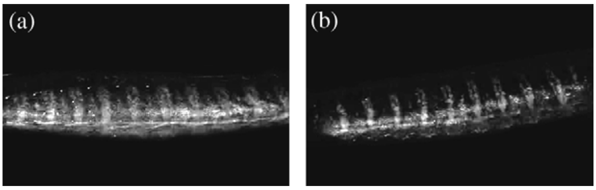

FIG. 2. Female 'amplifiable ornament' (a) in isolation and (b) interacting with another female (increasing contrast in the striped pattern).

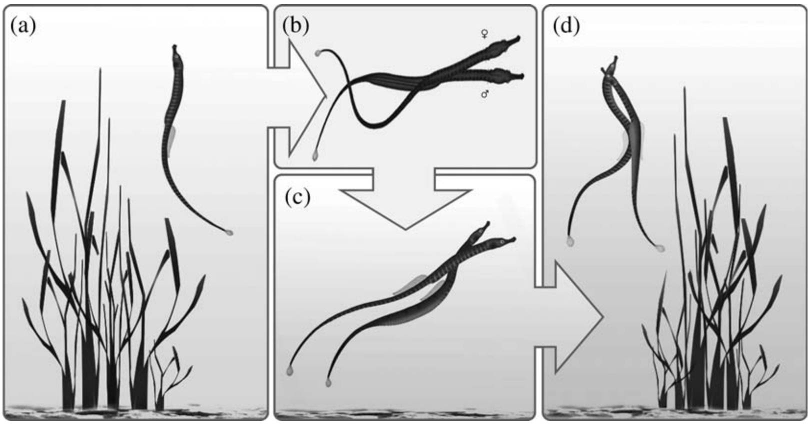

FIG. 3. Overview of the main behavioural stages of courtship and mating: (a) vertical swimming, (b) crossing, (c) parallel swimming and (d) spawning.

pair. Spawning [Fig. 3(d)]: after the initial courtship phase, the female placed its distended genital papilla in the anterior area of the male's marsupium, whose pouch folds were separated and visibly swollen. As soon as the genital areas were in close contact, the mating pair slowly ascended in the water column (up to 45 cm), vigorously vibrating the dorsal fin and usually performing a few rotations (11 out of 17 documented matings). During this spawning phase, that lasted, mean ± S.D. 16 ± 6 s ($n = 17$, range: 6–25 s), the female rapidly transferred a few eggs into the male's brood pouch ($n = 14$, mean ± S.D. = 17 ± 6 eggs, range: 4–30 eggs). Falling eggs, outside the marsupium, were visible in 24% of the observed transfers. Swaying: immediately after egg transfer, the female swam directly into the substratum while the male continued to swim in the water column performing quick and violent body contractions. This behaviour was probably responsible for the packing of the eggs in the posterior end of the marsupium. Even though sperm ejaculation was not directly visible, fertilization probably occurs inside the marsupium, immediately after egg transfer (Kvarnemo & Simmons, 2004). At the end of this phase ($n = 17$, mean ± S.D. = 26·0 ± 3·0 s, range: 21–31 s), the male slowly descended to the substratum where it stayed motionless (mean ± S.D. 95·0 ± 85·4 s), sometimes assuming an S-like position (seven in 17 matings), as also observed in *Syngnathus acus* L. and *Syngnathus typhle* L. (Vincent *et al.*, 1995).

After a variable latency period (mean ± S.D. = 15·0 ± 6·4 min), a new courtship ritual might occur. In aquaria, some pairs (three in eight) were observed to spawn up to three consecutive times and, interestingly, one female was observed spawning with three different males within <30 min. Even though the number of mating pairs was low, a significant correlation was observed between male and female size ($n = 17$, $r = 0{\cdot}920$, $P < 0{\cdot}01$). In the case of courting pairs that did not achieve copulation, a non-significant correlation was observed ($n = 11$, $r = 0{\cdot}042$, $P < 0{\cdot}05$).

The general pattern of courtship behaviour seems reasonably conserved within the family, basically consisting of three main phases: prespawning, egg transfer and postspawning, with fertilization probably occurring during or shortly after egg transfer from the female to the male's incubating surface (Fiedler, 1954; Vincent *et al.*, 1995; Masonjones & Lewis, 1996; Matsumoto & Yanagisawa, 2001; Monteiro *et al.*, 2002; Berglund & Rosenqvist, 2003). Although behaviour analysis was based solely on diurnal observations, the courtship ritual observed in *S. abaster* falls within this general framework. This general pattern found in diverse genera representing distinct phylogenetic lineages (Wilson *et al.*, 2003) seems to be secondarily modified in some species that live in habitats where displays in the water column are probably disadvantageous. Indeed, a comparison of two species of the genus *Nerophis* seems informative in this respect: *Nerophis ophidion* (L.) spawns in relative calm waters, rising up before, during and after egg transfer (Fiedler, 1954), whereas *N. lumbriciformis*, which spawns in the rock intertidal, shows a complete absence of vertical displays (Monteiro *et al.*, 2002). A similar evolutionary reduction of courtship displays in the water column was also documented by Almada & Santos (1995) for blenniids that spawn in habitats subjected to strong wave action.

Another widespread phenomenon seems to be the occurrence of disruptions during the first phase of the courtship ritual. During the aquarium observations, females were observed approaching the courting pair and beginning to actively flicker or merely following the pair in a parallel motion, a behaviour that might be viewed as a form of competition (Matsumoto & Yanagisawa, 2001). Curiously, the intruding female was usually unable to mate with the courting male. Only in one occasion was the opposite observed. Nevertheless, these disrupting females were far more successful in redirecting the other female's attention, causing the apparent end of the ongoing courtship display. Similar observations have been described for *Corythoichthys haematopterus* (Bleeker) (Matsumoto & Yanagisawa, 2001), *Syngnathus schlegeli* Kaup (Watanabe *et al.*, 2000) and *N. lumbriciformis* (Monteiro *et al.*, 2002), suggesting that the still poorly understood female–female interactions, that greatly vary among syngnathid genera, play an important role in the competition for access to mates (Berglund & Rosenqvist, 2003). As in *N. ophidion* and *S. typhle* (Berglund & Rosenqvist, 2003), the apparent absence of overtly aggressive interactions among *S. abaster* females suggests that female dominance occurs mainly through sexual signalling, namely a more contrasted colouration in the trunk (see Fig. 2). This ornamentation seems to be an amplification of the normal colouration, similar to what has been described both for *S. typhle* (Berglund & Rosenqvist, 2003) and *N. lumbriciformis* (Monteiro *et al.*, 2002), a phenomenon that might be directly involved not only in male attraction but also in female–female interactions.

The observed sexual dimorphism in size (females being larger than males) and behaviour suggest sex-role reversion in the sampled population of *S. abaster* (at least under an even sex ratio), a phenomena already documented for several other syngnathids (Kornienko, 2001; Monteiro *et al.*, 2002; Berglund & Rosenqvist, 2003) as well as other fish families [Kuwamura, 1985 (Apogonidae); Balshine-Earn & McAndrew, 1995 (Cichlidae); Swenson, 1997 (Gobiidae)].

Recently, several hypotheses have been presented on the evolution of sex-role reversal in syngnathids, superimposing behavioural information on phylogenetic data (Wilson *et al.*, 2003). Still far from a definitive result, genetic evidence suggests a pattern still difficult to analyse within syngnathids, with sex-role reversal appearing to be largely independent of pouch complexity (Wilson *et al.*, 2003). These difficulties may well arise from two distinct factors: 1) observations do not take into account the natural variation in the degree of sex-role reversal that might exist in geographically distinct populations within a species. Factors such as the operational sex ratio (Vincent *et al.*, 1994), mate quality (Berglund & Rosenqvist, 2003), differences between sexes in the relative 'time in' (availability to mate) *v*. 'time out' period (Masonjones & Lewis, 2000), as well as predation, breeding resources and mate rate encounters are thought to locally modulate the final expression of the sex-role reversal, not only in syngnathids (Almada *et al.*, 1995; Forsgren *et al.*, 2004). For example, *S. schlegeli*, from Vostok and Amurskii bays, is described as presenting conventional sex roles by Kornienko (2001), while Watanabe *et al.* (2000) present data suggesting that the same species, from Otsuchi and Funakoshi Bays, is a sexually reversed syngnathid; 2) observations largely depend on a binary definition of sex-role reversal (reversed *v*. conventional). Although *S. abaster* proved to be sex-role reversed, there is a considerable difference in the magnitude of some measured variables, such as the role of the male during courtship ritual, when compared with other Western European syngnathids, also reported as sex-role reversed species (Kornienko, 2001; Monteiro *et al.*, 2002; Berglund & Rosenqvist, 2003). *Syngnathus abaster* males have a more active role in courtship, flickering and sometimes approaching females, when compared to *N. lumbriciformis* (Monteiro *et al.*, 2002), a species where males are much more passive. Moreover, sexual dimorphism is more pronounced in *N. lumbriciformis* (Monteiro *et al.*, 2002) and *N. ophidion* (Berglund & Rosenqvist, 2003), with differences in colouration among the sexes being observed not only in the middle region of the body (amplifiable ornament display) but also in the head of the females. *Nerophis ophidion* females present bright blue colour markings along the sides of the head while *N. lumbriciformis* females exhibit an intricate pattern of bright facial spots (Monteiro *et al.*, 2005*a*). A moderate sex-role reversion has also been reported for the tidewater goby, *Eucyclogobius newberryi* (Girard), where females display a secondary sexual trait and compete for mates more intensely than do males. Nevertheless, males also engage in intrasexual competition for territories and sometimes also initiate courtship (Swenson, 1997).

More detailed information on syngnathid courtship and mating patterns, encompassing population heterogeneity and what seems to be the clinal nature of reproductive behaviours, would certainly allow for a more conclusive phylogenetic analyses of the evolution of the reproduction within the Syngnathidae family.

Although genetic data are lacking and observations reported in this paper are from captive fish, available evidence suggests that this pipefish is polygynandrous since females were observed mating with different males, sometimes within a very short time period, and males were also observed receiving

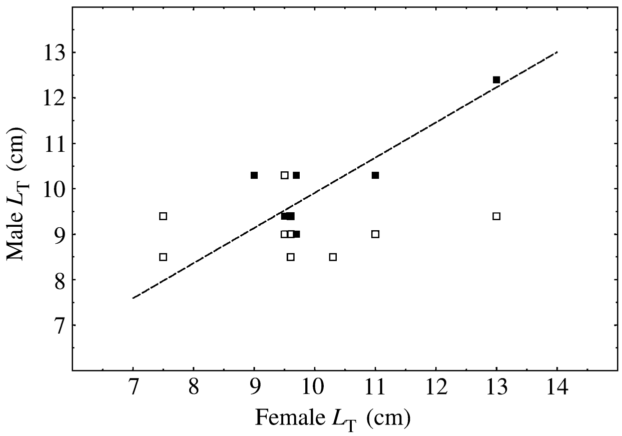

FIG. 4. Male and female total length relationships in successful (■; $y = 0{\cdot}7774x + 2{\cdot}16$) and unsuccessful (□; NS) *Syngnathus abaster* mating pairs.

eggs from distinct females. In general, more polyandrous species show greater degrees of sexual dimorphism, as a result of an increased intensity in sexual selection (Avise *et al.*, 2002). Polygynandrous mating systems, however, have been reported for two sex-role reversed species with intermediate degrees of sexual dimorphism [*S. typhle* and *Syngnathus floridae* (Jordan & Gilbert) Jones & Avise, 2001], as well as for the strongly sexual dimorphic sex-role reversed *N. lumbriciformis* (Monteiro *et al.*, 2006), possibly suggesting a more elaborated pattern than the generally accepted continuum sexual selection-mating system (Avise *et al.*, 2002).

Clutch size of pregnant *S. abaster* males (10–64 eggs) are at the lower end of the range reported for other syngnathids, with *Hippocampus reidi* Ginsburg and *Hippocampus erectus* Perry presenting the largest reported clutch size (Monteiro *et al.*, 2005*b*).

The observed significant correlation between male size and egg number may reflect a $L_T$-dependent reproductive fitness (Fig. 1), with larger males accommodating more offspring, as also reported for *S. typhle* (Berglund & Rosenqvist, 2003) and *S. schlegeli* (Watanabe & Watanabe, 2002). Accordingly, $L_T$ seems also to affect mate choice, as suggested by the observed significant correlation between male and female size ($n = 17$ successful mating pairs, $r^2 = 0{\cdot}846$; Fig. 4). Courting pairs with more asymmetrical $L_T$ seemed less successful in achieving mating ($n = 11$ unsuccessful mating pairs, $r^2 = 0{\cdot}002$, $P > 0{\cdot}05$).

We would like to thank the anonymous referees for their criticism and suggestions as well as everyone who helped during the fieldwork, especially A. Silva, A. Jorge and P. Correia. V. Almada's participation was partially funded by Programa Plurianual de Apoio às Unidades de Investigação. N. Monteiro's participation was funded by Fundação para a Ciência e a Tecnologia. K. Silva's participation was funded by Fundação para a Ciência e a Tecnologia (FCT-SFRH/BD/13171/2003).

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