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Early life history of *Syngnathus abaster*

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The embryonic and larval development of the pipefish *Syngnathus abaster* is described, based on *ex situ* observations. The full development sequence lasted 24–32 days (at 18–19° C), which was shortened to 21 days at higher temperatures (21–22° C). Newborn juveniles, with a uniform dark brown colouration, immediately assumed a benthic spatial distribution. This vertical distribution pattern remained unchanged at least during the first 4 weeks, after the release from the marsupium. The apparent absence of a pelagic life phase might have important repercussions in terms of population connectivity given increasing fragmentation and degradation of the eelgrass habitat in the species' range.

INTRODUCTION

The Syngnathidae (pipefishes, pipehorses, seadragons and seahorses) exhibits one of the most specialized forms of parental care, with females depositing eggs in a specialized incubating area, located either on the abdomen (Gastrophori) or tail (Urophori) of the males (Herald, 1959). Even though male pregnancy is a widespread characteristic in all syngnathids, the anatomical complexity of the brooding structures varies among species, from the simplest incubating ventral surface of the Nerophinae, where eggs are glued without any protective plates or membranes, to the Hippocampinae sealed brood pouch. This gradient in conspicuous male parental care structures was used by Herald (1959) in order to propose a phylogeny of the family, and the main results were later confirmed by Wilson *et al.* (2001, 2003), using mitochondrial DNA.

A comparative study on three anatomically distinct brooding structures highlighted different ultrastructures, suggesting distinct functions related to different reproductive strategies (Carcupino *et al.*, 2002). The underlying anatomical variations visible among the various pouch types may also signal distinct early life histories since the interactions between the male body and the developing embryos are inversely proportional to the degree of egg exposure to the external

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environment. The relative degree of development exhibited by newborns after release from the parent may be crucial to immediate survival and larval dispersal.

The main aim of this work was to extend understanding on syngnathid biology by describing, for the first time, the developmental sequence, from egg to newborn, of captive black striped pipefish *Syngnathus abaster* Risso, a data deficient species (IUCN, 2004). Simultaneously, the vertical distribution of newborns was analysed, as a means to evaluate the benthic or pelagic nature of the juveniles, a variable that has profound implications in the population dynamics of pipefishes that inhabit increasingly fragmented habitats, such as eelgrass meadows.

MATERIAL AND METHODS

Syngnathus abaster is a euryhaline species with a restricted distribution area that includes the Mediterranean and Black Seas, northward to the southern Bay of Biscay (northern Spain). The fish can be found either in coastal areas or in brackish and fresh waters (Cakic *et al.*, 2002), mainly among sand, mud or eelgrass meadows, between depths of 0·5 and 5 m, within a temperature range of 8–24° C (Dawson, 1986; Froese & Pauly, 2005). Males can easily be distinguished from females by the presence of the brood pouch (marsupium) located on the tail, formed by two skin-folds that contact medially with their free edges.

Eggs and larvae were obtained (August 2003 to March 2004) from wild specimens collected in a salt pond reservoir, at the Ria de Aveiro estuarine lagoon (40° 45′ N; 8° 40′ W), in Portugal, that successfully copulated in aquaria (15 male pregnancies were followed). Captive fish were fed daily with fresh *Artemia franciscana* nauplii and maintained in 80 l aquaria, illuminated with natural light, supplemented with 18 W fluorescent lamps. Due to the 'gas bubble disease', common in pipefishes (A. Berglund, pers. comm.), aeration was performed outside the fish tanks. Continuously running sea water was physically and biologically filtered and temperature was maintained within two different ranges, 18–19° C and 21–22° C. Substrata consisted mainly of sand and plastic seagrass laid in order to mimic the original habitat from where the fish were caught. Once copulation occurred, pregnant males were isolated and eggs were removed daily from the marsupium (except during the first day, when egg extraction occurred several times) and immediately preserved in 4% formalin. Both eggs and larvae were observed and photographed with a Leica stereomicroscope attached to a digital video camera.

In order to further extend knowledge on syngnathid early life history, newborn juveniles were maintained in the same conditions as adults, since they were already capable of eating *A. franciscana* nauplii. Juvenile total length (L_T) was recorded using a ruler (25 juveniles were continuously measured during the first 4 weeks). Since this was a simple and quick handling procedure, no mortality was observed due to fish manipulation. The vertical distribution of juveniles was also studied by separately placing individuals inside small 4 l aquaria (40 × 10 × 10 cm), distinct from those used for pregnant adults and with no water circulation, divided in three sections: surface (10 cm), middle section (20 cm) and bottom (10 cm). After an initial 'resting' period (*c.* 45 min), during which black striped pipefish were allowed to familiarize with the new surroundings, 10 min observation periods were conducted on the time juveniles spent in the 'surface' and 'bottom' sections. In order to assure data independence, each observation was conducted using different individuals, from different broods, that were only used once (10 trials measuring time spent near surface and another 10 trials aimed at measuring time spent in the bottom section). This experimental design was repeated during 3 consecutive weeks, always using different individuals. An orthogonal ANOVA with two factors [time (three levels = 3 weeks) and vertical position (two levels = surface and bottom)] was conducted. Data were transformed ($\ln x + 0{\cdot}5$) in order to obtain homogeneity of variances (Cochran's test; $P > 0{\cdot}05$). All statistical analyses were performed using Statistica 6.1 (Statsoft).

RESULTS

The bright orange eggs of *S. abaster* were almost spherical when spawned, measuring *c.* 1·6 mm ($n = 45$; mean ± S.D. 1·58 ± 0·24 mm; range = 1·09–2·06 mm), but became quite variable in shape due to different degrees of compression caused by adjacent eggs. Males carried embryos for *c.* 24–32 days (18–19° C; $n = 24$, mean ± S.D. number of eggs per males = 40·67 ± 10·10; range = 18–64) and gave birth, during a 2–3 day period, to completely independent young measuring *c.* 18 mm ($n = 64$; mean ± S.D = 17·67 ± 0·22 mm; range = 14–23 mm). At higher temperatures (21–22° C) embryonic and larval development was concluded within 21 days (four male pregnancies were observed). The main ontogenetic stages are summarized in Fig. 1 and representative pictures of the developmental process (18–19° C) are given in Fig. 2.

Cleavage in *S. abaster*, as in all bony fishes, is meroblastic (incomplete), leading to a formation of a mass of cells sitting atop of the yolk. Embryos entered the blastula stage *c.* 24 h after fertilization with the blastodisc presenting a regular hemispheric shape. Gastrulation began 72 h after fertilization and was characterized by the flattening of the blastodisc (epiboly) and the formation of the embryonic shield as a thickening of the germ ring [Fig. 2(a)]. By this time, the two edges of the marsupium folds strongly adhered to each other. The neural tube and the dorsal corda were apparent in the anterior region of the embryo body from the half epiboly stage (7 days after fertilization) [Fig. 2(b)]. During this period the embryo presented a well developed head, body and tail. The cephalic region was already discernable and the optic vesicles were visable. The anlagen of the crystalline lens and the ocular pigmentation were visible by day 9. Somite formation started *c.* 8 days after fertilization and, as development continued, the rudiments of the organs became visible, with the tail region now free from the yolk as the embryo elongated and fins became visible. Melanogenesis started on the head region after 8 days of development and, by day 10, some pigmented cells were visible along the antero-posterior embryonic axis [Fig. 2(c)]. After 14 days of development the heart beat and blood vessels were

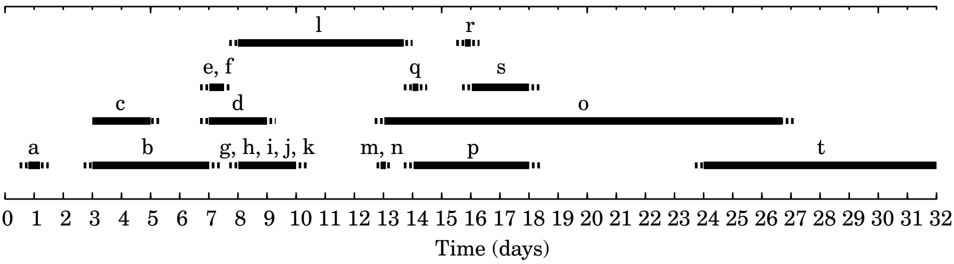

FIG. 1. Ontogenetic events occurring during the embryonic and larval development of *Syngnathus abaster* in order of first appearance (a, blastula stage; b, epiboly; c, embryonic shield recognizable; d, cephalic and caudal dilatation; e, optic vesicles; f, notochord and neural tube differentiation; g, beginning of somite formation; h, crystalline lens; i, ocular pigmentation; j, tail region free from yolk; k, beginning of melanogenesis; l, fin differentiation; m, heart beats and visible blood vessels; n, embryo motility; o, development of mouth apparatus; p, development of fin rays; q, dermal plates; r, brownish colouration; s, hatch from egg envelope; t, release from marsupium).

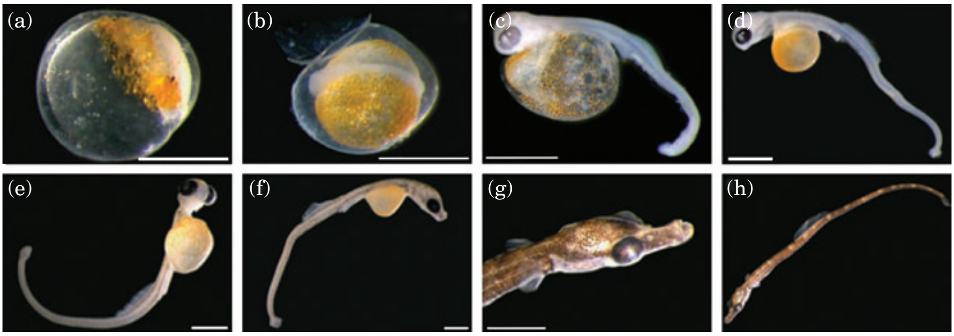

FIG. 2. Different developmental stages in *Syngnathus abaster*: (a) 3 days, (b) 7 days, (c) 13 days, (d) 17 days, (e) 19 days, (f) 21 days, (g) newborn head detail and (h) newborn juveniles. Scale bars 1 mm.

visible and embryos exhibited some motility, responding to touch. During the subsequent days fin rays began to differentiate [Fig. 2(d)] and the embryos started hatching from the egg envelopes, but remaining within the marsupium whose edges increased in volume and were now easily separable. As development proceeded, the yolk was gradually absorbed, with the elements of the dermal plates visibly developing and the mouth apparatus becoming elongated, acquiring the typical adult form [Fig. 2(e), (f)]. Finally, 24–32 days after fertilization, the embryonic development of *S. abaster* was completed and fully formed dark brown juveniles, resembling adult individuals [Fig. 2(g), (h)], were released from the marsupium by sharp bending movements of the male's body.

A significant difference was found between time spent near the surface or bottom of the aquaria (Table 1). Newborn juveniles spent most of the time near the bottom with only some sporadic movements towards the surface, followed by a return to the bottom section of the aquaria. The observed vertical distribution did not significantly change during the following weeks, but a slight reduction in time spent near the surface was observed (Fig. 3). Juveniles used for the calculation of the growth ($n = 25$) doubled in L_T within the first 4 weeks after abandoning the parental marsupium (mean ± S.D. initial size = 15·60 ± 0·22 mm; 4 weeks old = 3·14 ± 0·28 cm).

No evidence of schooling behaviour was ever observed when juveniles were maintained together. In fact, corroborating the results of the vertical distribution

TABLE I. ANOVA results on the vertical distribution of *Syngnathus abaster* juveniles during the first 3 weeks after abandoning the marsupium

Source	d.f.	*MS*	*F*	*P*
Time	2	5·7270	1·45	0·2426
Vertical distribution	1	487·5689	123·80	<0·001
Time × vertical distribution	2	3·4449	0·87	0·4228
Residuals	54	3·9385		
Total	59			

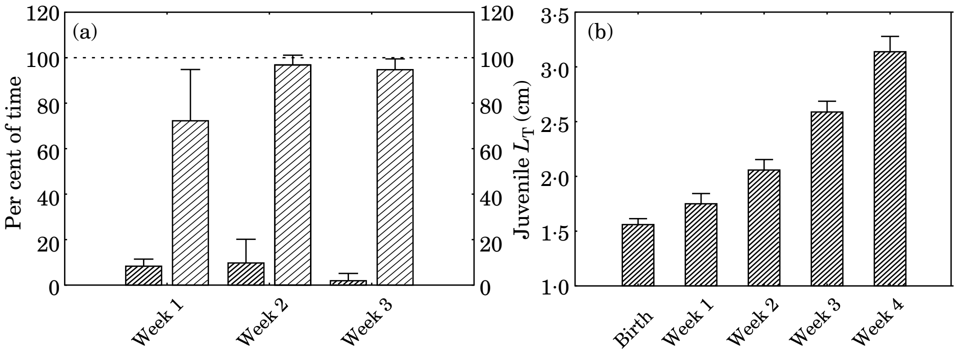

FIG. 3. (a) Vertical distribution in the water column [mean ± S.D. per cent time spent at the surface (▨) and bottom (▨)] displayed by juvenile *Syngnathus abaster* during the first 3 weeks after abandoning the marsupium and (b) mean ± S.D. juvenile total length during the first 4 weeks.

experiment, the juveniles, with a cryptic colouration, were usually observed in close contact with the substratum laid in the bottom of the aquarium.

DISCUSSION

The observation of the embryonic and larval development of *S. abaster* reinforces the notion that the presence of a marsupium contributes to a higher degree of newborn development, when compared to a marsupium-lacking pipefish such as *Nerophis lumbriciformis* (Jenyus) (Monteiro *et al.*, 2003) or *Nerophis ophidion* (L.) (Russell, 1976). As also observed in *Syngnathus acusimilis* Günther (Drozdov *et al.*, 1997), *S. abaster* larvae hatch from the egg envelope before complete formation of the mouth apparatus, still exhibiting the final remains of the yolk sack. The final stages of development occur inside the marsupium, without the physical constraints of the limited egg space. As a result, marsupium presenting pipefishes of the genus *Syngnathus* give birth to fully developed juveniles while marsupium-lacking pipefishes of the genera *Nerophis* and *Entelurus* produce less developed larvae (Russell, 1976; Monteiro *et al.*, 2003), whose morphology is still some way from the adult form. The observed differences in the final degree of development might have an important role in larvae early life history behaviour. Contrary to *N. lumbriciformis* newborn larvae, that immediately showed vertical swim-up and drift behaviour, using the pectoral fins to rotate along the body axis (Monteiro *et al.*, 2003), *S. abaster* juveniles occupy the bottom of the aquaria shortly after birth, rapidly growing, thus suggesting a benthic strategy (Fig. 3). During regular monthly samples conducted for >1 year, aimed at collecting fish larvae in the water column of a Portuguese river estuary (Lima River, northern Portugal), only two juveniles were captured, even though *S. abaster* is a common inhabitant of the estuary (S. Silva, unpubl. data).

Early life history behaviour is of great importance for the species ecology as it might determine population demography and 'connectivity' (Cowen *et al.*, 2000). Since, mainly due to habitat reduction and environmental degradation (Nagelkerken *et al.*, 2000), the total area occupied by eelgrass meadows is currently receding in southern Europe (Duarte, 2002), black striped pipefish populations face new and challenging problems. The observed behaviour of newborn juveniles, unlike that registered for *N. lumbriciformis* or *N. ophidion*, whose larvae display a clear pelagic life phase (Russell, 1976; Monteiro *et al.*, 2003), may imply a limited dispersion capability, thus contributing to the ongoing isolation of geographically distant populations (*S. abaster* populations seem to be confined to estuaries and salt pond reservoirs within mainland Portugal; N.M. Monteiro, pers. obs.). An increasing number of factors (the destruction and reduction of the habitat, the increasing geographical distance among populations and the apparent benthic behaviour of the juveniles) interact and contribute to a decrease in connectivity between populations.

The role of connectivity patterns *via* larval dispersal in structuring marine populations has been a central issue in marine ecology (Palumbi, 1999; Cowen *et al.*, 2000; Armsworth, 2002) and increasing knowledge of syngnathid early life history would increase the understanding of how developmental and behavioural processes may affect population persistence and evolution. Within pipefishes, species without a marsupium seem to produce smaller and less developed larvae, having a primordial fin and transparent colouration, that display pelagic early life history behaviour, *e.g. N. lumbriciformis*, *N. ophidion* and *Entelurus aequoreus* (L.) (Russell, 1976; Monteiro *et al.*, 2003). Pipefish species with a marsupium, with the exception of the small juveniles (13–14 mm) produced by *Syngnathus rostellatus* Nilsson (Froese & Pauly, 2005), seem to produce more developed juveniles that immediately take a bottom life on release from the parent (*Syngnathus acus* L., *Syngnathus typhle* L. and *S. acusimilis*) (Russell, 1976; Drozdov *et al.*, 1997). In the specific case of the genus *Hippocampus*, even though some authors state that seahorses avoid a planktonic larval phase (Schmid & Senn, 2002), as might be the case of *Hippocampus fuscus* Rüppell (Golani & Fine, 2002), several species are recurrently observed in open sea, (*e.g. Hippocampus mohnikei* Bleeker, *Hippocampus comes* Cantor, *Hippocampus spinosissimus* Weber and *Hippocampus abdominalis* Lesson (Kanou & Kohno, 2001; Foster & Vincent, 2004).

An interesting observation that, as far as is known, has not yet been described, deals with filial cannibalism. Even though juveniles were already able to feed on *A. franciscana* nauplii (supplied on a daily basis), the density of newborn was initially observed to dramatically decrease. The direct observation of adults feeding on juveniles (sometimes their own) confirmed the hypothesis that filial cannibalism does occur, since newborn are sufficiently slender to pass through an adult's mouth opening (K. Silva, pers. obs.). Even though potentially negligible in the wild, this phenomenon might be a serious concern in aquarium-reared individuals whose larvae are smaller than an adult's mouth. Thus, the type of substratum used (with plants and crevices) might play an important role in the rate of juvenile survival during the first development stages.

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