

# Implications of different brood pouch structures in syngnathid reproduction

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The evolutionary radiation of the family Syngnathidae was accompanied by a diversification of structures involved in male parental care whose anatomical variations may signal differences in reproductive strategies, with increasing egg protection possibly affecting female investment in offspring or larvae quality at the end of the embryonic development phase. An analysis of egg numbers showed significant differences between syngnathids with and without marsupium, suggesting that the brood pouch, besides increasing protection to the male and its eggs, also introduced the ability to carry an additional number of offspring per pregnancy, from one or more females. Curiously, even though larvae sizes do not significantly differ between the considered brooding structures (controlling for male length), seahorse larvae were smaller than expected, given the large volume of the pear-shaped eggs. These observations suggest that the sealed seahorse pouch, although allowing the transport of a great number of offspring and capable of multiple consecutive pregnancies, may impose serious functional constraints, namely those related with oxygenation of the developing larvae, thus explaining the increased egg surface together with the presence of specific salinity control mechanisms inside the marsupium.

## INTRODUCTION

The family Syngnathidae (pipefish, pipehorses, sea-dragons and seahorses) exhibits one of the most specialized forms of parental care, with the female depositing eggs in a specialized incubating area, located either on the abdomen (Gastrophori) or tail (Urophori) of the male who, according to each species, undergoes a variable incubation period (Herald, 1959). The phylogenetic relationships within this family have been studied both by Herald (1959), based on morphological observations, namely the position and closure method of the incubating area, and Wilson et al. (2001, 2003), who proposed a new mitochondrial DNA-based phylogeny. Both approaches agree that the evolutionary radiation of this group was accompanied by a diversification of structures involved in parental care, from an ancestral pipefish, that probably presented a rather simple brooding structure. In fact, various degrees of brood pouch complexity are still visible today in the family Syngnathidae, from the simplest incubating area, typical of the Nerophinae to the sealed pouch of the Hippocampinae (Herald, 1959). A comparative anatomical study of three different brood structures has been presented by Carcupino et al. (2002), describing the morphology and ultrastructure of the incubating surface of *Nerophis ophidion*, *Syngnathus abaster* and *Hippocampus hippocampus* (pouch types B1, A4i and A5, respectively (Herald, 1959)). The only common feature to all studied brood pouches was the presence of a vascularized dermis, thus suggesting distinct functions related to different reproductive strategies (Carcupino et al., 2002). As also stated by Carcupino et al. (2002), the interactions

between the male brood structures and the developing embryos are inversely proportional to the degree of egg exposure to the external environment. In marsupium-lacking pipefish, such as *Nerophis* or *Entelurus*, the open pouch functions primarily as an adequate substratum for egg development, even though some modifications in the brood area epithelium are visible, such as the convoluted microridges, that suggests an extension in the surface area that contacts with the eggs. Other complex morphological and ultrastructural modifications are visible in species that present a brood pouch, namely the presence of MR cells (mitochondria-rich cells), usually associated with an important osmoregulatory role (Watanabe et al., 1999), and a more vascularized dermis beneath the epithelium that contacts with the eggs.

Evolutionary theory predicts that organisms should attempt to maximize their reproductive success (Darwin, 1871; Clutton-Brock & Parker, 1992) but it does not point out the right direction to follow, since the number of paths is surely immense and different possibilities might just produce equally favourable results. Hence, the simple attachment of eggs occurred in a male pipefish ancestor that carried the eggs away from nest predators and sneaker fertilizations (Wilson et al., 2001) was just the starting point to a new set of evolutionary possibilities and constraints within the Syngnathidae family.

A positive correlation between egg size and the amount of parental care provided to embryos has long been assumed and documented in fish (Gross & Sargent, 1985; Kolm & Ahnesjö, 2005). Also, it is also generally accepted that egg size influences offspring quality. Thus, within the family Syngnathidae, data were gathered on

**Table 1.** Metric data gathered for several *Syngnathidae* species, distributed by three distinct brooding structure groups.

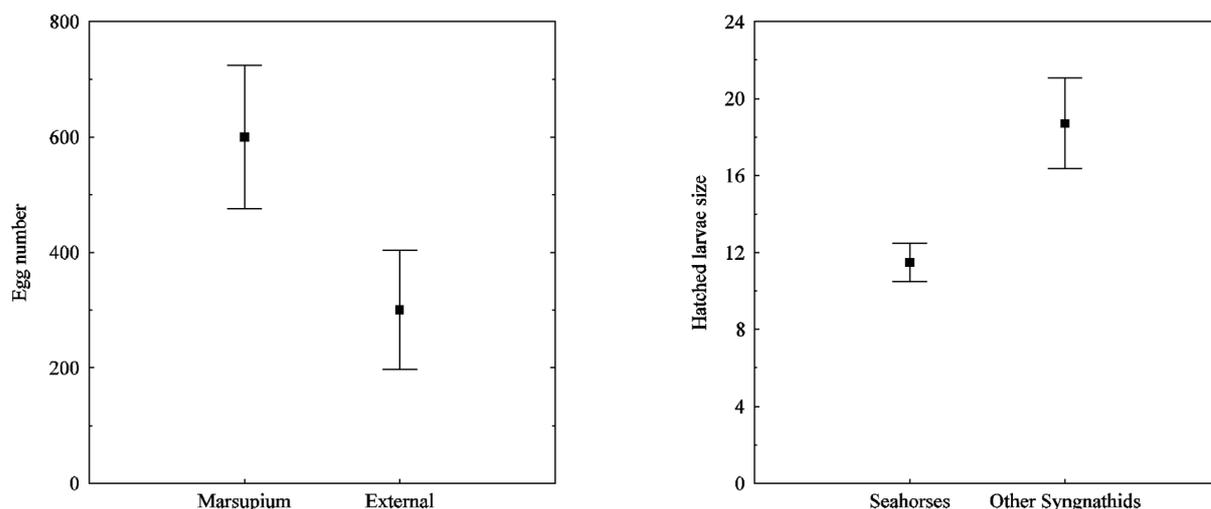
Species name	Marsupium type and location	Maximum reported male length (mm)	Maximum reported number of eggs	Maximum reported egg size (mm)	Maximum reported hatch size (mm)	Information source*
<i>Hippocampus abdominalis</i>	SP (U)	350	1116	2.0	16.2	1,2,3,4
<i>Hippocampus breviceps</i>	SP (U)	100	100	1.6	8.9	1,2,3,4
<i>Hippocampus comes</i>	SP (U)	187	350	1.4	9.5	1,2,3,4
<i>Hippocampus erectus</i>	SP (U)	190	1552	1.5	10.0	1,2,3,4,5
<i>Hippocampus fuscus</i>	SP (U)	144	150	1.8	7.5	1,2,3,4
<i>Hippocampus guttulatus</i>	SP (U)	180	581	2.0	11.8	1,2,3,4
<i>Hippocampus hippocampus</i>	SP (U)	150	865	1.6	9.3	1,2,4
<i>Hippocampus kuda</i>	SP (U)	280	1405	2.1	7.0	1,2,4
<i>Hippocampus reidi</i>	SP (U)	175	1572	1.2	6.7	1,2,3,4
<i>Hippocampus trimaculatus</i>	SP (U)	150	1783	1.8	6.1	1,2,3,4
<i>Hippocampus whitei</i>	SP (U)	130	250	1.8	8.5	1,2,3,4
<i>Hippocampus zosterae</i>	SP (U)	40	55	1.3	7.9	1,2,3,4
<i>Syngnathus abaster</i>	IP (U)	210	100	1.8	23.0	2,6,7,8
<i>Syngnathus acus</i>	IP (U)	460	400	2.5	26.0	2,6,7,8
<i>Syngnathus affinis</i>	IP (U)	218	570	–	–	2,6
<i>Syngnathus auliscus</i>	IP (U)	180	–	0.7	–	2,6,8
<i>Syngnathus californiensis</i>	IP (U)	500	–	1.2	–	2,6,8
<i>Syngnathus carinatus</i>	IP (U)	230	–	1.0	–	2,6,8
<i>Syngnathus euchrous</i>	IP (U)	250	–	1.2	–	2,6,8
<i>Syngnathus exilis</i>	IP (U)	250	–	1.1	–	2,6,8
<i>Syngnathus floridae</i>	IP (U)	270	1000	0.9	–	2,6,8
<i>Syngnathus folletii</i>	IP (U)	200	181	–	–	2,6,8,9
<i>Syngnathus fuscus</i>	IP (U)	330	200	0.8	9.0	2,6,8
<i>Syngnathus insulae</i>	IP (U)	204	–	1.3	–	2,6,8
<i>Syngnathus leptorhynchus</i>	IP (U)	330	225	1.3	4.8	2,6,8
<i>Syngnathus louisianae</i>	IP (U)	380	900	0.8	–	2,6,8
<i>Syngnathus phlegon</i>	IP (U)	200	400	1.4	18.0	2,6,7,8
<i>Syngnathus rostellatus</i>	IP (U)	190	100	1.5	13.5	2,6,7,8
<i>Syngnathus schlegeli</i>	IP (U)	300	1000	1.1	12.0	2,10,11
<i>Syngnathus scovelli</i>	IP (U)	183	296	1.2	13.0	2,6,8
<i>Syngnathus schmidti</i>	IP (U)	110	500	–	–	2,6
<i>Syngnathus springeri</i>	IP (U)	350	1400	–	–	2,6
<i>Syngnathus taenionotus</i>	IP (U)	190	100	–	–	2,6,7
<i>Syngnathus typhle</i>	IP (U)	350	105	2.0	23.0	2,6,7,8
<i>Heraldia</i> sp.	EB (G)	100	100	–	–	6
<i>Dunckerocampus baldwini</i>	EB (G)	150	200	–	–	6
<i>Dunckerocampus chapmani</i>	EB (G)	100	30	–	–	6
<i>Entelurus aequoreus</i>	EB (G)	400	1000	1.2	11.5	2,7
<i>Maroubra perserrata</i>	EB (G)	85	60	–	–	6
<i>Maroubra yasudai</i>	EB (G)	150	140	–	–	6
<i>Nerophis lumbriciformis</i>	EB (G)	150	80	1.1	10.0	2,7,12
<i>Nerophis maculatus</i>	EB (G)	300	186	1.4	11.5	2,7
<i>Nerophis ophidion</i>	EB (G)	300	150	1.2	12.0	2,7
<i>Phycodurus eques</i>	EB (U)	305	300	4.0	35.0	6,13
<i>Phyllopteryx taeniolatus</i>	EB (U)	470	310	5.8	32.0	14
<i>Solegnathus dunckeri</i>	EB (U)	470	120	–	–	15
<i>Solegnathus hardwickii</i>	EB (U)	520	210	4.0	33.0	15
<i>Solegnathus spinosissimus</i>	EB (U)	500	170	5.4	31.0	14

SP, sealed pouch; IP, inverted pouch; EB, external brooders; U, Urophori; G, Gastrophori.

\*Information sources: 1, Foster & Vincent (2004); 2, Froese & Pauly (2005); 3, Lourie et al. (1999); 4, Project Seahorse Life Tables; 5, Teixeira & Musick (2001); 6, Kuitert (2000); 7, Dawson (1986); 8, Vincent (1990); 9, Teixeira & Vieira (1995); 10, Drozdov et al. (1997); 11, Watanabe & Watanabe (2002); 12, Monteiro et al. (2003); 13, Dawson (1985); 14, Davey & Martin-Smith (unpublished data from Tasmanian populations); 15, Connolly et al. (2001).

egg size and number, as well as hatch size, in order to ascertain if the increasing elaboration of brooding structures positively correlates with an increase in offspring quality or female investment per offspring. Moreover, the new possibilities, such as the possibility to carry additional

number of eggs, introduced by the emergence of a more complex brooding structure, the marsupium, were also investigated. It could be expected that, according to both proposed (morphological or molecular) phylogenies, seahorses and external brooders should occupy opposite



**Figure 1.** Mean egg numbers in syngnathids with and without a marsupium (left) and the comparison of mean larvae hatch size in seahorses when compared with all other considered syngnathid species (error bars represent standard errors).

extremes in the results emerging from the several conducted analyses on egg and larvae development, since they represent, respectively, the most evolved and primitive form of egg bearing within the Syngnathidae family. Nevertheless, the low level of structural similarities, visible among different brood pouches, may indicate that the trend 'from pipefish to seahorse', suggested by Herald (1959), could be misleading.

## MATERIALS AND METHODS

Metric data gathered for several syngnathid species with distinct brooding structures were taken from various sources, as indicated in Table 1. Species information was also supplemented with new data collected from aquarium captive syngnathids and information kindly sent both by Project Seahorse and Davey & Martin-Smith (unpublished data). The combined dataset was broken down into three major groups according to pouch anatomy (sealed pouch, inverted pouch and exposed eggs), as summarized in Table 1.

Since seahorse length is measured differently (see Lourie et al., 1999), a conversion factor was calculated both for the newborn larvae and adult individuals. Four seahorse species were used (*Hippocampus hippocampus*, *H. ingens*, *H. mohnikei* and *H. reidi*) due to a shortage of newborn larvae images. In order to maintain some coherence, the same four species were also used for the adult conversion factor, even though many more images are currently available. The following equations were used: larvae recalculated size = larvae height  $\times$  1.26 and adult recalculated size = adult height  $\times$  1.20.

The adult length presented in Table 1 reflects the maximum reported size for the male of that species since some genera, such as *Nerophis lumbriciformis* (Monteiro et al., 2001) or *Entelurus aequoreus* (Dawson, 1986), are clearly dimorphic, with females attaining larger sizes. The number of eggs (in the male), hatch and egg size also reflect the maximum number of eggs reported for each species. The conversion of seahorse heights (adult and larvae) is not included in Table 1.

In order to test if the marsupium allowed syngnathids to carry an additional number of eggs per pregnancy episode, an analysis of covariance (ANCOVA) was undertaken considering the maximum number of eggs, controlling for male size, in two groups that differ in the presence or absence of a marsupium [genera *Hippocampus* and *Syngnathus*] (with a brood pouch) versus external brooders (Table 2A). Data were  $\ln$  transformed. Also, a Spearman correlation was conducted on the number of eggs carried and the degree of marsupium development, with seahorses coded as the most evolved brooding structure (sealed pouch), the genus *Syngnathus* in an intermediate position (inverted pouch) and external brooders as the most simple brooding structure.

Product-moment correlations were calculated for the number of eggs and male dimensions as well as male length and egg size. A linear regression was conducted on the reported hatch size for each species and egg size and the residuals analysed. Taking these results into consideration, in order to test the hypothesis that the sealed environment of a seahorse sealed pouch might have direct repercussions on larval size, an ANCOVA was performed trying to evaluate differences in hatch size, controlling for egg size, in syngnathids that presented a sealed pouch (genus *Hippocampus*) versus all other species with different brooding structures (Table 2B). An ANCOVA was also undertaken in order to evaluate hypothetical differences between the hatch size of species presenting a sealed pouch, inverted pouch and external brooders, controlling for male size (Table 2C).

Information was found on the major and minor axis dimensions of the eggs of *H. abdominalis*, *H. erectus*, *H. trimaculatus*, *Phycodurus eques* and *Solegnathus hardwickii*, syngnathids that exhibit pear-shaped eggs. If we compare the general shape of a pear-shaped egg to a prolate spheroid, we can obtain a reasonably precise measure of its volume and surface [volume =  $4/3\pi ab^2$ ; surface =  $2\pi b^2 + 2\pi(ab/e) \times \arcsin(e)$ ; where  $a$  = major semiaxis,  $b$  = minor semiaxis and  $e = \sqrt{1 - (b^2/a^2)}$ ]. Calculating the egg volume, we may also determine the radius of a sphere with the same volume, and calculate its surface

**Table 2.** Analysis of covariance (ANCOVA) results for the comparison between the number of eggs of syngnathids with or without a marsupium, controlling for male size (A), comparison between hatch size between seahorses and all other considered syngnathid species, controlling for egg size (B) and comparison between hatch size of syngnathids with a sealed pouch, inverted pouch and external brooders, controlling for male size (C).

A. One-way ANCOVA results on egg numbers (controlling for male size).

Source	SS	df	MS	F	P
Adjusted means	9.52	1	9.52	12.77	0.001
Adjusted error	29.05	39	0.74		
Adjusted total	38.56	40			

Homogeneity of regressions

Source	SS	df	MS	F	P
Between regressions	0.00	1	0.00	0.00	0.962
Remainder	29.05	39	0.74		

B. One-way ANCOVA results on hatch size (controlling for egg size).

Source	SS	df	MS	F	P
Adjusted means	125.94	1	125.94	6.97	0.014
Adjusted error	469.60	26	18.06		
Adjusted total	595.54	27			

Homogeneity of regressions

Source	SS	df	MS	F	P
Between regressions	1.58	1	1.58	0.09	0.769
Remainder	468.02	26	18.00		

C. One-way ANCOVA results on hatch size (controlling for male size).

Source	SS	df	MS	F	P
Adjusted means	55.42	2	27.71	0.64	0.537
Adjusted error	1085.80	25	43.43		
Adjusted total	1141.22	27			

Homogeneity of regressions

Source	SS	df	MS	F	P
Between regressions	97.66	1	48.83	0.64	0.308
Remainder	988.15	25	39.53		

(volume= $4/3\pi r^3$ ; surface= $4\pi r^2$ ; where  $r$ =radius). Using this reasoning, it was possible to calculate the surface differences between a pear-shaped egg and a spherical egg with identical volumes. Statistical analyses were performed in Statistica 7.0 (StatSoft).

## RESULTS

Syngnathids with a marsupium (genera *Hippocampus* and *Syngnathus*) significantly differed from external brooders (genera *Dunckerocampus*, *Entelurus*, *Heraldia*, *Maroubra*, *Nerophis*, *Phycodurus*, *Phyllopteryx* and *Solegnathus*) in the number of carried eggs, adjusting for male size (Table 2A). Marsupium presenting syngnathids were able to carry more eggs than external brooders (Figure 1). In fact, the number of eggs that syngnathid males carry was found to be correlated with the degree of marsupium development (Spearman rank order correlation,  $N=42$ ;  $r=0.426$ ;  $P<0.005$ ). Seahorses were able to carry more eggs than pipefish with a marsupium and external brooders (see Table 1).

The number of eggs was found to be independent of the male dimensions (product-moment correlation;  $N=42$ ;  $r=0.135$ ;  $P=0.393$ ). These results were consistent in species that present a closed or inverted marsupium (*Hippocampus*  $N=12$ ;  $r=0.491$ ;  $P=0.105$ ; *Syngnathus*  $N=16$ ;  $r=0.312$ ;  $P=0.240$ ). In marsupium-lacking pipefish that brood embryos in the abdominal area (Gastrophori), a high correlation value was found between male length and the number of carried eggs ( $N=9$ ;  $r=0.771$ ;  $P<0.05$ ). Inversely, in pipefishes and seadragons (Urophori), no significant correlation was observed ( $N=5$ ;  $r=-0.535$ ;  $P=0.353$ ) thus possibly highlighting a different trend observed among external brooders (Urophori and Gastrophori).

Even though egg size was found to be correlated with male length among all considered syngnathids (product-moment correlation;  $N=37$ ;  $r=0.518$ ;  $P<0.005$ ), this trend was not observed within seahorses ( $N=12$ ;  $r=0.437$ ;  $P=0.154$ ) nor in pipefish with a marsupium ( $N=17$ ;  $r=0.280$ ;  $P=0.275$ ). A positive correlation was, however, observed in external brooders ( $N=8$ ;  $r=0.717$ ,  $P<0.05$ ).

A positive correlation was observed between larvae hatch size and egg size (product-moment correlation;  $N=29$ ;  $r=0.838$ ;  $P<0.05$ ), indicating that bigger larvae hatch from the largest eggs. Nevertheless, the analysis of the residuals highlighted the fact that seahorse larvae are consistently smaller than those of the other syngnathids. In fact, seahorse larvae are smaller than expected (Figure 1), taking into consideration the large eggs produced by the genus *Hippocampus* (Table 2B), when compared with the other syngnathid larvae. Interestingly, however, seahorse larval sizes are not different from those of external brooders or inverted pouch syngnathids, if the male length is considered instead of the egg size (Table 2C).

Finally, pear-shaped eggs showed an average 9% increased surface when compared with spherical eggs of identical volumes.

## DISCUSSION

The analysis on the number of carried eggs showed not only that syngnathids with a marsupium were able to carry an additional number of eggs, that is to say, were able to produce a greater number of offspring per pregnancy episode, but also that the number of eggs was positively correlated with marsupium development. This difference, within the Syngnathidae family, between

species with and without a marsupium poses an interesting question: what kind of new possibilities were introduced by the brood pouch?

#### *Increased protection*

The first logical and consensual explanation might be the increased protection to the developing eggs from the surrounding physical or biotic environment. If, as suggested by Carlisle (1982), parental care patterns are adjusted according to prevailing and projected conditions, the syngnathid brood pouch, with the ability to physiologically maintain the prenatal embryos, could have evolved as a means to respond to environmentally variable coastal waters (Watanabe & Watanabe, 2001). Thus, creating a more stable developing milieu for the larvae, the marsupium could have helped stabilize the investment in sequential broods, more or less independently of the external conditions. Furthermore, the masking of the bright orange eggs (Vincent, 1990), that increase the predation risk of the pregnant males (Svenson, 1988), readjusts the fish's ability to efficiently mimic its natural environment.

#### *Increased progeny*

The development of the different brood structures increased the surface of contact between the eggs and the paternal body. A wider brooding surface area could be translated in more eggs. In marsupium-lacking pipefish, such as *Nerophis lumbriciformis*, the female may produce more eggs than the male can carry (Monteiro et al., 2002). Sometimes, during the egg transfer to the male's incubating surface, the female releases a vast number of eggs that surpass the brooding area of the male. These eggs become quickly unattached and fall off the male's body (which rotates several times along its major axis actively promoting this process). Only the eggs that directly contact the brooding surface (one layer thick) remain attached and continue the developing process (this feature is also visible for *Nerophis ophidion*; N.M. Monteiro, personal observation). Therefore, just by measuring the brooding area of a marsupium-lacking male, knowing the average egg dimensions, we can estimate the maximum number of eggs a male can carry. Thence, the positive relationship found between male length and the number of eggs carried by external brooders (Gastrophori) may be a direct result of their rectangular 'bi-dimensional' incubating area comprehended within the abdominal area.

In pipefish species that present a marsupium (e.g. *Syngnathus acusimilis*, see Kornienko, 2001), and particularly in seahorses, the eggs can be distributed inside the brood pouch in several layers, all in close contact with a parental tissue responsible for proper embryonic development. Therefore, within the same area of the male's body, syngnathids that present a marsupium may carry more eggs and still develop a close contact with each egg, thus increasing the number of produced offspring.

#### *Genetic mating systems and fertilization modes*

In species where a marsupium is present, fertilization occurs after egg transference. The male releases sperm

inside the brood pouch, therefore gaining complete confidence on the paternity of the embryos. Not surprisingly, the direct examination of the gonads of these syngnathids showed minute testis (Carcupino et al., 1999), since there is no apparent need for large quantities of sperm, due to the absence of sperm competition. Accordingly, Jones & Avise (2001) suggested that the elaborated brood pouch morphology might have evolved in order to ensure high paternity confidence. Nevertheless, the observation of the testis of *N. lumbriciformis* (N.M. Monteiro, unpublished data) also showed minute testis, indicating that syngnathids might have already had high paternity confidence before the development of the brood pouch (see Kvarnemo & Simmons, 2004).

It seems that both in *N. ophidion* (Berglund et al., 1986; McCoy et al., 2001) and *N. lumbriciformis* (Monteiro, unpublished data), the males carry only a batch of eggs from a single female per pregnancy, while females are polyandrous. Available evidence suggests that external brooders may be unable to receive and fertilize more than one clutch of eggs. In both *Corythoichthys haematopterus* (Matsumoto & Yanagisawa, 2001) and *C. intestinalis* (Gronell, 1984), two pipefish species with incomplete brood pouches (laterally walled brood pouch), the males only accept postures from a single female. Hence, it can be argued that the development of the brooding pouch might have introduced (or at least potentiated) the opportunity to copulate with several females (polygynandry), broadening the opportunity to display new mating systems that ultimately influenced the evolution of the family Syngnathidae.

#### *Associated problems*

Apart from all these new possibilities, specific constraints had to be overtaken in a closed environment such as the marsupium. Probably, the most important of all is oxygen concentration (see Kolm & Ahnesjö, 2005) and the requirement to continuously remove harmful metabolites produced by the developing embryos. These problems seem proportional to the degree of brood pouch sealing, attaining its maximum levels in seahorses. An important feature, found in syngnathids with a brood pouch is the ability to osmoregulate the milieu that involves the eggs (Masonjones & Lewis, 2000; Carcupino et al., 2002). Numerous interpretations have been suggested in order to adequately explain this feature, namely as being a mechanism responsible for the protection of the developing larvae until they are physiologically capable of enduring greater salinities (Berglund et al., 1986; Watanabe et al., 1999). Another, non-exclusive, hypothesis, based on the fundamental requirement of a correct and constant oxygenation of the eggs can also be proposed. High salt concentrations tend to help separate the organic from the aqueous phase, a process usually used by biochemists in the purification of proteins named 'salting out'. Since oxygen concentration is inversely proportional to the salinity level (Clesceri et al., 1998), syngnathid males actively contributing to reduce the salinity are indirectly increasing the oxygen concentration levels inside the brood pouch. It seems that the osmoregulation observed in marsupium-presenting syngnathids, at least during the first stages of embryonic development,

might play an important role on the oxygen solubility and, consequently, on oxygen levels actually available for the growing embryos, rather than acting as a defence mechanism against high salinity levels. Otherwise, why would only the larvae of certain syngnathid species (those with a marsupium) lose the ability to endure the normal seawater salinity, which poses no problem to the marsupium lacking pipefish, together with the vast majority of marine teleosts?

As stressed by Kolm & Ahnesjö (2005), a common, yet largely untested, hypothesis dealing with eggs incubated in water states that, larger eggs suffer relatively more from problems with obtaining sufficient oxygen than smaller eggs. The biggest eggs within the Syngnathidae family are present in seahorses, seadragons and pipehorses. Some of these species present pear-shaped eggs, a fact that might be interpreted as a way to increase the surface to volume ratio. One may argue that smaller eggs could present even more advantageous surface/volume ratios and, at the same time, be more energetically inexpensive to produce. Bearing in mind that embryo oxygenation is partially carried out by the male through its vascularized dermis, it is possible that the increased egg surface reflects the need for a minimum contact zone with the paternal tissue, with this overlapping area acting as a limiting factor. Even though no available data were found on syngnathid yolk dimensions, it is possible that the bigger egg volume, observed in seahorses, is largely independent from yolk volume. Otherwise, seahorse embryonic development would have to be energetically inefficient, with large quantities of yolk producing smaller larvae than would be expected given the large egg volume (see Figure 1).

It would be predictable that the sophisticated seahorse brood pouch implied significant differences at the end of the embryonic development when compared with other syngnathids. At least, it could be expected that external brooders would present the smallest larvae since the eggs are directly exposed to the external milieu and no intermediate environment, such as the marsupium, can grant subsequent developmental opportunities (the lack of the marsupium implies that the entire development of the individual must occur inside the confined egg space) since permanence in the egg shell may be an advantage in a species that lacks protection for larvae, while hatching soon and remaining inside a protective environment can provide more space to grow and less constraint metabolic exchanges in species that possess effective means of protecting their free embryos. Contrary to what could be expected, seahorse larvae were found to be smaller than the larvae of the other syngnathids, when controlling for egg size (Table 2) even though no significant differences were found among syngnathids when hatch size was compared controlling for male length. These observations seem to point out the fact that the volume of a seahorse egg might not reflect an extra female investment in offspring but the need for a bigger surface area. Since egg dimensions explained a total of 96.2% of young size, and seahorse eggs are heavier than young (Vincent, 1990), the nurturing role of the seahorse marsupium may have been overstated while its role in increasing oxygen concentration systematically overlooked.

Even though some confounding effects of phylogeny with pouch type might occur (a single genus is considered

for the sealed and inverted pouch: *Hippocampus* and *Syngnathus*, respectively), it seems that the measurement of simple characters, such as brood structure, egg and newborn size, are able to provide powerful insights into the mechanisms and strategies involved in syngnathid reproduction, highlighting a distinct approach displayed by seahorses that show several specific adaptations for the continual production of a great number of offspring due to the extra brooding space provided by the brood pouch, together with a high number of consecutive pregnancies [e.g. seven consecutive pregnancies reported for *H. whitei* (Vincent & Sadler, 1995)] even if some constraints due to the highest degree of marsupium closure had to be overcome.

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