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Early life history of the pipefish *Nerophis lumbriciformis* (Pisces: Syngnathidae)

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The embryonic and larval development of the pipefish *Nerophis lumbriciformis* is described. The full development sequence lasted 30 d (at 14–15°C), being shortened to 25–26 d at higher temperatures (16–17°C), and hatching occurred throughout a 2–3 d period. Unlike species of the genus *Syngnathus*, the newly hatched larvae presented a functional mouth apparatus and the yolk sac completely absorbed.

INTRODUCTION

The family Syngnathidae (pipefishes and seahorses) is characterized by especially pronounced adaptations for male parental care, with the female depositing eggs directly on a specialized incubation area or brood pouch on the tail or trunk of the male (Herald, 1959). This particular mode of reproduction, together with the presence of sex role reversal in some syngnathid species (Berglund et al., 1986; Vincent et al., 1992), has converted this family to an exceptional model for the study of sexual selection. *Nerophis lumbriciformis* Jenyns (1835) is a small and slender pipefish, found on the rocky intertidal to about 30 m, usually among seaweeds at high tide or underneath loose stones during the ebb tide, where it feeds on small crustaceans (Monteiro et al., 2001). Its distribution ranges from the Atlantic coast of Norway to the Kattegat and from Belgium southwards to Morocco (Dawson, 1986). Although locally common, the reproductive ecology of this species is still poorly known. Based on several successful spawnings that occurred in aquaria, the embryonic and larval development of this pipefish is described.

MATERIALS AND METHODS

Eggs were obtained both from captive males that underwent pregnancy in aquaria as well as from wild specimens collected at Viana do Castelo (northern Portugal). Captive fish were fed daily with fresh *Artemia nauplii*. Tanks (90 l) were illuminated with fluorescent lights (18 W) with a photoperiod of 13L:11D. Due to the 'gas bubble disease', common in pipefish, aeration was performed in an external tank connected to the used aquaria. The circulating seawater temperature was maintained constant at two different temperatures, 14–15°C and 16–17°C. The bottom of the tank was covered with sand, boulders forming several holes and crevices, and algae. Once spawning had occurred, eggs were carefully removed from the male's ventral surface with the aid of pincers, with a daily periodicity (except during the first day, when the egg extraction occurred several times). Eggs were immediately preserved in buffered 5% formalin, and observed under a Leica stereomicroscope attached to a digital video camera. The embryos were removed from the eggs and digitalized images were taken to allow subsequent description of the several stages of development. Figure 1 summarizes a sequence of the most relevant changes that occurred during embryonic development.

The complete sequence of embryonic development was based on three spawnings (by different males and females) that occurred in aquaria (temperature: 14–15°C). Two additional spawnings, of two other pairs, were also monitored at a slightly higher temperature (16–17°C). As the reproduction of this species seems to be inhibited by temperatures above 16°C (Monteiro et al., 2001), the fish were kept at 15°C and the temperature was raised to 16–17°C just after the beginning of pregnancy.

RESULTS

Nerophis lumbriciformis is a sex-role reversed species. During courtship females are the courting sex. Sexually dimorphic (Figure 1A), the females are more active and more coloured than the males. After egg transfer, parental care is exclusively paternal. Males tend to abandon large concentrations of individuals and are very sensitive to manipulation, especially near its incubating ventral surface (●● personal observation). This incubating surface, flat at the beginning of the incubation period, progressively develops alveoli in order to individually accommodate each egg (Figure 1A). The eggs, with its major axis measuring approximately 1.3 mm (N=59; range=0.7–1.72; average=1.298; SD=0.183), are spherical when spawned but become quite variable in shape due to different degrees of compression caused by adjacent eggs. The outer membrane is transparent, allowing a clear view of the bright orange yolk sac. This coloration is probably

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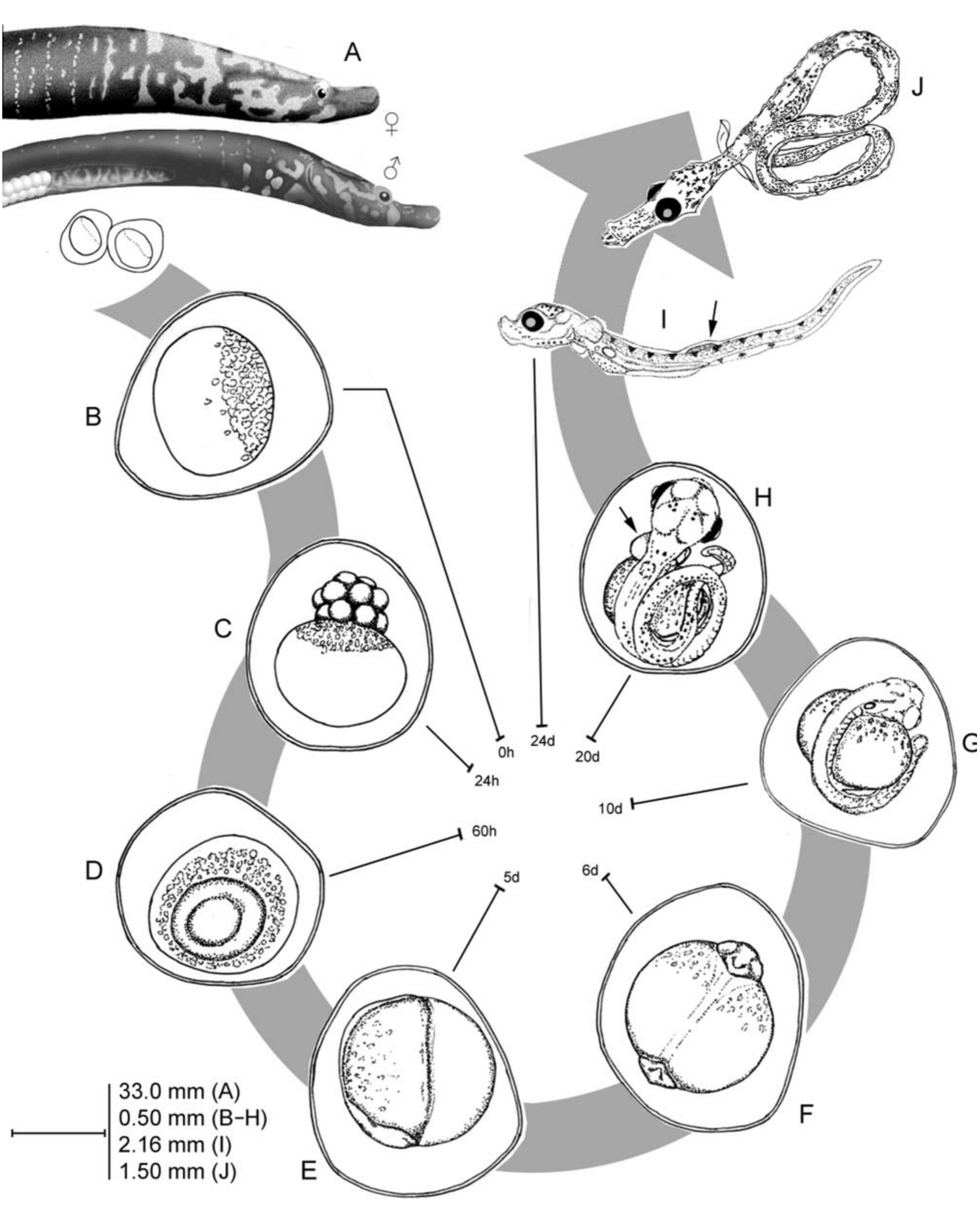

Figure 1. Development stages of the pipefish *Nerophis lumbriciformis*, from fertilization to hatching (B–I). Adult individuals (A) and a newborn juvenile (J) are also represented.

due to the high concentration of carotenoids present in syngnathid eggs (Vincent, 1990).

The eggs are spawned already forming long strings that may have a length greater than 20–25 eggs, arranged in two to four parallel rows, with a mucilaginous layer in the portion that is in contact with the male's incubating ventral surface (see Monteiro et al., 2002).

The duration of the full embryonic development varied, as expected, according to the tested temperature. At 14–15°C, hatching occurred 30 d after fertilization while at 16–17°C the whole process was shortened to only 25–26 days. Hatching occurred, for both tested temperatures, during a 2–3 d period. An abbreviated view of the different stages of development and of the newly hatched larvae (14–15°C), is presented in Figure 1. The first cleavage was visible two hours after fertilization and the blastomeres did not seem to differ from one another in size. Gastrulation began approximately 36 h after fertilization, with the flattening of the blastodisc that presented a regular hemispheric shape. Soon after the onset of epiboly, the embryonic shield was visible as a thickening of the germ ring (Figure 1E, 120 h). As epiboly continued, and just before its completion, the chorda and neural tube were now differentiated in the anterior region of the embryo body. During the period of segment formation the somites differentiated, the rudiments of the primary organs became visible, the tail bud became more prominent and the embryo elongated (Figure 1F–G, 144–240 h). Melanogenesis began 12 d after fertilization (Figure 1G, 288 h) and a few pigmented cells were then visible along the anteroposterior embryonic axis. The eyes were also pigmented. At this stage, the embryo was capable of performing some movements and responded to touch. Furthermore, heart beats were now discernible. The larval primordial marginal fin appeared bordering the trunk and tail and the sculpturing of the brain primordium was also visible. Pectoral fins, absent in adult individuals of the genus *Nerophis* and *Entelurus*, appeared as little folds (Figure 1H [arrow], 480 h). After 24 d of development, the embryo presented 12 pigmentation marks in the upper part of the trunk and tail and four to five marks in the ventral side of the primordial marginal fin (Figure 1 I, 576 h). The mouth apparatus did not seem to be fully formed and the dorsal fin, still partially undeveloped (Figure 1I [arrow], 576 h), was now perceptible. The release of premature juveniles, still exhibiting the final remains of the yolk sac, may occur at this time if the male is confronted with a stressful event (●●● personal observation). During subsequent development, the mouth apparatus gradually became elongated, acquiring the adult form, with the tip of the snout characteristically pointing upwards. Once released from the male's body, the juveniles were free swimming and no further care was provided (Figure 1J, juvenile of ~5 days). The juveniles showed vertical swim-up and drift behaviour, using the pectoral fins to rotate along the body axis. Young feed on zooplankton, actively seeking food in the water column, especially near the surface. Juveniles, of ~4 cm and a homogenous dark brown/red coloration, start to appear in the rocky intertidal zone by October. It is possible that these juveniles settle at an age of about three to four months, in contrast to the two months suggested by Russell (1976), since the reproduction peak occurs in Portugal in May to July (see Monteiro et al., 2001).

DISCUSSION

Unlike the species of the genus *Syngnathus* that hatch before completion of the formation of the mouth apparatus (Drozdov et al., 1997), in *Nerophis lumbriciformis* the mouth is already functional and the dorsal fin rays are clearly visible. This fact could be related to the absence of a marsupium in the genus *Nerophis*. Since the eggs are directly exposed to the external milieu, no intermediate environment, such as the marsupium, can grant subsequent developmental opportunities, consequently forcing the complete development of the individual inside the confined egg space.

Particular findings that we would like to comment on concern the presence in *Nerophis* larvae of some traits that are absent in the adults but are present in adult *Hippocampus*. These two genera markedly differ in morphology and were consistently recovered in different clades in a phylogenetic study that used different DNA mitochondrial fragments (Wilson et al., 2001). These traits include the ability to move the head to a right angle to the trunk and the use of this posture when feeding. Indeed, we observed newly hatched larvae bending the head down, forming a right angle with the trunk, just before prey aspiration, much like seahorses. The adult specimens of *N. lumbriciformis* are structurally incapable of bending their heads towards their ventral surface, a condition that forces them to lie down laterally or even turn upside down in order to capture prey that is in close contact with the bottom. Moreover, we described two paired structures that we interpreted as pectoral fin rudiments. These are capable of beating movements and are effectively used by the larvae to rotate the body. It is necessary to study these structures histologically to test the hypothesis that they are analogous and not true pectoral fins. If future studies confirm their homology with the pectoral fins of adult seahorses and most pipefish (except in the genus *Entelurus* and *Nerophis*), these characters will fall in the same patterns as those of the mobility of the head. These findings suggest the hypothesis that the presence of these traits in adult *Hippocampus* may represent a persistence of larval characters, being eventually neotenic or, alternatively, the absence of pectoral fins in adult *Nerophis* and *Entelurus* may represent a secondary loss.

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