

## Embryonic and larval development of *Lipophrys pholis* (Pisces: Blenniidae)\*

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**SUMMARY:** Information on the early ontogeny of *Lipophrys pholis* is scattered and incomplete. In this paper we describe for the first time the full developmental sequence from egg to juvenile in controlled conditions. In addition, some notes on the spawning behaviour of adults and the behaviour of larvae are provided. During oviposition, the female follows the male's path, suggesting that the male may apply sperm on the nest before spawning. Embryonic development lasted 16 days (17°C) and larval development to settlement lasted 29 days (15.5-17.5°C). At hatching, mean larval total length was 5.0 mm. The larvae hatched with the mouth and anus opened, with pigmented eyes and almost no yolk, and started to feed within one day. They first settled 29 days after hatching (13-14 mm TL) and showed full juvenile pigmentation and behaviour 8 to 9 days later (17-19 mm TL).

*Key words:* *Lipophrys pholis*, early ontogeny, reproductive behaviour, larval behaviour.

### INTRODUCTION

*Lipophrys pholis* (Linnaeus, 1758) is a very common rocky intertidal fish species in the northeastern Atlantic (Zander, 1986). Many papers have been published concerning its reproductive biology and ecology (e.g. Qasim, 1956, 1957; Dunne, 1977; Shackley and King, 1977; Laming *et al.*, 1982; Milton, 1983; Almada *et al.*, 1990a, 1990b, 1992; Faria *et al.*, 1996; Gonçalves, 1997).

In Great Britain, *L. pholis* breeds during spring and early summer (Qasim, 1957), while in Portugal the breeding season occurs in the cooler months, from October/November to May (Almada *et al.*, 1990a; Faria *et al.*, 1996). During the breeding peri-

od the males establish territories in crevices where spawning takes place (Lebour, 1927; Qasim, 1957; Dunne, 1977; Almada *et al.*, 1990b). Males defend and ventilate the developing eggs until hatching (Qasim, 1956; Almada *et al.*, 1990b). Breeding males guard multiple clutches and exhibit a typical dark coloration pattern (see Qasim, 1956; Almada *et al.*, 1990b, 1992). Almada *et al.* (1990b) presented an ethogram of the breeding males of *L. pholis* but never observed a complete courtship sequence and, as spawning occurred inside rock cavities, provided little information on the spawning process. Qasim (1956) described the spawning behaviour of a pair of fishes maintained in captivity.

In spite of all this information, little is known about the developmental biology of *L. pholis* and the available information is scattered and incomplete.

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Qasim (1956) described the embryonic development of this species in captivity. Brief descriptions of the eggs and larvae were provided by Hefford (1910) and Lebour (1927). Hefford (1910) presented a brief description of the pigmentation of a larvae 4.4 mm TL, which is probably a developing embryo that hatched precociously. Lebour (1927) provided a detailed description of the pigmentation of the newly hatched larvae (5.4 mm TL). Ford (1922) presented a brief description of the pigmentation of larvae 5.0 mm, 5.5 mm, 9 mm and 17.5 mm TL. Finally, McIntosh (1905) described the pigmentation and morphology of post-settlement individuals (TL > 19 mm).

In this paper we present the full developmental sequence of *L. pholis* from egg to juvenile. Some notes on the behaviour of the spawning pair and the behaviour of larvae are also presented.

## MATERIAL AND METHODS

Eggs and larvae were obtained from a captive group of 5 fishes (3 females: 8.6 cm, 9.8 cm and 10.5 cm TL; 2 males: 11.3 cm and 13.4 cm TL) maintained since November 1997 at the Vasco da Gama Public Aquarium, Lisbon. The fishes were fed daily with fish and shrimp. The tank was illuminated with fluorescent light (60 W) from 09:00 h to 19:00 h. The bottom of the tank was covered with a sand layer and several large flat stones and shells were provided as shelter and breeding sites.

The complete sequence of embryonic development is based on a spawning that occurred on 2 November 1998 (temperature: 17°C). We used three other batches that, although they did not survive until hatching, allowed replication of the first developmental stages. Eggs were removed from the stone immediately after spawning by aspiration with a tube. They were maintained in a glass recipient with aeration. To prevent infections methylene blue was added. Eggs were collected daily for description.

Larval development is based on one batch that hatched on 2 March 1999 (temperature range: 15.5–17.5°C). We used five other incomplete sequences for confirmation. Upon hatching larvae were collected by aspiration from the progenitors' aquarium and were reared in 30 l glass tanks illuminated with fluorescent light (18 W) for 24 h per day. A constant flow of seawater was maintained. Larvae were fed three times a day with *Brachionus* sp. enriched with Selco (Artemia Systems), which were gradually replaced by *Artemia* sp. nauplii 14 days after hatch-

ing. Larvae were collected daily until the 14<sup>th</sup> day after hatching. After that, they were collected every two or three days. After being anaesthetised (Hypnodil, Janssen Pharmaceutica), both eggs and larvae were observed under a Nikon stereomicroscope, photographed by a Nikon FX-35DX camera and preserved in 5% buffered formalin. The egg capsules were opened and the embryos were distended to allow more detailed observations. All larval measurements presented are total length.

The observed spawning was videotape recorded (with a Sony Hi8 CCD-V600 E camera). Behavioural descriptions were made using *ad libitum* and focal observations (sensu Martin and Bateson, 1986).

## RESULTS

### Spawning

Spawning lasted more than 9 h (time observed). When our observations started (at 9 a.m.), the female was over the nest wall. The male approached, touched the female with the snout and rotated until the genital papilla touched the female's back. After touching the female, the male performed pectoral fin beatings and high amplitude movements of the tail and posterior part of the body, rubbing the nest wall with the genital papilla. This movement ended with a brief body shaking. This process has a mean duration of 18.0 sec (s.d.=12.1, range 5.0–40.0 sec, n=10). Following the male's path, the female applied the belly to the nest wall and skimmed over the nest surface with slowly pectoral fin movements while quivering the tail. The genital papilla touched the nest wall several times with the eggs being laid one at a time in a single layer (duration of oviposition: mean=45.0 sec, s.d.=10.9, range 25.0–65.0 sec, n=10). This sequence was repeated several times, alternating with resting periods. In general, both fishes alternated their movements over the stone.

During spawning, the male presented a general black coloration with white lips, while the female showed a light coloration, with fins almost transparent. Both sexes had swollen genital papilla.

### Embryonic development

Eggs were golden-brown and transparent, with a spherical shape (Fig. 1) except at the attachment disk. The diameter was 1.30 mm (s.d.=0.04, range: 1.21–1.41 mm, n=52), which is in agreement with

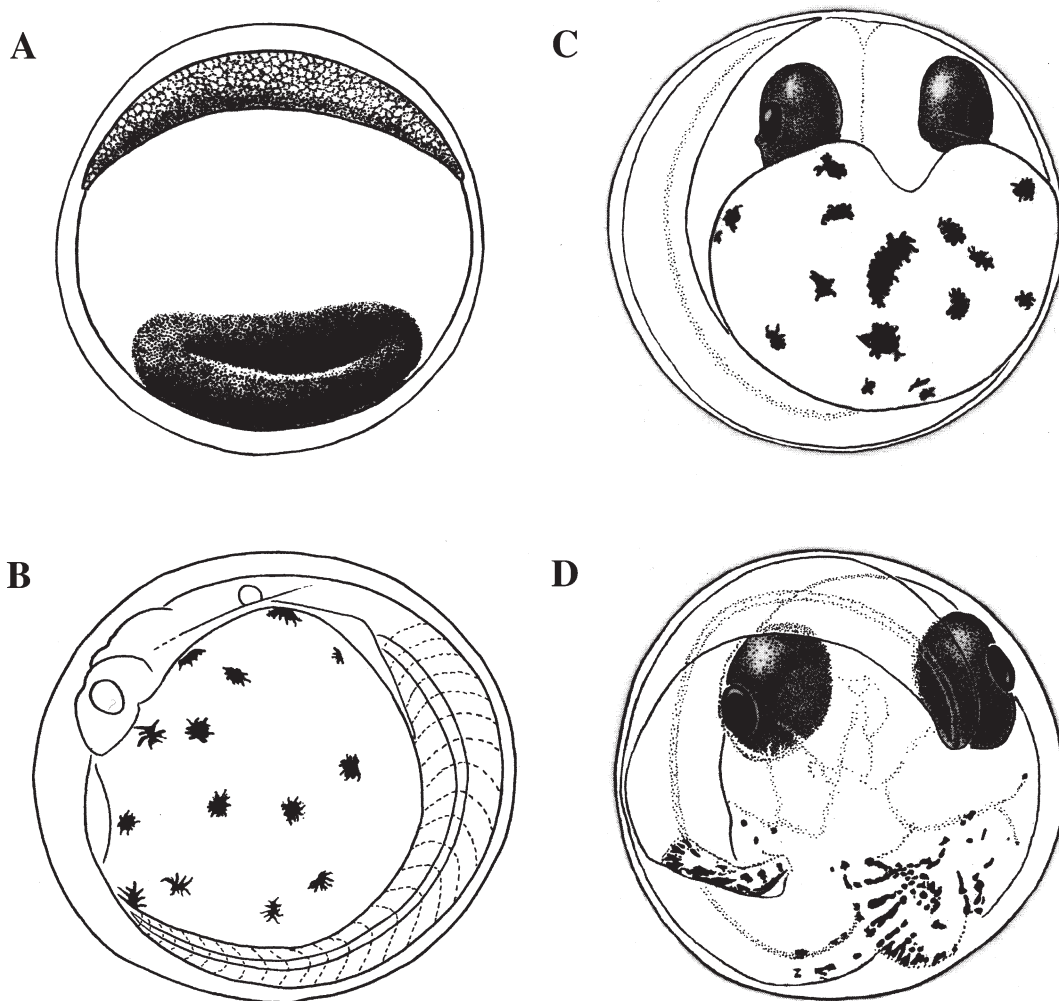


FIG. 1. – Eggs collected at different developmental stages: (A) Day 1; (B) Day 5: embryo almost reaching the margin of the yolk; (C) Day 8: embryo longer than egg major axis; (D) Day 15: embryo prior to hatching (dorsal view).

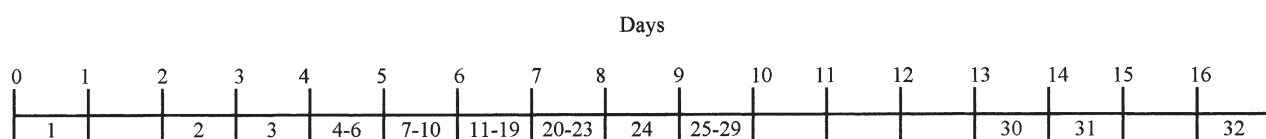


FIG. 2. – Ontogenetic events of embryonic development of *Lipophrys pholis* in order of first appearance: (1) blastodisc; (2) embryo recognizable; (3) cephalic and caudal dilatation; (4) eye lens; (5) brain; (6) notochord differentiation; (7) brain lobes; (8) notochord; (9) myomeres; (10) heart beatings; (11) pigmented eyes; (12) embryo reaches the margin of the yolk; (13) tail bud free of the yolk; (14) gut differentiation; (15) auditory vesicles; (16) pectoral fin buds; (17) mouth differentiation; (18) median fin fold; (19) hatching glands; (20) anus visible but closed; (21) mouth visible but closed; (22) embryo longer than egg major axis; (23) otoliths; (24) embryo movements; (25) gas bladder; (26) pectoral fins developed; (27) anus opened; (28) mouth opened; (29) mandibles differentiation; (30) eye movements; (31) liver differentiation; (32) hatching.

published measurements: 1.18-1.60 mm (McIntosh, 1903; Hefford, 1910; Lebour, 1927; Qasim, 1956).

Hatching occurred on the 15th to 16th day after spawning (Fig. 2). There were two peaks of hatching, the first in the morning and the second and most intense at the end of the day. This disagrees with

Qasim (1956), who observed maximum hatching during the morning. Qasim (1956) described hatching with the embryo emerging tail first. In our observations of 12 hatching events the larvae always emerged head first, after rapid shaking movements of the body.

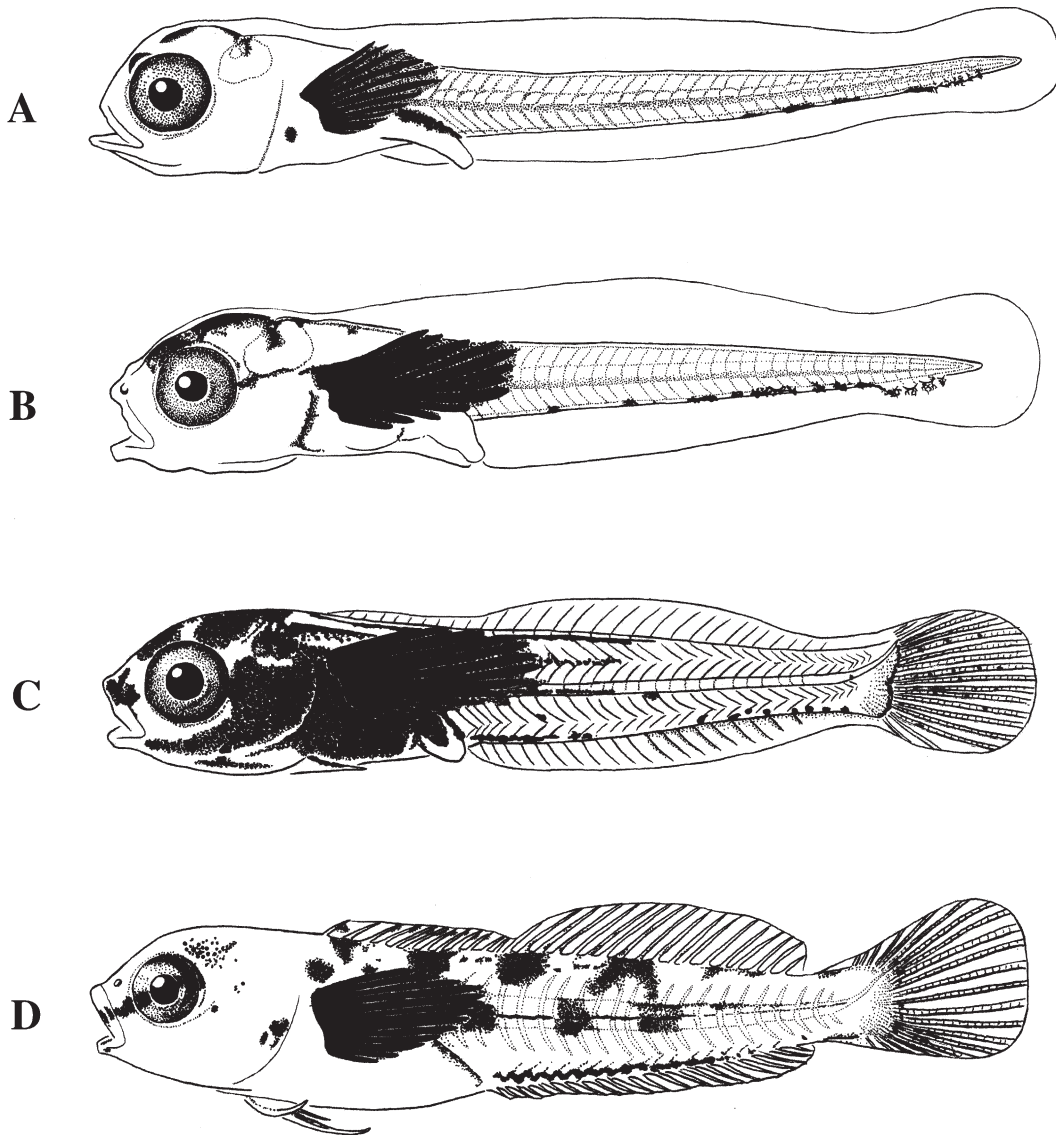


FIG. 3. – Larvae collected at different developmental stages: (A) Day 1: newly hatched larva (5.5 mm TL); (B) Day 5: 6.3 mm TL; (C) Day 25: 13.0 mm TL; (D) Day 41: juvenile (17.0 mm TL).

### Larval development

Newly hatched larvae measured 5.03 mm (s.d.=0.19; range: 4.73-5.33 mm; n=16), which is in agreement with published values presented by Ford (1922), Lebour (1927) and Fives (1986). The anus and mouth were open, with formed lips, teeth and differentiated jaws (Fig. 3). The yolk was almost fully absorbed. The liver was developed, the eyes were fully pigmented, and the gas bladder was formed but not completely filled. The pectoral fins were differentiated and all three otoliths were present. The opercula were open with four branchial arches present.

At hatching the larvae presented peritoneal pigmentation, and twelve rows of melanophores on the pectoral fins. Ventrally, there were 2-4 melanophores on the throat and 7-9 on the last myomeres. Dorsally, there were some sparse melanophores over the brain and the upper lip and there was one melanophore between the inner ear vesicles (Fig. 3).

The pigmentation pattern was maintained during development with an increase in the number and intensity of melanophores at the ventral row (from behind the anus to the caudal peduncle), and at the cephalic region, with melanophores extending from between the eyes to the dorsal region (Fig. 3).

Days

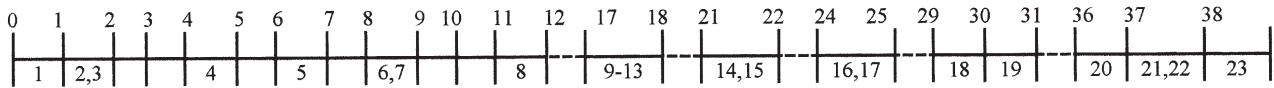


FIG. 4. – Ontogenetic events of larval development of *Lipophrys pholis* in order of first appearance: (1) nostrils closed; (2) exogenous feeding; (3) filled gas bladder; (4) caudal fin bud; (5) hypurals; (6) caudal fin rays; (7) ventral fin bud; (8) notochord starts to flex; (9) anal fin bud; (10) anal fin rays; (11) 2<sup>nd</sup> dorsal fin rays; (12) notochord flexion completed; (13) ventral fin rays; (14) segmented caudal fin rays; (15) first dorsal fin rays; (16) median fin fold reabsorption; (17) ossified vertebrae; (18) larvae begun to contact the aquarium bottom; (19) larvae started to settle; (20) nostril tentacles; (21) most larvae settled on the bottom; (22) juvenile behaviours; (23) typical juvenile pigmentation.

At day 9 after hatching (6.5-7 mm) diffuse yellowish pigmentation, which subsequently extended all over the head, was present. At day 12 after hatching (8mm) there were some melanophores over the midline and the neural tube. Their number and intensity increased and two dorsal and two lateral rows were formed on each side of the body. Between day 24 and day 30 (13.5-14 mm) all fin rays were present (D=XI-XIII +18-20; A=II + 18-20; V=I + 3; P=13, n=20).

After metamorphosis (17-19 mm) the fish developed juvenile pigmentation (Figs. 3 and 4). A ventral row of melanophores at the base of the anal fin was present and the other fins were also pigmented (less intensely at the caudal fin). The head was extremely pigmented and there was some pigmentation at the throat. Dorsally there were three dark bands (large spots) that extended through the midline, and alternated with three other blotches situated laterally (on each side of the body).

### Larval behaviour

After hatching, the larvae immediately swam towards the surface. They avoided sinking by swimming actively until day 4 when the gas bladder was filled. Feeding behaviour began one day after hatching and was characterised by an impulse forward towards the prey item, sometimes preceded by an “S” posture of the posterior part of the body. When two larvae approached, they avoided each other by changing direction with rapid caudal and pectoral fin movements.

Larvae began to settle to the bottom of the tank 29 days after hatching (13-14 mm) and 8 to 9 days later they were benthic (15-16 mm). However, juvenile behaviour such as turning movements of the head and hiding under objects in close contact with the surfaces by flexing the body against them (tigmotaxis) was observed only at 17-19 mm.

These results agree with our field observations (unpublished data), since some larvae of this species

captured in the plankton measured 16.6 mm (s.d.=1.3, range: 15.0-18.9 mm, n=7) and the smallest fishes found in monthly sampled tidepools (some still lacking juvenile characters) averaged 17.4 mm (s.d.=0.1, range=15.0-19.0mm, n=60). McIntosh (1905) also found 19 mm TL individuals in tidepools.

### DISCUSSION

Our observations of spawning behaviour contrast with that provided by Qasim (1956) in an important detail. While Qasim’s description implies that the male fertilises the eggs after attachment, our observations based on videotape recordings point to the contrary. The male first rubs the substratum with the genital papilla and the female follows the male’s path while laying eggs, suggesting that the female spawns over a surface that is likely to already contain sperm. Patzner (1984) showed that the micropyle of the eggs of blenniids is in the middle of the adhesion disc and thus faces the substratum when the eggs are attached. Qasim (1956) reported that in the ovary, the position of the eggs is such that they must be extruded with the adhesion disc facing the substratum. This means that it is very likely that contact with sperm must precede attachment, either through the presence of sperm in the water column or by a sperm layer previously attached to the rocks by the male, as described for some gobiids (Marconato *et al.*, 1996; Ota *et al.*, 1996; Faria *et al.*, 1998). Our observations suggest that the male probably applies sperm to the rock surface before egg attachment.

The embryonic developmental sequence described here generally agrees with Qasim (1956), except that the timing of events that we observed was much shorter. While Qasim (1956) recognised the differentiation of the embryo at day 8 after hatching, the presence of eye rudiments at day 14, and the formation of myomeres and heart beatings at



day 24, we observed these events at day 2, day 4, and day 5 respectively. In our study, embryonic development lasted 16 days at 17°C, while Qasim reported an embryonic developmental time of 43 days at 11.5-15.0°C, and 61 days at 9.5-14°C. These differences are probably due to the incubation temperature since the decrease of the developmental time with higher temperatures is known for many fish species (Blaxter, 1969). Nevertheless, the difference of almost 50% in the timing of developmental events is noteworthy.

The newly hatched larvae of *L. pholis* showed the typical pattern of features characteristic of marine fishes with male parental care (Thresher, 1984). They swam actively immediately after hatching and the onset of exogenous feeding occurred one day after. This pattern is also found in other coastal species with demersal eggs and contrasts with the one generally described for species with pelagic eggs (see e.g. Russel, 1976; Moser *et al.*, 1984).

After metamorphosis and settlement the juveniles showed typical behaviours associated with a benthic mode of life, like lateral movements of the head and hiding behaviour, which could be important for survival in a highly irregular substrate.

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