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# Ontogenetic development of *Lipophrys trigloides* (Pisces: Blenniidae), with some notes on the spawning behaviour

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The full developmental sequence from egg to juvenile of *Lipophrys trigloides* in controlled conditions is described. In addition, some notes on the spawning behaviour of adults are provided. Embryonic development lasted 13–14 days at 21.00–23.00°C, 16–17 days at 17.25–18.50°C and 17–18 days at 14.00–16.50°C. Newly hatched larvae measured 4.8 mm, had the mouth and anus opened, pigmented eyes and almost no yolk. Most larvae settled between day 39 and 42 after hatching at 16.0–17.0 mm total length (TL) and showed full juvenile pigmentation patterns and behaviour 5 to 10 days later (17.0–18.0 mm TL). A summary of the characteristics of the eggs and larvae that may help in the distinction between *L. trigloides* and *Lipophrys pholis*, a very close related species, is also provided.

## INTRODUCTION

*Lipophrys trigloides* (Valenciennes, 1836) is a relatively common rocky intertidal fish species along the Portuguese rocky shores. Although there is some information about the biology of this species (e.g. Nieder & Zander, 1993; Nieder, 1997), nothing is known about its breeding behaviour and development.

In this paper the full developmental sequence of *L. trigloides*, from egg to juvenile, is described for laboratory reared fish. Some notes on the behaviour of the breeding pair are also presented.

## MATERIALS AND METHODS

Eggs and larvae were obtained from a captive group of six fish (2 males with 7.0 cm and 7.7 cm TL respectively; 4 females between 6.6 and 8.7 cm TL), maintained since June 2001 at Vasco da Gama Aquarium (Lisbon). The 600-l tank was illuminated with fluorescent light (60 W), from 0900 to 1900 hours. 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 three batches spawned on vertical stones and the tank wall (for temperature ranges see Table 1). A sample of eggs was removed daily for description from the stone guarded by the male, by aspiration with a long pipette. The eggs were observed under a Nikon stereomicroscope, photographed by a Nikon FX-35DX camera and preserved in buffered 5% formalin. The egg capsules were opened and the embryos were distended to allow more detailed observations.

Full larval development is described from one batch that hatched on 15 December 2003. We used three other batches to confirm the patterns of larval development (see Table 1). Upon hatching, larvae were reared in glass 30-l tanks, illuminated with fluorescent light (18 W) 24 h per day. A constant flow of seawater was maintained. Larvae were fed twice a day with *Brachionus* sp. enriched with protein Selco (Artemia Systems) and algae, which were replaced by *Artemia* sp. nauplii by day 58. Larvae were collected daily, anaesthetized (ethylene glycol monophenyl ether—Merck) and photographed daily until metamorphosis. All larval measurements correspond to total lengths.

## RESULTS

Captive males guarded batches from September to July. Breeding males presented a general black coloration with white swollen tips of the dorsal fins. During the breeding period, both males established territories in crevices and under stones, where spawning took place. On some occasions males were observed excavating the substrate under the nest stone. Males defended and ventilated the developing eggs with undulating movements of the body and tail, until hatching. On one occasion it was possible to observe the spawning event. The female presenting normal coloration pattern and the black male rubbed the nest wall with the genital papilla and performed pectoral fin beatings and high amplitude movements of the tail. These movements were repeated several times, alternating

**Table 1.** *Temperature range of the batches used for (A) embryonic development and (B) for larval development.*

| | Mean (°C) | Range (°C) | SD | N |
|---|---|---|---|---|
| A. *Spawning*. | | | | |
| 17/02/2003 | 15.21 | 14.00–16.50 | 0.07 | 19 |
| 22/04/2003 | 17.96 | 17.25–18.50 | 0.47 | 19 |
| 29/05/2003 | 21.71 | 21.00–23.00 | 0.51 | 14 |
| B. *Hatching*. | | | | |
| 14/02/2003 | 18.25 | 16.50–20.50 | 1.31 | 37 |
| 24/04/2003 | 21.55 | 18.50–24.75 | 1.83 | 35 |
| 29/05/2003 | 16.98 | 16.00–18.00 | 0.57 | 20 |
| 15/12/2003 | 17.25 | 17.00–17.50 | 0.24 | 41 |

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

| °C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14.00–16.50 | d1 | d2 | d3 | d3 | d3 | d4 | d4 | d4 | d5 | d5 | d5 | d7 | d7 | d7 | d7 | d7 | d8 | d8 | d9 | d9 | d10 | d10 | d10 | d10 | d11 | d11 | d13 | d14 | d18 |
| 17.25–18.50 | d2 | d3 | d3 | d3 | d3 | d3 | d4 | d4 | d4 | d4 | d4 | d5 | d5 | d5 | d5 | d5 | d7 | d8 | d9 | d9 | d10 | d10 | d10 | d10 | d11 | d11 | d13 | d14 | d17 |
| 21.00–23.00 | d1 | d2 | d2 | d2 | d2 | d2 | d3 | d3 | d3 | d3 | d3 | d3 | d4 | d4 | d4 | d4 | d5 | d6 | d6 | d6 | d7 | d7 | d7 | d7 | d9 | d9 | d9 | d12 | d14 |

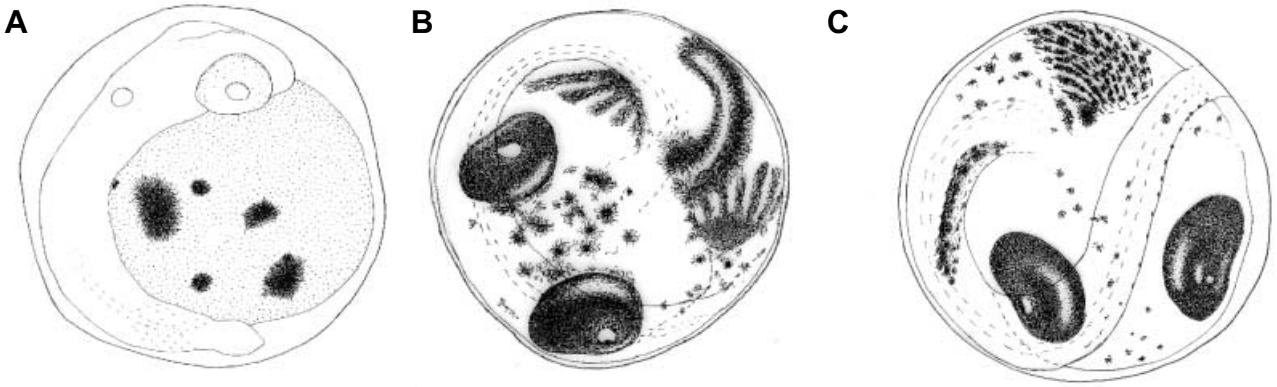


**Figure 1.** Eggs collected at different developmental stages: (A) day 6: beginning of the eyes pigmentation; (B) day 9: embryo with developing pectorals; (C) day 14: embryo prior to hatching (from batch spawned at 14.00–16.50°C).

with resting periods. In general, both individuals alternated their movements over the nest wall.

Both males guarded multiple clutches. From February to June, one of the males was observed guarding eggs for seven distinct periods of time, separated by 1–12 days without eggs. During each guarding period eggs spawned at different times were present. Some batches were placed adjacent to recently laid eggs and others filled the areas left vacant by hatching.

Recently laid eggs are golden brown and transparent. They are spherical with a somewhat flattened surface (Figure 1). The major axis is 1.3 mm (SD=0.16, range: 1.12–1.50 mm, N=51), and the minor axis is 0.86 mm (SD=0.06, range: 0.80–0.92 mm, N=51). From the base to about a half, a white and opaque cup-like structure is present.

The main ontogenetic events of embryonic development at different temperatures are shown in Tables 2 and 3 respectively. Figures 1 and 2 present eggs and larvae collected at different developmental stages. The length of the embryonic period varied with temperature: 13–14 days at 21.00–23.00°C, 16–17 days at 17.25–18.50°C and 17–18 days at 14.00–16.50°C.

Newly hatched larvae (Figure 2) measured 4.8 mm TL (SD=0.19, range: 4.5–5.0 mm TL, N=10). The anus and mouth were opened, with formed lips and differentiated jaws. The yolk was almost fully absorbed or absent. The liver was developed, the eyes were fully pigmented and the gas bladder was formed, but not completely filled.

**Table 3.** *Ontogenetic events of larval development of* Lipophrys trigloides *in order of first appearance (days after hatching): (1) exogenous feeding; (2) filled gas bladder; (3) caudal fin rays; (4) nothocord starts to flex; (5) ventral fin buds; (6) anal fin rays; (7) 2nd dorsal fin rays; (8) notochord flexion completed; (9) first dorsal fin rays; (10) ventral fin rays; (11) median fin fold reabsortion; (12) ossified vertebrae; (13) larvae begun to contact the aquarium bottom; (14) most larvae settled on the bottom; (15) first development of juvenile pigmentation. Size-ranges are also indicated.*

| °C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16.00–18.00 | d2 | d2 | d11 | d13 | d17 | d17 | d17 | d17 | – | – | – | – | – | – | – |
| | 4.8–6.9 mm | | | 7.0–8.9 mm | | | | | | | | | | | |
| 17.00–17.50 | d2 | d2 | d9 | d10 | d10 | d15 | d15 | d16 | d16 | d20 | d21 | d21 | d31 | d42 | d53 |
| | 5.0–6.9 mm | | | 7.0–8.9 mm | | | | | | 11.0–12.9 mm | | | | >16.0 mm | |
| 16.50–20.50 | d2 | d2 | – | – | – | – | – | – | – | – | – | – | d24 | d39 | d40 |
| | 5.0–6.9 mm | | | | | | | | | | | | | >16.0 mm | |
| 18.50–24.75 | d2 | d2 | – | – | – | – | – | – | – | – | – | – | d36 | d40 | d42 |
| | 5.0–6.9 mm | | | | | | | | | | | | ? | ? | ? |



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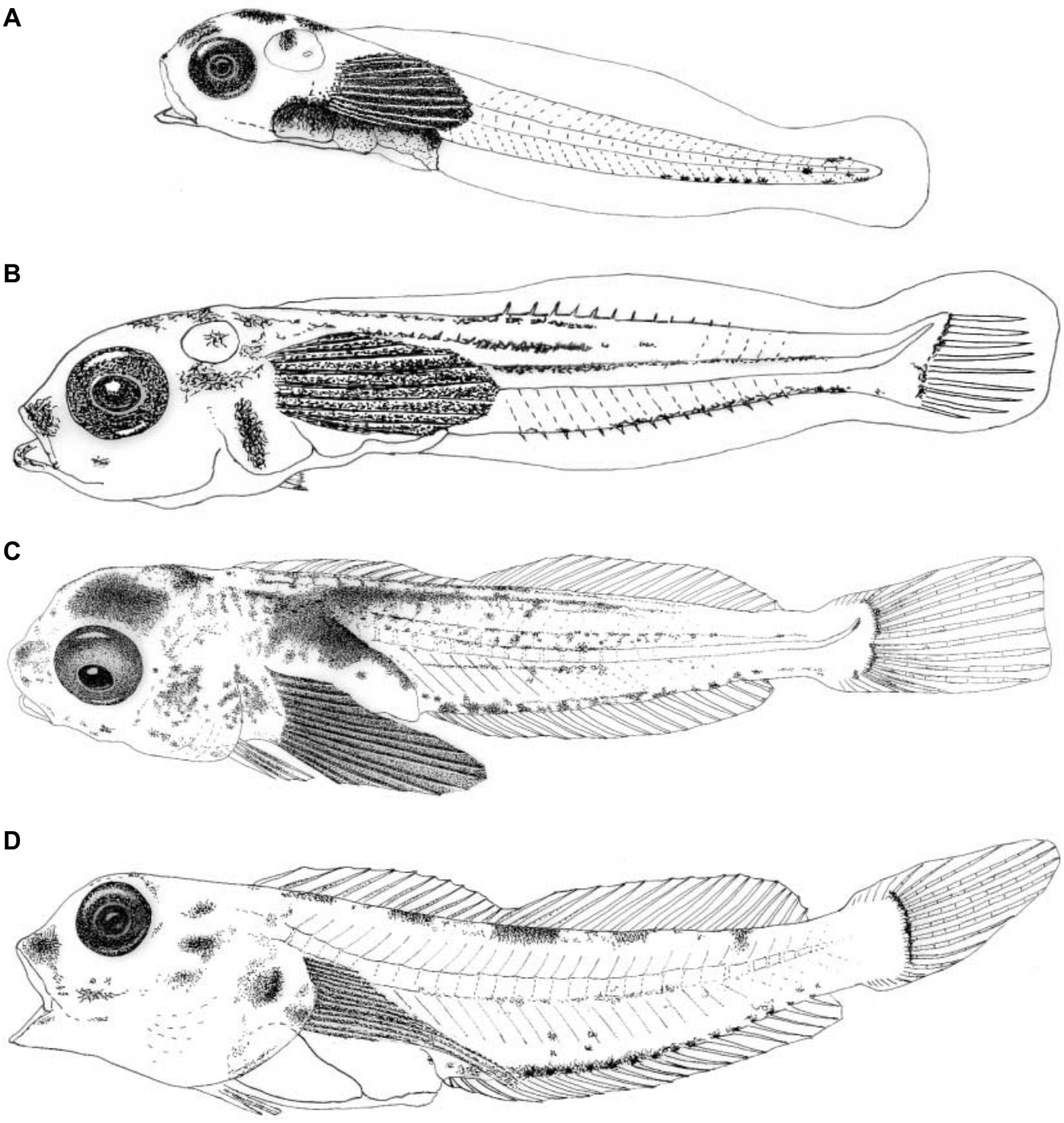


**Figure 2.** Larvae collected at different developmental stages: (A) day 1: newly hatched larva (5.0 mm TL); (B) day 14: 8.5 mm TL; (C) day 35: 12.8 mm TL; (D) day 41: juvenile (16.5 mm TL).

Pectoral fins were differentiated and the sagittae and lapilli otoliths were visible. The opercula were opened. Larvae presented heavy peritoneal pigmentation and 8–10 rows of melanophores on the pectoral fins. Ventrally, there were 1–2 melanophores on the base of the pectoral fins and 8–14 on the last myomeres, with 1–6 near the caudal tip. Dorsally, there were some sparse melanophores over the brain (between the eyes) and the upper lip, and one melanophore in the optic capsule (Figure 2).

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), on the throat, and at the cephalic region where melanophores extended from between the eyes to the dorsal region and opercula. At day 3 (5.8 mm), diffuse yellowish pigmentation, which subsequently extended all over the head and body, was present. Between day 6 and day 9 (6.2–6.9 mm) there was a small melanophore in the angle of the lower jaw, 2–3 on the opercula which increased in number and intensity during development, and 2–4 over the midline and the neural tube. The number and intensity of these melanophores increased, forming two dorsal and two lateral rows on each side of the body between day 25 and day 33



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(12.0–14.0 mm). At this time, all fin rays were present (D=XII+16–17; A=II+18; V=I+3; P=13), and there was a row of melanophores on the base of the caudal and anal fin and 3–4 at some dorsal fin rays.

After metamorphosis (16.0–17.0 mm) the juvenile pigmentation appeared. A ventral row of melanophores at the base of the anal and caudal fins was present, and almost all fin rays were pigmented. The head was heavily pigmented, with three large spots on the opercula, one at the angle of the lower jaw and one at the upper jaw. There was also some sparse pigmentation on the throat. Dorsally, at the base of the dorsal fin, there were 4–6 dark bands which extended to the midline, alternating with three other lateral dark bands present over the midline.

The change to a benthic mode of life was gradual. Between day 24 and day 36 after hatching (10.0–12.5 mm), fish began to contact the aquarium bottom. Gradually they spent longer times at the bottom, until definitely settling. Most fish settled between day 39 and day 42 after hatching (16.0–17.0 mm). Juvenile pigmentation only appeared between day 40 and day 53 (16.5–17.0 mm) and juvenile behaviour, such as thigmotaxis was observed only in fish older than 45 to 50 days (17.0–18.0 mm). These results agree with observations in the field, where the smallest fish collected in tide-pools were about 18.0 mm (SD=1.20, range: 16.0–19.0 mm, N=12), with some fish still lacking full juvenile pigmentation (C.F., personal observations).

## DISCUSSION

Nest sites are very similar to those used by the closely-related species *Lipophrys pholis*, both in captivity (Faria et al., 2002) and in the field (Portuguese rocky shores), where one nest site was observed to be occupied by males of the two species in different years (C.F., personal observations).

The basic sequence of embryonic and larval development described for *Lipophrys trigloides* largely agrees with the known descriptions for other related species of this family (e.g. Qasim, 1956; Sabatés, 1994; Faria et al., 2002). The duration of the larval period found in this study (42 days) is in accordance with the estimated period of larval duration described by Raventós & MacPherson (2001), based on otoliths readings of new-settlers collected in the north-western Mediterranean (37–71 days).

Since *L. trigloides* and *L. pholis* could live and breed in the same habitat, we believe that is worthwhile to attempt a summary of characteristics that may help in the identification in the field of eggs and larvae of these two species. In both cases, eggs have similar size. They can be distinguished however by their shape, the eggs of *L. pholis* are spherical (1.10×1.00 mm for major and minor axis) while those of *L. trigloides* are somewhat flattened above (1.30×0.86 mm for major and minor axes), and by the presence of the cup-like structure surrounding the base of *L. trigloides* eggs, which is absent in *L. pholis* (Faria et al., 2002).

The basic pattern of pigmentation of newly hatched larvae of both species consists of melanophores over the cephalic region and peritoneal regions, pectoral fins and a ventral post-anal row. Since both larvae present similar length at hatching (4.50–5.00 mm in *L. trigloides*; 4.73–5.33 mm in *L. pholis*), the only visible difference is the appearance with development of some melanophores at the angle of the lower jaw, which appear in *L. trigloides* with 6.0 mm, and are always absent in *L. pholis* (Faria et al., 2002).

The similarities of these two species are remarkable. Both have similar size eggs, present similar embryonic (16–17 days at 18°C in *L. trigloides*; 15–16 days at 17°C in *L. pholis*) and larval developmental times (42 days until settlement at 17.25°C in *L. trigloides*; 37 days until settlement at 16.50°C in *L. pholis*). Newly hatched larvae are quite similar in size and pigmentation, and settlement occurs at the same size (16–17 mm TL in *L. trigloides*; 15–16 mm TL in *L. pholis*).

Raventós & MacPherson (2001) noted that developmental patterns of fish of the same genus tend to be similar, and remarked that *L. trigloides* seems to be an exception, having a longer planktonic larval duration than that of other *Lipophrys* species, namely *Lipophrys canevae* (Vinciguerra, 1880) and *Lipophrys adriaticus* (Steindachner & Kolombatovć, 1883). Interestingly, Almada et al. (in press) analysed genetic material from these species and found that *L. trigloides* and *L. pholis* are sister species, while *L. canevae* and other small *Lipophrys* species formed a very distinct clade, questioning the monophyly of the genus *Lipophrys* which, according to the authors, must include only *L. pholis* and *L. trigloides*.

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