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Ontogenetic development of *Parablennius pilicornis* (Pisces: Blenniidae) in controlled conditions

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SUMMARY: The full developmental sequence from egg to juvenile of *Parablennius pilicornis* in controlled conditions is described. Embryonic development lasted 9 days at 18.0 to 21.0°C, 14 days at 17.0 to 20.0°C, 16 days at 16.0 to 19.0°C and 17 days at 14.0 to 18.5°C. Newly hatched larvae measured 3.1 mm total length (TL), had the mouth and anus open, pigmented eyes, almost no yolk, and the pectoral fins were small and unpigmented. Most larvae settled between day 66 and 69 after hatching (27.0 mm TL) and showed full juvenile pigmentation patterns between day 91 and 93 (30.0 mm TL). However, on day 61/62 after hatching (when they were more than 25.0 mm) they began to show a complex agonistic repertoire.

Keywords: *Parablennius pilicornis*, early ontogeny, Blenniidae.

RESUMEN: DESARROLLO ONTOGÉNICO DE *PARABLENNIUS PILICORNIS* (PISCES: BLENNIIDAE) EN CONDICIONES CONTROLADAS. – La secuencia de desarrollo, desde el huevo hasta el estadio juvenil, de *Parablennius pilicornis* se describe en condiciones controladas. La duración del desarrollo embrionario fue de 9 días a 18.0-21.0ºC, 14 días a 17.0-20.0ºC, 16 días a 16.0-19.0ºC y 17 días a 14.0-18.5ºC. La talla de las larvas en el momento de la eclosión era de 3.1 mm TL, la boca y el ano ya estaban abiertos, los ojos pigmentados, prácticamente sin vitelo y las aletas pectorales pequeñas y sin pigmentación. El asentamiento, en la mayor parte de las larvas, tuvo lugar entre el día 66-69 después de la eclosión (27.0 mm TL) y mostraban el patrón de pigmentación característico de de la fases juvenil entre el día 91-93 (30.0 mm TL). No obstante, a partir del día 61-62 después de la eclosión (TL> 25.0 mm) empezaban a mostrar un repertorio agonístico complejo.

Palabras clave: *Parablennius pilicornis*, ontogenia, Blenniidae.

INTRODUCTION

Parablennius pilicornis (Cuvier, 1829) is a common rocky subtidal fish species in the western Mediterranean and eastern Atlantic, from Biscay to South Africa and off Brazil and Argentina (Zander, 1986). In Portugal (Arrábida) the breeding period of this species is from February/March to September (Gonçalves and Almada, 1998). As in all blenniid studied so far (e.g. Wirtz, 1978; Almada *et al*., 1983; Heymer, 1995), the eggs are guarded by the male until hatching and the nest can contain eggs from different females and be at distinct developmental stages (Almada *et al*., 1987). Although there is some literature concerning the developmental biology of this species (e.g. Almeida *et al*., 1980; Denoix, 1984; Olivar, 1986), the information available on embryonic development is scattered and incomplete. Olivar (1986) provided a detailed description of larval development, but her study was based on fish collected from plankton and there is no chronology of the different events. In this paper the full sequence of embryonic development of *P. pilicornis* is presented for the first time, and a chronology of larval development is provided based on laboratory reared fish.

Eggs and larvae were obtained from a group of 4 fish (2 males: 10.0 cm TL and 11.0 cm TL; 2 females: 8.0 cm TL and 9.0 cm TL), kept in captivity since November 2003 at a public aquarium, Aquário Vasco da Gama (Lisbon). We also used four other batches of a pair of fish kept in captivity in 1994 and 1995 (for temperature ranges see Table 1), which provided replicates of some embryonic developmental events. The 600 l tank was illuminated with fluorescent light (60W) from 9:00 h to 18:00 h. The bottom of the tank was covered with a layer of sand and several large flat stones were provided as shelter and breeding sites.

The complete sequence of embryonic development was based on two batches spawned on vertical stones on 21 June 2004 and 22 July 2004. A sample of eggs was removed daily 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% formaldehyde. The egg capsules were opened and the embryos were removed to allow more detailed observations.

Full larval development was described from two other batches that hatched on 4 September 2004 and 23 November 2004. Upon hatching, larvae were reared in 30 l glass tanks, illuminated with fluorescent light (18W) 24 h a day. A constant flow of seawater was maintained. Larvae were fed two times a day with *Brachionus* sp. enriched with protein Selco (Artemia Systems) and algae, which was replaced by *Artemia* sp. nauplii by day 71-73. Larvae were collected, anaesthetized (Ethylene Glycol Monophenyl Ether – Merck) and photographed until metamorphosis. All specimens were preserved and deposited in the Aquarium Vasco da Gama collection. Eggs and larval measurements were taken in fresh material. Larval measurements correspond to total lengths and were taken from anaesthetized larvae.

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

	Mean (ºC)	Range (ºC)	SD	N
(A) Spawning				
28/11/1994	18.07	16.00–19.00	1.02	17
15/12/1994	16.83	14.00–18.50	1.54	17
09/01/1995	16.50	15.00–18.50	0.92	17
31/01/1995	18.56	17.00–20.00	0.91	14
21/06/2004	19.50	18.00–20.00	0.69	9
22/07/2004	20.60	20.00–21.00	0.45	9
(B) Hatching				
04/09/2004	18.09	16.00–19.00	0.74	73
23/11/2004	17.67	16.00–19.00	0.79	91

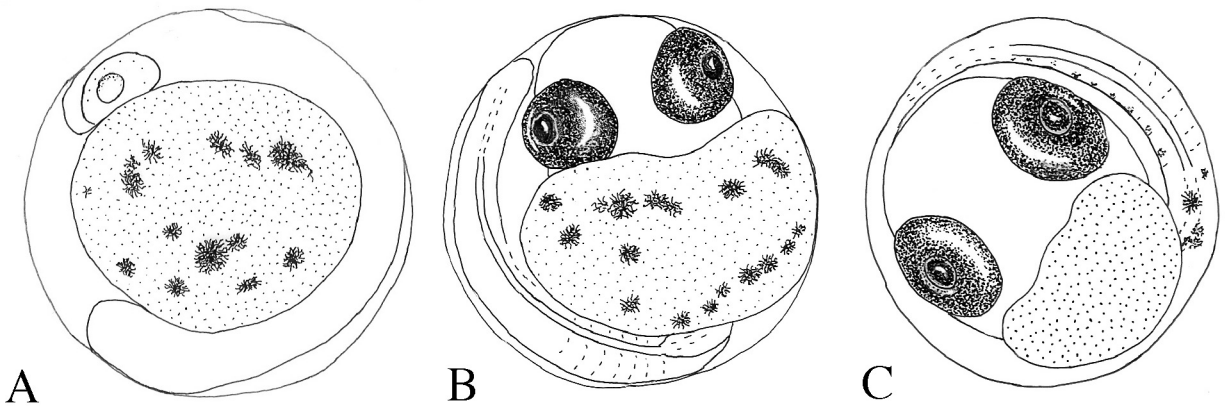

FIG. 1. – Eggs collected at different developmental stages: (A) Day 2: embryo differentiation; (B) Day 4: embryo with differentiated gut; (C) Day 7: embryo just prior to hatching (from batch spawned at 18.00 to 20.00˚C).

TABLE 2. – Ontogenetic events of embryonic development of *Parablennius pilicornis* in order of first appearance: (1) embryo recognizable; (2) cephalic and caudal dilatation: (3) eye lens; (4) brain; (5) notochord differentiation; (6) brain lobes; (7) nothocord; (8) myomeres; (9) beginning of pigmented eyes; (10) tail bud free of the yolk; (11) auditory vesicules; (12) otoliths; (13) gut differentiation; (14) median finfold; (15) embryo movements; (16) mouth differentiation; (17) anus visible but closed; (18) pectoral fin buds; (19) mouth visible but closed; (20) hatching glands; (21) anus open; (22) opercula differentiation; (23) mouth open; (24) opercula open; (25) gas bladder; (26) hatching. d is days following spawning.

ºC	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
18.00-20.00	d1	d2	d2	d2	d2	d3	d3	d4	d3	d3	d3	d4	d4	d4	d4	d5	d5	d7	d6	d7	d7	d8	d8	d9	d9	d9
20.00-21.00	d1	d2	d2	d2	d2	d3	d3	d3	d3	d3	d3	d4	d3	d4	d3	d5	d5	d6	d6	d6	d7	d8	d8	d9	d9	d9

TABLE 3. – Ontogenetic events of larval development of *Parablennius pilicornis* in order of first appearance (days after hatching): (1) exogenous feeding; (2) filled gas bladder; (3) teeth; (4) caudal fin rays; (5) notochord starts to flex; (6) pre-opercular spines; (7) anal fin rays; (8) dorsal fin rays; (9) pectoral fin rays; (10) notochord flexion completed; (11) segmented caudal fin rays; (12) ventral fin buds; (13) ossified vertebrae; (14) ventral fin rays; (15) larvae begin to make contact with the aquarium bottom; (16) most larvae settled on the bottom; (17) juvenile behaviours; (18) head tentacles; (19) typical juvenile pigmentation. (TL) Total Length. The two batches used for larval development were merged. Temperature range: 16.00 to 19.00ºC.

events	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
days	1-2	3-4	10-12	13	13	15-16	20	20	19-20	24-26	24-29	24-32	26-38	33-40	54-58	66-69	66	89-91	91-93
TL (mm)	3.0-3.2		5.0-6.2			7.0-8.2				10.0-11.5			13.0-14.0		20.0	?	?	?	30.0

Captive fish spawned repeatedly during the entire year. Recently laid eggs were transparent and golden brown, becoming greyer in subsequent days. They were almost semi-spherical (Fig. 1), although somewhat flattened on top, and had a flat attachment disk. The major axis was 0.60 mm (SD=0.07, range: 0.50 to 0.70 mm, N=10) and the minor axis was 0.52 mm (SD=0.04, range: 0.50 to 0.60 mm, N=10).

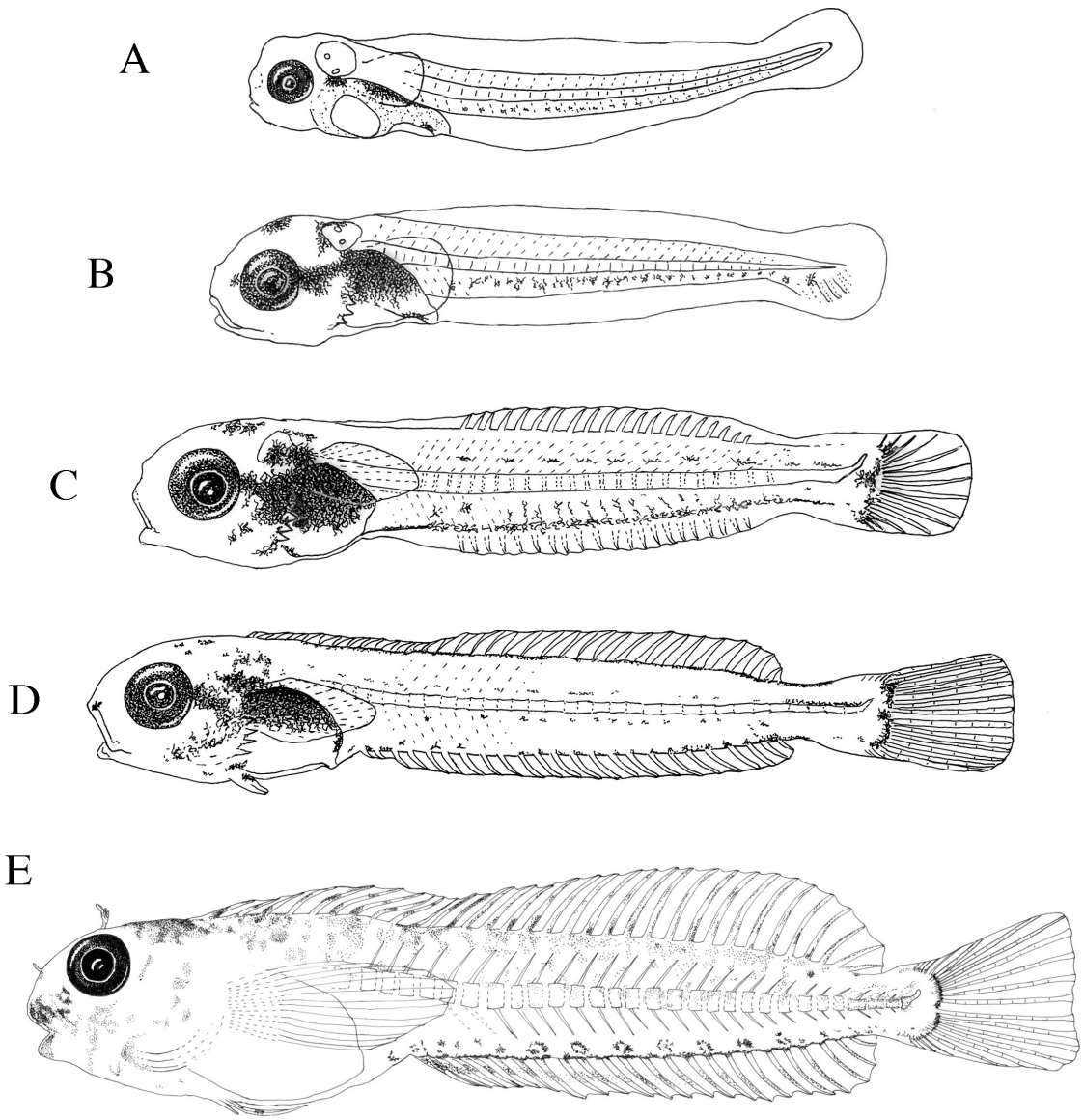

FIG. 2. – Larvae collected at different developmental stages: (A) Day 1: newly hatched larvae (3.1 mm TL); (B) Day 13: 6.0 mm TL; (C) Day 25: 10.0 mm TL; (D) Day 38: 14.0 mm TL; (E) Day 91: juvenile (30.0 mm TL).

The main ontogenetic events of embryonic and larval development at different temperatures are shown in Tables 2 and 3 respectively.

The length of the embryonic period varied with temperature: 9 days at 20.0 to 21.0°C, 14 days at 19.0°C, 17 days at 17.0 to 18.0°C (mean temperatures).

Newly hatched larvae (Fig. 2) measured 3.1 mm TL (SD=0.07, range: 3.0 to 3.2 mm, N=10). The anus was open. The mouth was also open, although it was relatively small and already had well defined lips and differentiated jaws. The yolk was almost fully absorbed. The opercula were open and the sagittae and lapilli otoliths were visible. The gas bladder was formed, but not completely filled. The eyes were fully pigmented and the pectoral fins were small and rounded (10% of TL), without any rays or pigmentation.

The larvae of this species presented some characteristic features like the presence of 6 or 7 preopercular spines (5 of which were well developed) from a length of 6.0 mm until metamorphosis and a relatively small pre-anal length (25 to 33% of TL from the earliest stages to juveniles). At 10.0-11.0 mm TL the notochord flexion was completed. At 13.0-14.0 mm TL the total number of vertebrae was 38, excluding the urostyle (10 pre-anal vertebrae) and all fin rays were present (D=XII+21; A=II+23; V=I+3; P=14).

The pigmentation patterns were distinct. Newly-hatched larvae had heavy peritoneal pigmentation. Ventrally, there was one melanophore near the anus and a series of melanophores (ca. 30) on the ventral midline of the tail. After day 2 there was an internal row of melanophores from behind the eyes to the gut. The pigmentation pattern was maintained during development, with an increase in the number and intensity of melanophores in the ventral row (from behind the anus to the caudal peduncle), on the liver and on the head and opercula. On day 1 or 2 after hatching (3.1-3.2 mm TL) there were melanophores on the base of the pectoral fins and in some larvae there was one on the upper lip. Between day 4 and day 7 after hatching (3.2-3.8 mm TL) diffuse yellowish pigmentation, which subsequently extended all over the head, was present. The melanophores of the ventral midline of the tail became more and more ramified and extended dorsally, almost reaching the level of the notochord on day 12-13 after hatching (6.0 mm TL). Between day 14 and day 19 after hatching (5.8-8.1 mm TL) two lines of melanophores appeared on the lateral walls of the tail (one under and one over the notochord), and on day 29 after hatching (10.5 mm TL) other dorsal melanophores appeared and formed one dorsal line. The number and intensity of these melanophores increased, forming four longitudinal lines along the entire body (one ventral line; two lateral lines, one under and one over the notochord; and one dorsal line). On day 27 to 32 after hatching (10.0 to 11.7 mm TL) another melanophore was also visible on the lower lip. On day 33–34 after hatching (13.0 mm TL) there was a row of melanophores at the base of the dorsal, anal and caudal fins. Some melanophores appeared at the pelvic and caudal rays on day 24-32 after hatching (10.0-11.7 mm TL) and at dorsal and anal rays on day 42-46 (16.0 mm TL).

The juvenile pigmentation appeared on day 91 to 93 after hatching (30.0 mm TL). A ventral row of melanophores at the base of the anal and caudal fins was present. There was a longitudinal band of melanophores along the dorsal and anal rays. The head and opercula were heavily pigmented. Melanophores were present on the upper and lower lip, at the throat and at the base of the pectoral fins. Dorsally, at the base of the dorsal fin, some dark bands extended to the midline, alternating with some other lateral dark bands present over the midline.

The change to a benthic mode of life was gradual. Between day 54 and 58 after hatching (19.0 to 19.8 mm TL), fish began to make contact with the aquarium bottom. Gradually, they spent longer times there, until definitively settling. Although we did not quantify this, most fish settled between day 66 and day 69 after hatching (27.0 mm TL), still without juvenile pigmentation. After that, they showed juvenile behaviours, such as thigmotaxis and turning movements of the head. However, on day 61 to 62 after hatching, the larvae (with more than 25 mm TL), although not fully benthic, began to show many of the typical agonistic behaviour patterns of the species, like charging, biting, chasing, butting, fleeing and lying flat on the bottom.

The basic sequence of larval development described in this paper for *P. pilicornis* fully confirms and completes the descriptions provided by Olivar (1986) based on larvae collected in the field. However, the newly-hatched larvae contrast sharply with those described by Almeida *et al.* (1980), which were smaller (2.6 mm) and did not have a fully developed mouth. Olivar (1986) also collected smaller larvae in the plankton. Perhaps the condi-

tions in the field (e.g. turbulence or temperature variations) are conducive to hatching at an earlier stage than in ideal conditions where the larvae hatch at a more advanced stage.

The chronology of larval development of *Lipophrys trigloides* (Valenciennes, 1836), for which there are data from laboratory reared fish (Faria *et al*., 2005) and otolith readings from specimens collected in plankton (Raventós and Macpherson, 2001), were remarkably congruent. Thus we feel reasonably confident that the chronology obtained in this study provides useful estimates for the timing of the different developmental events of *P. pilicornis* for the temperature range considered. Future studies based on otolith readings of plankton collected specimens would be of great help for assessing the validity of laboratory studies.

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