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Embryonic and larval development of the brown wrasse *Labrus merula* (Pisces: Labridae)

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In March 1997 one male and one female brown wrasse *Labrus merula* spawned spontaneously in aquaria conditions. Eggs were incubated at ambient temperature, salinity, oxygen and pH. The development of the eggs, yolk-sac larvae and larvae is described and illustrated with particular emphasis on features of practical value for identification of specimens from plankton. The ripe egg of brown wrasse is a typical labrid egg with a mean diameter of 0.93 ± 0.05 mm. The incubation period was 106 h 45 min at a mean temperature of 14.3°C. Newly hatched yolk-sac larvae were 3.8 ± 0.02 mm, while the yolk-sac was resorbed when larvae reached 4.68 ± 0.15 mm in total length. Some characteristics which may be useful for identification are described and compared with some other labrid yolk-sac larvae and larvae. The length of newly hatched yolk-sac larvae of brown wrasse was significantly larger (*t*-test, $P < 0.05$) than those of the other labrid species, but it is similar to that of *Labrus bergylta*. During the first 16 d (after resorption of yolk-sac) brown wrasse larvae does not possess a double crescent of melanophores on top of the head, but has a few melanophores on the anal fin which is very similar to the pigmentation of *Symphodus* (*Crenilabrus*) *melops* larvae, although there is a difference in length between them. Larvae older than 16 d have a double crescent of melanophores on the top of the head with melanophores on the anal fin-fold identical to *L. bergylta* larvae, but the difference in larvae length also exists.

INTRODUCTION

Brown wrasse, *Labrus merula* Linnaeus, 1758, is the object of intensive commercial and sport fisheries along the eastern Adriatic coast and has became as a consequence rare (Jardas, 1996). The fish are distributed throughout the Mediterranean, absent in the Black Sea, and in the eastern Atlantic from Portugal to Morocco including the Azores (Quignard & Pras, 1986). They inhabit areas around rocks and seaweed in coastal waters at depths down to 50 m (Jardas, 1996). It is an obligatory carnivore, preying on sea urchins, ophiuroids, molluscs, crabs and worms (Quignard & Pras, 1986; Jardas, 1996).

As reported by Sordi (1962), Onofri (1975) and Jardas (1996) the species is protogynous hermaphrodite and this is shown by the fact that 50% of the population are males that have changed sex, and the other 50% of the population consists of younger females. The brown wrasse eggs are adhesive (Quignard & Pras, 1986). In nature spawning takes place at the end of winter and beginning of spring (Onofri, 1970, 1975; Jardas, 1996). The scientific literature pertaining to the biology and ecology of this species in the eastern Adriatic and elsewhere is rather sparse. Onofri (1970, 1975, 1975a) presented data on morphological and meristic characteristics of brown wrasse in the middle Adriatic. This author (1980) reported data about the behaviour of this species in the southern Adriatic. Quignard (1966) presented data about the general biology of brown wrasse from European coastal waters.

Information on early developmental stages of brown wrasse is lacking. Species identification of early life history stages is critical for systematics as well as for studies using larval abundance for population estimates. Data obtained by larval rearing can provide essential identification information and permit an evaluation of variation in diagnostic characters of larvae from different ages, broods, and species. The relative importance of these diagnostic characters may then be evaluated.

This paper presents the first data about the embryonic and larval development in aquaria conditions and the first descriptions of the early life history stages of *Labrus merula*. The objectives are to describe the early life history and to assist in the identification of planktonic stages of this species.

MATERIALS AND METHODS

Parental stock (three males and five females) were kept in aquaria at the Institute of Oceanography and Fisheries, Biological Station in Dubrovnik, at ambient salinity, temperature and natural photoperiod. A temperature range between 12.8 and 15.1°C (mean $14.27° \pm 0.542$), salinity 37.1 and 39.8 psu (mean 39.0 ± 0.623), oxygen 7.3 and 8.4 mg l^{-1} (mean 7.92 ± 0.212), pH 7.7 and 8.2 (mean 7.97 ± 0.056). This stock was collected at Elafiti Archipelago (southern Adriatic) near Dubrovnik between May and June 1996. During one year fish were fed food predominantly composed of small pelagic fish, molluscs, sea

urchins and worms. In March 1997 one male and one female spawned spontaneously. From 10 to 30 March the same female spawned three times. Eggs, yolk-sac larvae and fed larvae from the first spawn were used for the measurements and descriptions. Ages of parental fish were determined by a technique described by Onofri (1975). The male was 8-y old (total length = 34.2 cm, total weight = 530.2 g) and the female was 7-y old (total length = 34.0 cm, total weight = 510.4 g). The spawning began when males changed body colour from greenish to dark-blue, while females developed dark vertical stripes and enlarged their bellies. The adhesive eggs were placed by female at the bottom of the aquaria and fertilized with sperm from one male. The fertilized eggs were then moved to tanks (~300-l) with a constant flow of 50–70% seawater daily through a 50-μm mesh net. The experiment was carried out under an artificial photoperiod (12:12 h, 2000 lux). The water was gently aerated from the bottom of the tank. To control bacterial concentrations, streptomycin sulphate (30 mg l^{-1}) was added at the initiation of the incubation period. Tanks were cleaned once daily by siphoning so that stagnant water in the deeper parts of the tanks was removed.

Embryonic development was observed under a binocular microscope and photos of individual stages were taken. The diameters of the eggs were measured. One hour after fertilization a sample of 10–20 eggs was taken every 5–7 min to determine the exact time of first cleavage. Embryogenesis was examined at different time intervals. Anaesthetized larvae in live condition were measured at an accuracy of a few hundredths of a millimetre using an ocular micrometer attached to a binocular microscope. The following measurements were taken: total length, the distance along the midline of the body from the tip of the snout to the end of the caudal fin; notochord length, the distance along the midline of the body from the tip of the snout to the end of the notochord; preanal distance, the distance along the midline of the body from the tip of the snout to the vent; head length, the distance between the tip of the upper jaw and the cleithrum; body depth, the perpendicular depth of the trunk at the anus; greatest body depth, body depth at its widest point; yolk-sac volume; and horizontal eye diameter. About 10–15 living yolk-sac larvae and larvae were used for each measurement. The time to yolk-sac resorption as well as to mouth opening were recorded. The mouth width of larvae was also measured. Cardiac contractions per minute were also recorded at each measurement. One day before hatching, the flow (50–70%) was opened through the 125-μm mesh outlet filter.

For the first 15 d larvae were fed exclusively on rotifers, *Brachionus plicatilis*, cultured in a thermostatic chamber at 26°C and 25–28 psu. Feed of rotifers consisted of the green alga, *Chlorella* sp., supplemented with baker's yeast (15–20%). Rotifers were added to tanks with larvae, to a density of 7–10 ind ml^{-1}. At the initation of feeding 60 rotifers were measured and a size range of 90–280 μm was established. With the introduction of AF Grade *Artemia*, rotifers were still given but at a reduced density. *Artemia* nauplii (San Francisco Bay, USA) were in the size range 240–365 μm. They were supplied (early in the morning and an hour before turning off the light in the evening) until a concentration between 1–3 ind ml^{-1} was reached. On day 27 (after resorption of the yolk-sac) all larvae died, so observations and measurements were stopped.

RESULTS

Mature unfertilized eggs were spherical and attached by mucus to the bottom of the aquaria. The yolk was homogeneous and unsegmented (Figure 1A). Eggs ranged in diameter from 0.83 to 1.05 mm, with a mean (±SD) of 0.93 ± 0.5 mm. There were no visible oil globules in the eggs. Table 1 illustrates changes observed during embryonic development. After fertilization a narrow periviteline space developed. Dead eggs were removed. The first cleavage occurred at about 2 h 52 min after fertilization (Figure 1A), the second after 3 h 27 min, and the third, which was parallel to the second, at 4 h 11 min. At 17 h the blastoderm was in advanced stages of cleavage or 'mulberry stage', while gastrulation started 30.5 h after fertilization. Formation of the embryo began after 41 h and somatic segmentation after 56 h. Between 58 and 65 h, 10–13 myomeres were clearly visible, the optic vesicles and olfactory lobules were clearly visible and the pericardial cavity was developing. With the formation of the embryo, dendritic melanophores appeared on the yolk sac surface and on the dorsal and ventral surface of the embryo. An increase in pigment and migration of the embryonic pigment to the pattern later shown by yolk-sac larvae took place during later stages of egg development. The heart was observed beating after 65–66 h, while movements of the embryo were observed 68 h after fertilization. At 100 h, the embryo occupied three-quarters of the yolk-sac circumference, and dendritic melanophores were clearly visible on the yolk-sac and ventral–dorsal surface of the embryo (Figure 1B).

Hatching started at 104 h 25 min, and after 2 h 20 min all yolk-sac larvae hatched. The newly hatched were transparent and floated at the surface with yolk-sac uppermost and sometimes in a lateral position. The head and the anterior part of the body were curved around the yolk-sac and the fin-fold invested much of the body (Figure 2A). The body is segmented into 38–39 myomeres. The mouth was undeveloped, but a straight, simple tubular gut was observed. There were black and yellow-green chromatophores covering most of the trunk, but leaving the caudal region free. The distance from the

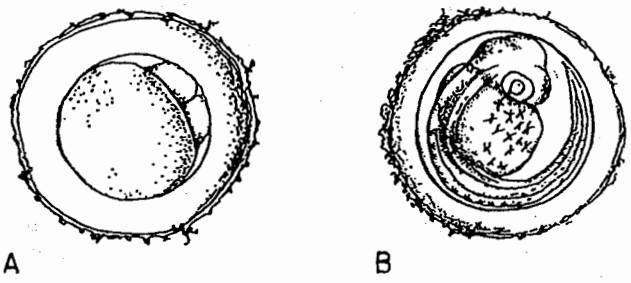

Figure 1. Embryonic development of *Labrus merula*: (A) meridional first cleavage; (B) embryo—tail tip almost touches the head.

Table 1. *Embryonic development of* Labrus merula *at mean temperature 14·3°C.*

Time (h)	Time (min)	Stage	Description
	0	Fertilization	
2	52	2 cells	Meridional first cleavage
3	27	4 cells	Second cleavage
4	11	8 cells	Cleavage parallel to the second
5	20	16 cells	Cleavage parallel to the first
17	05	Morula	'Mulberry stage'
27	10	Blastula	Visible blastocel, germinative ring
30	30	Gastrula	Gastrulation starts
33	34	Gastrula	Invagination of blastomeres ends, embryo stretched (head towards the vegetative pole)
36	05	Neurula	Formation of neural groove starts
41	12		Formation of embryo begins, notochord
42	10	Embryo	
55	52		Somatic segmentation begins, formation of optic vesicles and forebrain
58	38		Optic vesicles formed, olfactory lobes differentiated
59	15		Somites clearly visible, melanophores appear along the dorsal side and on the yolk-sac
62	48		Somite differentiation completed, optic vesicles and olfactory lobules clearly visible
65	05		Cardiac contractions (66 min^{-1})
68	12		Embryo well developed and connected with the yolk-sac, head close to tail, melanophores very marked on the yolk-sac
73	35		Cardiac contractions (75 min^{-1})
76	45		Tail lifted clear of the yolk-sac
83	09		Tail movement begins
89	15		Tail tip almost touches the head
92	11		Rhythmical movements every 12–14 s
98	15		Very marked and frequent movements every 4–6 s
100	21		Cardiac contractions (86 min^{-1})
104	25	Free yolk-sac larvae	Hatching begins
106	45		All yolk-sac larvae hatched

snout to the posterior end of the pigmented area was 3.02±0.1 mm. The anus opened slightly further than halfway along the body. The melanophores were arranged in four longitudinal rows. They were situated on the myomeres, four above the notochord on each myomere and four below. The yellow chromatophores were in similar rows but situated on the lines between the myomeres. There were 2–3 melanophores in the otocystic region and 1–2 at the snout. There were 55–60 dendritic melanophores on the yolk-sac, about 6–8 around the anus. Table 2 shows changes in length and shape of yolk-sac larvae during the first 6 d from hatching. At the beginning of day 2 (25 h after hatching) yolk-sac larvae are still heavily pigmented (Figure 2B). There were no melanophores on the dorsal fin-fold, but there was a row of 7–8 on the anal fin-fold just behind the anus. The eyes were not pigmented. At the end of the second day, granular pigmentation had developed in the eyes. By day 3 the maxillaries and lower jaw were forming but were not distinct. Faint granular pigmentation of the eye was completed. The development of the caudal fin, indicated by a thickening of tissue on the ventral side of the notochord, began at 4.3 mm TL. By the end of day 3 (about 72 h after hatching), the foregut and hindgut were visible. Two-thirds of the yolk sac had already been absorbed which strongly affected the yolk-sac larval growth. The anus was open by day 4. The differentiation of pectoral fins had started. The maxillaries and lower jaw were distinct but the mouth was not open. A number of melanophores increased to 15–18 in the row on the anal fin-fold just behind the anus. By the end of day 5 (118 h after hatching) the mouth was completely open and functional (Figure 2C). It ranged from 0.32 to 0.44 mm. The eye was completely pigmented. The body and notochord were straight. The yolk sac was resorbed by day 6 (Figure 2C). On the seventh day the passage of food along the digestive tract of the larvae was clearly visible (Figure 2D). Larvae were mobile at that time, and able to swim on the surface. Head length increases from 11.8% of total length in 3.76 mm to 16.3% in 4.52 mm yolk-sac larvae. Greatest body depth ranged from 0.64 mm in 3.76 mm to 0.94 mm in 4.52 mm yolk-sac larvae, while body depth ranged from 0.23 mm in 3.76 mm to 0.44 mm in 4.52 mm yolk-sac larvae.

Total length of larvae ranged from 4.72 to 5.68 mm at day 27 (after absorption of yolk-sac). Preanal length ranged from 2.52 mm in 4.72 mm to 3.46 mm in 5.68 mm larvae. Head length increases from 17.0% of total length in 4.68 mm to 29.8% in 5.68 mm larvae. Greatest body depth ranged from 0.66 mm in 4.68 mm to 0.80 m in 5.68 mm larvae. Body depth ranged from 0.41 to 0.56 mm in larvae of the same size. When the larvae reached 4.92 mm the posterior end of the notochord is fully bent up and the dorsal and anal fins analages were recognizable. Larvae were characterized by intensive pigmentation in the same regions as like as yolk-sac larvae, with heavy internal

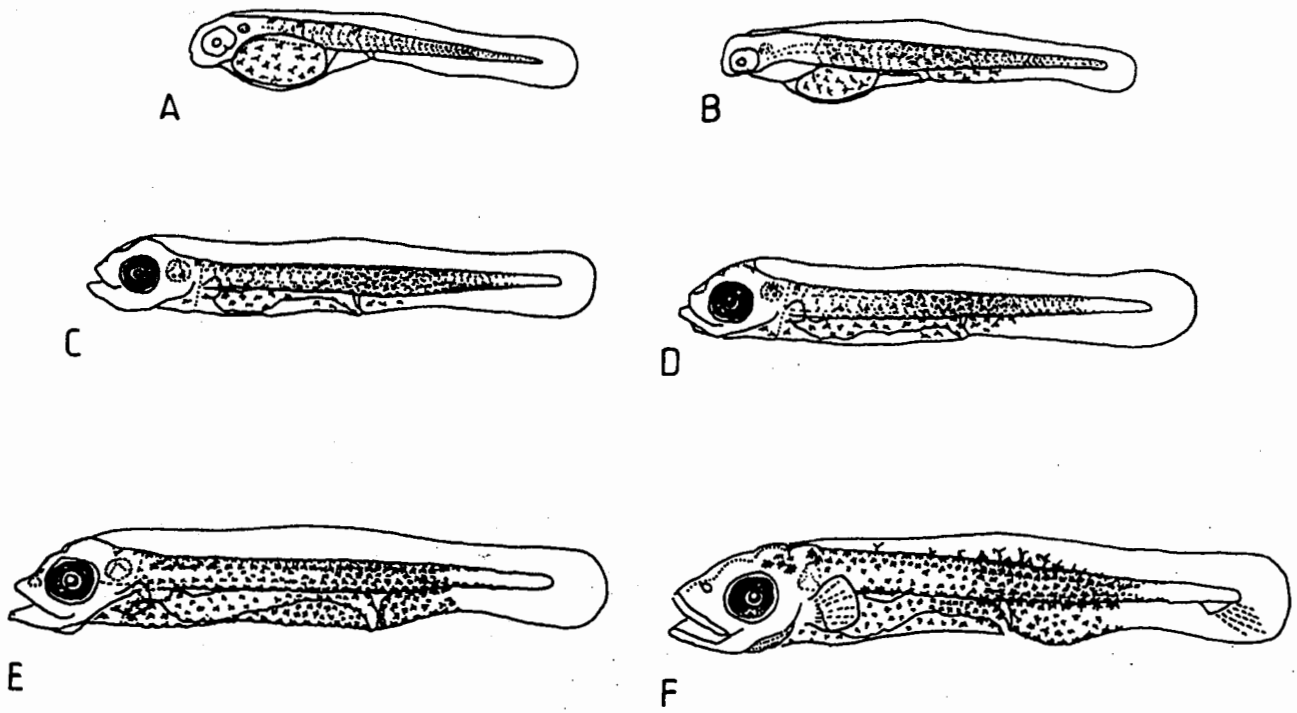

Figure 2. Yolk-sac larvae and larvae of *Labrus merula*: (A) newly hatched yolk-sac larvae; (B) 24-h old yolk-sac larvae; (C) 6-d old yolk-sac larvae; (D) 7-d old larvae; (E) 16-d old larvae; (F) 30-d old larvae.

Table 2. *Changes in length and shape of* Labrus merula *yolk-sac larvae during the first 7 d from hatching at mean temperature 14.3°C.*

Days from hatching	Total length (mm)	Notochord length (mm)	Yolk-sac volume (mm^3)	The eye diameter (mm)	The distance from the snout to the anus (mm)
0	3.76±0.2	3.59±0.2	0.376	0.29±0.02	1.98±0.1
1	3.99±0.3	3.81±0.3	0.291	0.30±0.02	2.08±0.0
2	4.14±0.2	3.94±0.2	0.240	0.29±0.02	2.13±0.1
3	4.44±0.1	4.22±0.1	0.195	0.32±0.01	2.35±0.0
4	4.52±0.1	4.29±0.1	0.055	0.33±0.02	2.43±0.1
5	4.68±0.2	4.45±0.1	Resorbed	0.34±0.02	2.51±0.1
6	4.72±0.1	4.45±0.1		0.34±0.01	2.52±0.1

Table 3. *Comparison of data for eggs, yolk-sac larvae and larvae of different labrid species.*

Species	Source	Diameter of egg (mm)	Hatched yolk-sac larvae (mm)	Larvae- start of active feeding (mm)	Preanal length (mm)	Egg diameter (mm)
Ctenolabrus rupestris	Ehrenbaum (1905–1909)			3·14		
	Heincke & Ehrenbaum (1900)		1.95–2.19			
	Holt (1899)	0.72–1.01				
Labrus bergylta	Matthews (1887)	1.0	3.8		2.1	0.27
	Danois (1913)	0.7–0.8		~6.5		
Symphodus cinereus	Quignard (1962)	0.72–0.73	3.00	3.14		
Crenilabrus ocellatus	Sparta (1931)	0.68	3.00			
Symphodus (Crenilabrus) melops	Quignard (1967)	0.80–0.85	2.5–3.0	2.9–3.1		
Labrus merula	Present study	0.83–1.05	3.76	4.45	2.0	0.29

pigmentation on the swim bladder. Pigmentation was characterized by the small number of melanophores (spots) on the head, without a definite crescent-shaped arrangement, and without melanophores along the anal fin-fold until they reached 16-d old (Figure 2E). Then larvae were characterized by the paired crescent-shaped groups of melanophores on the head and melanophores on the dorsal fin (Figure 2F). There were no melanophores on the caudal region, the snout nor lower jaw.

DISCUSSION

Since 25% of the fish of our parental stock matured spontaneously in aquaria it may be concluded that *Labrus merula* are able to complete their sexual cycle in captivity. Fish were sexually mature by mid March, which is in agreement with the data of Grubišić (1962) and Jardas (1996). Release of ripe eggs lasted for 20 d. This indicates that brown wrasse is a partial spawner in which the maturation of oocytes is not synchronized, as in some sparids: *Sparus aurata* (Katavić, 1984), and *Dentex (Dentex) dentex* (Glamuzina et al., 1989).

A comparison of egg diameters and total lengths of newly hatched yolk-sac larvae and larvae in some labrids is presented in Table 3. It is difficult to identify morphological characteristics by which the eggs of brown wrasse differ from those of other labrid species. However, only two of labrid species *Ctenolabrus rupestris* and *Coris julis* spawn pelagic eggs (Russell, 1976) in the Mediterranean. The eggs of the remaining species including *L. merula* being demersal and laid down in nests or shallow depressions (Onofri, 1970). The level of relative variation of egg sizes in marine fish populations is shown to be consistent across a wide taxonomic range of species and much of this initial variation appears to be due to maternal effects (Chambers & Leggett, 1996). The length of newly hatched larvae of brown wrasse was significantly larger (t-test, $P<0.05$) than those of the other labrid species (Table 3), but it is similar to that of *Labrus bergylta*. This information may assist in identifying newly hatched yolk-sac larvae; however, it should be of limited value since during the first few hours after hatching yolk-sac larvae grew very rapidly and the length changed very quickly. At the temperatures at which eggs were taken in the present study (mean temperature 14.3°C) embryonic development would take 106 h 45 min. The duration of the egg and larval phases vary with temperature (Lasker, 1981). In addition to effects on the rate of development, temperature has been reported to alter the relative timing of the appearance of morphological characters. Quignard (1967) studied the effects of different temperatures on the development of the egg of *Symphodus (Crenilabrus) melops* and found this to be the case. At the temperatures at which eggs were taken in Quignard's study (15 and 21°C) embryonic development required 144 h and 88 h, respectively.

Ford (1922) provided a clear key for identification of (state geographical area) labrids based on the pigment patterns alone. He confirmed his identifications by rearing the larvae in aquaria until the adult characters could be observed. Ford (1922) showed that larvae could be grouped according to their pigmentation patterns as follows:

A. With many melanophores covering the sides of the body, except the tail region.
   1. With a double crescent of melanophores on the top of the head.
      — With melanophores on anal fin-fold ..... *Labrus bergylta*
      — With no melanophores on anal fin-fold ..... *Centrolabrus exoletus*
   2. No double crescent of melanophores on top of head, but a few only; with melanophores on anal fin-fold ..... *Symphodus (Crenilabrus) melops*

B. No pigmentation on sides of body, and only limited numbers of melanophores.
   1. Five dorsal and three postanal ventral body contour melanophores ..... *Labrus mixtus*
   2. One postanal ventral body contour melanophore ..... *Ctenolabrus rupestris*

Pigmentation of brown wrasse larvae is similar to that of *L. bergylta* and the differences in preanal length and eye diameter are negligible. During the first 16 d brown wrasse larvae lack a double crescent of melanophores on top of the head, and possess few melanophores on the anal fin-fold similar to the pigmentation in *Symphodus (Crenilabrus) melops* larvae, but a difference in length exists (Table 3). Larvae older than 16 d have a double crescent of melanophores on the top of the head and melanophores on the anal fin-fold identical to *L. bergylta* larvae, but the difference in larval length also exists (Table 3). It is also possible that in their early stages the larvae of labrid species with pigmented sides may be confused with those of the goby *Lebetus* (Russell, 1976).

Spawning period and geographical distribution could also assist in determining the early life history stages of labrids. Labrids recruited mainly between July and September, with a pronounced peak between the end of July and the beginning of August (Jardas, 1996). *Labrus merula*, *L. viridis*, *L. bimaculatus* and *Thalassoma pavo* spawn from March to April (Jardas, 1996), while mass recruitment in labrids started in June (*Symphodus roissali*) and July (*Symphodus melanocercus* and *Symphodus tinca*), and continued through August (*Coris julis*, *Ctenolabrus rupestris*, *Symphodus cinereus*, *S. doderleini*, *S. mediterraneus*, *S. ocelattus* and *S. rostratus*) (Dulčić, 1993). Distribution of *L. bergylta* is in the eastern Atlantic from Norway to Morocco including Madeira, Azores and Canaries; while *L. merula* occurs in the Mediterranean and eastern Atlantic from Portugal to Morocco including the Azores (Quignard & Pras, 1986). Problems associated with the identification of early life stages of these two species could arise only in the last three areas mentioned.

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