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Egg and early larval development of laboratory reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) (Picies, Serranidae)*

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SUMMARY: The embryonic and early larval development of the laboratory-reared dusky grouper, *Epinephelus marginatus* (Lowe, 1834) are described and illustrated. The eggs, with a mean diameter of 846.68 ± 41 μm and a range from 736-940 μm, were spherical and transparent with transparent chorion. Embryonic development lasted 30 hours at 23°C. Newly-hatched larvae were 1.52 ± 0.066 mm in length. Absorption of the yolk sac was complete after the fourth day, when larvae reached 2.63 ± 0.123 mm in total length. The mouth opened 72 hours after hatching, and was in function after 96 hours, with an opening diameter ranging from 250-300 μm. Larvae had two fields of intensive pigmentation, one above the intestine, and the other between the anus and the end of the notochord.

Key words : Dusky grouper, *Epinephelus marginatus*, egg and embryonic development, characteristics of larvae, pigmentation.

INTRODUCTION

The dusky grouper, *Epinephelus marginatus* (Lowe, 1834), is distributed throughout the Mediterranean Sea, and in the Atlantic, from the coast of the British Isles to Southern Africa, and along the coast of Southern Brazil. It inhabits rocky bottoms, from shallow waters to depths of 50 meters and lives in caves of stone blocks, where it spends most of its time (Jardas, 1996). The early life history is unknown, especially that describing egg and larval ecology, and it is difficult to determine these stages in ecological investigations.

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In Southeast Adriatic waters the dusky grouper spawns during late August and early September. Eggs were collected at the beginning of September, excluding larval stages (Skaramuca *et al*., 1989). Discoveries recording ripe eggs sites are rare, based on ichthyoplanktonic research (Sparta, 1935). In recent times, spawned eggs have been described (Barnabe, 1974 : Zabala *et al*., 1997). Little data whatsoever are available regarding larval stages, except for the description by Barnabe (1974), and one picture of larva published later (Zabala *et al*., 1997).

For the systematic study of larval abundance in population estimates, the identification of early stages is critical. This paper presents results of egg and embryonic development, including description of early larval stages, for laboratory spawned and reared dusky grouper.

MATERIALS AND METHODS

Broodstock were collected from southeastern Adriatic waters and were held from one to ten years in aquarium conditions, at ambient seawater temperatures (12-24°C) and salinities (36-38 ppt), pumped at eight meter depths and 30 meters distance from the shore. The fish were spawned using hormonal treatment (Glamuzina *et al.*, 1998a) and eggs were fertilised with sperm from sex-reversed males (Glamuzina *et al.*, 1998b). Dry fertilisation lasted for 15 minutes and the remaining spermatozoa were rinsed through a 350 μm sieve with a light spout of fresh seawater. The rinsed eggs were transferred to a glass jar and floating eggs were collected. Eggs and larvae were incubated at 23°C , flow-through sea water, with aeration from the bottom. Samples of 30 eggs were taken every hour, and samples of 30 larvae every six hours, for description and measurement, using an ocular microscope. Careful examinations were carried out, supported by photography and drawings. Later on, the samples were fixed in 8% buffered formalin for more detailed morphological studies.

The characteristics of newly-spawned ripe and fertilised eggs were noted, together with the duration of each embryonic stage. Embryogenesis characteristics were monitored.

Larval development was described using measurements of total length: the distance along the midline of the body from the tip of the snout to the end of caudal fin rays; standard length: the distance along the midline of the body from the tip of the snout to the end of the urostyle; preanal distance: the distance along the midline of the body from the tip of the snout to the anus; 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 as its widest point; eye diameter, longer diameter of the yolk sac and diameter of oil globule.

RESULTS

Egg characteristics and embryonic development

The average egg diameter of the dusky grouper was 846.68 ± 41 μm, with sizes varying from 738-940 μm. However, within one hour following fertilisation, the egg diameter became more uniform. Samples of surviving eggs showed that there were no eggs diameters less than 840 μm and egg size increased to an average size of 869.6 ± 35.1 μm and remained so up to the hatching of larvae.

The eggs were transparent and spherical. Developing eggs had only one oil globule. Those of poorer quality were slightly opaque and had two or more oil globules. The eggs were buoyant, with oil globules having an average diameter of 160 ± 21 μm (range 115-220 μm).

TABLE 1. – Embryonic development of dusky grouper, *Epinephelus marginatus,* at 23°C

Time Hour	Time Minutes	Stage	Description
0	00	Fertilisation	
1	05	2- cells	first cleavage
1	20	4- cells	second cleavage, plane perpendicular to the first
1	40	8- cells	cleavage parallel to the second
2	10	16- cells	cleavage parallel to the first
2	30	32- cells	
3	20	64- cells	
5	00	morula	
8	15	gastrula	gastrulation starts
15	20	neurula	formation of neural groove starts, formation of embryo begins, notochord
21	00	embryo	embryo well developed, somites clearly visible, optic vessicles formed
27	00	embryo	sporadic movements of embryo, heartbeat rate 80 per minute
29	00	embryo	tail tip almost touches the head, rhythmical movements every 10 seconds, heartbeat rate 90 per minute
30	00	free larva	hatching begins
31	30	larva	50% larvae hatched
33	00	larvae	more of 95% larvae hatched

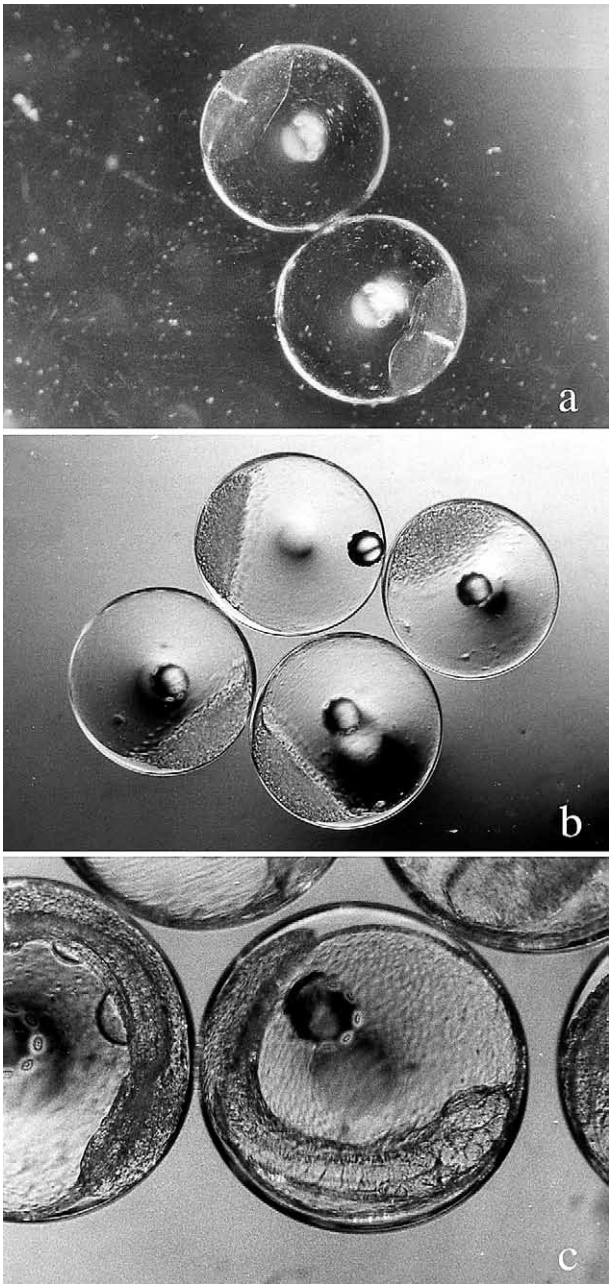

FIG. 1. – Pictures of dusky grouper eggs at different stages of embryogenesis: a) two-cell stage, b) gastrula, and c) embryo.

Shortly after stopping aeration in the tank, all eggs grouped at the water's surface.

One hour and five minutes after dry fertilisation, the blastodisk divided into two cells for the first time. Eggs developed in a manner typical for teleosteans (Ahlstrom and Ball, 1954). Table 1 details the next key phases of embryonic development. Figure 1 presents photographs of eggs.

Larval development

The average net total length of newly-hatched, dusky grouper larvae was 1.52 ± 0.07 mm. Larvae varied from 1.40- 1.67 mm in total length, and were characterized by huge yolk sacs, along almost the entire body, except for the small tail part (Figs. 2a and 3 a). The body was somewhat curved around the yolk sac. After hatching, larvae floated in the water column, without significant movement, except for sporadic tail thrusts. If aeration of the tank was stopped, all larvae rose to the surface and collected in large formations.

A few hours after hatching, the larvae showed significant growth. The growth rate was highest during the first 24 hour, afterwhich it decreased significantly. Table 2 shows changes in all measured characteristics of the larvae during the first five days. Drawings and photographs of the larvae during this period are presented in Figures 2 and 3.

The appearance of two areas of pigmentation represents the basic morphological characteristic of the dusky grouper in its early larval stage. The first area, located in the middle between the anus and above and bellow the posterior edge of the notochord appeared on the second day following hatching (Fig. 2d). During the next few days, these two areas enlarged and finaly joined one another. A second area of pigmentation was above the front intestine and stomach. Pigmentation occurred here a day later and in a few days

TABLE 2. – Changes in lengths and shape of the dusky grouper, *Epinephelus marginatus* yolk sac larvae during the first five days from hatching at constant temperature of 23°C.

hours after hatching	total length (mm)	standard length (mm)	preanal length (mm)	head length (μm)	maximal width (μm)	minimal width (μm)	the eye diameter (μm)	longest yolk sac diameter (μm)	oil globule diameter (μm)
0	1.52							890	160
15	2.14	1.81	1.07		530	287	152	840	140
39	2.41	2.23	1.16	401	564	245	160	450	100
65	2.49	2.15	1.12	463	537	301	192	190	50
89	2.55	2.41	1.18	557	471	247	229	yolk in	20
110	2.63	2.26	1.08	524	538	284	236	traces resorbed	resorbed

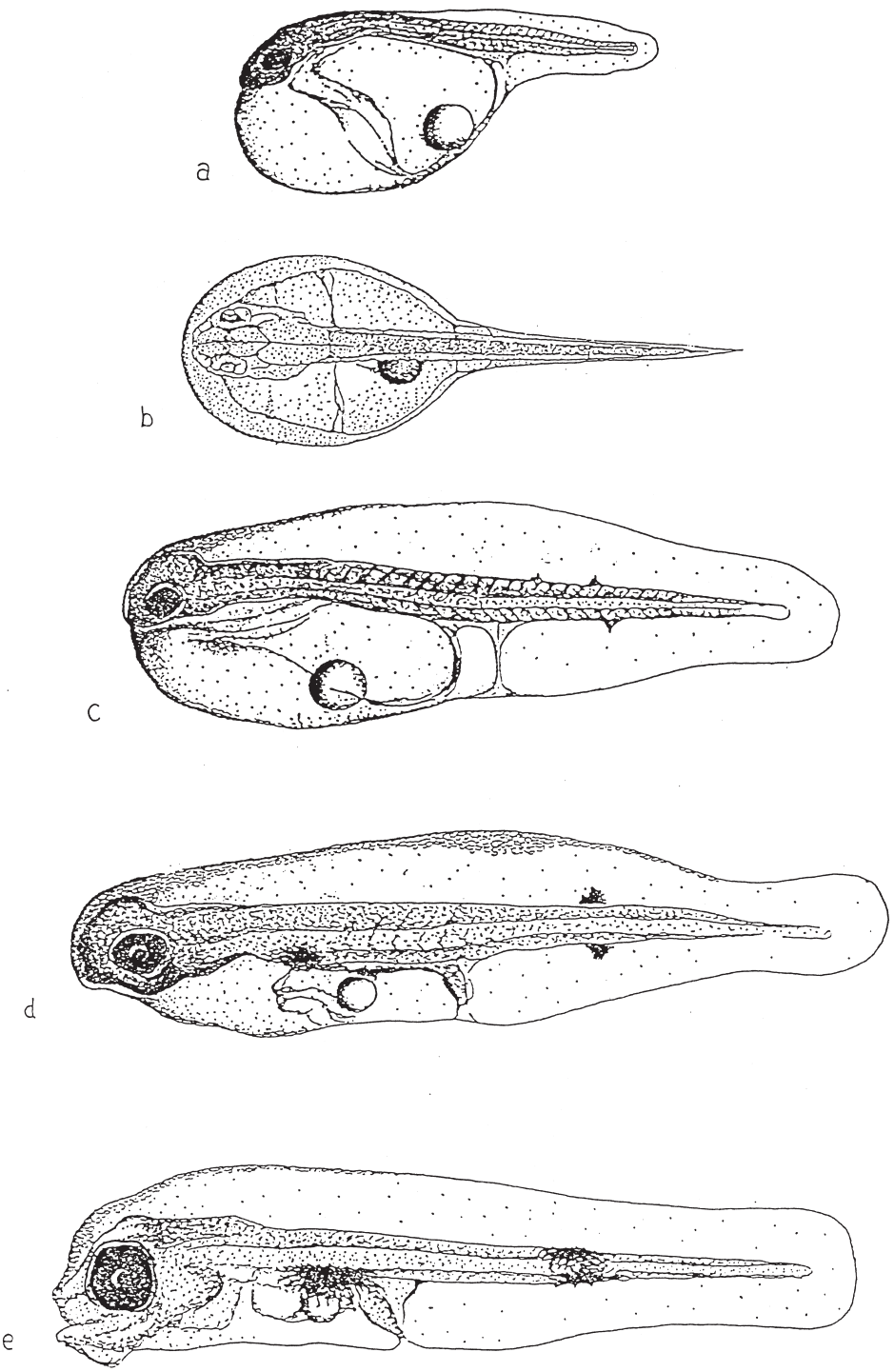

FIG. 2. – Drawings of dusky grouper larvae: a,b) newly-hatched larva, c) 24-hour old larva, and d) 60-hours old larva, e) 96-hours old larva.

became so intense that it covered the entire top area of the front intestine and stomach, totally hiding the swimbladder which started to develop (Fig. 2e). During the first five days of development, there was no visible pigmentation to be seen on any other area of the body. All larvae examined during the first five days of development were characterized by these two fields of pigmentation. Figures 2 and 3 show the morphological development of larvae in detail.

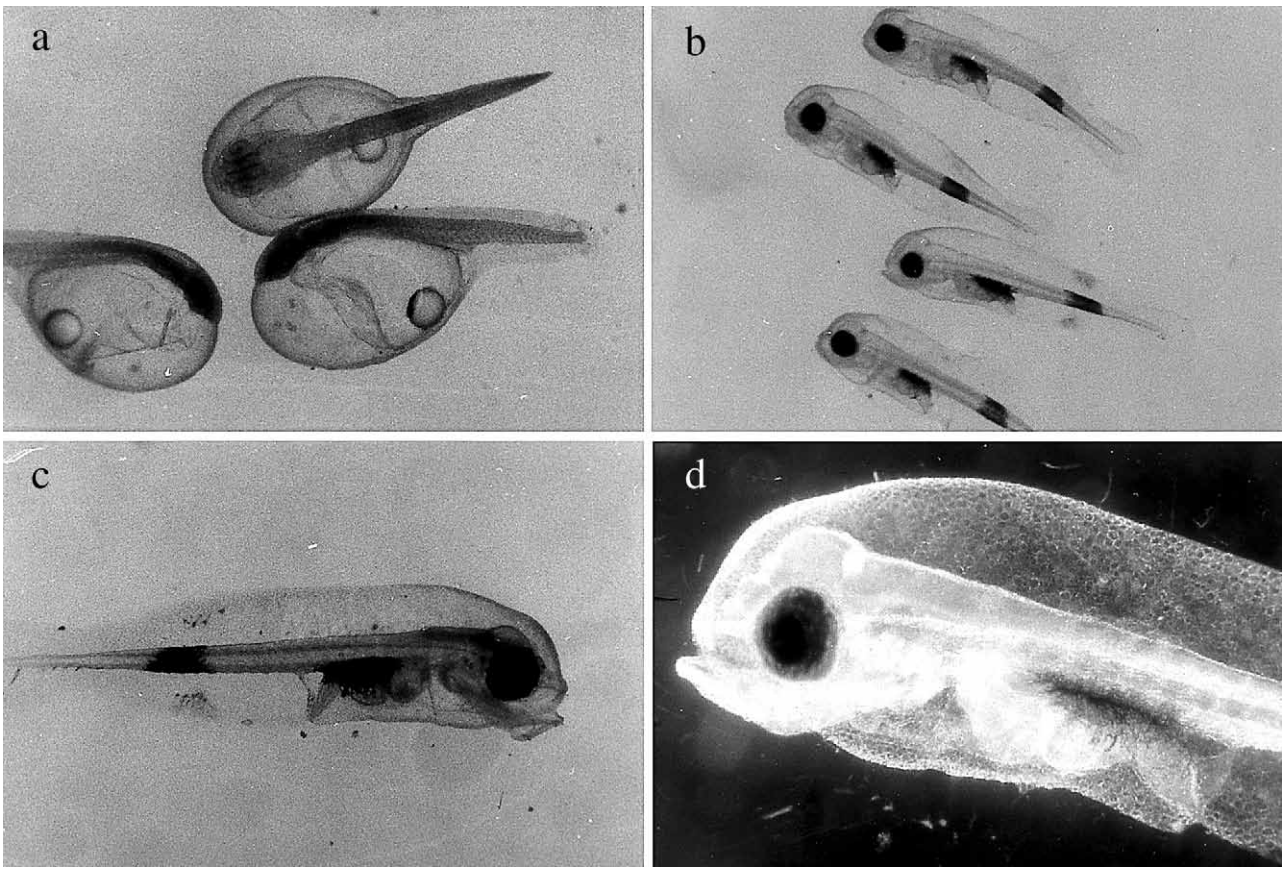

FIG. 3. – Pictures of dusky grouper larvae: a) newly-hatched larva, b) 72-hours old larvae, c) 96-hours old larva, and d) head of larva.

The mouth opened after 72 hours, becoming fully functional after 96 hours when larvae started to feed. The mouth opening was between 250-300 μm.

DISCUSSION

The egg size of dusky grouper described by other authors is significantly smaller, ranging from 0.67 mm for unfertilized and unhydrated eggs in Spanish waters (Zabala *et al*., 1997), to 0.75 mm for fertilised eggs in Andalusian waters (Barnabe, 1974). The eggs from southeastern Adriatic waters, as described by Skaramuca *et al*.(1989) with a diameter of 836 μm, were of a comparable size to those obtained in our experiments. But, the eggs obtained in captivity in Southern Italy were bigger with a diameter of 888 μm (Spedicato *et al*., 1995)

The eggs of the dusky grouper, *Epinephelus marginatus* are among the smallest eggs described up to now for the genus. Such a small egg was reported for the camouflage grouper *Epinephelus polyphekadion* from the Red Sea waters with a average diameter of 757.3 μm (James *et al*., 1997) and for the marbled grouper *Epinephelus microdon* from Micronesian waters with a range from 769-832 μm (Tamaru *et al*., 1996). The egg size of other groupers is bigger (Tucker, 1991; Watanabe *et al*., 1995; Chen, 1990; etc.). There is no other data on egg size for other species of the genus *Epinephelus* in Mediterranean waters, especially for the Adriatic.

The same situation exists with the net size of newly-hatched larvae, as dusky grouper larvae are the smallest of those described in this genus. For Mediterranean species, there is no recorded data available on sizes of larval stages.

However, most other characteristics, including large yolk sac, head and body shapes, location of oil globules, short intestine tracts and especially, characteristic pigmentation, are almost identical for most of the species described in this genus. The areas showing pigmentation in the early developmental stages, above the digestive system and between the anus and the end of the notochord, are also noted in the species *E. tauvina* (Hussain and Higguchi, 1980), *E. striatus* (Powell and Tucker, 1992), as well

as in *E. fuscoguttatus* (Kohno *et al*., 1993). Alongside the general similarities of the larvae, this offers the best morphological characteristic used in separating early grouper larvae from other fish. Problems can arise if the spawning season of more than one species of the genus *Epinephelus* overlaps, for example, the species *Epinephelus costae* spawning season in Adriatic coincides with the spawning of *E. marginatus* (Jardas, 1996). Additional research on this species, now in progress, should offer a solution to this situation.

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