SCI. MAR., 64 (3): 341-345 SCIENTIA MARINA 2000

Egg and early larval development of laboratory reared goldblotch grouper, *Epinephelus costae* (Steindachner, 1878) (Pisces, Serranidae)*

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SUMMARY: The embryonic and early larval development of the laboratory-reared goldblotch grouper, *Epinephelus costae* (Steindachner, 1878), are described and illustrated. The eggs, with a mean diameter of 926±19 μm and a range from 890-950 μm, were spherical and transparent with transparent chorion. Embryonic development lasted 24 hours at 25.5°C. Newly-hatched larvae were 1.762±0.047 mm in length. Absorption of the yolk sac was complete after the third day, when larvae reached 2.95±0.231 mm in total length. The mouth opened 60 hours after hatching, and was in function after 80 hours, with an opening diameter ranging from 280-320 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: goldblotch grouper, *Epinephelus costae,* egg and embryonic development, characteristics of larvae, pigmentation.

INTRODUCTION

The goldblotch grouper, *Epinephelus costae* (Steindachner, 1878), previously known as *Epinephelus alexandrinus* (Valenciennes, 1828) is distributed throughout the Mediterranean Sea, and in the Atlantic, from the coast of Portugal to Namibia. It inhabits sandy, muddy and rocky bottoms at depths of 10 to 300 meters (Jardas, 1996). There is little information about the goldblotch grouper in the scientific literature, and there is no data about either spawning or characteristics during early stages. We lack any data about this species in the Adriatic. Only literature about the female reproduction cycle and fecundity in Tunisian waters (Bouain and Siau, 1983), age and growth in Egyptian (Wadie *et al*., 1981) and Tunisian waters (Bouain, 1986) could be found for the Mediterranean Sea.

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

MATERIALS AND METHODS

Broodstock were collected from southeastern Adriatic waters and held from one to seven years in

*Received December 9, 1999. Accepted April 3, 2000.

aquarium conditions, at ambient seawater temperatures (12-25°C) and salinities (36-38 ppt). The fish were spawned using the same hormonal procedure applied earlier on dusky grouper. Females were injected with 2000 IUkg^{-1} of human chorionic gonadotropin (HCG) with two injections of hormone spaced 24-h apart (Glamuzina *et al.*, 1998a). Eggs were fertilised with sperm from three sex-reversed males. 17-α Methyltestosterone (5 mg kg^{-1}) mixed with food was used to induce sex reversal (for details see 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 25-25.5°C, flow-through sea water, with aeration from the bottom. Samples of 30 eggs were taken every hour and following hatching, 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 ot the yolk sac and diameter of oil globule.

RESULTS

Egg characteristics and embryonic development

The average diameter of newly spawned eggs was 938 ± 29 μm, with sizes varying from 709-1057 μm. However, within two hours following fertilisation, the egg diameter became more uniform. Samples of surviving eggs showed that there were no eggs diameters less than 890 μm and more than 950 μm, and average egg diameter was 926±19 μm. Smaller and bigger dead eggs were unripe and overripe, respectively. Good 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 at 38 ppt salinity, with oil globules having an average diameter of 199±36 μm (range 148-244 μm). Shortly after stopping aeration in the tank, all eggs became hyponeustonic.

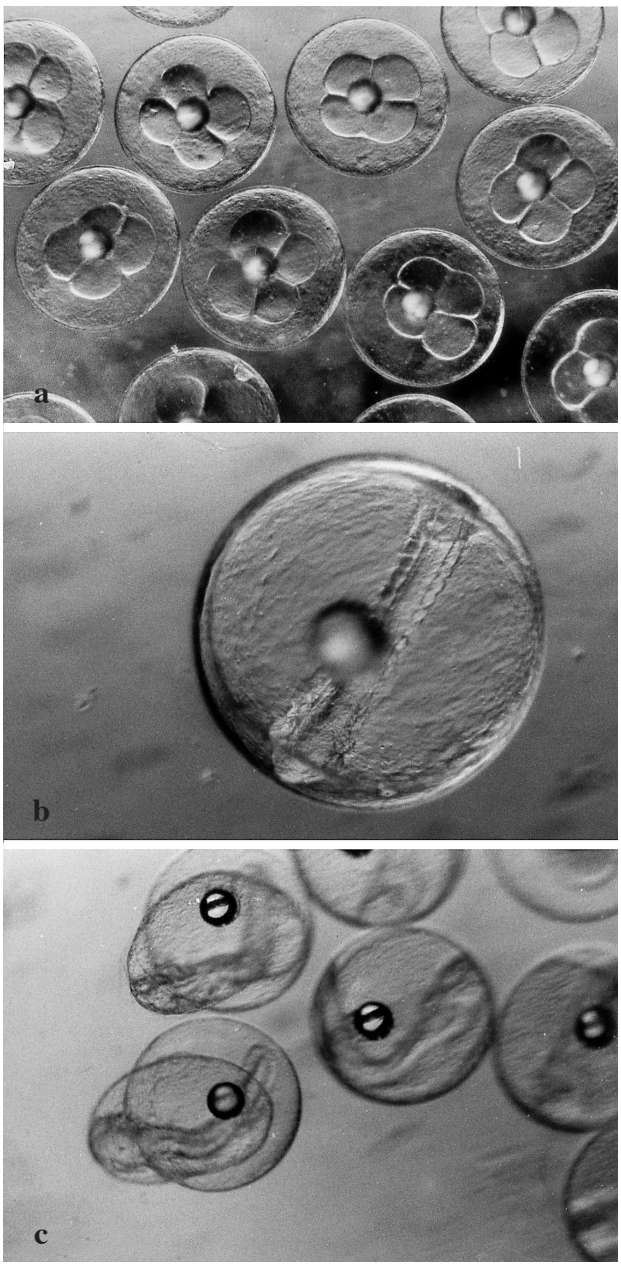

FIG. 1. – Goldblotch grouper eggs at different stages of embryogenesis: a) four-cell stage, b) early embryo, c) hatching.

TABLE 1. – Embryonic development of the goldblotch grouper, *Epinephelus costae,* at 25-25.5°C.

Time Hours	Minutes	Stage	Description
0	00	Fertilisation	
1	00	2- cells	first cleavage
1	15	4- cells	second cleavage, plane perpendicular to the first
1	30	8- cells	cleavage parallel to the second
1	55	16- cells	cleavage parallel to the first
2	20	32- cells	
2	50	64- cells	
4	40	morula	
7	50	gastrula	gastrulation starts
12	00	neurula	formation of neural groove starts, formation of embryo begins, notochord
15	00	embryo	embryo well developed, somites clearly visible, optic vesicles formed
21	00	embryo	sporadic movements of embryo, heartbeat rate 80 per minute
23	00	embryo	tail tip almost touches the head, rhythmical movements every 10 seconds, heartbeat rate 95-105 per minute
24	15	free larva	hatching begins
26	00	larva	50% larvae hatched
28	00	larvae	more than 95% larvae hatched

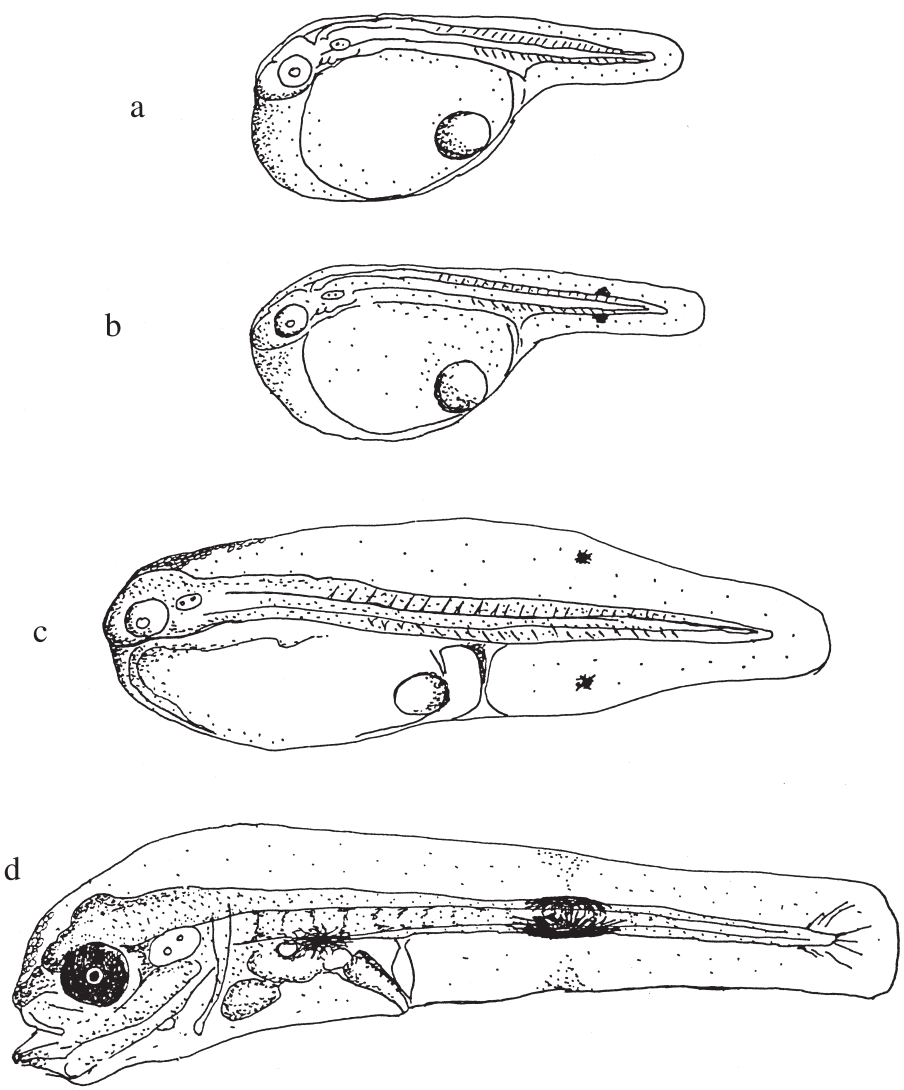

FIG. 2. – Drawings of the goldblotch grouper larva: a) newly-hatched larva, b) 5-hours old larva, c) 24-hours old larva, d) 80-hours old larva.

TABLE 2. – Changes in lengths and shape of the goldblotch grouper, *Epinephelus costae,* yolk-sac larvae during the first four days from hatching at temperatures of 25-25.5°C.

hours after hatching	total length (mm)	standard length (mm)	preanal length (mm)	head length (mm)	maximal width (μm)	minimal width (μm)	eye diameter (μm)	longest yolk sac diameter (μm)	oil globule diameter (μm)
0	1.762							0.870	0.20
+11	2.475	2.32	1.234	0.568	0.508	0.35	0.252	0.681	0.20
+15	2.634	2.58	1.337	0.6	0.557	0.351	0.249	0.89	0.187
+23	2.851	2.656	1.347	0.649	0.612	0.27	0.238	0.577	0.174
+47	3.03	2.81	1.29	0.611	0.614	0.287	0.252	0.22	0.09
+59	3.121	2.898	1.37	0.692	0.613	0.323	0.281	res.	0.02
+83	2.956	2.749	1.341	0.662	0.607	0.317	0.283	res.	res.

One hour after dry fertilisation, the blastodisk divided into two cells for the first time. 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, goldblotch grouper larvae was 1.76±0.048 mm. Larvae varied from 1.69-1.85 mm, and were characterized by huge yolk sacs, along almost the entire body, except for the small tail part (Fig. 2a). 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 swarm in large formations.

During the first 12 hours after hatching, the larvae showed significant growth. The growth rate was highest during the first 24 hour, after which, it decreased significantly (Table 2). Table 2 shows changes in all measured characteristics of the larvae during the first four days. Drawings and photographs of the larvae during this period are presented in Figure 2 and 3.

The appearance of two areas of pigmentation represents the basic morphological characteristic of the goldblotch grouper in its early larval stage. The first area, located in the middle between the anus and above and below the posterior edge of the notochord appeared on the second day following hatching. During the next few days, these two areas enlarged and finally 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 became so intense that it covered the entire top area of the front intestine and stomach, totally hiding the swimbladder, which started to develop (Figs. 2d, 3b, 3c). During the first four days of development, there was no visible pigmentation

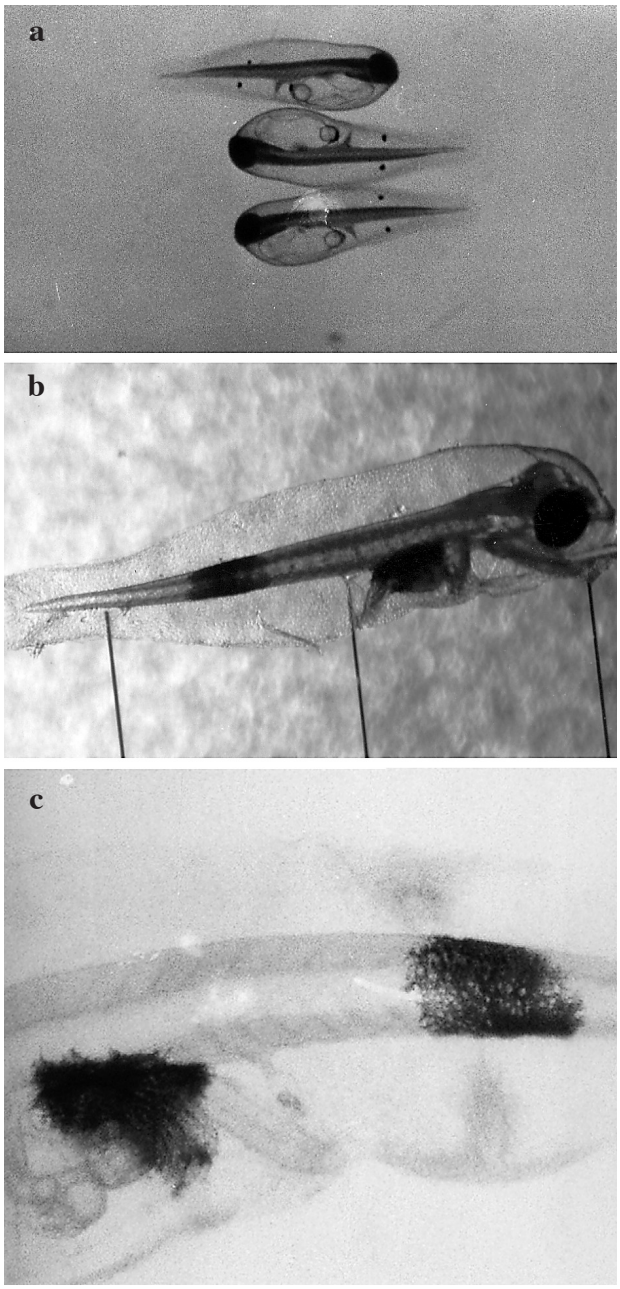

FIG. 3. – Pictures of the goldblotch grouper larva :a) 10-hours old larvae, b) 80-hours old larva, c) areas of intensive pigmentation in mouth-opened larvae of goldblotch grouper.

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 the larvae in detail.

The mouth opened after 60 hours, becoming fully functional after 80 hours when larvae started to feed. The mouth opening was between 280-320 μm.

DISCUSSION

There are no data about egg and larvae of goldblotch grouper in the literature. The descriptions of larvae by Bertolini (1933) and Sparta (1935) according to Heemstra and Randal (1993), are actually *Mycteroperca rubra*. So, comparison is possible only with related Mediterranean species.

The egg size of the dusky grouper from southeastern Adriatic waters, as described by Skaramuca *et al*. (1989) and Glamuzina *et al*. (1998c) is significantly smaller than those described for the goldblotch grouper. The eggs of the goldblotch grouper are among the biggest eggs described up to now for the genus and comparable with the Nassau grouper, *Epinephelus striatus* (Powell and Tucker, 1992) and the brown spotted grouper, *E. tauvina* (Chen, 1990).

The same situation exists with the net size of newly-hatched larvae. Goldblotch grouper larva is bigger than dusky grouper larva obtained under similar conditions of captivity in the southeastern Adriatic. However, most other characteristics, including the large yolk sac, head and body shapes, location of oil globules, the short intestinal tracts and especially, characteristic pigmentation, are almost identical for goldblotch and dusky grouper, as well for the most of other 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 seen in dusky grouper, *E. marginatus* (Glamuzina *et al.,* 1998a), as well as *E. tauvina* (Hussain and Higguchi, 1980), *E. striatus* (Powell and Tucker, 1992) and *E. fuscoguttatus* (Kohno *et al*., 1993). Alongside the general similarities of the larvae, this offers the best morphological characteristic to separate early grouper larvae from other fish.

As mentioned in earlier an paper (Glamuzina *et al.,* 1998c), problems can arise if the spawning season of more than one species of the genus *Epinephelus* overlaps. The goldblotch grouper spawning season in the Adriatic coincides with the spawning of the dusky grouper *E. marginatus* (Jardas, 1996). The only difference between egg and early larvae of these species is their size, but this characteristic and its application in ecological studies is limited and in most cases not useful. During 1999 we caught seven grouper fingerlings near Dubrovnik, with body characteristics of goldblotch and pigmentation characteristics of dusky grouper (Glamuzina, unpublished data). These facts show that ecological studies on early stages of these two grouper species will be very difficult.

ACKNOWLEDGEMENTS

This work is financed by the Ministry of Science and Technology, Republic of Croatia.

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Scient. ed.: M. Harmelin-Vivien