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EMBRYONIC AND LARVAL DEVELOPMENT OF STRIPED SEA BREAM *(LITHOGNATHUS MORMYRUS* L 1758)

Kursat Firat*, Sahin Saka and H. Okan Kamaci

Aquaculture Department, Faculty of Fisheries, Ege University, 35440 Urla-Iskele, Izmir, Turkey

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Abstract

Development of the embryonic and yolk-sac larvae of striped sea bream *(Lithognathus mormyrus)* was investigated. The average egg and oil globule diameters were 0.71±0.1286 mm and 0.18±0.1344 mm, respectively. The morula, gastrula, and neurula stages, somite formation, and hatching occurred 2:10, 5:15, 8:45, 9:45, and 21:15 hours after fertilization. The total length, yolk-sac volume, oil globule volume, digestive tube length, otolith diameter, head length, eye diameter, preanal and postanal lengths of newly hatched larvae were 1.74±0.03 mm, 0.12±0.008 mm^3, 0.003±0.0002 mm^3, 0.47±0.02 mm, 54±8 µm, 299±11 µm, 157±8 µm, 911±0.3 µm, and 827±0.03 µm. When the mouth opened 40 h after hatching, these measurements were 2.87±0.014 mm, 0.003±0.001 mm^3, 0.001±0.0001 mm^3, 0.61±0.015 mm, 134±8 µm, 376±10 µm, 221±13 µm, 1.02±0.03 mm, and 1.84±0.03 mm. During the first 18 hours, the larvae reached 84% of its final total length, the yolk sac was 65% absorbed, and the oil globule was 62% absorbed. Statistical analysis showed negative allometry for the relationships between total length and yolk-sac volume (r^2 = 0.88) and total length and oil globule volume (r^2 = 0.76), and positive allometry for the relationship between total length and digestive tube length (r^2 = 0.89).

Introduction

The demersal striped sea bream *(Lithognathus mormyrus)* is a Sparidae with high commercial value. It inhabits various types of sea bottoms, especially rocky and sandy bottoms and sea grass beds, at depths of 0-150 m (Bauchot and Hureau, 1986, 1990). The species is native to the west Indian and east Atlantic Oceans, from the Bay of Biscay to the Cape of Good Hope and around the Canaries and Cape Verde. It is also present in the Mediterranean, Black, Azov, and Red Seas (Harmelin et al., 1995; Lorenzo et al., 2001). There is much interest in

* Corresponding author. E-mail: kfirat@sufak.ege.edu.tr

its fisheries (Kraljevic et al., 1995, 1996), morphometry (Palma and Andrade, 2002), and reproduction (Lorenzo et al., 2001) but literature concerning its early life history is scarce or limited to embryonic and larval development under captivity (Mater, 1976; Divanach and Kentouri, 1983).

Knowledge of the ontogeny of a species is important not only for basic embryology but also for fishery and aquaculture applications as it serves in understanding the functioning and environmental preferences during different developmental stages (Fukuhara, 1992). An understanding of normal larval morphology is critical, and can be used to evaluate culture conditions for mass production of high quality juveniles (Koumoundouros et al., 1999).

This paper describes the embryonic and larval development of striped sea bream.

Materials and Methods

The study was carried out in July 2002 at the Teknomar Sea Fish Broodstock Facility in Izmir, Turkey. The eggs were spawned spontaneously by wild breeders without hormonal treatment. The eggs were stocked into 20-l incubators at a density of 2500 eggs/l. The incubators were put into a fully-controlled tank to stabilize the physical and chemical parameters of the water. Hatched larvae were restocked at 200 larvae/l in 1-m^3 tanks. The water flow was adjusted to exchange 5% of the total volume of the tank hourly and aeration was 40 ml/min. Temperature was 25°C and salinity 38‰. Experiments took place in a dark room; temperature, salinity, and oxygen were measured hourly.

Thirty randomly selected eggs were taken from the incubators and embryonic development was determined every 5 min until the morula stage. After this stage, eggs were sampled every 30 min and the diameters of the eggs and oil globules were measured. After hatching, 30 individuals were taken from the center of the water column of the tank every six hours. The total body length, length and width of the spheroid yolk sac, and diameter of the spherical oil globule were measured using a microscope with an ocular micrometer to the nearest 0.01 mm. The volumes of the yolk sac (V_{ys}) and oil globule (V_{og}) were calculated using the formulae of Blaxter and Hempel (1966) and Cetta and Capuzzo (1982) as follows: $V_{ys} = 4/3\pi \times (L/2) \times (H/2)^2$ and $V_{og} = 4/3\pi \times (d/2)^3$. The following characters were measured: digestive tube length, otolith diameter, head length, eye diameter, preanal length, and postanal length. Observations and measurements were made on anesthetized (phenoxy-2 ethanol, 0.5 ml/l) specimens using a binocular with an ocular micrometer. Curled larvae were not evaluated.

The experiment terminated when the first mouth openings were observed. Each observation was triplicated. Regression analyses of total length/digestive tube, total length/yolk sac volume, and total length/oil globule volume were carried out. Measurements of eggs and larvae were expressed as means±standard deviations.

Results

The temperature averaged 25.2±0.2°C and the salinity averaged 37.1‰. During incubation, dissolved oxygen varied 6.1-6.3 mg/l. Oxygen saturation was over 85% and the mean pH was 7.6±0.05. Ammonia and nitrite components were always less than 0.012 mg/l.

Eggs were spherical, transparent, and had a clean chorion. The vitellus was homogeneous and unsegmented. Egg diameters ranged 0.69-0.73 mm, with a mean of 0.71±0.1286 mm. Eggs were buoyant and contained a single unpigmented oil globule of 0.15-0.20 mm (mean 0.18±0.1344 mm). A small perivitelline space developed during embryonic development. During incubation, oscillatory contractions caused the peripheral cortical cytoplasm to migrate toward the animal pole where it formed a convex, lens-shaped blastodisc 15 min after fertilization. Usually, eggs were transparent except for this formation. There was an oil droplet at the center of the egg. Egg development is shown in Table 1.

At the end of the gastrula stage, the yolk sphere was nearly covered by the thin blastoderm leaving exposed a small area around

Table 1. Embryonic development of striped sea bream eggs at 25ºC.

Time (h after fertilization)	*Stage Description*	*Stage Description*
0:00	Fertilization	
0:35	2-blastodisc stage	Meridional first cleavage
0:40	4-blastodisc stage	Second cleavage
0:45	8-blastodisc stage	Cleavage parallel to the second
1:00	16-blastodisc stage	Cleavage parallel to the first
2:10	Morula stage	64-128 blastomer
3:50	Blastula stage	Nuclei from the marginal cells migrate out of the cells
5:15	Gastrula stage	
8:45	Neurula stage	Head formation, kupffer
9:45	2-somite stage	
11:45	9-somite stage	Embryo covered 1/2 of yolk-sac
14:00	16-somite stage	Premordial formation
15:15	18-19-somite stage	Formation of optic lens, embryo covered 2/3 of yolk-sac
15:45		Otolith formation
18:15		Embryo covered 3/4 of yolk-sac
19:45		Frequent body movements
21:15	Newly hatched larvae	Embryo tears the chorion and hatching.

the vegetal pole (Fig. 1). The head was recognized anteriorly in the distinct embryonic body. Kupffer's vesicles appeared at this stage.

About 9:45 h after fertilization, a groove appeared in the dorsal area of each optic lobe, the blastopore completely closed, and there were two somites on the embryo (Fig. 2). By 11:45, the embryo covered half of the inner yolk-sac circumference and at 18:15 h embryo activity was noted. At 19:45, the embryo was active and the head region extremely developed. At 21:15, the embryo pressed against the chorion with sinusoidal movements, melting its inner layers and tearing the single outer layer to escape head-first.

At hatching, the yolk-sac larvae measured 1.74±0.03 mm. The newly hatched larvae were transparent and floated at the surface with the yolk-sac upwards. The head and anterior parts of the body curved around the yolk sac (Fig. 3). The width of the yolk-sac was 4-5 times larger than the body and put pressure on the anterior part of the body. The oil globule was in the posterior area of the yolk-sac. The body was almost covered by a primordial fin and there were 34 or 35 somites. A straight and simple digestive tube reached the primordial fin. The distance of the terminal section of the tube from the posterior and yolk-sac was approximately equal to the radius of the oil globule. Melanophores were clearly visible in the hind brain region, the oil globule (1 or 2 somites in width), the mid-gut

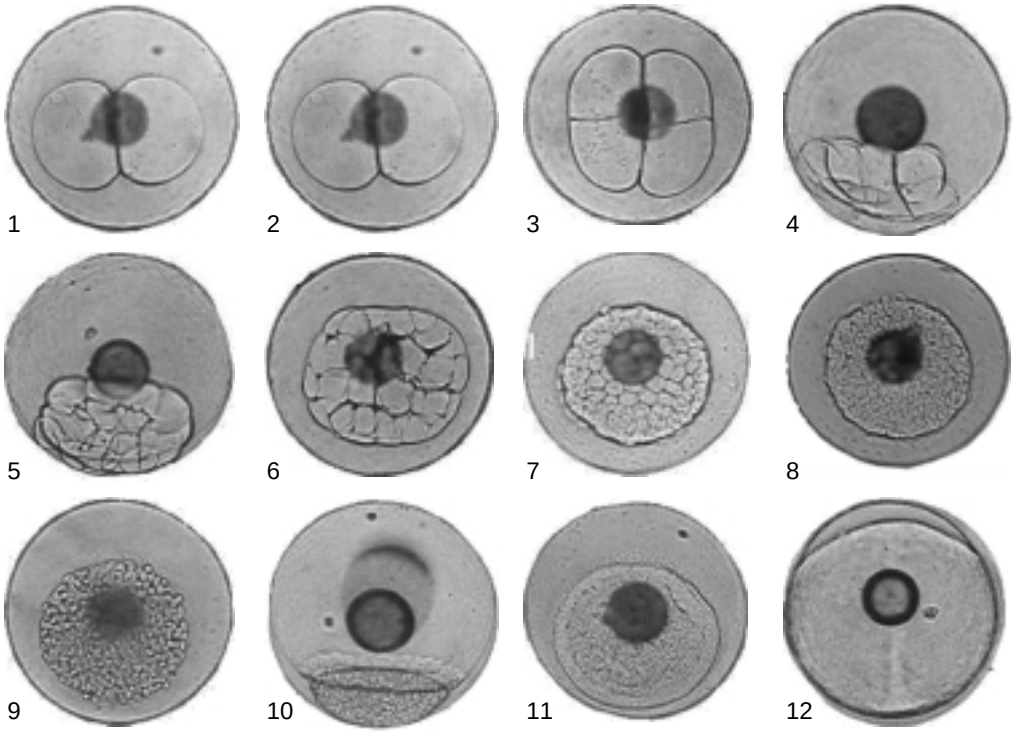

Fig. 1. Embryonic development of striped sea bream eggs: (1) germinal disc collapse, (2) 2-cell stage, (3) 4-cell stage, (4) 8-cell stage, (5) 16-cell stage, (6) 32-cell stage, (7) beginning of morula stage, (8) end of morula stage, (9) beginning of blastula stage, (10) end blastula stage, (11) beginning of gastrula stage, (12) end of gastrula stage.

region (1 or 2 somites in width), and the anal region (9-11 somites).

Body contractions decreased six hours after hatching. The anterior side of the yolk-sac was larger than the posterior side (Fig. 4). The digestive tube clearly appeared; the terminal side became thicker and closer to the body with respect to the ventral side of the oil globule. The primordial fin became thinner and wider. Melanophores increased and reached a width of 4 somites on the anal side. Fifty-two percent of the body development up to mouth opening and the highest resorption of the yolk-sac (37%) and oil globule (23%) occurred during this period (Fig. 5).

Twelve hours after hatching, there was no sharp increase of melanophore distribution on the body. The head and anterior parts were still slightly raised toward the yolk-sac. The eyes were not pigmented. The digestive tube appeared clearly, the terminal side was thicker and the same distance from the vertical side as from the oil globule. As yolk-sac absorption decreased, body development and digestive tube length increased. More than 75% of the total length development was completed by 12 h, and development of the digestive tube was more than 73%. At 18 h, the total length development relatively decreased and absorption of the oil globule increased. More than 60% of the yolk-sac and oil globule were absorbed during this period. The eyes were pigmented and digestive tube thickened.

At 24 h, the maxillaries and lower jaw were forming but not yet distinct. Faint granular pigmentation of the eye was completed. This was

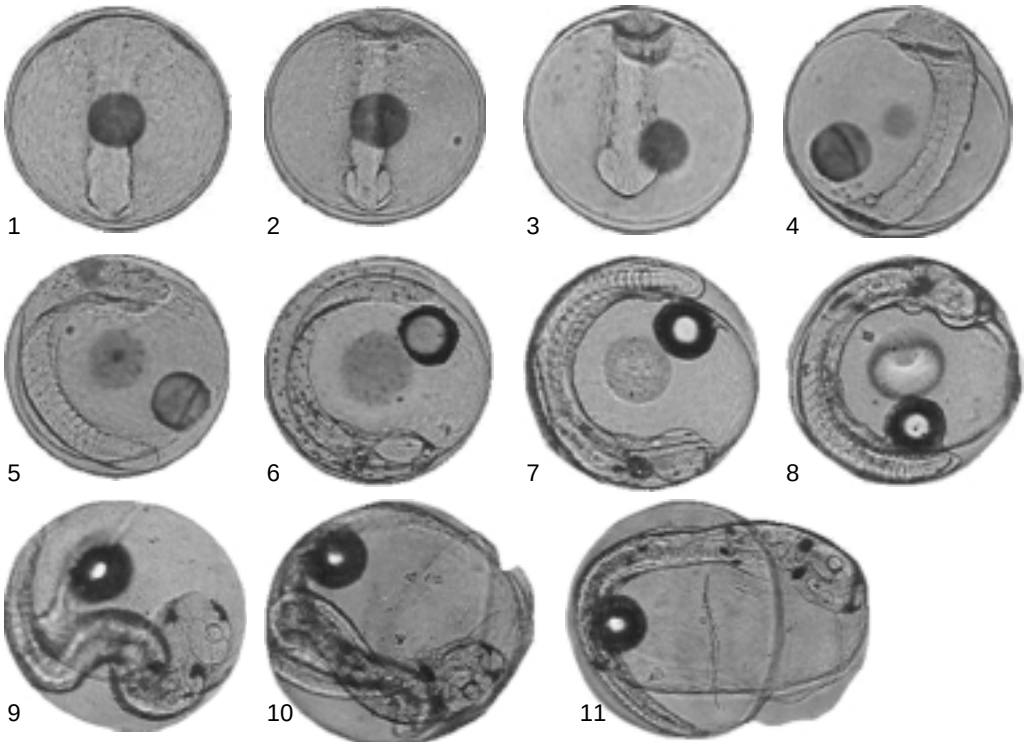

Fig. 2. Embryonic development of striped sea bream eggs. (1) beginning of neurula stage, (2) end of neurula stage, (3) 2-somite stage, (4) 9-somite stage, (5) 16-somite stage, (6) 18-19-somite stage, (7) appearance of otoliths, (8) embryo extended 3/4 of egg circumference, (9) repulsive movement, (10) solving of chorion, (11) hatching.

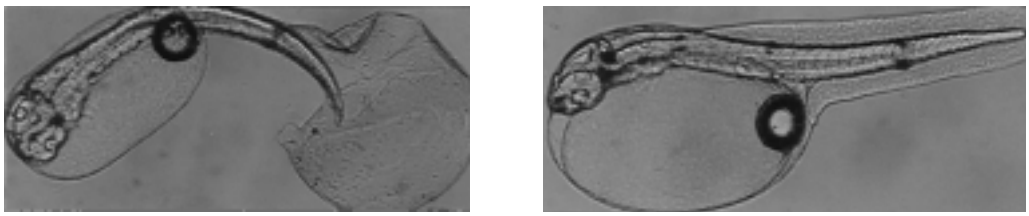

Fig. 3. Embryonic development of striped sea bream eggs. (1) newly hatched larva, (2) 30 min after hatching.

the period of least total length development, about 81% of yolk-sac was absorbed, and absorption of the oil globule significantly decreased. At 30 h, the foregut and hindgut were visible. Pectoral fin differentiation began. Oil globule absorption started to increase again and continued steadily 11% after each 6-hour period.

At 36 h, the oil globule was located near the center of the yolk-sac. The maxillaries and lower jaw were distinct but the mouth had not yet opened. At 40 h, the mouth completely

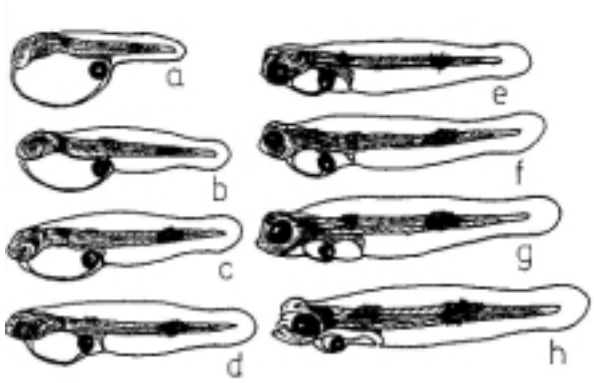

Fig. 4. Morphological changes of striped sea bream larvae prior to mouth opening: (a) hatching, (b) 6 h after hatching, (c) 12 h, (d) 18 h, (e) 24 h, (f) 30 h, (g) 36 h, (h) 40 h.

opened and was functional. Anus development was complete and there was a projection on the terminal side of the digestive tube. The body and notochord were straight and the body was pigmented.

Analysis shows negative allometry for the relationships between total length and yolk sac volume ($y = -0.098 \times 0.2964$, $r^2 = 0.88$, $n = 240$; Fig. 6) and total length and oil globule volume ($y = -0.0019 \times 0.0068$, $r^2 = 0.76$, $n = 240$; Fig. 7), and positive allometry for the relationship between total length and digestive tube length ($y = +0.1217 \times +0.2567$, $r^2 = 0.89$, $n = 240$; Fig. 8).

Measurements of head length, otolith and eye diameters, and preanal and postanal lengths are shown in Table 2.

Discussion

The striped sea bream egg is a typical Sparidae egg. It is difficult to identify morphological characteristics in these eggs that differ from those of the gilthead sea bream, *Sparus aurata* (Alessio and Gandolfi, 1975), white sea bream, *Diplodus sargus* (Kentouri et al., 1980), sharp snout sea bream, *Diplodus puntazzo* (Faranda et al., 1985), common two banded sea bream, *Diplodus vulgaris* (Jug-Dujakovic and Glamuzina, 1988), or common dentex, *Dentex dentex* (Jug-Dujakovic et al., 1995; Firat et al., 2003). Therefore, further information is needed to identify eggs for ichthyoplanctonic studies.

Generally, striped sea bream eggs are pelagic, have a single oil globule and eggs with a diameter of 0.55-1.02 mm. The chorion is transparent and thin, and the diameter of the micropyle is about 10 µm (Mater, 1976; Divanach and Kentouri, 1983). Previously used incubation temperatures of 23°C (Altay, 1997) and 25°C (Satar, 1998) had no negative effects on egg development. In this study, egg diameters varied 0.69-0.72 mm and hatched 21:15 h after fertilization. In earlier studies, egg diameters measured 0.55-0.77 mm (Divanach and Kentouri, 1983) and 0.85-0.98 mm (Altay, 1997; Satar, 1998) and hatched 24-28 h after fertilization at 23°C (Altay, 1997) and 22 h at 25°C (Satar, 1998).

Newly hatched larvae developed very quickly during the first 18 h. Larvae reached 84% of their final total length during the first 18 h and the remaining 16% was reached by mouth opening. The yolk-sac was absorbed in parallel with the development of total length; 65% was absorbed within 18 h and the remaining 35% by mouth opening. Absorption of the oil globule was slower than absorption of the yolk-sac, as in other teleosts. At 18 and 22 h, respectively, 62% and 38% of the oil globule volume were absorbed and it seems the oil globule was not completely absorbed at mouth opening. Slow absorption of the oil globule might be related to slow swimming action of the larvae.

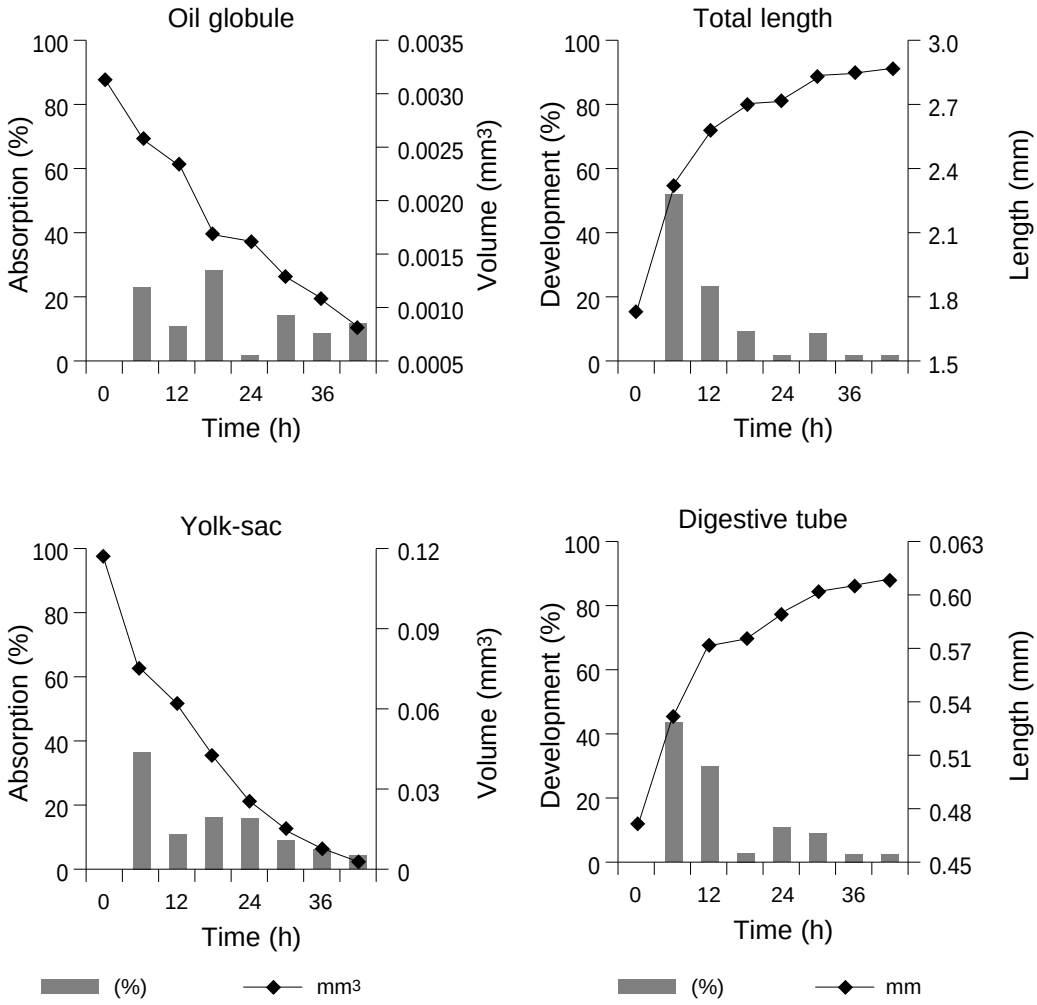

Fig. 5. The volume of oil globule, the volume of yolk-sac, total length and the length of digestive tube of striped sea bream and periodically absorption ration.

The digestive tube lengthened rapidly during the first 18 h. Thereafter, it slowly thickened. Absorption of the yolk-sac was relatively slow compared to length development and organogenesis began rapidly on the second day, as seen by mouth development, differentiation of the pectoral fin, rapid development of the otolith, and thickening of the digestive tube. As in previous studies, most of the yolk-sac was used to develop body length. Development of the digestive tube paralleled total length development (Saka et al., 2001).

In this study, we investigated embryonic and yolk-sac development of striped seabream larvae in captivity. We evaluated morphometric changes compared to total length. Information on the development of morphometric characters in larvae and juveniles can be used to improve culture condi-

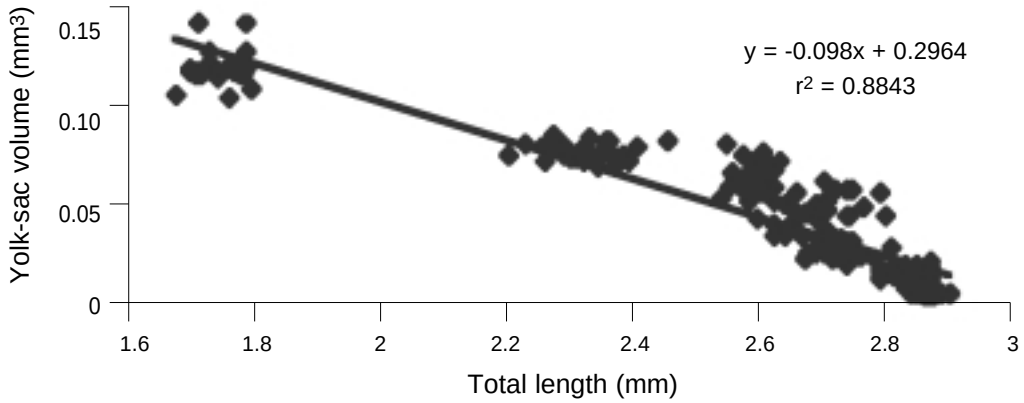

Fig. 6. The relationship between total length and yolk-sac volume of striped sea bream.

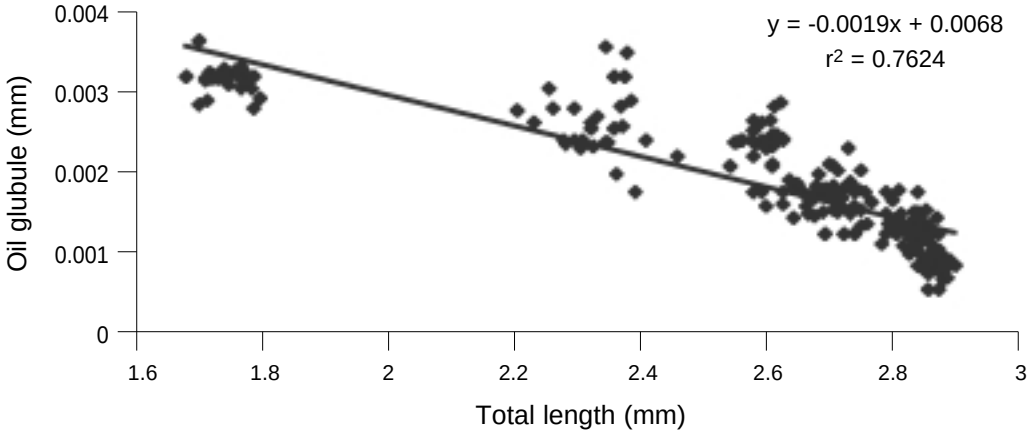

Fig. 7. The relationship between total length and oil globule volume of striped sea bream.

tions (production plan, feeding schedule, techniques) and for quality assessment and control of larvae and juveniles (Koumoundouros et al., 1999).

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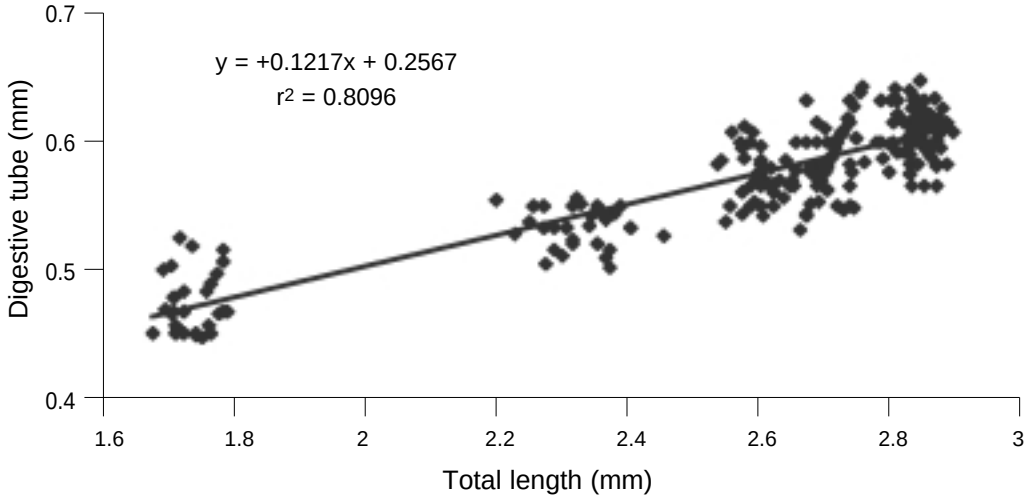

Fig. 8. The relationship between total length and digestive tube length of striped sea bream.

Table 2. Development of striped sea bream larvae (±SD).

Time (h after hatching)	*Head length (µm)*	*Otolith diameter (µm)*	*Eye diameter (µm)*	*Preanal length (mm)*	*Postanal length (mm)*
0	299±11.30	54	157±8.24	0.91±0.03	0.82±0.032
6	323±16.90	58	167±6.90	1.05±0.03	1.27±0.036
12	328±14.15	61	200±16.37	1.08±0.31	1.50±0.032
18	345±15.30	65	208±16.82	1.04±0.02	1.65±0.035
24	349±12.04	81	218±17.23	1.00±0.03	1.71±0.032
30	351±10.46	93	222±19.24	1.01±0.02	1.80±0.032
36	361±15.39	109	224±13.83	1.01±0.02	1.83±0.022
40	376±9.931	134	227±17.01	1.02±0.02	1.84±0.028

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