Embryonic and larval development of largescaled scorpionfish *Scorpaena scrofa* (Scorpaenidae)

by

Jakov DULČIĆ (1), Jurica JUG-DUJAKOVIĆ (2), Vlasta BARTULOVIĆ (3), Branko GLAMUZINA (3), Edhem HASKOVIĆ (4) & Boško SKARAMUCA (3)

ABSTRACT. - The embryonic and early larval development of the laboratory-reared largescaled scorpionfish, *Scorpaena scrofa* is described. Fertilized eggs were pelagic and elipsoidal with a homogeneous and unsegmented yolk (no oil globule) surrounded with transparent gelatinous layer. Eggs ranged in diameter from 0.83 to 0.95 mm, with a mean (± SD) of 0.89 ± 0.121 mm. Embryonic development lasted 31 h 45 min at a mean temperature of 25.6°C. Newly hatched larvae measured 2.09 ± 0.07 mm total length in average (range 2.0-2.25 mm). The yolk was completely absorbed by day 4 after hatching. Changes in length and shape of yolk-sac larvae and larvae during the first seven days after hatching are also presented.

RÉSUMÉ. - Développement embryonnaire et larvaire de la rascasse rouge, *Scorpaena scrofa* (Scorpaenidae).

Le développement embryonnaire et larvaire en laboratoire de la rascasse rouge, *Scorpaena scrofa* est décrit. Les œufs fertilisés sont pélagiques et ellipsoïdaux avec un vitellus homogène et non-segmenté (sans globule lipidique) entouré par une couche gélatineuse transparente. Le diamètre des œufs varie de 0,83 à 0,95 mm. Le développement embryonnaire a duré 31 h 45 min à une température moyenne de 25,6°C. Après l'éclosion, les larves ont une longueur totale de 2,09 ± 0,07 mm. Le vitellus a été complètement absorbé 4 jours après l'éclosion. Des changements de longueur et de forme des larves pendant les sept premiers jours après l'éclosion sont également présentés.

Key words. - Scorpaenidae - *Scorpaena scrofa* - Egg - Yolk-sac larvae - Larvae - Embryonic development.

Largescaled scorpionfish, *Scorpaena scrofa* Linnaeus, 1758, is a subtropical species distributed throughout the Mediterranean Sea (except Black Sea), and in the eastern Atlantic, from British Isles (rare) to Senegal including Madeira, the Canary islands, and Cape Verde (Eschmeyer, 1986). It is solitary and sedentary species over rocky, sandy or muddy bottoms to depths of 500 m. It feeds on fishes, crustaceans and mollusks (Hureau and Litvinenko, 1986). Largescaled scorpionfish is a highly commercial species in the coastal fisheries along the Croatian coast (eastern Adriatic) (Jardas, 1996). Information on early development stages of largescaled scorpionfish is sparse. Description of egg and larval stages was presented only by Sparta (1942).

This paper presents the data about the embryonic and larval development in aquaria conditions at *in situ* temperature and the descriptions of early life history stages of *S. scrofa*. The objectives are to describe the early life history, to assist in the identification of planktonic stages of this species and to compare this species to other scorpaenid taxa in an attempt to contribute to a taxonomic resolution of the scorpaenids.

MATERIAL AND METHODS

Samples of *S. scrofa* were obtained by gill-net from the Pelješac channel (eastern middle Adriatic) on 15 July 2005. Males (four specimens) were ranged between 24.4 and 27.7 cm, while females (7 specimens) between 18.0 and 25.6 cm. Specimens were returned alive to laboratory of Development Research Centre in Aquaculture in Ston (Croatia) and placed in a 250-l tank with the addition of pure oxygen. The fish spawned spontaneously during night (between 00 h 00 and 03 h 00). The fertilized eggs were transferred to several 150-l incubators (about 350 ml each) with a constant flow of 100-120% seawater filtered daily through a 50 µm mesh net at *in situ* salinity, temperature and natural photoperiod. A temperature range between 24.8 and 27.2°C (mean 25.6 ± 0.429°C), salinity 37.6 and 38.2 psu (mean 37.95 ± 0.159 psu), oxygen 7.8 and 8.3 mg l^{-1}.

The characteristics of newly-spawned ripe and fertilized eggs were noted, together with the duration of each embryonic stage. Embryonic development was observed under a binocular microscope. The diameters of the eggs were mea-

(1) Institute of Oceanography and Fisheries, POB 500, 21000 Split, CROATIA. [dulcic@izor.hr]
(2) University of Dubrovnik, Research and Development Centre in Mariculture, Ston, CROATIA.
(3) University of Dubrovnik, Department of Aquaculture, Ćire Carića 4, 20000 Dubrovnik, CROATIA.
(4) Faculty of Natural Sciences and Mathematics, Sarajevo, BOSNIA AND HERZEGOVINA.

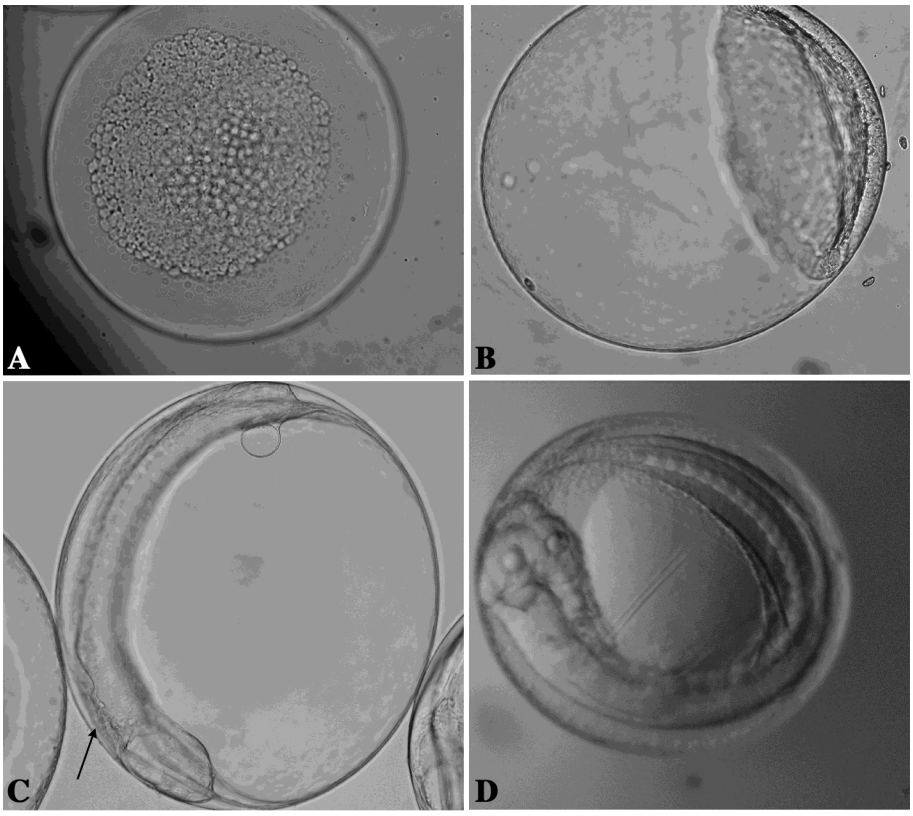

Figure 1. - *Scorpaena scrofa*. **A**: Mullbery stage; **B**: Early gastrula; **C**: Somatic segmentation starts and presence of Kupffer's cells (arrow); **D**: Stage 30 h. *[**A** : Stade morula ; **B** : Stade gastrula ; **C** : Début de la seg - mentation somatique et présence de cellules de Kupffer (flèche) ; **D** : Stade à 30 h.]*

sured. One hour after fertilization a sample of 10-15 eggs was taken every 7-10 min to determine the exact time of first cleavage. Embryogenesis was examined at different time intervals. Anaesthetized larvae in live condition were measured to an accuracy of 0.01 mm 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 tip of the snout to the end of the notochord; preanal length, distance along the midline of the body from the tip of the snout to the vent; body depth, the perpendicular depth of the trunk at the anus; greatest body depth, body depth at its widest point; length of pectoral fin, yolk-sac volume and horizontal eye diameter. The mouth width of larvae was also recorded. About 10-15 living yolk-sac larvae and larvae were used for each measurement. The time of yolk-sac resorption as well as to mouth opening were recorded. Cardiac contraction per minute was also recorded at each measurements. One day before hatching, the flow (40-60%) was opened through 125-µm mesh outlet filter. Upon resorption of the yolk-sac and opening of the mouth, the larvae were fed on *Artemia salina* and rotifers, *Brachionus plicatilis*, cultured in a thermostatic chamber at 26°C and 25-28 psu. On day 8 after fertilization, all larvae have died, so observations and measurements were ended.

RESULTS

Egg characteristics and embryonic development

Fertilized eggs were pelagic and elipsoidal with a homogeneous and unsegmented yolk surrounded with transparent gelatinous layer. Eggs ranged in diameter from 0.83 to 0.95 mm, with a mean (± SD) of 0.89 ± 0.121 mm. There were no oil globule(s) in the eggs. Table I illustrates changes observed during embryonic development at an average of 25.6°C. The first cleavage occurred at about 1 h after fertilization, the second after 2 h 15 min, and the third at 3 h 40 min. At 5 h 15 min, the blastoderm was in advanced stages of cleavage indicating a "mulberry stage", while gastrulation started 11 h 10 min after fertilization. Neurula stage started 12 h 45 min after fertilization, periblast still not closed. Formation of the embryo began after 16 h, and somatic segmentation after 18 h 15 min. The presence of Kupffer cells is registered 17 h 10 min after fertilization. Between 20 and 23 h, optic vesicles formed, olfactory lobes differentiated and the pericardial cavity was developing. After 26 h, somite differentiation was completed while optic vesicles and olfactory lobules were clearly visible. The heart was observed beating after 26 h 15 min, while movements of the embryo were observed 28 h after fertilization. After 29 h 30 min, the embryo occupied three-quarters of the yolk-sac circumference. Hatching started

Time	Stage	Description
0:00	Fertilization	
1:00	2 cells	Meridional first cleavage
2:15	4 cells	Second cleavage
3:40	16 cells	Cleavage parallel to the second
4:10	32 cells	Cleavage parallel to the first
4:45	64 cells	
5:15	Morula	Mulberry stage (Fig. 1A)
7:15	Blastula	Visible blastocel; oil bead is concentrate; germinative ring
11:10	Gastrula	Gastrulation starts (Fig. 1B)
11:40	Gastrula	Yolk being englobed by the blastodisc
12:15	Gastrula	Invagination of blastomeres ends
12:45	Neurula	Formation of neural groove starts
16:00	Neurula	Formation of embryo begins; notochord
17:00	Embryo	Optic vesicles and forebrain formation begin
18:15		Somatic segmentation is started; presence of Kupffer's cells (Fig. 1C)
20:30		Optic vesicle formed
21:40		Olfactory lobes differentiation, melanophores appear along dorsal side
22:50		Somites and melanophores on the body clearly visible
26:00		Optic vesicle and olfactory lobules clearly visible; somite differentiation completed
26:15		Heart beating visible
26:30		The embryo occupies half the circumference of the egg
27:05		Cardiac contractions (45 min^{-1}); blood circulatory system is visible on the yolk-sac
28:00		Embryo movements
28:15		Cardiac contractions (64 min^{-1})
28:40		Tail lifted on the yolk-sac
28:55		Tail movement begins every 2 min
29:15		Cardiac contractions (105 min^{-1})
29:30		The embryo occupies three-quarters of the yolk-sac circumference
29:45		Tail movements every 20 s; jaw formation is completed; cardiac contractions (118 min^{-1}); melanophores are visible
30:00		Tail (overgrown) is behind the head; progressive development of interior organs (Fig. 1D)
30:10		Cardiac contractions (117 min^{-1}); embryo with yolk-sac turning around axis of egg; blood is red
30:15		Mouth is open; cardiac contractions (126 min^{-1}),
30:25	Free yolk-sac larvae	Hatching begins
31:45		All yolk-sac larvae hatched

Table I. - Embryonic development of *Scorpaena scrofa* at mean temperature 25.6°C. *[Développement embryonnaire de* S. scrofa *à la température moyenne de 25,6°C.]*

after 30 h 25 min, and after 31 h 45 min all yolk-sac larvae hatched.

Larval development

The newly hatched yolk-sac larvae were transparent and floated at the surface with the yolk-sac uppermost and sometimes in a lateral position without significant movement, except for sporadic tail thrusts (Fig. 2A). Total length varied between 2.00 and 2.25 mm (average length 2.09 ± 0.07 mm). The finfold invaded much of the body. The body was segmented into 24-25 myomeres. The eyes were unpigmented. The mouth was undeveloped, but a short and simple gut was observed. The anus is situated close behind the posterior of the yolk-sac. The pectoral fin started to be visible. At the beginning of day 2 (about 25 h after fertilization) eyes pigmentation started (Fig. 2B), and the average length of pectoral fin is 0.11 ± 0.05 mm. Two thirds of yolk-sac were absorbed. At the end of the second day, granular pigmentation had developed in the eyes and formation of the maxillaries and lower jaw started. Table II shows changes in length and shape of yolk-sac larvae during the first seven days after hatching. About 46 h after hatching, the mouth was completely opened and functional (Fig. 2C). The mouth opening was between 250 and 290 µm. By the end of day 3 (about 67 h after hatching), the foregut and hindgut were functional. The anus was open. Pectoral fins (average length 0.25 ± 0.04 mm) had three marginal rows of melanophores. The eye was completely pigmented. By the beginning of day 4, yolk-sac was completely absorbed. Pectoral fins had four marginal rows of melanophores. The body and notochord were completely straight. The passage of food along the digestive tract of larvae was clearly visible. Larvae were mobile at that time, and able to swim at the surface. One row of melanophores was visible on the ventral contour of the

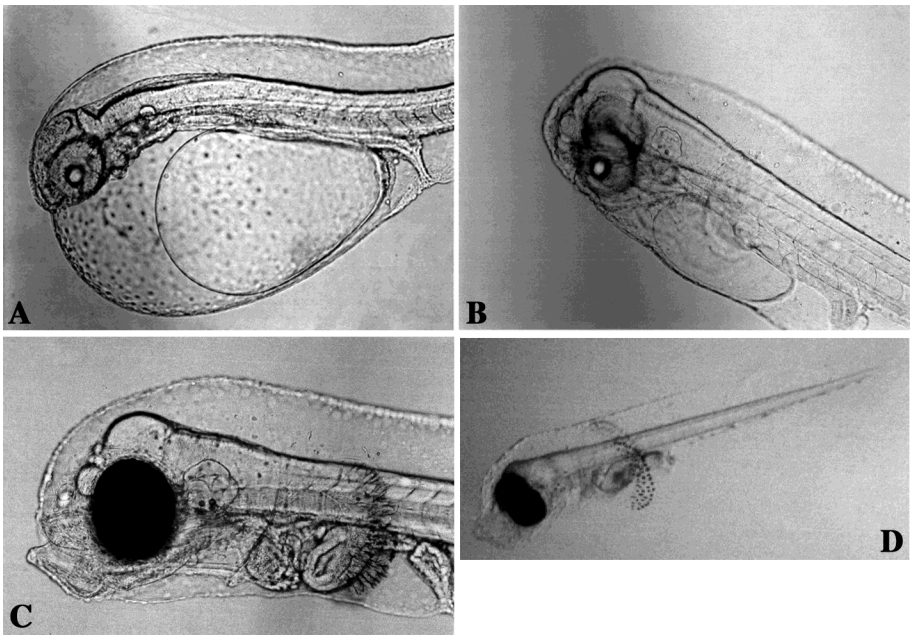

Figure 2. - *Scorpaena scrofa*. **A**: Newly hatched larva; **B**: 1 day-old yolk-sac larva, **C**: Larva with completely opened mouth and pectoral fin with melanophores; **D**: Pectoral fins with rows of melanophores and melanophores visible on the dorsal and ventral contours of the body. *[**A** : Larve récemment éclose ; **B** : Larve âgée d'un jour ; **C** : Larve avec bouche complètement formée et nageoires pectorales pigmentées ; **D** : Nageoires pectorales avec rangées de mélanophores et mélanophores visibles sur les bordures dorsale et ventrale du corps.]*

Table II. - Changes in length and shape of *Scorpaena scrofa* larvae during the first six days from hatching at mean temperature 25.6°C. *[Changements de longueur et de forme des larves de* S. scrofa *pendant les six premiers jours suivant l'éclosion, à la température moyenne de 25,6°C.]*

Days from hatching	Total length (mm)	Notochord length (mm)	Yolk-sac volume (mm^3)	Eye diameter (mm)	Distance snout-anus (mm)
0	2.09 ± 0.07	1.93 ± 0.04	0.67 ± 0.06	0.12 ± 0.02	1.06 ± 0.02
1	2.25 ± 0.08	2.11 ± 0.03	0.41 ± 0.04	0.17 ± 0.02	1.10 ± 0.01
2	2.51 ± 0.11	2.34 ± 0.03	0.22 ± 0.02	0.19 ± 0.04	1.11 ± 0.03
3	2.54 ± 0.12	2.40 ± 0.05	0.09 ± 0.01	0.21 ± 0.05	1.12 ± 0.02
4	2.60 ± 0.11	2.45 ± 0.05	-	0.20 ± 0.04	1.10 ± 0.02
5	2.65 ± 0.09	2.48 ± 0.04	-	0.20 ± 0.05	1.11 ± 0.03
6	2.68 ± 0.07	2.52 ± 0.05	-	0.21 ± 0.03	1.13 ± 0.03

body from anus to the end of notochord. On the end of day 5, pigmentation is clearly and strongly visible from the stomach to the end of notochord. Few melanophores were visible on the peritoneal and anus region. About one-third of pectoral fin was covered with melanophores (5-6 rows). Formation of caudal fin started (visible rays). At day 6, formation of spinal armature in the cephalic region was visible. Rows of melanophores started to be visible on the dorsal and ventral contour of the body. Pectoral fins with 18 rays and with 5-6 marginal rows of melanophores. Formation of rays in caudal fin started. By the end of day 6, formation of head and opercular spines were clearly visible. Rows of melanophores were visible on dorsal, ventral and lateral contour of body (Fig. 2D). At the beginning of day 7, all larvae have died according to presence of the urinary calculi.

DISCUSSION

According to the fact that spawning of *S. scrofa* occurred in aquarium conditions, it could be stated that this species could finish its reproductive cycle in controlled aquarium conditions without any hormonal treatment. Parental stock was mature at the end of July, in agreement with statements of Jardas (1996). Spawning of the specimens occurred during the night, that some authors connected with the adaptation of species (spawned eggs are protected from the predators since the visibility for them is lower during night) (Colin and Clavijo, 1998). *S. scrofa* is a partial spawner (asynchronic development of oocytes) as scorpaenid *Helicolenus dactylopterus dactylopterus* (Muñoz and Casadevall, 2002) and sparids *Sparus aurata* (Katavić, 1984) and *Dentex (Dentex) dentex* (Glamuzina *et al.*, 1989).

The egg of largescaled scorpionfish was first described by Sparta (1942). The egg size described by this author is smaller (0.88 x 0.68 mm), than in this study (0.95 x 0.83 mm). There is no other data on egg size for this species, but some exist for other scorpaenids (Tab. III). Jug-Dujaković *et al.* (1995) gave 1.09 to 1.14 mm for egg diameters for *Scorpaena porcus*, while Sparta (1941) gave 0.92 x 0.84 mm, what are the values higher then those already reported for *S. scrofa*. Tåning (1961) pointed out that the egg

Table III. - Comparison of data for eggs and newly hatched larvae of *Scorpaena scrofa* and other scorpaenid species. *[Comparaison des données pour les œufs et les larves à l'éclosion de* S. scrofa *et d'autres espèces de scorpaenids.]*

Species	References	Egg diameter (mm)	Presence of oil globule	Hatched yolk-sac larvae (mm)
Scorpaena scrofa	Sparta (1942) Present study	0.88 x 0.68 0.95 x 0.83	No	2.80 2.0-2.25
Scorpaena porcus	Jug-Dujaković *et al.* (1995)	1.09-1.14	No	2.27
Scorpaena notata	Sparta (1941) Dekhnik (1973) Sparta (1942)	0.92 x 0.84 0.90-1.15 x 1.08-1.30 0.88 x 0.76	 No	1.72 2.1-2.4 2.70
Sebastes marinus	Tåning (1961)	2.3 x 1.6	Yes (1)	usually longer than 6.0
Sebastes viviparus	Tåning (1961)	2.0 x 1.25	Yes (1)	just under 6.0

of *Sebastes viviparus* was slightly smaller than that of *Sebastes marinus*, the sizes just before hatching being 2.0 x 1.25 mm and 2.3 x 1.6 mm, respectively. It is clear that eggs of the above mentioned species are bigger than those of largescaled scorpionfish and have an oil globule which is not observed for *S. scrofa*. 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. According to available data, characters for identifying eggs of largescaled scorpionfish among scorpaenid species could be diameter and presence/absence of oil globule.

Incubation period was 31.45 hours in this study, while Sparta (1942) observed between 4 and 5 days in eggs sampled on 15th September. The duration of incubation period varies with temperature so this difference could be connected with the period of spawning (in this case: summer or autumn spawning period). 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. In this study temperature ranged from 24.8 and 27.2°C in July, while during the sampling period on 15th September in the Gulf of Napoli, the temperature was probably lower. Sparta (1942) also explains the difference according to temperature effect, i.e. sampling period (May and September) of *S. scrofa* eggs.

The length of newly hatched yolk-sac larvae of *S. scrofa* from this study is significantly lower (t-test, $p < 0.05$) then those reported by Sparta (1942). This information could assist in identifying newly hatched yolk-sac larvae of scorpaenids; 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. However, most other characteristics, including head and body shapes, characteristic pigmentation, are very similar for most of the species described in scorpaenids. Spawning period and geographical distribution of species could also assist in determining the early life history stages (Glamuzina *et al.*, 1998), since the problems can arise if the spawning season of more than one species of the scorpaenids overlaps.

Comparisons of embryonic and larval morphologies may, in certain cases, elucidate phylogenetic relationships, and may therefore contribute to taxonomical clarification (Cohen, 1985). Such comparisons should be based on larval characters that exhibit minimal flexibility to environmental cues. Meristic and morphometric characters and developmental patterns that are extremely plastic, should be avoided. Among the characters studied, only the pigmentation patterns exhibited a considerable stability and thereby were valid for comparisons with other taxa. Developmental characters displayed higher variability, which was probably environmentally induced, and therefore could be of limited comparative value. Even though additional research on this species and other scorpaenids, now in progress, should offer a solution to this situation.

REFERENCES

COHEN D.M., 1985. - Ontogeny, systematics, and phylogeny. *In*: Ontogeny and Systematics of Fishes. *Am. Soc. Ichthyol. Her - petol*., Spec. Publ., 1: 7-11.

COLIN P. & I.E. CLAVIJO, 1988. - Spawning activity of fishes producing pelagic eggs on a shelf edge coral reef, southwestern Puerto Rico. *Bull. Mar. Sci*., 43: 246-279.

DEKHNIK T.B., 1973. - Ichthyoplankton of the Black Sea. 234 p. Kiev: Naukova Dumka Press. (In Russian)

ESCHMEYER W.N., 1986. - Scorpaenidae. *In*: Smiths' Sea Fishes (Smith M.M. & P.C. Heemstra, eds), pp. 463-478. Grahamstown: J.L.B Smith Institute of Ichthyology.

GLAMUZINA B., JUG-DUJAKOVIĆ J. & I. KATAVIĆ, 1989. - Preliminary studies on reproduction and larval rearing of common dentex, *Dentex dentex* (Linnaeus, 1758). *Aquaculture*, 77: 75-84.

GLAMUZINA B., GLAVIĆ N., SKARAMUCA B. & V. KOŽUL, 1998. - Induced sex reversal of dusky grouper, *Epinephelus marginatus* (Lowe). *Aquacult. Res*., 29(8): 563-567.

HUREAU J.-C. & N.I. LITVINENKO, 1986. - Scorpaenidae. *In*: Fishes of the North-Eastern Atlantic and the Mediterranean (Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nielsen J. & E. Tortonese, eds), pp. 1211-1229. Paris: UNESCO.

JARDAS I., 1996. - The Adriatic Ichthyofauna. 533 p. Zagreb: Školska knjiga. (In Croatian)

JUG-DUJAKOVIĆ J., DULČIĆ J. & M. KRALJEVIĆ, 1995. - Preliminary data on embryological and larval development of black scorpionfish *Scorpaena porcus* (Linnaeus, 1758). *Bilješke-Notes, Inst. Oceanogr. Fish.*, 78: 1-8.

KATAVIĆ I., 1984. - Induced spawning and culture of early life history stages of sea bass *Dicentrarchus labrax* (Linnaeus, 1758) and gilthead sea bream *Sparus aurata* (Linnaeus, 1758). PhD thesis, 197 p. Univ. of Zagreb. (In Croatian)

LASKER R., 1981. - Marine Fish Larvae: Morphology, Ecology and Relation to Fisheries. 131 p. Washington: Univ. of Wahington Press.

MUÑOZ M. & M. CASADEVALL, 2002. - Reproductive indices and fecundity of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae) in the Catalan Sea (western Mediterranean). *J. Mar. Biol. Ass. U.K.*, 82: 995-1000.

SPARTA A., 1941. - Contributo alla conoscenza di uova, stadi embrionali e post-embrionali negli Scorpenidi. I. *Scorpaena porcus* L. *Arch. Oceanogr. Limnol*., 1: 109-115.

SPARTA A., 1942. - Contributo alla conoscenza di uova, stadi embrionali e post-embrionali negli Scorpenidi. II. *Scorpaena scrofa* III. *Scorpaena ustulata* Lowe. IV. *Scorpaena dactylopte - ra* De La Roche. *Arch. Oceanogr. Limnol*., 1: 116-134.

TÅNING V., 1961. - Larval and postlarval stages of *Sebastes* species and *Helicolenus dactylopterus*. *Rapp. P.-V. Réun. Cons. Perm. Int. Explor. Mer*, 150: 234-240.

Reçu le 25 septembre 2006.
Accepté pour publication le 21 septembre 2007.