# EMBRYONIC DEVELOPMENT OF SEA BASS, *DICENTRARCHUS LABRAX*, AFTER HORMONAL TREATMENT IN EGYPT

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# Abstract

Human chorionic gonadotropin alone has been successfully used to induce spawning in *D. labrax* species. It is possible to obtain full ripe eggs during the spawning season with cumulative doses ranging from 3.37 to 4.90 IU of HCG per gram of total body weight of fish. Milt could be flown from male after injection with one or two doses ranging from 1.32 to 2.90 IU of HCG per gram. The fertilized eggs pass through various stages of embryonic development until the embryo becomes fully formed. By age  $72 \pm 4.0$  hours after fertilization, the hatching process occurred (mean temperature: $15 \pm 0.60$ °C). On the third day after hatching, larvae start to take exogenous food of rotifers, at water temperature of  $16.4 \pm 4.0$  °C.

Keywords : Fish, induced spawning, Egypt.

### Introduction

The European sea bass, *Dicentrarchus labrax*, is an euryhaline species and can be found in the open sea as well as in lagoons and near river estuaries. Recently in Egypt, sea bass has been introduced in commercial aquaculture. Numerous studies on eggs and larval development of sea bass, *D. labrax* were carried out by many authors (1, 2). The control of reproduction, the various problems of larval rearing, and growth of *D. labrax* have also been studied. The present paper reports the results of induced spawning of *D. labrax* using chorionic gonadotropin injection, artificial fertilization and embryonic development. This knowledge may help to overcome problems in commercial aquaculture production by increasing the available amounts of sea bass juveniles.

## Materials and methods

The species *Dicentrarchus labrax* studied in the present work were collected alive from the Mediterranean sea along El-Maadeia coast, during the period of spawning season (1995). After the collection of samples, the fish were transported in special aerated tanks in the hatchery located at Alexandria, Egypt. The ripe fish were held in tanks supplied with flowing sea water, good aeration and siphoned out daily for about (2-5) weeks prior to the beginning of each experiment. Their body length ranged from 33.2 cm to 45.0 cm, while their body weight ranged from 500 to1400 g. The fish were divided into two groups : The first group composed of 12 fish which were injected with two doses, ranging from 1000-2500 IU HCG/fish, with 48 hours interval. In the second experiment, 11 fishes (Females and Males) were injected with one single high dose ranged from 1000-5000 IU/fish, which was enough to cause ovulation. The response to the hormone treatment was studied.

The hand stripped method was used also for marking out different stages of embryos and their duration. After the start of the spawning, the individuals were stripped by the dry method. The eggs and milt were stripped manually with abdominal pressure. The milt was mixed gently with eggs without adding any sea water. After fertilization, eggs were washed with sea water and placed in aerated aquaria. The eggs were reared until hatching and their development was studied. The natural water temperature and salinity during the experiments fluctuated between 13 to 17°C and 36 to 38 ppt, respectively. Results and discussion The results of these experiments showed that it was possible to obtain

The results of these experiments showed that it was possible to obtain full ripe eggs (40% of total eggs spawned) from female sea bass, D. *labrax* during spawning seasons with cumulative doses ranging from 3.57 to 4.9 IU of HCG per grams of total body weight. On the other hand, milt could be flown from males after injection with one or two doses if required, with a total dose ranging from 1.32 to 2.9 IU of HCG per gram.

#### Embryonic development and hatching larvae

I. Blastodisc stage. When eggs were discharged by the female, they were translucent, spherical in shape and about  $1.12 \pm 0.02$  mm in diameter. The whole egg was full of undifferentiated yolk material. The eggs exhibited two or four oil globules, with diameter ranging from 0.1 to 0.35 mm. A perivitelline space was formed at 30 minutes after fertilization, and comprised about 6-8% of the egg size. One hour after fertilization, the protoplasm was gradually differentiated from yolk so as to form a circular blastodisc which was about 0.75 mm in diameter, and 0.32 mm height.

**II.** Cleavage stages. At 1 hour and 50 minutes after fertilization, the first cleavage had been completed, giving two distinct elliptical blastomeres, with a length of 0.75 mm and height about 0.32 mm. Within 2 hours and 30 minutes, the second cleavage occurred at right angles to the first one, and four blastomeres became clearly defined. The third cleavage producing the 8-cell stage. The fourth cleavage occurred after 4 hours and 30 minutes, parallel to the second giving rise to 16 blastomer stage. After 4 hours and 45 minutes the fifth cleavage produced 32 cells. Cell division continues and after 7 hours, the morula stage appears. The blastoderm, has a flat, multicellular body similar to the germinal disc. Blastula stage appeared after 9 hrs after fertilization. The blastomeres, were spread over half the

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surface of the yolk, while the germ ring appeared to be slightly getting thicker in the deeper layer at the edge of the blastodisc.

**III.** Gastrulation stage. At the 12 hr stage, the blastoderm had become symmetrical and spread over the whole surface of the yolk during the process of epiboly. During this stage, the embryonic shield was visible. By 15 hr, the embryo now apceared as a slight fold (keel) of the blastoderm in the lower hemisphere of the egg.

**IV. Organogenesis.** At the 18 hour, the head had begun to be slightly differentiated. At stage 19 hr, the embryo was distinct throughout its length with the optic lobes, while the caudal swelling was evident on either side of the posterior extremity. The anterior part of the head is still fused with the yolk sac. At 22 hour the optic and olfactory lobes became more prominent. The melanophores start to appear on the head and trunk of the embryo.

*V. Tail separation and embryo movement stages.* At the 27 hour stage, the various parts of the brain were visible. A first trace of the olfactory placode could also be seen. The auditory capsules were plainly discernible as pits on either side of the posterior part of the head. The rudiment of the heart may be seen just below the olfactory lobe. At this stage, the tail was separated from the yolk. More or less, about 47 hr, the embryo was fully formed. The heart beats were more regular and the frequency of the movement of the embryo inside the egg membrane increased.

*Larval stage*. At temperature ranging from 15 to 17°C hatching occurred at the age of  $72 \pm 4$  hours. The newly hatched larvae tended to float near the surface of the water. The larvae measured  $3.0 \pm 0.15$  mm in length. The yolk sac was  $1.025 \pm 0.12$  mm in length and  $0.7 \pm 0.10$  mm in width; it contained one oil globule (about  $0.35 \pm 0.02$  mm in diameter). The eyes were unpigmented and large. The body of the larvae was pigmented with melanophores. Two rows of melanophores were situated dorso-laterally on each muscle segment of trunk and tail regions.

On the third day after hattching, the mouth and the anus open, and the larvae started to take rotifers as exogenous food. The well developed mouth parts allowed the larvae feed on a mixture of Clorella salina and Brachionus plicatilis. The full absorption of the yolk occurred on the 6th day after hatching, almost at the same time as the oil globule absorption began. The convolution of the digestive track occurred on the 7th day after fertilization.

For two decades, *D. labrax* has proved to be an important species for marine fish culture(2). Knowledge of the pattern of early larval development, particularly in reared fish, is important, as it facilitates aquaculture research and fish resources management(3). In the present study, *D. labrax* in captivity did not spawn naturally. Chorionic gonadotropin hormone was used to enhance gonadal maturation and to induce spawning.

In the present study, the fertilized eggs of *D. labrax* were transparent, buoyant and measured about  $1.12 \pm 0.02$  mm in diameter with yolk diameter about  $1.04 \pm 0.03$  mm. The same result has been recorded for this species(1).

The hatching occurred at about  $72 \pm 4$  hours after the fertilization with temperature ranging from 15 to 17°C. In the present study, the newly hatched larvae measured  $3.0 \pm 0.15$  mm and had a mean total length of 3.20 mm.

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