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POSTER

**Study of reproduction in captivity of blackspot sea bream (*Pagellus bogaraveo* B.).
Embryonic development and consumption of vitelline sac.**

J.B. Peleteiro, M. Olmedo, C. Gómez & B. Alvarez-Blázquez.
Instituto Español de Oceanografía, C.O. de Vigo. Cabo Estay, Canido. Apdo. 1552 Vigo.36080
Pontevedra. Spain.

ABSTRACT

Blackspot sea bream is one of the species in the world of aquiculture with more possibilities for culture on an industrial scale. This is due to the high price brought in the market and to an increasingly greater scarcity in Spanish fishing grounds.

For the purposes of this paper, a stock of 48 Blackspot sea bream with an average weight of 1118 g were used. These were taken from wild waters, maintained in a 32 m³ tank in two different periods, the first during 1991/1996 at a controlled temperature of 14°C, and the second during 1996/1997 at a room temperature of between 12 and 21°C.

In march 1996, 21 individuals from this stock were induced to spawn with three progressive doses of LHRHa of 5, 10 and 15 µg/kg, administered intramuscularly, at two week intervals, but no positive response was obtained.

The stock reproduced naturally in march 1997, one year after temperature monitoring was withdrawn. The fertilized eggs were gathered in a 500 µm collector and were sampled (20000 eggs/spawning), measured (1.188±0.021 mm diameter) and incubated at room temperature (16.37±0.261°C). The duration of embryonic development from the blastula stage, in which they were collected, to eclosion, was 37.1 degrees/day.

Follow-up was performed on embryonic development every 8 hours and on vitelline sac consumption every 24 hours by photography.

The larvae were measured from the moment of eclosion (total length: 3.70±0.0935mm) every twenty four hours until the vitelline sac was consumed (5th day of life) when they were measured (total length: 4.798±0.107 mm) and weighed (mean weight: 48.564±10.8 µg). The length and width of the vitelline sac were also measured daily until complete consumption.

The period from eclosion (day 0 of life) and the opening of the mouth (day 6 of life), when the total length was 4.920±0.052mm, was 94.3 degrees/day.

INTRODUCTION

In response to industrial demand looking for farmable species as alternatives to traditional species such as turbot (*Scophthalmus maximus*), sea bream (*Sparus aurata*) or sea bass (*Dicentrarchus labrax*), a research project was initiated in the early '90's to determine the possibility of culturing new commercial species in Spanish and international waters.

One of the species proposed offering most possibilities for being cultured, both for its biological characteristics, its high value on the market and its scarcity in fishing grounds was blackspot sea bream (*Pagellus bogaraveo*), a traditional species in Spanish and Mediterranean country markets.

On the basis of data, from fisheries, such as sexual behaviour, maturity stage or size-weight ratio (Sánchez F., 1982; Sánchez F., 1983; Krug, H.M., 1986), the first experiences with fattening and reproduction were conducted on individuals caught in wild waters.

The results in terms of adaptation to captivity, fattening and behaviour were promising (Chereguini O. *et al.*, 1990; Peleteiro, J.B. *et al.*, 1994). The data show a similar behaviour to other known sparides as *Diplodus sargus* (Divanach P. *et al.*, 1982; Cejas J. *et al.*, 1987), *Dentex gibbosus* (Fernández-Palacios *et al.*, 1994), *Dentex dentex* (Jug-Dujaković *et al.*, 1995) and *Pagellus erythrinus* (Cejas J. *et al.*, 1993), or previously cultured sparides such as sea bream (*Sparus aurata*) (Alessio G. *et al.*, 1975; Cejas J. *et al.*, 1993).

One of the more important difficulties, however, found in culturing sea bream was reproduction in captivity. The only documentation found on the subject (Fernández-Pato C., *et al.*, 1990), relates natural spawnings in captivity with individuals stabled in 10 m³ tanks. Apart from this reference, we have information of the occurrence of abundant spawnings from reproducers kept under natural conditions in tanks of over 100 m³ volume (Fernández A., 1994, pers. com.) which bears considerable influence on the installations.

The purpose of this study was to define the initial stages of culturing blackspot sea bream (*Pagellus bogaraveo*) and to gain more detailed knowledge of its reproduction cycle in captivity.

MATERIAL AND METHODS

BROODSTOCK

The batch of reproducers used for this experience comprised 48 individuals from wild waters, with an average weight of 1118 g (1:3 male to female ratio), kept in a 32 m³ tank with a natural photoperiod. Water temperature was controlled at 14±1°C during the period 1991-1996 and, from June 1996, maintained at room temperature (12-21°C).

Fish feed comprised pellets made from fish meal and oil, mussel, squid and crab which were well accepted by the fishes.

Samplings were taken of the diameter of the oocytes obtained in individuals from the fish market and laboratory to determine the spawning season in our study zone (NW Spain).

When spawning season was determined in the natural environment and in wild waters, a batch of 21 individuals was separated out for spawning by hormonal induction. Prior to anaesthesia with MS222, SANDOZ (0.08 g/l) to avoid possible damage in handling, the fishes were injected intramuscularly with LHRHa (des-Gly¹⁰, [D-Ala⁶] - LUTEINIZING HORMONE RELEASING HORMONE, SIGMA), in three progressive doses of 5, 10 and 15 µg/kg at an interval of two weeks between each.

In June 1996, the water temperature was changed and the first natural spawnings were obtained in March 1997.

SPAWNINGS

The fertilized eggs were gathered in the conventional method, placing a collector with a 500 micra mesh in the overflow of the tank. The first spawning was gathered on 17/3/97 and, from here on, daily batches of approximately 20000 eggs, supposedly from one single female.

The eggs were separated from the organic matter in suspension by decantation. They were then sampled and 100 eggs were gathered to calculate diameter.

A batch of 20000 eggs gathered at 09:00 a.m. at the blastule stage was used to study embryonic development.

INCUBATION

Eggs were placed in batches of 5000 eggs/l in 2 liters cylindrical incubators with a 500 mm mesh at the bottom to allow for water circulation (see photo). These containers were suspended inside a 150 l tank with water entering from the top, discharging at the bottom at room temperature, ranging from 16.3 to 16.71C throughout this period. Dead eggs were collected and sampled daily from the bottom of the incubators.

A sample of eggs was collected from the incubators every 8 hours (08:00, 16:00 and 24:00 h) for the purposes of embryonic development, to observe the different stages and to compile a photographic record.

After hatching, the bad eggs were removed from the bottom and the larvae were maintained in the same containers under the same conditions.

Daily samples of 25 larvae were taken to study the development of the biometric parameters. Total length of the larvae, the length and width of the vitelline sac and the diameter of the oil drop were measured daily. On day 5 of age, when the digestive system becomes functional and the vitelline sac has been practically consumed, the length (mm) and dry weight (µg) of the larvae were measured.

RESULTS

1.- As a result of the distribution of the ovocytes achieved by sampling two females from the stock, it was decided to induce spawning by hormonal injection, and no positive response was obtained.

2.- Due to the results obtained through induction, the environmental conditions of the reproducers were changed, maintaining the natural photoperiod and removing temperature control, changing to room temperature thus obtaining the first natural spawnings.

3.- The embryonic development of these eggs and the evolution of the larvae until the vitelline sac was consumed are shown in the following photographs:

EMBRYONIC DEVELOPMENT

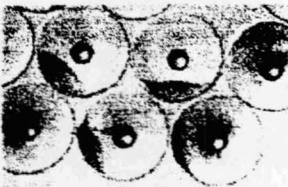


Photo N.1 . Eggs recently collected (hour 0) from the broodstock tank. Blastule stage. Perivitelline space increases on the blastodisc edges and the spherical shape of the egg changes slightly to an oval morphology. Average egg diameter was 119 ± 0.0215 mm.

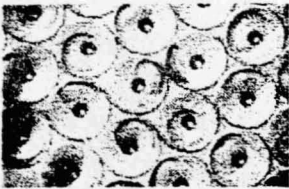


Photo N.2 . Gastrule stage (hour 6). Edges of the germinal disc have increased in thickness due to invagination. A wider zone appears, indicating the start of formation of embryonic sketch.

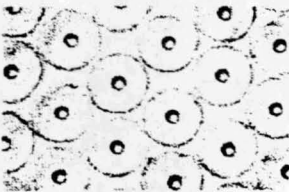


Photo N.3 . Appearance of the embryonic sketch (hour 14) as an incomplete patch in the rear part from the animal to the vegetative pole. The segmented part tends progressively to cover the entire egg.



Photo N.4 . Forming of the embryonic body (hour 22) occupying practically half of the circumference of the eggs. Internally, neural formation occupying $1/3$ of the width of the body may be observed. In the anterior part of the body, an arrow-shaped expansion is observed, the sketch of the optic capsules.



Photo N.5. Appearance of the first chromotaphores (hour 30). At this stage, the ovoidal shaped optic capsules become evident.



Photo N.6. The embryo takes 3/4 of the egg (hour 38). In this stage the heart beat and a more developed pigmentation can be observed.



Photo N.7. Completed embryo development (hour 46). The embryo takes almost the total volume of the egg. The tail begins to separate and moves frequently. The frequent contractions of the larva, with enzymatic secretion, break the egg membrane in a very short time, to come out.

CONSUMPTION OF VITELLINE SAC



Photo N.8. Eclosion (hour 54). The vitelline sac takes the third part of the length of the larva and the oil globule is situated in the end of the sac, next to the anus. Total length: 3.70 ± 0.0935 mm. Vitelline sac length: 1.27 ± 0.0561 mm. Vitelline sac width: 0.52 ± 0.0663 mm. Oil globule diameter: 0.25 mm.

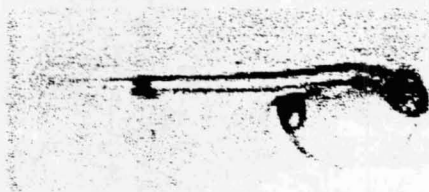


Photo N.9. 24 hours age larva. The vitelline sac is reduced to a 77%. Total length: 4.13 ± 0.1983 mm. Vitelline sac length: 0.98 ± 0.0438 mm. Vitelline sac width: 0.43 ± 0.0335 mm. Oil globule diameter: 0.25 mm.



Photo N.10. 41 hours age larva. The vitelline sac is reduced to a 76%. Total length: 4.28 ± 0.0718 mm. Vitelline sac length: 0.98 ± 0.6455 mm. Vitelline sac width: 0.44 ± 0.0391 mm. Oil globule diameter: 0.25 mm.



Photo N.11. 65 hours age larva. The vitelline sac is reduced to a 47%. Total length: 4.50 ± 0.1089 mm. Vitelline sac length: 0.60 ± 0.0320 mm. Vitelline sac width: 0.41 ± 0.0421 mm. Oil globule diameter: 0.22 mm.

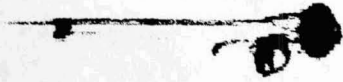


Photo N.12. 95 hours age larva. The vitelline sac is reduced to a 37%. Total length: 4.72 ± 0.0893 mm. Vitelline sac length: 0.47 ± 0.0244 mm. Vitelline sac width: 0.31 ± 0.0294 mm. Oil globule diameter: 0.20 ± 0.0302 mm.

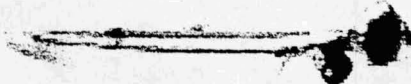


Photo N.13. 115 hours age larva. The mouth is now opened, and the exogenous feeding starts. Total length: 4.80 ± 0.1065 mm. Dry weight: 48.56 ± 0.0108 μ g. Vitelline sac length: 0.30 ± 0.0456 mm. Vitelline sac width: 0.27 ± 0.0238 mm. Oil globule diameter: 0.15 mm.

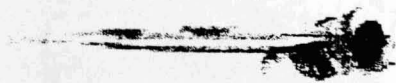


Photo N.14. 138 hours age larva. The vitelline sac is totally consumed. The digestive system is totally functional and the larva shows normal feeding habit. Total length: 4.92 ± 0.0520 mm.

CONCLUSIONS

Despite the objective of this study not being to obtain concrete data but rather to further knowledge of the behaviour of sea bream in captivity, the results obtained when achieving natural spawnings in captivity from wild individuals largely solves possible doubts regarding the feasibility of culturing sea bream. It also became evident that there is a need to conduct further studies on behaviour in captivity, particularly as regards reproduction.

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