

**Protocol C - Determination of egg quality and spawning performance
[Practical guide of protocols: broodstock management]**

in

Felip A. (ed.), Carrillo M. (ed.), Herráez M.P. (ed.), Zanuy S. (ed.), Basurco B. (ed.).
Advances in fish reproduction and their application to broodstock management: a practical manual for sea bass

Zaragoza : CIHEAM / CSIC-IATS

Options Méditerranéennes : Série B. Etudes et Recherches; n. 63

2009

pages 23-25

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=800909>

To cite this article / Pour citer cet article

Protocol C - Determination of egg quality and spawning performance [Practical guide of protocols: broodstock management]. In : Felip A. (ed.), Carrillo M. (ed.), Herráez M.P. (ed.), Zanuy S. (ed.), Basurco B. (ed.). *Advances in fish reproduction and their application to broodstock management: a practical manual for sea bass*. Zaragoza : CIHEAM / CSIC-IATS, 2009. p. 23-25 (Options Méditerranéennes : Série B. Etudes et Recherches; n. 63)



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Protocol C

Determination of egg quality and spawning performance

I - Introduction

Egg quality, defined as those traits of the egg that determine its capacity to survive, is a key issue to be determined in fish species reared in captivity. Generally, for many fish, egg mortality rates are high with low post-weaning survival. Consequently, to assess the quality of the progeny, the evaluation of egg quality as well as spawning quality are crucial for aquaculture purposes. Many factors have been suggested to influence spawning and egg quality (Bromage, 1995; Carrillo *et al.*, 1995; Carrillo *et al.*, 1997). However, the lack of standardized methods and criteria to evaluate gamete quality makes this issue difficult to address (Bromage, 1995).

A first consideration to obtain eggs of good quality is to keep good broodstocks and to know how to manage them properly. Thus, broodstocks have to be kept in quiet isolated units in order to avoid disturbance during spawning. On the other hand, since broodstock nutrition affects the quality of the progeny, sea bass broodstock are generally fed with a natural diet of trash fish (e.g. *Boops boops*) (Carrillo *et al.*, 1995). Therefore, this particular situation requires the breeders to be hand-fed.

At present, there are a variety of criteria that may be used to define gamete and progeny quality as well as several characteristics which evaluate some factors affecting fecundity and egg quality in fish (Carrillo *et al.*, 2000). Particularly, the assessment of fecundity and egg buoyancy is crucial for aquaculture. Fecundity is the number of eggs produced by a broodfish and its egg buoyancy can be easily calculated by placing the eggs obtained from a broodfish in a graduated cylinder to determine the volume of the floating (viable) and sinking (non-viable) eggs.

However, as the number of eggs may be different in a specific volume depending on the egg size, broodfish size or spawning, a reliable method of calibration for counting the number of eggs per unit of volume (fecundity) needs to be conducted (Navas *et al.*, 1998; Carrillo *et al.*, 2000). To do this, the measurement of the egg diameter and the counting of the number of eggs in several sub-samples collected from the spawns of different broodfish during the reproductive season need to be made. Later, the application of a typical regression equation can be used to evaluate fecundity. For sea bass, the use of the formula $N = 2865 - 1796D$ (where N = the number of eggs in 1 ml, and D = the mean diameter of 100 eggs) is a reliable way to evaluate this trait. The regression line for this species has been obtained using 25 different spawnings, three sub-samples per spawn and the mean diameter of 100 eggs related to the number of eggs observed in 1 ml ($r^2 = 0.94$) (Navas *et al.*, 1998; Carrillo *et al.*, 2000). It should be noted that the measurement of fecundity for new species in aquaculture might be of interest as an indicator of broodstock fitness of each specie under cultivation. In the case of sea bass, the parameters commonly used to monitor broodstock fitness include the volume of good (floating) and poor (sinking) eggs, the hatching rate, the larval deformation rate and the larval survival rate. Consequently, the monitoring of these parameters may be useful for the proper assessment of the spawning, gamete and progeny quality (Navas *et al.*, 1998; Carrillo *et al.*, 2000). Most studies use these criteria routinely. In this Protocol, a microplate system for egg incubation is shown as a reliable approach to determine egg quality characteristics and spawning performance in sea bass.

II - Purpose

The objective of this Protocol is to show a variety of criteria in order to evaluate spawning performance and egg quality in sea bass. A microplate system for incubation is shown to determine hatching rate, deformation rate and larval survival rate at different times after hatching.

III - Procedure

- (i) Collect each spawn and add eggs into a 2-l graduated cylinder using seawater.
- (ii) Calculate the percentage of buoyant (viable) to sinking (dead) eggs (buoyancy rate) volumetrically filling the graduated cylinder with seawater.
- (iii) Take a portion of buoyant eggs and measure the diameter ($n = 100$ eggs / spawn) using a stereoscope.
- (iv) Take 400-500 buoyant eggs and rinse them with sterile seawater three times.
- (v) Distribute the eggs in triplicate in 96-well microtitre plates (one egg and 200 μ l sterile seawater / well).
- (vi) Cover the plates with a lid and place them into a plastic zip-lock bag.
- (vii) Incubate the plates at 16°C for 10 days.
- (viii) Determine hatching rate, deformation rate and larval survival rate at 1 day after hatching (DAH), 3 DAH and 10 DAH.

IV - Materials and equipment

- 2-l graduated cylinders
- 2-ml Pasteur plastic pipettes
- Excavated glass slides
- Stereoscope
- 96-well microtitre plates and lids
- Plastic zip-lock bags
- Incubator at 16°C
- Gloves, lab coat and boots

V - Reagents and solutions

- Sterile seawater.

VI - Results and discussion

Assessment of egg quality of any teleost species is necessary to determine the quality of spawnings. An incubation system based on a 96-well microtitre plate has been developed to monitor the early stages of sea bass egg development from fertilization to several days after fertilization (Fig. C.1) (Carrillo *et al.*, 2000). At 10 days after fertilization, early mortality (7.15%) and deformation rates (0.06%) of sea bass reared in this incubation system have shown to be low suggesting it is an easy and reliable system for the evaluation of physiological broodstock fitness in sea bass. Starting with good quality eggs and after incubation of fertilized eggs in a

reliable incubation system, several indicators can be measured to determine the quality of the progeny.

These indicators are: (i) hatching rate (hatched eggs / fertilized eggs x 100); (ii) deformation rate (deformed larvae / hatched eggs x 100); (iii) larval survival rate (live larvae / hatched eggs x 100). In order to assess the quality of the progeny in the sea bass, these factors are usually monitored at three different times during the incubation period (i.e., 1, 3 and 10 DAH).

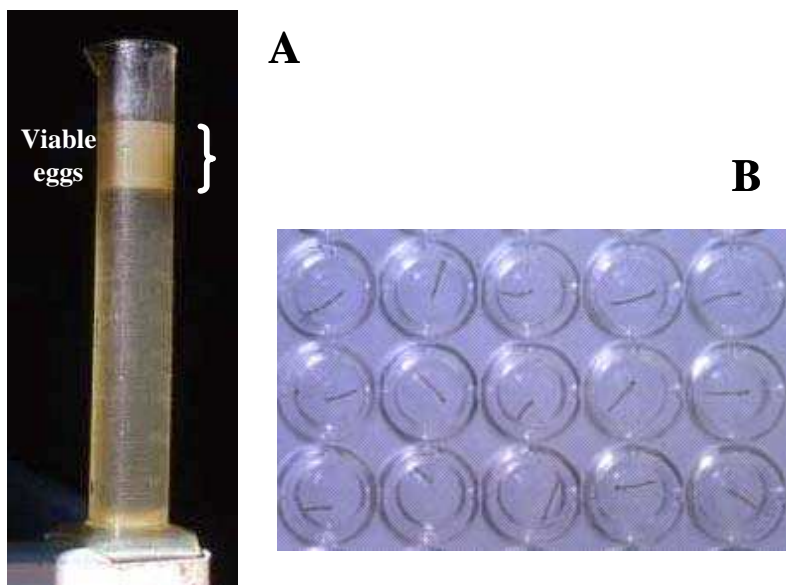


Fig. C.1. Quality of the progeny. (A) Evaluation of the quality of spawnings (percentage of floating eggs). (B) A microplate system for egg incubation until hatching (percentage of larval survival).

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