

SPONTANEOUS SPAWNING OF THE BLACK SEA- CUCUMBER *Holothuria forskali* (DELLE CHIAJE, 1823). PRELIMINARY RESULTS OF FERTILIZATION, EGGS INCUBATION AND LARVAL REARING

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Introduction

Holothuria forskali (Delle Chiaje, 1823) is a native species in the Cantabrian Coast (Spain) that can also be found in the North East Atlantic Area and Mediterranean Sea. It is a detritivore species that feeds mainly at night. This gonochoric species has an annual reproduction cycle with a synchronous spawning for male and female in spring in the Atlantic Area (Tuwo and Conand, 1992). *H. forskali* is an interesting species from an aquaculture point of view, not only as a gastronomic product, but also for its bioremediation potential in integrated multitrophic (IMTA) configurations. Therefore, breeding control in captivity is basic for its integration in IMTA systems. With this aim we planned to create a stock of breeders obtaining them from their natural environment.

Material and methods

In April 2021, coinciding with the end of the reproductive season of the species in the Atlantic, 33 *H. forskali* adults were collected at La Maruca's Beach (Cantabrian Sea, Spain: 43°28'47"N 3°50'11"W) during low tide (seawater temperature at 12.9 °C). Specimens were transferred in a 20 L cooler to the hatchery of the "El Bocal" Marine Aquaculture Station of the Oceanographic Spanish Institute of Santander (Cantabria, Spain). At all time, the sea-cucumbers were carefully handled to avoid expulsion of Cuvierian tubules which would induce stress.



Specimens were weighted (wet weight) and placed in a rectangular tank (3 m long x 1 m wide and an average depth of 0.25 m: aprox.600 L) with flowing seawater at 13 °C.

Unexpectedly, two hours after arrival at our facilities, a spontaneous spawning event occurred, and 5 specimens (3 males and 2 females) began to release their gametes (Fig. 1). Naturally fertilized eggs were rapidly removed from the tank with 20 µm sieve and washed with seawater. Also, eggs and sperm were collected directly from the gonopore of those specimens that remained releasing their gametes naturally, and artificial fecundation was performed in a flask. Egg diameter was measured in one hundred fertilized eggs.



Figure 1.- Female (I) and male (II) of *Holothuria forskali* performing natural spawning.

Swiftly and with the available means, egg incubation and larval rearing was planned. The seawater used for embryonic rearing was filtered at 1 µm and sterilized with a UV light system. Eggs were incubated at 14 °C (±2 °C) in 1 L flask with soft aeration and natural photoperiod.

Once embryonic development was completed, lyophilized *Nannochloropsis gaditana* at a final concentration of 3.10³ cell .mL⁻¹ was used to feed the larvae.

Results and discussion

- The mean weight of the *Holothuria forskali* collected was 120.24 ± 33.50 g.
- No mortality was recorded in adult specimens.
- The average diameter of the fertilized eggs of the natural spawning was 156.37 ± 7.35 µm (n = 100), while the average diameter of the eggs of the dissected females was 200±11.27 µm y 199±10.25 µm.
- Embryonic development of *H. forskali* started with elevation of the fertilization envelop and expulsion of the polar bodies (figure 2.C),. The morula stage took place 2 day after fertilized (daf) (Figure 2.D , Table 1)...

Table 1. Duration of the embryonic development of *H. forskali* (daf) day after fertilized at 14 °C..

Embryonic and larval stage	Time day after fertilized (daf)
Morulla	3
Early auricularia	4
Mid auricularia	5-10

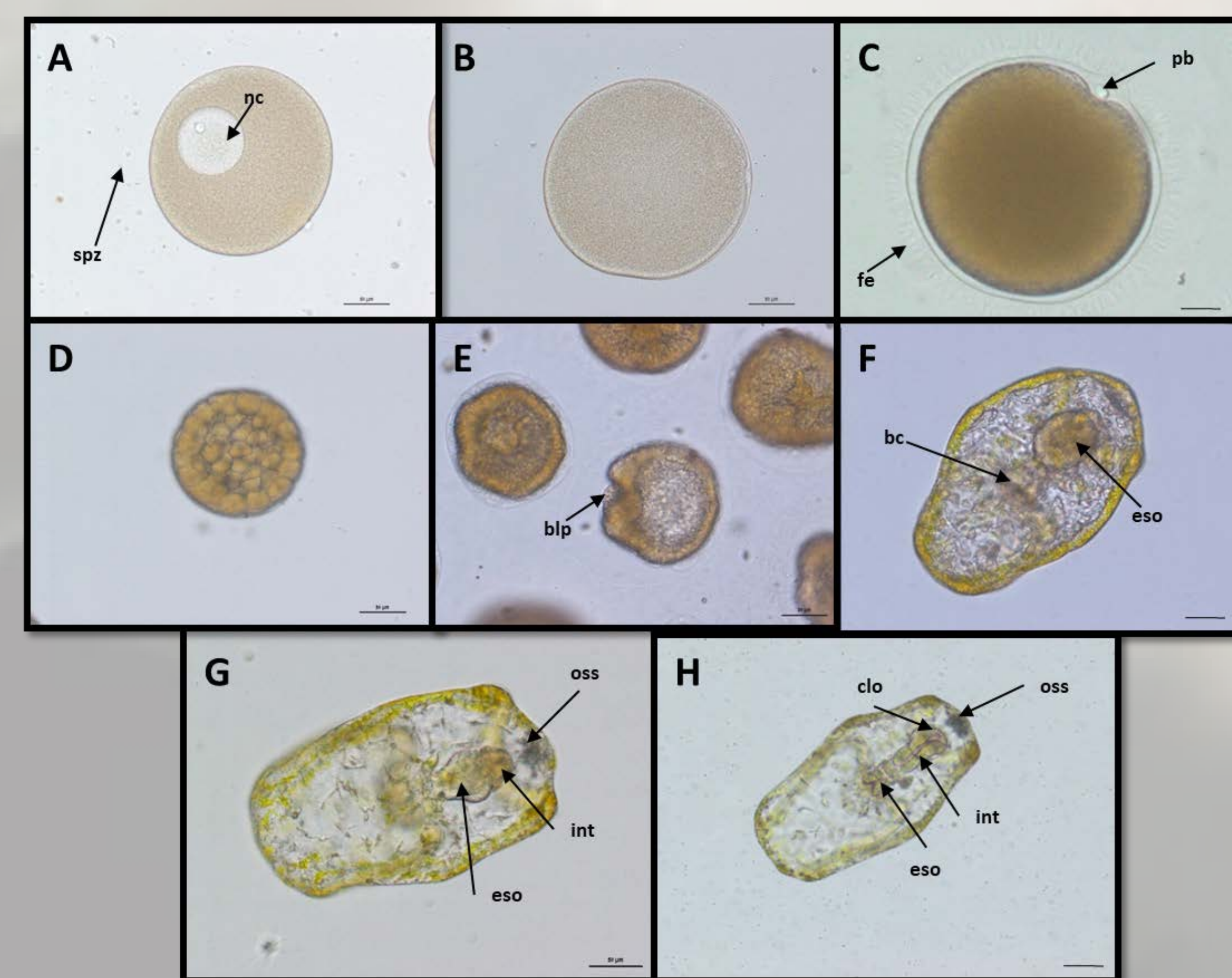


Figure 2. Embryonic development of *H. forskali*. A: Spawned oocyte with clearly visible nucleus (nc) and spermatozoons (spz). B: early fertilized egg. C: fertilized egg with the fertilization envelop developed (fe) and differentiated polar bodies (pb). D: Morula. E: Gastrula with blastopore (blp). F: early auricularia, 4 days after fertilization; buccal cavity (bc), esophagus (eso). G: Mid auricularia, 5 days AF; intestine (int) ossicle (oss). H: Mid auricularia 6 days AF; cloaca (clo). Scale bars= 50 µm.

After mid auricularia larval stage, larval rearing collapsed. Until that moment, embryonic development was slower than as Laguerre et al. (2020) described it. This could be due to our lower water temperature.

References

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