

Description of reproductive performance and ovarian histology of wild *Farfantepenaeus paulensis* from shallow waters in southern Brazil.

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Abstract

The present study reports on the reproductive performance of shallow water-caught *Farfantepenaeus paulensis* animals and relate it to ovarian histological analysis. Males and females were captured in shallow waters (5-10 m) at the north bay of Florianópolis (Santa Catarina, Brazil) during springtime. Reproductive performance was assessed through successive spawns of unilaterally eyestalk-ablated females maintained during 50 days under controlled environmental conditions. Histological analysis of the ovaries collected just before spawning were performed in five randomly selected females (35.5 ± 7.6 g). The mean number of eggs per spawning event ($75,384 \pm 31,526$) were considered well within the range proposed for *F. paulensis* spawning performance. However, the egg fertilization rate (72.4%) was higher when compared to the egg hatching rate (31.9%) and percentage of metamorphosis to zoea I stage (13.1%). The histological and visual analysis of ovaries indicated that full development was not reached, which might be related to the poor offspring quality observed. This is the first report that *F. paulensis* females captured in shallow waters were able to spawn even though full oocyte maturation was not attained.

Key words: Reproductive performance, ovarian histology, penaeid, *F. paulensis*

Introduction

The reproductive performance of penaeid broodstock from different sources has been reported over the past few years. It is generally assumed that wild shrimp provide superior performance in terms of fecundity (Browdy *et al.*, 1986; Menasveta *et al.*, 1993; Ramos *et al.*, 1995; Cavalli *et al.*, 1997), egg quality (Primavera and Posadas, 1981; Hameed, 1997) and larval survival (Preston *et al.*, 1999). Despite their supposedly advantageous performance, the unpredictable supply and high capture costs of wild broodstock are major problems that discourage their use in aquaculture.

Since the early 80's, nauplii production of *Farfantepenaeus paulensis* in southern Brazil has been based on the capture of wild broodstock in deep-sea waters (40-70 m) off Santa Catarina State (Marchiori and Boff, 1983), where adult populations are usually found (D'Incao, 1999). In the last few years, due to lower costs, animals have also been captured in shallow water (5-10 m) bays. Although presenting an acceptable spawning performance (Peixoto *et al.* 2003), a marked decrease in egg quality and nauplii survival has been observed when using animals from this source. Additionally, ovaries of spawning females showed a less intense green color when compared with the pattern established for deep-sea animals (Peixoto *et al. in press*), which might be associated with incomplete ovarian maturation. Nevertheless, this observation should be validated by histological analyses for reliable association with the maturation stage.

Histological analysis is one reliable procedure to evaluate females quality through microscopic determination of their gonadal maturation. This approach has been successfully applied to evaluate the capability of broodstock from different sources to attain a complete oocyte development (Tan-Fermin, 1991; Medina *et al.*, 1996; Palacios *et al.*, 1999). Hence, we report in the present study on the reproductive performance of shallow water-caught *F. paulensis* animals and relate it to ovarian histological analysis.

Materials and Methods

Shrimps were captured in shallow waters (5-10 m) at the north bay of Florianópolis (Santa Catarina, Brazil) during springtime. In our maturation facilities, males (22.4 ± 2.7 g) and unilaterally eyestalk-ablated females (35.6 ± 4.5 g) were maintained for 50 days in four circular tanks (3.5 m diameter) filled with 5,000 L. Stocking density was six animals/m² at a female to male ratio of 1.5. Environmental conditions were controlled to maintain water temperature ranging from 24-26 C, salinity from 33-35 ppt and artificial lighting for 14 h/d.

Food was offered to satiation four times daily (0900, 1100, 1400 and 1700) and consisted of with fresh frozen shrimp (*Artemesia longinaris*), crab (*Callinectes* sp.), squid (*Illex* sp.) and a commercial diet (Purina MR35, Brazil), respectively.

Ovarian color and shape were examined daily as a criteria to select females ready to spawn. Females with green-colored ovaries (mature) were transferred in the late afternoon to separate spawning tanks (filled with 90 L) and returned to their respective maturation tanks on the following morning. The total number of eggs was estimated from three 100 ml replicated samples of the spawning tank water collected after homogenization. Fertilization rate was determined microscopically following Primavera and Posadas (1981). Since all fertilized spawns were pooled for incubation, the production of nauplii and zoea were monitored daily according to standard hatchery procedures (Marchiori, 1996).

For histological analysis, tissue samples from ovaries were taken from five randomly selected females (35.5 ± 7.6 g) that were considered as "ready to spawn". The gonadal tissue (middle portion) was fixed with Davidson solution for 24 h, embedded in paraffin, sectioned (6mm) and stained with hematoxylin-eosin (Bell and Lightner, 1988). Histological sections were observed under a microscope and the diameter of at least 30 oocytes per female was measured with an eyepiece micrometer. Only oocytes showing nuclei sectioned approximately at the equatorial plane were measured. The ovarian maturation stage was determined according to the criteria proposed for deep-sea *F. paulensis* broodstock, which is based on histological analysis and visual comparison to a widely available chromatic scale catalogue (Pantone Matching System, Coated Simulation, www.pantone.com) (Peixoto *et al. in press*).

Results

The reproductive performance in terms of percentage of fertilized spawns, number of spawns per female and mean number of eggs per spawning event are presented in the table I. The egg fertilization rate was much higher when compared to the egg hatching rate and percentage of metamorphosis to zoea I stage (Table I). Thus, the total zoea I production declined sharply compared to total nauplii and egg production (Figure 1).

Table I: Reproductive performance parameters (mean \pm SD) of *F. paulensis* females from shallow waters during 50 days.

Number of spawns	270
Fertilized spawns (%)	41.5
Spawns / female *	1.35
Eggs / spawning event	75,384 \pm 31,526
Fertilization rate (%)	72.4 \pm 25.3
Hatching rate (%)	31.9
Metamorphosis to zoea I stage (%)	13.1

* Per female initially stocked that spawned at least once.

Nauplius

The ovarian histology for all samples showed acidophilic oocytes with yolk granules in the cytoplasm, nucleoli located on the periphery of cell nucleus and absence of peripheral bodies, corresponding to the previous stage of full maturation (Figure 2). In accordance, it was also observed visually that the full development was not reached, since the ovaries presented a light green color (catalogue 578 PC). The oocyte diameter varied from 165 to 210 μ m (mean \pm SD = 181 ± 13 μ m).

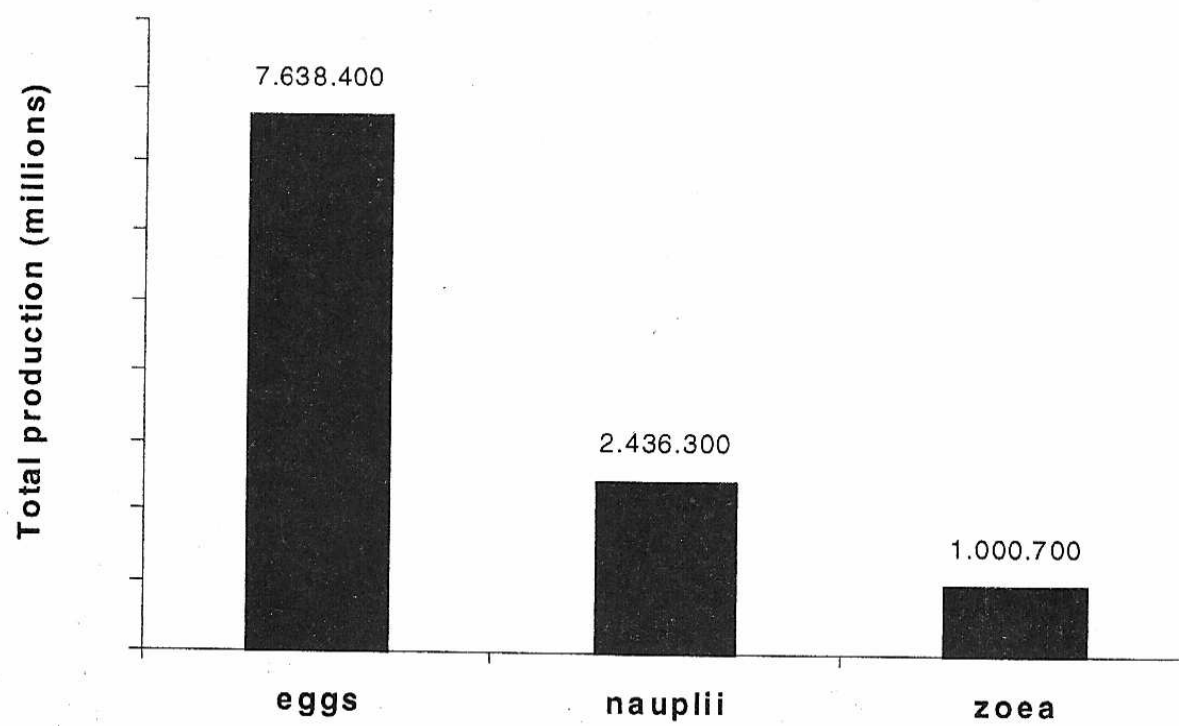


Figure 1: Total eggs, nauplii and zoea production of *F. paulensis* females from shallow waters during 50 days.

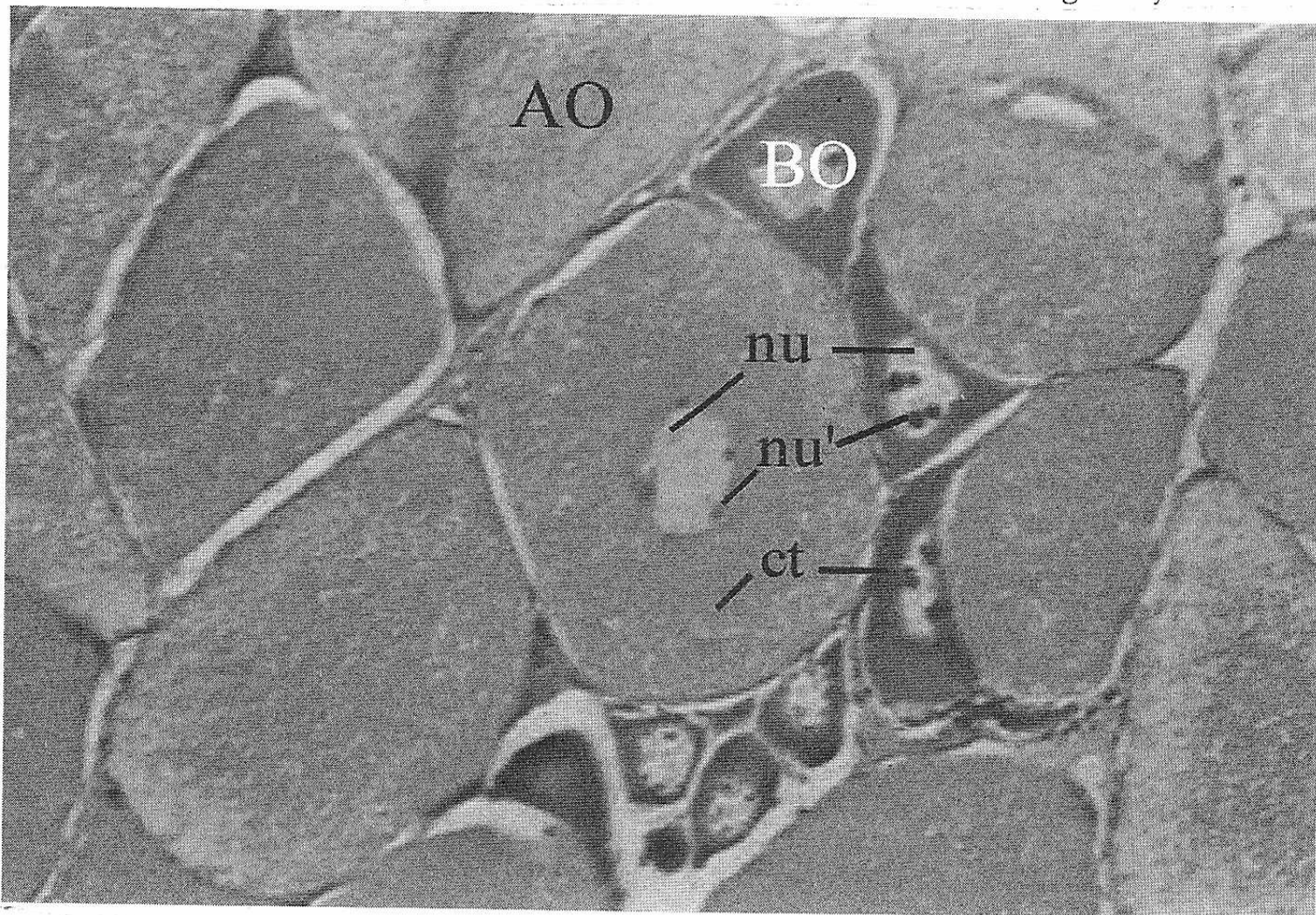


Figure 2: Cross section ($6\mu\text{m}$) of *F. paulensis* ovary showing acidophilic oocytes in development stage II (AO) surrounded by basophilic oocytes (BO) (400x). Basophilic oocytes show nucleus (nu) and nucleoli (nu') greater than cytoplasm size (ct). In stage II, acidophilic oocytes with yolk granules in the cytoplasm are dominant. In these oocytes, the nucleoli is on periphery of the cell nucleus with a considerable increase in cytoplasm size. Note the absence of peripheral bodies in the cytoplasm. Scale bar = $200\mu\text{m}$.

Discussion

In previous studies conducted under similar conditions, the mean number of eggs per spawning event of deep-sea *F. paulensis* ranged from 100,000 to 160,000 and fertilization rates were higher than 65% (Marchiori and Cavalli, 1993; Cavalli *et al.*, 1997; Cavalli *et al.*, 1998). On the other hand, Peixoto *et al.* (2003) found 78,000 eggs per spawning event and mean fertilization of 68.4% to be acceptable for shallow water *F. paulensis*. Although our results of spawning performance are in accordance with the ranges reported for the species, the offspring viability of shallow water *F. paulensis* during hatchery was not analyzed in previous studies.

In addition to the usual reproductive performance parameters, histological analysis is another way to evaluate the quality of females through determination of their gonadal maturation. This approach has been successfully applied to microscopically evaluate the ovarian condition of broodstock from different sources (Tan-Fermin, 1991; Medina *et al.*, 1996; Palacios *et al.*, 1999). For wild *F. paulensis* captured in deep-sea waters of southern Brazil, earlier histological classification of ovarian development by Worsmann

et al. (1971) described four stages, namely: immature (stage I), developing (stage II), mature (stage III) and spent (stage IV). These stages were later confirmed by Peixoto *et al.* (*in press*), who visually associated each stage to a specific color.

In the present study, the ovarian histology for all samples showed the prevalence of acidophilic oocytes and absence of peripheral bodies in the cytoplasm. This description corresponds well to the gonadal maturation stage II determined in previous studies (Worsmann *et al.*, 1971; Peixoto *et al.* *in press*). Histological observations were reinforced by the ovarian color, which presented a light green color (stage II) instead of the olive green color (catalogue 5747 PC) characteristic of fully mature, ready-to-spawn (stage III) females (Peixoto *et al.* *in press*). Furthermore, the oocyte diameter in the present study are well within the range determined for maturation stage II (100 to 220 μm) by Peixoto *et al.* (*in press*).

A somehow similar phenomenon was reported by Medina *et al.* (1996), who found that the ovaries of pond-reared *Melicertus kerathurus* passed directly from late vitellogenic stage to the spent stage, suppressing the mature stage. The authors associated the inability to form peripheral bodies (mature oocytes) with lower hatching performance of this broodstock in captivity. In accordance, it was reported that the formation of the peripheral bodies occurs in the final step of the maturation process and is probably associated with the egg hatching success, through egg activation and hatching envelop processes (Tan-Fermin and Pudadera, 1989; Clark *et al.*, 1990). Therefore, the present results suggest that the low offspring viability observed here might be related to the inability of the females to attain fully matured oocytes, as indicated by the absence of peripheral bodies and the less intense ovarian color.

A possible explanation for the inconsistent reproductive performance of shallow water *F. paulensis* might be related to their natural history and local environmental conditions. Similar to most penaeids, *F. paulensis* presents two different phases in its life cycle: an oceanic one, marked by reproduction and larval development, and another represented by growth in estuarine areas (D'Incao, 1999). Hence, it is possible to speculate that the individuals captured in shallow waters might be remainders of the migration from the estuaries to deeper waters or perhaps they might represent an intermediate step in this process. As such, *F. paulensis* from shallow water bays are probably younger than those captured in deep-sea waters. Nevertheless, it was reported that five month *Penaeus monodon* could mature and spawn after eyestalk ablation, but produced poor quality larvae (Primavera, 1978). This may suggest the need for older females to provide a sustainable production of high quality larvae.

The present study is the first report that *F. paulensis* females captured in shallow waters were not able to attain full oocyte development, which might be related to the poor offspring quality observed. However, further studies are necessary to elucidate this problem and to improve the larvae production from shallow water *F. paulensis*.

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