Ovarian development of wild pink prawn *(Farfantepenaeus paulensis)* females in northern coast of Santa Catarina State, Brazil

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Abstract

The pink prawn *(F. paulensis)* is one of the most important fishery resources in SE/S Brazil and overexploitation has led to stock collapse in 90's. The aim of this investigation was to apply histological techniques and gonadsomatic index (GSI) to validate macroscopic classification of ovaries from *Farfantepenaeus paulensis,* in attempt to provide a practical color standard to define maturity levels and biological information to properly manage this overexploited stock. Histological sections and GSI were used to validate macroscopic classification of ovaries. Four different development stages were suggested (immature, developing, ripe and spent). Mature oocytes present cortical rods and spent ovaries were characterized by atretic oocytes. Size frequency of oocytes were analyzed and polymodal distribution was observed, suggesting partial spawning in the wild. This investigation provided a practical scale to classify ovaries of *F. paulensis* as well as base information related to reproduction dynamics for this species.

Keywords: *F. paulensis,* reproduction, ovary development, histology, GSI.

Introduction

The pink prawn (*Farfantepenaeus paulensis* Pérez-Farfante, 1967) is one of the most valuable fisheries resources in southern and southeastern Brazil, occurring from Bahia (Ilhéus-14°50'S) to Mar del Plata (Argentina-38°30'S) (D'Incao, 1999). This species has a life cycle where spawning occurs at sea and after few weeks postlarval prawns settle in estuarine waters, used as nursery grounds (Dall *et al.,* 1990). After four or five months, the sub-adults start their emigration offshore to develop the gametes and complete life cycle back in marine waters. Spawning can occur all year long in muddy soft bottoms, however highest abundances of ripe females have been observed during spring and autumn in depths varying from 40 to 60 meters (D'Incao, 1999).

The maximum sustained yield (MSY) estimated for the adult stock of *F. paulensis,* exploited by commercial double-rig fleet in offshore waters, decreased from 7165 (1965-1972) to 1963 tons (1987-1985). Mean relative abundance values decreased from 12.42 kg/h (1965-1972) to 3.15 kg/h (1987-1995), when this fishery finally collapsed due to overfishing. This scenario led the double-rig fleet to search for different demersal resources as an alternative to maintain economic yield of this activity (D'Incao *et al.,* 2002).

The pink prawn is also exploited inside estuaries and shallow bays along the coast, where post-larvae enter to spend the growout phase. The most important nursery ground of this species is the Estuary of Lagoa dos Patos, where a maximum landing of 8000 ton has been recorded. Cap-

ture of juveniles inside the estuaries is performed by artisanal fishermen, by using stationery traps with light attraction. One of the most important characteristics of this resource is the interannual oscillation in recruitment pattern, mainly caused by oceanographic conditions and pluviosity rates (Valentini, *et al.* 1991; D'Incao *et al.,* 2002).

Biological and aquaculture issues were previously investigated (Worsmann *et al.,* 1971; Worsmann and Sesso, 1977; Marchiori and Boff, 1983; Valentini *et al.,* 1991; D'Incao *et al.,* 2002; Peixoto *et al.,* 2003); however a detailed analysis of ovary development in the wild is fundamental to understand the reproductive dynamics of this species. The aim of this investigation is to apply histological techniques and gonadsomatic index (GSI) to validate macroscopic classification of ovaries, in attempt to provide a practical color standard to define maturity levels and biological information to properly manage this overexploited stock.

Material and Methods

Sampling was performed during autumn and late spring to assure that females participating on the most important breeding events were captured. Fishing gear used was an otter trawl, and investigation cruises were conducted by IBAMA onboard of the N/P Soloncy Moura (Instituto Brasileiro do Meio Ambiente e dos Recursos Hídricos Renováveis, IBAMA – CEPSUL). A total of 76 trawl fishing operations, with duration of 30 minutes each, was performed in depths ranging from 10 to 100 meters (Figure 1). Each station was sampled during daylight and night since spawning occurs mainly during dusk and night.

Total length (TL) was measured from the tip of rostrum to the end of telson. Total weight (TW) and gonadal weight (GW) were recorded to the nearest 0.001 g. At least 15 ovaries, from each development stage, were dissected and weighted to estimate gonadsomatic index (GSI%). Equation used to estimate gonadsomatic index was GSI = (GW/TW)*100. A sample of ovarian tissue was collected from anterior part of gonad for histological sectioning since homogeneous development along the ovary has been reported for this species (Peixoto *et al.,* 2003). Shape and color (Pantone, 1999) of ovaries were digitally recorded under the approximated quantity of light, to establish a macroscopic classification validated by subsequent histological sections. To define the ovary color accurately, an automated tool was used to match the predominant ovary color to Pantone's reference table (Figure 2). This tool samples a square of 12 x 12 pixels and provides the mean reference color obtained within this area.

Tissue was fixed in Davidson's, paraffin embedded, sectioned (6μm) and Hematoxilin-Eosin stained. At least 30 oocytes diameters per female were measured and a One-Way ANOVA and Tukey test was applied to verify significant differences ($p < 0.05$) among mean oocyte size pooled by development stage.

Results

Considerable differences in color and shape of the ovaries were observed for *F. paulensis* in the wild. According to histological sections and macroscopic analysis, four gonadal stages were recorded (Figure 3). The suggested stages are as follows: Stage I (immature): Ovary is white trans-

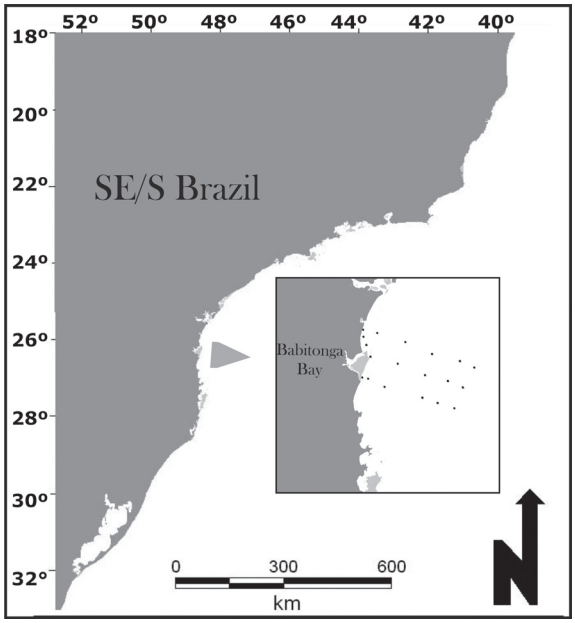

Figure 1. Southeastern and south Brazil coast, where fishery targeting pink prawn *(F. paulensis)* is conducted, highlighting the sampling area of Babitonga Bay. Black dots indicate sampling stations, in depths varying from 10-100 meters.

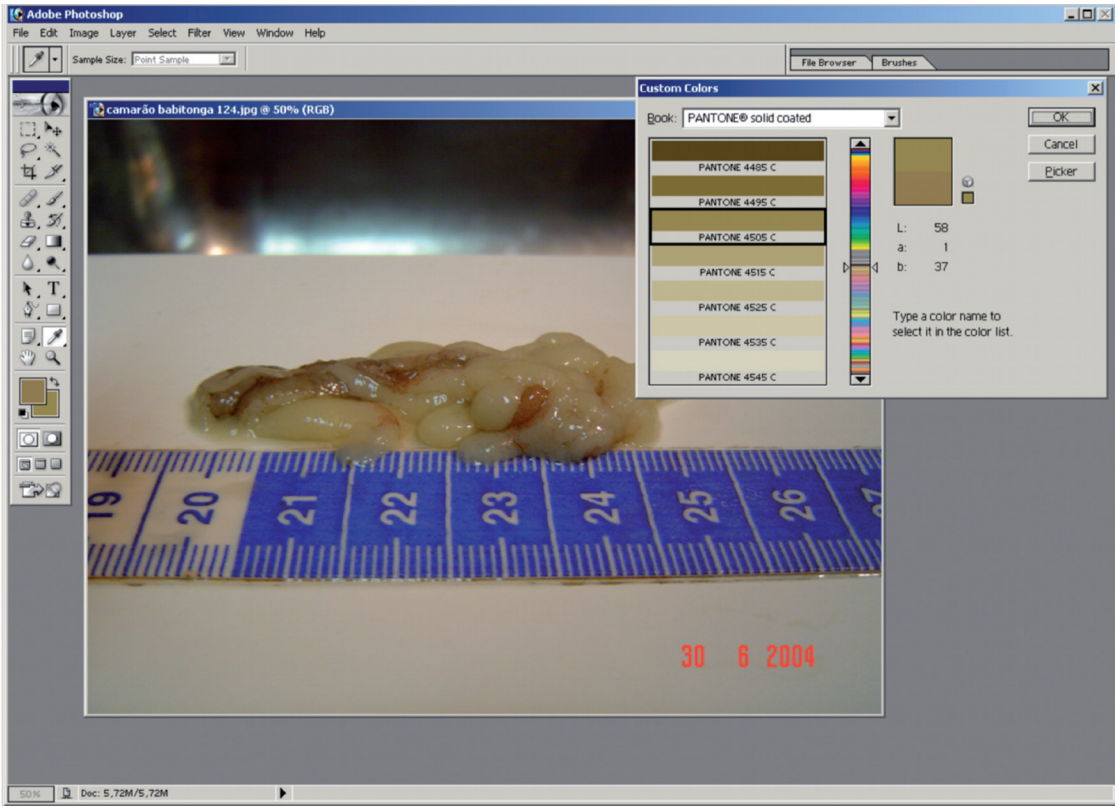

Figure 2. Screen capture of the automated tool used to recognize the ovary color. An area of 12x12 pixels was sampled and automatically compared to digital Pantone's reference color table.

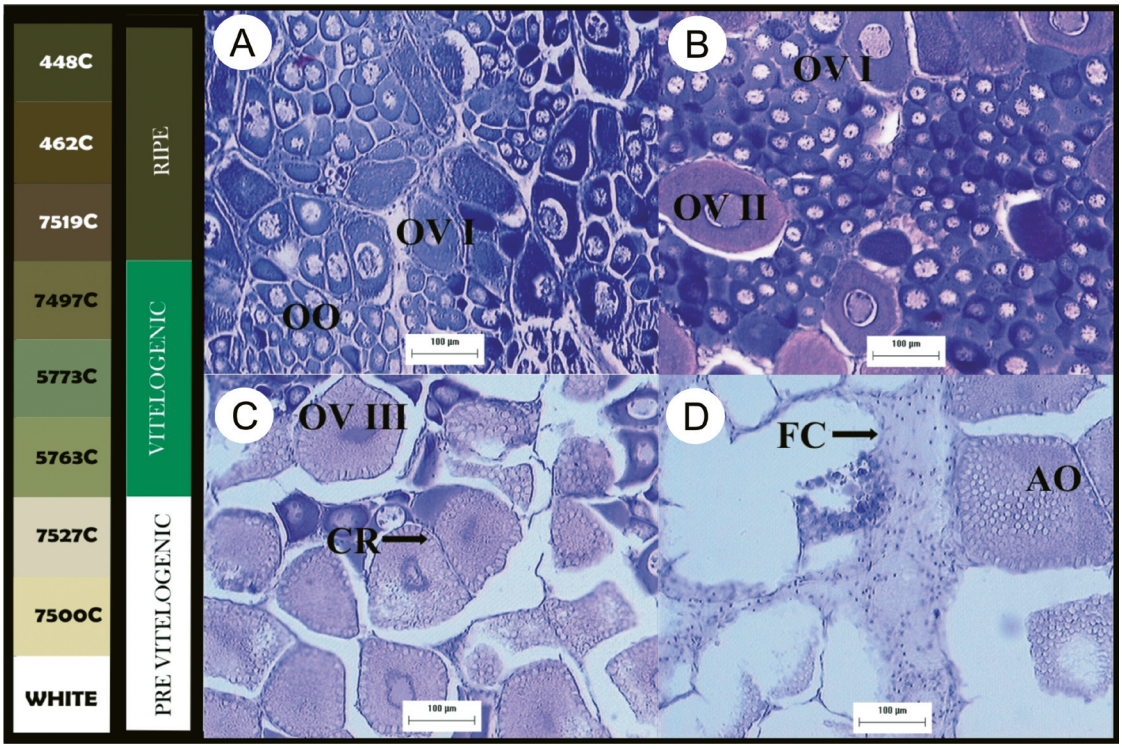

Figure 3. Histological sections (100x) and representative color of each ovarian maturation stage for *Farfantepenaeus paulensis*. (A) Stage I (immature): small basophilic oocytes (OVI) color ranged from white-translucent to light gray (7527C); (B) stage II (developing): acidophilic oocytes with yolk granules in the cytoplasm (OVII), light to neutral green color (5763C-7479C); (C) stage III (ripe): acidophilic oocytes (OVIII) with cortical rods (CR), dark green to pale black color (7519C-448C); (D) stage IV (spent): atretic oocytes (AO) with collapsed ovarian follicles cells (FC) I. Scale bars:100 µm.

lucent to light gray (white-7527C) and can not be observed through the carapace. Cephalotoracic portion of the ovary is reduced to posterodorsal part of stomach and do not present developed lobes. Abdominal region of the gonad is reduced and usually do not extend further than third abdominal somite. Only previtelogenic oocytes (OVI) and oogonies (OO) were observed during this stage. These cells are small (87.39 mm ± 17.66) and basophilic, suggesting the absence of yolk production. Stage II (developing): Ovary increases in size, occupying most of abdominal cavity, presenting two longitudinal and parallel lobes. In cephalic region, the ovary covers part of stomach and presents approximately 4 lobes. The ovary can be observed through carapace and color ranges from light to neutral green (5763C-7479C). During this stage developing oocytes (OVII) increase in size (138.89 mm ± 17.23), vitelogenesis starts (yolk granules fill cytoplasm) and oocytes are now eosin stained in histological sections. Stage III (ripe): Ovary entirely occupies abdominal cavity and presents colors ranging from dark green to pale black (7519C-448C). Cephalotoracic portion of ovary covers the entire stomach and presents several developed lobes. Microscopically, ripe cells (OVIII) are larger (195.11 mm ± 21.15) and present cortical rods (CR), a structural modification that indicates final maturation of oocytes in most of penaeid species. These structures are important during egg activation process, and help to avoid polyspermy and to create a microenvironment suitable for egg development (Clark *et al.,* 1980). Stage IV (spent): The spent stage is usually similar to immature, even though is very difficult to distinguish macroscopically from the other stages. However, spent ovary can be identified microscopically by the presence of reabsorbing cells or atretic oocytes (AO). During this stage, some ripe and developing oocytes remain in ovarian follicle indicating that partial spawning may occur for this species in the wild (Figure 3).

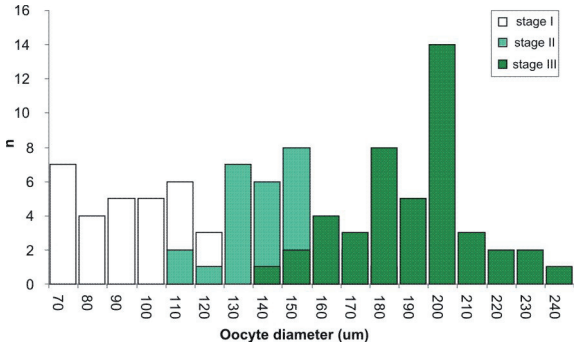

Figure 4. Relative frequency of size distribution of oocytes (µm) pooled by development stage obtained from histological sections of *F. paulensis* ovaries.

Size overlapping of oocytes was recorded between different developing stages (Figure 4), however significant differences ($p < 0.005$) related to mean oocyte diameter were observed (Table I). Visual analysis of size frequency of oocytes showed a polymodal pattern, with two peaks for immature oocytes and three for developing and ripe (Figure 4). Mean gonadsomatic index (GSI) varied from 0.476 (immature) to 4.73 (ripe) and significant differences in this parameter were observed among different stages ($p < 0.005$) (Figure 5, Table II).

Table I. Statistic summary of oocyte size analysis, containing number of oocytes measured (n) for each development stage, mean values (X), confidence intervals (CI ± 95%), range (minimum and maximum) and standard deviations of means (std. deviation).

n	X	CI (± 95%)		minimum	maximum	std. deviation
30	87.39	80.80	93.99	61.30	115.66	17.66
30	138.89	132.46	145.32	105.69	172.65	17.23
30	195.11	187.21	203.00	144.73	234.14	21.15

Table II. Statistic summary of GSI analysis, pooled by development stage, obtained from *F. paulensis*. TL = total length, TW = total weight, GW = gonadal weight, GSI = gonadsomatic index.

	Stage I			Stage II			Stage III		
	mean	CI (± 95%)		mean	CI (± 95%)		mean	CI (± 95%)	
TL (mm)	153.32	117.49	189.22	191.05	172.27	209.83	195.78	180.32	211.24
TW (g)	33.33	12.17	54.49	57.73	42.52	76.51	57.79	40.16	75.39
GW (g)	0.121	0.07	0.17	1.69	0.864	2.52	2.86	0.856	4.87
GSI (%)	0.476	0.33	0.63	2.69	1.539	3.84	4.74	2.18	7.29

Discussion

Macroscopic classification based on color and shape of ovaries, as well as the GSI showed a close relationship with cell development and ovary structure, obtained from histological sections. However, the spent ovaries presented a more problematic macroscopic classification due to a great visual variation among samples. Spent ovaries showed different levels of recovering and it was reflected macroscopically, making visual detection of spawning activity difficult for this species in the wild. However, color, shape and GSI can be used to determine reproductive stages of the ovary during routine practices in laboratory to a certain extent. These visual traits have been widely used to identify ovary development, whether to fishery management or aquaculture purposes (Vogt, *et al.,* 1989; Quinitio and Millamena 1992; Castille and Lawrence, 1991; Medina, *et al.,* 1996; Palacios, *et al.,*1999; Peixoto *et al.,* 2003).

Many previous investigations suggested at least five development stages for penaeid prawns (Vogt, *et al.,* 1989; Quinitio and Millamena 1992; Castille and Lawrence, 1991; Medina, *et al.,* 1996; Palácios, *et al.,*1999), including *F. paulensis* (D'Incao, 1999; Marchiori and Boff, 1983). Usually these stages are named as immature (I), initial developing (II), incipient maturity (III), mature (IV) and spent (V) and determined through macroscopic features of the ovaries. However, most of the authors have not found significant histological differences between stage I and II related to cell diameter and only three different oocytes types were observed (Worsmann *et al.,* 1971; Worsmann and Sesso, 1977; Quintero and Garcia, 1998; Peixoto *et al.,* 2003). Thus, the present investigation suggests the adoption of four development stages to be used for *F. paulensis* ovary identification, since no histological differences were observed that could justify the use of more than four stages. Moreover, both controversial phases (I and II) represent a rest ovary, provided that no vitelogenesis occurs during this phase and no reproduction can be attained.

Macroscopic classification of ovaries showed great variability related to color and shape, which was not always reflected in histological traits. Thus, our suggestion is to keep the four stages observed in histological sections (Peixoto *et al.,* 2003) and provide a more accurate color classification, by adopting many reference colors for each stage instead of a single one. This investigation recorded at least three different colors for the same histological feature, and most frequent colors within each stage were adopted to describe the maturation level.

Previous investigations concerning *F. paulensis* ovary development have reported difficulties in distinguishing stages I (immature) and IV (spent) for captive stocks, especially when using spawning induction techniques (e.g. ablation, temperature) (Marchiori and Boff, 1983; Peixoto, *et al.,* 2003). During present investigation, same difficulties were observed, however the classification difficulties were extended to the other stages. Prawns caught in the wild showed different ovary recovery levels after spawning and atretic oocytes could be found in individuals with different macroscopic traits, from stage I to III. One possible explanation is that ovary development in the wild (unablated and under lower temperatures) seems to be slower than in captivity and spawning process more gradual. Previous investigations on *F. paulensis* recorded different spawning quality and oocyte traits when ablated and non ablated prawns were compared (Peixoto *et al.,* 2005). According to Peixoto *et al.* (2002), ablated *F. paulensis* attained final maturation much faster than unablated individuals did. Advanced maturation of ablated and temperature induced individuals for this species was observed just few hours after spawning, confirming that ovary development is highly affected by traditional induction methods applied in aquaculture (Peixoto *et al.,* 2005).

Polymodal distribution of oocytes size, as well as ripe cells remaining in ovary after spawning, provided another evidence to conclude that the species performs partial spawning as a reproductive strategy in the wild. Modal progression analysis of oocytes size frequency suggests that eggs are gradually released, since it is possible to follow at least two "cohorts" of oocytes along the entire ovary development process. Laboratory reared *F. paulensis* presented different spawning pattern, with complete release of oocytes after being induced by ablation and temperature usually above those found in the ocean (Peixoto *et al.,*

2005). The hypotesis suggested is that *F. paulensis* may perform partial spawning in the wild, releasing packs of eggs during environment favorable periods, as observed for other penaeids (Christiansen and Scelzo, 1971; Dumont and D'Incao, 2004).

The diameter of oocytes was similar to previous investigations concerned on penaeid prawns, including overlapping sizes of stages II (developing) and III (ripe) (Guitart and Quintana, 1978; Ramos and Torras, 1986; Peixoto *et al.,* 2003). However, consistently lower size values were recorded for this species in the wild when compared to reared individuals (Peixoto *et al.,* 2003), except by measures obtained from stage I (immature). The GSI also showed lower values when compared to values from individuals subjected to spawning induction techniques (Peixoto *et al.,* 2003). Briefly, the ovary development and spawning strategy of *F. paulensis* seems to be strongly affected by captivity conditions, with slower development in the wild, evidenced by, partial release of eggs, smaller oocytes and lower GSI. Using an accurate visual classification, established based on histological sections and GSI, was possible to define a practical scale to classify maturity of females, as well as provide base information on spawning for this species in the wild.

References

Castille, F. L. and Lawrence, A. L. 1991. Reproductive studies concerning natural shrimp populations: a description of changes in size and biochemical compositions of the gonads and digestive glands in penaeid shrimps, Pp. 17-32 *In* de Loach, P. F.; Dougherty, W. J. and Davidson, M. A. (eds.). Frontiers of shrimp research. Elsevier, Amsterdam.

Christiansen, H. E. and Scelzo, M. A. 1971. Ciclo de maduración sexual y observaciones sobre la morfologia del aparato genital del camarón *Artemesia longinaris* Bate. Carpas, 16(5/D): 1-22.

Clark Jr., W.H., Lynn, J.W., Persyo, H.O., 1980. Morphology of the cortical reaction in the eggs of *Penaeus aztecus.* Biological Bulletin, 158, 175– 186.

D'Incao, F., Valentini, H. and Rodrigues, L. F. 2002. Avaliação da pesca de camarões nas regiões Sudeste e Sul do Brasil. 1965-1999. Atlântica, 24(2): 49-62.

D'Incao, F. 1999. Subordem Dendrobranchiata (camarões marinhos). Pp. 271-299 *In* Buckup, L. and Bond-Buckup, G. (eds.). Os camarões do Rio Grande do Sul. Editora da Universidade UFRGS, Porto Alegre.

Dall, W.; B. J., Hill, P. C., Rothlisberg, and D. J., Staples, 1990. The biology of the penaeidae. Advances in Marine Biology. Academic Press, 489p.

Dumont, L. F. C. and D'Incao, F. 2004. Estágios de desenvolvimento gonadal de fêmeas do camarão-barba-ruça (*Artemesia longinaris* – Decapoda: Penaeidae). Iheringia, 94(Suppl. 4): 389-393.

Guitart, B. and Quintana, M. 1978. Estadios de maduración gonadal en las especies importantes del género Penaeus em el Banco del Campeche. Revista Cubana de Investigaciones Pesqueras, 3: 82-126.

Marchiori, M. A. and Boff, M. H. 1983. Induced maturation, spawning and larvae culture of the pink shrimp *Penaeus paulensis* Pérez-Farfante, 1976. Memorias Asociacion Latinoamericana Acuicultura, 5: 331-337.

Medina, A.; Vila, Y.; Mourente, G. and Rodríguez, A. 1996. A comparative study of the ovarian development in wild and pond-reared shrimp, *Penaeus kerathurus* (Förskal, 1775). Aquaculture, 148: 63-75.

Palacios, E.; Rodriguez-Jaramillo, C. and Racota, I. S. 1999. Comparison of ovary histology between different-sized wild and pond-reared shrimp *Litopenaeus vannamei (= Penaeus vannamei).* Invertebrate reproduction and development, 35(3): 251-259.

Pantone. 1999. Pantone Professional Color System. 14th ed. New Jersey, Carlstad. 260p.

Peixoto, S.; Cavalli, R. O.; D'Incao, F.; Wasielesky, W. and Milach, Â. 2002. A comparative study of the ovarian histology of eyestalk ablated and unablated *Farfantepenaeus paulensis* after spawning. Boletim do Instituto de Pesca, 28: 71-76.

Peixoto, S., Cavalli, O. R., D'Incao, F., Milach, A. M. and Wasielesky, W. 2003. Ovarian maturation of wild *Farfantepenaeus paulensis* in relation to histological and visual changes. Aquaculture Research, 34: 1255-1260.

Peixoto, S., Cavalli, R. O and Wasielesky, W. 2005. Recent developments on broodstock maturation and reproduction of *Farfantepenaeus paulensis.* Brazilian Archives of Biology and Technology, 48(6): 997-1006.

Quintero M.E.S. and Garcia A. 1998. Stages of gonadal development in the spotted pink shrimp *Penaeus brasiliensis.* Journal of Crustacean Biology, 18:680-685.

Quinitio, E. T. and Millamena, O. M. 1992. Ovarian changes and female-specific protein levels during sexual maturation of the white shrimp *Penaeus indicus.* The Israeli Journal of Aquaculture, 44(1): 7-12.

Ramos, L. and Torras, E. 1986. Histologia del ovário maduro del camarón rosado *Penaeus notialis* com ablacion del pedúnculos oculares. Revista de Investigaciones Marinas, 7: 53-61.

Valentini, H.; D'Incao, F.; Rodrigues, L. F.; Rebelo-Neto, J. E. and Rahn, E. 1991. Análise da pesca do camarão-rosa *(Penaeus paulensis e Penaeus brasiliensis)* nas regiões e sul do Brasil. Atlântica, 13(1): 143-157.

Vogt, G.; Quinitio, E. T. and Pascual, F. P. 1989. Interaction of the midgut gland and the ovary in vitellogenesis and consequences for the breeding success: a comparison of unablated and ablated spawners of *Penaeus monodon*. Pp. 581-592 *In* de Pauw, J. N.; Ackefors, H.; Wilkins, N. E. (eds.). Aquaculture: A biotechnology in progress. European Aquaculture Society, Bredene.

Worsmann T. U. and Sesso, A. 1977. Observations ultrastructurales sur la maturation des cellules germinales de l'ovaire de *Penaeus paulensis*. Archives D'Anatomie D'Histologie D'Embryologie Normales et Experimentales, 60: 175-188.

Worsmann T. U., Neiva G. S., Oliveira M. T. and Valentini H. 1971. Contribuição ao estudo da maturação da gônada feminina do 'camarão-rosa' (*Penaeus paulensis* Pérez-Farfante,1967). Boletim do Instituto de Pesca, 1: 23-28.

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