

# Ovarian development and length at first maturity of the sea-bob-shrimp *Xiphopenaeus kroyeri* (Heller) based on histological analysis

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## Abstract

The sea-bob-shrimp *Xiphopenaeus kroyeri* (Heller, 1862) is a penaeid prawn widely distributed throughout the Western Atlantic, occurring from North Carolina (USA) to Rio Grande do Sul, Brazil. The present work was motivated by the need of a better knowledge on the biology and life cycle of sea-bob-shrimp in Tijucas Bay, Tijucas, Santa Catarina State. Population parameters, such as reproduction were estimated. Samples were obtained from trawl net artisanal fishery. Sexual maturity of females was established based on a chromatic scale, developed from ovary histological sections and the length at first maturity was estimated for females was 24 mm (CL).

Key words: *Xiphopenaeus kroyeri*, ovarian development, histology, length at first maturity, Southern Brazil.

## Introduction

*Xiphopenaeus kroyeri* (Heller, 1862) is a penaeid prawn widely exploited for human consumption in Southern and Southeastern Brazil (D’Incao *et al.*, 2002). The species has a wide distribution in Southwestern Atlantic, occurring from North Carolina (USA) to Rio Grande do Sul (Brazil), inhabiting shallow coastal waters (0-30 m) with muddy and sandy soft bottoms (D’Incao, 1999). As most of penaeid prawns, *X. kroyeri* presents fast growth (Gulland and Rotschild, 1981) and lifespan suggested is around 18 months (Neiva and Wise, 1963). Its life cycle does not demand an estuarine grow out phase, spending all life in marine waters (Iwai, 1973).

Double-rig prawn trawlers, operating in Brazil, used to have the pink prawn species (*Farfantepenaeus paulensis* and *F. brasiliensis*) as the main target. However, overexploitation of these resources resulted in a remarkable decrease of yields, which led to a demand for new prawn stocks. With this new scenario, the sea-bob-shrimp became one of the most valuable resources, suggesting that new investigations on its biology must be performed (Valentini *et al.*, 1991; D’Incao *et al.*, 2002).

Among population parameters, the knowledge on reproductive dynamics and ovary maturation can be considered between the most important to effectively manage an exploited population, since they can be used to determine minimum allowable size for catch (Vazzoler, 1996; King, 1997). However, the accurate determination of the ovarian maturation stages is not always easily attained, since estimates based only on macroscopic observations may result in misclassification of the actual development of the ovaries (Peixoto *et al.*, 2003; Dumont *et al.*, 2007). Therefore, the aim of this investigation is to estimate the length at first maturity for the sea-bob-shrimp in Southern Brazil based on histological sections, providing a practical classification scale to define ovary maturation for this species.

## Material and Methods

Sampling took place in Tijucas Bay, off Santa Catarina coast (27°14’46”S-48°36’47”W).

Biological samples were obtained on a monthly basis by using a typical artisanal boat (10 meters long and a central engine of 30 Hp’s) trawling at

approximately 2 knots. Carapace length (CL-mm) was taken from the posterior margin of cephalothorax to the tip of the rostrum. Total weight (TW) was taken to the nearest 0.001 g.

The *X. kroyeri* females ovaries were initially classified according to a standard Pantone's color catalog (Pantone, Inc. 1999) to establish a chromatic reference point (Peixoto *et al.*, 2003; Dumont and D'Incao, 2004; Dumont *et al.*, 2007).

Ten ovaries from each development stage were used to establish a chromatic scale to classify the ovaries of the sea-bob-shrimp, summing 40 females. Samples were fixed in formalin, in such a way that 10 volumes of fixative were used to preserve 1 volume of sample (Bell and Lightner, 1988). Tissue was paraffin embedded, hematoxylin-eosin stained and submitted to histological sections (0.6  $\mu\text{m}$ ) (Bell and Lightner, 1988). At least 30 oocytes were measured from each development stage, always considering the largest diameter of each oocyte. Oocytes sizes, at each stage, were grouped in 5  $\mu\text{m}$  intervals to perform size-frequency analysis. To test significant differences ( $p < 0.05$ ) in mean size of oocytes, at each stage, an ANOVA and *a posteriori* Tukey's test were performed (Zar, 1984; Peixoto *et al.*, 2003; Dumont and D'Incao, 2004; Dumont *et al.*, 2007).

Length at first maturity was estimated by fitting the frequency of mature females to a logistic model, described by the following equation:

$$y=1/(1+\exp^{-(r(CL-LM))}),$$

where  $r$  is the slope,  $CL$  is the carapace length and  $LM$  the size at first maturity. Additionally, a linear regression was fit to CL and TW data to show possible differences in relative growth in weight by development stage.

## Results

According to histological sections we defined four different ovary maturation stages, which are described as follows:

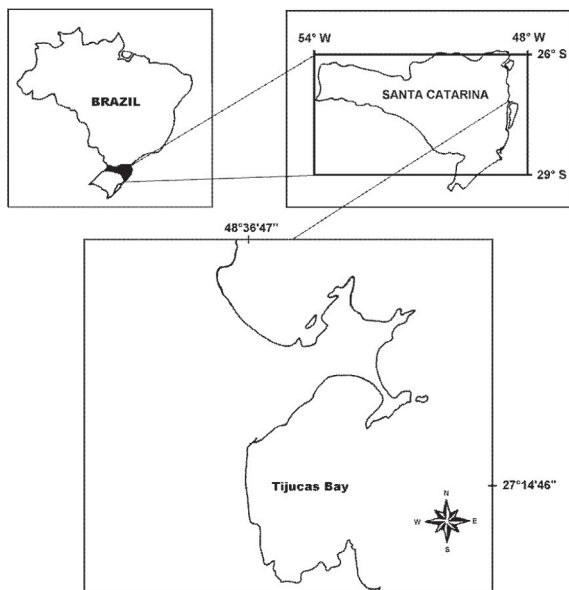
**Stage I (pre-vitelogenic or immature):** Most frequent cells observed are the previtelogenic oocytes and the oogonies, which form the germinative epithelium. These cells are basophilic (stained by hematoxylin) indicating the lack of yolk production during this stage. Macroscopically, the ovaries vary from white-translucent to light-grey (white/427C/7534), occupying a reduced portion of the abdomen cavity. Mean oocyte diameter is 72.03  $\mu\text{m}$  ( $\pm 2.42$ ) and size-frequency was polymodal (Fig. 2).

**Stage II (vitelogenic):** The cells are acidophilic and stained by eosin. Star of vitelogenesis is observed with small yolk granules in the cytoplasm. Mean cell diameter during this stage is 113.46  $\mu\text{m}$  ( $\pm 6.01$ ) (Tab. I), (Fig. 2). Macroscopically the ovaries are larger, occupying the entire abdominal cavity, as well as part of cephalothoracic portion and color ranges from light to olive green (5803C/5773C/5763C).

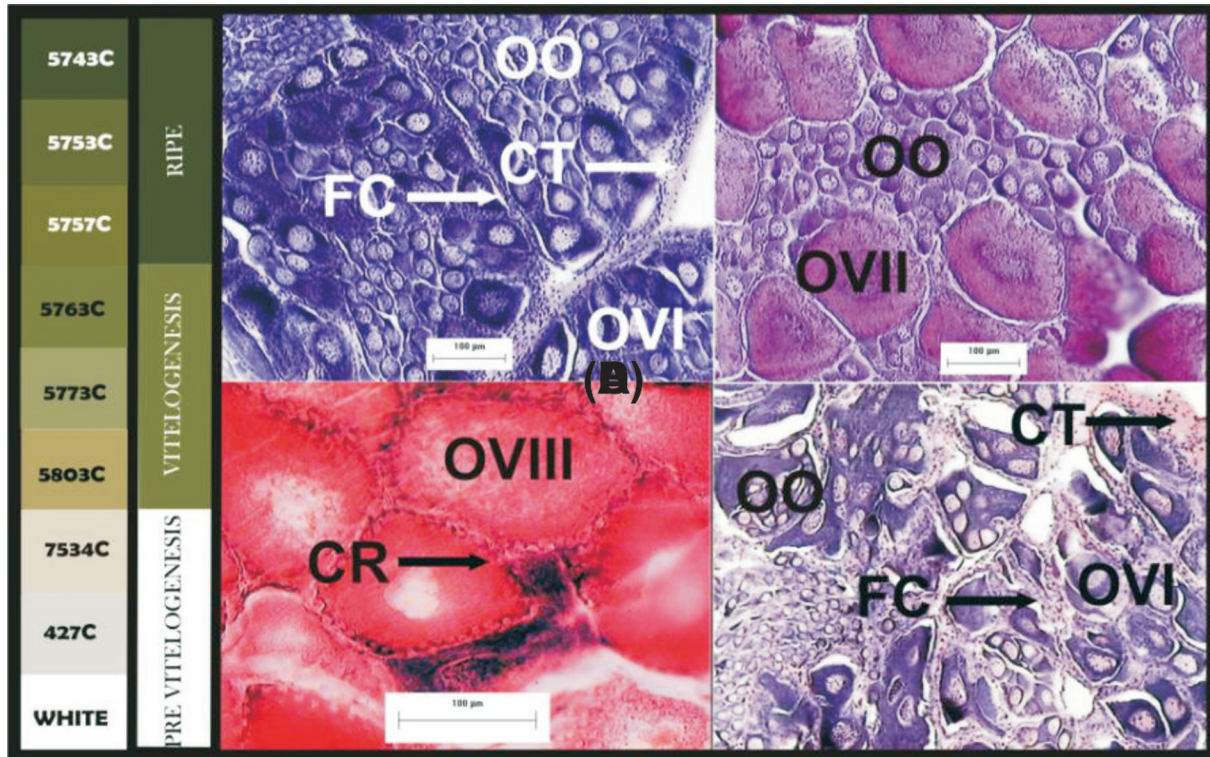
**Stage III (ripe):** An increase in mean cell diameter is also observed during this stage (171.30  $\mu\text{m}$   $\pm 6.01$ ) (Tab. I). The vitelogenesis is intense during this phase, with cytoplasm entirely covered by yolk granules. Round shape of oocytes is altered due to the important yolk concentration, presenting, in some cases, cortical rods in periphery of the cell (5757C/5753C/5743C).

**Table I.** Summary of mean oocyte diameter, containing number of measured cells (n), mean size of the cells, standard deviation (SD), standard error (SE) and confidence intervals of means (CI 95%).

Stage	n	Mean	SE	SD	CI 95%	
1	30	72.03	16.09	2.94	66.02	78.04
2	537	113.46	27.56	1.19	111.13	115.80
3	32	171.30	26.40	4.67	161.78	180.82
4	63	98.42	29.93	3.77	90.88	105.95



**Figure 1.** Study area, Tijuca Bay, Santa Catarina (27°14'46\"S, 48°36'47\"N).

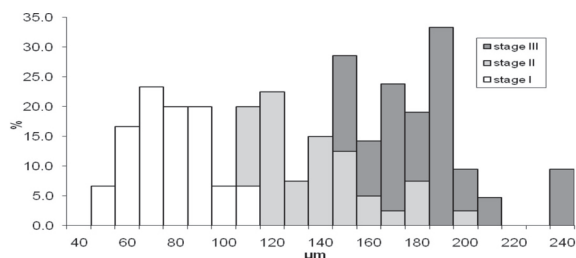


**Figure 2.** Histological sections obtained from *Xiphopenaeus kroyeri* ovaries. A- Stage I (previtelogenic): small basophilic oocytes (OVI) and oogonies (OO) are the dominant cells in the ovary. B- Stage II (vitelogenic): Oocytes are stained by eosin indicating the start of yolk production (OVII). C- Stage III (ripe): Oocytes attained final maturation (OVIII), presenting cortical rods (CR). D- Stage IV (spent): Some ovarian follicles are empty and organization of female's gonads is absent, remaining only immature cells.

Stage IV (spent): Ovaries loose the cell organization, presenting empty follicles due to release of ripe oocytes. The presence of atretic (reabsorbing) oocytes is noticed (Fig. 2).

Size distribution of cell diameter was polymodal for stages II and III, suggesting progressive maturation of oocytes and a gradual release of the eggs in water column (Fig. 3).

The length of first maturity estimated for females was 24 mm (CL). From the class interval of 33 mm onwards, all the females presented ripe ovaries. The coefficient of determination estimated for the adjustment ( $R^2$ ) was 0.99 (Fig. 4).



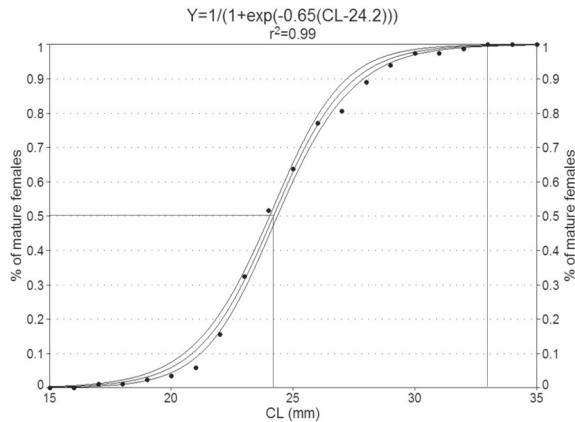
**Figure 3.** Size distribution of cell diameter according to maturation stage of *Xiphopenaeus kroyeri* females in Southern Brazil. The X axis shows the oocytes diameters, while the Y axis shows the percentage.

Slopes obtained from TW and CL linear relationships showed increasing values from immature ( $b=0.55$ ) to ripe ( $b=0.87$ ).

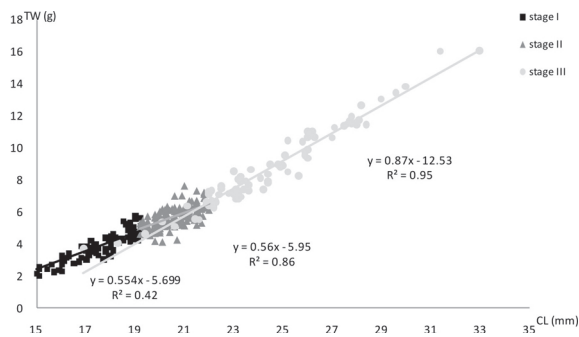
### Discussion

Histological analysis of ovaries showed a narrow relationship with macroscopic traits observed, providing a routine method to classify *X. kroyeri* ovaries in field and laboratory procedures (Peixoto *et al.*, 2003; Dumont and D’Incao, 2004; Dumont *et al.*, 2007).

Four maturation stages were suggested based on macroscopic and histological features. However, distinguishing stages I (immature) from stage IV (spent) macroscopically can be a daunting task. The same observation has been extensively reported in the literature for penaeid prawns (Peixoto *et al.*, 2003; Dumont and D’Incao, 2004; Dumont *et al.*, 2007), since observation of the ovaries during both phases is difficult in most cases. Conversely, the color attribution, based on a reference color table, provided an accurate method to differentiate vitelogenic and ripe ovarian development stages for this species.



**Figure 4.** Logistic curve of sexual maturity estimated for *Xiphopenaeus kroyeri* females in Tijucas Bay, SC. The solid lines represent the class interval where the probability of being mature is 50% and 100% (CL).



**Figure 5.** Linear regressions of TW and CL estimated for *Xiphopenaeus kroyeri* females in Santa Catarina State, Southern Brazil, pooled by ovary development stage.

Another important finding was the presence of cortical rods indicating final maturation for this species. This structure is formed just before fertilization and is important to create a suitable environment for egg development, as well as to prevent polyspermy to occur (Clark *et al.*, 1980). Previous investigation on ovary maturation of *X. kroyeri* did not report the presence of such structures, which may be an effect of the water temperature in which studied population inhabits (Clark *et al.* 1980).

Variation in size at first maturity (LM) of *X. kroyeri* has been reported for Brazilian coast. Population studied inhabits the southernmost limit of occurrence for this species. As a likely consequence of lower water temperature, slow ontogenetic growth may lead to delayed ovary maturation explaining larger LM estimated (Gulland and Rotschild, 1981). However, the use of a more accurate method to classify the ovary development

used in present investigation may also have influenced the estimations of LM.

The straight relationship between macroscopic and microscopic traits observed in *X. kroyeri* ovaries in present study, it will allow the implantation of a practical method to classify maturation of this species in southern Brazil.

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