

# Gonad development in females of fiddler crab *Uca rapax* (Crustacea, Brachyura, Ocypodidae) using macro and microscopic techniques

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**ABSTRACT.** The morphology of the ovaries in *Uca rapax* (Smith, 1870) was described based on macroscopic and microscopic analysis. Females were collected in Itamambuca mangrove, Ubatuba, state of São Paulo, Brazil. In the laboratory, 18 females had their ovaries removed and prepared for histology. Each gonad developmental stage was previously determined based on external and macroscopic morphology and afterwards each stage was microscopically described. The ovaries of *U. rapax* showed a pronounced macroscopic differentiation in size and coloration with the maturation of the gonad, with six ovarian developmental stages: immature, rudimentary, developing, developed, advanced and spent. During the vitellogenesis, the amount of oocytes in secondary stage increases in the ovary, resulting in a change in coloration of the gonad. Oogonias, primary oocytes, secondary oocytes and follicular cells were histologically described and measured. In female's ovaries of *U. rapax* the modifications observed in the oocytes during the process of gonad maturation are similar to descriptions of gonads of other females of brachyuran crustaceans. The similarities are specially found in the morphological changes in the reproductive cells, and also in the presence and arrange of follicle cells during the process of ovary maturation. When external morphological characteristics of the gonads were compared to histological descriptions, it was possible to observe modifications that characterize the process in different developmental stages throughout the ovarian cycle and, consequently, the macroscopic classification of gonad stages agree with the modifications of the reproductive cells.

**KEYWORDS.** Macroscopic analysis, histological analysis, oogonias, primary and secondary oocytes, *Uca rapax*.

**RESUMO.** Desenvolvimento da gônada em fêmeas do caranguejo-violinista *Uca rapax* (Crustacea, Brachyura, Ocypodidae) utilizando técnicas macro e microscópicas. A morfologia dos ovários de *Uca rapax* foi descrita baseada nas análises macroscópica e microscópica. As fêmeas foram coletadas no manguezal de Itamambuca, Ubatuba, Estado de São Paulo, Brasil. No laboratório, os ovários de 18 fêmeas foram retirados e preparados para histologia. Os estágios gonadais foram caracterizados previamente por meio de análises da morfologia externa e macroscopia e posteriormente cada estágio foi descrito microscopicamente. Os ovários das fêmeas de *U. rapax* apresentaram uma diferenciação macroscópica pronunciada no tamanho e coloração durante o processo de maturação, sendo caracterizados seis estágios de desenvolvimento ovariano: imaturo, rudimentar, em desenvolvimento, desenvolvido, avançado e esgotado. Microscopicamente, estes estágios diferiram quanto ao tamanho, cor e proporção de oogônias e oócitos presentes. Na análise histológica, oogônias, oócitos primários e secundários e células foliculares foram descritos e mensurados. As modificações observadas nos oócitos durante o processo de maturação dos ovários das fêmeas de *U. rapax* são similares às descrições de gônadas de outras fêmeas de braquiúros. As semelhanças foram especialmente observadas nas mudanças morfológicas das células reprodutivas e, também no arranjo das células foliculares durante o processo de maturação ovariana. Quando as características morfológicas externas das gônadas foram comparadas com as descrições histológicas, foi possível observar modificações que caracterizam o processo em diferentes estágios de desenvolvimento do ciclo ovariano e conseqüentemente, a classificação macroscópica das gônadas coincide com as modificações nas células reprodutivas.

**PALAVRAS-CHAVE.** Análise macroscópica, análise histológica, oogônias, oócitos primários e secundários, *Uca rapax*.

Reproductive cycles of a crustacean include a series of morphological and physiological events. In specimens completing the juvenile stages, these events include proliferation of gonial cells (activation of gametogenesis), differentiation and growth of gametes to maturation (gamete production), reproductive behavior associated with mating, release of gametes; ovulation, spawning, and incubation of embryos until hatching to release larvae or juveniles (GIESE & PEARSE, 1974; SASTRY, 1983). This cycle is basic to all sexually reproducing crustaceans, but the time relationship between certain events and duration of these events varies for different species (SASTRY, 1983).

The process of oogenesis is quite similar for most decapods, but the period and frequency of oogenic cycles vary greatly from a few days to 2 years for other

species. The reproductive traits as the rate of oocyte growth, the amplitude of ovarian growth, the frequency of oogenic cycles, the timing within the year and the number of oogenic cycles during one intermolt period appears to be specific. As reported by SASTRY (1983), all these aspects have important consequences to the reproductive ecology of species.

During the course of gonad maturation the ovaries undergo a sequence of macroscopic changes in its morphology (mainly in its relative size), which are easily detectable by a naked-eye (ADIYODI & SUBRAMONIAN, 1983; MEUSY & CHARNIAUX-COTTON, 1984; ARCULEO *et al.*, 1995). The change in colour is the result of modifications in carotenoid content occurring during the oogenesis, which play an important role during embryogenesis (GOODWIN, 1951).

The macroscopic characterization of the gonads among decapods has been commonly investigated by several authors. Most of the papers dealing with the determination of the onset of the sexual physiological maturity or histological maturity, taking into account the degree of gonad development (COSTA & NEGREIROS-FRANZOZO, 1998; PINHEIRO & FRANZOZO, 1998; LÓPEZ-GRECO & RODRÍGUEZ, 1999a; SANTOS & NEGREIROS-FRANZOZO, 1999; CASTIGLIONI & SANTOS, 2001; SWINEY & SHIRLEY, 2001; FLORES *et al.*, 2002; CASTIGLIONI & NEGREIROS-FRANZOZO, 2006 among others). However, studies of histological description of gonads are less common (CRONIN, 1947; ADIYODI & SUBRAMONIAN, 1983; MEUSY & CHARNIAUX-COTTON, 1984; WENNER *et al.*, 1987; KROL *et al.*, 1992; LÓPEZ *et al.*, 1997; ANDO & MAKIOKA, 1998), the majority being on Caridea and Penaeoidea (RODRÍGUEZ, 1985; TAN-FERMIN & PUDADERA, 1989; QUINITIO & MILLAMENA, 1992; MORAES, 1994; QUINTERO & GRACIA, 1998; OLIVEIRA *et al.*, 1999; PALACIOS *et al.*, 1999; SAKAJI *et al.*, 2000; SAKAJI, 2001; MOSSOLIN & BUENO, 2002 and DUMONT & D'INCAO, 2004).

The fiddler crab *Uca rapax* (Smith, 1870) is one of the most abundant species, living in burrows excavated in coarse wet sediment in mangrove habitats along the northern coast of the state of São Paulo, Brazil. It occurs from Florida south through the Gulf of Mexico, Antilles, and Venezuela to Brazil (from states of Pará to Santa Catarina) (MELO, 1996). Although many aspects of this species' behavior, physiology, growth and populational biology are known (SALMON, 1971; GREENSPAN, 1980; McNAMARA & MOREIRA, 1983; GENONI, 1985, 1991; SALMON & KETTLER, 1987; ZANDERS & ROJAS, 1996a,b,c and CASTIGLIONI & NEGREIROS-FRANZOZO, 2004a, b, 2005), scarce information is available on its reproduction (CASTIGLIONI & NEGREIROS-FRANZOZO, 2006; CASTIGLIONI *et al.*, 2006). This paper was accomplished in order to match data from macroscopic and microscopic analysis of females' gonad development of *U. rapax* in order to check the validity of the macroscopic technique.

## MATERIAL AND METHODS

Fiddler crabs were obtained in April 2002 at Itamambuca mangrove, Cavallo river (23°24'S and 45°00'W), Ubatuba, state of São Paulo, Brazil. For sampling we used the procedure of capture per unit effort (cpue), by two collectors during 15 min. at low tide periods. In the studies performed by CASTIGLIONI & NEGREIROS-FRANZOZO (2004a) and CASTIGLIONI & NEGREIROS-FRANZOZO (2006), it was observed that the females of *U. rapax* attained the morphologic and physiologic sexual maturity with 12.1 mm of carapace width (CW) (size in which 50% of females can be considered sexually mature).

Collected crabs were sorted and packed in labeled plastic bags and were maintained during 30 min. in a freezer to be anesthetized. Afterwards, the gonad developmental stages in females were analyzed removing the carapace of the specimens on the dorsal region. The shape, size and coloration were analyzed and classified in 6 stages of development (macroscopic maturity criterion): immature, rudimentary, developing, developed, advanced and spent, following a modification of the

procedures proposed by HAEFNER (1976), CHOY (1988), ABELLÓ (1989) and NEGREIROS-FRANZOZO *et al.* (2002).

A total of 18 females (3 at each stage of gonad development) larger than 12.1 mm of CW were processed for microscopic characterization of the ovaries. Each female was sacrificed and the gonads were quickly dissected and fixed in Bouin's solution for 24 hours at 20°C. Afterwards, the gonads were dehydrated through an alcohol series (80%, 90% and 95% - one hour in each). Gonads were placed and kept for two hours in a pre-infiltration solution and transferred to an infiltration solution for three to four hours or overnight. In the following day, blocks were made using a blocking solution and an accelerator. Finally, the gonads were embedded in resin, the sections were prepared using a microtome (3 to 4 µm), stained using haematoxylin-eosin (3 min.), and mounted in slides using Entelan.

Cell types found in the ovary of *U. rapax* were described, and for each macroscopical stage histological descriptions were also accomplished. Diameter of germinative cells (oogonias and oocytes) show in the ovaries was measured (largest and smallest diameter) and a mean of the two diameters obtained for each cell was calculated. They were measured by means of a micrometric ocular lens, calibrated against a Leitz Wetzlar plate with 10 µm spacing.

## RESULTS

Female's reproductive system in *U. rapax* follows the anatomical pattern observed in Brachyura. They have pair ovaries (dorsum-lateral to the digestive system) and connected in the middle, showing an "H" shape.

Different cellular types were observed during the ovary development of *U. rapax*, as follows:

**Oogonia:** these cells are characterized by the presence of young germinative cells that are grouped in the center of the ovary. This type of cell shows a disperse chromatin attached to the nuclear membrane and a basophilic cytoplasm (Fig. 1). Oogonia diameter ranged from 10.0 to 22.5 µm with a mean ( $\pm$  standard deviation) of  $16.1 \pm 3.0$  µm.

**Primary oocytes (pre-vitellogenics):** these cells are found generally close to the oogonia. Early primary oocytes have a basophilic homogeneous cytoplasm without follicle cells. Late primary oocytes have a heterogeneous cytoplasm that presents small vacuoles. The cytoplasm of late primary oocytes is less basophilic than the cytoplasm of early primary oocytes. Late oocytes are surrounded by follicle cells that are round in shape (Fig. 1 and Fig. 2). Pre-vitellogenic oocytes have a well defined nucleus with prominent nucleoli on the border, and a dispersed chromatin attached to the nuclear membrane. The size of the primary oocytes ranged from 40 to 80 µm ( $59.1 \pm 9.1$  µm).

**Secondary oocytes (vitellogenics):** these cells are acidophilic, showing high content of yolk and lipid drops. In these cells, nucleus:cytoplasm ratio is very low. All vitellogenic oocytes are surrounded by flat follicle cells that allowed the observation of the complete process of follicle formation (Fig. 2). The vitellogenic oocytes showed a size range from 160 to 280 µm in diameter ( $224.8 \pm 35.3$  µm) (Fig. 3).

The macroscopic and microscopic evaluation of female gonads of *U. rapax* enabled the ovaries to be grouped into five distinct developmental stages, characterized as follows:

**Rudimentary:** undeveloped, thin, and transparent gonads with a filamentous aspect. In this stage oogonias were numerous and surrounded by follicle cells and the primary and secondary oocytes cannot be observed and the animal is physiologically classified as immature.

**Developing:** ovary coloration yellow to orange-reddish. Gonad: hepatopancreas ratio is about 1:10. Oogonias and pre-vitellogenic oocytes could be observed at this phase surrounded by follicle cells. In

this stage, a small number of vitellogenic oocytes could be observed. Oogonias are grouped and oocytes are dispersed in the ovary (Fig. 3).

**Developed:** ovary with a lobate shape and dark red coloration. Gonad: hepatopancreas ratio is about 1:2. Oogonia and pre-vitellogenic oocytes could be observed in the histological analysis. The oocytes showed a homogenous and basophilic cytoplasm, chromatin located on the borders of the cell, and only one nucleolus in the first stages of primary vitellogenesis. These pre-vitellogenic oocytes are starting to be surrounded by follicle cells. In well developed pre-vitellogenic oocytes, the cytoplasm is less basophilic with lipid drops. In these

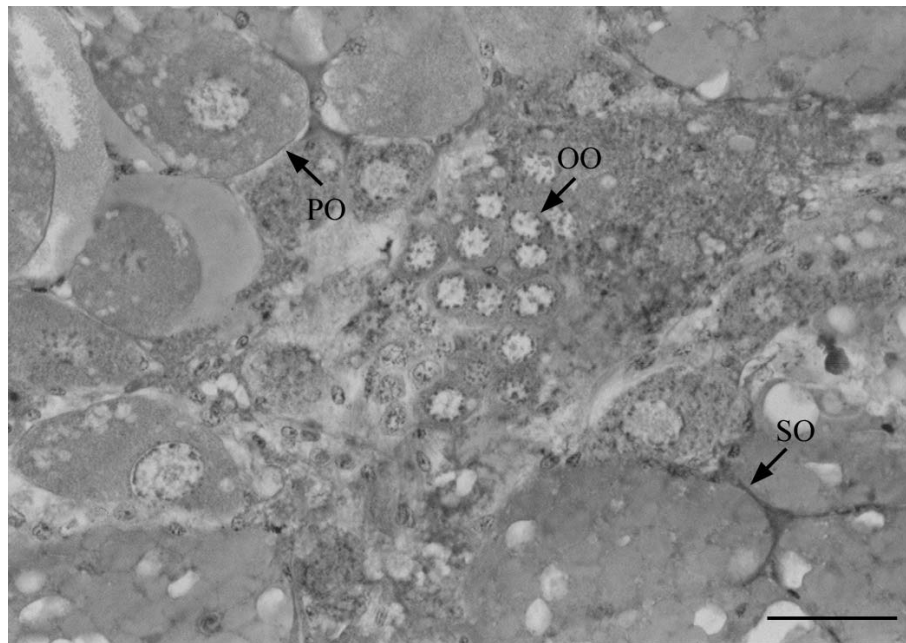


Fig. 1. Developing gonads of *Uca rapax* (Smith, 1870) showing the oogonias (OO), primary oocytes (PO) and secondary oocytes (SO). Bar, 0.04 mm.

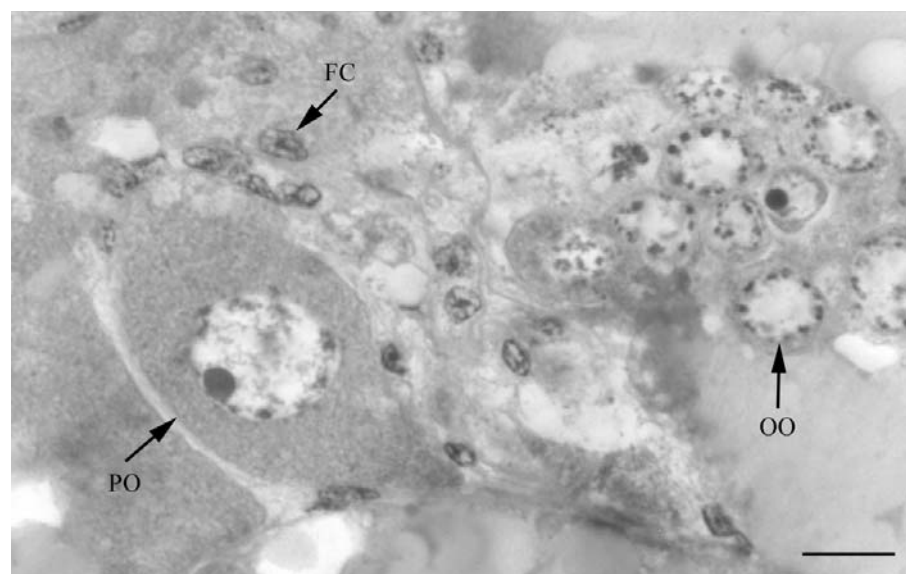


Fig. 2. Developing gonads of *Uca rapax* (Smith, 1870) showing the oogonias (OO), primary oocytes (PO) and some follicular cells (FC). Bar, 0.07 mm.

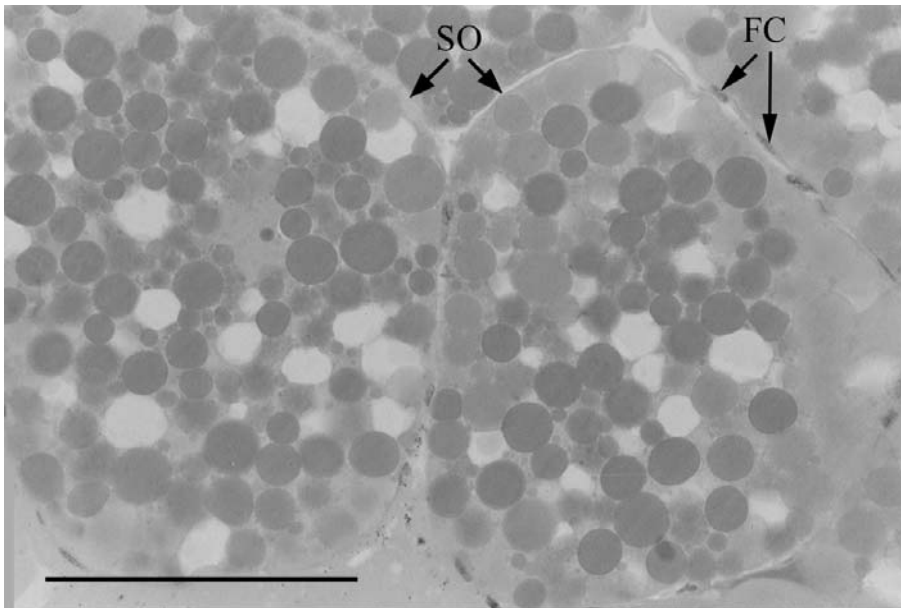


Fig. 3. *Uca rapax* (Smith, 1870): secondary oocytes (SO) surrounded by follicular cells (FC). Bar, 0.1 mm.

oocytes, follicle cells with a round or oval shape start to surround the cells. Vitellogenic oocytes are abundant and present acidophilic cytoplasm, yolk, and lipid drops. Follicle cells surround the secondary oocytes forming a sheet of flat cells.

Advanced: ovary occupies the whole thoracic cavity, shows a lobate shape more pronounced than the observed in the previous stage, and a very dark red coloration. Gonad:hepatopancreas ratio is about 9:10. The gonad reaches its higher level of development, and its largest volume. This gonad stage is characterized by the abundance of mature secondary oocytes, and by the high number of vacuoles in the yolk. Oogonias and pre-vitellogenic oocytes are rare. Vitellogenic oocytes are surrounded by flat follicle cells.

Spent: thin, flaccid, filamentous, transparent ovary. Gonad: hepatopancreas ratio is about 1:10. Oogonias and few primary oocytes are present.

It was not possible to describe microscopically the immature gonad due to its reduced size, which hindered the dissection and processing.

## DISCUSSION

Ovaries color change during vitellogenesis is a common characteristic in crustaceans and has been recorded for *Macrophthalmus hirtipes* (Jacquinot, 1853), *Crangon crangon* Linnaeus, *Callinectes danae* Smith, 1869, *Portunus spinimanus* Latreille, and *Cyrtograpsus angulatus* Dana, 1851 by SIMONS & JONES (1981), HAEFNER & SPAARGAREN (1983), COSTA & NEGREIROS-FRANZOZO (1998), SANTOS & NEGREIROS-FRANZOZO (1999), and CASTIGLIONI & SANTOS (2001), respectively. This variation in coloration has been used to elaborate macroscopic scales in the ovarian development (ARCULEO *et al.*, 1995; PINHEIRO & FRANZOZO, 1998; LÓPEZ-GRECO & RODRÍGUEZ, 1999b; FLORES *et al.*, 2002). These scales allow a quick evaluation of the reproductive stage in a population in

time and space, as well as the study of ovarian cycles in a species or population without performing histological analyses (ARCULEO *et al.*, 1995; LÓPEZ-GRECO & RODRÍGUEZ, 1999b).

The ovaries of *U. rapax* showed a pronounced macroscopic differentiation in size and coloration during the maturation process of the gonad. During the vitellogenesis, the amount of oocytes in secondary stage increases in the ovary, resulting in a change in coloration of the gonad. According to CHARNIAUX-COTTON (1980), ovary coloration is a result of the storage of vitellogenine presenting carotenoid pigments. According to BROWDY *et al.* (1990), the yolk is composed of water, proteins, and lipids, being an essential nutrient for the embryo development, a matrix for tissue formation, and a fuel for the new animal.

Three cellular stages of the gonad development of *U. rapax* were characterized in the present study: oogonias, pre-vitellogenic, and vitellogenic oocytes. The description of cellular stages agrees with the characteristics described by ADIYODI & SUBRAMONIAN (1983) and MEUSY & CHARNIAUX-COTTON (1984). These characteristics were posteriorly identified in other species of decapods (LÓPEZ *et al.*, 1997, ANDO & MAKIOKA, 1998; ELORZA & DUPRÉ, 2000).

According to KROL *et al.* (1992), the ovary germinative epithelium is located on the border or in the middle of the ovaries in decapod crustaceans. The oogonias and pre-vitellogenic oocytes are found in the middle region of the ovarian lobes, while the vitellogenic oocytes can be observed in the periphery. KING (1948) observed that the rapid multiplication of the central germinative cells pushes the first oocytes to the periphery of the ovarian lobes, which explains the occurrence of more developed oocytes in this region. This cellular arrangement was observed for *U. rapax* in the present work and also for *Gecarcinus lateralis* (Fréminville, 1835) (WEITZMAN, 1966), *Pachygrapsus marmoratus* (Fabricius,

1787) (ROUQUETTE, 1970), *Carcinus maenas* (Linnaeus, 1758) (LAULIER, 1974), *Chasmagnathus granulata* Dana, 1851 (LÓPEZ *et al.*, 1997) and *Penaeus penicillatus* (Alcock, 1905) (OLIVEIRA *et al.*, 1999).

In the histological analyses of the female's ovaries, the modifications observed in the oocytes during the process of gonad maturation are similar to the descriptions in the literature for other females of decapod crustaceans (ADIYODI & SUBRAMONIAN, 1983; LÓPEZ *et al.*, 1997; OLIVEIRA *et al.*, 1999; ANDO & MAKIOKA, 1998; ELORZA & DUPRÉ, 2000). The similarities are specially found in the morphological changes in the reproductive cells, and also in the presence and arrange of follicle cells during the process of ovary maturation. Thus, the morphology and development of females' gonad has intensively been utilized as a tool for reproductive cycle studies.

When external morphological characteristics of the gonads were compared to histological descriptions, it was possible to observe modifications that characterize the process in different developmental stages throughout the ovarian cycle. Consequently, the macroscopic classification of gonad stages agrees with the modifications of the oocytes. However, the macroscopic differentiation between rudimentary and senescent gonads could demonstrate a significant difference, since in both stages the gonads present the same size, differing only in the flaccid appearance of the senescent gonad. On the other hand, the spent gonad is generally more voluminous than the immature gonad. In the histological analysis, the senescent gonad shows several oogonias, a small number of primary and secondary oocytes, and empty follicles. However, these cell types were not observed in immature ovaries. Thus, the macroscopic analysis of the size and coloration of gonads can be used to identify correctly the different stages of gonad development of females of *U. rapax*, since these stages showed also differences in their reproductive cells at the microscopic analysis.

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