INTRODUCTION

*Litopenaeus schmitti* (Burkenroad, 1936) is known as the white shrimp, occurring in Western Atlantic from Antilles, Virgin Islands and Cuba (23° 30'N), through Honduras, Western Coast of Caribbean, Venezuela and along the Atlantic coast of South America until the northern border of Rio Grande do Sul State (29° 45'S) (Pérez-Farfante, 1970a,b). This species has the typical penaeid shape and it is usually found from littoral to 30 meters depth, despite the fact that occasional occurrences have been reported for 50 meters (D’Incao, 1995).

As well as the pink shrimp (*Farfantepenaeus spp.*), this species is largely dependent on the estuarine regions to complete its life cycle (Neiva & Wise, 1963; Garcia & Le Reste, 1981; D’Incao, 1999), showing continuous reproduction that usually presents two seasonal peaks (Coelho & Santos, 1995b). Thus, fishing effort applied over the both phases of its life cycle may lead to the collapse of either, small scale, or industrial exploitation of these resources (Garcia & Le Reste, 1987; D’Incao, 1991).

The main target, of penaeid fishery in Brazil, used to be the pink shrimps (*Farfantepenaeus paulensis* and *F. brasiliensis*). The reduction of yields, observed in commercial fleet, resulted in a multi-target activity, in such a way that other penaeid species became commercially important (Valentini et al., 1991; D’Incao et al., 2002). Among the recent exploited shrimp species, the white shrimp is one of the most valuable ones in Southern and Southeastern Brazil. As a result, the impact of this activity over the white shrimp was also altered and the need for novel population assessment is noticeable.

The comparison of macroscopic traits to the features observed in histological sections allows creating a practical reference table to classify ovary development in laboratory routines, to be used in ecological and fishery investigations. This technique has been proved to be useful in many penaeid species, such as *F. paulensis* and *F. brasiliensis* (Peixoto et al., 2003; Dumont et al., 2007), *A. longinaris* (Dumont & D’Incao, 2004), *Penaeus indicus* (Castille & Lawrence, 1991; Quinitio & Millamena, 1992), *Penaeus kerathurus* (Medina et al.,...
Size or age at first maturity is a valuable reference point to properly manage a shrimp stock, since reproduction is the phenomenon responsible for the stock renewal (Coelho & Santos, 1995; Castilho et al., 2007). Therefore, ovarian maturation stages, as well as the mean length at first maturity are vital to investigate reproductive dynamics of *L. schmitti*. Thus, the aim of this investigation is to create an accurate chromatic scale to classify ovary development of the white shrimp from Southern Brazil based on histological sections and to estimate the size at first maturity.

**MATERIAL AND METHODS**

Sampling was performed during autumn and late spring to assure that females participating on the most important breeding events were captured, since most of subtropical penaeid species are reproducing during these periods (Gulland & Rotschild, 1981). 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 in the surrounding area of Babitonga Bay, Santa Catarina State, Brazil (26°S) (Figure 1). Each station was sampled during daylight and night since spawning occurs mainly during the dusk and the night.

The reliability of macroscopic scale was validated by performing histological techniques that provided valuable information on the relationship between color and shape of the ovaries and the cell traits. The ovaries were preferred due to larger size and easier macroscopic identification than the testes.

Figure 1. Study area in Southern Brazil, highlighting the Babitonga Bay and the adjacent area where sampling took place.
Additionally, the reproduction process occurring in a population largely depends on the females that are usually used as an indicative of this population process.

Total length (TL) was measured from the tip of rostrum to the end of telson. At least 15 ovaries, from each development stage, were sampled. 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 a similar species (Peixoto et al., 2003). Shape and color (Pantone, 1999) of ovaries were digitally recorded under the approximated same quantity of light, to establish a macroscopic classification validated by subsequent histological sections. To define the ovary color accurately, an automated tool was used, comparing the predominant ovary color to a Pantone’s reference table. This tool samples a square of 12 x 12 pixels and provides the mean reference color obtained within this area (Dumont et al., 2007). Tissue was fixed in Formalin (10%), paraffin embedded, sectioned (6µm) and Hematoxilin-Eosin stained. At least 30 oocytes diameters per female were measured and a One-Way ANOVA and Tukey’s test were applied to verify significant differences (p < 0.05) among mean oocyte size grouped according to development stage (Zar, 1984).

Mean size at first maturity (LM) was considered as the size class interval (1mm interval) in which frequency of ripe females is 50% (King, 1997). Frequency of ripe females was fitted to the logistic model by an automated least square procedure, in such a way that:

\[ P = \frac{1}{1 + \exp(-r(CL1 - LM))} \]

Where P is the percentage of ripe females in a given length class, r is the logistic curve slope, CL1 is the upper limit of carapace size interval and LM is the mean length at first maturity.

RESULTS

Considerable differences in color and shape of the ovaries were observed for *L. schmitti* in the wild. Four different stages were observed, named stage I (immature), stage II (developing), stage III (ripe) and stage IV (spent).

![Figure 2. Litopenaeus schmitti. Histological sections of the ovary. A. stage I (immature); B. stage II (developing); C. stage III (ripe); D. stage IV (spent). Og= oogonies; OvI= pre-vitellogenic oocyte; OvII= vitellogenic oocyte; OvIII= mature oocyte; Sf= spent follicle. Scale= 100µm.](image)
Stage I (immature): Ovary is white translucent to warm gray (3C) and is difficult to distinguish it through the carapace. Cephalotoracic lobes of the ovary are not developed and are reduced to the posterodorsal part of the stomach. The abdominal region of the gonad is reduced and usually does not extend further than third abdominal somite. In terms of cell traits, only the previtellogenic oocytes (OVI) and oogonies (OO) were observed during this stage. These cells are small (54.01 mm ± 3.54) and the absence of yolk production is confirmed by the affinity of the cytoplasm for the hematoxilin, suggesting basophily.

Stage II (developing): Filling most of the abdominal cavity, the ovary is clearly better developed, when compared to stage I. Two longitudinal and parallel lobes are observed along the abdominal portion of the gonad. In the cephalic region, the ovary covers part of stomach. Now, the ovary can be observed through carapace and color ranges from gray to green (417C-451C). During this stage, developing oocytes (OVII) significantly increase in size (136.8 mm ± 8.8), the start of vitellogenesis is observed indicated by eosin stained oocytes in histological sections.

Stage III (ripe): Ovary fills the entire abdominal cavity and presents colors ranging from dark green to black (418C-BlackC). Cephalotoracic portion of ovary covers the entire stomach and presents several developed lobes. Microscopically, ripe cells (OVIII) are larger (199.8 mm ± 6.4) and present cortical rods (CR), a structural modification that indicates final maturation of oocytes in most of penaeid species.

Stage IV (spent): The spent stage is usually similar to immature, even though it is very difficult to distinguish them macroscopically. 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. Size overlapping of oocytes was recorded between different developing stages 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).

![Figure 4. Litopenaeus schmitti. Size frequency of cell diameter according to development stage of ovaries.](image)

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%) and size range (minimum and maximum).

<table>
<thead>
<tr>
<th>N</th>
<th>mean</th>
<th>CI -95%</th>
<th>CI 95%</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>54.5</td>
<td>51.0</td>
<td>58.0</td>
<td>35.0</td>
<td>93.2</td>
</tr>
<tr>
<td>46</td>
<td>136.8</td>
<td>128.0</td>
<td>145.6</td>
<td>80.8</td>
<td>187.9</td>
</tr>
<tr>
<td>30</td>
<td>199.8</td>
<td>193.4</td>
<td>206.3</td>
<td>166.5</td>
<td>226.6</td>
</tr>
</tbody>
</table>

The smallest ripe female measured 146 mm (TL) and the largest 180 mm (TL). Estimated mean length at first maturity (LM) was 152 mm (TL) (Figure 5, Table II) and the length at which 100% of females are mature is 170 mm (TL).
Table II. Summary of fit obtained from logistic model, containing the parameter fit (Parm), the value estimated (Value), the standard error (se), the t value (t), the confidence limits to 95% of significance as well as the p value (limit of significance adopted was p<0.005).

<table>
<thead>
<tr>
<th>Parm</th>
<th>Value</th>
<th>se</th>
<th>t</th>
<th>CI (95%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.35</td>
<td>0.01</td>
<td>297</td>
<td>0.32</td>
<td>0.38</td>
</tr>
<tr>
<td>LM</td>
<td>152</td>
<td>0.10</td>
<td>1.487</td>
<td>151</td>
<td>152.5</td>
</tr>
</tbody>
</table>

DISCUSSION

The comparison of macroscopic traits to the features observed in histological sections allowed to create a practical reference table to classify ovary development in laboratory routines to be used in ecological and fishery investigations. However, slightly different colors were attributed to each stage, when compared to the previous investigation for this species. The ovary of *L. schmitti* presented a thin layer covering the entire organ, which may have caused distortion in the color reference when analyzed without dissecting. Therefore, unlike most of penaeids, we strongly recommend the dissection of the ovaries obtained from this species to classify the development stage of maturity, since the layer covering it, may represent a source of misinterpretation. In contrast, the microscopic analysis prevents the common mistakes performed in ovary classification of penaeids, including the errors associated to the presence of color pigments that are not directly associated to the ovary development.

The ovary development of penaeid shrimps is usually divided in 5 different stages, named immature, developing, incipient, ripe and spent (Vogt et al., 1989; Castille & Lawrence, 1991; Tan-Fermin, 1991; Medina et al., 1996; Quintero & Garcia, 1998; Palacios et al., 1999). Recently, the investigations intending to classify the ovaries for fishery management purposes have suggested the simplification of the development stages, reducing it to only 4, named immature, developing, ripe and spent (Peixoto et al., 2003; Dumont et al., 2007). It does happen due to high similarity between developing and incipient maturity stages, which show similar stage of cell development, but slightly different organization. Nonetheless, the indication of final maturation is given by the presence of the cortical rods, since these structures are important during egg activation process, and help to avoid polyspermy as well as to
create a microenvironment suitable for egg development (Clark et al., 1980).

Once the ovary development is well determined, a reliable classification of the ovaries allows estimating the mean length at first maturity (LM). This parameter has been used as a reference point to manage exploited penaeid stocks and may indicate ecological process if monitored through time and/or space. When the LM estimated for this species in Southern Brazil is compared to Southeastern Brazil (SP) very similar values were recorded (158 mm CL), suggesting that the same LM value may be used as a reference point for this species in Southern and Southeastern Brazil. Slightly lower values were estimated for the Gulf of Venezuela, for a population inhabiting warmer waters, which is coherent with the general pattern of latitudinal variation in the size at first maturity reported for penaeids (Castilho et al., 2007).

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