SEXUAL MATURITY OF THE SMALL SHRIMP Potimirim brasiliana Villalobos, 1959 (Crustacea, Atyidae), from the southeastern coast of Brazil

Marina Machado da Costa
Patrícia Hoffmann
Geslaine Rafaela Lemos Gonçalves
Maria Lucia Negreiros-Fransozo

University of the São Paulo State, UNESP, Institute of Biosciences, Department of Zoology, Rua Prof. Dr. Antônio Celso Wagner Zanin, 250, Distrito de Rubião Junior, 18618-689, Botucatu, São Paulo, Brazil, dorettomarina@outlook.com

Abstract: The shrimp Potimirim brasiliana plays an important role in streams along the Brazilian coast as it feeds on the organic debris from the surrounding ecosystem. We determined the morphological sexual maturity of P. brasiliana by means of relative growth. Monthly throughout a year, we used sieves (2 mm mesh diameter) to collect the specimens in a coastal stream. A total of 3,521 specimens had their carapace length (CL) measured, as well as their second pleura length (PL) and the length (AML) and width (AMW) of the appendix masculina. The studied relationships were the following: CL vs. PL; CL vs. AML and CL vs. AMW, with the data adjusted to a logistic equation. All the relationships tested among the demographic groups differed statistically. The CL50 of females measured 3.45 mm, while the CL50 of males measured 2.75 mm. Two adult morphological groups were set apart for each sex. The CL vs. AMW relationship is the best at demonstrating the separation of the morphological groups for males. This fact is an intriguing issue as it suggests the presence of morphotypes in males, probably, playing different roles in the reproductive process.

Keywords: Caridea, morphology, reproduction, Ubatuba.
INTRODUCTION

Studies on the relative growth of animals have begun mid last century, mainly on crustaceans, by Teissier (1935, 1960), Huxley & Teissier (1936), Newcomb (1948) and Hartnoll (1978, 1982). These authors showed that the relationship between the growth of one body part or organ in relation to the whole body can be described by the power equation \( Y = aX^b \), where \( Y \) is the variable dimension, \( X \) the reference dimension, while \( a \) and \( b \) are constants (Huxley & Teissier, 1936).

The growth of crustaceans is well known due to its rigid exoskeleton that allows for accurate measurements and also because the molting process (exoskeleton shedding) delimits their well-defined life cycle stages (Hartnoll, 1978 and Petriella & Bosch, 1997). Generally, there are few studies on caridean shrimps because the majority of them addresses the relative growth of species with economic interest, particularly, many studies on the genus Macrobrachium Bate, 1868 such as Valenti et al. (1989), Moraes-Riodades & Valenti (2002, 2004), Mossolin & Bueno (2003), Mantelatto & Barbosa (2005), Konan et al. (2010), Pantaleão et al. (2012), Bueno et al. (2019) and Nogueira et al. (2019). In addition, there are few studies focused on other caridean genera, such as: Anger & Moreira (1998), Martínez-Mayén et al. (2000), Lozano-Alvarez et al. (2007), Paschoal et al. (2013a), Herrera et al. (2018) and Pescinelli et al. (2018).

The changes in the proportions of body size and its parts are noted on the relative growth of the secondary sexual characters, mainly those associated with the reproduction of decapods (Hartnoll, 1974; Gonçalves et al., 2017). For instance, thalassins, anomurans and brachyurans show morphological changes in the male chelipeds and the female abdomen, which commonly characterize the transition frommorphologically immature to the mature stage (Flores & Negreiros-Franozo, 1999; Bertini & Fransozo, 1999; Negreiros-Franozo & Fransozo, 2003; Hirose & Negreiros-Franozo, 2007 and Bertini et al., 2007).

In carideans, both sexes have morphological variations related to reproduction. According to Bauer (2004) in caridean males, “the endopod of the first pleopod pair is usually different from other pleopods, indicating some sexual function” (Bauer, 2004); while “females show distinct variations in the structure of the pleopods when in breeding condition, which are related to incubation of embryos, i.e., “brood pouch”.

One can assess the sexual maturity of decapod crustaceans grounded on three lines: morphological maturity, which is usually based on changes in the relative growth of secondary sexual characters; physiological or histological maturity, evidenced by the presence of mature oocytes, spermatozoon or spermatophores; and functional maturity, the capacity to copulate and carry embryos (López-Greco et al., 1999; Viau et al., 2006 and Gonçalves et al., 2016).

According to De Grave & Fransen (2011), the infraorder Caridea, the second most species-rich group within Decapoda, corresponds to half as many species as in Brachyura. The same authors also mentioned that this infraorder is dominated by Palaemonidae (981 species), followed by Alpheidae (663), Atyidae (469), Hippolytidae (338) and Crangonidae (219), in addition to 12 monogeneric families, for instance, Physetocarididae.

The Atyidae family comprises freshwater shrimps (De Grave et al., 2008, 2009; De Grave & Fransen, 2011) and shows a remarkable diversity. They are represented in the Neotropical region by 19 species (De Grave et al., 2008).

The genus Potimirim Holthuis, 1954, whose meaning in a Brazilian native language (Tupi-Guarani) is “small shrimp”, occurs in coastal rivers and streams from Mexico, Central America and South America (Torati & Mantelatto, 2012). This genus comprises five valid species, among them Potimirim brasiliana Villalobos, 1959 and Potimirim potimirim (Müller, 1881). These species are native to rivers along the Brazilian coast, i.e., Bahia, Rio de Janeiro, São Paulo, Paraná and Santa Catarina as mentioned by Almeida et al. (2008), Hoffmann & Negreiros-Franozo (2010) and Torati & Mantelatto (2012). Recently, Moraes et al. (2017) recorded P. potimirim in Rio Grande do Norte, Brazil, extending its geographic distribution.

The shrimp P. brasiliana lives amongst macrophytes, partially submerged along the river margins, and on the vegetal material deposited on the bottom of clear-water rivers flowing towards the sea (Barros & Fontoura, 1996b). Therefore, P. brasiliana is an important component of the freshwater environment it inhabits, due to its fundamental role in the transportation and retention of debris, and in the recycling of nutrients (Covich et al., 1999; Croll et al., 2001). It also contributes to the renewal and resuspension of sediments (Moulton et al., 2004) and it cleans the hard substratum exerting a negative influence on the periphyton (Souza & Moulton, 2005). We believe that such activities are related to the feeding habits and the existence of certain morphological structures, i.e., specialized setae for capturing organic debris from the water and substratum.
A few studies on the genus *Potimirim* are available in the literature such as: population biology by Lima et al. (2006), molecular phylogeny by Torati & Mantelatto (2012), evaluation of the role of some macrophytes in populations of carideans by Paschoal et al. (2013b), reproductive and population aspects of *P. brasiliana* by Rocha et al. (2013), and the sexual system by Grilli et al. (2014).

Nevertheless, additional data on the general biology of *P. brasiliana* is scarcely found. The reproductive biology and growth of *P. brasiliana* (published as *P. glabra*) were described by Barros & Fontoura (1996a) and Hoffmann & Negreiros-Fransozo (2010) for populations of Santa Catarina and São Paulo, respectively.

Under these circumstances, we analyzed a population of *P. brasiliana* from a coastal stream in Ubatuba, São Paulo, southeast Brazil, regarding its morphological sexual maturity, based on its relative growth. The results obtained here will allow a better understanding of specie’s biology in order to set better guidelines to protect it against human interference, such as sewage or industrial discharge and the construction of piers in areas of procreation and development for many species.

**Material and methods**

The shrimps were sampled monthly, from March 2005 to February 2006, in a small river in the Camburi district (23º22'07"S; 44º46'02"W), a touristic and residential area of Ubatuba, São Paulo, Brazil. The Camburi River has a clear and turbulent water due to a narrow riverbed, large algae-covered rocks, and a very irregular margin (Hoffmann, 2007). The marginal vegetation is very rich, comprised of bushes and trees that offer shade in a landscape with little human influence along the river within the Atlantic forest (Hoffmann, 2007).

We captured the shrimps using a conical sieve (2 mm mesh) to hand-sieve under water, very near the rocks. We placed the specimens in plastic bags with water from the sampling site, labelled and kept them in thermal boxes. After that, we identified the shrimps by the diagnostic features indicated by Villalobos (1959) and Grilli et al. (2014). In the laboratory, we separated the specimens into demographic groups (juveniles, males, females and ovigerous females) and conserved them in ethanol (70%).

Individuals were sexed according to the presence of an *appendix masculina* on the second pair of pleopods, which is a very conspicuous morphological structure that characterizes males in species of the genus *Potimirim* (Villalobos, 1959; Hoffmann, 2007 and Hoffmann & Negreiros-Fransozo, 2010).

In the case of shrimps with lost or broken pleopods, sexing was based on the presence (males) or absence (females) of a pair of spines close to the proximal articulation of the carpus on the third pair of pereiopods, which in taxonomical descriptions are considered to be the "male sexual organs" (Villalobos, 1959; Hoffmann & Negreiros-Fransozo, 2010).

The measurements used for the morphometric analysis in this study were adopted from Hoffmann & Negreiros-Fransozo (2010). We measured the carapace length (CL) of both sexes. For females, we measured the pleura length of the second abdominal somite (PL) that is associated with reproduction as it protects the embryos during incubation (Bauer, 2004). For males, we measured the *appendix masculina* of the second pair of pleopods in two portions: length (AML) and width (AMW). Measurements were made using a microscope stereoscope (SV6, Zeiss) equipped with an image system (Axiovision, Zeiss: 0.01 mm).

In the relative growth analysis, we adopted as dependent variables the dimensions PL, AML and AMW (Y axis), and as independent variable (X-axis), the dimension CL.

**Statistical analysis**

We used the allometric technique described by Huxley (1950) to determine the morphologic sexual maturity of *P. brasiliana*, based on the measurements of their body parts. For the allometry analysis, we selected a higher number of individuals than those mentioned in Hoffmann (2007) due to the high number of shrimps caught. Separate equations were calculated for each sex. This technique allows us to observe variation in the growth pattern of certain body parts through a slope change on the straight line.

The data were log-transformed and fitted to a linear model. The degree of allometry (positive > 1, negative < 1 or isometric = 1) was checked using a Student’s T-test, with a significance level of 5% (α = 0.05) (Sampedro et al., 1999).

The process to isolate data in groups of juveniles and adults did not vary between sexes. Before the classification into juveniles or adults, we isolated the set of data in two groups using the "K-means clustering" method (Legendre & Legendre, 2012) in the bivariate set of data that consists of a standardized residual of the variables used for each sex. Subsequently, we proceeded to the discriminant analysis for reallocation of any incorrectly classified datum.

The specimens belonging to the Morphotype-I were designated as adults I, and Morphotype-II as adults II. We used the same process for each studied relationship. The
separation of data into groups of juveniles and adults (I and II) adopted the analytical procedures described in detail by Bueno & Shimizu (2009) and Hirose et al. (2012). Linear regression was applied for each dependent variable in each group of data, previously distinguished in the K-means clustering analysis. When absolute value data corresponding to the residual pattern were higher than 2.57 (p < 0.01) (Sokal & Rohlf, 1995), they were considered not belonging to the distribution (= outlier), a measure also taken by Bueno & Shimizu (2009).

When we found a significant difference in the regression parameters (between juveniles and adults), we determined the CL at the onset of morphologic sexual maturity for both sexes and for each size. Then, in order to verify the precision for each group, the slopes (b) and the intercepts (a) of the equations of every demographic group were tested using a covariance analysis (ANCOVA), at the significance level of 5% (= 0.05) (Zar, 1999). Statistical calculations were performed with the software Past (version 1.62) (Hammer et al., 2001) and Statistica (version 8.0).

RESULTS

THE SAMPLED POPULATION

We measured 618 juveniles (males and females), 1482 adult females and 1421 adult males (Tab. 1). Among the specimens of adult females, 482 of them were carrying embryos in the setae of their pleopods, and we named them as ovigerous females. Shrimps were not checked with respect spermatozoon presence by means smear for sexual classification.

SIZE OF THE SPECIMENS

We considered the size of the shrimps as the carapace length. The males varied in size from 1.8 to 4.6 mm CL, while the females varied from 1.5 to 5.5 mm CL and the ovigerous females from 3.2 to 5.5 mm CL (Tab. 2).

Tab. 1. *Potimirim brasiliana* Villalobos, 1959. Results of the relative growth based on the allometric technique of a population sampled in Ubatuba, São Paulo, Brazil.

<table>
<thead>
<tr>
<th>Relationships</th>
<th>Sex</th>
<th>Demographic category</th>
<th>N</th>
<th>logY = a + b logX</th>
<th>t</th>
<th>Allometry</th>
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<td>J</td>
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<td>539</td>
<td>-0.24</td>
<td>1.09</td>
<td>0.86</td>
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<tr>
<td>CL vs. PL</td>
<td>♀</td>
<td>F I</td>
<td>1000</td>
<td>-0.29</td>
<td>1.25</td>
<td>0.72</td>
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<tr>
<td></td>
<td></td>
<td>F II</td>
<td>482</td>
<td>-0.13</td>
<td>1.08</td>
<td>0.87</td>
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<td></td>
<td></td>
<td>79</td>
<td>-0.38</td>
<td>1.29</td>
<td>0.18</td>
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<tr>
<td>CL vs. AML</td>
<td>♂</td>
<td>M I</td>
<td>405</td>
<td>-0.12</td>
<td>0.99</td>
<td>0.59</td>
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<td></td>
<td></td>
<td>M II</td>
<td>706</td>
<td>0.21</td>
<td>1.31</td>
<td>0.39</td>
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<td></td>
<td></td>
<td></td>
<td>77</td>
<td>-1.05</td>
<td>1.69</td>
<td>0.13</td>
</tr>
<tr>
<td>CL vs. AMW</td>
<td>♂</td>
<td>M I</td>
<td>715</td>
<td>-0.41</td>
<td>1.31</td>
<td>0.63</td>
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<tr>
<td></td>
<td></td>
<td>M II</td>
<td>706</td>
<td>0.17</td>
<td>1.13</td>
<td>0.41</td>
</tr>
</tbody>
</table>

N = number of shrimps; a = intercept; b = slope; CL = carapace length; PL = pleura length; AML = length of the *appendix masculina*; AMW = width of the appendix masculina; F I = female morphotype I; F II = female morphotype II; M I = male morphotype I; M II = male morphotype II; and J = juvenile; *significant at 5%.
STATISTICAL RESULTS

Supported by the allometric technique, the relative growth analyses revealed positive allometry in the majority of the studied relationships (CL vs. PL; CL vs. AML and CL vs. AMW), except for the demographic category of males M I (CL vs. AML), which showed isometry (see Tab. 1). As we can observe in the regressions (Fig. 1, 2 and 3), the shrimp *P. brasiliana* had morphotypes in the adult stage for both sexes (Tab. 1). This means that the studied dimensions (PL, AML and AMW) grow at a higher rate than the carapace size (CL), with exception of the M I stage (males) in which the *appendix masculina* increases proportionally to the carapace length (CL).

The comparison of equations among the demographic categories (juveniles, morphotype I and morphotype II) showed differences between males and females in all combinations (see Tab. 2). Thus, it is necessary to represent the relative growth graphically for each sex and maturity stage (Fig. 1, 2 and 3; Tab. 2 and 3).

The size at which 50% of males were morphologically mature (CL50) was 2.75 mm CL; while for females it was 3.45 mm CL. Thus, males mature at smaller sizes than females.

DISCUSSION

MORPHOMETRY AND MORPHOLOGICAL SEXUAL MATURITY

Caridean shrimps are a very diverse group of decapods, inhabiting several aquatic
environments. However, so far, they have been scarcely studied with regards to their morphometry and sexual maturity associated with their relative growth. Species of the genus *Macrobrachium* (Palaemonidae family) are an exception, probably because some of them are of economic interest (Moraes-Riodades & Valenti, 2002; Mossolin & Bueno, 2003; Mantelatto & Barbosa, 2005; Konan et al., 2010; Pantaleão et al., 2012; Bueno et al., 2019 and Nogueira et al., 2019). As stated by Hoffmann & Negreiros-Franzo (2010), the attainment of sexual maturity involves the development of secondary sexual characters, the maturation of the gonads and, in females, the capacity of generating offspring.

In most decapods studied, the relative growth of the chelipeds usually shows a positive allometry that represents the attainment of the sexual maturity in males (Flores & Negreiros-Franzo, 1999; Mantelatto & Barbosa, 2005; Bertini et al., 2007; Hirose et al., 2012, 2017; Pantaleão et al., 2014, Nogueira et al., 2019). This was not observed in some of previously studied carideans, but both sexes probably have morphological variations linked to reproduction events, mainly mating behavior and incubation.

Berkeley (1930) and Descouterelle (1971) proposed that the *appendix masculina* has a key role in the spermatophore transfer. Mating experiments with other carideans confirm this view, for instance, Bauer (1976), Berg & Sandifer (1984) and Nakashima (1995) on *Heptacarpus sitchensis* (Brandt, 1851), *Palaemonetes pugio* Holthuis, 1949 and *Athanas komintoensis* (Kubo, 1942). In those studies, males lacking the endopod and the *appendices*
masculina of the second pleopods (they were removed) readily mated with females, but frequently failed to pass on spermatophores. According to Bauer (2004), caridean males have the endopod of the first pair of pleopods morphologically different from the other pleopods, indicating some kind of sexual function. Recently, Ahamed & Ohtomi (2014) proposed that the appendix masculina in Plesionika izumiae Omori, 1971 has a secondary sexual character linked with morphological sexual maturity. Also, when studying six species of Alpheus Fabricius, 1798, Costa-Souza et al. (2019) found that the appendix masculina grows at a higher rate during the adult stage than the juvenile one.

Morphometric analysis of P. brasiliana confirmed the occurrences of different groups in both sexes after the sexual maturity (CL50). The results obtained here indicate that the natural adult population of this species in Camburi River is composed of two groups for each sex: females with different sizes of the second pleura and males with different sizes of the appendices masculina, a separation evidenced by the allometric constant from the morphometric relationships presented here. As shown by Moraes-Riodades & Valenti (2004) and Pantaleão et al. (2014), a few carideans of the genus Macrobrachium have different morphotypes based on the growth of their chelipeds. Similarly, in the literature, it is suggested that these morphological differences are related to distinct behaviors between individuals, predominantly mating behavior. For instance, Barki et al. (1991) previously mentioned the importance of the morphological and allometric differences of the cheliped among morphotypes of Macrobrachium rosenbergii (De Man, 1879), regarding the dominance hierarchy. In M. rosenbergii, sexually mature males appear in three main morphotypes and several inter-
mediate forms with different morphology, anatomy, physiology and behavior (Karplus & Sagi, 2010).

More recently, Augusto & Valenti (2016) compared the metabolism, nitrogenous excretion, growth, substrate, ingestion rate, and fecal production of three male morphotypes (CC= cinnamon claw, GC1= green claw 1, and GC2= green claw 2) of Macrobrachium amazonicum (Heller, 1862). These authors concluded that the three morphotypes have different physiology regarding the parameters mentioned above, which might indicate their pattern of growth, activity, role within the population and the differences in their reproductive behavior.

Considering the results presented here for Potimirim brasiliana, further studies on the hypotheses of distinct behaviors between morphotypes should help us better understand the actual role of the appendix masculina and the second pleura. Aside from Palinemonidae, other caridean shrimps such as Alpheidae, Hippolytidae and Atyidae do not have similar mating behavior. In other words, adults of those three groups showed a less complex mating behavior and the males did not use the chelipeds to fight or compete with other males for a receptive female. Thus, the size of the cheliped in some species did not show differential growth between the juvenile and adult stages.

The functions of chelipeds or even of some pereiopods that can indicate growth in ontogenetic stages (morphotypes) of other decapods are not usually found in the present studied species of Caridea. However, the growth at different stages during the adult life of P. brasiliana could indicate a more efficient role in the morphotype II. We believe the appendix masculina is used in the moment of the sperm transfer, i.e., the male touches the pair of appendages next to the sternal area of the female to facilitate the deposition of the sperm mass near the gonopores. After that, the female

Fig. 3. Potimirim brasiliana Villalobos, 1959. Relationship between “carapace length vs. appendix masculina width” for males (Juveniles, Adults - M I and M II) and the adjusted equations. (CL = carapace length; AMW = appendix masculina width).
spawns and the fertilization can occur in sequence. The type of mating behavior seen in shrimps living in huge groups in nature (like the species of the genus *Potimirim*) is named “Pure search” (Bauer, 2004). They mate among several individuals and the use of the *appendix masculina* could guarantee that the sperm mass does not drift in the water. The Atyidae, *Atyephyra desmaresti* (Millet, 1931), showed positive allometry regarding the sexual endopod, in
In short, a new behavioral study using photo and video records associated with electronic scanning techniques are needed to test such hypothesis.

RELATIVE GROWTH

The relative growth of the appendix masculina studied here for *P. brasiliana* (regressions CL vs. AML and CL vs. AMW) indicated positive allometry that conforms to the indication of some sexual function, mentioned by Bauer (2004). However, further studies on its mating behavior are needed to clarify that function. There might be an early (genetic) factor, operating long before sexual maturation that in combination with social factors contributes to the determination of morphotypes (Karplus & Barki, 2019).

Analysis of the relative growth of the length of pleurae in females (CL vs. PL) revealed a conspicuous allometric rate that translates to an increase in pleura length from the juvenile to the adult stage. During the adult stage, the space below the pleura works as a brood pouch, when females start carrying embryos/eggs. Thus, as the space increases, more eggs are incubated, optimizing the reproductive process (Nazari et al., 2003; Bauer, 2004; Mortari et al., 2009; Paschoal et al., 2013a).

This study found no sign of slope changes when analyzing the size at sexual maturity of *P. brasiliana* (Tab. 3), along the latitudinal gradi-
ent. Thus, in order to evaluate whether the relationship is latitudinal or local, we need further studies on the populations of *P. brasiliena* at different latitudes and different local environmental factors, mainly the quality of food resources.

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**REFERENCES**


Hoffmann, P. 2007. Ecologia populacional e reprodutiva de Potimirim glabra (Kingsley, 1954) (Caridea, Atyidae) em riachos da região de Ubatuba-SP. Master Science Dissertation (Zoology), Institute of Biosciences, São Paulo State University (UNESP), Botucatu, SP, Brazil. 96 p.


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