



BIOLOGICAL SCIENCES

Size at onset of sexual maturity in *Macrobrachium amazonicum* (Heller, 1862) phenotypes: an integrative approach

LUCAS R.P. PASCHOAL & FERNANDO J. ZARA

Abstract: The size at the onset of sexual maturity (SOM) was determined in four hololimnetic populations of *Macrobrachium amazonicum* using morphometric, physiological and functional criteria. Male prawns from two populations analyzed had hypertrophied chelipeds and large body proportions, showing the presence of four morphotypes, i.e. large-size phenotype (LS). However, the other two populations showed smaller males without morphotypes, i.e. small-size phenotype (SS). The development of sexual weapons in males modulated the mating system and SOM in this species. It was verified that there is a synchrony between physiological and functional maturities in SS males. On the other hand, functional maturity in LS males occurred after physiological and morphological maturities. In females, we verified synchronization between morphological and functional maturities. The results obtained indicated that, in both sexes, physiological maturity anticipates the others criteria. There were no differences between the sequential scheme of sexual maturity for female phenotypes, being: physiological → functional \cong morphological. However, the evaluation of sexual maturity in males must take into account the phenotype of population, since that was modulated by functional maturity. The sequential scheme for LS males is: physiological → morphological → functional, while for SS males it is: physiological = functional → morphological.

Key words: Amazon River prawn, morphometric variation, size at maturity, reproduction.

INTRODUCTION

Some prawn species of the genus *Macrobrachium* Spence Bate, 1868 can inhabit coastal and inland waters (without marine influence), as is the case of the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862). This species displays a great environmental adaptability and wide intraspecific variability, with populations showing significant differences in morphological and reproductive traits (Odinetz-Collart & Rabelo 1996, Vergamini et al. 2011, Anger 2013, Hayd & Anger 2013, Augusto & Valenti 2016, Paschoal et al. 2019). The Amazon River prawn has a wide distribution in the Americas, occurring from Costa

Rica to Argentina. In Brazilian basins, it occurs in estuarine and/or freshwater ecosystems from the state of Roraima to Paran (Vergamini et al. 2011, Pileggi et al. 2013).

In estuaries, *M. amazonicum* has an amphidromous life history, where adults live and reproduce in freshwater environments, while their larvae develop in estuarine environments, returning to freshwater when juveniles (Bauer 2013). In these environments, individuals of this species have higher dimensions (body size) than those recorded for animals of inland populations. Furthermore, amphidromous females show high fecundity values (maximum fecundity range: 1,344 - 7,417

eggs) when compared to females of inland populations (maximum fecundity range: 315 - 4,264 eggs) (Maciel & Valenti 2009, Hayd & Anger 2013, Pantaleão et al. 2018, Paschoal et al. 2019). In addition, amphidromous populations have a social hierarchy, where males with dominant morphotypes (GC1 and GC2) have selective advantages over submissive morphotypes (TC and CC) (Moraes-Valenti & Valenti 2010, Ibrahim 2011). However, some populations with such characteristics were recently recorded in lentic environments of the Paran Basin (southeastern Brazil) (Pantaleão et al. 2014, Paschoal & Zara 2017, 2018, Paschoal et al. 2019), indicating the shift of amphidromy by the entirely freshwater life cycle (i.e. hololimnia), due to the biological and physical impossibility of migrations to estuaries (Anger 2013).

The knowledge of the life history for different populations of *M. amazonicum* is fragmented and has gaps to be filled as reproductive and morphological aspects, since it is focused on studies dealing with amphidromous populations (Anger et al. 2009). In addition, the phenotypic plasticity of *M. amazonicum* populations is not yet fully understood (Anger 2013). Three types of phenotypes are recorded for the species: (a) large-size amphidromous populations, and (b) large-size and (c) small-size hololimnetic populations (Moraes-Riodades & Valenti 2004, Vergamini et al. 2011, Pantaleão et al. 2012, 2014, 2018, Paschoal & Zara 2017, 2018, Paschoal et al. 2019). These phenotypic differences recorded for this species are attributed to intrinsic factors (i.e. physiological and behavioral differences) (Moraes-Riodades & Valenti 2004, Augusto & Valenti 2016) and/or the availability of nutritional resources in different areas (Pantaleão et al. 2012, 2014).

The wide phenotypic and physiological plasticity of *M. amazonicum* makes the size at the onset of sexual maturity (SOM) of the

species being variable between populations (Pantaleão et al. 2012). Thus, it is necessary to use several criteria to estimate the SOM, since this information is crucial for the maintenance and improvement of the Amazon River prawn stocks (Moraes-Valenti & Valenti 2010). This species is very important in artisanal fisheries and is highly consumed in northern and northeastern Brazil (Odinetz-Collart & Moreira 1993, Maciel & Valenti 2009), and considered a species with great potential for world aquaculture (New 2005). Consequently, several studies have been conducted in recent years in order to improve, maintain and increase the quality of stocks, larviculture and sustainable farming (Preto et al. 2010, Henares et al. 2013, Maciel & Valenti 2014, Dutra et al. 2016, Henry-Silva et al. 2015, Santos et al. 2016).

Despite its importance, information about sexual maturity in *M. amazonicum* is limited to morphometric relationships (relative growth) and the determination of morphometric maturity (see Odinetz-Collart & Rabelo 1996, Moraes-Riodades & Valenti 2004, Maciel & Valenti 2009, Pantaleão et al. 2012, 2014). Thus, there is a gap related to physiological maturity (i.e. maturation of the reproductive systems) for the species. Also, there were no studies analyzing functional maturity (i.e. ♂: capability to mate and copulate, ♀: to carry eggs) in *M. amazonicum*. In this context, the present study analyzes the morphometric differences of four hololimnetic populations of *M. amazonicum* showing distinct phenotypes and determines the SOM in these populations, correlating morphometric, physiological and functional criteria.

MATERIALS AND METHODS

Sampling

Monthly samples were collected from October 2014 to December 2015, in Grande River (Paraná Basin) at four sampling sites. Two points in the Marechal Mascarenhas de Morais Hydroelectric Power Station (HPS) reservoir - municipalities of Cássia (CAS - 20° 30' 53.6" S, 46° 50' 16.4" W) and São João Batista do Glória (SBG - 20° 39' 43.5" S, 46° 32' 5.8" W); another two points in the Furnas HPS reservoir - municipalities of Carmo do Rio Claro (CRC - 20° 57' 20" S, 46° 9' 14" W) and São José da Barra (SJB - 20° 43' 10.6" S, 46° 11' 26.4" W) in the state of Minas Gerais, southeastern Brazil.

Prawns were collected by active sampling, a sieve (60 cm in diameter and 1.25 mm mesh) was swept through marginal vegetation and macrophyte banks for 30 minutes by one person; and passive collection, where six baited-traps (85 cm length x 35 cm width/height) were placed near the margin (0.5 - 2 m), baited with small pieces of beef liver and removed after four hours. Immediately after capture, the animals were anesthetized by chilling (ten minutes in ice) and preserved in 70% ethanol (except for animals used in histological analysis that were kept alive). After that, they were placed in properly labeled bottles and transported to the laboratory.

All animals collected during the monthly samplings were counted, and subsamples were obtained following Wenner et al. (1991): if $N \leq 80$ individuals were collected, all animals were analyzed; when $80 < N < 160$ individuals were collected, 80 individuals were randomly selected to be sexed and measured. If $160 < N < 320$ were collected, 50% of the specimens were checked; and in the case of samples with $N \geq 321$ individuals, 25% of the animals were selected. Individuals randomly selected from the

subsamples were sexed by the (a) verification of the secondary sexual characters: absence (♀) or presence (♂) of the *appendix masculina* on the endopodites of the second pair of pleopods, (b) verification of the positions of the gonopores: openings in the coxopodites of the 3° (♀) or 5° (♂) pair of pereopods, and (c) dissection of the reproductive organs: ovaries (♀) and testes (♂) (Paschoal & Zara 2017).

Measurement, morphotyping and morphometric maturity

The specimens of *M. amazonicum* had body structures measured with an analogic caliper (0.02 mm), according to Moraes-Riodades & Valenti (2004) and Pantaleão et al. (2012, 2014): carapace length (CL), total length (TL), width of the second abdominal pleura (2° PL), abdomen length (AL), major cheliped length (MCL), propodus length (PPL) and dactyl length (DCL) (Fig. 1). For the measurement of MCL, PPL and DCL, the right cheliped of the animals was assessed. The fresh weight (W) of each individual was obtained with an analytical scale (± 0.0001 g).

Male prawns were visually divided into four morphotypes: translucent claw (cheliped) (TC), cinnamon claw (CC), green claw 1 (GC1) and green claw 2 (GC2). These morphotypes were classified according to Moraes-Riodades & Valenti (2004) and Pantaleão et al. (2014), based on size and color of the chelipeds, angles of the spines on the carpus and propodus and the pubescence on dactyls. Subsequently, principal component analyses (PCA's) were performed to separate different morphological groups (morphotypes) in males for each population, using all body dimensions measured (\log_{10}) in these animals (Sampedro et al. 1999).

The morphometric maturity was estimated by the relative growth patterns of the species, which was determined by allometric equations

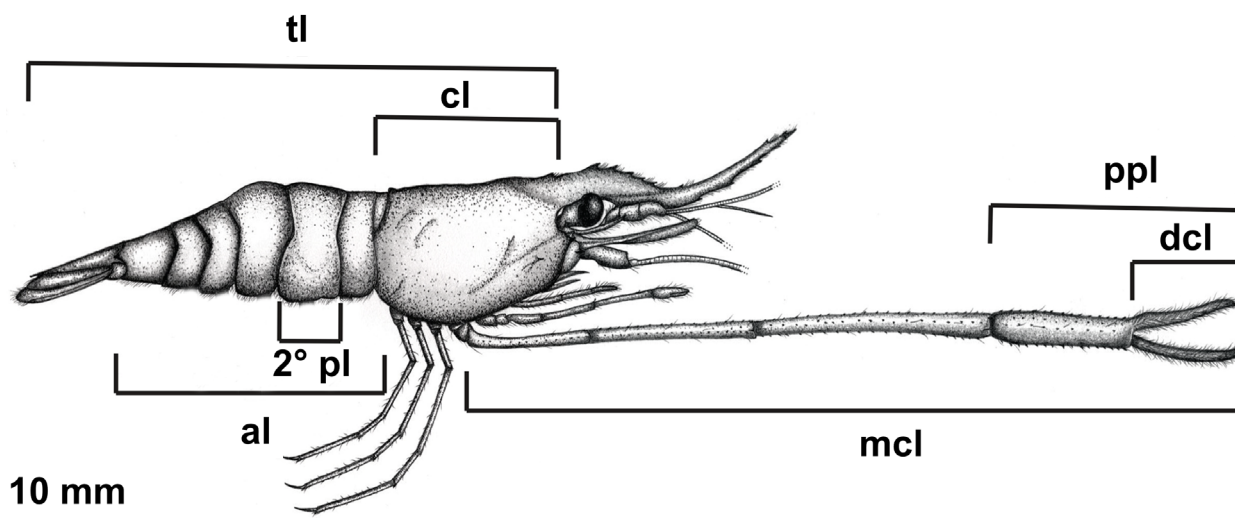


Figure 1. General scheme for prawn size dimensions used in the morphometric analysis in *Macrobrachium amazonicum* (modified from Pantaleão et al. 2014). 2° pl, width of the second abdominal pleura. al, abdomen length. cl, carapace length. dcl, dactyl length. mcl, major cheliped length. ppl, propodus length. tl, total length.

$Y = aX^b$ (Huxley 1950). These equations were adjusted, having as independent variable (X) the CL and relating it to the other body dimensions of the animal (dependent variables, Y). Values of the allometric constant (b) were tested using the t -test, as $H_0: b = 1$ (or 3 in the case of weight), and used to determine the growth patterns of a specific body part in relation to CL. Equations were subsequently linearized ($\log_y = \log_a * \log_x - b$). The morphometric relationships that presented differences in the growth patterns were submitted to a non-hierarchical analysis of K-means clustering, minimizing the variability within the groups and maximizing the variation between them. The result of this analysis was refined by applying a discriminant analysis. The inflection point between the groups identified by the discriminant analysis was considered as the morphometric SOM, i.e. the smallest adult in population (Sampedro et al. 1999, Pantaleão et al. 2012). After the determination of groups, differences between slopes (b) and intercepts (a) of lines at each development stage were compared using Covariance analysis (ANCOVA) ($\alpha = 0.05$). All calculations and statistical analyzes

were performed with the software R version 3.3.1. (R CORE TEAM 2016).

Histology and physiological maturity

For the determination of the physiological maturity, all males and females of the subsamples of each population had their reproductive systems dissected. The macroscopic morphology of the reproductive systems was analyzed under stereomicroscope. The physiological SOM in the different populations was established by the smallest CL recorded for the animals that reached the following criteria: (a) males with spermatozoa in their testes and vasa deferentia (VD), and (b) females with oocytes in exogenous or secondary vitellogenesis (with yolk granules) (Viau et al. 2006, Poljaroen et al. 2010, Soonklang et al. 2012, Zara et al. 2012, 2013, Nascimento & Zara 2013). The presence of spermatozoa in the testes and VD, as well as that of vitellogenic oocytes in the ovarian lobes, was verified by smears of these structures (obtained from animals preserved in ethanol) under light microscope (Cuzin-Roudy & Amsler 1991, Paschoal & Zara 2017, 2018). In order to confirm

the smears results, ten recently-collected individuals of both sexes from each population showing different macroscopical stages of sexual development were anesthetized by chilling (-20 °C / 5 min.) and dissected. After the dissection, the animals had fragments of their reproductive systems fixed in 4% buffered-paraformaldehyde (24 hours). Subsequently, they were washed twice in the same 0.2 M sodium phosphate buffer (pH 7.2), dehydrated in an increasing series of ethanol (70 to 95%), and embedded and included in glycol-methacrylate historesin Leica®. Blocks were cut in 4-5 µm sections with a rotary microtome. After the microtomy, the slides were stained with hematoxylin and eosin (H&E) for general histological description (Zara et al. 2012, 2013).

Functional maturity

For *M. amazonicum* females, functional maturity was determined by the smallest CL recorded for ovigerous females captured in the field for each population (Viau et al. 2006). In males, functional maturity was considered as the ability to copulate and transmit ejaculate (Elner & Beninger 1995). For this purpose, animals of the SJB and CRC populations were analyzed by *ad libitum* sampling in the laboratory (one set per population), in order to verify the mating behavior and copulation. Due to the gregarious behavior of these SS populations (L.R.P. Paschoal & F.J. Zara personal data), 90 prawns of each population (N: 180) were kept for a month in six aquariums with dark bottoms (45 cm x 25 cm x 30 cm), with basalt gravel as substrate and macrophytes and fragments of rocks for shelter. Each aquarium contained 15 females and 15 males. Male prawns had their cephalothorax marked with a mixture of fast-drying glue gel (Super Bonder Power Flex Gel®) and silver purpurin powder. Prawns were acclimated and conditioned to a 12 hour photoperiod and

26 °C (± 0.5). For CAS and SBG populations, showing the presence of males with submissive and dominant morphotypes (see below), and nocturnal and cryptic habits (L.R.P. Paschoal & F.J. Zara personal data), the LS prawns were observed in the field for six hours (18:00 to 0:00 h) at each monthly sample, for observation of pair formation and copulation. For nocturnal observations, LED spotlights (up to 5 m in range) were positioned near to the margins of the aquatic environments. When the copulation success was verified, the pairs were immediately captured with a hand net (0.13 mm mesh). All four morphotypes were examined with regard to mating behavior and reproductive probabilities. After the evaluation of the mating behavior in the populations, the functional SOM in males was established by the smallest CL recorded for the individual captured, showing the necessary characteristics for the successful copulation. The types of mating systems observed in *M. amazonicum* were analyzed and classified according to Correa & Thiel (2003).

RESULTS

Morphometric analysis, morphotypes and morphometric maturity

A total of 14,375 individuals were collected during the 15 months analyzed at the four sampling sites. Subsamples containing 5,335 animals were analyzed: CAS population - 307 ♂ and 1,276 ♀, SBG population - 664 ♂ and 559 ♀, SJB population - 1,090 ♂ and 553 ♀, and CRC population - 441 ♂ and 445 ♀. Table I summarizes the morphometric variables measured for all individuals. Females were larger and heavier than males in all populations, and they also possess larger body structures. The only exception was the chelipeds and their articles (propodus and dactyls) in males of CAS and SBG populations (Table I). The presence of morphotypes in males of these

Table I. Mean (\pm standard deviation - SD), minimum (Min) and maximum (Max) values of the analyzed morphometric variables of male and females of *Macrobrachium amazonicum* in populations with large (LS) and small (SS) size phenotypes. 2° PL, width of the second abdominal pleura. AL, abdomen length. CAS, Cássia. CL, carapace length. CRC, Carmo do Rio Claro. DCL, dactyl length. MCL, major cheliped length. PPL, propodus length. SBG, São João Batista do Glória. SJB, São José da Barra. TL, total length. W, fresh weight.

Population (phenotype)	Variables	Males				Females			
		Mean	SD	Min	Max	Mean	SD	Min	Max
CAS (LS)	CL (mm)	13.05	5.08	3.60	24.90	14.80	3.41	5.10	28.70
	TL (mm)	42.10	14.00	12.10	76.40	48.23	10.13	17.20	85.90
	AL (mm)	22.43	7.10	6.70	40.50	25.79	5.23	10.20	43.70
	2° PL (mm)	5.08	1.73	1.30	9.22	7.06	1.75	2.00	12.10
	MCL (mm)	31.63	20.31	10.20	108.90	28.33	8.16	7.00	79.40
	PPL (mm)	8.20	7.24	1.80	39.00	6.24	2.27	1.30	25.20
	DCL (mm)	3.91	3.39	0.80	19.80	2.95	1.06	0.90	11.90
	W (g)	1.685	1.694	0.018	8.668	1.979	1.217	0.072	10.045
SBG (LS)	CL (mm)	9.02	2.56	4.20	23.50	10.59	3.08	4.50	19.10
	TL (mm)	32.45	7.56	15.30	68.80	36.96	9.68	11.90	63.00
	AL (mm)	17.72	3.90	8.10	34.70	20.10	5.20	8.80	33.60
	2° PL (mm)	3.83	0.96	1.75	8.10	5.02	1.82	1.50	9.80
	MCL (mm)	18.02	9.56	7.30	87.60	19.64	6.44	6.55	40.40
	PPL (mm)	3.95	3.23	1.20	28.80	4.35	1.72	1.90	10.50
	DCL (mm)	1.86	1.55	0.70	12.90	2.00	0.79	0.80	4.90
	W (g)	0.586	0.629	0.039	6.001	0.967	0.786	0.043	4.581
SJB (SS)	CL (mm)	6.90	1.05	3.70	11.30	8.15	1.24	4.10	13.40
	TL (mm)	25.33	3.52	11.90	40.70	28.55	4.34	10.09	48.90
	AL (mm)	13.98	1.79	6.30	21.30	15.60	2.27	8.60	21.60
	2° PL (mm)	2.89	0.44	1.30	4.70	3.90	0.88	1.80	7.60
	MCL (mm)	13.32	2.36	5.60	28.20	15.39	2.63	5.10	23.30
	PPL (mm)	2.72	0.44	1.50	5.40	3.33	0.56	1.40	5.10
	DCL (mm)	1.27	0.20	0.70	2.20	1.49	0.25	0.65	2.70
	W (g)	0.222	0.100	0.035	0.967	0.358	0.160	0.051	1.829
CRC (SS)	CL (mm)	7.31	1.14	4.10	10.50	8.65	1.72	4.10	14.30
	TL (mm)	26.11	3.94	15.10	37.20	30.07	5.73	14.50	43.80
	AL (mm)	14.55	2.43	8.20	26.80	16.43	3.14	8.00	24.00
	2° PL (mm)	3.10	0.53	1.70	4.45	4.18	1.13	1.60	7.00
	MCL (mm)	13.41	2.60	2.50	20.20	15.79	3.62	7.50	24.40
	PPL (mm)	2.83	0.50	1.00	4.60	3.43	0.76	1.50	5.55
	DCL (mm)	1.32	0.23	0.70	2.00	1.56	0.37	0.70	3.15
	W (g)	0.263	0.117	0.042	0.846	0.446	0.244	0.046	1.345

populations was confirmed by PCA's, resulting in a clear separation of morphological groups (Supplementary Material - Fig. S1). The first two axes of PCA's retained most of the data variability and provided good explanations (CAS: 98.05%, SBG: 97.92%, SJB: 89.85% and CRC: 87.35%). Major cheliped length (MCL) was the morphometric variable with the largest contribution in Axis 1 for CAS and SBG populations, while for SJB and CRC populations it was the total length (TL) (Appendix I). Linear regressions were adjusted using the variable with the greatest contribution

in PCA's (Fig. 2). Due to this, it was possible to verify the presence of two phenotypes, referred here as large-size phenotype (LS) in CAS ($\bar{CL} = \text{♂}: 13.05 \pm 5.08, \text{♀}: 14.80 \pm 3.41 \text{ mm}$) and SBG ($\bar{CL} = \text{♂}: 9.02 \pm 2.56, \text{♀}: 10.59 \pm 3.08 \text{ mm}$) populations, and small-size phenotype (SS) for SJB ($\bar{CL} = \text{♂}: 6.90 \pm 1.05, \text{♀}: 8.15 \pm 1.24 \text{ mm}$) and CRC ($\bar{CL} = \text{♂}: 7.31 \pm 1.14, \text{♀}: 8.65 \pm 1.72 \text{ mm}$) populations.

For males of LS populations, the relationship that best demonstrated a clear difference between juveniles and adults, and separated the morphotypes was MCL vs. CL. The

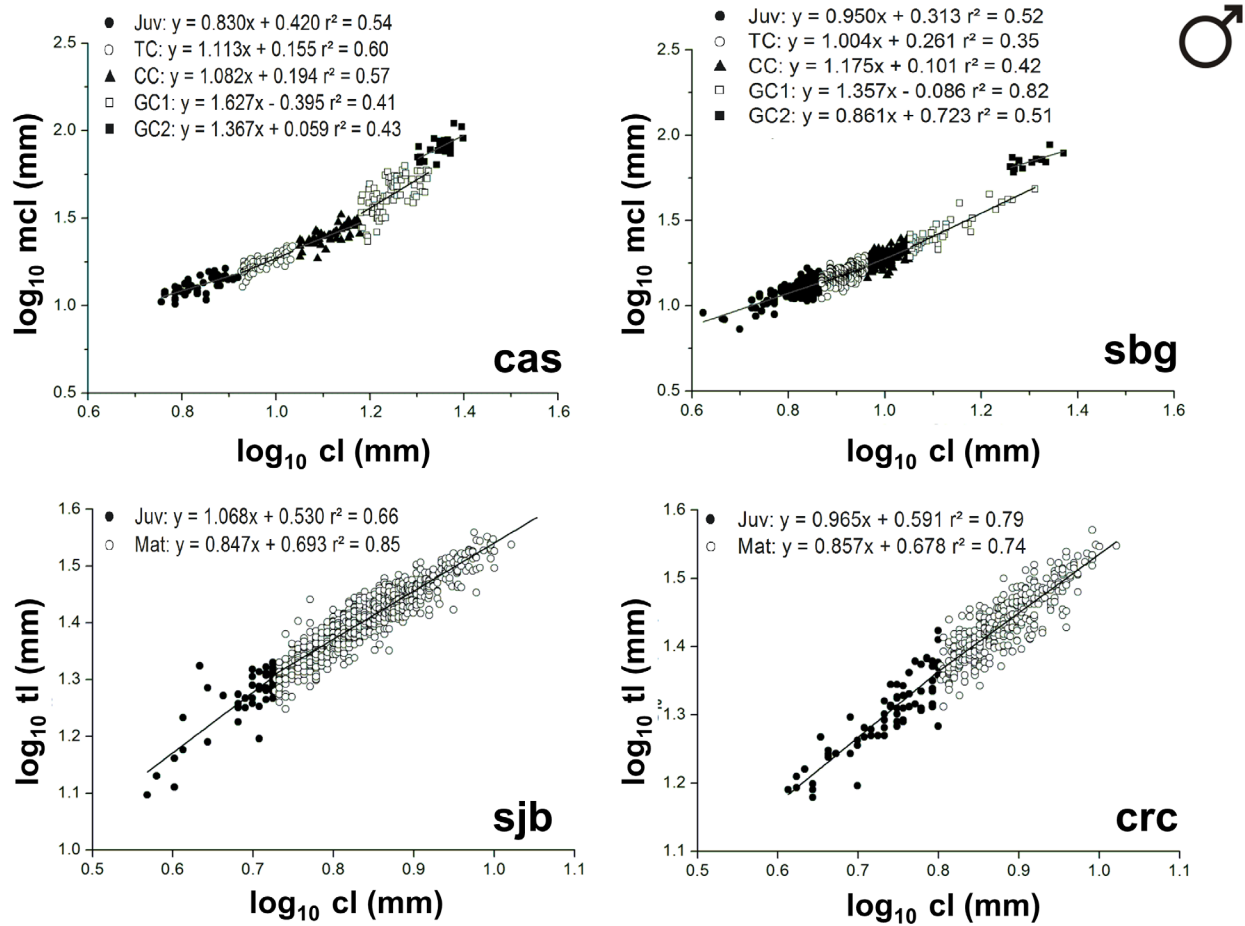


Figure 2. Dispersion of points for the morphometric relationship mcl vs. cl for *Macrobrachium amazonicum* males in large-size phenotype populations (cas and sbg), showing the separation of morphotypes and the size at onset of morphological maturity. For the small-size phenotype populations (sjb and crc) the morphometric relationship tl vs. cl shows the morphological maturity, without the presence of morphotypes. cas, Cássia. CC, cinnamon claw. cl, carapace length. crc, Carmo do Rio Claro. GC1, green claw 1. GC2, green claw 2. Juv, juveniles. Mat, matures. mcl, major cheliped length. sbg, São João Batista do Glória. sjb, São José da Barra. TC, translucid claw. tl, total length.

morphometric SOM in LS males was estimated at 8.40 mm CL for CAS population, and at 7.40 mm CL for SBG population (Fig. 2 and Table II). The ANCOVA results showed that there were significant differences for the intercepts (*a*) in all morphotypes of LS populations. However, only the morphotypes CC vs. GC1 in both populations showed differences in slopes (*b*) indicating that the other morphotypes have similar relative growth rates when compared to each other (Appendix II). Additionally, it was possible to demonstrate that only the GC1 morphotype showed positive allometry for MCL vs. CL relationship (Appendix III). Both LS populations showed similar relative growth parameters and allometric levels, demonstrating the investment in sexual weapons (chelipeds and their articles) (Appendices II and III). On the other hand, in SS males without morphotypes, the TL vs. CL relationship demonstrated the morphometric SOM at 5.30 mm CL in SJB population, and at 6.30 mm CL in CRC population (Fig. 2 and Table II). The results of ANCOVA's show that in SJB population, immature and mature SS males have different relative growth rates for almost all morphometric relationships, whereas in

CRC population these rates are quite similar (Appendix II). The allometric levels of these populations are similar, showing that adult animals tend to invest less energy for body growth than juveniles (Appendix III).

Similar to LS males, LS females also invested energy on growth of the chelipeds. The relationship that best demonstrated the differences between juveniles and adults was MCL vs. CL. The morphometric SOM was recorded at 10.50 mm CL in CAS population, and at 10.30 mm CL in SBG population (Fig. 3 and Table II). The results of the ANCOVA's showed that for the SBG females, the growth rates for chelipeds and their articles are divergent along maturation, whereas for CAS females the allometric coefficient rates are similar (Appendix IV). Adult females of both LS populations showed positive allometry for chelipeds and their articles (Appendix V). In females of SS populations, the relationship that best demonstrated the sexual maturity was 2° PL vs. CL. The morphometric SOM was recorded at 7.30 mm CL in SJB population, and at 8.30 mm CL in CRC population (Fig. 3 and Table II). In both populations, the body growth (TL) is similar between immature and mature females,

Table II. Morphometric (Morp.), physiological (Phys.) and functional (Func.) maturities values in both sexes of *Macrobrachium amazonicum* in large-size (LS) and small-size (SS) phenotype populations, as well as the number of ovigerous females captured under to the size estimated for morphometric maturity (OVG). CAS, Cássia. CRC, Carmo do Rio Claro. N, number of animals. SBG, São João Batista do Glória. SJB, São José da Barra.

Sex	Population (phenotype)	N	Morp.	Phys.	Func.	OVG
Male	CAS (LS)	307	8.40	5.70	14.40	-
	SBG (LS)	664	7.40	4.60	15.20	-
	SJB (SS)	1,090	5.30	3.70	3.70	-
	CRC (SS)	441	6.30	4.00	4.00	-
Female	CAS (LS)	1,276	10.50	6.70	9.40	6
	SBG (LS)	559	10.30	6.70	9.40	2
	SJB (SS)	553	7.30	5.00	7.10	1
	CRC (SS)	445	8.30	5.00	7.40	12

however they exhibit differences in abdominal pleura width (2° PL) growth rates during the maturation (Appendix IV). Adult females of the SS populations presented positive allometry for the width of the second abdominal pleura (2° PL), while in the LS populations, this condition was registered only in females of the SBG population. In CAS population, females showed negative allometry for this relationship (Appendix V).

Physiological maturity

The smallest size at the onset of physiological (histological) maturity recorded in LS males was 5.70 mm CL in CAS population, and 4.60 mm CL in SBG population, whereas for the SS males it was 3.70 mm CL in SJB population, and 4.00 mm CL in CRC population (Table II). No anatomical or histological differences were observed in the male reproductive system for studied populations or between the four morphotypes, except for CC males that show a very low concentration of spermatozoa in their

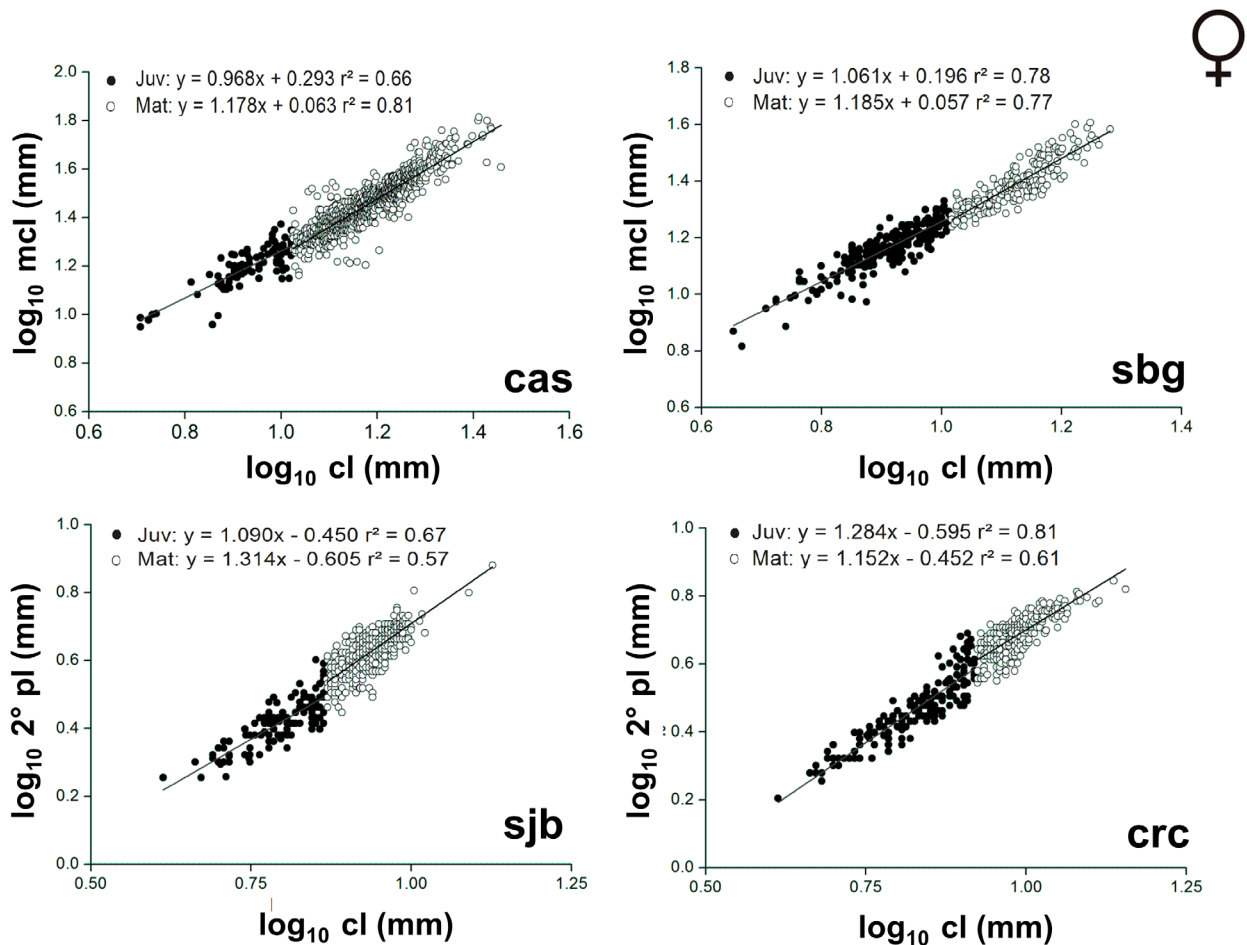


Figure 3. Dispersion of points for the morphometric relationship mcl vs. cl in immature and mature females of *Macrobrachium amazonicum* in large-size phenotype populations (cas and sbg), and 2° pl vs. cl for small-size phenotype populations (sjb and crc). 2° pl, width of the second abdominal pleura. cas, Cássia. cl, carapace length. crc, Carmo do Rio Claro. Juv, juveniles. Mat, matures. mcl, major cheliped length. sbg, São João Batista do Glória. sjb, São José da Barra.

testes (Supplementary Material - Fig. S2). In *M. amazonicum*, the reproductive system consists of a pair of testes and vasa deferentia (VD) connected to the gonopores of the coxopodites of the fifth pair of pereopods (Fig. 4a-b). The testes exhibit thick anterior lobes arranged over the hepatopancreas or perigastric organ (Fig. 4a), while the posterior lobes are thinner, located below the heart. These organs are connected to the VD, which are arranged laterally in the cephalothorax, connecting to the gonopores of the fifth pair of pereopods (Fig. 4b). In mature animals, the testes and VD are whitish and quickly identified macroscopically (Fig. 4a-b). These organs in immature animals are thin, colorless and cannot be distinguished macroscopically. Testes smears from immature animals showed only spermatogonia and the spermatozoa were absent in VD. In physiologically mature males, it is noted by histology that the testes are formed by several seminiferous tubules grouped in lobules with cells in spermatogenesis and spermiogenesis (Fig. 4c-d), and the VD are filled by secretion and spermatozoa (Fig. 4e-f). Testes smears and histology in mature animals show typical tack-shaped spermatozoa with a very prominent spike filling the seminiferous tubules, whereas only a small peripheral area of the tubules shows spermatogonia (Fig. 4c-d). The seminiferous tubules are surrounded by accessory (Sertoli) cells and involved by connective tissue (Fig. 4d). The VD is divided into three regions: proximal, medial and distal, these regions are differentiated only by the increase of the vessel caliber. All regions show the same histological aspect, vary from squamous to cubic epithelium with basal nuclei enveloped laying on the musculature (Fig. 4e-f). Inside the lumen of VD in physiologically mature animals, a large amount of centrally compacted spermatozoa is surrounded by secretion forming a single sperm mass involved by acidophilic secretion,

easily noted in the distal region of the VD, the ejaculatory ducts (ED) (Fig. 4e-f). This final portion of VD is much dilated (i.e. bulbs of the ED), which have thicker musculature than the other regions (Fig. 4e-f) and the androgenic glands are arranged perpendicularly to the muscular layer on ED (Fig. 4e). These glands are well-developed in mature animals, whereas in immature animals they are extremely reduced or absent.

The smallest size at the onset of physiological maturity recorded for females of the two LS populations was 6.70 mm CL, while for the two SS populations it was 5.00 mm CL (Table II). The physiological maturity is marked by the colorimetric and volumetric transformation of the ovaries throughout their development and progressive accumulation of yolk granules in the cytoplasm of oocytes (Fig. 5a-i). The immature ovaries are colorless or translucent before reaching physiological maturity (Fig. 5a). These organs are characterized by the presence of many oogonia, previtellogenic oocytes (meiosis) and oocytes in primary vitellogenesis without mature yolk granules (Fig. 5b-c). At this stage, the oocytes in primary vitellogenesis show a large rounded nucleus with few blocks of heterochromatin dispersed and one or two well evident nucleoli. The cytoplasm is strongly basophilic and may have small vesicles or acidophilic dilatations that characterize the endogenous yolk (Fig. 5b-c). When females attain the physiological maturity, they show for the first time the greenish coloration of the mature ovaries, a macroscopic evidence of sexual maturity in this species (Fig. 5d). This color is due to the presence of oocytes in exogenous (secondary) vitellogenesis, with mature yolk granules accumulated in the cytoplasm (Fig. 5e-f). At the beginning of this stage, large follicular cells surround the secondary vitellogenic oocytes. The nucleus becomes smaller, due to

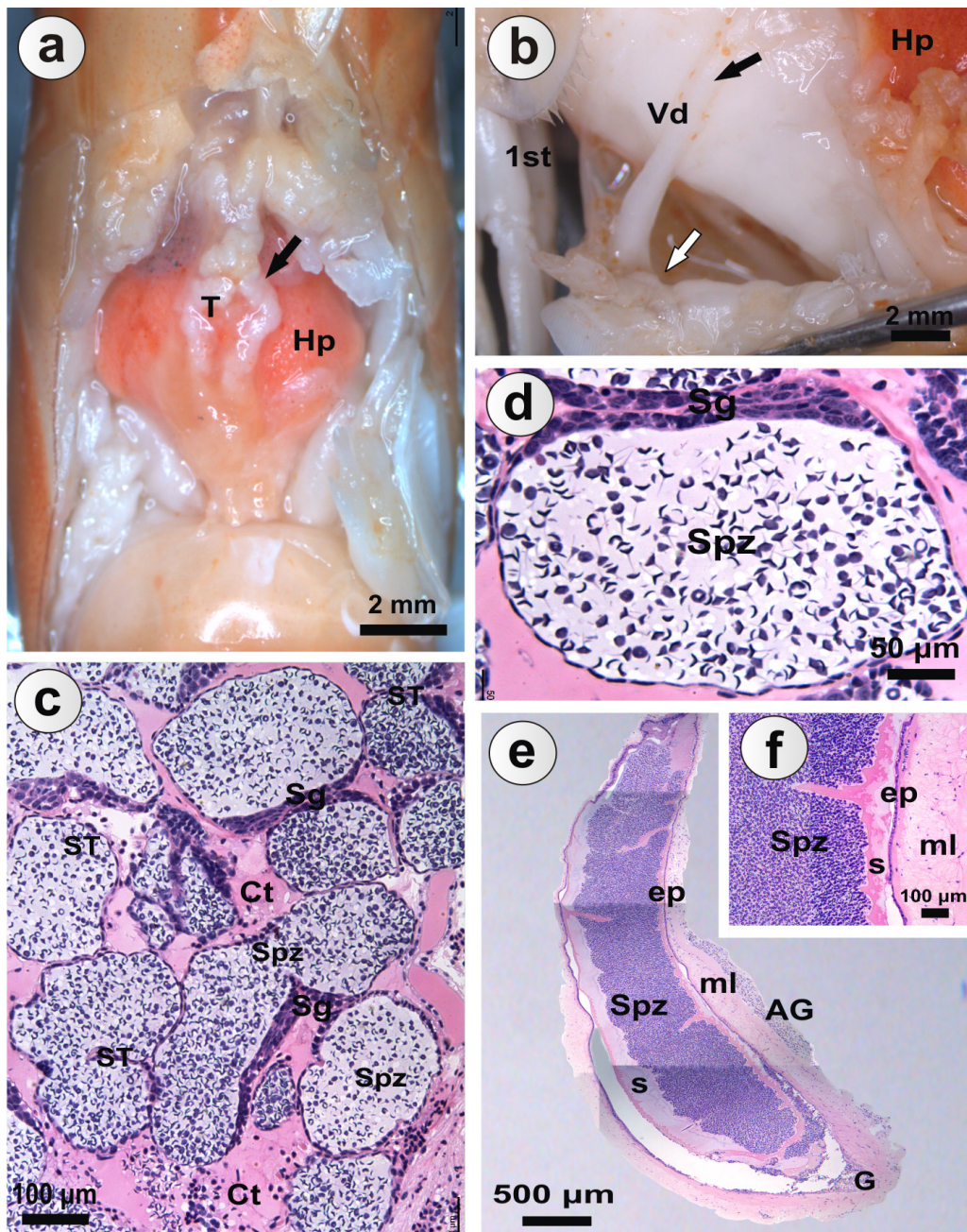


Figure 4. a. Dorsal view of the male reproductive system in *Macrobrachium amazonicum*, showing the testes with their thick anterior lobes (black arrow) arranged over the hepatopancreas. b. Lateral view of the cephalothorax of a male, showing the vas deferens (black arrow) opening into the gonopore of the coxopodite of the fifth pereiopod (white arrow). c. General aspect of the seminiferous tubules of the testis, with a large quantity of spermatozoa filling the lumen and a small peripheral area containing spermatogonia. d. An isolated seminiferous tubule filled with the tack-shaped spermatozoa in the lumen. e-f. Longitudinal section of the distal region of the vas deferens filled with spermatozoa surrounded by secretion. This region has a thick muscular layer and shows large amounts of centrally compacted spermatozoa. 1st, first pleopods. AG, androgenic gland. ep, epithelium. Ct, connective tissue. G, genital pore. Hp, hepatopancreas. ml, muscle. s, secretion. Sg, spermatogonia. Spz, spermatozoa. ST, seminiferous tubules. T, testes. Vd, vas deferens.

the process of compaction promoted by the yolk granules. These granules are acidophilic and few lipid droplets are detected at this stage. The previtellogenic oocytes are still easy to find in the ovary (Fig. 5e). At the end of maturation, the ovaries occupy a large area of the coelomic cavity in the cephalothorax and exhibit an intense greenish coloration (Fig. 5g). At this stage, the oocytes are well-developed and have a larger diameter when compared to the previous stages (Fig. 5h-i). The main feature of these oocytes is the cytoplasm filled with acidophilic mature yolk granules and many lipid droplets. In this ovarian stage, the oocytes are surrounded by flattened follicular cells and are well adhered to each other, producing a more compact appearance (Fig. 5h-i).

Functional maturity

In all studied populations, ovigerous females were captured during the 15 months of study, indicating a pattern of continuous reproductive activity. The smallest size recorded for ovigerous females in SS populations was 7.10 mm CL in SJB and 7.40 mm CL in CRC, whereas for LS females the smallest ovigerous had 9.40 mm CL in both populations. It is noteworthy that these females carrying eggs with eye pigmentation, i.e. fertilized eggs. Functional maturity was close to the morphometric maturity, with few ovigerous females captured under to the size estimated for morphological maturity (Table II). Laboratory mating (copulation) experiments carried out with SS populations were characterized by the “pure search” pattern (*sensu* Correa & Thiel 2003), with no signs of agonistic behavior among males. The animals tended to concentrate in one area and there were no pair formation. Males of this phenotype

had functional maturity simultaneously to histological maturity (Table II). Besides that, males with small sizes (CL \geq 3.70 mm) had a well-developed and functional *appendix masculina*, corroborating the functional maturity. On the other hand, copulations in LS populations were observed only after the pair formation in field. Thirty couples were captured and evaluated in CAS population, while in SBG population 20 couples were analyzed. It was possible to verify that only males with dominant morphotypes (GC1 and GC2) were able to copulate, although submissive males (TC and CC) were more abundant in both populations (morphotypes ratio: 4 TC: 3 CC: 2 GC1: 1 GC2). GC1 and GC2 males were observed near to the margin (up to 2 m) in aquatic environments with sandy sediment, and placed themselves behind or beside (in parallel) of receptive females. Dominant males protected and guarded females before and after copulation, and it was common to observe agonistic behaviors (fights) between males with hypertrophied chelipeds. During these fights, the male elevated its pereopods and the anterior portion of the body, and attacked its opponent with the dactyls of the second pair of chelipeds. Commonly, this event was short ranging from three to fifteen seconds (\bar{X} : 12 ± 3 seconds). Sneak copulation strategy was not observed in the LS populations during the 15 months of sampling. The mating system of these populations has the “neighborhoods of dominance” pattern (*sensu* Correa & Thiel 2003). The smallest size of males in LS populations captured after the observed copulation was 14.40 and 15.20 mm CL for CAS and SBG, respectively (Table II).

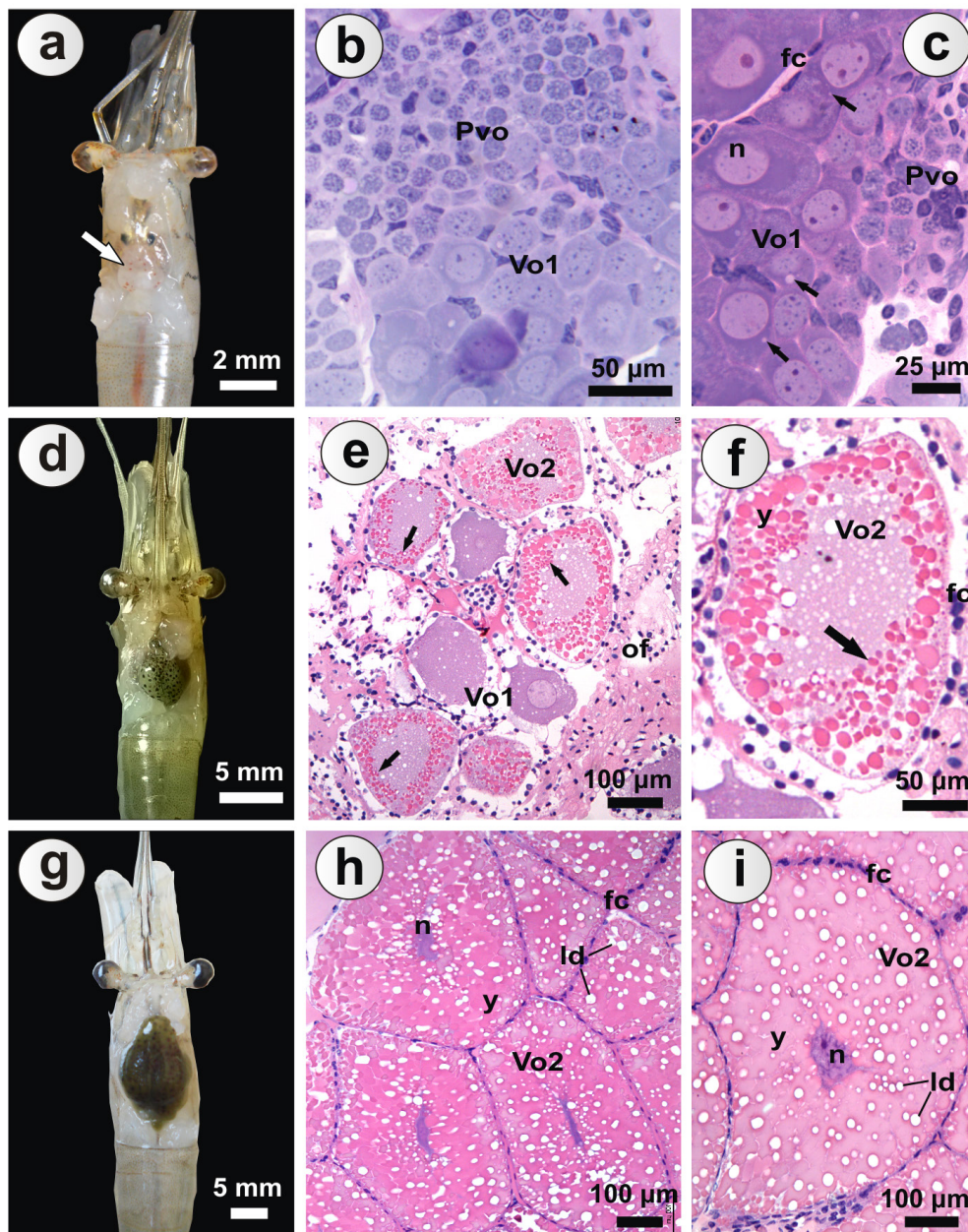


Figure 5. a. Dorsal view of the immature, colorless and small-sized ovary (white arrow) in *Macrobrachium amazonicum*. b-c. Cross-section of immature ovary with large quantity of previtellogenic and primary vitellogenic oocytes showing a strongly basophilic cytoplasm with small unstained vesicles (black arrows). d. Dorsal view of the ovary at the onset of sexual maturity. These organs occupy a small proportion in the cephalothorax and have a greenish coloration. e-f. Secondary vitellogenic oocytes showing mature acidophilic yolk granules (black arrows) accumulated in the cytoplasm. These oocytes are surrounded with large and ovoid follicular cells. G. Dorsal view of the mature ovary. These organs occupy a large area at cephalothorax and have an intense greenish coloration. H-I. Mature oocytes showing nucleus in the central region surrounded by many acidophilic yolk granules and lipid droplets. The follicular cells of these oocytes are flattened. fc, follicular cell. ld, lipid droplet. n, nucleus. of, ovarian follicle. Pvo, previtellogenic oocytes. Vo1, primary vitellogenic oocyte. Vo2, secondary vitellogenic oocyte. y, mature yolk granules.

DISCUSSION

The populations of *Macrobrachium amazonicum* analyzed in this study showed very distinct morphological and reproductive characteristics, indicating the great environmental adaptability and wide phenotypic plasticity of the species. Intraspecific variation of the reproductive strategy and SOM maximizes the reproductive success of the species in different aquatic environments (Odinetz-Collart 1991).

In two of the four populations analyzed, both adult males and females exhibited large body proportions, and these animals spent energy for the growth of chelipeds (i.e. large-size phenotype). In addition, the presence of morphotypes in males of these populations was verified through the analysis of this morphometric variable (MCL). In general, males of the genus *Macrobrachium* have higher body proportions than females, since they tend to invest more energy for somatic growth and the development of robust chelipeds (i.e. sexual weapons), increasing the success in intra/interspecific competitions (Correa & Thiel 2003, Moraes-Riodades & Valenti 2004, Paschoal & Zara 2018). The pronounced sexual dimorphism in *M. amazonicum* with the presence of social hierarchy confers adaptive advantages for dominant males (GC1 and GC2), such as: more success at obtaining food resources and dominating territories (Correa & Thiel 2003, Ibrahim 2011), and access to females available for mating, as observed in the present study. Thus, animals with larger sizes and robust sexual weapons would have greater reproductive success, explaining the positive allometric growth for MCL registered only for the GC1 morphotype. Since the morphotype development is sequential, it could be raised that animals with GC1 morphotype will concentrate more energy for the acquisition

of sexual weapons during the passage to the morphotype GC2, and after entering in this final morphotype they cease the somatic growth, allocating energy only for the maintenance of metabolic processes (Moraes-Riodades & Valenti 2004, Augusto & Valenti 2016). On the other hand, the increase of chelipeds size in females from LS populations would be related to the optimization of the reproductive events, as mating choices, protection and maternal care for hatching and spawning (Viau et al. 2006).

Currently, only the study of Pantaleão et al. (2014) found morphotypes in males of *M. amazonicum* from inland populations (Tietê River Basin, São Paulo - southeastern Brazil), being the present work the second record of this condition. These same authors estimated the morphometric SOM in males with morphotypes at 8.8 mm CL, a higher value than recorded here. However, the functional maturity analyzed here in populations with morphotypes was recorded with significantly higher values, showing that neither the physiological nor the morphological maturities actually express the moment when the LS males are able to copulate and transfer their gametes and/or defend females and territories. Regarding females, there are no studies evaluating morphometric maturity in populations with dominant morphotypes from inland waters or using the same methodology used here, which hinders comparison with other populations. However, the morphometric SOM in LS females is similar among the studied populations.

The absence of morphotypes, the lower energy investment for somatic growth and, consequently, the smaller body sizes were the main characteristics of males from the other two studied populations (i.e. small-size phenotype). However, the females of these populations invest energy for the growth of the second abdominal pleura. This pattern is

typical in caridean shrimps that adopted the “pure search” mating system, where males that do not protect or defend females invest energy for the production of gametes by shifting the energy that would be used for body growth, while females accumulate energy for the growth of the brood chamber (abdomen and pleuras) optimizing the fecundity and protection of embryos (Bauer 2004, Paschoal et al. 2013, 2016).

Pantaleão et al. (2012) also analyzed the morphometric aspects of small-size phenotype of the Amazon River prawn from inland environments (Tietê River Basin, São Paulo - southeastern Brazil). As recorded here, these authors did not register morphotypes in males, correlating the absence of morphotypes in SS populations to the fish predation and/or to the adoption of “pure search” mating system. Pantaleão et al. (2012) estimated the morphometric SOM for this species at 4.26 mm CL for males and 5.39 mm CL for females. These values are lower than those recorded in the present study. It can be verified that the morphometric maturity estimated here, when compared to the other criteria of maturity (physiological and functional) shows higher values, overestimating the SOM in *M. amazonicum*. Males of SS populations are mature and functional even with small sizes, thus the analysis of sexual maturity in *M. amazonicum* should take into account the general phenotype of the population. The wide variability of SOM values would justify the use of the physiological maturity in populations without morphotypes, because this is similar to functional maturity.

The male and female reproductive systems of *M. amazonicum* show the anatomical organization similar to that commonly described for gonochoric caridean shrimps (Sandifer & Lynn 1980, Bauer 2004). Histologically, it is possible to verify that the reproductive systems of the Amazon River prawn are similar to the

other species of the genus *Macrobrachium* (Chow et al. 1982, Sagi et al. 1988, Mossolin & Bueno 2002, Poljaroen et al. 2010, Soonklang et al. 2012). In both sexes of *M. amazonicum*, the physiological maturity anticipates the others maturities and is attained at small sizes (CL) within the analyzed populations. This shows that in these animals the physiological and morphological maturities are not synchronized, and they need their reproductive systems fully functional before the pubertal molt. This pattern is similar to that of other species of the genus *Macrobrachium* (Chow et al. 1982, Sagi et al. 1988, Mossolin & Bueno 2002, Poljaroen et al. 2010, Soonklang et al. 2012) and some brachyurans (Zara et al. 2012, 2013, Nascimento & Zara 2013). However, it is the opposite of that recorded for some aeglids (Viau et al. 2006) and penaeid shrimps (Heckler et al. 2013), where the morphological maturity anticipates the physiological maturity. In *M. amazonicum*, males become physiologically mature before females, and this may be associated with the short lifespan of male prawns. Gavio et al. (2006) and Paschoal et al. (2016) suggested that the caridean males reach their optimal reproductive condition at small sizes and are capable to copulate with larger females, but they disappear from the population due to the low longevity, while females with longer life spans remain in the population and continue to grow.

Only males with GC1 and GC2 morphotypes were able to copulate. Also, the sneak copulation was not observed in the LS populations of *M. amazonicum*. Ra'anán & Sagi (1985) suggested that the high frequency of large prawns in populations favors small sneaking males, since dominant males will be occupied with fights and territorial protection, which consumes a large amount of energy and time. However, if the frequency of dominant males is low (as observed in the present study), their relative reproduction

advantage over the submissive males increases, promoting a reproductive-dominance hierarchy (i.e. only dominant males are capable to mate and copulate). The mating system that best fits the LS populations of *M. amazonicum* would be the “neighborhoods of dominance” (*sensu* Correa & Thiel 2003), which would explain the high values of functional maturity recorded for males of these populations. Dominant males (GC1 and GC2 morphotypes) of these populations have hypertrophied chelipeds and it is possible to observe that these animals protect and guard females during the mating period. Moreover, these males destined a smaller portion of energy to sperm production, while a greater portion is directed to somatic growth and the production and maintenance of sexual weapons, increasing the fitness and improving the success of males in intraspecific competitions (Moraes-Riodades & Valenti 2004, Paschoal & Zara 2018). In contrast, males from the SS populations invest much less energy for somatic growth leading to absence of sexual weapons. However, they attain physiological and functional maturities at smaller sizes when compared to animals of LS populations, indicating that the energy that would be spent on growth is better used in reproductive events, such as sperm production (Paschoal et al. 2016, Paschoal & Zara 2018). Additionally, SS males are gregarious, without display both agonistic behavior and female guard. Thus, the mating system of SS populations has the “pure search” pattern (*sensu* Correa & Thiel 2003). In all populations of *M. amazonicum* analyzed, few ovigerous females (i.e. functionally mature) were captured under to the size estimated for morphometric maturity, indicating that there is synchronization between morphological and functional maturities in females. Thus, the use of relative growth or the evaluation of the smallest ovigerous female is indicated to estimate the SOM in female

palaemonid shrimps, as suggested by Paschoal et al. (2013, 2016).

CONCLUSIONS

The use of morphometric data showed to be a useful tool for the determination of distinct phenotypes and population stocks in *M. amazonicum*. The three criteria used for the evaluation of sexual maturity showed that, in both sexes, physiological maturity anticipates the others and that males reach sexual maturity in smaller sizes than females in all criteria, except for functional maturity in males of LS populations. The development of chelipeds (sexual weapons) in males, in addition to change the mating strategies in *M. amazonicum*, modulated the SOM in this species, since LS prawns needed robust chelipeds for pairing and copulation success. Thus, the sequential scheme of sexual maturity in *M. amazonicum* may be summarized as: ♀ - physiological → functional ≅ morphological, ♂_{LS} - physiological → morphological → functional, and ♂_{SS} - physiological = functional → morphological. The sexual maturity in *M. amazonicum* varies from one criterion to another, as well as between the two evaluated phenotypes, so we suggest that the best criterion used to estimate the SOM for the maintenance and preservation of the Amazon River prawn stocks is that registered with the highest value of CL. The information presented here may also be used in the evaluation and maintenance of farmed specimens and breeders, improvement of stocks, assessment of temporal series of breeders, as well as may be used in studies involving the population biology of the species in freshwater environments.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior

- Brazil (CAPES) - Finance Code 001. We thank CAPES program Ciências do Mar II (CIMAR II #1989/2014 proc. 23038.004309/2014-5) for the great support to FJZ during this study. FJZ also thanks the São Paulo Research Foundation (FAPESP) program JP 2005#04707-5 and Biota (Intercrusta #2018/13685-5) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq PQ2# 30331/2017-6). We thank Dr. Fernando L. Mantelatto, Dr. Rogério Costa, Dr. João Pantaleão and Dr. Abner Carvalho Batista for the constructive reviews and suggestions that improved the manuscript. This study complied with Brazilian regulations (MMA-ICMbio, license #47653-1 to LRPP, and MMA-ICMbio, permanent license #34587 to FJZ).

REFERENCES

- ANGER K. 2013. Neotropical *Macrobrachium* (Caridea: Palaemonidae): on the biology, origin, and radiation of freshwater-invading shrimp. *J Crust Biol* 33: 151-183.
- ANGER K, HAYD L, KNOTT U & NETTELMANN U. 2009. Patterns of larval growth and chemical composition in the Amazon River prawn, *Macrobrachium amazonicum*. *Aquaculture* 287: 341-348.
- AUGUSTO A & VALENTI WC. 2016. Are there any physiological differences between the male morphotypes of the freshwater shrimp *Macrobrachium amazonicum* (Heller, 1862) (Caridea: Palaemonidae)? *J Crust Biol* 36: 716-723.
- BAUER RT. 2004. Remarkable shrimps. Adaptations and Natural History of the Carideans. Oklahoma: University of Oklahoma Press, 282 p.
- BAUER RT. 2013. Amphidromy in shrimps: a life cycle between rivers and the sea. *Lat Am J Aquat Res* 41: 633-650.
- CHOW S, OGASAWARA Y & TAKI Y. 1982. Male reproductive system and fertilization of the palaemonid shrimp *Macrobrachium rosenbergii*. *Bull Jpn Soc Sci Fish* 48: 177-183.
- CORREA C & THIEL M. 2003. Mating systems in caridean shrimp (Decapoda: Caridea) and their evolutionary consequences for sexual dimorphism and reproductive biology. *Rev Chil Hist Nat* 76: 187-203.
- CUZIN-ROUDY J & AMSLER MOL. 1991. Ovarian development and sexual maturity staging in Antarctic krill, *Euphausia superba* Dana (Euphausiacea). *J Crust Biol* 11: 236-249.
- DUTRA FM, FORNECK SC, BRAZÃO CC, FREIRE CA & BALLESTER ELC. 2016. Acute toxicity of ammonia to various life stages of the Amazon River prawn, *Macrobrachium amazonicum*, Heller, 1862. *Aquaculture* 453: 104-109.
- ELNER RW & BENINGER PG. 1995. Multiple reproductive strategies in snow crab, *Chionoectes opilio*: physiological pathways and behavioral plasticity. *J Exp Mar Bio Ecol* 193: 93-112.
- GAVIO MA, ORENSANZ JM & ARMSTRONG D. 2006. Evaluation of alternative life history hypotheses for the sand shrimp *Crangon franciscorum* (Decapoda: Caridea). *J Crust Biol* 26: 295-307.
- HAYD L & ANGER K. 2013. Reproductive and morphometric traits of *Macrobrachium amazonicum* (Decapoda: Palaemonidae) from the Pantanal, Brazil, suggests initial speciation. *Rev Biol Trop* 61: 39-57.
- HECKLER GS, SIMÕES SM, LOPES M, ZARA FJ & COSTA RCD. 2013. Biologia populacional e reprodutiva do camarão sete-barbas na Baía de Santos, São Paulo. *Bol Inst Pesca* 39: 283-297.
- HENARES MNP, LIMA PRETO B, ROSA FRT, VALENTI WC & CAMARGO AFM. 2013. Effects of artificial substrate and night-time aeration on the water quality in *Macrobrachium amazonicum* (Heller 1862) pond culture. *Aquaculture Res* 46: 618-625.
- HENRY-SILVA GG, MAIA CSP, MOURA RST, BESSA JUNIOR AP & VALENTI WC. 2015. Integrated multi-trophic culture of Nile tilapia (*Oreochromis niloticus*) and Amazon River prawn (*Macrobrachium amazonicum*) in brackish water. *Arq Bras Med Vet* 67: 265-273.
- HUXLEY JS. 1950. Relative growth and form transformation. *Proc R Soc Lond B Biol Sci* 137: 465-469.
- IBRAHIM ANAF. 2011. Controle social do crescimento do camarão-da-amazônia *Macrobrachium amazonicum*. Master Thesis, Universidade Estadual Paulista - UNESP Jaboticabal, São Paulo, Brazil. (Unpublished).
- MACIEL CR & VALENTI WC. 2009. Biology, fisheries, and aquaculture of the Amazon River prawn *Macrobrachium amazonicum*: a review. *Nauplius* 17: 61-79.
- MACIEL CR & VALENTI WC. 2014. Effect of tank colour on larval performance of the Amazon River prawn *Macrobrachium amazonicum*. *Aquaculture Res* 45: 1041-1050.
- MORAES-RIOADES PMC & VALENTI WC. 2004. Morphotypes in male Amazon River prawns, *Macrobrachium amazonicum*. *Aquaculture* 236: 297-307.
- MORAES-VALENTI PM & VALENTI WC. 2010. Culture of the Amazon River prawn *Macrobrachium amazonicum*. In: New MB, Valenti WC, Tidwell JH, D'Abromo LR and Kutty MN (Eds). *Freshwater prawns: biology and farming*. Wiley-Blackwell, Oxford, p. 485-501.

- MOSSOLIN EC & BUENO SLS. 2002. Reproductive biology of *Macrobrachium olfersi* (Decapoda, Palaemonidae) in São Sebastião, Brazil. *J Crust Biol* 22: 367-376.
- NASCIMENTO FA & ZARA FJ. 2013. Development of the male reproductive system in *Callinectes ornatus* Ordway, 1863 (Brachyura: Portunidae). *Nauplius* 21: 161-177.
- NEW MB. 2005. Freshwater prawn farming: global status, recent research and a glance at the future. *Aquaculture Res* 36: 210-230.
- ODINETZ-COLLART O. 1991. Strategie de reproduction de *Macrobrachium amazonicum* en Amazonie Centrale (Decapoda, Caridea, Palaemonidae). *Crustaceana* 61: 253-270.
- ODINETZ-COLLART O & MOREIRA LC. 1993. Potencial pesqueiro de *Macrobrachium amazonicum* na Amazônia Central (Ilha do Careiro): variação da abundância e do comprimento. *Amazoniana* 122: 213-227.
- ODINETZ-COLLART O & RABELO H. 1996. Variation in egg size of the freshwater prawn *Macrobrachium amazonicum* (Decapoda, Palaemonidae). *J Crustacean Biol* 16: 684-688.
- PANTALEÃO JAF, CARVALHO-BATISTA A, TEODORO SSA & COSTA RC. 2018. The influence of environmental variables in the reproductive performance of *Macrobrachium amazonicum* (Heller, 1862) (Caridea: Palaemonidae) females in a continental population. *An Acad Bras Cienc* 90: 1445-1458.
- PANTALEÃO JAF, HIROSE GL & COSTA RC. 2012. Relative growth, morphological sexual maturity, and size of *Macrobrachium amazonicum* (Heller 1862) (Crustacea, Decapoda, Palaemonidae) in a population with an entirely freshwater life cycle. *Invertebr Reprod Development* 56: 80-190.
- PANTALEÃO JAF, HIROSE GL & COSTA RC. 2014. Occurrence of male morphotypes of *Macrobrachium amazonicum* (Caridea, Palaemonidae) in a population with an entirely freshwater life cycle. *Braz J Biol* 74: 223-232.
- PASCHOAL LRP, GUIMARÃES FJ & COUTO ECG. 2013. Relative growth and sexual maturity of the freshwater shrimp *Palaemon pandaliformis* (Crustacea: Palaemonidae) in northeast of Brazil (Canavieiras, Bahia). *Iheringia, Ser Zool* 103: 31-36.
- PASCHOAL LRP, GUIMARÃES FJ & COUTO ECG. 2016. Growth and reproductive biology of the amphidromous shrimp *Palaemon pandaliformis* (Decapoda, Caridea) in a Neotropical river from northeastern Brazil. *Zoologia* 33: epub 1-14.
- PASCHOAL LRP, OLIVEIRA LJF, ANDREOLI GC & ZARA FJ. 2019. Reproductive biology of *Macrobrachium amazonicum* (Heller, 1862) populations with distinct phenotypes in Neotropical reservoirs during the “El Niño” event. *Mar Freshwater Res* 70: 1465-1479.
- PASCHOAL LRP & ZARA FJ. 2017. First record of intersexuality in the Amazon River shrimp *Macrobrachium amazonicum* (Heller, 1862) (Caridea: Palaemonidae). *J Crust Biol* 37: 507-511.
- PASCHOAL LRP & ZARA FJ. 2018. Sperm count of *Macrobrachium amazonicum* (Heller, 1862) populations with distinct life histories, with introduction of a simple counting method. *Aquaculture* 491: 368-374.
- PILEGGI LG, MAGALHÃES C, BOND-BUCKUP G & MANTELATTO FL. 2013. New records and extension of the known distribution of some freshwater shrimps in Brazil. *Rev Mex Biodivers* 84: 563-574.
- POLJAROEN J, VANICHVIRIYAKIT R, TINIKUL Y, PHOUNGPETCHARA I, LINTHONG V, WEERACHATYANUKUL W & SOBHON P. 2010. Spermatogenesis and distinctive mature sperm in the giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879). *Zool Anzeiger* 249: 81-94.
- PRETO BL, KIMPARA JM, MORAES-VALENTI P & VALENTI WC. 2010. Population structure of pond-raised *Macrobrachium amazonicum* with different stocking and harvesting strategies. *Aquaculture* 307: 206-211.
- RA'ANAN Z & SAGI A. 1985. Alternative mating strategies in male morphotypes of the freshwater prawn *Macrobrachium rosenbergii* (De Man). *Biol Bull* 169: 592-601.
- R CORE TEAM. 2016. R (The R Project for Statistical Computing). Software version 3.3.1. URL: <<https://www.r-project.org/>>. Accessed 01.12.16.
- SAGI A, MILNER Y & COHEN D. 1988. Spermatogenesis and sperm storage in the testes of the behaviorally distinctive male morphotypes of *Macrobrachium rosenbergii* (Decapoda, Palaemonidae). *Biol Bull* 174: 330-336.
- SAMPEDRO MP, GONZÁLES-GURRIARÁN E, FREIRE J & MUIÑO R. 1999. Morphometry and sexual maturity in the spider crab *Maja squinado* (Decapoda: Majidae) in Galicia, Spain. *J Crustacean Biol* 19: 578-592.
- SANDIFER PA & LYNN JW. 1980. Artificial insemination of caridean shrimp. In: Clark Jr WH and Adams HS (Eds). *Recent Advances in Invertebrate Reproduction*. Elsevier, Amsterdam, p. 271-288.
- SANTOS MR, RODRIGUES CG & VALENTI WC. 2016. Effect of habitat diversity on population development of the Amazon River prawn. *J Shellfish Res* 35: 1075-1081.

SOONKLANG N, WANICHANON C, STEWART MJ, STEWART P, MEERATANA P, HANNA PJ & SOBHON P. 2012. Ultrastructure of differentiating oocyte and vitellogenesis in the giant fresh water prawn, *Macrobrachium rosenbergii* (de Man). *Microsc Res Tech* 75: 1402-1415.

VERGAMINI FG, PILEGGI LG & MANTELATTO FL. 2011. Genetic variability of the Amazon River prawn *Macrobrachium amazonicum* (Decapoda, Caridea, Palaemonidae). *Contr Zool* 80: 67-83.

VIAU VE, LÓPEZ-GRECO LS, BOND-BUCKUP G & RODRIGUEZ EM. 2006. Size at the onset of sexual maturity in the anomuran crab, *Aegla uruguayana* (Aeglididae). *Acta Zool* 87: 253-264.

WENNER EL, CONN III WP, SANDIFER PA & SHEALY JR MH. 1991. A comparison of species composition and abundance of decapod crustaceans and fishes from the North and South Edisto rivers in South Carolina. South Carolina Marine Research Center: Technical Report No. 78, 48 p.

ZARA FJ, GAETA HH, COSTA T, TOYAMA MH & CAETANO FH. 2013. The ovarian cycle histochemistry and its relationship with hepatopancreas weight in the blue crab *Callinectes danae* (Crustacea: Portunidae). *Acta Zool* 94: 134-146.

ZARA FJ, TOYAMA MH, CAETANO FH & LÓPEZ-GRECO LS. 2012. Spermatogenesis, spermatophore and seminal fluid production in the adult blue crab *Callinectes danae* (Portunidae). *J Crust Biol* 32: 249-262.

LUCAS R.P. PASCHOAL^{1,2}

<https://orcid.org/0000-0003-2461-4675>

FERNANDO J. ZARA^{1,3}

<https://orcid.org/0000-0002-7664-7674>

¹Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Estadual Paulista/UNESP, Instituto de Biociências de Rio Claro, Av. 24A, 1515, 13506-900 Rio Claro, SP, Brazil

²Faculdade de Tecnologia Nilo de Stéfani/FATEC, 14883-130 Jaboticabal, SP, Brazil

³Universidade Estadual Paulista/UNESP, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, Laboratório de Morfologia de Invertebrados/LMI, Departamento de Biologia Aplicada e Centro de Aquicultura (CAUNESP), Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884-900 Jaboticabal, SP, Brazil

Correspondence to: **Lucas Rezende Penido Paschoal**

E-mail: lucasrezende20@gmail.com

Author contributions

Each author have contributed significantly to the intellectual content of the work, specifically: Dr. LRPP - Conceptualization, Data sampling, analysis and interpretation, Writing original draft preparation, Writing-Reviewing and Editing, and Dr. FJZ - Conceptualization, Supervision and Writing-Reviewing last version.

SUPPLEMENTARY MATERIAL

Figure S1. and Figure S2.
Appendices I - V.

How to cite

PASCHOAL LRP & ZARA FJ. 2020. Size at onset of sexual maturity in *Macrobrachium amazonicum* (Heller, 1862) phenotypes: an integrative approach. *An Acad Bras Cienc* 92: e20180560. DOI 10.1590/0001-3675202020180560.

Manuscript received on June 4, 2018;
accepted for publication on November 6, 2018

