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Morphometric differences between two exotic invasive freshwater caridean species (genus *Macrobrachium*)

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ABSTRACT

The prawns *Macrobrachium amazonicum* and *Macrobrachium jelskii*, have been introduced in the southeastern Brazilian region. Thus, it becomes necessary knowing the reproductive aspects of these invasive species. This study aimed to analyze the relative growth and to estimate the morphological sexual maturity of the freshwater prawn species *M. amazonicum* and *M. jelskii*. We collected monthly samples from January through December 2010 in th Rio Grande riverbank. Prawns were measured to their cephalothorax length (CL), length of the pleura of the second abdominal somite in females (PL) and length of the appendix masculina (AML). We analyzed 2937 specimens of *M. amazonicum* (628 males and 2,309 females) and 2167 specimens of *M. jelskii* (504 males and 1663 females). We observed different allometric patterns in the relative growth of the AML and PL (both more accentuated in *M. amazonicum*) between the species. The present results indicate that these species adopt different reproductive strategies, possibly representing adaptations that may have facilitate their establishment in the Rio Grande riverbank. When comparing our results to the ones obtained in previous studies, we observed that the reproductive traits of both species indicate a marked phenotypic plasticity.

Introduction

Caridean shrimps have a great ecological importance in aquatic ecosystems (Magalhães 1999) due to the role they play as predators of other invertebrates (Kensley and Walker 1982) and fish eggs (Silva and Taddei 2014), besides serving as a prey for vertebrates (Magnusson et al. 1987). The genus Macrobrachium Bate, 1868 encompasses around 243 described species worldwide (De Grave and Fransen 2011), from which 45 are found in the American continent. According to Holthuis (2000), the species included in this genus have a circumtropical distribution, with native species in all the continents, except Europe. There are around 20 species registered in Brazil (Mantelatto et al. 2016), from which several are among the most economically important ones, such as Macrobrachium carcinus (Linnaeus 1758), Macrobrachium acanthurus (Wiegmann 1836) and Macrobrachium amazonicum (Heller 1862), (Valenti 1985), and the exotic invasive species Macrobrachium rosenbergii (De Man 1879), which was accidentally introduced as a consequence of their cultivation (Iketani **ARTICLE HISTORY**

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et al. 2016). *Macrobrachium* specimens are used as a protein resource by human obtained in nature-fishing activities or shrimp farming (Maciel and Valenti 2009; Bentes et al. 2014; Costa et al. 2016; Taddei et al. 2017a).

Among the native species of the genus *Macrobrachium* occurring in the Brazilian territory, *M. amazonicum* (Heller 1862), popularly known as 'Amazon river prawn' or 'canela prawn', has a wide geographical distribution, occurring in most of South American river basins (Holthuis 1952; Bialetzki et al. 1997; Anger 2013). However, it is native species from Amazonian rivers of the South Atlantic Basin and in the northern, north-eastern, and eastern coasts of Brazil, and thus, it is considered an exotic species in the Rio Grande river basin, where their introduction occurred as a consequence of anthropogenic dispersion (Magalhaes et al. 2005). Furthermore, there is a study emphasizing the impact that *M. amazonicum* may cause in fish species populations (Silva and Taddei 2014), and thus it may be considered an invasive exotic species.

Besides the possibility of disturbances in the food web, exotic species may act as competitors to species

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that occupy similar niches. In this sense, the 'Sossego prawn' *M. jelskii* (Miers 1877) is another Amazonian species introduced in the Rio Grande basin (Magalhaes et al. 2005). This species occupy the same niche with *M. amazonicum* in the Rio Grande river basin. Both species present economic interest, once they are used as baits in the hook fishery, as food source for the riverside populations, and for ornamental purposes in aquaria (Cirilo et al. 2011).

Concerning decapod crustaceans that inhabit continental waters, knowledge of their biology and the effects of invasive species in natural habitats is still poorly known. Facing this scenario, knowledge of their reproduction is important not only for natural stock maintenance (Valenti 1996), but also helping us in comprehending the mechanisms through which a species may generate a considerable environmental impact.

The reproductive biology of *M. amazonicum* and *M. jelskii* was previously investigated from other Brazilian regions: Scaico (1992) and Da Silva et al. (2004) to the North-eastern; Odinetz-Collart and Magalhães (1994), Lima et al. (2014) and Taddei et al. (2017b) to the Northern; Hayd and Anger (2013) to the Midwestern; Bialetzki et al. (1997) to the Southern; and Pantaleão et al. (2012) and Barros-Alves et al. (2012) to the South-eastern. These studies suggest that each species present a certain level of reproductive plasticity, that is the same species showed different reproductive aspects, which vary according to the studied area. Therefore, their reproductive aspects must be investigated, focusing the attention to the space-specific aspects.

This study provides reproductive information for the freshwater carideans *M. amazonicum* and *M. jelskii* sampled at the Rio Grande river basin, based on the relative growth and the sexual morphological maturity, using the secondary sexual morphometric features. Such features could serve as a tool for management and conservation proposals. Moreover, they could increase our knowledge on the influence of introduced species.

Material and methods

The *M. amazonicum* and *M. jelskii* specimens were sampled monthly from January through December 2010, in the Rio Grande river, south-eastern Brazil (20°11′03 "S-48°41′25"W). The sampling area is upstream of the Marimbondo Hydroelectric Plant, and downstream to the Porto Colômbia Plant. This area is semi-lotic, with

inner waters with typical characteristics, that is with no influences from the estuarine environment.

Sampling was performed during the night, trawling with a net along the marginal vegetation. The net we used had a 2-m width opening with 0.5-m height, 1-m length and mesh size 1 mm. For each sample, we performed 100-m trawls, against the river flow, covering a 200-m² sampled area. Prawns were conditioned in plastic bags, frozen and then, transported to the crustacean biology laboratory (Zoology Department, IBB, UNESP).

In the laboratory, specimens were identified to species (Melo 2003; Magalhaes et al. 2005). Afterwards, we took a 100-g subsample, of which individuals were measured. We assessed prawns' sex by observing the presence of the appendix masculina in the endopod of the second pleopod for males, using a stereomicroscope provided with an ocular micrometer (precision: 0.1 mm). We measured the individuals' cephalothorax length (CL), the highest length of the pleura of the second abdominal somite (PL), in females, and the appendix masculina length (AML).

We adopted CL as the independent variable, for both sexes; and AML and PL as the dependent variables, for males and females, respectively. Values were then log transformed and submitted to the K-Means hierarchic classification method, aiming to classify individuals into two groups: juveniles and adults (about K-Means, see more details in Sampedro et al. 1999; Corgos and Freire 2006; and Pantaleão et al. 2012).

The morphometric relationships were submitted to a regression analysis, adjusted to the exponential function $Y = aX^b$. The equations were linearized, resulting in the following formula: $\log Y = \log a + b \cdot \log X$, where 'X' corresponds to the independent variable (CL), "Y" represents the dependent variables (AML or PL), 'a' corresponds to the intercept in the y axis; and 'b' represents the allometric coefficient. When b = 1, growth is considered as isometric; when b > 1 or b < 1, it is considered as positive or negative allometric, respectively. We tested the b values using a Student's T test, with a 0.05 α -significance, as well as we tested the similarity between the intercept and the slope of the line, using a covariance analysis (ANCOVA, $\alpha = 0.05$) (Zar 2010).

To estimate the size at morphological sexual maturity, males and females of each species were analyzed separately. The size (CL) in which 50% of the individuals reached sexual maturity was estimated by the relative frequency (percentage) of adults in each size class, adjusted to a sigmoid curve based on the following equation: $y = \frac{1}{1+e^{(-r(CW-CWS0))}}$; where y is the estimated proportion of mature individuals and r is the logistic curve angulation coefficient. The equation was adjusted by the minimum squares method (Vazzoler 1996).

Results

With respect to *M. amazonicum*, we analyzed 2937 specimens of which 628 males and 2309 females; while to *M. jelskii* there were 2167 specimens, being 504 males and 1663 females.

The body size of *M. amazonicum* specimens varied from 3.8 through 15.6 mm CL (average size: 6.59 ± 1.39 mm); while the body sizes observed for *M. jelskii* ranged from 3.2 through 11.2 mm CL (average body size: 6.17 ± 1.71 mm). The difference observed between the average body size of each species was statistically significant (t = 9.671; p < 0.01).

Based on the K-Means hierarchic classification, the sampled population of *M. amazonicum* was sorted into 231 juvenile males, 397 adult males, 1091 juvenile females and 1218 adult females. The prawn *M. jelskii* sampled population was sorted into 214 juvenile males, 290 adult males, 1033 juvenile females and 630 adult females.

We observed an overlapping size range between adults and juveniles, for both sexes of each species. The largest *M. amazonicum* juvenile male had a CL of 7.4 mm, while the smallest adult, 5.4 mm CL; and the largest juvenile female had a CL of 7.6 mm, while the smallest adult a CL of 6.0 mm, coinciding with the size of the smallest ovigerous female. The largest *M. jelskii* juvenile male had a 7.1 mm CL, while the CL of the smallest adult was 4.1mm; the largest juvenile female was had a CL of 7.5 mm, while the smallest adult a CL of 5.5 mm, and the smallest ovigerous female, 6.5 mm CL (Table 1).

For *M. amazonicum*, the growth of the appendix masculina in relation to the carapace length presented a positive allometry in juveniles (b = 1.31; t = 4.77) and isometry in adults (b = 0.92; t = 1.61) (Figure 1(a)). The juveniles of *M. jelskii* presented isometry (b = 1.07; t = 0.72), while adults, negative allometry (b = 0.71; t = 4.81) (Figure 1(b)).

Concerning the females' growth of the pleura of the second abdominal somite, we observed a positive



Figure 1. Morphometric relation CL vs. AML in males, indicating the different straight lines for juveniles and adults, based on the k-means analysis. (a) *Macrobrachium amazonicum* (Heller, 1862); (b) *Macrobrachium jelskii* (Miers, 1877).

allometry in *M. amazonicum*, for both juvenile (b = 1.32; t = 16.87) and adult (b = 1.14; t = 8.18) specimens (Figure 2(a)). The juvenile females of *M. jelskii* showed isometry (b = 1.01; t = 0.40), while adult females, positive allometry (b = 1.37; t = 12.41) for this structure (Figure 2(b)).

All the growth relations revealed significant differences between juveniles and adults, for each species (p < 0.01, Table 2), indicating a difference in the growth rates of each analyzed structure, between juveniles and adults of each sex.

The estimated morphological sexual maturity sizes for *M. amazonicum* species were 6.2 and 6.5 mm CL for males and females, respectively (Figure 3); while for *M. jelskii*, 50% of the males and females were morphologically mature,

Table 1. Minimum and Maximum size of carapace length reached by *M. amazonicum* and *M. jelskii* from the Rio Grande River, north-western of Minas Gerais State, Brazil.

		CL (CL (mm)	
		M. amazonicum	M. jelskii	
Total	Size	From 3.8 through 15.6	From 3.2 through 11.2	
	Mean \pm SD	6.6 ± 1.4	6.2 ± 1.7	
Male	Largest juvenile	7.4	7.1	
	Smallest adult	5.2	4.1	
Female	Largest juvenile	7.6	7.5	
	Smallest adult	6.0	5.5	
	Smallest ovigerous female	6.0	6.5	

CL = carapace length; SD = standard deviation



Figure 2. Morphometric relation CL vs. PL in females, indicating the different straight lines for juveniles and adults, based on the k-means analysis. (a) *Macrobrachium amazonicum* (Heller, 1862); (b) *Macrobrachium jelskii* (Miers, 1877).

from 5.4 (Figure 4(a)) and 6.7 mm CL (Figure 4(b)) on, respectively (Figure 4).

Discussion

In *M. amazonicum* juveniles, the positive allometry observed for the appendix masculina may be related

Table 2. Results of the allometric relations studied in *M. amazonicum* and *M. jelskii*. We used ANCOVA to test the straight-line inclination and intercept for each sex that indicates the beginning of maturity in relation to the body size. (Numbers in parentheses mean number of individuals.)

Species	Relationship	Dependent Morphometric dimension	Maturity (CL ₅₀)	p
M. amazonicum	JF vs. AF	PL	6.5 mm	< 0.001*
	(1091) (1218) IM vs AM	AMI	62 mm	< 0.001*
	(231) (397)	, uvie	0.2 11111	< 0.001
M. jelskii	JF vs. AF	PL	6.7 mm	< 0.001*
	JM vs. AM	AML	5.4 mm	< 0.001*
	(214) (290)			

Notes: JF = juvenile female; AF = adult female; JM = juvenile male; AM = adult male.

The values of the significance (p) are given. PL = female's pleura of the second abdominal somite; AML = appendix masculinae. *Significant (p < 0.01).

to a higher energetic output into the growth of this secondary sexual character; thus, individuals may become reproductively able, even with a smaller body size. Such a redirection in the energetic output occurs more frequently in populations inhabiting inner continental areas (as observed by Pantaleão et al. 2012), unlike what is seen in estuarine areas, where males tend to direct their energy toward body growth (Hayd and Anger 2013).

Concerning the studied population, the isometry we observed for *M. amazonicum* adult males suggests that the growth of the appendix masculina tends to decrease in this life phase, thus growing proportionally to the body size from this moment on. It probably happens because, after reaching sexual maturity, males begin to perform reproductive activities. It is a phase when behavioural and ecological factors (such as the search and fight for females, the ability to handle



Figure 3. *Macrobrachium amazonicum* (Heller, 1862). Fit of the logistic function, indicating the CL in which 50% of the prawns are morphologically mature. (a) Males; (b) Females.



Figure 4. *Macrobrachium jelskii* (Miers, 1877). Fit of the logistic function, indicating the CW in which 50% of the prawns are morphologically mature. (a) Males; (b) Females.

them and the territory defence) modulate the reproductive success. In both cases, body size would be an important factor. Furthermore, Bauer (2004) suggests that, usually, morphologic and physiologically mature palaemonid males direct a greater energy output into producing a larger number of spermatozoa. Thus, growth for both body and other structures would be less intense.

Similarly, the appendix masculina's growth intensity in M. jelskii also tends to decrease after males reach sexual maturity. However, since this structure did not present a positive allometry in any developmental phase, it is suggested that this species has different reproductive strategies. It means that the size of the appendix masculina, compared to the individual's body size, may not have such a great importance for *M jelskii* as it has for M. amazonicum. It may indicate that M. jelskii has a more intense energetic output into the production of spermatozoa when compared to M. amazonicum, as proposed by Bauer (2004) for carideans with low energetic investment on appendix masculina. Possibly, this is a reproductive strategy that allows these species to survive in sintopy (i.e. at the same environment, without repelling each other).

Populations of *M. amazonicum* with traits similar to the ones we observed are common in semi-lotic environments, in which individuals occur in high population densities, with 'pure search' copulating systems, or with low-intensity pre-copulatory interactions, or agonistic combats among males (Bauer 2004; Pantaleão et al. 2012).

It is noteworthy that the results obtained for *M. amazonicum* are local peculiarities, especially if we consider that, in other regions, males of this species may show a greater investment in growing their body

size and other secondary sexual characters. In some cases, they can even display different morphotypes within a single population (Pmc and Valenti 2004; Pantaleão et al. 2014).

According to Maciel and Valenti (2009), the occurrence of such morphotypes is related to the local population traits. The morphotypes may mostly occur in populations with intense intraspecific competition and sex ratio close to 1:1 (Bentes et al. 2014). The absence of such morphotypes is more common in populations in which individuals show an aggregate distribution, as it occurs in muddy environments (Pantaleão et al. 2012), with a great abundance of food and females. This may decrease intraspecific competition (Taddei et al. 2017b), as seen for the individuals addressed here.

Females of both species showed positive allometry for the growth of the pleura of the second abdominal somite, after reaching maturity, revealing that this structure is especially important when individuals become adult. Besides providing space for incubation, the abdominal chamber may provide egg protection, optimizing the reproductive events (Bauer 2004).In this sense, one may observe extra difference between both species. While M. amazonicum shows positive allometry in both developmental stages, M. jelskii only does so during the adult stage. It demonstrates that females of *M. amazonicum* start driving energy into developing the pleura of the second abdominal somite since their juvenile stage. This characteristic, added to the largest size reached by this species, results in more space available to storing eggs (Bauer 2004).

Variations in the growth rate of the secondary sexual characters are frequently used to provide knowledge about the morphological sexual maturity in decapod crustaceans (Flores et al. 2002; Negreiros-Fransozo and Fransozo 2003; Silva et al. 2014). For both species, we observed that males reach sexual maturity with smaller body sizes when compared to females. This a common pattern concerning carideans, since larger females would be able to incubate a higher number of eggs (Anger and Moreira 1998; Bauer 2004). Furthermore, this pattern can be evidence that, in the Rio Grande region, males of both species probably do not get into aggressive combats for females.

In the studies carried out by Sampaio et al. (2007) and Pantaleão et al. (2012), both males and females of M. amazonicum reached morphological sexual maturity in smaller sizes when compared to the results presented here (5.0 mm CL for females (Sampaio et al. 2007); and 4.2 and 4.7 mm CL for males and females, respectively (Pantaleão et al. 2012)). By contrast, Freire et al. (2012) observed M. amazonicum at the Brazilian northern region, that both males and females of reached their maturity with larger sizes, i.e. 12.5 and 12.0 mm CL, respectively. Such variations were also observed for *M. jelskii* in the studies carried out in the South-eastern region by Taddei (2006) and the Midwest region by Soares et al. (2015), in which males and females became mature with 7.0 and 8.5 mm CL, respectively. The differences in the size at the onset of sexual maturity among populations of the same species may be related to their high phenotypic and physiologic plasticity, which can show variations related with environmental features (Hines 1989). Besides this, environmental factors such as temperature and salinity can also modulate the size at the onset of sexual maturity (Hines 1989).

Concerning this aspect, studies addressing reports on the size of ovigerous females may be useful when comparing the morphological sexual maturity size, since Bauer and Abdalla (2000) considered the presence and/or the extrusion of eggs in the females' pleopods as indicators of sexual maturity. Thus, we highlight the fact that the size of the smallest ovigerous female (*M. amazonicum* = 6.0 mm CL; *M. jelskii* = 6.5 mm CL) is similar to the morphological sexual maturity, calculated for *M. amazonicum* and *M. jelskii* females in our study (6.5 and 6.7 mm CL, respectively).

Despite the similarity concerning the morphological sexual maturity characteristics of both species, we could also observe a few differences which have contributed to the success of the colonization and establishment of these species in the area. Firstly, when comparing the CL_{50} between males and females of *M. amazonicum*, we observed very close values (difference: 0.3 mm), just the opposite of *M. jelskii* (difference = 1.3 mm). It means that *M. jelskii* couples may be formed with a smaller

male. Therefore males attaining sexual maturity in smaller sizes represent an advantage; since they are likely ready to copulate earlier. The females of *M. amazonicum* compensate by becoming sexually mature at smaller sizes, and after that, they keep growing, reaching a final larger body size (consequently, with more space available for egg incubation) when compared to *M. jelskii*. Therefore, *M. amazonicum* takes a certain reproductive advantage when compared to *M. jelskii*. Furthermore, *M. amazonicum* has shown higher fecundity (Da Silva et al. 2004; Costa et al. 2016) when compared to *M. jelskii* (Mossolin et al. 2013; Soares et al. 2015). It may justify the difference in the abundance between the two species, observed in this study.

However, it is not feasible estimating a model on how the interaction and dominance process will be taken between the studied species. Even though inexistent, studies addressing the temporal approach of these topics are of great importance predicting the environmental impacts resulting from the species' colonizing, replacing and competing capabilities. The results presented here concerning patterns on growth and size at morphological sexual maturity of *M. amazonicum* and *M. jelskii* may contribute to a better understanding of the reproductive biology of both species. Furthermore, it may be useful for further studies aiming to achieve the minimum capture size, improving the management of natural stocks and the sustainable use of the resources in the studied area.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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