

## Research Article

## Successful invasion of the Amazon Coast by the giant river prawn, *Macrobrachium rosenbergii*: evidence of a reproductively viable population

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### Abstract

*Macrobrachium rosenbergii* has been introduced into a number of countries, and in some of these, it has already been frequently captured in the wild. As for any other exotic species, its success in new habitats is related primarily to its reproductive capacity. For the present study we evaluated the reproductive capacity of an introduced population of *M. rosenbergii* in estuaries of the Amazon Coast, north Brazil. A total of 588 specimens (305 males and 283 females) were captured during 24 months. Of the females, 82 (28.9%) were incubating eggs, and 62 (21.9%) had recently spawned. In addition, more than 70% of the females had mature or maturing gonads. Mean fecundity was 55,000 eggs, with a minimum of 9,086, and a maximum of 192,172 eggs. Oviparous females were captured throughout the study period, indicating continuous reproduction. Adult males are known to present three morphotypes, all of which were observed in the present study, but with a predominance of the intermediary Orange Claw morphotype (48%). The body length and weight of males were greater than in females. Different size classes were observed for both sexes during the study period suggesting a continued recruitment of new individuals to the population over the time. The species has been harvested from the wild for more than ten years, being interrupted during the period when the river is flooded. This may function as a natural interval for the recovery of stocks, which may favor the establishment of a sustainable population over the long term. We conclude that the exotic population of *M. rosenbergii* in eastern Amazonia is capable of reproducing itself and probably has the capacity to persist over future generations, causing a strong impact on native species. Given this, it is important to continue monitoring the progress of this species and its potential effects on the biodiversity of the Amazon basin.

**Key words:** exotic species, biological invasion, prawn reproduction, Palaemonidae and Amazonia

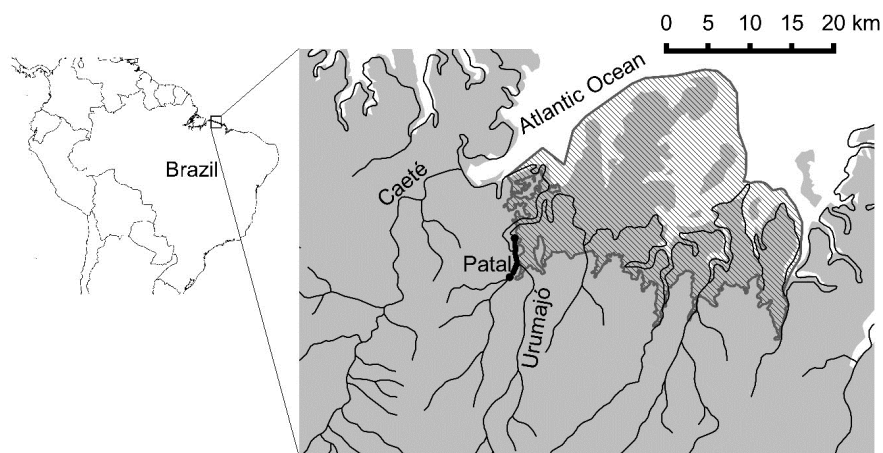
### Introduction

The giant river prawn, *Macrobrachium rosenbergii* (De Man, 1879), is a freshwater prawn known in Brazil as the Malaysian giant, Malaysian prawn or Hawaiian prawn. The natural range of this species is the South and Southeast Asia, as well as Indochina (Wowor and Ng 2007). But, the size, robust nature, high market value, and the availability of efficient culture techniques for this species, led to widespread aquaculture in many countries in the Americas, Africa, and parts of Europe (New and Kutty 2010). It is now one of the world's most heavily farmed freshwater species, with a total annual production of more than 200.000 tons in 2013 (FAO 2015).

The species was introduced into Brazil in the late 1970s by the Oceanography Department of the Federal University of Pernambuco, which imported post-larvae from Hawaii (Cavalcanti 1998). A decade later, no less than 32 commercial larviculture operations were active throughout Brazil (Valenti 1993). Now, outside of captivity, *M. rosenbergii* has been recorded in several Brazilian states (Cintra et al. 2003; Magalhães et al. 2005; Gazola-Silva et al. 2007; Loebmann et al. 2009; Iketani et al. 2011; Silva-Oliveira et al. 2011).

In Pará state, in the eastern Amazon Basin, the first locally produced post-larvae were distributed in 1985; in 1991 a governmental hatchery start activities, and the peak of production was reached in 1995 (Morales-Riodades et al., unpublished data).

**Figure 1.** Study area of Amazon coast, between the Patal and Urumajó rivers (thick line represent the fishing area, i.e., the interval between the coordinates: 01°04'29.1"S, 46°38'8.7"W to 01°02'01.3"S, 46°37'47.8"W). The diagonal lines cover the area of Arai-Peroba Marine Extractive Reserve in northeastern Pará, Brazil.



However, as local supplies were irregular, many prawn farmers have been forced to import post-larvae from other regions of the country. Currently, post-larvae are not produced in Pará state anymore, and there are only five working hatcheries in the rest of the country (New and Kutty 2010). The development of the prawn farming industry in Amazonia was also hampered by problems such as the difficulties of obtaining specialized equipment and personnel.

Soon after *M. rosenbergii* had been introduced for farming in Pará, specimens were captured in natural aquatic habitats in the northeastern coastal region of the state. These animals had escaped from poorly-maintained and unregulated holding tanks (Barros and Silva 1997). More recently, the number of records of the occurrence of *M. rosenbergii* in natural environments has increased considerably (Cintra et al. 2003; Iketani et al. 2011; Silva-Oliveira et al. 2011), and the species is now harvested and sold in local markets all over the coastal areas (Freire and da Silva 2008). However, it is still unknown whether a sustainable population has been established, i.e. a population capable of reproducing and surviving over the long term.

Given the expansion of *M. rosenbergii* in natural habitats on the Amazonian coast, the monitoring of its reproductive biology in this new environment is essential for the understanding of its potential threat to native ecosystems. Among the species of *Macrobrachium*, *M. rosenbergii* is one of the largest: males can reach a total length of 320 mm, the females of 250 mm (Brown et al. 2010). This great size allied to agonistic behavior (see Karplus and Sagi 2010 for review) can lead to competition for

space and food with at least seven native species of *Macrobrachium* (see geographic distribution in Pimentel and Magalhães 2014). The northeastern extreme of the Pará state has been the source of the largest number of records of the species in the wild. Thus, the present study focused on a medium sized estuary in this region, the Patal River (Figure 1), for the analysis of the reproductive biology of the species' feral population.

## Methods

### *Reproductive biology*

*M. rosenbergii* was collected within an area that lies between the Patal and Urumajó rivers (01°04'29.1"S, 46°38'8.7"W to 01°02'01.3"S, 46°37'47.8"W) in the Arai-Peroba Marine Extractive Reserve on the Amazon Coast, in northeastern Pará (Figure 1). This area is located between the Caeté River and the settlement of Emboraí. The mean annual precipitation is approximately 2500 mm, with a marked rainy season between January and June, and a dry season between July and December.

The specimens were collected using a cast-net, during low neap tides (diurnal ebb tide) until the water started rising. A canoe was used to travel between the two rivers (about 7km); with the assistance of an experienced local fisherman, resident in Patal village, whose primary source of income is the fishery of *M. rosenbergii*. Samples were obtained monthly (one day soon after the waxing crescent moon) between September 2007 and August 2009. Once captured, prawns were stored on ice and transported to the Aquaculture Laboratory at the Bragança Campus of the Federal

University of Pará. In the laboratory, the specimens were identified according to the classification of Dore and Frimodt (1987), and then sexed based on the presence of the sexual appendage on the second pair of pleopods of the males. Thus, all specimens were measured using a digital caliper (precision of 0.01 mm) and weighed on a digital balance (precision of 0.2 g). The total length (TL) was measured from the tip of the rostrum to the tip of the telson, and the length of the carapace (CL), from the orbit to the extremity.

The females were examined for the definition of their gonadal maturity, and presence of eggs adhered in the abdomen or possible signs of recent oviposition. When present, the eggs were removed and placed in a 2% solution of sodium hypochlorite for the complete dissociation of the material for counting and the calculation of fecundity. The number of eggs was estimated using sub-samples (a quarter of the material collected) extracted in a Falsom sub-sampler designed for studies of zooplankton (Boltovskoy 1981).

According to Ra'Anan and Sagi (1985) and Kuris et al. (1987), adult males of *M. rosenbergii* can be classified in three distinct morphotypes, which vary in terms of body size, coloration, and the arrangement of the spines on the second pair of pereopods: SM (Small Male), OC (Orange Claw), and BC (Blue Claw). Associated with morphotypes there is a dominance hierarchy (BC > OC > SM). This seemed to regulate priority of access to preferred areas (e.g. shaded protected crevices) (Karplus and Sagi 2010), which may impact in the native species. So, given the importance of this variation to understand the reproductive scenario of the population, all male specimens were carefully assigned to one of these morphotype classes.

Abiotic parameters, such as temperature and pH were also measured using a HACH multiparameter apparatus (HACH, Loveland, Colorado, USA); salinity was determined using a refractometer (Atago, Bellevue, WA, USA); precipitation data were obtained from the Tracuateua meteorological station (82145: 01°01'00" S, 46°54'00" W), located approximately 30 km from the study area.

### *Fisheries*

Fishermen resident within the Patal-Urumajó river system were interviewed using a questionnaire. This survey provided information on the time spent fishing *M. rosenbergii*, the equipment used, the mean daily catches, and the income obtained from the sale.

### *Data analysis*

The sex ratio was analyzed using a Chi-square ( $\chi^2$ ) test. Measurements relating to body length and weight were tested for homogeneity of variances and normality using the Kolmogorov-Smirnov statistic. Data were not normally distributed, so the Kruskal-Wallis analysis of variance was applied followed by Dunn's test multiple comparisons for time series. The relationship between carapace length and the number of eggs in the females was obtained by linear regression. To evaluate the TL variation through the year, histograms were made grouped sampling of two months, except between January to July of 2009 (for male) and January to June of 2009 (for females), due to the low number of individuals sampled, these months were put together.

## **Results**

### *Environmental parameters*

Generally, the rivers studied contained freshwater, while between November 2008, and February 2009, the water was oligohaline, with salinity between 1 and 3. The water temperature varied between 24.0°C and 29.5°C, with a mean of 27.0±1.4°C. The water was generally acid (mean pH = 6.24 ± 0.66), except in November 2008, and April 2009, when the pH was 7.4.

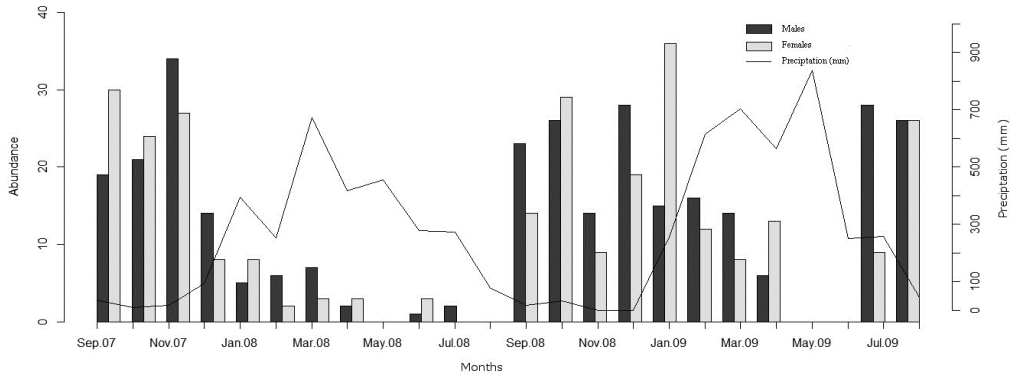
### *Reproductive biology*

#### Abundance and sex ratio

A total of 588 specimens of *M. rosenbergii* was collected, 305 (51.9%) males and 283 (48.1%) females. The species was most abundant in November 2007, when 61 prawns were sampled. Then, the catches declined and no animals were obtained in May and August 2008. In September 2008, 37 specimens were captured, and the numbers were relatively high until January 2009, when 51 specimens were sampled. Subsequently, the number of specimens captured each month decreased as the level of the river rose (in the middle of the rainy season) and none were captured in May and June 2009, but 38 and 53 prawns were obtained in July and August, respectively (Figure 2). The sex ratio (male:female) considering all prawns sampled during the two years of study was 1.08:1 ( $\chi^2 = 0.82$ ;  $P = 0.36$ ).

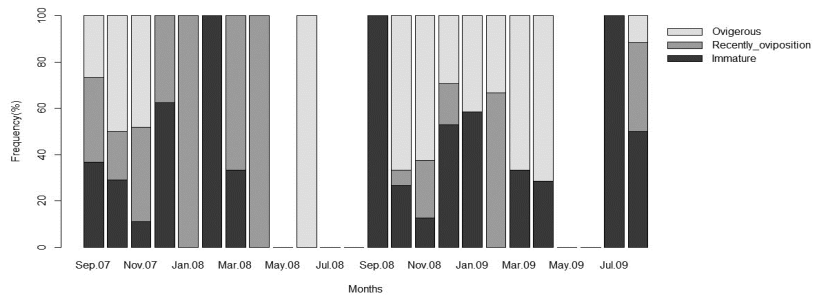
#### Abundance of ovigerous females and adult male morphotypes

Twenty-nine percent (n = 82) of the 282 female specimens collected were ovigerous and 23% (n = 68)

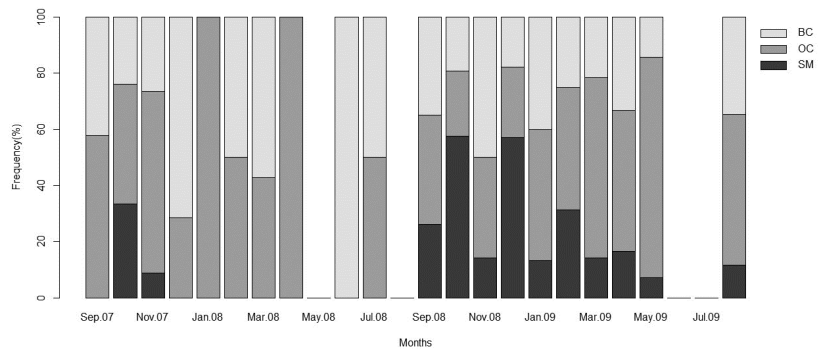


**Figure 2.** Number of males and females of *M. rosenbergii* collected between September 2007 and August 2009. The bars represent the number of specimens, and the line the total precipitation recorded each month at Tracuateua, 30 km from the study area.

**Figure 3.** Reproductive condition of *M. rosenbergii* females captured between September 2007 and August 2009 in Patal/Urumajó Rivers. Legend: Ovigerous - females incubating eggs; Recently oviposition - females with signs of recent spawning; Immature - females with different degrees of gonadal maturation, but not yet made the first spawning.



**Figure 4.** Relative frequency of the different male *M. rosenbergii* morphotypes collected each month in Patal/Urumajó Rivers. Legend: BC - Blue Claw, OC - Orange Claw, and SM - Small Male.



had recently undergone oviposition. The majority of non-ovigerous females were either mature (55%) or maturing (22%), while the remainder (23%) were immature (Figure 3). Mean fecundity was  $55,341 \pm 29,347$  eggs, with a minimum value of 9,086 (November 2007), and a maximum of 192,172 (August 2009). The number of eggs recorded per female was strongly correlated to carapace length (Pearson's  $r = 0.59$ ;  $P = 0.0001$ ).

The carapace of the smallest ovigerous female was 48 mm long, while most females had a carapace length of 70–100 mm. All three adult male

*M. rosenbergii* morphotypes were observed during the study. Most specimens (48%) were of the OC type, followed by BC (31%) and SM (21%). The OC morphotype was also the most abundant during the majority of months, except for October and December 2008, when BC was the most common (Figure 4). Small males (SM) were not captured between December 2007 and August 2008, but were collected in all other months. The BC males were the largest, with a mean TL of  $233.96 \pm 32.82$  mm, against  $203.27 \pm 34.93$  mm in the OC forms, and  $133.28 \pm 24.50$  mm in SM males.

Relationship between body weight, length and TL variation over the time

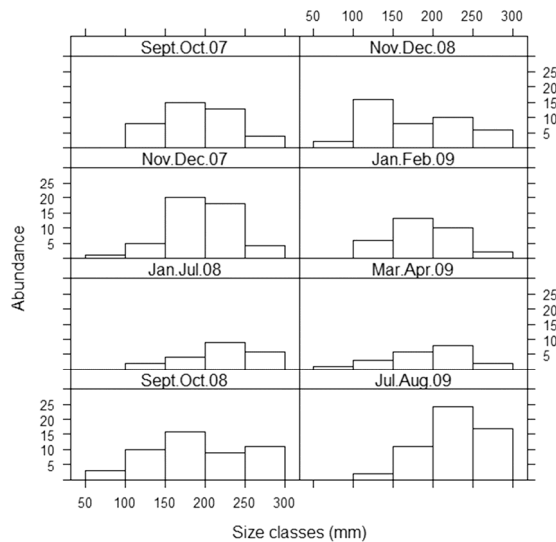
The body length of the male specimens varied from 74.05 to 289.77 mm, with a mean value of  $201.46 \pm 48.87$  mm, significantly higher ( $H = 44.38, P < 0.0001$ ) than that of the females ( $172.95 \pm 28.68$  mm, range: 81.61–249.03 mm). Similarly, the mean weight of the males ( $112.96 \pm 81.34$  g; range: 4.29–331.49 g) was significantly higher ( $H = 59.94, P < 0.0001$ ) than that of the females ( $59.35 \pm 30.66$  g; range: 5.47–211.58 g). For male and females the histograms showed a predominance of prawn with TL between 160 – 203 mm and 148–182 mm, respectively. And, for both sexes, a large range of different size classes was present in all collection periods showing the recruitment of new individuals to the population (Figure 5 and 6). The Kruskal-Wallis analysis according to Dunn’s test confirmed this results showing significant differences between several collection periods (for males:  $H=30.79, P < 0.05$ ; for females:  $H=21.22, P < 0.05$ ).

Fisheries activities

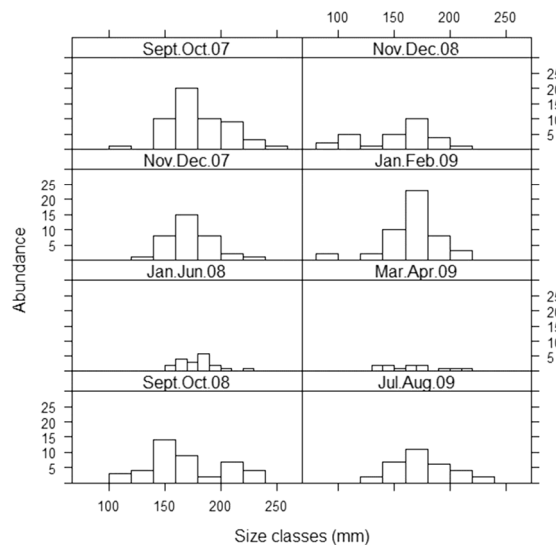
*M. rosenbergii* has been caught in the Patal River area during the past 10 years. During the survey period, six artisanal fishermen from different settlements were involved in the activity. Each one catches 70 to 90 kg of prawn per month, which are sold at US\$4.00 per kg (US\$1.00 = R\$ 3.91). Fisheries stop when the rivers are in flood, usually from January to June.

**Discussion**

The introduction of *M. rosenbergii* in several countries for aquaculture purpose has emerged as a potential hazard. The record of the species in the wild, outside of their natural area has increased over the time. The first record was 1996 in Venezuela (Pereira et al. 1996) and 1997 in Brazil (Barros and Silva 1997). Since the 2000s, new records of some feral individuals were made in China, Dominican Republic, Jamaica, Panama, Madagascar, Russia, Saint Lucia, USA (Hawai’i and Mississippi) and Taiwan (Yan et al. 2001; Woodley et al. 2002, Kairo et al. 2003, Hanamura et al. 2008; FAO-DIAS 2015). Not surprisingly, the first exotic populations of *M. rosenbergii* established (with natural reproduction) have also been registered in Venezuela and Brazil. Moreno et al. (2012) captured a total of 591 shrimp over 18 months in the Morocoto River (Benítez



**Figure 5.** Variation of the total length (TL) of males of *M. rosenbergii* captured in Amazon coast. The prawns were grouped for each two months, except January to July of 2008 due to the small number of specimens sampled.



**Figure 6.** Variation of the total length (TL) of females of *M. rosenbergii* captured in Amazon coast. The prawns were grouped for each two months, except January to June of 2008 due to the small number of specimens sampled. No female was captured in both July and August 2008.

municipality, Sucre State, Venezuela). In the present work, the analyses of monthly samples during 24 months revealed a population (588 prawn sampled) comprised of mature males and females, continuous reproduction and high female fecundity in the Brazilian Amazon estuaries (Patal/Urumajó Rivers).

Previous reports of the species in Brazil represented isolated records of a few individuals, presumably animals recently escaped from captivity. In addition to records from Pará (Barros and Silva 1997; Cintra et al. 2003; Iketani et al. 2011; Silva-Oliveira et al. 2011), the species has been recorded further along the north coast in the Parnaíba Delta (Loebmann et al. 2010), in São Paulo (Magalhães et al. 2005) in southeastern Brazil, and in the southern state of Paraná (Gazola-Silva et al. 2007). Silva-Oliveira et al. (2011) recorded *M. rosenbergii* at nine sites in Pará and the Patal River accounted for the largest number of specimens captured (n = 64). Although ovigerous females were captured, these authors did not consider the possibility of a wild breeding population because *M. rosenbergii* had been locally farmed until 2002 and the specimens were collected in 2003 and 2005. The fact that the species has been caught on the Patal River for at least 10 years indicates that the introduction of these animals into the wild is not a recent event as previously assumed.

The present study provides convincing evidence on the establishment of a breeding population of *M. rosenbergii* in Patal-Urumajó river system. Adult specimens were captured systematically over 24 months (except those at the end of the rainy season). The first report of the species from the Patal River (Maciel et al. unpubl. data), recorded a male vs female sex ratio of 1:8.5 and all the male specimens collected were of the BC morphotype, suggesting a declining population, given that the BC forms are larger, slower, and more vulnerable to predators (Rao 1967). According to Karplus et al. (2000), in the absence of other male morphotypes, there may be a tendency for the population to become exclusively female. On the contrary, the present study indicated that population is not declining, because the number of specimens captured increased progressively as the level of the river ebbed lower, and the population included relatively large animals (body weight > 100 g), and a diversity of size classes, varying from 70 to 290 mm in length. As the time elapsed between the period of reduced capture rates and the increase in the abundance of the species was not sufficient to allow the animals to grow to such a large size, it seems that individuals of the same cohorts were caught before and after the rainy season. The sex ratio recorded in the present study was well balanced and all three adult male morphotypes were observed, which indicates a well-structured reproductive population, despite the fact that it has been subject to fisheries by local fishermen during the past 10 years.

The decrease of the capture coincides with the rainy season, when the Patal-Urumajó river system is flooded. It may be due to the increase in the flooded area, which allows the dispersion of animals, avoiding capture. A scarcity of captured prawns at this time of year has been recorded for *Macrobrachium amazonicum* (Heller, 1862) in other rivers of the Amazon region (Odinetz-Collart 1993, see review of Maciel and Valenti 2009), and it seems likely that a similar process affected *M. rosenbergii* on the Patal River. A similar result was found by Moreno et al. (2012), which correlated to water turbidity and the decrease in river currents facilitating the movement of prawn. This river flooding period may act as a natural interruption in fisheries, which allow the recovery of stocks. In fact most of the local fishermen move to agriculture at this time. In addition, fishermen reported that they normally returned the ovigerous females captured to the river to preserve stocks.

In natural populations in India, Rao (1967) showed that the extreme limit of salinity tolerance was 0 to 16. According to George (1969) *M. rosenbergii* generally inhabit freshwaters, and migrate down to the estuarine regions to spawn in areas where salinity fluctuates between 5 to 20. In the Hooghly estuary, females either in advanced stages of maturity or berried (ready to release eggs) were obtained only in the middle zone of the river where, during the peak of spawning, the salinity ranged from 0.61 to 1.65 (Rajyalakshmi 1961). In the Patal-Urumajó river system, a novel environment for *M. rosenbergii*, female specimens with a TL of more than 100 mm were sexually mature and reproducing. These females were catch in water oligohaline with the highest salinity was 3. But, at the mouth of the Urumajó river the salinity fluctuates between 6.0 to 20.0 (Cavalcante 2007). A recent study in the laboratory has shown that the females of this species are more fertile and present better weight gain when maintained at lower salinity (0 to 6) (Yen and Bart 2008). This suggests that the oligohaline environment typically found in Amazonian estuaries may be suitable for the development of *M. rosenbergii* embryos. The large size of the females collected in the Patal-Urumajó river system, and their mean fecundity of over 55 thousand eggs appear to indicate that the low salinity of this environment is not a limiting factor to the development of the population of this exotic species, confirming the findings in laboratory experiments. It is also possible that the females migrate to Urumajó mouth for spawning.

Despite the increase in record of feral populations of *M. rosenbergii*, this is the first study to observe a large number of ovigerous females. Fifty percent of the female *M. rosenbergii* captured during the present study were ovigerous, or had recently oviposited, and virgin females were also observed throughout the year, which indicates that both reproduction and the recruitment of new individuals are continuous. Ismael and New (2000) recorded continuous reproduction in tropical areas within the species' natural range, with seasonal breeding in temperate regions. In natural population of India, the breeding peaks occurs just after the rainy season (Rajyalakshmi 1961; Raman 1967; Rao 1967). Reproductive peaks during or just after the rainy season appear to be common, and have been recorded in a number of other species, such as *M. borellii* (Bond and Buckup 1982), *M. acanthurus* (Valenti et al. 1989), *M. amazonicum* (Odinetz-Collart 1993, Odinetz-Collart and Moreira 1993), *M. olfersi* (Mossolin and Bueno 2002), *M. potiuna* (Antunes and Oshiro 2004), and *M. dux* (Arimoro and Meye 2007).

The females from the Patal River has a high fecundity, with a maximum individual value of ~200,000 eggs. Fecundity was positively related to female body length, and was similar to the pattern recorded in both captive and wild populations of the species (see review of Brown et al. 2010). The number of eggs observed in natural populations ranges between 7,000 and 111,400 eggs in the Hooghly estuary (Rajyalakshmi 1961) and between 139,600 and 503,000 eggs in Central Kerala, both in India. In a recent study, Habashy (2013) under laboratory conditions observed the number of eggs ranging from 2,050 to 150,500. With a mean of 98,746 eggs per female, *M. carcinus* (Lara and Wehrtmann 2009), the largest native species of the genus found in South America, is the most similar to *M. rosenbergii* in terms of fecundity. Other medium size native species from the same area are far less fecund, what is the case of *M. acanthurus* (mean of 8,483 eggs) and *M. amazonicum* (varied between 40 and 3,375) (Albertoni et al. 2002; Lucena-Fredou et al. 2010). Egg counts from 13 females collected in November 2007 (the month when ovigerous females were most common) indicated that the females together produce approximately 700,000 eggs. Thus, considering fecundity as an indicator of the risk of colonization, *M. rosenbergii* at Patal River showed the same high fecundity of the natural populations, which suggest it is well established in this new environment.

The above conclusion is reinforced by the fact that relatively large specimens (TL = 300 mm, weight = 300 g) can be found in the wild, which suggests that *M. rosenbergii* suffers little predation in this new environment. The males did not present a normal distribution of body size, due to the presence of the three different morphotypes, given that the SM males are generally smaller than the females. By contrast, the females were normally distributed, with high frequencies in the 148 mm to 182 mm size classes (Figure 6). Virgin females of large size were observed, indicating high growth rates. Recently, Thanh et al. (2009) evaluated the performance of diallel crosses of three strains of *M. rosenbergii*, two wild Vietnamese river populations (Dong Nai and Mekong) and the domesticated Hawaiian population, showing that the cross between females originating from Vietnam with Hawaii's males resulted in females with a significantly higher growth rate when compared with purebred strains. In another study, Thanh et al. (2010) suggested an association between the single nucleotide polymorphisms (SNPs) in intron 3 of the crustacean hyperglycemic hormone (CHH) with this high growth rate. Iketani et al. (2011) using molecular data showed the admixture of individuals from genetically distinct native populations (Mekong in Vietnam and either Bangladesh or Thailand) in Brazil. As the populations from Vietnam are more closely related to Malaysian populations (origin of Hawaii Thanh's strains) than with Thailand and Bangladesh populations (De Bruyn et al. 2005) and prawns of the Hawaiian stock have also been introduced in Brazil (New 2002) it is likely that the larger size of females captured in the Patal river has been caused by hybridization between these genetically different origins. Therefore, it is necessary to evaluate the CHH gene SNPs in both the Patal/Urumajó Rivers and in other exotic populations found in Brazil.

According to Ra'Anan et al. (1991), immature females and OC males present the highest rates of growth, whereas SM males grow more slowly. While the BC males grow to a large size, growth ceases after initial maturation. Overall, then, the characteristics of the feral population of *M. rosenbergii* in eastern Brazilian Amazonia appears to be very similar to those of both captive and native populations of the species (see review of Karplus et al. 2000). A predominance of OC males was recorded in the present study, followed by the BC morphotype and small males (SM) (2:5:3 ratio SM:OC:BC). This situation contrasts considerably with that reported by Cohen et al. (1981), who

recorded a 5:4:1 ratio for the SM, OC, and BC categories, respectively. Curiously, this ratio has never been reported in captive populations in Brazil (Valenti 1998). Karplus and Sagi (2010) found that the proportions of the different morphotypes in captive populations may vary significantly according to the stocking density. In this case, the proportion of SM males may double when the density increases from 1 to 4 animals per m<sup>2</sup>, with the opposite trend occurring in BC males. We postulate that, as the density of wild populations is probably much lower than that of captive ones, agonistic encounters may be less frequent, leading to a relatively lower frequency of smaller males (SM). Either way, the presence of the three male morphotypes is probably a positive factor for the establishment of the population, given that all three are able to copulate (Karplus and Sagi 2010), and that the BC males tend to suffer higher mortality, leading to a sex ratio biased in favor of the females, especially in the larger size classes (Karplus and Sagi 2010; Rao 1967). The presence of smaller morphotypes may also reflect the ongoing replacement of individuals in a continually reproducing population.

The criteria used to determine the status of the population (i.e. abundance and sex ratio close to 1:1, presence of ovigerous females and all males morphotypes, large variation of the length) can be adopted as a parameter to characterize other exotic populations of *M. rosenbergii* and even other exotic species of Palaemonidae prawns, like *Palaemon macrodactylus* found in Argentina, Australia, great part of Europe and United States (Ashelby et al. 2004; Spivak et al. 2006; Ashelby et al. 2013) and *M. nipponense* recorded in Iran, Iraq and Ukraine (De Grave and Ghane 2006; Salman et al. 2006; Son et al. 2013).

Richardson et al. (2000) divided the invasion process into a series of stages, determined by ecological barriers or filters, which the species must overcome before it can be established permanently. It includes geographic, environmental (local), reproductive, dispersal and two other environmental barriers (disturbed and natural habitats) (see Richardson et al. 2000). Following their criteria, our results appear to indicate that the *M. rosenbergii* population of the Patal/Urumajó Rivers has overcome at least the first three or four of these barriers and has already reached the naturalization stage, at which the population is able to survive and reproduce, and is thus self-sustaining after only 10 years (about 20 generations) of being released in the area.

Silva-Oliveira et al. (2011) demonstrated by modeling analyses that the estuaries of the Amazon Coast region provide suitable environmental conditions for the adaptive success of *M. rosenbergii*. The results in the present study indicate high reproductive performance of the species in this area, corroborating that the area is suitable for the establishment of the species. The temperature, precipitation patterns and coastal configuration of the Amazonian estuaries are highly similar to those regions where *M. rosenbergii* is native in tropical Asia (Sage-Atlas 1998; Hijmans et al. 2005; USGS 2008); thus, it is not surprising that its reproductive biology is also very similar in this new environment. The genetic analyses performed previously by our group (Iketani et al. 2011) allied with the evidence from the present study can favor the expansion of *M. rosenbergii* across the estuaries of the Amazon Coast. This situation requires careful monitoring to avoid damages to local biological communities in a mega-diverse environment, as the Amazonian rivers.

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