The invasive status of *Macrobrachium rosenbergii* (De Man, 1879) in Northern Brazil, with an estimation of areas at risk globally

Glaúcia C. Silva-Oliveira¹,², Jonathan Stuart Ready¹, Gabriel Iketani¹, Sandra Bastos¹, Grazielle Gomes¹, Iracilda Sampaio¹ and Cristiana Maciel¹*

¹Instituto de Estudos Costeiros, Campus Universitário de Bragança, Universidade Federal do Pará, Al. Leandro Ribeiro S/N Bairro: Aldeia, CEP 68600-000, Bragança, Pará – Brasil
²Câmpus do Marajó, Universidade Federal do Pará, Avenida Anajás, S/N – Bandeirantes, Aeroporto, CEP 68800-000, Breves, Pará – Brasil
E-mail: gcso007@yahoo.com.br (GCSO), jonathan.ready@gmail.com (JSR), iketani.g@gmail.com (GI), sbastos@ufpa.b (SB), grazielle@ufpa.br (GG), ira@ufpa.br (IS), macielufpa@gmail.com or cmaciel@ufpa.br (CM)

*Corresponding author

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Abstract

Introduction of *Macrobrachium rosenbergii* to Brazil for prawn farming has resulted in escapes to the local natural environment. In some areas risk of feral populations establishing in local environments is considered low due to a lack of suitable habitat for completing the life cycle. In contrast, introduction of this species along the mangrove coasts of northern Brazil can result in escape to highly suitable habitat for reproduction. We confirm colonization of these habitats by *M. rosenbergii* and define this species as a casual exotic in Brazil. We highlight that natural reproduction is now occurring, meaning that feral populations may soon become self-sustaining. Ecological niche models using the Maxent software were developed to assess potential areas at risk of invasion in Brazil and more widely across the world. These models indicate that there are large areas of suitable habitat available in northern Brazil where introduced populations of *M. rosenbergii* could expand. The effect of such introductions could extend to a very large area, from Maranhão in Brazil to the Orinoco delta in Venezuela.

Key words: giant river prawn, bioinvasion, exotic species, Brazil

Introduction

Introduction of exotic species for culture to regions with highly productive wild fisheries has been a common outcome of development of aquaculture all over the world (Naylor et al. 2001; Minchin 2007). This practice usually involves introduction to regions with similar environments to the native distribution of the organism in order to maximise performance in aquaculture. Exotic species are often introduced specifically to areas where individuals are capable of surviving after escape or release from captivity. The Giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879) (Figure 1) is now cultured widely around the world, both across the species native distribution and elsewhere (New 2005). Insufficient monitoring of introduced exotic populations is the principle cause of escape from captivity and the main source of establishment of feral populations in non-native environments (Gajardo and Laikre 2003; Carr and Whoriskey 2006).

In Brazil, cultivation of *M. rosenbergii* was initiated at the end of the 1970’s (Cavalcanti 1998; New 2000) and by 1998 escapees to the wild were detected in natural habitats in hydrographic basins in the north-east, the São Francisco, and in the upper and lower Paraná rivers (IBAMA 1998). In Pará state, *M. rosenbergii* was introduced during the period 1995-1998 to 15 municipalities namely; Aperetuba, Ananindeua, Belém, Bragança, Castanhã, Curuça, Goianésia do Pará, Igarapé-açu, Marabá, Mê do rio, Paraängebas, Paragominas, Salinópolis, São Miguel do Guamá and Santo Antônio do Taurá (Moraes-Riodades et al. 1999). Following these introductions, some specimens were found in local wild environments (Barros and Silva 1997; Cintra et al. 2003).
Among conditions required to allow self-sustaining wild populations of *M. rosenbergii* to develop in natural habitats, is the ability of berried females to reach estuarine conditions to release their larvae, a process that allows completion of the life cycle (Kurup and Harikrishnan 2000). Females in natural environments are capable of making long migrations in search of brackish water in which to release their eggs (New and Singholka 1984). Females have been recorded to release up to approximately 500,000 eggs (depends on individual size and weight), and in tropical regions they can reproduce throughout the year (Pinheiro and Hebling 1998), there is a significant potential for a feral population to expand rapidly from even a small starting population. Sexually mature males present a hierarchy of morphotypes (Ra’anani and Cohen 1985). Small males (SM) have short delicate claws, usually translucent. Orange-clawed males (OC) are of an intermediate size, spineless and orange-coloured claws. Blue-clawed males (BC) are the largest and possess long blue claws, covered with spines (Kuris et al. 1987). The phases of these morphotypes are dynamic and depend on the social environment, with males developing through forms SM, and then OC before ultimately becoming BC (Sagi and Aflalo 2005). All mature male morphotypes are capable of fertilizing females (see Karplus and Sagi, 2010 for review).

The northern coast of Brazil, where *M. rosenbergii* has been introduced to develop a culture industry, has a tropical climate and large estuarine areas comprising conserved mangrove forests (Cohen et al. 1999). Ecological conditions in this region are very similar to native habitat of *M. rosenbergii* in south and south-east Asia, though the presence of potential competitors could mean that introduced populations die out slowly after any initial introduction. The aim of the current study was to confirm that feral populations of *M. rosenbergii* in Brazil can still complete their reproductive cycle in the wild and hence characterise their invasive status in natural environments in northern Brazil. Additionally we aimed to determine the total geographical area that could potentially be affected by self-sustaining introductions by using an ecological niche modelling approach. The latter prediction could also be used to determine other areas of risk of invasion, both in Brazil and at a more global scale.

### Methods

#### Time and area of study

Study areas were chosen based on information provided by local fishermen. Surveys were carried out from 2002 to 2007 between the months of July to December when rainfall was lowest and relative prawn density was highest. Surveys initially focused on collecting only adult prawns. The focus changed later during sampling to also include earlier developmental stages in 2005. Collections were made using cast nets, seine nets and traps as well as purchasing samples from local fishermen, with two collections at each locality, with each collection lasting 1 day. They took place in the estuaries of the following municipalities in Pará state, Brazil: Viseu (Gurupi River), Augusto Corrêa (Patal River), Bragança (Caeté River), Tracuateua (dam and tidal channel Ponta Alta), Capanema (tidal channel Paissandu), Quatipuru (Quatipuru River), Irituia (Irituia River), Benfica (Benfica River) and Soure (Paracauari River) (Figure 2, see Appendix 1 for geo-referenced records).

#### Adult identification and condition

Adult specimens were preserved in 70% ethanol before being identified using specific taxonomic keys (Dore and Frimodt 1987). Total weight (g), total length (mm), sex (based on presence or absence of the male appendix on the second pair of pleopods, Ismael and New 2000) and male morphotype stage (following the colouration of the claws: SM- transparent, OC- orange, BC- blue (Kuris et al. 1987) were recorded for each individual.

#### Ovigerous females

Eggs of each ovigerous female were removed and preserved in 70% ethanol before being identified using specific taxonomic keys (Dore and Frimodt 1987). Total weight (g), total length (mm), sex (based on presence or absence of the male appendix on the second pair of pleopods, Ismael and New 2000) and male morphotype stage (following the colouration of the claws: SM- transparent, OC- orange, BC- blue (Kuris et al. 1987) were recorded for each individual.
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**Figure 1.** A male of *Macrobrachium rosenbergii* (total length of 260 mm) sampled in Patal River, Augusto Corrêa, Pará, Brazil. Photograph by Gabriel Iketani.

**Figure 2.** Places in Brazil with previous feral records of *Macrobrachium rosenbergii*. Red circles represent the new records obtained by this work. Bragança (BR) samples had already been captured by Barros and Silva (1997) and again by this work. Salvaterra (ST), Soure (SO), Icoaraci (IC), Benfica (BF), Irituia (IR), Quatipuru (QU), Capanema (CP), Tracuateua (TR), Augusto Corrêa (AC), Araioses (AR), Delta do Parnaíba estuary (DP), Cajueiro da Praia (CJ), Brejo Alegre (BA) and Pontal do Paraná (PA).
Juvenile animals and molecular identification

Juveniles were captured using hand nets. After collection, individuals representing the genus *Macrobrachium* were separated based on the presence of a hepatic spine (Holthuis 1952; Gomes-Corrêa 1977). To complete this identification, two samples of adults of each of the species *Macrobrachium rosenbergii*, *M. acanthurus*, *M. amazonicum*, *M. carcinus*, *M. jelskii*, *M. nattereri* and *M. olfersii* were also processed to provide comparative gene sequences. Genomic DNA was extracted from tissues using a phenol-chloroform method (Sambrook et al. 1989). The Cytochrome Oxidase C subunit 1 (CO1) was amplified from genomic DNA samples using PCR with the primer pair COI-F and COI-A (Palumbi and Benzie 1991). See Iketani et al. (2011) for details of PCR and sequencing reactions.

Sequences were aligned and corrected using the program BioEdit (Hall 1999). To evaluate the degree of similarity between sampled juveniles and known adult individuals representing each described species we constructed a Neighbour-Joining (NJ) tree using PAUP* 4.0 (Swofford 2002). The evolutionary model used in the analyses was first determined using the Modeltest 3.7 (Posada and Crandall 1998). The significance of branch nodes on trees were estimated using bootstrap analysis based on 1000 pseudoreplicates (Felsenstein 1985). A single individual of *Potimirim potimirim*, a freshwater prawn belonging to the family Atydae, was used as an outgroup in all analyses.

Statistical analyses

For the morphometric analysis (body length, weight and egg number) descriptive statistics were applied. Data were expressed in terms of mean and standard deviation. Normality of the data was confirmed using the Kolmogorov-Smirnov test. The chi-squared test ($\chi^2$) was used to test for significant differences in sex ratio. All analyses were performed using Bioestat version 5.0 (Ayres et al. 2007).

Distribution modelling

Distribution models were used to assess the potential range of spread of *M. rosenbergii* and were developed using Maxent software (Phillips et al. 2006) and (continuous variable) GIS raster data reformatted to half degree resolution:

1.) elevation and 2.) downstream distance (a measure of proximity to saline waters), both extracted from the Hydro1K dataset (USGS 2008); 3.) mean annual temperature, 4.) January precipitation, 5.) July precipitation from the Worldclim dataset (Hijmans et al. 2005); 6.) soil pH and 7.) soil carbon (IGBP-DIS, 1998), accessed through the Atlas of the Biosphere webpages (SAGE-ATLAS 2008).

Distribution spread predictions were based on input occurrence data for *M. rosenbergii* populations taken only from the western part of the species’ natural range in Malaysia, Indonesia, Vietnam and surrounding areas, (excluding data from the Philippines, Papua New Guinea and Australia). This restriction was made as a recent systematic study has challenged the taxonomic status of eastern populations (Wowor and Ng 2007) and there is clear evidence that wild populations in the eastern part of the natural distribution constitute genetically distinct lineages (de Bruyn et al. 2004). There is little doubt however, that the cultured form in most parts of the world (including that in Brazil) originated from Malaysia (Cavalcanti 1998; New 2000). Data on natural occurrences were obtained from as many published articles as were available, including only sites with distinct georeferenced positions. A total of 38 points were identified using these references (de Bruyn et al. 2005; Wowor and Ng 2007, Appendix 2).

Settings for the Maxent predictions included: a random seed, removal of duplicate presence records, removal of a 25% test sub-sample, a regularization multiplier of 1,500 maximum iterations, 0.00001 convergence threshold, and a 10,000 background point maximum. Automatic features were used to create response curves. Receiver operating characteristic curves and (threshold-dependent) binomial tests based on omission and predicted area were also implemented automatically in Maxent.

Results

Adult identification and condition

In total 111 adult *M. rosenbergii* were collected from natural habitats in 9 municipalities from north-eastern Pará state (~ 40% male and 60% female) (Figure 2). The municipality of Augusto Corrêa was notable as the location where most specimens were collected (n=64, of which 26 were male and 38 female, Appendix 1).
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Individual weights varied from 2.64 g to 354.75 g (mean = 68.59, S.D. = 65.13). Body length varied from 51.4 mm to 285.5 mm (mean = 157.99, S.D. = 59.46 mm). The sex ratio of males to females collected did not deviate significantly from 1:1 (sex ratio = 1:1.6; P > 0.05, χ²-test). The field survey recovered all three male morphotypes in the wild (SM, OC, and BC) with the BC morphotype in highest frequency (Figure 3). All three morphotypes were only present at a single site; the municipality of Augusto Corrêa (24 SM, 1 OC, and 1 BC). Other localities were dominated almost entirely by BC males.

**Ovigerous females**

Ovigerous females were encountered in three municipalities: Augusto Corrêa (n = 11), Viseu (n = 3) and Bragança (n = 2) (Figure 4). Mean observed fecundity was 36,303 (S.D. = 17,556) eggs. The minimum and maximum number of eggs/female observed were 514 at Augusto Corrêa and 75,440 at Bragança, respectively. Two large females collected at Bragança and maintained to release eggs in the laboratory, both produced viable PLs. Individually they produced 52,720 and 138,000 PLs, respectively.

**Molecular identification of juvenile animals**

Samples came from 5 municipalities: Viseu, Augusto Corrêa, Bragança, Tracuateua, and Capanema, respectively. Of these, a single individual from Augusto Corrêa was identified as being 100% similar to *Macrobrachium rosenbergii* (Figure 5). Only *M. carcinus* was not identified among the sampled juveniles. Juveniles of *M. amazonicum* were detected at all locations.

**Distribution modelling**

Results of the Maxent prediction modelling show that a large area of potentially suitable habitat for *Macrobrachium rosenbergii* to establish feral populations is available in tropical coastal areas in northern Brazil. In particular, very large areas of highly suitable habitat are available at the mouth of the Amazon and more generally along the northern coast of South America (from Maranhão, Brazil to the mouth of the Orinoco River in Venezuela). In addition, areas along most coastal regions from the Isthmus of Panama to some areas in the Caribbean Sea (Florida, Cuba and the Yucatan Peninsula), and also along the southern coast of West Africa (from Sierra Leone to Cameroon) (Figure 6), were identified as being suitable for feral *M. rosenbergii* populations to establish. Remaining surrounding coastline areas examined in the modelling study are generally predicted to be of lower suitability for establishment, except that patches of suitable habitat could extend southwards along the coastline to Rio de Janeiro in Brazil, and the Congo in west Africa and as far north as Florida in the USA, and Senegal in Africa (Figure 6). Probability of suitable habitat in East Africa (Kenya and Tanzania) and Madagascar in contrast was low (Figure 7). For the Maxent modelling exercise, 24 actual presence records were used to train the model and 6 presence records were used to test the model. Eight points were discarded as duplicate presence points because they were in the same raster grid cell in
the model. The relative contributions that different variables contributed to the model were: mean annual temperature, 31.5%; downstream distance, 26.3%; July precipitation, 22.9%; January precipitation, 9.6%; elevation, 7%; soil pH, 2.4%; and soil carbon, 0.5%.

Receiver operator characteristic curve statistics showed that the area under curve (AUC) was 0.99 for the training data and 0.98 (S.D. 0.013) for the test data, showing that the predictions were significantly better than random. Binomial tests, based on omission and predicted area, were all highly significant, indicating that test points had been predicted significantly better than by a random prediction with the same fractional area.

Discussion

In the current study feral *Macrobrachium rosenbergii* were found in a diverse array of natural water bodies in north-east Pará State in Brazil, including both estuarine as well as freshwater habitats including dams. In coastal areas in Paraná State in the south of Brazil, this prawn has also been observed (moulted carapaces) outside of captivity. This is considered to be a cause for concern due to proximity of these sites to coastal areas and therefore to brackish waters where the species could complete its lifecycle in the wild (Gazola-Silva et al. 2007). In São Paulo State the risk of feral populations establishing is considered less as the prawn has only been found in inland waters, very far away from coastal estuarine waters, therefore reducing any potential for the species to reproduce (Magalhães et al. 2005).

Two specimens of *M. rosenbergii* recently found in estuarine areas of the states of Piauí and Maranhão have been considered escapes from farms (Loebmann et al. 2010). In Pará, a small number of prawns were found in the municipalities of Belém, Bragança, Colares and Salvaterra (Figure 2, Appendix 1) (Barros and Silva 1997; Cintra et al. 2003).

Although ovigerous feral *M. rosenbergii* females were only encountered at three sites with the majority of these collected in a single river
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**Figure 6.** Maxent prediction map showing the probability of encountering suitable environments for *Macrobrachium rosenbergii* (based on presence data from the Malaysian, Thai and Indonesian populations deemed to be the source populations of farmed prawn) in South America and West Africa.

**Figure 7.** Maxent prediction map showing the probability of encountering suitable environments for *Macrobrachium rosenbergii* (based on the presence data from the Malaysian, Thai and Indonesian populations deemed to be the source populations of farmed prawn) in East Africa, Asia and Australasia (prediction for Australia is not shown due to lack of environmental data from Hydro1K dataset).
Relative fecundity estimates of *M. rosenbergii* are higher than those recorded for native species to the region, for example *M. amazonicum* only produces approximately 2,000 eggs per female (da Silva et al. 2004). It would be necessary, however, to determine whether this high reproductive potential would be converted into population growth through successful natural recruitment. Genetic identification of one juvenile in the wild in Augusto Corrêa strongly supports the contention that *M. rosenbergii* can at least reproduce successfully in natural environments of the Patal River in Pará State. This approach was very useful in confirming the possible recruitment of exotic species, as suggested by Armstrong and Ball (2005). The small number of juveniles identified can be explained by two possibilities. Firstly, the choice of sampling area, i.e., juveniles maybe have been captured outside the spawning area. Secondly, the relatively small sample number used for genetic identification. Therefore further analyses should expand the area of study and include more samples, particularly in the municipality of Augusto Correa, to search for the spawning area and the period of peak recruitment.

Here, positive identification of more than 100 *M. rosenbergii* individuals in estuarine areas suggests that natural habitats in north-east Pará State have probably been colonised successfully, given the presence of all male morphs, ovigerous females which hatched larvae in the lab and the presence of at least 1 juvenile detected by molecular means captured from the natural environment (See Appendix 1 for more details). Even given the growth in reports of occurrence outside captivity however, it is not yet possible to confirm the existence of any truly self sustaining feral populations across the region given the recent cessation of shrimp farming in the region (2002), relative to the collection period (2003-2005) and also given the possibility that the population may be reproducing but in decline. There are also only three populations with ovigerous females and only two where the SM morphotype was detected, which may either represent sampling bias or differences between populations in terms of recruitment (Figures 3 and 4). Persistence may require periodical introductions to continue (Richardson et al. 2000).

Using the framework suggested for Colautti and MacIsaac (2004) we could classify *M. rosenbergii* in Brazil as a stage IVa category exotic, an invasive species found in small numbers, but, widespread over a great part of North and Northeastern Brazil from the Amazon coast to the Delta of the Parnaíba Environmental Protection Area (Figure 2) (Loebmann et al. 2010; this work). The occurrence of *M. rosenbergii* in the region demands attention. Despite the fact that no confirmed impacts resulting from its introduction have yet been observed, the biology of this species (see Brown et al. 2010 for review) suggests that some negative effect to the native biota is likely to occur, and this has been hinted at by local fishermen who claim that some species of fishes have not been seen since the introductions. The greater body size of the prawns sampled (51.4mm to 285.5mm) and the territorial behaviour could result in competition with native prawns for space. Also, the higher fecundity and fertility of *M. rosenbergii* also gives it a competitive advantage over native prawns in terms of its population dynamics. In addition, the species’ larval diet of zooplankton and the omnivory of the adult mean that competition for food with many species may occur.

While the distribution of cultivation localities for *M. rosenbergii* could be taken as an indication of suitable habitats and areas at risk from invasion, such an approach suffers from two flaws. Firstly, it is possible to farm shrimp in regions well outside those in which they should survive naturally by either controlling the environment (e.g. heating water), or by isolating them from potential competitors (a species may be able to survive and grow at lower temperatures than its native range, but would not compete as well at these temperatures with native fauna). Secondly, not all potentially suitable habitats may have had commercialization of shrimp initiated due to political, commercial or other social reasons. As such, modelling the potential distribution using a niche modelling method outlines specifically the regions most at risk, and more importantly indicates the connections between these regions, such that the potential routes of invasion from a single starting point can be assessed.

The Maxent model predicted the distribution based principally on the factors of mean annual temperature, downstream distance and
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precipitation in July and January. The climatic variables are frequently important for niche models at such a geographical scale, and the downstream distance likely represents the importance of estuarine environments to the reproduction biology of the species and the subsequent distance effects on migration which result in a reduced encounter likelihood further away from estuaries. The distribution of potential suitable habitat predicted by the Maxent niche modelling program (Figure 6) indicates that a large area of potentially suitable habitat exists for *M. rosenbergii* in the north of South America. In combination with evidence of the invasive status of *M. rosenbergii* in estuarine areas just to the south east of the mouth of the Amazon River, the model suggests that any impact on biological diversity across the region would potentially occur at a large scale. Any measures implemented to control the likely spread and expansion of this invasive species will therefore require both a coordinated effort across a large area and co-operation among different nations. Further study and monitoring of the invasion of *M. rosenbergii* in this area and neighbouring regions will be required to confirm the persistence of the invasive populations and hence to develop a suitable management plan.

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