

Reproductive and morphometric traits of *Macrobrachium amazonicum* (Decapoda: Palaemonidae) from the Pantanal, Brazil, suggests initial speciation

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Abstract: The palaemonid shrimp *Macrobrachium amazonicum* shows an unusually large geographic range (ca. 4000km across) living in coastal, estuarine, and limnic inland habitats of the upper Amazon, Orinoco, and La Plata basins. This raises doubts whether allopatric, ecologically diverse populations belong to the same species. While shrimps from estuarine and Amazonian habitats have been studied in great detail, very little is known about hololimnetic inland populations. In the present study, biological traits related to growth (maximum body size, fresh weight, morphometric relationships) and reproduction (sex ratio; occurrence of male morphotypes; minimum sexable size; minimum size of ovigerous females; fecundity; egg size), were studied in *M. amazonicum* collected from a pond culture and two natural freshwater habitats (Rio Miranda; Lagoa Baiazinha) in the Pantanal of Mato Grosso do Sul, Brazil. In total, 2270 shrimps were examined (603 males; 1667 females, 157 of these ovigerous). Sex ratio (males:females) was at all sampling sites strongly female-biased, ranging from 0.2-0.6. Maximum body size was larger in natural habitats compared to the pond culture, suggesting reduced growth or a shorter life span under artificial mass rearing conditions. Maximum fecundity observed in our material was 676 eggs, reached by the largest female (TL=65mm; Lagoa Baiazinha). A significant difference between slope parameters of linear regressions describing fecundity, either in terms of numbers of eggs laid or of larvae released, in relation to female fresh weight, indicates egg losses. This may be due in part to a 2.4-fold increase in egg volume occurring during the course of embryonic development, while the available space under the abdomen remains limited. Size-weight relationships differed significantly between males and females, indicating sex-specific morphometric differences. Males appear to have a more slender body shape than females, reaching thus lower weight at equal TL. When reproductive and morphometric traits are compared with literature data from estuarine and inland populations living in the Amazon and Orinoco plains, shrimps from the Pantanal show conspicuous peculiarities differing from other populations: (1) maximum body size is far smaller, suggesting shorter longevity; (2) females are consistently larger than males; (3) different male morphotypes are absent; (4) minimum sexable size and (5) minimum size of ovigerous females are smaller. These traits suggest a heterochronic shift (predisplacement) of sexual maturation and r-selection. In summary, our data show biologically relevant differences in life-history traits of shrimps from the Pantanal compared to *M. amazonicum* populations in other regions. All these differences persist also in long-term cultures maintained under constant conditions. Altogether, our data support the hypothesis that *M. amazonicum* in the Paraná-Paraguay drainage basin has phylogenetically diverged from allopatric populations that are hydrologically separated by continental watersheds, implying an at least incipient vicariant speciation. Rev. Biol. Trop. 61 (1): 39-57. Epub 2013 March 01.

Key words: Caridea, freshwater shrimp, growth, reproduction, fecundity, life history.

In the evolution of the caridean shrimps, the Palaemonidae and, in particular, clades belonging to the palaemonid genus *Macrobrachium* have been highly successful invaders in brackish and freshwater ecosystems (Jalihal *et al.* 1993, Freire *et al.* 2003, Bauer 2004). Among the currently recognised ca. 240 species of *Macrobrachium*, at least 57 live in the neotropical region, and new species are still being discovered, especially in land-locked freshwater habitats such as continental rivers, lakes, and subterranean caves (de Grave *et al.* 2009, de Grave & Franssen 2011, Mejía-Ortiz & López-Mejía 2011).

One of the most widely distributed monophyletic clades within this neotropical group of shrimps is currently assigned a single species, *M. amazonicum* (Heller 1862) (see Pileggi & Mantelatto 2010, Vergamini *et al.* 2011). According to the taxonomic and biogeographic literature (e.g. Holthuis 1952; for recent review see Maciel & Valenti 2009), this species has been recorded from a geographic area about 4000km across, ranging from the Northern coasts of South America (Venezuela, Columbia, Guayana) to Northern Argentina and Paraguay, and from the foothills of the Andes (Ecuador, Peru, Bolivia) to the Atlantic coasts of Northeastern Brazil. This vast area of distribution comprises the estuarine reaches of coastal rivers draining to the Caribbean and Atlantic Ocean as well as fully limnic inland waters of the Orinoco, Amazon, São Francisco, and La Plata basins, which are hydrologically separated from each other by continental watersheds (Albert *et al.* 2011).

While adult morphology (Holthuis 1952) as well as recently published data of molecular genetics (Pileggi & Mantelatto 2010, Vergamini *et al.* 2011) support the unity of this species, there is increasing evidence from studies of larval biology (Anger & Hayd 2009, Anger *et al.* 2009, Anger & Hayd 2010, Urzúa & Anger 2011) and of the ontogeny of physiological functions (Charmantier & Anger 2011), which suggests that at least the populations living in the La Plata (Paraná-Paraguay) basin must have phylogenetically separated from the remaining

clades assigned to *M. amazonicum*. Earlier studies of molecular genetic diversity (Bastos 2002), extensive morphometric analyses (Porto 2004), as well as consistent differences in larval morphology and developmental patterns (K. Anger unpublished) corroborate the hypothesis of an at least incipient speciation.

This controversial discussion raises the question whether *M. amazonicum* living in the upper La Plata basin differs also in other life-history traits from tentatively conspecific populations in other hydrogeographic regions of South America. Such comparisons, however, are still severely hampered by poor availability of life-history data from the Paraná-Paraguay river system, which is hydrologically separated from the Amazon and São Francisco basins in the North (Buckup 2011, Carvalho & Albert 2011). The present study therefore provides preliminary data for a population living in the Pantanal of Mato Grosso do Sul, Brazil, comparing patterns of growth and reproduction with previously reported data from other populations of *M. amazonicum*.

MATERIALS AND METHODS

The Pantanal, brief characterization of the study region: The Pantanal is the world's largest wetland, covering ca. 365 000km², 140 000km² of which comprise the central floodplains in Brazil, Bolivia, and Paraguay (Swarts 2000, Iriondo *et al.* 2007). According to the standard Köppen-Geiger climate classification system, this region is characterized as a sub-humid tropical savannah (AW category; Köppen 1936, Peel *et al.* 2007), with an average rainfall of 1 100mm per year and an annual average temperature of 26°C. Physical, chemical, biological, and other environmental conditions in the Pantanal plain have in detail been described in various books and reviews, in particular the seasonal patterns of flood pulses and dry periods that are typical of this region (Heckman 1998, Willink *et al.* 2000, Junk *et al.* 2011a, Junk *et al.* 2011b, Nunes da Cunha & Junk 2011a).

Sampling sites, collection of shrimps:

During the dry season (July-November) in 2008 and 2009, shrimps were collected from two natural freshwater habitats located in the Pantanal of Miranda, State of Mato Grosso do Sul (MS), Brazil (Fig. 1): (1) Miranda River ($20^{\circ}09' S - 56^{\circ}30' W$); (2) Lagoa Baiazinha ($20^{\circ}16' S - 56^{\circ}23' W$), an adjacent shallow lake hydrologically connected to the river. Additional material was obtained from an artificial pond culture maintained at the State University (UEMS) at Aquidauana, MS ($20^{\circ}28' S - 55^{\circ}48' W$).

At all sampling sites, shrimps were collected from shallow habitats (<1m depth) near the water margins, mostly near floating mats of water hyacinth (*Eichhornia* spp.), using hand nets (70cm diameter; 2mm mesh size). Shrimps (in total 2 270 individuals) were subsequently fixed and stored in labeled plastic containers

with 70% ethanol. On sampling days, measurements of water temperature, pH and conductivity were carried out using a digital YSI 63 (Yellow Spring Instruments, USA) apparatus, those of dissolved oxygen concentration with an HI-9146 (Hanna, Germany) probe. Since we do not attempt to show here small-scale local or seasonal differences in environmental conditions, only minimum and maximum values of these parameters are given (Table 1) to briefly characterize the habitats from which our material was sampled.

The Miranda River (or Rio Miranda) is characterized by moderate to strong currents, entangled side branches, transiently exposed sand banks, and floating mats of aquatic plants (*Eichhornia azurea*, *E. crassipes*, *Alchornea castaneifolia*), while fallen trees and over-reaching riparian vegetation provide local shelter and some shade near the river banks (for

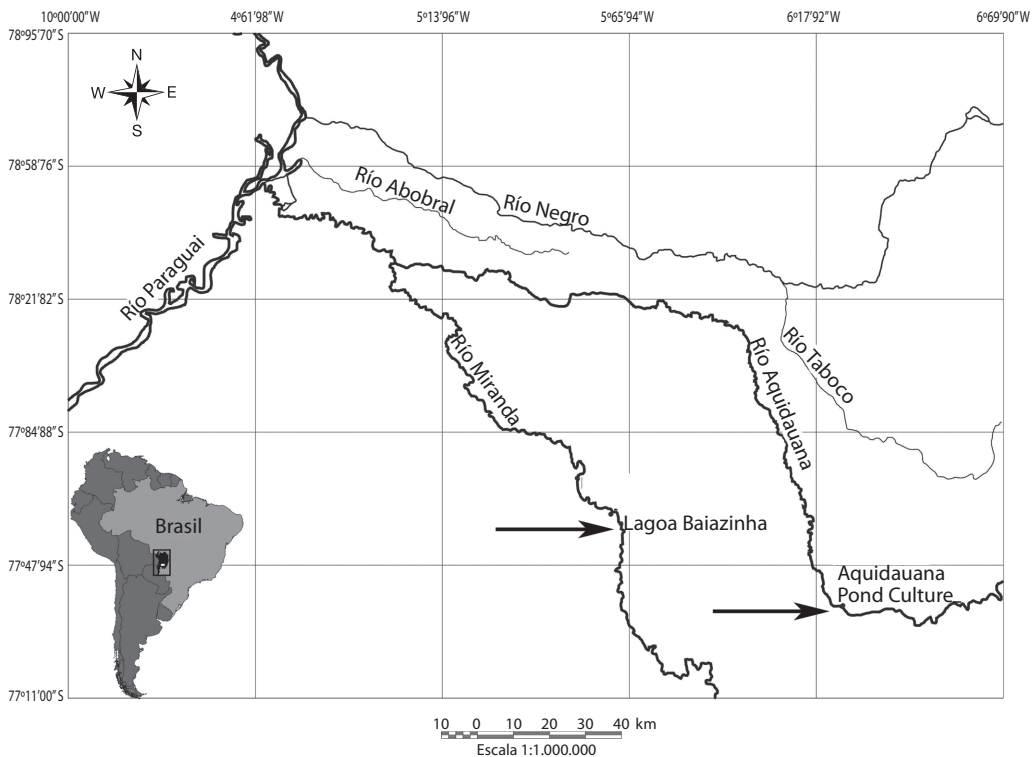


Fig. 1. Study area (with UTM coordinates) in the Pantanal of Mato Grosso do Sul, Brazil; locations of sampling sites on the Miranda River, nearby Lagoa Baiazinha, and artificial pond culture at the State University (UEMS) at Aquidauana marked with arrows.

TABLE 1
 Number of shrimps (empty and ovigerous females; total number of females and males; grand total for each site, Σ)
 collected from two natural freshwater habitats in the Pantanal of Mato Grosso do Sul, MS, Brazil,
 and from one artificial pond culture (State University of MS at Aquidauana)

	Rio Miranda	Lagoa Baiazinha	Pond culture	Total
Females	779	323	565	1 667
(empty)	(762)	(278)	(470)	(1 510)
(ovigerous)	(17)	(45)	(95)	(157)
Males	289	191	123	603
Σ shrimps	1 068	514	688	2 270
T (°C)	17-30	25-31	20-31	
Cond (μ S/cm)	10-51	26-43	15-50	
pH	7.0-8.2	4.9-7.9	5.7-8.4	
DO (mg/l)	7.0-8.3	3.7-5.9	4.2-7.7	

Minimum and maximum values of temperature (T), conductivity (Cond), pH, and dissolved oxygen (DO) measured during the study period.

further ecological characterization of the Rio Miranda, see Depaula *et al.* 1995, Resende *et al.* 2000, Hayd & Nakagaki 2002, Mendes *et al.* 2004, Resende *et al.* 2006, Pivari *et al.* 2008, Soares & Oliveira 2009). Lake (or Lagoa) Baiazinha is connected, and thus, hydrologically similar to the Rio Miranda, but with almost stagnant water and denser mats of floating water hyacinth (for hydrological and ecological characteristics of shallow Pantanal lakes, see Heckman 1998, Willink *et al.* 2000, Suarez *et al.* 2004, Wantzen *et al.* 2008, Ton-dato *et al.* 2010).

The shrimps kept for aquaculture research at the UEMS in Aquidauana originated from the Rio Miranda (same site as above). This culture is maintained in an earthen pond (300m²; 1.5 depth) with flow-through conditions, receiving freshwater from a well (flow rate ca. 600L/min). Its surface is partially covered with floating mats of *Eichhornia crassipes*, whose roots provide shelter for shrimps. These are fed twice daily with commercial feeds for carnivorous fish (Mar & Terra and Douramix), offering an equivalent of about 3.5% of shrimp biomass per day (for details and references on cultivation techniques, see Moraes-Valenti & Valenti 2010).

Examination of shrimps, eggs and larvae; and statistical analyses: In the laboratory,

shrimps were identified using keys and further morphological information available for neotropical *Macrobrachium* (Holthuis 1952, Gomes Corrêa 1977, Kensley & Walker 1982, Melo 2003). Some specimens were also sent to Dr. Célio Magalhães (Instituto Nacional de Pesquisas da Amazônia in Manaus, Brazil), who checked and confirmed previous identifications. Shrimps identified as *M. amazonicum* were counted and sexed (Ismael & New 2000, Brown *et al.* 2010). As the smallest recognizable males (with appendix masculina expressed on the second pair of pleopods) had a total body length of 19mm, shrimps with smaller body size were regarded as immature juveniles, which were not considered in this study. The total numbers of shrimps that were collected from the different sampling sites and later used in this study (classified as males, non-ovigerous females, or ovigerous females) are given in table 1.

Total length (TL, distance between the anterior margin of the rostrum and the distal tip of the telson) and carapace length (CL, posterior margin of the eye orbit to the midpoint of the posterior carapace margin) were measured to the nearest 0.01mm under a dissecting microscope (Labor SE2200) equipped with a digital vernier caliper (Digimes 100.174BK Plus). Shrimps with broken rostrum or telson

were disregarded. After brief blotting on filter paper, shrimp fresh weight (FW) was determined to the nearest 0.1mg on a Toledo (Ohays Adventurer AR2140) balance.

Male shrimps were checked for the presence of different morphotypes (Moraes-Riodades & Valenti 2004). The sex ratio was calculated as number of males divided by the number of females (cf. Castillo *et al.* 2011). In ovigerous females, eggs were removed and counted under a dissecting microscope. Fecundity (number of eggs per female) was described in relation to TL, CL and FW (see below). A sample of 12 eggs per female was inspected under a compound microscope to measure egg size and classify the stage of embryonic development (Anger & Moreira 1998, Ituarte *et al.* 2005). Three developmental stages were distinguished: (I) almost 100% of the egg volume occupied by yolk, no eye pigments visible, embryo showing little or no differentiation; (II) incipient eye formation visible as a reddish line, beginning embryonic differentiation (segmentation, development of appendices), heart beat visible but often irregular, yolk occupying ca 50-60% of egg volume; (III) eye fully developed, heart beat regular, differentiation of appendages in the final phase, yolk largely depleted. Egg volume (V) was calculated using the formula for an ellipsoid, $V = \pi \cdot l \cdot h^2 / 6$, where l=longest diameter (length) and h=shortest diameter (height).

In addition to the analysis of sampled and fixed materials (Table 1), 76 ovigerous females with eggs in a late developmental stage (III) were collected from the pond culture, and individually transferred to aquaria with freshwater (2L volume; aeration; ca 30°C; natural light conditions). Larvae hatching within five days were counted under a stereo microscope in order to detect possible differences between the initial egg number (“realised fecundity”) and the number of larvae that eventually were released by females with a given body size; differences were considered as an indication of egg losses occurring during the time of embryogenesis (Anger & Moreira 1998, Briggs *et al.* 2002, Ituarte *et al.* 2007). Fecundity,

in terms of either egg or larval numbers per female, could be described as a linear function of female FW, $Fec = a + b \cdot FW$, or as a power function of size dimensions ($x = CL$ or TL), $Fec = a + x^b$. Slope parameters (b) were statistically compared with ANCOVA (Sokal & Rohlf 1995).

Morphometric relationships between CL and TL were described separately for females (regardless whether ovigerous or non-ovigerous) and males, applying linear regression, $TL = a + b \cdot CL$ (Fig. 2). Non-linear models were also tested, but the coefficients of determination (r^2) were consistently lower. Again, the slope parameters (b) obtained for males and females, respectively, were compared with ANCOVA.

For all three categories of shrimp distinguished in this study (males, females with and without eggs; Table 1), relationships between TL and FW (in ovigerous females including the egg mass) were described using power functions, $FW = a \cdot TL^b$ (Hartnoll 1982). This analysis was initially carried out separately for each sampling site to test for possible differences in size-biomass relationships due to local variations in nutritional or other ecological conditions. However, when the slope (b) and intercept parameters (a) of the linearized power functions (after log-log transformation of data sets; for review of biometric functions describing fecundity, see Somers 1991) were compared by means of ANCOVA, no significant differences were detected among sampling sites. Consequently, the data from each sampling site were subsequently pooled for each category of shrimp, so that generalized size-weight relationships could be given, and slopes of allometric functions could be statistically compared between shrimp categories using ANCOVA (for results, see Fig. 3D).

RESULTS

Habitat conditions: This study concentrated on life-history traits in a little known shrimp population from the Pantanal, which is currently assigned to the widely distributed species *M. amazonicum*. Therefore, only

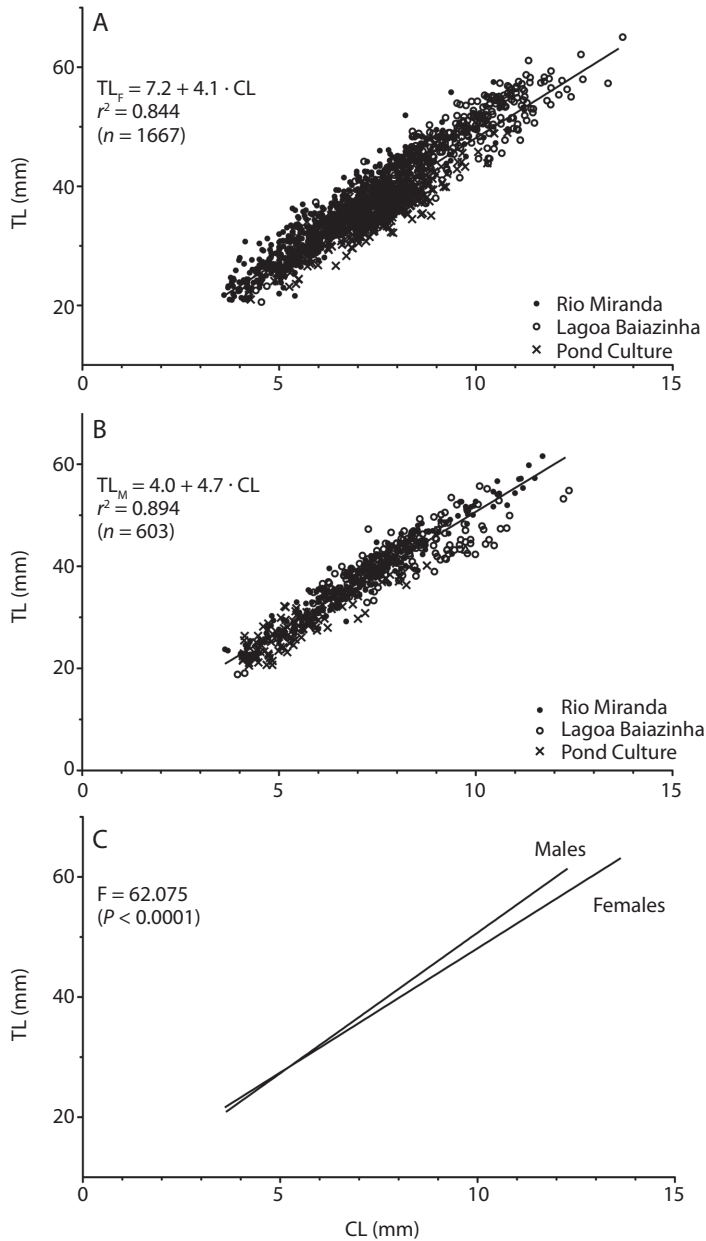


Fig. 2. Linear regressions describing the relationships between carapace length (CL) and total length (TL) of female (A) and male (B) *Macrobrachium amazonicum* from the Pantanal (pooled data from three sampling sites); r^2 , coefficient of determination; n, number of observations; F statistic and level of significance, P, for statistical comparison between slopes (ANCOVA; graph C) indicate a highly significant morphometric difference between males and females.

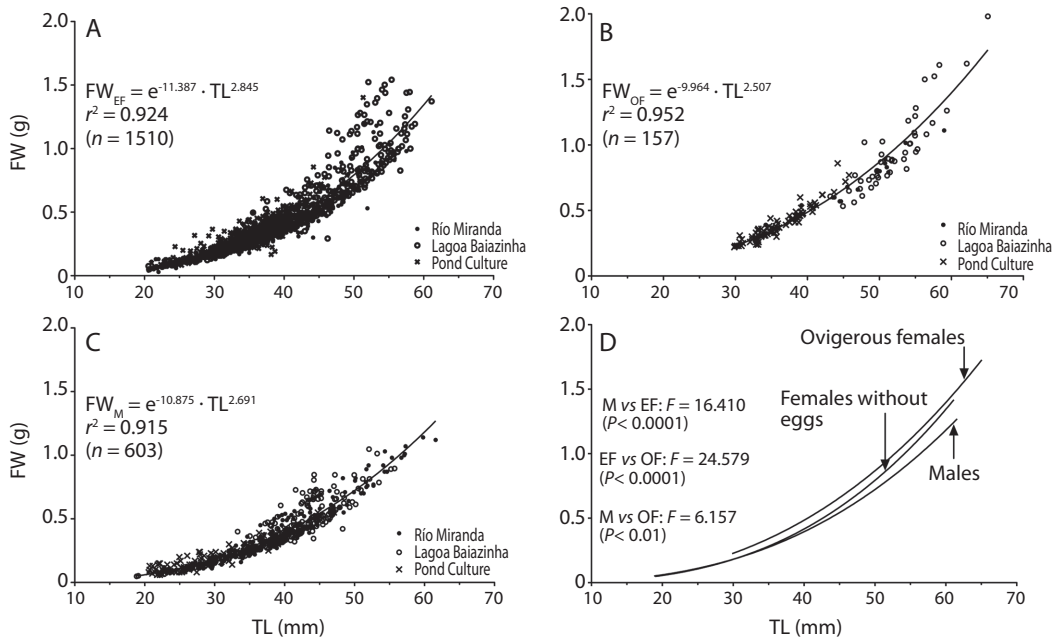


Fig. 3. Power functions describing the relationships between total length (TL) and fresh weight (FW) in non-ovigerous (or empty) females (EF; graph **A**), ovigerous females (OF; graph **B**), and males (M; graph **C**) of *Macrobrachium amazonicum* from the Pantanal (pooled data from three sampling sites); r^2 , coefficient of determination; n , number of observations; F statistics and levels of significance, P , for statistical comparisons between slopes (ANCOVA, with log-transformed data sets; graph **D**) indicate significantly different size-weight relationships in males, non-ovigerous females, and ovigerous females.

a brief summary of preliminary data characterizing the environmental conditions at three different sampling sites is given here, and no attempts are made to compare in detail ecological or seasonal variations in small-scale local populations. While the maximum temperatures observed on collection days were similar (30-31°C; Table 1), minimum values varied more strongly among sites (17-25°C), with lowest variability at Lake Baiazinha. Likewise, maximum conductivity measurements varied only little among sites (43-51 $\mu\text{S}/\text{cm}$), while the minimum values ranged from 10-26 $\mu\text{S}/\text{cm}$. The pH values varied in general more strongly, probably due to short-term changes in primary production and weather conditions, ranging from 4.9-8.4; in this physical parameter, both the highest average level and greatest stability occurred in a lotic habitat, the Miranda River. The same applies to dissolved oxygen, which was mostly higher and less variable in the river

as compared to shallow lentic habitats (Lagoa Baiazinha, culture pond; Table 1).

Population structure: sex ratio, maximum body size, morphometric traits: In total, 2270 shrimps with sexable size were collected during this study, most of them from the Miranda River (1 068), followed by materials from the pond culture (688), and Lake Baiazinha (514 individuals; Table 1). In all samples and at all three sampling sites, consistently more females than males were collected. The sex ratio (males:females) observed at these sites was thus always strongly female-biased, with average values of 0.37, 0.22 and 0.59, respectively (overall ratio:0.36).

As another consistent pattern, female shrimps reached generally a larger body size than males (overall maximum values in our materials: 65 vs. 58mm total length, TL, respectively; Table 2). In general, the smallest

TABLE 2
Maximum size (carapace length, CL; total length, TL) reached in *Macrobrachium amazonicum* populations living in estuarine and continental waters with lotic or lentic conditions

	Maximum body size				Reference
	Males		Females		
	TL (mm)	CL (mm)	TL (mm)	CL (mm)	
ESTUARINE WATERS					
Amapá, Brazil	140	28.9*	136	31.3*	Vieira 2003
Ceará, Brazil	100	20.4*	110	25.1*	Guest 1979
Ceará, Brazil	94	19.2*	92	20.6*	Santos <i>et al.</i> 2006
Pará, Brazil	144	29.8*	130	30.0*	Silva 2002
Pará, Brazil	132	27.2*			Moraes-Riodades & Valenti 2002
Pará, Brazil	141	29.1*	109	24.8*	Silva <i>et al.</i> 2007
Pará, Brazil	160	33.2*	140	32.4*	Moraes-Valenti & Valenti 2010
Pará, Brazil			106	24.1*	Lucena-Fredou <i>et al.</i> 2010
CONTINENTAL WATERS					
Amazon & Orinoco, lotic habitats					
Amazon River, Brazil	98	16.5	94	19.4	Kensley & Walker 1982
Amazon River, Brazil			89	20.0*	Magalhães 1985
Amazon River, Brazil	106	22.0			Odinetz Collart & Moreira 1993
Amazon River, Brazil	93*	19.0	97*	22.0	Odinetz Collart & Magalhães 1994
Amazon River, Brazil	122*	25.1	89*	20.0	Porto 2004
Amazon River, Brazil	70**	10.8*	73	16.1*	Farias 2009
Amazon River, Peru	91	18.5*	95	21.4*	García-Dávila & Magalhães 2004
Tocantins River, Brazil	105	21.5*	98	22.1*	Flexa <i>et al.</i> 2005
Tocantins River, Brazil	136*	28.0	114*	26.0	Odinetz Collart & Magalhães 1994
Tocantins River, Brazil	132	28.0	126	23.0	Odinetz Collart 1987
various Rivers, Colombia	79	17.8	89	16.7	Valencia & Campos 2007
Amazon & Orinoco, lentic habitats					
Amazon lakes, Brazil	84*	17.0	81*	18.0	Odinetz Collart & Magalhães 1994
Amazon lakes, Brazil			93*	21.0	Odinetz Collart 1991a
Tucuruí reservoir, Brazil	89*	18.0	81*	18.0	Odinetz Collart & Magalhães 1994
Tucuruí reservoir, Brazil			65*	14.1	Odinetz Collart 1991b
Orinoco lakes, Venezuela			72	15.8*	Romero 1982
Paraná-Paraguay, lotic habitats					
Rio Miranda, Pantanal	58	11.1	60	11.2	present study
Paraná-Paraguay, lentic habitats					
Lagoa Baiazinha, Pantanal	56	10.9	65	13.7	present study
Pond culture, Pantanal	47	9.1	51	11.3	present study
Tietê reservoir, S. Paulo, Brazil	44*	8.5	54*	11.4	Pantaleão <i>et al.</i> 2012

* = CL or TL values estimated from regression equations (Fig. 2).

shrimps were collected from the artificial pond culture, whereas larger individuals occurred in natural habitats, Lagoa Baiazinha and Rio Miranda (Table 2). While only few male individuals reached a body size above 50mm TL, numerous females were found also in the size

class 50-60mm. Individuals with TL>60mm occurred only exceptionally in females and never in males.

When TL was plotted against CL, a linear regression model described this morphometric relationship in both sexes consistently better

than a non-linear (logarithmic, exponential or allometric) model. This indicates that the various body parts grow at similar rates, and morphometric proportions change only gradually throughout juvenile and adult growth (Fig. 2). However, when these linear regressions were statistically compared between males and females, a small but highly significant difference in the slope parameters was detected (Fig. 2C). A significantly steeper slope indicates that body parts other than the carapace tend to grow in males at a slightly faster rate than in females. As a consequence, our regressions imply that large males (e.g. at TL=60mm) show a somewhat smaller CL compared to females with an equally large TL (in this example, CL=11.9 vs. 12.9mm, respectively).

When the proportion of CL:TL is compared between size classes and sexes, this morphometric quotient changes in both sexes throughout juvenile and adult growth, increasing from 0.16-0.17 (in females and males, respectively) at the smallest sexable size (TL=20mm) to 0.20-0.21 at maximum body size (TL=60mm). In summary, measurements of CL (which are technically easier and much more precise than those of TL) can reliably be used for studies of shrimp growth, but sex-related differences in body proportions should be considered.

Size-biomass relationships: When linear dimensions of body size (CL, TL) were plotted against biomass (measured here as fresh weight, FW), consistently an allometric growth pattern was obtained (TL-FW relationships shown as examples, Fig. 3). Again, statistical comparisons of slope parameters showed that these relationships varied significantly between males and females, with or without eggs, respectively (Fig. 3D). While practically no differences in FW occurred between small males and females (<30mm TL), larger individuals became increasingly dissimilar. When FW is compared between males and females (without eggs) at approximately maximum TL (60mm), females show significantly higher FW than males. This indicates morphometric differences

between sexes, confirming the appearance that males are more slender than females with equal TL. When ovigerous females are considered separately, one can see that the egg masses (included in female FW) further enhance their total biomass, so that their TL-FW relationship shows a significantly steeper slope compared to both males and non-ovigerous females (Fig. 3D).

Reproductive traits: The smallest sexable males observed in our materials had a CL of 2.5mm and a TL of 19mm. This size was in our study therefore considered as the lower limit for safe sex identification, and it may be considered as minimum estimate of the "size at the onset of maturity" (SOM; Anger & Moreira 1998) for males. For females, SOM is indicated by minimum body size of ovigerous individuals. In our materials, the smallest egg-carrying female had a TL of 29.8mm (or 6mm CL; Table 3).

Among the 603 male shrimps collected in our study, there was no indication whatsoever of an occurrence of different morphotypes. All males corresponded to the form "translucent claw" (TC; Moraes-Riodades & Valenti 2004).

Fecundity, expressed as number of eggs carried per female, shows allometric relationships to linear dimensions of female body size (CL, TL), while it increases linearly with female FW (Fig. 4).

In ovigerous females isolated from the pond culture, an average of 186 ± 74 (range: 87-387) larvae per female was released, the largest hatch being produced by the largest individual (TL=53.7mm). From this experiment, fecundity can be quantified as number of larvae released per female, and statistical relationships to CL, TL or FW may be obtained. When the fecundity-FW regression obtained from these data is compared with the equivalent relationship between egg number and FW, a significantly weaker slope is calculated for larval numbers regressed on FW (Fig. 4D). The increasing gap between regression lines obtained for numbers of egg or larvae, respectively, in relation to FW represents egg losses,

TABLE 3
Reproductive traits of *Macrobrachium amazonicum* populations living in estuarine and continental waters with lotic or lentic conditions

	Minimum body size				Maximum Fecundity (eggs)	Egg size (l x h, mm)	Reference
	Males		Ovigerous females				
	TL (mm)	CL (mm)	TL (mm)	CL (mm)			
ESTUARINE WATERS							
Amapá, Brazil	46	8.0	69	15.0*	4 033		Vieira 2003
Ceará, Brazil					2 193		da Silva <i>et al.</i> 2004
Ceará, Brazil			59	12.7*	2 200	0.63 x 0.85	Guest 1979
Ceará, Brazil	39	7.5*					Santos <i>et al.</i> 2006
Pernambuco, Brazil			57	13.0	2 673	0.57 x 0.73	Vega Perez 1984
Pará, Brazil			38	7.5*	1 344		Lobão <i>et al.</i> 1986
Pará, Brazil					6 513	0.76	Ribeiro 2003
Pará, Brazil			52	10.9*	3 375		Lucena-Fredou <i>et al.</i> 2010
Pará, Brazil	57*	11.2					Moraes-Riodades & Valenti 2004
CONTINENTAL WATERS							
Amazon & Orinoco, lotic habitats							
Amazon River, Brazil	81	15.0	66	12.9	1 277	0.50 x 0.70	Kensley & Walker 1982
Amazon River, Brazil			49	10.2*	2 259	0.77 x 1.05	Magalhães 1985
Amazon River, Brazil			36*	7.0	2 165		Odinetz Collart 1991
Tocantins River, Brazil	22	3.8					Flexa <i>et al.</i> 2005
Tocantins River, Brazil			44*	9.0	5 706		Odinetz Collart & Magalhães 1994
Tocantins River, Brazil			36*	7.0	617		Odinetz Collart & Magalhães 1994
Apure, Venezuela					1 000	0.83 x 1.03	Gamba 1984
Amazon & Orinoco, lentic habitats							
Amazon lakes, Brazil			48*	10.0	2 850		Odinetz Collart & Magalhães 1994
Pernambuco, Brazil			57	12.7	2 673	0.57 x 0.73	Vega Perez 1984
Orinoco lakes, Venezuela			41	8.2*	953	0.60 x 1.00	Romero 1982
Paraná-Paraguay, lotic habitats							
Rio Miranda, Pantanal Lentic	22	4.1	34	6.7	479		present study
Lagoa Baiazinha, Pantanal	19	2.5	43	7.6	676	0.57 x 0.92	present study
Pond culture, Pantanal	21	4.1	30	6.0	485	0.53 x 0.93	present study
Goiás, Brazil	14	2.7	33*	6.4	315	0.66 x 0.83	Porto 1998
Tietê reservoir, SP, Brazil	23*	4.0	29*	5.3			Pantaleão <i>et al.</i> 2012

Minimum body size (carapace length, CL; total length, TL) of males with appendix masculina and of ovigerous females, considered as estimates of size at the onset of maturity (SOM); egg size given as average larger and smaller diameter (l, h; where data are available). * = CL or TL values estimated from regression equations (Fig. 2).

which seem to gradually increase with increasing female size.

Throughout the period of embryonic development, the average egg volume increased from stage I to II by about 30%, and it almost doubles during the final stage III, eventually reaching a 2.4-fold higher value than in the initial stage I (Table 4).

DISCUSSION

Our study provides preliminary data on the ecology and reproduction of shrimp populations (currently assigned to *M. amazonicum*) from the upper Paraná-Paraguay (or La Plata) basin. Since our data suggest both small-scale and short-term variability in physical

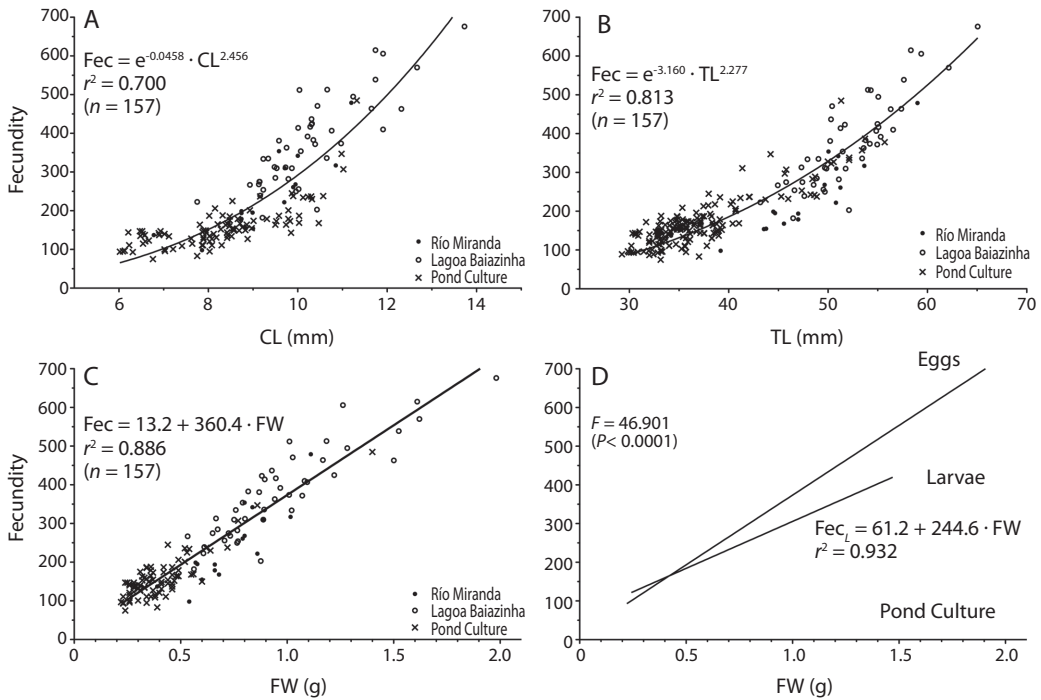


Fig. 4. Power functions and linear regressions describing fecundity (number of eggs per female) in relation to carapace length (CL, graph **A**), total length (TL, graph **B**) and fresh weight (FW, graph **C**) in *Macrobrachium amazonicum* from the Pantanal (pooled data from three sampling sites); r^2 , coefficient of determination; n , number of observations; F statistic and level of significance, P , for statistical comparisons between slopes (ANCOVA; graph **D**) indicate significant differences between linear FW-fecundity relationships in terms of numbers of eggs produced or larvae released, respectively.

TABLE 4

Macrobrachium amazonicum, egg size in three different stages of embryonic development (I-III)

Stage	h	l	V
I	0.53 ± 0.02 (0.47-0.55)	0.67 ± 0.03 (0.63-0.71)	0.10 ± 0.01 (0.07-0.11)
II	0.57 ± 0.03 (0.53-0.63)	0.80 ± 0.06 (0.73-0.90)	0.13 ± 0.02 (0.11-0.19)
III	0.72 ± 0.06 (0.60-0.78)	0.93 ± 0.08 (0.75-1.00)	0.24 ± 0.06 (0.14-0.32)

Smaller and larger diameters (height, h ; length, l ; in mm), volume, V (mm^3 , estimated from diameter assuming an ellipsoid form). Mean ± SD ($n=12$), range in parentheses.

conditions (temperature, conductivity, pH, dissolved oxygen concentration), future studies should further analyze this variability as well as large-scale regional, seasonal and annual variations in the context of seasonal flood pulses and dry periods in relation to differences in the elevation and connectivity of different aquatic habitats (see Junk *et al.* 2011a, Nunes da Cunha & Junk 2011b). In spite of

its preliminary nature, however, our study has already shown several remarkable biological differences between populations living in the Pantanal and those from estuaries or from limnic inland waters of other drainage basins in Northern South America.

Although numerous significant differences among populations had previously been observed in morphological and morphometric

traits (Porto 2004) as well as in molecular genetics (Bastos 2002), these variations have, so far, not been considered as sufficient to justify a taxonomic splitting of the *M. amazonicum* complex (Maciel & Valenti 2009, Vergamini *et al.* 2011). In contrast, recent findings of developmental and physiological differences between shrimps from estuarine (Northern Brazilian) and limnic inland (Pantanal) waters, respectively, have been interpreted as indications of an at least incipient phylogenetic divergence between allopatric populations (Anger & Hayd 2010, Charmantier & Anger 2011, Urzúa & Anger 2011).

One of the most striking differences between estuarine and land-locked inland populations, in general, is the capability of the latter to complete their life cycles in freshwater, while the former must “export” their larvae to waters with higher salt concentrations (for recent review, see Charmantier & Anger 2011). Shrimps from the Pantanal show a significantly higher capacity of hyper-osmoregulation in freshwater than those from an estuarine population, but they have lost the presumably ancestral function of hypo-osmoregulation in seawater (Charmantier & Anger 2011). These contrasting physiological traits are relevant in the context of evolutionary invasions of limnic environments by originally marine crustaceans, especially as they already appear in the earliest life-history stages, allowing for larval development in freshwater.

Likewise, also the present data corroborate the hypothesis that shrimps from the Paraná-Paraguay basin, which are currently assigned to *M. amazonicum*, may actually represent a separate, endemic species. One of the most striking differences was found in maximum body size. Adult shrimps reach in the Pantanal maximally only about one third to one half (males and females, respectively) of the size that is commonly observed in other drainage basins within the vast geographic distributional range of this taxon. Our data, obtained from >2 000 individuals, correspond very well with those available from other regions within the same drainage basin, namely from Goiás

(Porto 1998) and a reservoir receiving water from the Rio Tietê, São Paulo state (Pantaleão *et al.* 2012). This suggests general similarity in life-history traits of shrimps living in various parts of the La Plata drainage system, implying similar patterns of difference from the remaining populations.

Smaller maximum body size in a pond culture compared to natural habitats (47 vs. 56-58mm in males, 51 vs. 60-65mm in females) indicate that high population densities as well as nutritional or other ecological factors may also affect the body size that can be reached in a population (Moraes-Valenti & Valenti 2007, Moraes-Valenti *et al.* 2010). Size differences observed between cultured and natural populations in the Pantanal may be due either to lower growth rates or shorter life spans under mass culture conditions. However, these differences are very small compared to those among populations from different drainage basins. This suggests that striking interpopulational variations in maximum size, in particular in males, cannot be explained by phenotypic plasticity. Rather, they suggest particularly short longevity in Pantanal shrimps, especially in males, likely reflecting genetic drift or different selection pressures on life-history patterns (Graur 2001, Mousseau & Olvido 2005).

As another consistent difference in population structure and reproductive traits, females reach in the Pantanal and in other regions of the La Plata basin a larger maximum body size than males, while the opposite pattern has typically been observed in other populations. This may be related to variation in another reproductive trait, namely the presence or absence of male morphotypes with differentially expressed major chelipeds. Male morphotypes appear to be completely absent in the region of the Paraná-Paraguay basin (present study, Porto 1998, Pantaleão *et al.* 2012), while they are common in other populations assigned to the same species (Moraes-Riudades & Valenti 2004, Papa *et al.* 2004, da Silva *et al.* 2009). They occur also in some congeners, e.g. in *M. rosenbergii* de Man 1879 (Kuris *et al.* 1987) and *M. vollenhovenii* Herklots 1851 (Konan *et*

al. 2010), while they are absent in other species, e.g. *M. australiense* Holthuis 1950 (Lee & Fielder 1982) and *M. ohione* Smith 1874 (Bauer 2004). In species lacking male morphotypes, as in the shrimps from the Pantanal, females typically grow to larger maximum size than males (e.g. Truesdale & Mermilliod 1979, Bauer & Delahoussaye 2008).

Differences in growth and reproductive traits have significant implications for mating systems, which are generally considered as species-specific characteristics and barriers for gene flow (Bauer & Abdalla 2001, Correa & Thiel 2003, Bauer 2004, Bauer & Thiel 2011). The occurrence of strong sexual dimorphism and, particularly in *Macrobrachium* spp., of male morphotypes with extremely large chelae is associated with mate-guarding behaviour (Barki *et al.* 1991a, b, Bauer 2004, Moraes-Riodades & Valenti 2004). Weakly expressed sexual dimorphism with smaller males, by contrast, is typically associated with indiscriminate mate selection and lacking investment in the defence of females as potential mating partners (“pure search” or “sneak mating” tactics; Wickler & Seibt 1981, Laufer *et al.* 1994, Correa & Thiel 2003, Bauer & Abdalla 2001, Bauer 2004, Bauer & Thiel 2011). Differential mating behaviours, with mate-guarding in estuarine *M. amazonicum* (originating from Pará, Brazil) and pure search in Pantanal shrimps, have consistently been observed also in long-term “common garden” laboratory cultures (Anger & Hayd unpublished). In Pantanal shrimps, indiscriminate mating may favour a population structure with low proportions of males, i.e. lower sex ratios. Female-biased sex ratios were also observed in the Mississippi River system in Louisiana, with values close to 1 occurring only in the Atchafalaya River estuary during the breeding season, when females migrate downstream in order to release larvae (Bauer & Delahoussaye 2008).

Sex ratios reported from natural populations of *M. amazonicum* are consistently below 1.0, indicating a general preponderance of females. In our materials, the overall ratio was 0.4 (varying from 0.2-0.6 among sampling

sites), which falls within the range of 0.2-0.8 observed in various other populations assigned to *M. amazonicum* (Odinetz-Collart 1991, Vieira 2003, Flexa *et al.* 2005, Santos *et al.* 2006, Sampaio *et al.* 2007, Pantaleão *et al.* 2012). As the currently available data are too scarce to allow for safe conclusions, more field studies are necessary to analyze relationships between variations in sex ratio, mating systems, ecological conditions, and phylogenetic divergence.

Another difference between populations may be seen in the minimum sexable size of males (appearance of appendix masculina). In the Pantanal, males reached this reproductive stage at a smaller size compared to most other populations, especially to those from estuaries. The smallest identifiable males had in our materials a total length (TL) of 19mm, which is similar to the minimum values measured in the Rio Tietê (23mm; Pantaleão *et al.* 2012); an even smaller male size was observed only in Goiás (Porto 1998). Except for a single report of a similarly low value in a population outside the La Plata system (22mm; Rio Tocantins; Flexa *et al.* 2005), values provided in the literature are generally higher.

Similar to males, also female shrimps from the Pantanal seem to reach sexual maturity earlier than those from other drainage basins. The available data suggest that females in estuarine populations begin with egg laying mostly at >50mm TL, while generally smaller minimum values (down to 36mm) have been reported from limnic inland populations in the Amazon and Orinoco basins. The smallest ovigerous females found in our samples had a TL of 30mm, which is similar to minimum size observed in the Rio Tietê (29mm; Pantaleão *et al.* 2012) and in Goiás (33mm; Porto 1998). All these values correspond to about one half of maximum female size reached in the same populations (RSOM \approx 50%; Anger & Moreira 1998).

In crustaceans with similar body forms, fecundity depends primarily on body size. It is therefore not surprising that egg numbers observed in small-sized Pantanal shrimps were generally far lower than the numbers reported

for much larger shrimps from other populations. *M. amazonicum* from estuarine or Amazonian populations can reach about twice the maximum size observed in the Pantanal, while maximum fecundity differs by one order of magnitude (676 vs. 6 513 eggs per female; cf. present study, Ribeiro 2003). Although fecundity increases as a non-linear (allometric) function of size, this difference remains strikingly large. When regressions describing fecundity of Pantanal shrimps in relation to female size or weight are extrapolated to maximum size occurring in other populations (130mm), a theoretical maximum number of ca. 3 000 egg is obtained. This is only about half the maximum fecundity found in estuarine populations (Ribeiro 2003), suggesting spatial limitations due to larger average egg size in shrimps from the Pantanal.

Significant differences between the numbers of eggs produced and those of larvae eventually released indicate egg losses, as observed also in other species of *Macrobrachium* (see Anger & Moreira 1998, and references cited therein). These losses may in part be caused by parasites and bacterial or fungal infections (Kuris 1991). However, an increasing egg size occurring during the course of embryonic development may eventually also contribute to losses due to space constraints within the brooding chamber.

The available data on egg size in *M. amazonicum* from different populations are relatively scarce and also unreliable, because developmental changes occurring during embryogenesis have rarely been considered in the literature. A comparative study of different populations within the Amazon river system showed that egg size tends to increase with increasing distance from the sea (Odinetz-Collart & Rabelo 1996). This is congruent with observations of larger average egg size in limnic inland waters of the Orinoco basin (Venezuela) compared to estuarine populations from Northern Brazil (Gamba 1984). Also, freshly hatched larvae released by females from the Pantanal show significantly larger size as well as higher dry mass and protein contents than those from an

estuarine population, suggesting larger egg size in Pantanal shrimps (Urzúa & Anger 2011). Comparative field studies of egg size measured in precisely defined developmental stages will be necessary to scrutinize such tendencies.

Smaller body size of Pantanal shrimps at the onset of maturity observed in both sexes, especially in males, implies a heterochronic shift in the development of reproductive systems. This predisplacement of maturation, due either to processes of neoteny or paedogenesis (for concepts and theory of heterochrony, see Brusca & Brusca 1990, McKinney & McNamara 2001), may compensate for presumably shorter longevity indicated by smaller maximum size (see above). As a consequence, life-time reproduction should be enhanced, and the generation turnover is accelerated, which suggests an r-selected life-history pattern (Reznick *et al.* 2002).

All characteristic reproductive traits of *M. amazonicum* from the Pantanal compared to those from other populations (e.g. differential maximum body size of males and females, the presence or absence of male morphotypes, typical mating behaviours, minimum sexable size, minimum size of ovigerous females, different larval size and biomass at hatching) persist, at least throughout several years and generations, also in large-scale aquaculture (Preto *et al.* 2010) and in small-scale cultures of both populations maintained under identical conditions in the laboratory (Anger & Hayd 2009, Anger *et al.* 2009, Anger & Hayd 2010, Charmantier & Anger 2011, Urzúa & Anger 2011). Long-term stability of differential reproductive or other biologically relevant traits in “common garden experiments” is generally considered as evidence for divergence of genetically fixed characters (Mousseau & Olvido 2005).

Future comparative studies of reproductive and other life-history traits may show further characteristic differences between hydrographically and genetically isolated populations of *M. amazonicum*. While vicariant divergence between shrimps from the Pantanal and estuarine populations in Northern and Northeastern Brazil is obvious, the situation is not as

clear in the Amazon, Orinoco and other large South American rivers, where geographical and genetic isolation is incomplete. If future studies can show phylogenetic divergence also between distant populations living in the same river system, e.g. in the upper and lower Amazon, such findings may provide interesting examples of parapatric speciation due to reduced gene flow over enormous distances (Ritchie 2001). Regardless of vicariant or parapatric mechanisms, the *M. amazonicum* complex will remain a highly interesting model for studies of phylogenetic divergence and speciation in the context of evolutionary invasions of limnic environments.

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RESUMEN

El camarón palaemónido *Macrobrachium amazonicum* muestra una distribución geográfica inusualmente grande (aprox. 4 000km de diámetro), vive en las zonas costeras, estuarios y hábitats interiores límnicos del alto Amazonas, Orinoco y las cuencas de La Plata. Esto plantea dudas sobre si poblaciones alopátricas y ecológicamente diversas pertenecen a la misma especie. Mientras que los camarones de estuarios y de hábitats Amazónicos se han estudiado con gran detalle, se sabe muy poco acerca de las poblaciones holo-limnéticas del interior. En el

presente estudio, los rasgos biológicos relacionados con el crecimiento (tamaño máximo del cuerpo, peso fresco, relaciones morfométricas) y la reproducción (proporción de sexos; ocurrencia de morfotipos masculinos; tamaño mínimo sexable; tamaño mínimo de las hembras ovígeras; fecundidad; tamaño del huevo), fueron estudiados en *M. amazonicum* recolectados en un estanque de cultivo y dos hábitats naturales de agua dulce (Río Miranda; Baiazinha Lagoa) en el Pantanal de Mato Grosso do Sul, Brasil. En total, 2 270 camarones fueron examinados (603 machos, 1 667 hembras, 157 de estas ovígeras). La proporción de sexos (machos:hembras) fue en todos los sitios de muestreo fuertemente sesgada por hembras, desde 0.2 hasta 0.6. El tamaño corporal máximo fue mayor en los hábitats naturales comparado con el estanque de cultivo, sugiriendo un crecimiento reducido o un lapso de vida más corto en condiciones artificiales de crianza masiva. La fecundidad máxima observada en nuestro material fue 676 huevos, alcanzado por la hembra de mayor tamaño (largo total, LT=65mm). Una diferencia significativa entre los parámetros de pendiente de regresiones lineales que describen la fecundidad, comparando el número de huevos puestos o de larvas liberadas, en relación con el peso de la hembra, indican pérdida de huevos. Esto puede ser debido a un aumento de 2.4 veces en el volumen del huevo que ocurre durante el desarrollo embrionario, mientras que el espacio disponible bajo el abdomen sigue siendo limitado. Relaciones de tamaño-peso diferían significativamente entre machos y hembras, lo que indica diferencias morfométricas sexo-específicas. Los machos parecen tener una forma de cuerpo más delgado que las hembras, alcanzando por lo tanto el peso más bajo en la igualdad de LT. Cuando los rasgos reproductivos y morfométricos son comparados con datos de la literatura de poblaciones estuarinas y poblaciones del interior que viven en el Amazonas y en los llanos del Orinoco, los camarones del Pantanal muestran peculiaridades diferentes: (1) el tamaño máximo del cuerpo es mucho más pequeño, lo que sugiere menor longevidad; (2) las hembras son más grandes que los machos; (3) diferentes morfotipos masculinos están ausentes; (4) el tamaño mínimo sexable y (5) el tamaño mínimo de hembras ovígeras son más pequeños. Estas características sugieren un cambio heterocrónico (pre-desplazamiento) de la maduración sexual y la selección-r. En resumen, nuestros datos muestran diferencias biológicamente relevantes en los rasgos de la historia de vida de los camarones del Pantanal comparados con *M. amazonicum* en otras regiones. Todas estas diferencias persisten también en cultivos a largo plazo mantenidos bajo condiciones constantes. En conjunto, nuestros datos apoyan la hipótesis que *M. amazonicum* en la desembocadura de la cuenca Paraná-Paraguay ha divergido filogenéticamente de poblaciones alopátricas que están hidrológicamente separadas por cuencas continentales, lo que implica al menos una incipiente especiación vicariante.

Palabras clave: Caridea, camarones de agua dulce, crecimiento, reproducción, fecundidad, historia de vida.

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