Morphotypes in male Amazon River Prawns, *Macrobrachium amazonicum*

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Abstract

The occurrence of morphotypes in *Macrobrachium amazonicum* males was investigated. Prawns aged 4 to 24 months were taken from 10 aquaculture earthen ponds. Color and spination of right second cheliped were analyzed under a stereomicroscope. Post-orbital and carapace length were measured as well as the length of the cheliped and all limb joints on the right second pereiopods. Four distinct morphotypes were identified: Translucent Claw (TC), Cinnamon Claw (CC), Green Claw 1 (GC1) and Green Claw 2 (GC2). They differed in cheliped morphology and some morphometric relationships. Chelipeds were translucent in TC prawns while in CC they were generally cinnamon-color. Both showed a few spines and some low prominences similar to very small tubercles. GC1 and GC2 showed long moss green chelipeds provided with long and robust spines. However, in GC2, cheliped length was always greater than post-orbital length and the angles of spines on the carpus and propodus were more open, ranging from $51^\circ$ to $92^\circ$, while, in GC1 it varies from $34^\circ$ to $65^\circ$. Cheliped length, the cheliped length/post-orbital length ratio and the spine angle were significantly different among the four morphotypes. A description for the identification of each group is provided and the development of *M. amazonicum* males is discussed. Each morphotype may play a different role in the population and in the environment in which it lives. Therefore, the identification of morphotypes is advisable for future researches on the biology and culture of *M. amazonicum*.

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1. Introduction

The culture of freshwater prawns has been recognized as a way to produce crustaceans with a low environmental impact (New et al., 2000a; Moraes-Riodades and Valenti, 2001). This is one of the aquaculture sectors most rapidly growing in the world, with more than 10-fold (1000%) increase over the 1990s. A total of 118,500 mt *Macrobrachium rosenbergii* (see FAO, 2002) and about 100,000 mt *Macrobrachium nipponense* (see Miao and Ge, 2002) were produced in 2000. Thus, the world production of freshwater prawns exceeded 200,000 t in volume and more than US$1 billion in value.

The great development of *M. nipponense* in China over the past years shows the potential of local species other than *M. rosenbergii* for aquaculture purposes. Among South America native species, the most important one is the Amazon River Prawn *Macrobrachium amazonicum*. The species is widely distributed from Venezuela to Argentina (Davant, 1963; Pettovelo, 1996; Bialetzki et al., 1997) and is being intensively exploited for artisanal fisheries in Brazil (Odinetz-Collart, 1987; Odinetz-Collart and Moreira, 1993; New et al., 2000b). Post-larvae of this species have been produced since 1996 in the State of Pará, Brazil, and pilot grow-out projects have been performed (Moraes-Riodades and Valenti, 2001).

*M. amazonicum* shows a great potential for aquaculture (Kutty et al., 2000). It reaches a length of 16 cm and a weight of 30 g (authors’ personal observations) and its meat has a firmer texture and a more marked taste compared to *M. rosenbergii*, thus being better accepted by consumers (Moraes-Riodades and Valenti, 2001). The species is extensively consumed by populations in the Amazon region (Moraes-Riodades and Valenti, 2001) and in the Brazilian Northeast (New et al., 2000b).

Studies on natural *M. amazonicum* populations have shown wide variability in size (Odinetz-Collart, 1993; Odinetz-Collart and Moreira, 1993), probably caused by heterogeneous growth, among other factors. Heterogeneous growth is known to be common in crustaceans and occurs in *M. rosenbergii* (Ra’anan and Cohen, 1985). In this species, sexually mature male populations consist of distinct morphotypes which differ in size, morphology, physiology and behavior (Karplus et al., 2000). The existence of these morphotypes has great implications in aquaculture. The selection and management of broodstock must consider the existence of male categories, since virtually only dominant males reproduce successfully. In addition, the management of grow-out ponds should take into consideration the heterogeneous growth of males in order to maximize production. Management processes compatible with the growth characteristics of the morphotypes have been developed for *M. rosenbergii* (Karplus et al., 2000; Tidwell et al., 2001).

Thus, for the development of a technology for *M. amazonicum* culture it is desirable to know whether there is a population structure with different morphotypes, as observed in *M. rosenbergii*. If this is the case, a set of criteria should be defined to permit the unequivocal identification of each category. Investigations of this type should precede the studies in order to optimize the management of reproduction and grow-out. Thus, the objective of the present study was to test the hypothesis of the existence of morphotypes in *M. amazonicum* males. It is part of a multidisciplinary and multi institutional program for the development of technology for *M. amazonicum* farming recently (2000) started in Brazil.
2. Materials and methods

The study was conducted in the Crustacean Sector of the Aquaculture Center (CAUNESP) at the São Paulo State University, Jaboticabal. From March to October 2001, 140 adult male specimens of *M. amazonicum* from ten 0.01–0.02 ha earthen ponds were collected and selected. The first pond contained 2-year-old prawns, after metamorphosis, and was stocked with post-larvae brought from the Larviculture stations of Curupé, municipality of Curuçá, Pará, Brazil. One pond contained 1-year-old animals, six ponds contained 6-month-old prawns, and the last two contained 4-month-old prawns; these animals were produced in the Prawn Hatchery of CAUNESP from broodstock originating from northeastern Pará. Prawns were stocked at 10–20 PL/m² and a pelleted commercial diet (32% crude protein) were supplied at a rate of 5% of prawn biomass per day.

Five to ten adult males of each total length class (5 mm classes) were selected for the analysis of chelipeds and body dimensions. Prawns with appendix masculina in the second pair of pleopods were considered to be adults. The appendage appears when the animals measure 50–55 mm in total length (Moraes-Riodades and Valenti, 2002).

The color and spination of the chelipeds of all animals were analyzed under a model MZ-5 Wild-Leitz stereomicroscope at 40 × magnification. On the basis of these data, the prawns were divided into similar groups. The chelipeds of six animals per group were photographed with a Leica 150 digital camera (model GC-X1). The photographs were then used to measure the angles of 10 spines on the distal portion of the carpus and the proximal portion of the propodus by means of a protractor with 1° precision. The mean angle and standard deviation was then calculated for each group.

All males were measured by the method of Kuris et al. (1987) for morphotype definition in *M. rosenbergii*. The following dimensions were measured: post-orbital length (Lpo), defined as the distance between the posterior margin of the right eye and the distal tip of the telson with the animal stretched out; carapace length (Lcp), the distance between the posterior margin of the right orbit and the midpoint of the posterior margin of the carapace; length of the second right cheliped (Lcl), distance between the proximal margin of the ischium and the distal tip of the propodus; dactylus length (Ldc), distance between the proximal and distal tip, measured along the dorsal line; propodus length (Lpp), distance between the proximal lateral condyle and the distal end measured on the lateral surface with the propodus flexed; carpus length (Lc), distance between the proximal lateral condyle and the distal margin measured with the cheliped flexed; merus length (Lm), distance from the proximal to the distal margin measured along the dorsal line; ischium length (Li): distance from the proximal to the distal margin measured along the dorsal line.

All measurements were made with a digital vernier caliper with 0.05 mm precision. The measurements of the cheliped and its joints were made in the second right pereiopod. The means and standard deviation of Lpo, Lcp and Lcl and of the Lcl/Lpo ratio were calculated for each group. The mean values of these dimensions and of the spine angles were subjected to analysis of variance (F test) followed by the Waller–Duncan test using the S.A.S. software. Variance homogeneity was analyzed by the Brown–Forsythe test. Differences were considered to be significant when \( P \leq 0.05 \).
The equations representing the relationships between the cheliped (and its joints) and the carapace and between each limb of the cheliped were determined for each group and adjusted using log base 10 data. The allometric equation $y = ax^b$ (Hartnoll, 1982) was used in the logarithmic form, i.e.: $\log y = \log a + b \log x$, where $x =$ size of the reference dimension from which the size of the $y$ dimension will be estimated; $a$ is a constant related to the intercept on the ordinate axis, and $b$ is the allometric growth constant. The adjusted equations for each group were compared by analysis of covariance (Sokal and Rohlf, 1995) using the Statistics software, with the level of significance set at $P \leq 0.05$.

3. Results

Post-orbital length ranged from 43.2 to 105.8 mm. Four groups of males differing in color and spination pattern were identified in all age groups studied and in all ponds. They were called Translucent Claw (TC), Cinnamon Claw (CC), Green Claw 1 (GC1) and Green Claw 2 (GC2). Fig. 1 shows a specimen of each group originating from the same progeny, showing differences in body and cheliped size. Each group is described in detail below.

TC prawns presented a post-orbital length ranging from 43.2 to 73.0 mm and cheliped length ranging from 20.0 to 54.3 mm. Their ischium was translucent, colorless (sporadically presented some brown pigments distally) and devoid of spines. The merus was translucent with sometimes, but not always, a greenish tinge and devoid of spines. The carpus was semi-translucent brownish, with a few beige setae and low prominences, which we called small tubercles that seem to be spines buds; some larger animals also presented small spines with an inclination forming an angle of $7^\circ$ to $15^\circ$. The propodus was semi-translucent brownish, had a few beige setae and no spines, although small tubercles often occurred. The dactylus was opaque, slightly greenish and some beige setae were occasionally present.

![Fig. 1. Comparison of the animals in the four groups.](image-url)
CC animals presented a post-orbital length ranging from 49.9 to 73.6 mm and a cheliped length ranging from 24.7 to 54.8 mm. All joints presented beige setae and, except the dactylus, small tubercles. The ischium was translucent greenish and showed brown pigments distally, while green pigments were occasionally present; frequently, there were some spines. The merus was translucent greenish, with some small spines. The carpus was light green, with several brown spots, but when observed without a stereomicroscope showed a beige color; it is covered with small tubercles and translucent spines, forming angles ranging from $8^\circ$ to $39^\circ$. The propodus was greenish-beige, with many beige setae (more than in the TC) and a few spines forming an angle similar to that of carpus spines. It had a brown line, broken or continuous, from the middle portion to the joint with the dactylus. The dactylus was beige with some green portions and presented a brown or greenish-blue spot at the joint with the propodus.

GC1 prawns presented a post-orbital length ranging from 67.7 to 105.8 mm and cheliped length always shorter than post-orbital length ranging from 56.2 to 98.8 mm. All joints presented more beige setae than TC and CC, while fingers are distinctly velvety pubescent. The ischium was opaque, greenish or whitish, with small tubercles and opaque slender spines. The merus was green, with few small tubercles and short, but robust greenish spines. The carpus was moss green and covered with many robust greenish spines (sometimes fine and long ones), forming angles ranging from $34^\circ$ to $65^\circ$. The propodus was moss green, with many green spines, which sometimes are small and robust with an angle similar to that of the carpus and often having a brown pigmentation; it had a greenish-blue stripe extending from the middle portion to the joint with the dactylus. The dactylus was brown with some moss green portions and had a greenish-blue spot at the joint with the propodus.

GC2 animals presented a post-orbital length ranging from 65.4 to 104.0 mm and cheliped length ranging from 71.9 to 175.6 mm. The pattern of color, spines and setae was similar to that of GC1. However, cheliped length was always greater than post-orbital length and the angles of spines on the carpus and propodus were more open, ranging from $51^\circ$ to $92^\circ$.

All groups presented a large quantity of brown pigments in every joints (except the ischium of TC), which can be seen under stereomicroscope. The TC and CC groups differed in size, whereas GC1 and GC2 were of similar size and larger than TC and CC

| Table 1 | Post-orbital length (Lpo), carapace length (Lcp), cheliped length (Lcl), cheliped length/post-orbital length ratio, and spine angle for the four groups |
|---------|--------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|
|         | TC (57)                                          | CC (34)                         | GC1 (17)                        | GC2 (32)                        |
| Lpo (mm)| 55.1 ± 8.2 c                                    | 63.4 ± 7.0 b                    | 87.1 ± 11.6 a                   | 87.4 ± 10.5 a                   |
| Lcp (mm)| 15.8 ± 2.8 c                                    | 18.8 ± 2.6 b                    | 27.3 ± 3.8 a                    | 27.4 ± 3.8 a                    |
| Lcl (mm)| 30.8 ± 7.1 d                                    | 39.8 ± 8.6 c                    | 80.6 ± 13.9 b                   | 114.4 ± 25.9 a                  |
| Lcl/Lpo | 0.56 ± 0.07 d                                    | 0.62 ± 0.09 c                   | 0.92 ± 0.08 b                   | 1.30 ± 0.21 a                   |
| Angles  | 12.2 ± 2.3 d                                     | 21.0 ± 6.9 c                    | 49.6 ± 8.9 b                    | 61.6 ± 9.2 a                    |

Means in the same line followed by different letters are significantly different ($P < 0.05$).
Data are reported as means ± S.D. Number of specimens measured are presented in brackets. TC = Translucent Claw, CC = Cinnamon Claw, GC1 = Green Claw 1, GC2 = Green Claw 2.
animals (Table 1). The four groups differed significantly in cheliped size, Lcl/Lpo ratio and angles of the carpus/propodus spines (Table 1).

In general, the relationships between cheliped length and carapace length and between the length of each joint of the second right cheliped and carapace length differed in the four groups. The relationships of the length of the cheliped limbs with one another did not differ between the TC and CC groups, but did show a clear difference between GC1 and
4. Discussion

Morphometric analysis confirmed the division into groups according to color and spination of the second right cheliped. Thus, our results indicated that the cultivated male population of *M. amazonicum* is composed of four distinct groups differing in cheliped morphology and size and in various morphometric relationships. Each group presents a specific growth pattern demonstrated by the differences in allometric growth constant. Thus, the hypothesis of the existence of morphotypes in this species was confirmed, with the morphotypes corresponding to the groups described earlier (TC, CC, GC1 and GC2).

The existence of males with proportionally more developed and more colored chelipeds with more abundant spines was observed by Holthuis (1950, 1952) in various species of the genus *Macrobrachium*. This author called these animals dominant and also elaborated keys for species identification based on these males. However, only *M. rosenbergii* had been described in detail to date (Ra’anan and Cohen, 1985; Kuris et al., 1987), with the definition of morphotypes. Since the present study also described morphotypes in *M. amazonicum*, morphotypic differentiation may be a characteristic of the genus *Macrobrachium*. However, this genus has more than a hundred of known species around the world and, therefore, studies on several other species should be conducted to confirm this hypothesis.
Kuris et al. (1987) studied in detail the morphotypic differentiation of *M. rosenbergii* males and defined each morphotype on the basis of measurements of the ratios between the chelipeds joints and between the chelipeds and the body using techniques employed in the study of relative growth, as well as color and spination data for the second right cheliped. Small males (SM) are small animals with fine translucent or rose-colored chelipeds and with very low growth rate. Orange claw (OC) males are subdivided into weak orange claw (WOC) and strong orange claw (SOC) groups. WOC males are small with orange-colored chelipeds and present a high growth rate, while SOC males are large, have medium-sized orange-colored chelipeds, have a high growth rate and a low reproductive activity. Blue claw (BC) males are large and have very long blue chelipeds; they present a growth rate close to zero and a high reproductive activity (Ra’an an and Cohen, 1985). In a comparison of the results obtained in the present study on *M. amazonicum*, the TC morphotype may correspond to the SM morphotype of *M. rosenbergii*, CC may correspond to WOC, GC1 to SOC, and GC2 to the group of dominant BC males. Similar to *M. rosenbergii*, there is size overlap between TC and CC prawns and GC1 and GC2 prawns in *M. amazonicum*. On the other hand, in *M. rosenbergii*, SOC is morphologically more similar to WOC than to BC, whereas in *M. amazonicum*, GC1 is quite different from CC and is similar to GC2. Further studies are needed to determine whether there is a correspondence between the physiological, behavioral and functional characteristics of each morphotype in the two species.

The morphotype development in *M. amazonicum* adult males may be a sequential process as it occurs in *M. rosenbergii*. The TC morphotype comprises smaller animals presenting characteristics similar to those of juveniles, whose appendix masculina is being formed. In contrast, the CC morphotype is formed by slightly larger animals, with a slightly higher cheliped size/body size ratio (Table 1). In addition, they present greater development in cheliped color and spination. Morphotypes GC1 and GC2 are much larger and with considerably modified chelipeds compared to the others. Considering animal size and the development of cheliped spination, color and size, the developmental sequence seems to be: TC → CC → GC1 → GC2. The passage from one morphotype to other may occur through a single molt or through a gradual process. In addition, passage through all phases may not be obligatory and retrocession may occur. Several studies discussed by Karplus et al. (2000) have demonstrated that an obligatory sequence occurs in the development of *M. rosenbergii*, with progression through the four morphotypes (SM, WOC, SOC and BC). Rearing of the animals in individual chambers should be performed to determine how this process occurs in *M. amazonicum*.

Morphological variations along post-larval development are common in crustaceans. The morphometric relationships are often marked by two discontinuities or inflection points that correspond to the pre-puberty molt and to the puberty molt (Hartnoll, 1982). These molts condition the onset of secondary sex characteristics. All the animals studied here were adults and therefore the passage from one morphotype to another cannot be associated with the pre-puberty or puberty molt, but represents a change of morphotype within a population. In addition, the four morphotypes were observed in ponds stoked with post-larvae of the same age, demonstrating that they are not prawns of different age groups. This characterizes the existence of polymorphism in *M. amazonicum*, according to Hartnoll (1982) concept.
The chelipeds are appendages of great importance for prawns, having a function in food gathering (Valenti, 1985; Ismael and New, 2000), in the occupation and defense of the territory (Nagamine and Knight, 1980), in agonistic behaviors (Nagamine and Knight, 1980; Volpato and Hoshino, 1984; Ismael and New, 2000), in the maintenance of social structure (Volpato and Hoshino, 1984; Karplus et al., 1992), in courtship behavior, and during copulation (Ismael and New, 2000). Thus, changes in cheliped size, color and spination necessarily cause deep modifications of the intraspecific interactions and in the male’s interaction with the environment. Each morphotype certainly has a different function in the population and in the environment, in which it lives conferred by the characteristics of its chelipeds.

Males with highly developed chelipeds such as GC2 animals devote much energy and material to the growth and maintenance of this appendage at the expense of body growth. In addition, a very large cheliped reduces the ability to swim and even to move on the substrate. Thus, GC2 males, and perhaps also GC1 males, must be more sedentary. In contrast, TC and CC males must be more active, having greater mobility and expending more energy in the search for food and also to escape from GC1 and GC2 males.

Large chelipeds confer advantages to the individuals that carry them. They permit the capture of food at a longer distance from the body, allowing the exploration of a larger area without the need to move. In addition, they permit the capture of prey and the defense against predators and competitors, keeping the body distant and therefore more protected. Macrobrachium prawns have the habit of living in shelters (Valenti, 1985) and their long chelipeds permit them to defend their shelter without getting out of it or letting the competitor in. M. rosenbergii BC males are known to use their chelipeds during courtship in order to turn the female belly up for copulation (deposition of the spermatophore) and to protect her immediately after oviposition (Ismael and New, 2000). Thus, males of this morphotype have greater reproductive success. In this same species, it was demonstrated that the cheliped is essential for the maintenance of the social structure of the population, also in order to inhibit the growth of subordinate animals (Karplus et al., 1992). The greater energy needed to maintain the cheliped and the difficulty in locomotion may be probably compensated for by advantages in different situations, such as those cited above, provided by a large cheliped. On this basis, GC2 M. amazonicum males are expected to present these advantages and to represent the dominant and most actively reproducing males. Behavioral studies are needed to confirm this hypothesis.

Observations made by us have demonstrated that the four morphotypes live together in the same pond and originate from the same progeny. In addition, samples from rivers in the State of Pará, Brazil, have shown that these four morphotypes also occur in natural environments. The population structure and the mechanisms leading to the development of these four morphotypes should be investigated. These knowledge are important for broodstock selection and for management of the heterogeneous growth in grow-out ponds to maximize the production. The present study provided a description for the identification of each morphotype of M. amazonicum males, which may be useful for future researches on the biology and culture of this species.
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