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ARE THERE ANY PHYSIOLOGICAL DIFFERENCES BETWEEN THE MALE MORPHOTYPES OF THE FRESHWATER SHRIMP *MACROBRACHIUM AMAZONICUM* (HELLER, 1862) (CARIDEA: PALAEMONIDAE)?

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ABSTRACT

A comparison was made of the metabolism, nitrogenous excretion, growth, oxidized energy substrate, ingestion rate, and fecal production of the males of three morphotypes (CC, GC1, and GC2) of the palaemonid shrimp *Macrobrachium amazonicum* (Heller, 1862). The proportion of these morphotypes in the population is fixed, and individuals can change from one type to another (TC to CC, CC to GC1, and GC1 to GC2) to maintain this proportion. The three morphotypes were evaluated for 30 days, during which individuals were fed daily with commercial food. Food debris, feces, and exuviae were collected daily. Oxygen consumption was measured in a closed respirometer, and ammonia excretion was measured by colorimetry. The atomic ratio O:N was used to indicate the predominant energy substrate oxidized. Mass gain (% $\mathrm{WW_i}$) was higher in morphotypes CC and GCI (12.7 ± 3.2 and 16.0 ± 3.7%, respectively) than in GC2, in which it was nearly zero (1.9 ± 1.5%). Lost exuviae contained approximately 40% of the energy content of the individuals, and males did not cease feeding as post-molts as reported in some crustaceans. Despite the elevated growth of CC and GC1, the ingestion rates were similar in all morphotypes and corresponded to 3% of the total biomass. It is possible that CC and GC1 channel a higher percentage of ingested energy and nutrients into growth, whereas GC2 channels more energy into other pathways such as reproduction. Whereas the morphotypes CC and GC1 mainly use carbohydrates as their energy substrate, GC2 uses proteins. Given the elevated growth rate of CC and GC1, they appear to preferentially use amino acids in tissue building, whereas GC2 uses these substrates as an energy source. The feces eliminated by the morphotypes were always proportional to the ingestion rate (approximately 2%), suggesting utilization in terms of nutrient absorption was similar for them. Oxygen consumption in specific mass was similar for all three morphotypes (approximately $1.8\ \mu\mathrm{g}\ (\mathrm{mg\ dw})^{-1}\ \mathrm{h}^{-1}$), and ammonia excretion was approximately 180% higher in GC2 than in the other two morphotypes. These results might reflect the pattern of growth, activity, function in the population, and differences in reproductive behaviour in the morphotypes and could be evidence of a preparation for the subsequent morphotype.

KEY WORDS: excretion, fecal production, growth, metabolism, physiology, sex differences

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INTRODUCTION

The neotropical palaemonid shrimp *Macrobrachium amazonicum* (Heller, 1862) has a wide distribution, occurring in the Orinoco, Amazon, São Francisco, Paraná, and Paraguay rivers and in coastal regions of northern and northeastern South America, including the Paraguay River and the Miranda, Negro, and Taboco rivers (Maciel and Valenti, 2009). There is a geographical separation and, as a consequence, genetic isolation between different populations of *M. amazonicum* in the northern (including the Atlantic and Caribbean coasts) and southern (La Plata River system) regions of Brazil. This wide geographical distribution results in distinct physiological, reproductive, behavioural, and ecological patterns between many individuals of the populations. *Macrobrachium amazonicum* is of particular interest because males show distinctive morphotypes (TC, CC, GC1, and GC2) as a result of its wide distribution (Moraes-Riodades and Valenti, 2004).

The existence of morphotypes among males that occupy the same ecological niche has been described in other species of freshwater palaemonid shrimps such as *Macrobrachium rosenbergii* (De Man, 1879) (Ra'anan and Cohen, 1985; Kuris et al., 1987), *M. dayanum* (Henderson, 1893) (Langer et al., 2002), and *M. grandimanus* (Randall, 1840) (Wortham et al., 2012). These morphotypes perform distinct functions in the population and are characterized by their morphological differences (size, color, number of spines) and reproductive and behavioural patterns. The proportion of these morphotypes in the population is fixed and species-specific, and individuals can change from one type to another (TC to CC, CC to GC1, and GC1 to GC2) to maintain this proportion. Males of different morphotypes but of the same age can be descendants of the same female. According to Moraes and Valenti (2004), the passage from one morphotype to another may occur through a single molt or through a gradual process. Passage through all phases may

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not be obligatory, and retrocession may occur. Ra'anan and Cohen (1985) and Kuris et al. (1987) identified three morphotypes in populations of *M. rosenbergii* (SM, BC, and OC), which were characterized by morphological, reproductive, and behavioural differences. Genetic characterization of the males of the different *M. rosenbergii* morphotypes showed that there are significant differences at the molecular level between the morphotypes (Ranjeet, 2010). Moraes-Riodades and Valenti (2004) demonstrated the existence of four groups of males from a population of *M. amazonicum* in northeastern Pará State, northern Brazil: Translucent Claw (TC), Cinnamon Claw (CC), Green Claw 1 (GC1) and Green Claw 2 (GC2).

The TC morphotype originates from undifferentiated juveniles and comprises small and agile animals (approximately 5.5 cm postorbital length) that present some juvenile characteristics. The CC morphotype consists of slightly larger individuals than TC (approximately 6.3 cm) and with a higher ratio between the size of the chela and the rest of the body. The GC1 and GC2 morphotypes are larger (approximately 8.7 cm) with highly differentiated chelas; GC1 presents low reproductive activity, whereas GC2 is sedentary and is the morphotype with the highest reproductive activity in the population. The presence of males of different sizes in the population of shrimps of the genus *Macrobrachium* is evidence of the existence of a social hierarchy (Karplus et al., 1992). The adaptive value of a hierarchy among males is mainly related to the reproductive success of the species and protection against predation.

Papa (2007) observed the presence of a functional reproductive system in all *M. amazonicum* morphotypes, but they did not verify differences in the growth of the hepatopancreas, body weight, or chela length between GC1 and GC2, which suggested the existence of only three morphotypes in the population. Santos et al. (2014) detected differences in proteolytic activities among farmed morphotypes but not among wild individuals.

A clear definition of the morphotypes of caridean shrimps is extremely important both for understanding their adaptive value in the population and for aquaculture because once species such as *M. amazonicum* and *M. rosenbergii* are farmed, their rearing in fattening hatcheries must account for the heterogeneous growth of males to maximize production. In *M. rosenbergii*, some researchers have successfully developed a rearing method compatible with the growth characteristics of the morphotypes (Karplus et al., 2000; Tidwell et al., 2001).

We compared the physiological characteristics of three morphotypes (CC, GC1 and GC2) of *M. amazonicum* fed with commercial food by evaluating the metabolism, nitrogenous excretion, growth, energy substrate (proteins, carbohydrates or lipids) predominantly oxidized, ingestion, fecal production and energy expenditure from molting. These same physiological characteristics have already been described by Augusto and Masui (2014) for the TC morphotype and will be used for comparative purposes with the other morphotypes studied here.

MATERIALS AND METHODS

Collection and Acclimation of Laboratory Animals

Male *M. amazonicum* at the inter-molt stage were collected in hatcheries from the Aquaculture Center of UNESP (CAUNESP), Jaboticabal, São Paulo State, Brazil. These individuals came from a population in northeastern Pará State, Brazil (01°14′30″S, 48°19′52″W), and have been kept in hatcheries since 2001. All individuals collected were at the inter-molt stage. After collection, shrimp were maintained for 7 d in the Carciniculture Laboratory of the Aquaculture Center of UNESP (CAUNESP) to acclimate them to laboratory conditions. During this period, they were kept in individual tanks containing fresh water with constant aeration, a temperature of 30°C, and a photoperiod of 12 h light and 12 h of darkness. Approximately 50% of the water in tanks was changed every two days. Animals were fed daily with commercial shrimp food (Poli-Nutri®) in an amount corresponding to 7% of their body mass. Food was offered at 1800 h (6 pm) because a pilot experiment found that individuals kept in the laboratory feed mainly in the evening.

All experimental groups (CC, GC1, and GC2) consisted of at least seven individuals. The shrimp, which were at the pre-molt or post-molt stage at the end of the experiment, were discarded once their elevated hydration would alter the measurements of mass gain.

Experimental Procedures for the Evaluation of Physiological Parameters

The shrimp were weighed (Marte balance AS 2000C) after acclimatization to laboratory conditions and transferred to individual aquaria containing fresh water for the evaluation of daily ingestion and egestion rates and weekly growth rate for 30 d. Laboratory animals were kept as indicated above. At the end of this period, the individuals were used to evaluate oxygen consumption and ammonia excretion.

Evaluation of the Ingestion (C) and Egestion (F) Rates

Ingestion rate was calculated as the difference between the mass of the food offered and the mass of the food left in the tanks. Unconsumed food was removed from the aquaria in the morning daily by siphoning, dried in filter paper, weighed (wet mass), oven dried at 60°C for 48 h and weighed again to 1 μg (Mettler Toledo). Control samples of food were weighed, placed in tanks that were kept under the same experimental conditions, but without animals, stored in Eppendorf tubes, and kept in a freezer for further energy content analysis. Food energy content was determined by the wet combustion of samples from 8 to 12 mg dry weight (Karzinkin and Tarkovskaya, 1964). This method is based on the oxidation of organic matter with potassium iodate in the presence of sulfuric acid and using sodium thiosulfate as a titrant.

Egestion was measured by taking feces from the tanks every day in the morning using a plastic pipette, dried at 60°C for 48 h, and weighed as in unconsumed food.

Growth (P)

Males were weighed weekly for a period of 30 days. On the last day of the experiment, after oxygen consumption and ammonia excretion were evaluated, animals were killed by freezing, oven dried at 60°C for 48 h, and weighed again. Energy content was determined using a calorimetric pump (Parr, 6300). Samples of at least 1 g were macerated and burned with a calorimetric pump. Energy content was obtained by calculating the ratio between the heat released and the heating of the water around the pump. An increase of 1°C per kilogram of water is equivalent to 1 kcal of energy.

Exuviae (E_V)

Samples of exuviae were collected from tanks during the 30 day experimental period. Exuviae were dried with absorbent paper and weighed as indicated above. They were then oven dried at 60°C for 48 h and weighed again. Food energy content was determined by wet combustion of samples ranging from 8 to 12 mg dry weight (Karzinkin and Tarkovskaya, 1964).

Oxygen Consumption, Ammonia Excretion, and O:N Ratio

Oxygen consumption and ammonia excretion were measured on the last day of the experimental period (day 30^t). Shrimp were kept for 24 h without food to avoid the calorigenic effect of food and their gut content was observed with the purpose of verifying the absence of food (Rosas et al., 1999; Lemos and Phan, 2001). After this period, animals were placed in individual conical respirometric chambers containing 440 ml of filtered water and maintained in trays with water and an electrical heater at

30°C (Augusto and Masui, 2014). Individuals were kept in respirometric chambers for 30 min with aeration to acclimatize them and reduce the stress caused by manipulation. Aeration was then removed and the oxygen concentration within the chamber was measured using a dissolved oxygen meter (monitor and probe, YSI, Yellow Spring, OH, USA, models 53 and 5905, respectively). The oxygen concentration was measured again after 60 min. Control chambers without animals were kept under the same experimental conditions.

Variations in oxygen concentration were calculated as the difference between the values obtained in samples and controls (no animals). After incubation, shrimp were killed by freezing, oven dried at 60°C for 48 h and weighed (Marte balance AS 2000C). Ammonia excretion was measured in samples of 5 ml of water obtained from the respirometer chambers at the beginning and end of the experiment to measure oxygen consumption. The ammonia concentration in water samples was determined in triplicate by colorimetry (Koroleff, 1983). The O:N ratio was calculated by dividing the oxygen consumed (in mol) by the ammonia excreted (in mol) (Mayzaud and Conover, 1988). This method is based on the reaction of NH_3 in an alkaline solution with phenol to produce a blue color (indophenol blue) in the presence of a strong oxidizing agent such as hypochlorite. The absorbance of samples was measured using a spectrophotometer at 630 nm.

Statistical Analysis

We applied one-way ANOVA followed by the Student-Newman-Keuls multiple means test to locate statistically significant groups. All statistical analyses were performed after verifying the normality of the distribution and equality of variance using Sigma Stat 2.03 and a minimum significance level of $P = 0.05$.

Results

Data for mass gain, growth, ingestion, fecal release, oxygen consumption, and ammonia excretion in morphotypes CC, GC1, and GC2 of *M. amazonicum* shrimp are shown in Table 1.

Mass gain (% MU_i) was higher in CC (12.7 ± 3.2%) and GC1 (16.0 ± 3.7%). In GC2, the gain was only 1.9 ± 1.5% of the initial wet weight. Growth was higher in CC than in GC2, which showed almost no growth (0.01 ± 0.006%). There were no differences among morphotypes for ingestion rate (approximately 3% of initial biomass) or release of daily feces (approximately 2.5% of ingested food).

Oxygen consumption and individual ammonia excretion varied between the morphotypes (Table 1). Oxygen consumption was higher in GC1 and GC2 compared to CC. Ammonia excretion was different among all morphotypes, with the lowest values in CC (0.87 ± 0.18 mg ind^{-1} day^{-1}) and the highest in GC2 (13.53 ± 0.54 mg ind^{-1} day^{-1}). The atomic ratio of O:N was lower than 7 in GC2, which suggests the use of proteins as an energy substrate, whereas the results obtained for CC and GC1 indicated a mixture of proteins and carbohydrates.

The energy content of food was 13.8 ± 0.2 kJ $(g\ MS)^{-1}$ and 7.9 ± 0.2 kJ $(g\ MS)^{-1}$ for exuviae, which corresponds to approximately 40% of the energy value of the whole bodies of shrimp (Table 1).

The specific dry-mass oxygen consumption was similar for all morphotypes (Fig. 1). Ammonia excretion was approximately 180% higher in GC2 than in the other two morphotypes evaluated (Fig. 2).

Discussion

Results from this investigation suggest that the male morphotypes of *M. amazonicum* exhibit not only morphological differences but physiological differences as well. These differences, especially between CC and GC1 (and TC; Augusto and Masui, 2014) in relation to GC2, may be related to the physiological differences that characterize every morphotype, such as an unequal pattern of activity, growth, function in the population, and reproductive behavior. Because morphotypes were evaluated in isolation from the others, these differential responses may also be related to the physiological processes that precede the transformation of morphotypes. Some experiments, such as those outlined here, cannot be conducted with all morphotypes kept together in the same aquarium due to the impossibility of identifying the food ingested or the feces produced by each. Nevertheless, it is important that, whenever possible, future studies that investigate aspects of the biology of the different morphotypes are done with them kept together such that the studies will not miss the effects of social interactions.

The physiological aspects of *M. amazonicum* male morphotypes investigated suggest differences mainly between the CC and GC1 male morphotypes compared to GC2 morphotypes regarding mass gain, nitrogenous excretion, and oxidized energy substrate.

Growth

Heterogeneous growth in crustaceans often leads to the establishments of social hierarchies in a population (Barki et al., 1992; Silva and Arruda, 2015). Such development,

Table 1. Mass gain (% WW_i) by the end of 30 days and daily growth (P), ingestion rate (C), respiration (R), excretion (U), feces (F), and atomic O:N ratio in *M. amazonicum* morphotypes at 30°C. The energy content of morphotype bodies is also shown. WW_i: initial wet mass of animals. Values with different superscript letters within the same row are significantly different.

	CC	GC1	GC2
Mass gained (% WW_i)	12.7 ± 3.2^a	16.0 ± 3.7^a	1.9 ± 1.5^b
P (mg WW day^{-1})	24.6 ± 4.8^{ab}	53.7 ± 18.2^b	7.5 ± 5.8^a
C/WW_i (%)	3.22 ± 0.19	3.10 ± 0.22	3.08 ± 0.32
C (mg WW day^{-1})	198.0 ± 11.9^a	316.0 ± 30.1^{ab}	445.0 ± 88.0^b
F (%C)	1.95 ± 0.34	2.43 ± 0.40	2.8 ± 0.50
F (mg DW day^{-1})	2.95 ± 0.47^a	5.8 ± 0.88^{ab}	8.85 ± 1.78^b
R (g O_2 ind^{-1} day^{-1})	0.03 ± 0.001^a	0.06 ± 0.01^b	0.07 ± 0.003^b
U (mg NH_3-N ind^{-1} day^{-1})	0.87 ± 0.18^a	3.35 ± 0.48^b	13.53 ± 0.54^c
O:N	17.2 ± 2.9	11.1 ± 0.8	2.5 ± 0.1
Energy content (KJ g^{-1} DW^{-1})	19.2 ± 0.3^a	19.6 ± 0.2^a	19.2 ± 0.7^a

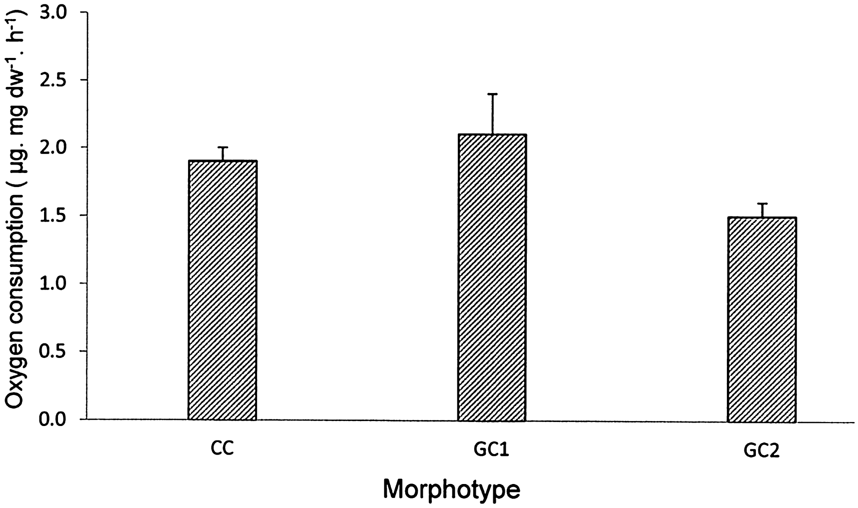

Fig. 1. Specific dry-mass oxygen consumption rate (μg (mg dw)$^{-1}$ h^{-1}) of the male morphotypes of *Macrobrachium amazonicum* kept at 30°C.

could be advantageous if fights among individuals of the same species end in death when the fighting is between individuals of the same size. We have verified that mass gain was higher in the CC and GC1 morphotypes and that GC2 had almost no gain (Table 1). These data show that CC and GC1 represent morphotypes with an elevated growth rate, whereas GC2 must use its ingested energy in other pathways, such as reproduction. According to Moraes-Riodades and Valenti (2004), GC2 is the morphotype with the largest overall size and with the largest chelae, and is dominant in the population. Augusto and Masui (2014) verified that the TC morphotype gains approximately 15 mg WW^{-1} day^{-1}, which corresponds to a gain of 22% of its initial wet weight after 30 days. These results suggest that the TC morphotype invests a great portion of the energy ingested in growth, as do the CC and GC1 morphotypes. The elevated growth rates observed in CC and GC1 (present work) and in TC (Augusto and Masui, 2014) are consistent because these morphotypes are smaller and are expected to require a significant investment in growth. GC1 males, however, have not yet developed large chelae that could serve both as a sex attractant and in agonistic behaviour. The three morphotypes of *M. rosenbergii* also present different growth patterns but the growth rate of males of the OC (orange claw) morphotype is higher than in the SM (small claw) and BC (blue claw; male dominant in population) morphotypes (Ra'anan et al., 1991).

Despite the elevated growth rate observed in the CC and GC1 morphotypes, the growth of several other aquatic animals can vary in nature due to factors such as social interaction, diet, and available space (Michaels et al., 2015; da Costa et al., 2016; Hellmann et al., 2016). For example, in fishes such as *Centropomus parallelus* (Poey, 1860) (Lemos et al., 2006), the presence of other individuals may reduce stress through social interactions, stimulating appetite and growth. Therefore, given the complex hierarchy that exists among species of *Macrobrachium* (Ra'anan and Cohen, 1985; Moraes-Riodades and Valenti, 2004; Ibrahim,

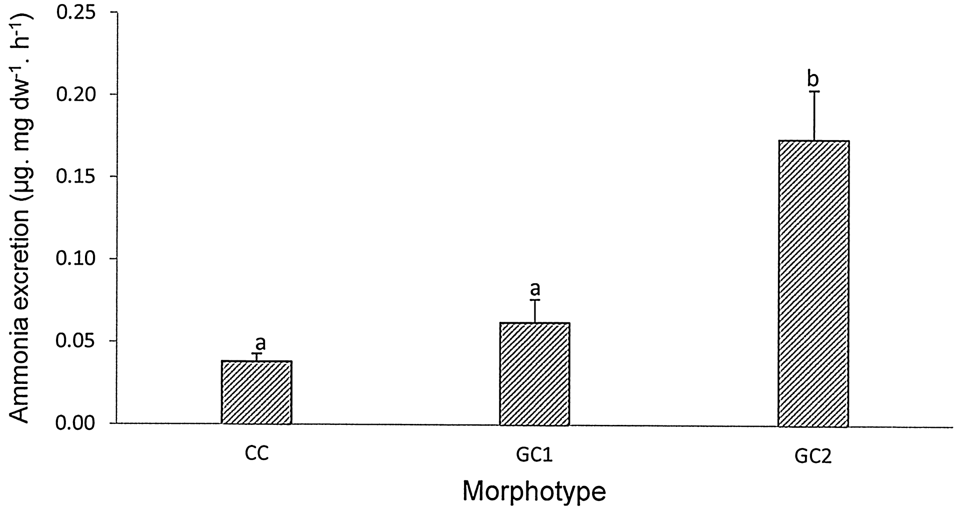

Fig. 2. Ammonia excretion (μg/mg MS/h) of the male morphotypes of *Macrobrachium amazonicum* kept at 30°C. Values with different letters are significantly different.

2011), it is possible that the growth rates may be higher in animals living in groups and in their natural habitat. In contrast, the growth of the TC, CC, and GC1 morphotypes might have been stimulated in laboratory experiments because the shrimp were isolated from one another, which may have favoured their transformation into the subsequent morphotype. When the dominant morphotypes of shrimps of the genus *Macrobrachium* are removed from the population, subordinate males show dramatic growth rates that replace the gap left by the dominant males (Moraes-Riodades and Valenti, 2004; Wortham et al., 2012). The crayfish *Cherax destructor* (Clark, 1936) reared in groups shows long periods between molts compared to individuals reared in isolation (Geddes et al., 1988). Shrimp in our experiments were isolated for 30 days in aquaria, and although transformations of morphotypes did not occur, it is possible that the elevated growth rate observed in CC and GC1 may be related to preparation for transformation into GC1 and GC2, respectively. Augusto and Masui (2014) also observed elevated growth rates in TC kept in isolation in aquaria over 30 days, but they did not observe transformation into different morphotypes.

Exuviae

Growth in crustaceans is marked by physiological alterations such as an increase in body hydration and the absorption of calcium from the molted exoskeleton and its storage in specific regions, such as in the hemolymph and gastroliths (Fernández et al., 2012). Although some crustaceans consume their molt, we did not observe individuals of *M. amazonicum* consuming their shed exoskeletons, which contain approximately 40% of the energy value of the body. Observations in the TC morphotype by Augusto and Masui (2014), CC, GC1 and GC2 showed that males did not cease feeding during pre- and post-molts, as has been reported in some crustaceans. Although males of *M. amazonicum* appear not to consume their exoskeletons, they do not stop eating, which perhaps compensates for the energy and nutrients lost in exuviae.

Ingestion Rate and Defecation

In a hierarchical population, dominant individuals can deprive their subordinates of food. According to Cobb et al. (1982), aggressive interactions can also cause a decrease in appetite of the subordinates in lobsters. The morphotypes of *M. amazonicum* were kept isolated in our experiment, so this type of inhibition by aggression was not tested. In these conditions of food availability and the absence of dominants and subordinates, all morphotypes (CC, GC1 and GC2) therefore consume the equivalent of 3% of their biomass. The daily ingestion rate is related to the mass of every morphotype, and the CG1 and GC2 morphotype consume, respectively, approximately 60% and 130% more than CC. The TC morphotype also has an ingestion rate that corresponds to approximately 3% of its biomass (Augusto and Masui, 2014). Although the TC (Augusto and Masui, 2014), CC, and GC1 morphotypes present elevated growth rates, these growth rates are not associated with an increase in ingestion rate compared to GC2, in which the investment in growth is almost nil.

The data set presented suggests that GC2 may be channelling a great portion of ingested energy and nutrients into reproduction. This morphotype nevertheless showed a high ammonia excretion rate (approximately 15% higher than the other morphotypes). The ammonia excretion of crustaceans can be altered by physiological factors such as molting cycle, size, diet, and environmental factors such as temperature, salinity, pH, dissolved oxygen and ammonia (Hayd et al., 2010; Augusto and Masui, 2014). It is possible that whereas TC, CC, and GC1 use amino acids in tissue growth, GC2 uses amino acids as an energy source. In addition, the food might have had an unbalanced composition in terms of free amino acids for the GC2 morphotype, or shrimp may have been fed in excess. The percentage of feces eliminated by the morphotypes, however, was always proportional to the ingestion rate (approximately 2%), thus suggesting a similar utilization in terms of absorption of nutrients among the morphotypes.

The O:N ratio is associated with the availability of energy reserves and the use of body protein (Mayzaud and Conover, 1988). Several factors may influence the use of the three different energy substrates (protein, carbohydrates, and lipids) by crustaceans, including diet (Pascual et al., 2004; Hayd et al., 2010; Rocha et al., 2013). In the diets of *M. rosenbergii* containing different levels of fibre, the O:N ratio varied from 10 (use of carbohydrates) to 49 (use of lipids) (González-Peña and Moreira, 2003). Chen and Nan (1994) observed that different species of marine shrimps fed the same commercial food used different energy substrates: *Penaeus japonicus* (Bate, 1888) and *M. ensis* (de Haan, 1844) used proteins, whereas *P. monodon* (Fabricius, 1798), *P. penicillatus* (Alcock, 1905), and *P. chinensis* (Osbeck, 1765) used lipids. Rosas et al. (2002) showed that the marine shrimp *Litopenaeus vannamei* (Boone, 1931) used proteins (O:N approximately 10) as a source of energy, having and the capacity to synthesize dietary carbohydrates through the gluconeogenic pathway. Although a large number of crustaceans use proteins as an energy substrate, Claybrook (1983) noted that proteins can be used when they occur in excess in the diet, when the profile of amino acids is unbalanced, or when the energy of lipids and carbohydrates is insufficient to supplement metabolic processes. Commercial foods for shrimp are produced based on marine shrimps, but the protein needs of freshwater species may be different.

The atomic O:N ratio suggests the use of proteins as an energy substrate in GC2 and a mixture of proteins and carbohydrates in CC and GC1. Augusto and Masui (2014) verified the use of proteins in TC fed the same food used in our experiments. Although the food exhibits a suitable calorie content (330 kcal $(100\ \mathrm{g})^{-1}$), which ranges from 310 to 410 kcal $(100\ \mathrm{g})^{-1}$ in crustaceans (Cuzon and Guillaume, 1997), it may be inappropriate in terms of raw materials for growth. The energetic content and the energy:protein ratio are important factors to be considered in the diet because if the diet is very rich in protein, it may lead to an increase in the ingestion rate, and an increase in energy content may reduce food consumption before the protein needs are met (Pezzato et al., 2003). Bautista (1986) and Shiau and Peng (1992) suggested that an energy:protein ratio of 125 mg protein kcal^{-1} is ideal for growth of the marine

Table 2. Oxygen consumption (μg (mg MS)$^{-1}$ h^{-1}) in selected natantian decapods. The studies did not evaluate oxygen consumption between males and females separately.

	Stage	Oxygen	Reference
Macrobrachium amazonicum	Adult	1.5 (GC2) to 2.1 (GC1) (30^{o}C)	This study
M. amazonicum	Adult	2.04 ± 0.18 (30°C) (TC)	Augusto and Masui (2014)
M. amazonicum	Adult	1.5 (30°C)	Zanders and Rodriguez (1992)
M. rosenbergii	Adult	1.3-1.8	Gonzales-Peña and Moreira (2003)
M. olfersii (Wiegmann, 1836)	Adult	0.6 (20°C)	Moreira et al. (1983)
M. olfersii	Post-larvae	5.0 (30°C)	Moreira et al. (1983)
M. acanthurus (Wiegmann, 1836)	Adult	0.6 (20°C)	Moreira et al. (1983)
M. heterochirus (Wiegmann, 1836)	Adult	0.4 (20°C)	Moreira et al. (1983)
M. nipponense (de Haan, 1849)	Adult	0.4-0.5 (25°C)	Wang et al. (2003)
Litopenaeus schmitti (Burkenroad, 1936)	Adult	0.4	Barbieri (2007)
L. vannamei	Juvenile	1.0 (17 S) and 2.4 (3 S)	Li et al. (2007)
Farfantepenaeus californiensis (Holmes, 1900)		0.7 (30°C)	Ocampo et al. (2003)
Penaeus japonicus (Bate, 1888)	Adult	0.3 (25 S, 25°C)	Kulkarni and Josh (1980)
P. monodon (Fabricius, 1798)	Post-larvae	3.5 (35 S, 27°C)	Gaudy and Sloane (1981)
P. chinensis (Osbeck, 1765)	Adult	1.0	Chen and Nan (1994)
P. peringueyi		1.0 (35 S; 30°C)	Allan et al. (2006)

shrimp *P. monodon*. Protein is a limiting growth factor and is needed in higher proportions compared to carbohydrates and lipids. Protein levels from 35 to 45% in the food have been suggested as ideal for the growth of marine shrimps (Chuntapa et al., 1999). Santos et al. (2014) verified higher proteolytic activities in the hepatopancreas of farmed *M. amazonicum* compared to wild shrimps and suggested that changes in farmed prawns may be an adaptation to aquaculture conditions.

Metabolism

The energy expended in metabolic processes, the total sum of all chemical reactions taking place in an organism, as measured by oxygen consumption, is used for the maintenance of homeostasis, including locomotion, growth, and reproduction (Diaz-Iglesias et al., 2012; McGaw et al., 2013; Mazzarelli et al., 2015). The individual oxygen consumption rate in *M. amazonicum* is directly related to the size of the animals, and it is higher in the GC1 and GC2 morphotypes (Table 1). The oxygen consumption verified in TC (0.01 ± 0.003 g O_2 ind^{-1} day^{-1}) by Augusto and Masui (2014) is even lower than that obtained here for CC (0.03 ± 0.001 g O_2 ind^{-1} day^{-1}). The oxygen consumption rates in dry specific-mass usually decreases with an increase in body mass. The GC2 morphotype of *M. amazonicum* had a tendency to have a lower specific dry mass oxygen consumption, but there is no statistically significant difference between the morphotypes (Fig. 1). Moraes-Riodades and Valenti (2004) suggested that the GC1 and GC2 morphotypes are more sedentary because they have a very large cheliped that reduces swimming ability. In contrast, the morphotypes TC and CC have smaller chelipeds, which allows them to be more active. The possibility cannot be ruled out that in nature as well as in hatcheries, TC and CC males spend additional energy running away from larger morphotypes such that energy is channelled into locomotion. Under laboratory conditions, however, there were no large differences between the morphotypes.

The oxygen consumption rates of *M. amazonicum* are similar to those of other freshwater decapods (Table 2), especially the carideans *M. rosenbergii* and *Palaemon peringueyi* (Stebbing, 1915) and the marine *Litopenaeus vannamei*. The differences observed in the literature may be the consequence of a species-specific response, or the ontogenetic stage evaluated, temperature, or diet. The size of respirometric chambers can also influence these values because animals that are kept in much smaller spaces will only have their basal metabolism evaluated, whereas those kept in larger spaces will have their intermediate metabolism measured.

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REFERENCES

Alcock, A. 1905. A revision of the "genus" *Penaeus*, with diagnoses of some new species and varieties. Annals and Magazine of Natural History (Series 7) 16: 508-532.

Allan, E. L., P. W. Froneman, and A. N. Hodgson. 2006. Effects of temperature and salinity on the standard metabolic rate (SMR) of the caridean shrimp *Palaemon peringueyi*. Journal of Experimental Marine Biology and Ecology 337: 103-108.

Augusto, A., and D. C. Masui. 2014. Comparison of growth, metabolism, feed, fecal production, excretion and energy budget in males and females of the Amazon River prawn (*Macrobrachium amazonicum*). Marine and Freshwater Behaviour and Physiology 47: 373-388.

Barbieri, E. 2007. Use of oxygen consumption and ammonium excretion to evaluate the sublethal toxicity of cadmium and zinc on *Litopenaeus schmitti* (Burkenroad, 1936, Crustacea). Water Environment Research 79: 641-648.

Barki, A., I. Karplus, and M. Goren. 1992. Effects of size and morphotype on dominance hierarchies and resource competition in the fresh-water prawn *Macrobrachium rosenbergii*. Animal Behaviour 44: 547-555.

Bate, C. S. 1888. Report on the Crustacea Macrura collected by Challenger during the years 1873-1876. Scientific Results of the Exploring Voyage of "Challenger" 1873-1876 24: 1-942.

Bautista, L. 1986. The response of *Macrobrachium rosenbergii* juveniles to varying protein/energy ratio ingest diets. Aquaculture 53: 229-234.

Boone, L. 1931. Anomuran, macruran Crustacea from Panama and Canal Zone. Bulletin of the American Museum of Natural History 63: 137-189.

Chen, J. C., and F. H. Nan. 1994. Comparisons of oxygen-consumption and ammnonia excretion of 5 penaeids. Journal of Crustacean Biology 14: 289-294.

Chuntapa, B. P., S. Piyatiratitivorakul, C. Nitithamyong, V. Viyakarn, and P. Menasveta. 1999. Optimal lipid:carbohydrate and protein:energy ratios in semi-purified diets for juvenile black tiger shrimp *Penaeus monodon* Fabricius. Aquaculture Research 30: 11-12.

Clark, E. 1936. The freshwater crayfishes of Australia. Memoirs of the National Museum of Victoria 10: 5-58.

Claybrook, D. L. 1983. Nitrogen metabolism, pp. 163-212. In, L. H. Mantel (ed.), Internal Anatomy and Physiological Regulation. D. E. Bliss (ed.), The Biology of Crustacea. Vol. 5. Academic Press, New York, NY.

Cobb, J. S., G. R. Tamm, and D. Wang. 1982. Behavioral mechanisms influencing molt frequency in the American lobster, *Homarus americanus*. Journal of Experimental Marine Biology and Ecology 62: 185-200.

Cuzon, G., and J. Guillaume. 1997. Energy and protein:energy ratio, pp. 51-70. In, L. R. D'Abramo, D. E. Conklin, and D. M. Akiyama (eds.), Crustacean Nutrition – Advances in World Aquaculture. Vol. 3. World Aquaculture Society, Baton Rouge, LA.

Da Costa, F. P., B. S. F. D. Gomes, S. D. D. A. Pereira, and M. D. Arruda. 2016. Influence of stocking density on the behaviour of juvenile *Litopenaeus vannamei* (Boone, 1931). Aquaculture Research 47: 912-924.

De Haan, H. M. 1823-1830. Crustacea, pp. i-xvii, i-xxxi, ix-xvi, 1-243. In, P. F. von Siebold (ed.), Fauna Japonica sive Descriptio Animalium, quae in Itinere per Japoniam, Jußu et Auspiciis Superiorum, qui Summum in India Batava Imperium Tenent, Suscepto, Annis 1823-1830 Collegit, Notis, Observationibus et Adumbrationibus Illustravit. Lugduni-Batavorum [= Leiden].

De Man, J. G. 1879. On some species of the genus *Palaemon* Fabr. with descriptions of two new forms. Notes Leyden Museum 1: 165-184.

Diaz-Iglesias, E., V. M. Castillo, M. Baez-Hidalgo, A. D. Re-Araujo, and A. Silva-Loera. 2012. A bioenergetics of juvenile red claw lobster, *Cherax quadricarinatus*, fed two artificial diets. Hidrobiologia 22: 147-160.

Fabricius, J. C. 1798. Supplementum Entomologiae Systematicae. Proft & Storch, Hafniae [= Copenhagen].

Fernández, M. S., C. Bustos, G. Luquet, D. Saez, A. Neira-Carrillo, M. Corneillat, G. Alcaraz, and J. L. Arias. 2012. Proteoglycan occurrence in gastrolith of the crayfish *Cherax quadricarinatus* (Malacostraca: Decapoda). Journal of Crustacean Biology 32: 802-815.

Gaudy, R., and L. Sloane. 1981. Effect of salinity on oxygen consumption in postlarvae of the penaeid shrimps *Penaeus monodon* and *P. stylirostris* without and with acclimation. Marine Biology 65: 297-301.

Geddes, M. C., B. J. Mills, and K. F. Walker. 1988. Growth in the Australian freshwater crayfish *Cherax destructor* (Clark, 1936), under laboratory conditions. Australian Journal of Marine and Freshwater Research 39: 555-568.

González-Peña, M. C. G., and M. G. S. Moreira. 2003. Effect of dietary cellulose level on specific dynamic action and ammonia excretion of the prawn *Macrobrachium rosenbergii* (De Man 1879). Aquaculture Research 34: 821-827.

Hayd, L. A., D. Lemos, and W. C. Valenti. 2010. Ontogenetic variation in ammonia excretion during the early life stages of the Amazon River prawn, *Macrobrachium amazonicum*. Journal of the World Aquaculture Society 41: 107-115.

Heller, C. 1862. Beiträge zur näheren Kenntnis der Macrouren. Sitzungsberichte der mathematisch-naturwissenschaftlichen Classe der Kaiserlichen Akademie der Wissenschaften in Wien 1862: 389-426, pls. 1, 2.

Hellmann, J. K., I. Y. Ligocki, C. M. O'Connor, A. R. Reddon, T. M. Farmer, S. E. Marsh-Rollo, S. Balshine, and I. M. Hamilton. 2016. The influence of status and the social environment on energy stores in a social fish. Journal of Fish Biology 88: 1321-1334.

Henderson, J. R. 1893. A contribution to Indian carcinology. Transactions of the Linnean Society of London (Series 2, Zoology) 5: 325-458.

Holmes, S. J. 1900. Synopsis of California stalk-eyed Crustacea. Occasional Papers of the California Academy of Sciences 7: 1-262.

Ibrahim, N. A. F. 2011. Controle social do crescimento do camarão da amazônia *Macrobrachium amazonicum*. Ph.D. Thesis, Centro de Aquicultura da Universidade Estadual Paulista, São Paulo.

Karplus, I., G. Hulata, D. Ovadia, and R. Jaffe. 1992. Social control of growth in *Macrobrachium rosenbergii*. The role of claws in bull-runt interactions. Aquaculture 105: 281-296.

———, S. Malecha, and A. Sagi. 2000. The biology and management of size variation, pp. 254-289. In, M. B. New and W. C. Valenti (eds.), Freshwater Prawn Culture: the Farming of *Macrobrachium rosenbergii*. Blackwell, Oxford.

Karzinkin, G. S., and O. I. Tarkovskaya. 1964. Determination of caloric value of small samples, pp. 122-124. In, E. N. Pavloskii (ed.), Techniques for the Investigation of Fish Physiology. Oldbourne Press, London.

Koroleff, F. 1983. Determination of ammonia, pp. 150-157. In, K. Grasshoff, M. Ehrhardt, and K. Kremling (eds.), Methods of Seawater Analysis. 2nd Edition. Verlag Chemie, Weinheim.

Kulkarni, G. K., and P. K. Josh. 1980. Some aspects of respiratory metabolism of a penaeid prawn, *Penaeus japonicus* (Bate) (Crustacea, Decapoda, Penaeidae). Hydrobiologia 75: 27-32.

Kuris, A. M., Z. Ra'anan, A. Sagi, and D. Cohen. 1987. Morphotypic differentiation of male Malaysian prawns, *Macrobrachium rosenbergii*. Journal of Crustacean Biology 7: 219-237.

Langer, S., R. Chalotra, and T. Kour. 2002. The occurrence and description of male morphotypes of *Macrobrachium dayanum*. Journal of Animal Morphology and Physiology 49: 49-54.

Lemos, D., and V. N. Phan. 2001. Energy partitioning into growth, respiration, excretion and exuvia during larval development of the shrimp *Farfantepenaeus paulensis*. Aquaculture 199: 133-145.

———, B. Netto, and A. Germano. 2006. Energy budget of juvenile fat snook *Centropomus parallelus* fed live food. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Biology 144: 33-40.

Li, E., L. A. Chen, C. Zeng, X. Chen, N. Yu, Q. Lai, and J. G. Qin. 2007. Growth, body composition, respiration and ambient ammonia nitrogen tolerance of the juvenile white shrimp, *Litopenaeus vannamei*, at different salinities. Aquaculture 265: 385-390.

Maciel, C. R., and W. C. Valenti. 2009. Biology, fisheries, and aquaculture of the Amazon River prawn *Macrobrachium amazonicum*: a review. Nauplius 17: 61-79.

Mayzaud, P., and R. J. Conover. 1988. O:N ratio as a tool to describe zooplankton metabolism. Marine Ecology Progress Series 45: 289-302.

Mazzarelli, C. C. M., M. R. Santos, R. V. Amorim, and A. Augusto. 2015. Effect of salinity on the metabolism and osmoregulation of selected ontogenetic stages of an Amazon population of *Macrobrachium amazonicum* shrimp (Decapoda, Palaemonidae). Brazilian Journal of Biology 75: 372-379.

McGaw, L. J., and D. L. Curtis. 2013. Effect of meal size and body size on specific dynamic action and gastric processing in decapod crustaceans. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Biology 166: 414-425.

Michaels, C. J., R. E. Antwis, and R. F. Preziosi. 2015. Impacts of UVB provision and dietary calcium content on serum vitamin D-3, growth rates, skeletal structure and coloration in captive oriental fire-bellied toad (*Bombina orientalis*). Journal of Animal Physiology and Animal Nutrition 99: 391-403.

Moraes-Riodades, P. M. C., and W. C. Valenti. 2004. Morphotypes in male Amazon River prawns, *Macrobrachium amazonicum*. Aquaculture 236: 297-307.

Moreira, G. S., J. C. McNamara, S. E. Shumway, and P. S. Moreira. 1983. Osmoregulation and respiratory metabolism in Brazilian *Macrobrachium* (Decapoda, Palaemonidae). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Biology 74: 57-62.

Ocampo, L., C. Rosas, and H. Villarreal. 2003. Effect of temperature on post-prandial metabolism of brown shrimp *Farfantepenaeus californiensis*. Journal of Shelf Research 22: 281-284.

Osbeck, P. 1765. Reise nach Ostindien und China. Nebst O. Toreens Reise nach Suratte und C. G. Ekebergs Nachricht von der Landwirthschaft der Chineser. Aus dem Schwedischen übersetzt von J. G. Georgi. Johann Christians Koppe, Rostock.

Papa, L. P. 2007. Caracterização estrutural do sistema reprodutor masculino e do hepatopâncreasdos diferentes morfotipos de *Macrobrachium amazonicum*. Ph.D. Thesis. Universidade Estadual Paulista, São Paulo.

Pascual, C., E. Zenteno, G. Cuzon, J. Suárez, A. Sánchez, G. Gaxiola, G. Taboada, T. Maldonado, and C. Rosa. 2004. *Litopenaeus vannamei* juveniles energetic balance and immunological response to dietary protein. Aquaculture 239: 375-395.

Pezzato, L. E., M. M. Barros, F. G. Sampaio, D. R. Falcon, G. S. Gonçalves, and H. Hisano. 2003. Relação energia:proteína dietária para pós-larvas de

Macrobrachium amazonicum (Crustacea, Decapoda). Acta Scientiarum: Animal Sciences 25: 235-241.

Ra'anan, Z., and D. Cohen. 1985. Ontogeny of social structure and population dynamics in the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). Crustacean Issues 3: 277-311.

———, A. Sagi, Y. Wax, I. Karplus, G. Hulata, and A. Kuris. 1991. Growth, size rank, and maturation of the fresh-water prawn, *Macrobrachium rosenbergii* – analysis of marked prawns in an experimental population. Biological Bulletin (Woods Hole) 181: 379-386.

Randall, J. W. 1840. Catalogue of the Crustacea brought by Thomas Nuttall and J. K. Townsend, from the West Coast of North America and the Sandwich Islands, with descriptions of such species as are apparently new, among which are included several species of different localities, previously existing in the collection of the Academy. Journal of the Academy of Natural Sciences of Philadelphia 8: 106-147, pls. 3-7.

Ranjeet, K. 2010. Genetic characterization of male morphotypes of *Macrobrachium rosenbergii* using RAPD markers. Uttar Pradesh Journal of Zoology 30: 107-113.

Rocha, A. J. S., V. Gomes, T. C. A. Santos, M. J. A. C. R. Passos, and V. N. Phan. 2013. Bioenergetic budget of juveniles of fat snook *Centropomus parallelus* (Perciformes, Centropomidae) as a function of salinity acclimation. Pan-Am Journal of Aquatic Science 8: 10-20.

Rosas, C., L. Ocampo, G. Gaxiola, A. Sanchez, and L. A. Soto. 1999. Effect of salinity on survival, growth, and oxygen consumption of post larvae (PL10-PL21) of *Litopenaeus setiferus*. Journal of Crustacean Biology 19: 244-251.

———, G. Cuzon, G. Gaxiola, C. Pascual, G. Taboada, L. Arena, and A. Van Wormhoudt. 2002. An energetic conceptual model of the physiological role of dietary carbohydrate and salinity on *L. vannamei* juveniles. Journal of Experimental Marine Biology and Ecology 268: 47-67.

Santos, F. M. S., K. Ribeiro, A. C. V. F. Júnior, L. B. C. Júnior, W. C. Valenti, and R. S. Bezerra. 2014. Digestive proteases from wild and farmed male morphotypes of the Amazon River prawn (*Macrobrachium amazonicum*). Journal of Crustacean Biology 34: 189-198.

Shiau, S., and C. Peng. 1992. Utilization of different carbohydrates at different dietary protein levels in grass prawn, *Penaeus monodon*, reared in seawater. Aquaculture 101: 241-250.

Silva, P. F., and M. D. Arruda. 2015. Social status and individual behavioral differences in juvenile *Macrobrachium rosenbergii*. Marine and Freshwater Behaviour and Physiology 48: 1-11.

Stebbing, T. R. R. 1915. South African Crustacea (part VIII of S. A. Crustacea, for the marine investigations in South Africa). Annals of the South African Museum 15: 57-104.

Tidwell, J. H., C. Shawn, A. Van Arnum, A. Leigh, and M. McCathy. 2001. The effect of photoperiod on growth and survival of freshwater prawn, *Macrobrachium rosenbergii* in nursery tanks. Journal of Applied Aquaculture 11: 41-47.

Wang, A., W. Wang, Y. Wang, L. Shang, Y. Liu, and R. Sun. 2003. Effect of dietary vitamin C supplementation on the oxygen consumption, ammonia-N excretion and Na^+/K^+ ATPase of *Macrobrachium nipponense* exposed to ambient ammonia. Aquaculture 220: 833-841.

Wiegmann, A. F. 1836. Beschreibung liniger neuen Crustaceen des Berliner Museums aus Mexico und Brasilien. Archiv für Naturgeschichte 2: 145-151.

Wortham, J. L., L. N. Van Maurik, and N. Lauren. 2012. Morphology and morphotypes of the Hawaiian river shrimp, *Macrobrachium grandimanus*. Journal of Crustacean Biology 32: 545-556.

Zanders, I. P., and J. M. Rodriguez. 1992. Effect of temperature and salinity stress on osmoionic regulation in adults and on oxygen consumption in larvae and adults of *Macrobrachium amazonicum* (Decapoda, Palaemonidae). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Biology 101: 505-509.

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