



From lecithotrophy to planktotrophy: ontogeny of larval feeding in the Amazon River prawn *Macrobrachium amazonicum*

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ABSTRACT: During downstream transport from rivers to estuaries, early larval stages of the Amazon River prawn *Macrobrachium amazonicum* (Heller 1862) are likely exposed to planktonic food limitation. In the laboratory, we studied the effects of presence or absence of food on larval survival, moulting and biomass (dry mass, and content of carbon, hydrogen and nitrogen). Unfed larvae developed successfully from hatching of the Zoea I (Z I) to the third zoeal stage (Z III). Complete absence of starvation effects indicated obligatory lecithotrophy in Z I, while significantly delayed moulting and reduced biomass in unfed Z II proved that this stage is facultatively lecithotrophic. Although unfed Z III did not develop any further (obligatory planktotrophy), they still showed a high endotrophic potential, surviving for up to another 10 d (in total 2 wk from hatching), and utilizing about two-thirds of their initial biomass. Dramatically decreasing C:N ratios in unfed larvae suggest an almost complete metabolic degradation of internal lipid stores (visible as fat droplets in the hepatopancreas region), but little protein mobilization. Larvae obtained from 2 different females differed significantly in biomass at hatching and, correspondingly, also in maximum survival time, delay of moulting to the Z III and average rates of biomass utilization. In conclusion, the early larval stages of *M. amazonicum* depend very little on food, shifting from completely non-feeding behaviour (Z I) through facultative lecithotrophy (Z II), to planktotrophy (later stages). This ontogenetic pattern may be an adaptation to initial food limitation associated with larval export from limnic to estuarine environments.

KEY WORDS: Palaemonid shrimp · Larval feeding · Lecithotrophy · Starvation · Larval growth · Dry mass · CHN

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INTRODUCTION

Among the decapod crustaceans, the Palaemonidae have been particularly successful in evolutionary transitions from life in coastal marine to brackish and, eventually, freshwater environments (Bauer 2004). Within this family, most estuarine and limnic species belong to the globally distributed genera *Macrobrachium* and *Palaemonetes* (Strenth 1976, Walker 1992, Jalihal et al. 1993, Murphy & Austin 2005). In contrast to *Palaemonetes* spp., many of the >200 extant species of *Macrobrachium* grow to large body sizes

and are therefore economically valuable targets for regional fisheries and aquaculture (New & Valenti 2000, Wickins & Lee 2002, Nwosu et al. 2007). Among these species, the Amazon River prawn *Macrobrachium amazonicum* (Heller 1862) has remained relatively unknown, although its potential for aquaculture and fisheries is highly rated (Kutty et al. 2000, Moraes-Valenti & Valenti 2007). It is widely distributed along the northern and northeastern coasts of South America (Holthuis 1952, Rodríguez 1982, Montoya 2003), and fully limnic inland populations live in the Amazon and Orinoco River basins (Walker 1992,

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Odinetz Collart & Rabelo 1996), as well as in the upper Parana and Paraguay systems (Pettovello 1996, Bialecki et al. 1997, Magalhães 2000, Hayd & Nakagaki 2002).

In contrast to numerous other freshwater-inhabiting congeners, which pass through an abbreviated mode of development (Murphy & Austin 2005), *Macrobrachium amazonicum* has an extended larval development through 9 to 11 stages, similar to those in most estuarine and marine relatives (Guest 1979, Vega Perez 1984, Magalhães 1985, Anger et al. 2009). In populations living in coastal rivers and adjacent estuaries, the larvae require for successful development a low or moderate salt concentration (Guest & Durocher 1979, Lobão et al. 1987, Rojas et al. 1990, Araujo & Valenti 2007). As a consequence of this physiological constraint, riverine populations living near the coast must follow an 'export strategy' (Strathmann 1982), which includes early larval transport to estuarine or coastal marine habitats and subsequent development in brackish or marine waters (Odinetz Collart 1991a). During the downstream transport with river currents, the early larval stages are exposed to food limitation due to typically low or unpredictable mesozooplankton production in lotic freshwater habitats with short residence times (Pedrosa et al. 1999, Akopian et al. 2002). This nutritional stress selects for an enhanced maternal energy investment into egg production, allowing a partially food-independent early larval development (Anger 2001, 2006). In *M. amazonicum*, the functional morphology of larval mouth parts (Odinetz Collart & Magalhães 1994), as well as behavioural observations (Araujo & Valenti 2007), suggest that Zoea I is most probably a non-feeding stage. However, it has remained unclear whether the first uptake of food occurs in the second or not until the third zoeal stage. This may be due in part to the experimental use of larvae from different populations (estuarine vs. fully limnic), with different criteria (morphology vs. behaviour), or because no rigorous quantitative techniques such as measurements of changes in biomass of fed and unfed larvae have been employed. Continuous microscopic observations of larval feeding behaviour are technically difficult and may be unreliable due to experimental artefacts (e.g. illumination effects). Thus, previous studies also could not rule out the possibility that larval feeding might actually begin even earlier, before the end of the first zoeal moulting cycle. Furthermore, intraspecific variability in larval biomass at hatching (Anger et al. 2009) may cause variation in the endotrophic potential and, as a consequence, in the onset of feeding in larvae produced by different females.

In the present investigation, we studied the ontogeny of feeding in the early zoeal stages (Z I to IV) of

Macrobrachium amazonicum originating from an estuarine population in northern Brazil. Larvae from different broods were reared in the presence or absence of food, and their survival, development and biomass were used as comparative criteria for the evaluation of nutritional vulnerability.

MATERIALS AND METHODS

Origin and maintenance of shrimps. Adult *Macrobrachium amazonicum*, including ovigerous females, were obtained from the Aquaculture Center CAUNESP (Jaboticabal, SP) of the State University of São Paulo, Brazil. The broodstock originated from a population living in coastal rivers and estuarine tidal creeks near Belém in the Amazon delta (1° 14' 30" S, 48° 19' 52" W; W. C. Valenti pers. comm.). In January 2006, 15 shrimps were transported in cooling boxes to the Helgoland Marine Biological Laboratory (BAH), Germany. Here, they were subsequently maintained in individual, aerated, flow-through aquaria with 30 l freshwater (total ion concentration: 0.2 mg l⁻¹), constant 29°C and a 12:12 h light:dark cycle (Anger et al. 2009). Pieces of frozen marine isopods (*Idotea* spp.) and commercial aquarium feeds (Novo Tab, JBL) were provided as food. The overflowing water from the aquaria was passed through a sieve (0.3 mm mesh size), where freshly hatched larvae were collected.

Experiments. Three rearing experiments, with and without food, were conducted with larvae obtained from different females (A, B and C). Larval hatching from egg batches occurred on 22 January, 5 and 7 March 2006, respectively. From each female, a sample of newly hatched larvae was taken for later determinations of initial biomass (see Table 1). The remaining larvae were individually pipetted into Nunc plastic bowls with 100 ml unaerated water. A tentatively optimal salinity for larval rearing (10 PSU; Araujo & Valenti 2007) was obtained by mixing appropriate amounts of tap water (<0.2 PSU) with seawater from the North Sea (32 to 33 PSU). Salt concentrations were checked to the nearest 0.1 PSU using a temperature-compensated electric probe (WTW Cond 330i).

In one treatment, the larvae were provided daily with fresh food (newly hatched Sanders Great Salt Lake *Artemia*; ca. 10 to 15 nauplii ml⁻¹), while the second group remained continuously unfed. During each water change (every 24 h), culture bowls were individually checked for moults or mortality and exuviae were removed. This tedious rearing technique was chosen to: (1) exclude possible cannibalism, especially among unfed larvae, and (2) precisely ascertain the stage and duration of development in each successive moulting cycle.

Rearing temperature varied among experiments: in Expt A (conducted with larvae from Hatch A), only the first zoal moulting cycle was studied to check whether larval feeding already begins at some point during the first zoal stage (Z I). In order to enhance the temporal resolution of our data (with daily observations of survival, moulting and biomass), the larvae were reared at a lower temperature (21°C). Moulting to Z II occurred here 4 d after hatching. The other 2 experiments (with Hatches B and C) were conducted at 29°C. Successive moulting cycles in these experiments required, on average, only 2 d.

Each treatment (with or without food) comprised initially $n = 48$ larvae; these were used exclusively for daily records of moulting and mortality. Expt A was terminated 4 d after hatching, when all larvae had reached the Z II stage. The other experiments (B, C) were terminated within 1 to 2 d after the death of the last unfed larvae. Samples for later determinations of larval biomass were taken from parallel cultures with sibling larvae reared under identical conditions of temperature, salinity and light (numbers of larvae per treatment depending on hatch size). Limitations in the number of larvae available for sampling, as well as high mortality in larvae kept for >1 wk without food, did not allow us to take samples for biomass determinations very close to the end of the experiments; as a consequence, our data for moulting and mortality cover a longer experimental period than those obtained for changes in larval biomass.

Biomass measurements. Biomass was measured as dry mass (W) and contents of carbon, hydrogen and nitrogen (collectively, CHN), following standard techniques (Anger & Harms 1990): samples were briefly rinsed in distilled water, blotted on fluff-free Kleenex paper for optical use, transferred to pre-weighed tin cartridges, and stored frozen at -18°C. Later, the samples were freeze-dried in a Lyovac GT-2E vacuum apparatus, weighed to the nearest 0.1 µg on a Sartorius SC microbalance, and analysed with an Elementar Vario Micro CHN Analyser using acetanilid as a standard. Each set of measurements comprised $n = 5$ replicate determinations with 3 to 6 individuals in each (depending on developmental stage).

Statistical methods. Statistical analyses were carried out following standard techniques (Sokal & Rohlf 1995) using a JMP software package (Version 5.1.2; SAS Institute). The data were first tested for normality and homoscedasticity (goodness-of-fit G -test; Durbin-Watson statistic). Average values are consistently given as arithmetic mean \pm SD. Multiple comparisons of mean values were made using ANOVA, which is fairly robust against deviations from normal distribution (Underwood 1997). For pairwise comparisons of mean-values, however, we applied a non-parametric Wil-

coxon test (Chi-squared approximation) rather than parametric Student's t -tests, as in several cases the data showed no normal distribution even after transformations. Correlation and regression coefficients were tested for significant deviations from zero (ANOVA), slopes of different regression equations were compared employing ANCOVA.

RESULTS

Internal lipid stores

At hatching, Z I of *Macrobrachium amazonicum* showed in the hepatopancreas region of the cephalothorax conspicuous amounts of droplets, most probably representing fat stores remaining from the egg yolk (Fig. 1). The number and density of these droplets decreased subsequently, regardless of the presence or absence of food. In fed larvae, significant amounts were detected in the Z III stage, while only few droplets remained through Z IV (Fig. 1) and thereafter. Effects of the complete absence of food on tentative lipid stores became microscopically visible for the first time when the Z III stage was reached. In this stage, the reduction of lipid droplets became more conspicuous in unfed than in fed larvae, resembling the appearance in fed Z IV (Fig. 1).

Larval biomass at hatching

We measured the initial dry mass and CHN on the day of hatching. Larvae from Female A showed similar dry mass values to those from Hatch B, but significantly higher CHN values (Table 1). Among the 3 hatches, larvae from Female A generally had the highest, those from C, the lowest biomass. The same pattern occurred in the C:N mass ratio (indicating similar variation in fat content), while the C:H quotient showed an opposite tendency (Table 1). In conclusion, our data provide evidence of significant intraspecific variability in the biomass and elemental composition of the freshly hatched larvae produced by different females (confirmed by ANOVA; all $p < 0.05$).

Expt A

The first experiment was conducted to ascertain whether Z I begin to consume and convert food at some point during their moulting cycle. In both treatments, all larvae moulted within the same night (4 d after hatching) to the second zoal stage, showing 100% survival. Hence, survival and development

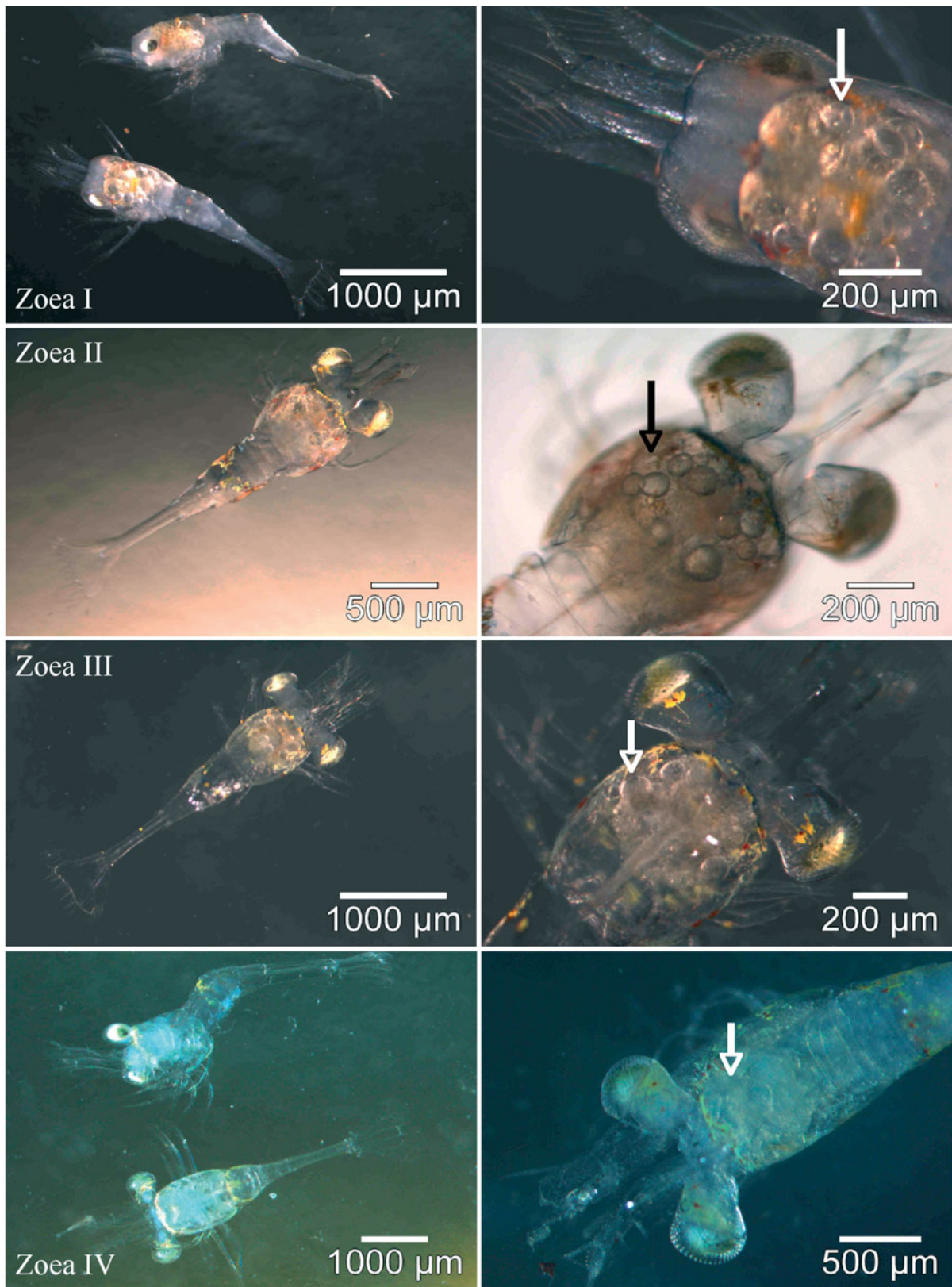


Fig. 1. *Macrobrachium amazonicum*. The first 4 larval stages (Zoea I to IV). Left panels: whole animals; right panels: lipid droplets (arrows) in the hepatopancreas region of the carapace

Table 1. *Macrobrachium amazonicum*. Variability in initial larval biomass and elemental composition at hatching among larvae produced by 3 different females (Hatches A to C): dry mass (W); carbon, nitrogen, hydrogen (C, N, H; in $\mu\text{g ind.}^{-1}$ and in %W); and C:N and C:H mass ratios; mean \pm SD; n = 5 replicate analyses

Hatch	W ($\mu\text{g ind.}^{-1}$)	C		N		H		C:N	C:H
		($\mu\text{g ind.}^{-1}$)	(%W)	($\mu\text{g ind.}^{-1}$)	(%W)	($\mu\text{g ind.}^{-1}$)	(%W)		
A	65.60 \pm 0.77	36.34 \pm 0.38	55.39 \pm 0.23	6.47 \pm 0.06	9.86 \pm 0.05	5.70 \pm 0.10	8.69 \pm 0.05	5.62 \pm 0.02	6.38 \pm 0.06
B	65.06 \pm 0.34	33.54 \pm 0.16	51.56 \pm 0.17	6.37 \pm 0.05	9.79 \pm 0.07	5.00 \pm 0.02	7.69 \pm 0.02	5.27 \pm 0.03	6.71 \pm 0.02
C	54.62 \pm 0.43	28.47 \pm 0.26	52.12 \pm 0.26	5.91 \pm 0.03	10.81 \pm 0.06	4.16 \pm 0.04	7.61 \pm 0.04	4.82 \pm 0.03	6.85 \pm 0.04

duration in the 2 treatments were identical, indicating complete larval independence from food.

Table 2 shows that all parameters of larval biomass (W, CHN) per individual decreased significantly during the 4 d of development during Z I, regardless of the presence or absence of food. W, for example, had decreased from an initial value of 66 (Table 1) to 59 $\mu\text{g ind.}^{-1}$ (or by ca. 10%) 4 d later (Table 2). The C content decreased concomitantly from 36 (Table 1) to 31–32 $\mu\text{g ind.}^{-1}$ (Table 2), i.e. by 13%. While the values of W, C and H generally showed high rates of decline, the N content decreased to a lesser extent (from 6.5 to 6.3 $\mu\text{g ind.}^{-1}$; or by <3%).

As a consequence of similar rates of decrease in W and C, the proportion of C (in percent of W) decreased only slightly throughout the course of this experiment from 55% (Table 1) to 52–53% (Table 2), while H showed a stronger decline (from 8.7% to 7.7–8.0%). The percentage of N, in contrast, increased significantly from ca. 9.9 to 10.6%. Due to these differential patterns in W and CHN, the C:N ratio decreased significantly from an initial value of 5.6 to final values of from 4.9 to 5.0. The C:H ratio, in contrast, increased slightly, from 6.4 (Table 1) to 6.7–6.8 (Table 2).

Generally insignificant differences observed between changes in the biomass of fed and unfed larvae,

respectively, revealed that Z I is a non-feeding stage, i.e. it does not take up and convert any food, even when this is available. The patterns of change in biomass are consistent with behavioural and microscopic observations, which consistently showed that Z I larvae never attempted to catch or eat *Artemia* nauplii. Correspondingly, the zoeal stomach and gut were always void of the characteristic red colouration that normally appears when brine shrimp nauplii have been ingested. This was observed only in fed Z II and later larval stages.

Expts B and C

As in Expt A, the Z I of Females B and C also moulted to Z II within the same night in both treatments (with and without food), showing 100% survival and identical stage duration (2 d at 29°C). These findings confirm the principal results of Expt A, indicating that the Z I of *Macrobrachium amazonicum* is a non-feeding (fully lecithotrophic) stage.

In the presence of food, all Z II larvae moulted after another 2 d to the Z III stage, while unfed larvae showed a slight but statistically significant delay (Z II durations: 2.13 \pm 0.04 and 2.33 \pm 0.48 d in Hatches B

Table 2. *Macrobrachium amazonicum*. Changes in larval biomass and elemental composition of first-stage larvae (Zoea I, Hatch A) during 4 d of development, with or without food (*Artemia* nauplii): dry mass (W); carbon, nitrogen, hydrogen (C, N, H; in $\mu\text{g ind.}^{-1}$ and in %W); and C:N and C:H mass ratios; mean \pm SD; n = 5 replicate analyses; for biomass at hatching see Table 1

Time	W ($\mu\text{g ind.}^{-1}$)	C		N		H		C:N	C:H
		($\mu\text{g ind.}^{-1}$)	(%W)	($\mu\text{g ind.}^{-1}$)	(%W)	($\mu\text{g ind.}^{-1}$)	(%W)		
Fed									
Day 1	65.05 \pm 1.22	35.08 \pm 0.54	53.93 \pm 0.24	6.42 \pm 0.09	9.87 \pm 0.06	5.49 \pm 0.08	8.44 \pm 0.05	5.46 \pm 0.02	6.39 \pm 0.02
Day 2	61.68 \pm 1.10	33.18 \pm 0.66	53.79 \pm 0.23	6.31 \pm 0.09	10.23 \pm 0.06	5.16 \pm 0.12	8.36 \pm 0.07	5.26 \pm 0.04	6.43 \pm 0.03
Day 3	60.88 \pm 0.72	32.08 \pm 0.37	52.70 \pm 0.09	6.26 \pm 0.07	10.27 \pm 0.02	4.96 \pm 0.08	8.14 \pm 0.07	5.13 \pm 0.01	6.47 \pm 0.05
Day 4	59.25 \pm 0.89	31.60 \pm 0.57	53.34 \pm 0.61	6.29 \pm 0.07	10.61 \pm 0.05	4.73 \pm 0.10	7.98 \pm 0.15	5.03 \pm 0.06	6.69 \pm 0.18
Unfed									
Day 1	64.14 \pm 0.69	35.11 \pm 0.34	54.75 \pm 0.13	6.43 \pm 0.05	10.03 \pm 0.03	5.52 \pm 0.07	8.61 \pm 0.06	5.46 \pm 0.01	6.36 \pm 0.04
Day 2	63.01 \pm 0.20	33.74 \pm 0.22	53.54 \pm 0.15	6.39 \pm 0.04	10.14 \pm 0.03	5.25 \pm 0.04	8.34 \pm 0.05	5.28 \pm 0.02	6.42 \pm 0.03
Day 3	61.12 \pm 0.53	31.86 \pm 0.23	52.13 \pm 0.21	6.23 \pm 0.07	10.19 \pm 0.06	4.94 \pm 0.03	8.08 \pm 0.05	5.12 \pm 0.02	6.45 \pm 0.02
Day 4	59.40 \pm 0.71	31.23 \pm 0.41	52.58 \pm 0.15	6.32 \pm 0.08	10.64 \pm 0.03	4.59 \pm 0.10	7.72 \pm 0.08	4.94 \pm 0.01	6.81 \pm 0.06

and C, respectively; $p < 0.01$ and $p < 0.0001$). In both experiments and both treatments, all larvae survived from hatching to the Z III stage. First mortality was consistently observed only in the Z III stage and thereafter (Fig. 2). Lack of mortality in both treatments (and in both hatches) shows consistently that the Z II stage is still, in principle, independent of food availability. Significantly delayed moulting to Z III, however, does show a negative effect of a lack of food, indicating that the Z II stage is not fully, but facultatively lecithotrophic.

While fed larvae moulted at regular intervals, on average every second day, to successive stages, the development of unfed larvae ceased at the Z III stage (Fig. 2). Although in the continued absence of food this stage was unable to moult to Z IV, it still showed a strong resistance against starvation, surviving through an extended additional period (up to another 10 d). Mortality increased dramatically about 8 to 10 d after reaching Z III, i.e. 12 to 14 d after hatching. When the last unfed Z III died in these experiments (14 to 15 d after hatching), fed siblings were already in the Z VII stage and showed low mortality (Fig. 2).

Larvae from Females B and C differed significantly,

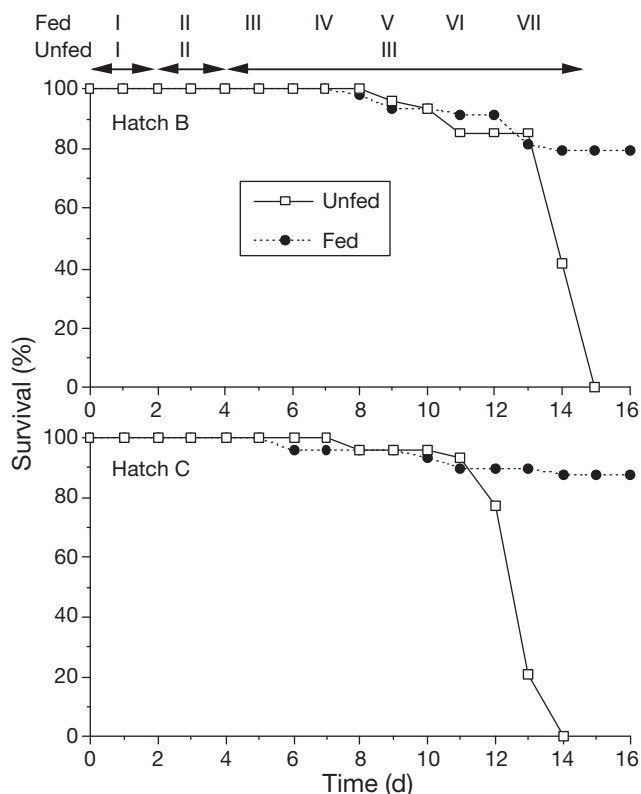


Fig. 2. *Macrobrachium amazonicum*. Survival (initial $n = 48$) of fed and unfed larvae originating from 2 different females (Hatches B and C). Roman numerals, arrows: successive larval stages (Zoea I, II, etc.) and periods of their occurrence

not only in their initial biomass at hatching (Table 1), but also in the developmental delay of the Z II stage and in the average time of survival in the continued absence of food. Unfed larvae from Hatch B showed, in comparison to those from Female C, a significantly weaker delay in Z II (2.13 vs. 2.33 d; $p < 0.02$), as well as a longer time of survival (13.9 vs. 12.7 d; $p < 0.0001$). These data show that Hatch B larvae were more resistant against starvation, which corresponded to significantly higher CHN and C:N values at hatching (Table 1).

When changes in biomass occurring throughout most of these experiments are compared between fed and unfed larvae originating from 2 different females (B,C), similar overall patterns can be seen in both the absolute biomass values (in $\mu\text{g ind.}^{-1}$; Fig. 3) and in the proportions of CHN (in %W) and the C:N mass ratios (Fig. 4). While unfed larvae from both hatches continually lost W and CHN per individual, fed larvae only initially showed a slight biomass loss, reflecting the non-feeding Z I development, followed by exponential growth (Fig. 3). The first significant biomass differences between fed and unfed larvae were consistently found in the Z II stage, confirming the conclusions drawn from developmental data (see above) that this stage is capable of feeding.

In both treatments and in the larvae from both females, the percentage values of C (Fig. 4) and H (not shown in the graph), as well as the C:N mass ratio, decreased significantly from hatching through the earliest zoeal stages (mostly during the development from Z I to Z III), followed by a more constant level thereafter. The N content, in contrast, showed an increasing trend in the early stages (especially in unfed larvae), followed by a decrease. Both the initial decrease in the percentage of C and in the C:N ratio, as well as the increase in the N content, were stronger in unfed than in fed larvae (Fig. 4).

When the losses in biomass of unfed larvae (using the C content as an example) are plotted against the time of development, an exponential pattern can be seen (Fig. 5). After logarithmic transformation of the C values, a linear relationship between $\log C$ and the time of development in the absence of food is obtained, allowing for a statistical comparison of the slopes of regression lines. Although the exponential curves appear to be practically parallel (Fig. 5), suggesting identical daily utilization rates, an ANCOVA revealed that the slopes differed significantly from each other ($p < 0.02$). Due to this difference, the intercepts cannot be compared (after removal of the interaction term). However, Table 1 shows that the initial C content differed greatly (33.54 ± 0.16 vs. $28.47 \pm 0.26 \mu\text{g ind.}^{-1}$). The subsequent rate of biomass degradation was thus very similar, but not identi-

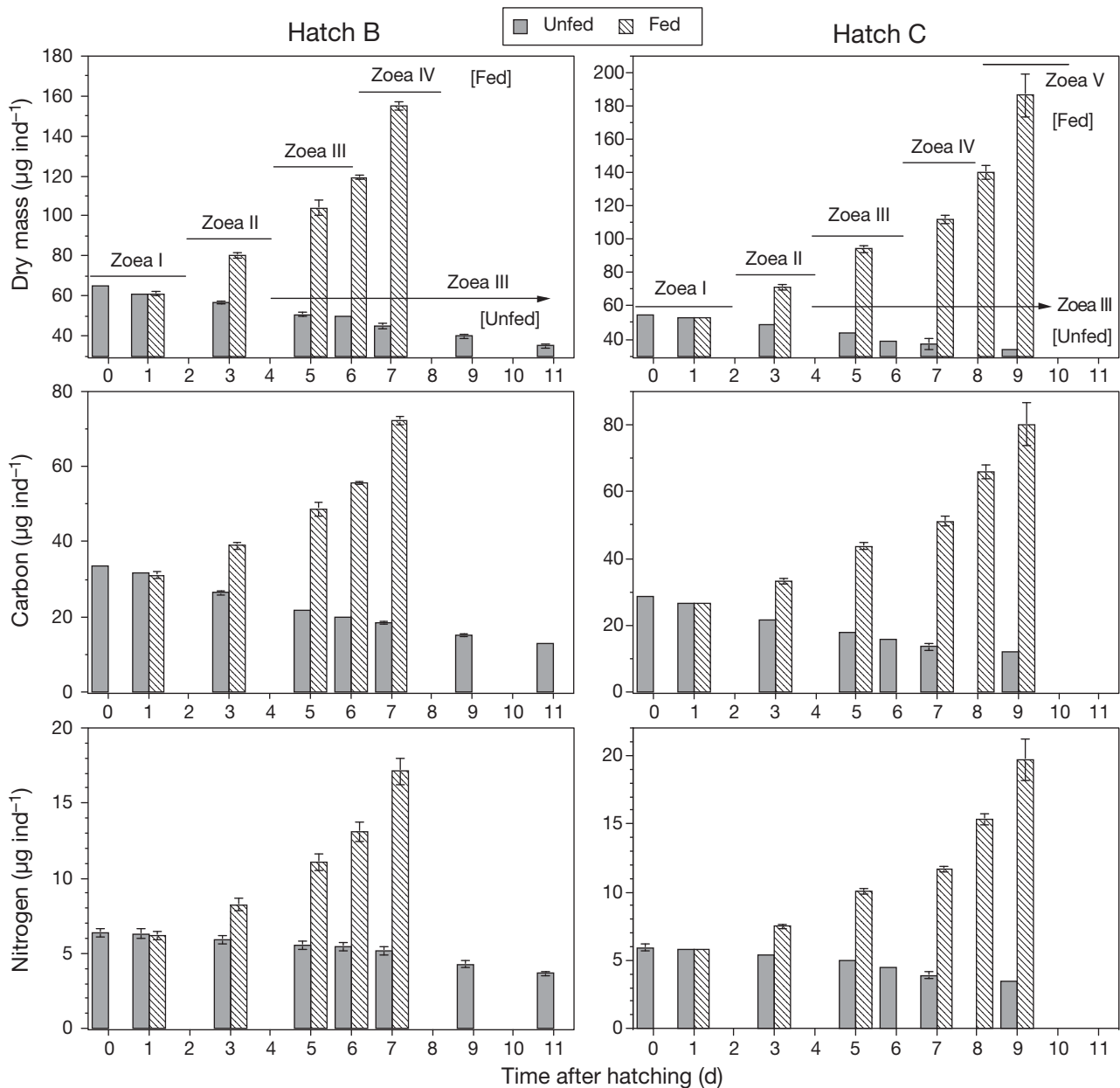


Fig. 3. *Macrobrachium amazonicum*. Changes in dry mass, carbon and nitrogen contents (mean \pm SD) of fed and unfed larvae from 2 different females (Hatches B, C). Horizontal lines indicate periods of development through successive stages (Zoea I, II, etc.); unfed larvae did not develop beyond the Zoëa III stage (arrows)

cal. These findings indicate that not only the initial biomass, but also the rate of metabolism during development in the complete absence of food varies significantly among larvae from different females.

The last biomass values that could be measured in unfed larvae amounted to 12.79 ± 0.29 and $12.05 \pm 0.11 \mu\text{g C ind.}^{-1}$ in Hatch B and C larvae, respectively (Fig. 3). When these values are compared with the initial C contents at hatching (Table 1), our data show that the larvae utilized within 11 or 9 d as

much as 62 and 58%, respectively, of their initial biomass. As some larvae were, at this time, still able to survive for another few days (maximally until Day 14 or 13; cf. Fig. 2), the final C values prior to death must have been even lower. The rate of biomass utilization from the day of hatching until dying may, thus, have exceeded two-thirds of the initial values at hatching; extrapolations from the 2 regression curves (Fig. 5) yield final carbon losses $>70\%$ for Days 14 and 13.

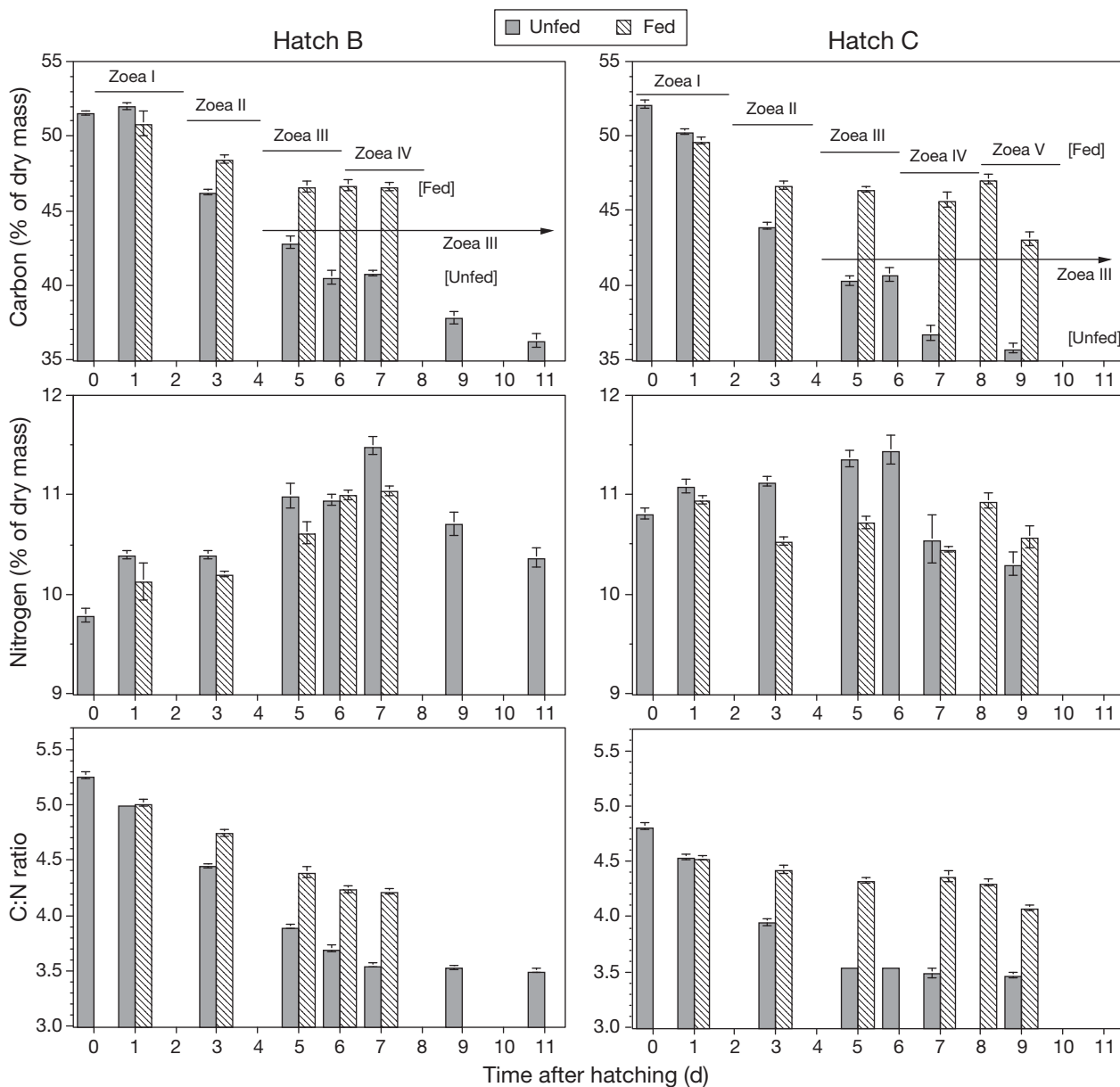


Fig. 4. *Macrobrachium amazonicum*. Changes in carbon and nitrogen contents and in the C:N mass ratio (mean \pm SD) of fed and unfed larvae from 2 different females (Hatches B, C). Horizontal lines, arrows: see Fig. 3

DISCUSSION

In the Amazon River prawn *Macrobrachium amazonicum* knowledge of the nutritional requirements in successive larval stages is important, not only to better understand the reproductive ecology of this widely distributed and regionally fished species (Odinetz Collart 1993), but also to develop rearing techniques for aquaculture (D'Abramo & New 2000, Kutty et al. 2000). Although no field studies of larval distribution have become available for coastal populations of *M. amazonicum*, experimental observations of salinity toler-

ance have consistently shown that a moderate salt concentration is necessary for successful larval development (Guest & Durocher 1979, McNamara et al. 1983, Moreira et al. 1986, Lobão et al. 1987, Rojas et al. 1990). Consistent with field observations of maximum reproductive activity during periods of falling water levels (Odinetz Collart 1991a), this physiological constraint indicates that this species follows an 'export strategy' (Strathmann 1982). Thus, larvae hatching in freshwater must soon be transported downstream, floating with river flow towards brackish estuarine or coastal marine waters.

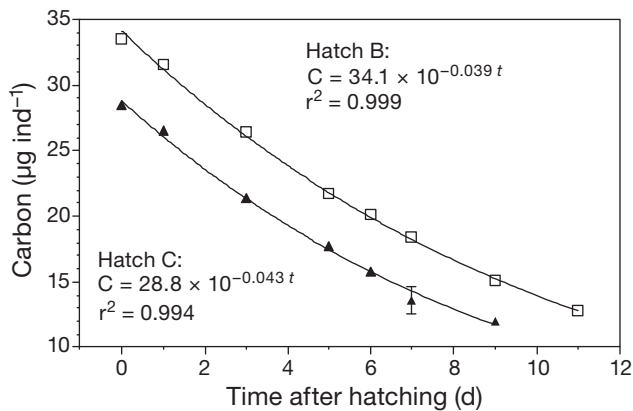


Fig. 5. *Macrobrachium amazonicum*. Exponential decrease in the carbon content (mean \pm SD) of larvae originating from 2 different females (Hatches B, C) reared in the continued absence of food (t : time). ANCOVA indicates highly significant effects of both variables (Day, Hatch; $p < 0.0001$); a significant interaction term (F -ratio = 17.792; $p < 0.02$) also indicates significantly different slopes of the linearized regressions (logarithmic transformation)

The initial phase of larval development in lotic freshwater habitats, where zooplankton production is very low (Pedrosa et al. 1999, Akopian et al. 2002), should have selected for an enhanced maternal energy investment into egg production (Anger 2001, 2006). This selection pressure may explain why the zoeae hatch with microscopically visible lipid stores remaining from the egg yolk (Fig. 1). Interestingly, both our microscopic observations and elemental analyses of larval biomass (decreasing C:N ratios; Fig. 4) show that the initial fat reserves are gradually utilized not only during the absence of food, but also (only at a slower rate) when enough food is available. This apparently programmed lipid degradation ensures that maternal energy previously invested in yolk-rich eggs is, sooner or later, converted to metabolic energy and to the chemical precursors needed for the synthesis of new tissues, developmental reconstruction processes and larval growth.

In all 3 larval hatches that we studied, the internal energy stores sufficed to support a completely non-feeding mode of development through the first zoeal stage, food-independent development through the Z II, and extended periods of survival during a continued absence of food in the Z III stage. The ontogenetic shift from obligatory lecithotrophy (Z I), through facultative lecithotrophy (Z II), to an eventually increasing degree of planktotrophy (Z III, later stages) represents a highly flexible strategy. This strategy must have a strong adaptive value under a scenario of initially poor, but then gradually improving nutritional conditions experienced during the course of ontogenetic migrations from riverine to estuarine or coastal marine environments.

Our data confirm previous studies by Odinetz Colart & Magalhães (1994) and Araujo & Valenti (2007), showing that the Z I is fully independent of planktonic food. As a consequence, this stage can survive and develop in unproductive lotic habitats. Under continued conditions of severe food limitation, Z II can also still successfully develop and moult to the next stage without showing reduced survival, but they pay for this capability with slightly delayed development and clearly reduced biomass as trade-offs. When food sources become available, Z II larvae also have the capability of utilizing external energy and investing it into growth. This flexibility should have an adaptive value in transitional environments between rivers and upper estuaries, where the physical and nutritional conditions may be unpredictable and plankton production temporally and spatially patchy. As soon as the Z III stage is reached, the nutritional vulnerability has increased, as the larvae are no longer able to moult to the following stage in the continued absence of food. Nevertheless, the remaining fat reserves still endow the Z III stage with an unusually strong tolerance for the food limitation that occurs in the transitional zones. Being able to survive for up to 2 wk in the complete absence of food, the larvae should have enough time to reach estuarine waters, where both a higher average salt concentration and higher plankton productivity (see Morgan 1995) favour the development through Z III and all later larval stages.

An ontogenetic shift from a completely non-feeding developmental mode through facultative lecithotrophy to a normal feeding stage has recently also been described for several endemic Jamaican crab species living and breeding in limnic or terrestrial habitats (Anger et al. 2007, and earlier papers cited therein). In these cases, however, the stepwise development from food-independent to feeding stages was not related to ontogenetic migrations through different habitats, as is presumably the case in *Macrobrachium amazonicum*, but rather to changes in functional morphology and behaviour, associated with ontogenetic variation in potential food sources. The peculiar breeding habitats of these crabs do not provide any planktonic food items, and, during the course of evolutionary invasion of such habitats, food limitation must have selected for full lecithotrophy in the zoeal stages. The terminal larval stage, the megalopa, in contrast, shows benthic behaviour and therefore has access to organic sediments, such as detritus and attached benthic microorganisms. Facultative lecithotrophy in the megalopa stage suggests, however, that this type of food source may also be scarce or unreliable. The juvenile crab is a feeding stage. It shows a much stronger developed locomotory activity than the megalopa and, being equipped with fully functional chelae and walking legs, is able to prey on larger benthic organisms.

All these unusual developmental modes with ontogenetic shifts in feeding and nutritional vulnerability have in common that planktonic food limitation prevailing during the early larval development has selected for evolutionary adaptations, in particular for an enhanced female energy investment in the production of yolk-rich eggs, from which lecithotrophic larvae hatch. As another common trait, the amounts of internal energy stores appear to be perfectly adjusted, so that the larvae can reach, independent of food availability, a later developmental stage that likely has sufficient access to external food sources.

The eventual mobilization of about two-thirds of the initially available energy pool (using the C content as a proxy for lipids and energy stores; see Anger 2001) shows that in *Macrobrachium amazonicum* most of the organic biomass present at hatching is metabolically available to ensure larval independence from food during early stages. The losses observed in the present study are higher than those in the initially lecithotrophic Jamaican crab larvae, where total lipid losses of up to 55% were measured (Anger et al. 2007), but they are similar to those in the fully lecithotrophic king crab larvae *Paralomis granulosa* and *Lithodes santolla* (Calcagno et al. 2003, Kattner et al. 2003). All these examples of partially or fully food-independent modes of larval development in decapod crustaceans have in common that lipids are primarily used as energy sources, while the protein fraction is largely conserved, remaining available for processes of developmental reconstruction and morphogenesis.

In palaemonid shrimps, including some marine species, the earliest larval stages frequently show a tendency of at least some independence from planktonic food (see Bauer 2004, Ituarte et al. 2005, Calado et al. 2007, 2008), and numerous other aquatic Decapoda also pass through non-feeding or facultatively lecithotrophic developmental phases (Anger 2001, Calado et al. 2008). In some cases, this initial lecithotrophy may thus be explained as a phylogenetic trait rather than an adaptation to specific life styles or ontogenetic migrations. Future comparative studies should investigate physiological mechanisms and adaptive implications, which would enhance our understanding of the life-history evolution of crustaceans in general.

In *Macrobrachium amazonicum*, it would also be interesting to test at which stage in the degradation of internal energy stores the larvae may reach a 'point of no return' (see Paschke et al. 2004, and earlier studies), losing their ability to recover after a delayed onset of feeding. Furthermore, possible interactions between nutritional and physical stress should be studied. This would provide us with ecologically meaningful and more realistic estimates of the physiological limitations involved in larval export strategies in lotic environ-

ments. In this context, the level of intraspecific variability in energy storage and utilization should also be studied in more detail, as these variable traits are subject to differential selection pressures in different habitats or climatic regions and, eventually, may drive segregation between populations and, ultimately, favour speciation. These aspects appear to be particularly interesting in *M. amazonicum*, which shows an extremely wide geographic and ecological distribution in South America, with several hydrologically (and hence, genetically) isolated inland populations. For instance, our data show significant variability in the initial larval biomass at hatching among different females from the same population, corresponding with variability in nutritional vulnerability (reflected by differences in the delay of development to the Z III stage and in the maximum time of survival under continued starvation). Variations among separate populations should thus be even more pronounced, possibly also explaining contrasting observations on the onset of feeding (non-feeding Z III development reported by Odinetz Collart & Magalhães 1994, but evidence for earlier feeding shown in the present study, corresponding with behavioural observations by Araujo & Valenti 2007).

We are still far from understanding, which specific adaptations to nutritional and osmotic stress allow fully limnic, land-locked inland populations of this species to develop through an extended mode of larval development in freshwater habitats (Odinetz Collart 1991a,b, Odinetz Collart & Magalhães 1994). It therefore would not be surprising if future comparative studies were to provide evidence for an incipient or ongoing speciation within *Macrobrachium amazonicum*, especially among the geographically separated populations living near the northeastern South American coasts, in central Amazonia, and in the upper Parana and upper Paraguay River basins.

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