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Feeding and growth in early larval shrimp *Macrobrachium amazonicum* from the Pantanal, southwestern Brazil

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ABSTRACT: The palaemonid shrimp *Macrobrachium amazonicum* (Heller 1862) lives in coastal rivers and estuaries along the northern coasts of South America as well as in inland waters of the Amazon, Orinoco, and upper La Plata (Paraguay-Paraná) River systems. In an experimental investigation on a little known, hydrologically isolated population from the Pantanal (upper Paraguay basin), we studied ontogenetic changes in early larval feeding and growth. Similar to a previously studied population from the Amazon estuary, the first zoeal stage (Z I) hatched with conspicuous fat reserves remaining from the egg yolk. While Z I is a non-feeding stage, Z II is facultatively lecithotrophic, and Z III is planktotrophic, requiring food for further development. Compared to estuarine larvae, those from the Pantanal hatched with lesser amounts of lipid droplets, and they survived for significantly shorter periods in the absence of food (maximally 8–9 d versus 14–15 d, at 29°C). Both populations moulted in short intervals (ca. 2 d) through larval stages Z I to VI. Biomass increased exponentially, with a higher growth rate observed in the Pantanal larvae. These develop in lentic inland waters, where high productivity allows for fast growth of planktonic predators. By contrast, the early larval stages of the Amazon population show a higher endotrophic potential and are thus better adapted to conditions of food limitation occurring during riverine downstream transport through lotic waters, towards coastal marine habitats. Initial larval independence from food in the Pantanal clade is interpreted as a plesiomorphic trait persisting from coastal marine ancestors.

KEY WORDS: Caridean shrimp · Larval feeding · Larval growth · Lecithotrophy · Starvation

INTRODUCTION

The palaemonid shrimp genus *Macrobrachium* Bate, 1868 comprises 239 extant species (De Grave et al. 2009), which inhabit freshwater, brackish estuarine, and coastal marine habitats in tropical and subtropical regions worldwide (Alekhnovich & Kulesh 2001, Bauer 2004, Murphy & Austin 2005). The South American species *M. amazonicum* (Heller 1862) shows a particularly wide geographic distribution, ranging from the Caribbean coast of Columbia (12° N) to northern Argentina and Paraguay (28° S), and from the subandine slopes of Ecuador, Peru, and Bolivia to the Atlantic coasts of northeastern Brazil, i.e. more than 4000 km across in both N–S and W–E directions (Holthuis 1952, Ramos-Porto & Coelho 1990, Odinetz Collart & Rabelo 1996, Pettovello 1996, Bialetzki et al. 1997, Magalhães 2000).

All northern populations are hydrologically connected to each other, living either in inland waters of the large river systems of the Amazon and Orinoco basins, or in coastal rivers and estuaries of the Caribbean and the Atlantic Ocean. Consequently, some gene flow remains possible, although restricted by vast distances and regional hydrological barriers. The southern populations, which live in the upper Para-

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guay and Paraná River basins (La Plata system), are hydrologically separated and thus genetically completely isolated from all others.

The occurrence of *Macrobrachium amazonicum* in the La Plata system has occasionally been considered to be the result of a recent anthropogenic introduction (Bialetzki et al. 1997, citing a technical report by Torloni et al. 1993). In a recent review, Maciel & Valenti (2009) also cited Magalhães et al. (2005) in this context. However, this reference is based on a misunderstanding of a statement, where Magalhães and coauthors are actually saying (p. 1933) that *M. amazonicum* was probably introduced into water reservoirs and rivers of the northeastern and eastern states of Brazil (citing Ramos-Porto & Coelho 1998), while the populations in the La Plata basin are explicitly referred to as belonging to the 'native fauna' (cf. Holthuis 1952). This is in fact strongly corroborated by much earlier records from northern Paraguay (dating back to A. Borelli's expedition in 1893–94, published by Nobili 1896) and from the Pantanal of Mato Grosso, southwestern Brazil (Moreira 1912, 1913).

Due to hydrological fragmentation within the enormous area of geographic distribution and genetic isolation of regional populations, *Macrobrachium amazonicum* shows great inter-populational variations in various biological traits including ecology, morphology, growth, reproduction, development, and physiology (for references, see Maciel & Valenti 2009). Based on morphological variation, Porto (2004) proposed to consider the coastal and fully limnic inland populations as 2 distinct species. This was supported by molecular genetic differences in the mitochondrial cytochrome oxidase subunit I (COI mtDNA; Bastos 2002). In a more recent study (Vergamini 2009), 16S rRNA and COI mtDNA data indicated that 3 monophyletic groups of populations may be distinguished: (1) eastern Amazonia, (2) coastal rivers in northern and northeastern Brazil, and (3) the upper La Plata (Paraná-Paraguay) basin. Although this pattern is quite plausible, genetic divergence rates calculated from these molecular data were interpreted as remaining within an intraspecific level, so that *M. amazonicum* is still being considered as a single species.

These inconsistent results and controversial interpretations show that more comparative studies of the biology, ecology, and genetics of different *Macrobrachium amazonicum* populations are necessary to resolve the degree of possible speciation. In particular, evolutionary key characteristics such as developmental and reproductive adaptations to differential environmental conditions in different habitat types should be identified to understand the distribution and phylogenetic divergence of populations within this clade.

In a previous study (Anger & Hayd 2009), an estuarine population of *Macrobrachium amazonicum* showed a high potential for food-independent larval development in the early postembryonic stages. This capability, which is based on an enhanced female energy investment into egg production, has also been observed in first-stage larvae of various other species of *Macrobrachium* and in other palaemonid shrimps living in coastal rivers and estuaries (e.g. Moreira et al. 1979, Mashiko 1983, Anger 2001). Initial independence from planktonic food sources has been considered an adaptive trait of planktonic larvae that are released into food-limited lotic freshwater habitats. This trait should enhance early larval survival while being transported downstream with the river currents, until the larvae reach lower estuarine or coastal marine waters where planktonic productivity is on average higher ('export strategy'; Strathmann 1982, Anger 2001). By contrast, in fully limnic inland populations that live and reproduce in lentic habitats such as the swamps of the Pantanal region, there should be no selection for such an energetically expensive adaptation.

Here we quantified the dependence on planktonic food as well as patterns of growth in the early larval stages of a shrimp population from the Pantanal of South Mato Grosso (upper Paraguay basin, southwestern Brazil) and compared these developmental traits to previous observations on the ontogeny of larval feeding and growth in an estuarine population from the mouth of the Amazon River (Anger & Hayd 2009, Anger et al. 2009).

MATERIALS AND METHODS

Origin and maintenance of shrimps. Adult *Macrobrachium amazonicum*, including ovigerous females, were obtained from a research hatchery maintained at the State University of Mato Grosso do Sul in Aquidauana (state of Mato Grosso do Sul, MS), southwestern Brazil. The broodstock originated from a population living in the Rio Miranda, Pantanal of Miranda, MS (sampling locations at 20° 8.9–10.7′ S, 56° 30.4–30.6′ W). This population will be referred to as the 'Pantanal population.' In February and November 2008, live shrimps were transported in cooling boxes to the Helgoland Marine Biological Laboratory (BAH), Germany. Here, they were maintained in aerated flow-through aquaria with 30 l of fresh water (total ion concentration: 0.2 mg l^{-1}), at constant 29°C, and an artificial 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. Freshly

hatched larvae were obtained from sieves (0.3 mm mesh size) receiving the overflowing water from the aquaria.

Experiments. Five rearing experiments were conducted with larvae obtained from different females (hatching on 26 February 2008, 23 September 2008, 15 November 2008, 17 February 2009, and 12 October 2009; referred to as Expts or Hatches A–E) in order to quantify rates of larval survival and development through successive stages. Hatches A–C (all from 2008) were produced by ovigerous females that had been brought directly from Brazil (see above), while later hatches originated from a laboratory culture built up and subsequently maintained at Helgoland. The larvae were individually reared in Nunc plastic bowls with 100 ml of unaerated water. A tentatively optimal salinity for larval rearing (5 PSU; K. Anger & L. Hayd unpubl. data) was obtained by mixing appropriate amounts of tap water (<0.2 PSU) with seawater from the North Sea (32–33 PSU). Salt concentrations were checked to the nearest 0.1 PSU using a temperature-compensated electric probe (WTW Cond 330i). The rearing temperature was 29 ± 0.5°C (temperature-controlled rooms).

Each experiment comprised 2 treatments. In one, the larvae were provided daily with newly hatched *Artemia* nauplii (Sanders Great Salt Lake; ca. 10–15 ind. 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. Depending on hatch size, each treatment initially comprised 24 to 48 larvae (37 in Expt A, 24 in Expts B–D, and 48 in Expt E). The experiments were terminated within 1 to 3 d after the death of the last unfed larvae. At this time, fed larvae reached the fifth or sixth zoeal stage. The successive zoeal stages (Zoea I, II, ...) are referred to in this paper as Z I, Z II, etc.

Larvae obtained from 3 other females (F–H; hatching on 23 September 2008, 17 January 2009, and 26 January 2009, respectively) were reared using the same cultivation techniques as described above to measure gains or losses in biomass (see below) during development with or without food, respectively. Only Female G produced sufficient larvae to study ontogenetic patterns of growth and elemental composition through the first 5 zoeal stages. Limitations in the number of larvae available for sampling as well as high mortality in larvae kept for more than 5 d without food did not allow us to take samples of unfed larvae for determinations of biomass close to the maximum time of survival under starvation; as a consequence, our data for survival and moulting cover a longer experimental period than those obtained for changes in larval biomass.

The present results on larval feeding and development in a Pantanal population were compared to those from a similar study on an estuarine population from northeastern Brazil (referred to here as the 'estuarine' or 'Amazon' population; see Anger & Hayd 2009, their Hatches B and C). Since the optimal larval rearing salinity for this population is 10 rather than 5 PSU (for references, see Anger et al. 2009), patterns of larval feeding and growth were not investigated at identical but rather at optimal salinity conditions (5 and 10 PSU, respectively).

Biomass measurements. Biomass was measured as dry mass (W) and contents of carbon, hydrogen, and nitrogen (collectively, CHN; Anger & Harms 1990) were determined. 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 analyzed with an Elementar Vario micro CHN analyzer using acetanilid as a standard. Each set of measurements normally comprised n = 5 replicate samples with 2 to 6 individuals each (depending on developmental stage). In the experiment with larvae from Female F, a technical failure of the CHN analyzer caused data losses, so that only 2 to 5 replicate analyses could be obtained in this case (Table 1). The most complete data set was obtained from Hatch G (data from hatching through the Z V stage), allowing for a comparison of the patterns of early larval growth in fed larvae from the Pantanal to those previously described for the estuarine population (Anger et al. 2009).

Statistical methods. Statistical analyses were carried out following standard techniques (Sokal & Rohlf 1995) using a JMP software package (version 5.1.2; SAS Institute). Average durations of survival in unfed treatments were given as median values (lethal time for 50%, LT_{50}), all other average values as arithmetic mean ± SD. Since our data deviated in some cases from normality or homoscedasticity even after transformations (goodness-of-fit *G* test; Durbin-Watson statistic), we generally applied non-parametric tests for pairwise and multiple comparisons of mean values (Wilcoxon and Kruskal-Wallis test, respectively; chi-squared approximation). For an overall comparison of the time of survival in unfed treatments, we used a nested 2-way ANOVA with Population (Amazon versus Pantanal) and Hatch (nested within Population) as factors, as this test is fairly robust against deviations from a normal distribution (Underwood 1997). Mortality data were compared with a contingency analysis (Pearson's test; chi-squared statistic). Correlation and regression coefficients were tested for significant deviations from zero (ANOVA).

Table 1. *Macrobrachium amazonicum*. Changes in biomass and elemental composition of early larval stages reared with or without food (*Artemia* nauplii; Hatches F–H; no data available for the zoea III of Hatch H): dry mass (W); carbon, nitrogen, hydrogen (C, N, H; in % of W; for absolute values per indivdual, see Fig. 5); C:N, C:H mass ratios; data are mean ± SD; n = 2–5 replicate analyses

Hatch	Zoea		W (µg ind.$^{-1}$)	C (% W)	N (% W)	H (% W)	C:N ratio	C:H ratio	n
F	I	Hatching	83.4 ± 2.4	41.3 ± 0.2	9.9 ± 0.1	6.9 ± 0.1	4.19 ± 0.01	5.96 ± 0.05	2
		Unfed	79.5 ± 0.8	42.8 ± 0.4	9.5 ± 0.1	7.0 ± 0.1	4.51 ± 0.02	6.15 ± 0.06	2
		Fed	75.2 ± 3.4	40.9 ± 0.4	10.0 ± 0.1	7.0 ± 0.1	4.10 ± 0.02	5.88 ± 0.02	3
	II	Unfed	62.2 ± 1.4	37.8 ± 0.5	10.7 ± 0.2	6.7 ± 0.1	3.53 ± 0.03	5.64 ± 0.02	5
		Fed	103.8 ± 0.6	39.4 ± 0.6	9.9 ± 0.1	6.7 ± 0.2	3.98 ± 0.10	5.92 ± 0.15	4
	III	Unfed	38.9 ± 2.8	34.6 ± 0.8	9.8 ± 0.2	6.7 ± 0.2	3.53 ± 0.01	5.17 ± 0.02	3
		Fed	126.2 ± 2.8	39.0 ± 0.6	10.5 ± 0.2	6.3 ± 0.1	3.73 ± 0.04	6.22 ± 0.04	4
G	I	Hatching	75.7 ± 1.1	49.4 ± 1.7	11.4 ± 0.4	8.6 ± 0.2	4.31 ± 0.02	5.75 ± 0.21	5
		Unfed	72.3 ± 2.3	47.5 ± 0.6	11.9 ± 0.2	8.0 ± 0.3	3.99 ± 0.05	5.98 ± 0.09	5
		Fed	73.2 ± 1.6	46.8 ± 0.4	11.8 ± 0.1	7.7 ± 0.1	3.96 ± 0.05	6.09 ± 0.04	5
	II	Unfed	61.4 ± 2.2	42.3 ± 0.4	12.2 ± 0.1	6.8 ± 0.3	3.48 ± 0.03	6.24 ± 0.26	5
		Fed	94.0 ± 1.4	45.1 ± 0.1	12.0 ± 0.1	7.2 ± 0.2	3.77 ± 0.04	6.23 ± 0.17	5
	III	Unfed	45.5 ± 1.1	40.7 ± 0.5	11.8 ± 0.1	6.8 ± 0.2	3.44 ± 0.02	5.97 ± 0.09	5
		Fed	138.7 ± 2.3	45.6 ± 0.2	12.0 ± 0.1	7.1 ± 0.4	3.79 ± 0.04	6.43 ± 0.37	5
	IV	Fed	178.8 ± 10.1	44.5 ± 0.5	11.8 ± 0.1	7.0 ± 0.2	3.75 ± 0.02	6.31 ± 0.10	5
	V	Fed	283.4 ± 15.5	44.7 ± 0.4	11.9 ± 0.2	7.1 ± 0.1	3.76 ± 0.03	6.32 ± 0.08	5
H	I	Hatching	84.1 ± 1.0	49.2 ± 0.2	10.9 ± 0.1	7.8 ± 0.3	4.53 ± 0.04	6.31 ± 0.21	5
		Unfed	74.4 ± 0.9	49.4 ± 0.4	12.0 ± 0.1	8.3 ± 0.1	4.12 ± 0.04	5.98 ± 0.02	5
		Fed	75.3 ± 1.0	50.3 ± 0.4	12.1 ± 0.2	8.4 ± 0.1	4.17 ± 0.04	6.02 ± 0.03	5
	II	Unfed	58.0 ± 1.3	42.8 ± 1.0	11.9 ± 0.2	7.4 ± 0.1	3.59 ± 0.04	5.76 ± 0.07	5
		Fed	102.7 ± 2.6	46.8 ± 1.0	12.3 ± 0.3	7.7 ± 0.1	3.81 ± 0.04	6.08 ± 0.04	5

RESULTS

Internal lipid stores

In the hepatopancreas region of their cephalothorax, freshly hatched larvae (Z I) showed fat droplets remaining from the egg yolk (Fig. 1). The numbers and densities of these droplets were conspicuously lower than in freshly hatched zoeae produced by a population of *Macrobrachium amazonicum* that originated from the mouth of the Amazon River (Anger & Hayd 2009, their Fig. 1).

During the first zoeal stage, the tentative lipid stores decreased regardless of presence or absence of food. Effects of starvation (i.e. a stronger fat degradation in unfed compared to fed larvae) became microscopically visible for the first time near the end of the Z II stage. These observations are consistent with observations of larval behavior, which showed that Z I is a completely non-feeding stage (never responding to food availability). As soon as the Z II stage had been reached, however, the larvae captured and ingested *Artemia* nauplii, showing that Z II is generally a feeding larval stage. On the other hand, unfed Z II were normally also able to successfully develop to the following stage (see below), which shows that Z II is facultatively lecithotrophic, as in the Amazon population (Anger & Hayd 2009).

Moulting and development

Both fed and unfed larvae moulted at regular intervals of about 2 d, until they reached the third zoeal stage (Z III). While unfed larvae remained in this developmental stage until they died (see below, Fig. 4), fed siblings continued to moult on average every 2 d. This highly regular moulting pattern can be described as a linear relationship between the number of stages and the time of development (Fig. 2; pooled data from all 5 experiments, fed larvae).

Presence or absence of food had no effect whatsoever on the survival or development through the first zoeal stage. In all 5 experiments (A–E), Z I showed no mortality at all, and stage duration was almost invariably 2 d. As the only exceptions, 4 individuals (2 fed and 2 unfed; out of a total of 314) already moulted to Z II after 1 d.

In the second zoeal stage, in some (but not all) experiments, continued lack of food caused a decrease in survival or a delay in the development to Z III (Fig. 3a,b). These effects were generally weak, with survival mostly exceeding 80% and a duration of the Z II stage ranging between 2 and 3 d. The strongest and most consistent effects of starvation were observed in larvae from Hatches A and E, while only a slight developmental delay occurred in Hatch B (weakly significant, $p < 0.05$), and no effects were

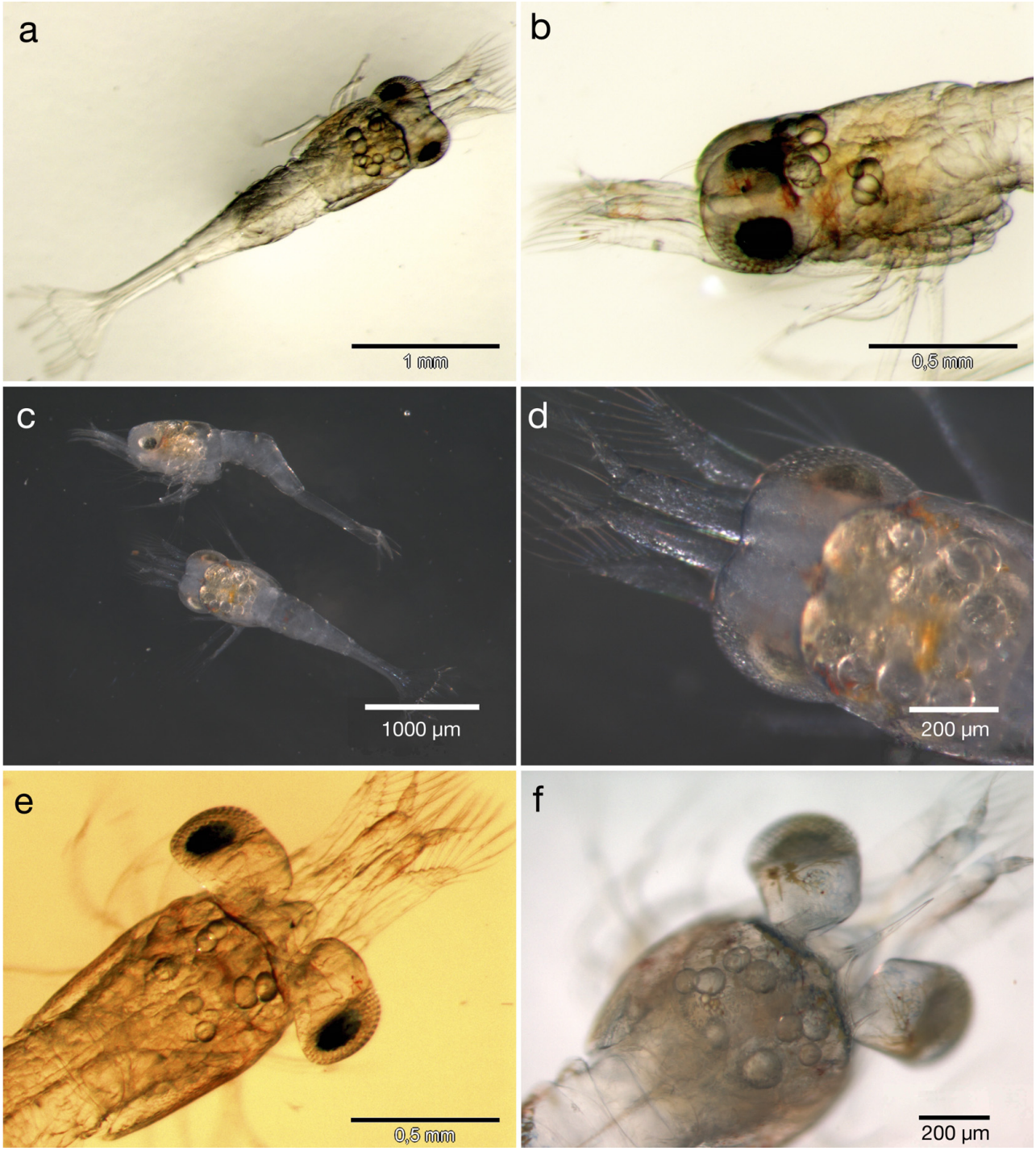

Fig. 1. *Macrobrachium amazonicum*. (a–d) Newly hatched zoea I and (e–f) zoea II with lipid droplets in the cephalothorax. (a,b,e) Larvae from the Pantanal (present study); (c,d,f) larvae from the Amazon estuary, from Anger & Hayd (2009, their Fig. 1)

found in Hatches C and D (Fig. 3). These observations indicate that larvae originating from different females varied significantly in their tolerance to starvation.

Significant differences among hatches also occurred in the average time of survival in unfed treatments (Fig. 4). Expressed as median value (or time of 50% mortality, LT_{50}), it varied between 5.8 and 7.3 d (Fig. 4). Hence, about one-half of the unfed larvae died on average 6 to 7 d after hatching, i.e. approximately at the same time when fed siblings moulted to Z IV. Com-

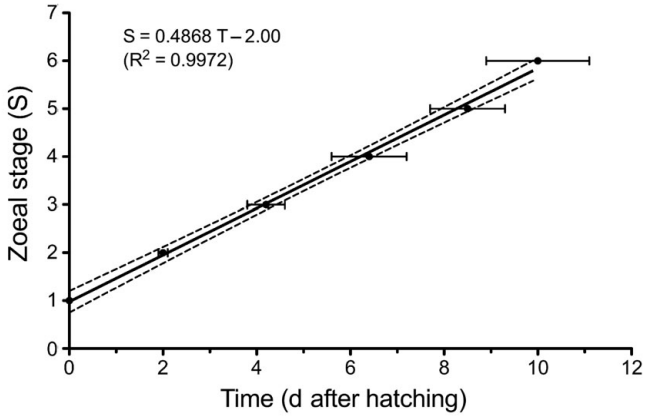

Fig. 2. *Macrobrachium amazonicum*. Pantanal population: pattern of early larval development with average time of moulting (T, mean ± SD) to successive stages (S); linear regression with 95% confidence intervals; R^2 = coefficient of determination

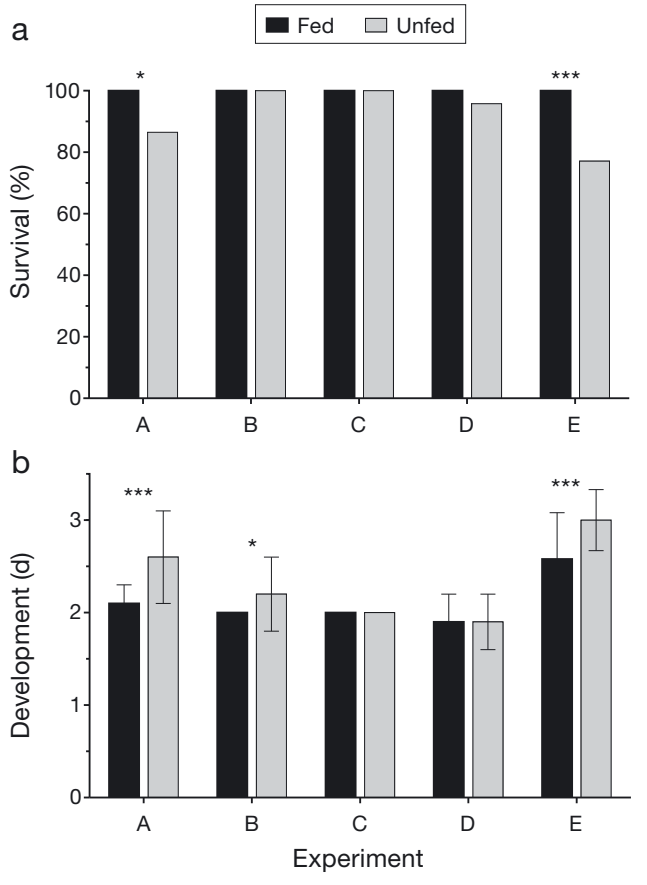

Fig. 3. *Macrobrachium amazonicum*. Pantanal population: (a) survival (%) and (b) duration of development (days) through the Zoea II stage reared with or without food; larvae obtained from 5 different females (A–E); significant differences between fed and unfed treatments marked with asterisks (*p < 0.05; ***p < 0.001)

plete mortality in unfed Z III was reached 8 to 9 d after hatching, while fed larvae already moulted to Z V. The shortest survival time was observed in Hatch E, which was congruent with significantly enhanced mortality and development duration in the Z II stage (Fig. 3). In Hatch A, by contrast, these different measures of larval starvation tolerance showed inconsistent patterns, with significant starvation effects on survival and development in Z II (Fig. 3), but longest time of survival in Z III (Fig. 4).

In Fig. 4, we also compare the average and maximum time of survival of unfed larvae from the Pantanal to previous observations on larvae from the Amazon estuary (Anger & Hayd 2009, their Expts B and C). This comparison shows that the larvae from the estuarine population survived about twice as long in complete absence of food compared to those from the Pantanal (LT_{50} = 12.5 to 13.8 versus 5.8 to 7.3 d; maximum survival time 14 to 15 versus 8 to 9 d). Mortality began in the Amazon larvae only 8 to 11 d after hatching, while the Pantanal larvae already showed complete mortality (Fig. 4). Nested 2-way ANOVA revealed highly significant effects (all p < 0.0001) of both the population of origin (Amazon versus Pantanal) and of the hatch within each population.

Changes in larval biomass during development and starvation

The patterns of change in larval biomass (measured as dry mass, W; and carbon, C; hydrogen, H; and nitrogen, N; collectively CHN) in treatments with or with-

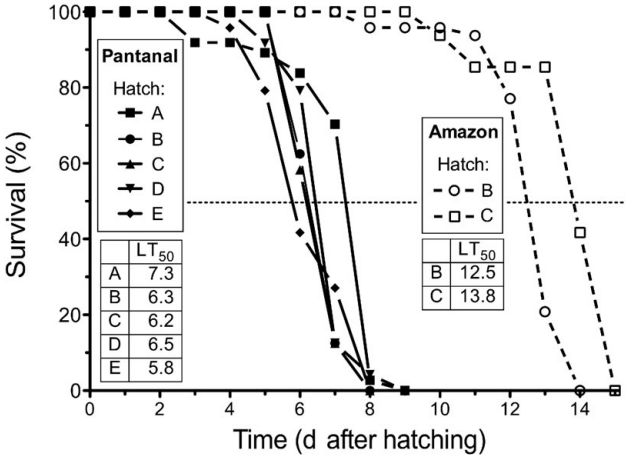

Fig. 4. *Macrobrachium amazonicum*. Larval survival (%) in continuous absence of food; comparison of larvae from the Pantanal (Hatches A–E, present study) with 2 hatches (B, C) from a previous study on a population from the Amazon estuary (Anger & Hayd 2009); LT_{50}: lethal time of starvation for 50% (dotted horizontal line)

out food (Fig. 5) were generally consistent with those observed in larval survival and development (Figs. 3 & 4). From hatching to the end of the Z I stage, both fed and unfed larvae lost small but significant amounts of W and CHN (in µg ind.$^{-1}$; see Fig. 5, showing W and C data as an example; Table 1). These initial biomass losses observed in both treatments reflect the non-feeding development through the first larval stage. Significant biomass differences between fed and unfed larvae were consistently detected only at the end of Z II and in late Z III larvae (Fig. 5, Table 1).

An increase in the biomass of fed larvae, concomitant with losses in unfed siblings, showed that Z II and Z III are feeding stages. From the end of Z I (2 d after hatching, i.e. shortly before the onset of feeding) to the end of Z III (6 d after hatching), fed larvae from Hatch G, for example, gained 89% in W and 85% in C per individual. By contrast, during the developmental period from hatching to the LT_{50} (i.e. within 6 d) unfed larvae lost about 40% of their initial W and as much as 69% of their initial C content per individual. Thus, biomass losses of unfed larvae prior to death (8 to 9 d after hatching; no data available; see Materials and Methods) must have been even higher.

Table 1 shows changes in the relative elemental composition of larval biomass (CHN in % of W) in Expts F to H. Independent of the presence or absence of food, the early larval stages generally showed decreasing tendencies in the percentage C and H values, while the percentage of N tended to increase (best visible in the most complete data set, Hatch G). As a consequence, the C:N mass ratio, which is considered an index reflecting the lipid:protein ratio, showed similar patterns as those observed in the percentage values of C and H. These changes in the elemental composition, especially the decreasing trends in C, H, and C:N,

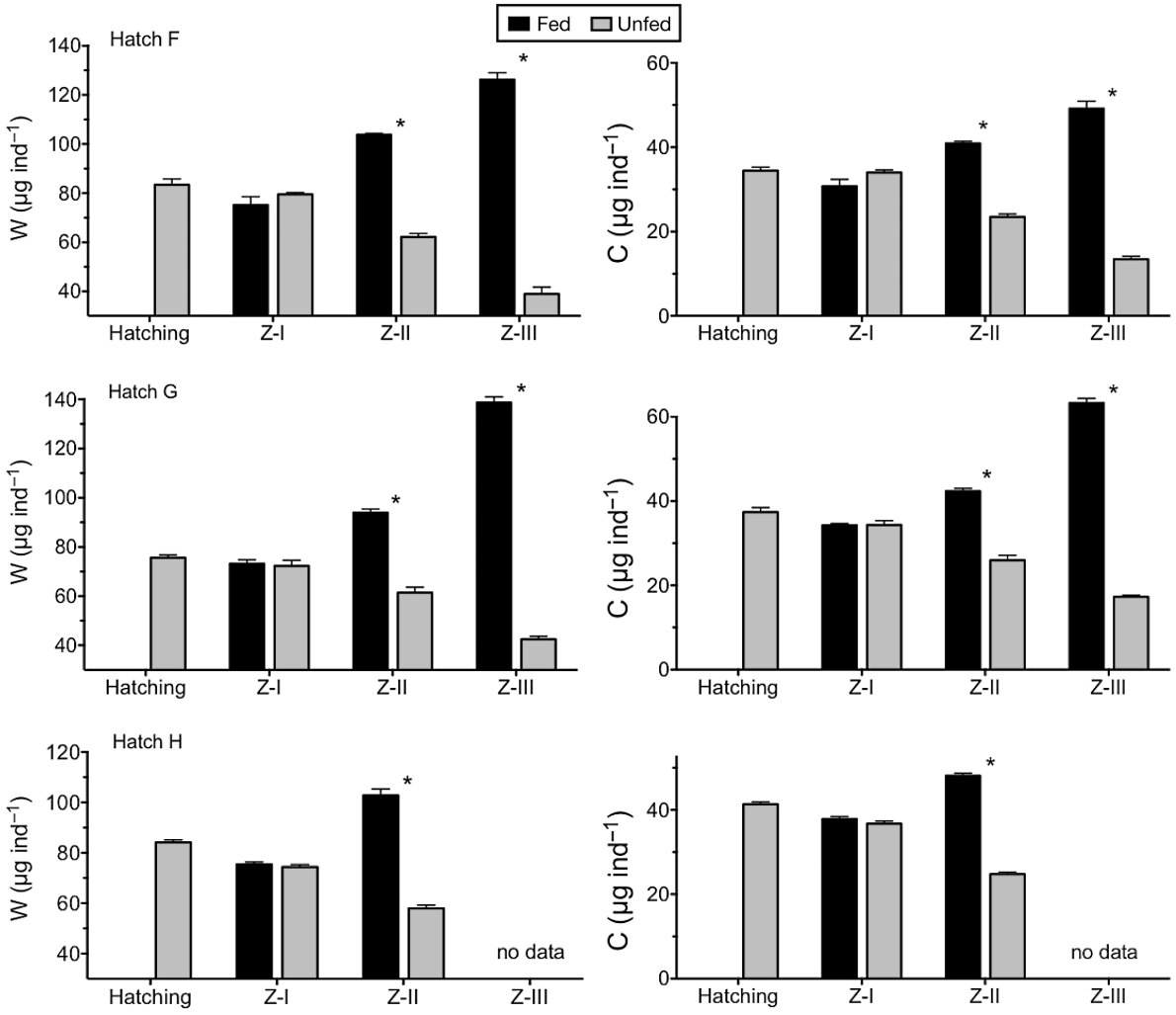

Fig. 5. *Macrobrachium amazonicum*. Pantanal population: changes in biomass (left graphs: dry mass, W; right: carbon content, C) of larvae from 3 hatches (F–H) reared from hatching to the zoea III stage, with or without food; significant differences between fed and unfed treatments marked with asterisks (*$p < 0.05$)

were generally stronger in unfed than in fed larvae. Overall, these patterns indicate a preferential utilization of lipid reserves as an energy source during initially lecitotrophic development (Z I; see Fig. 1) and starvation (later stages), respectively. Stable or increasing percentage N values, by contrast, showed that both fed and unfed larvae retained or increased their protein stores, which are required for the developmental reconstruction of new tissues and organs.

When the biomass of continually fed larvae is plotted against the number of successive stages (Fig. 6a), an exponential pattern of growth can be identified during the developmental period from the end of Z I through Z V (Fig. 6b). Hatch G larvae, for instance, showed a >3-fold increase in W and CHN per individual during this 8 d period (Table 1). Compared to the Amazon larvae, those from the Pantanal showed not only a clearly higher W in the Z I stage (cf. Table 1; Anger et al. 2009), but subsequently also a steeper biomass increase throughout the first 5 larval stages (slopes of the linearized regression equations: 0.359 versus 0.313, respectively; Fig. 6).

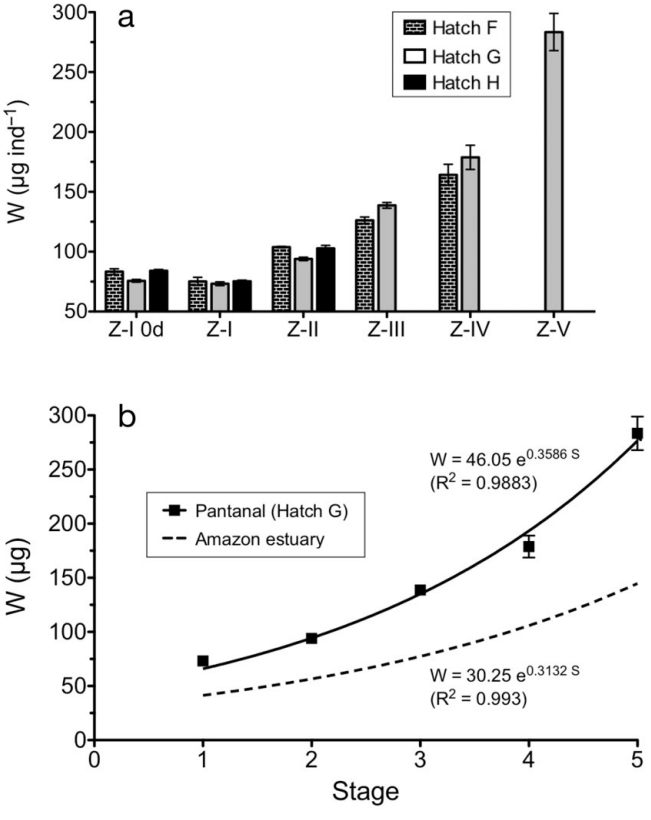

Fig. 6. *Macrobrachium amazonicum*. Pattern of early larval growth in fed treatments; biomass measured as dry mass (W, mean ± SD) at hatching and near the end of successive stages (zoea I to V); (a) comparison of W in 3 hatches of the Pantanal population (F–H); (b) exponential regression of premoult W as a function of the number of successive stages (S), comparison with the growth curve (dotted, below) obtained in a previous study on larvae from the Amazon estuary (Anger et al. 2009); R^2: coefficient of determination

The relative elemental composition of larval biomass (percentage values of CHN, C:N, C:H ratios) changed significantly during the initial lecithotrophic development from hatching through Z I, while later zoeal stages (Z II to V) showed a very constant chemical composition during their development and growth (Table 1).

DISCUSSION

Larvae of *Macrobrachium amazonicum* originating from a fully limnic inland population of the Pantanal of South Mato Grosso are morphologically similar at hatching (but not identical; A. dos Santos et al. unpubl. data) to freshly hatched zoeae produced by a population from the Amazon estuary (see Guest 1979, Vega Pérez 1984, Magalhães 1985). Both types of Z I larvae show fat droplets in the hepatopancreas region of the cephalothorax, representing lipid reserves that remain from the egg yolk. However, these droplets occurred in a conspicuously lower quantity in Pantanal larvae compared to those from the Amazon estuary (Fig. 1). This observation is congruent with a significantly lower larval lipid content at hatching, despite significantly larger body size, higher dry mass, and higher protein content in Pantanal larvae (A. Urzua & K. Anger unpublished).

Larvae from both populations have a completely non-feeding Z I stage, a facultatively lecithotrophic Z II, and extended survival times in Z III when food is continually absent (present study; cf. Odinetz Collart & Magalhães 1994, de Araujo & Valenti 2007, Anger & Hayd 2009). However, larvae from the Amazon estuary kept at the same temperature (29°C) in continued absence of food can survive almost twice as long as the Pantanal larvae (up to 14 to 15 versus 8 to 9 d), and in some hatches they can even reach the Z IV stage (K. Anger & L. Hayd unpubl. data).

Altogether, these differences indicate that the early larvae from the Amazon estuary can rely more on their internal energy stores. This should be advantageous when they are transported with fast-flowing river currents towards lower estuarine or coastal marine waters (Anger & Hayd 2009). In the Pantanal, by contrast, ovigerous females have been observed mostly near the margins of slowly flowing rivers and stagnant waters, where the larvae cannot possibly be exported towards the sea, but must hatch and develop in the same habitats where the adults live (L. Hayd & K. Anger unpubl. data). Shallow lentic waters, where Pantanal shrimps reproduce, are highly productive (Heckman 1998), as the residence time of those water bodies is far longer

than in lentic habitats, where hardly any planktonic food with appropriate particle size can be produced (Pedrosa et al. 1999, Akopian et al. 2002). Those different nutritional conditions prevailing in lentic and lotic habitats, respectively, should have selected for differential degrees of early larval independence from food, explaining differences in the amounts of lipid droplets and in the lipid content of dry mass at hatching, as well as in the tolerance of extended periods of food limitation (see Fig. 4).

If lentic waters in the Pantanal are not food-limited, this raises the question of why the early larvae of the Pantanal shrimps exhibit relatively large lipid stores reducing their initial dependence on planktonic food sources. Similar lecithotrophic tendencies have also been observed in the early larvae of other limnic palaemonid shrimp species, where no adaptive value of this trait is apparent (Anger 2001, Ituarte et al. 2005). Likewise, it is surprising that larval rearing is more successful in slightly brackish water (5 PSU, a condition that does not exist in the Pantanal), allowing higher survival and shorter development compared to pure freshwater (K. Anger et al. unpubl. data). Since both traits resemble those of estuarine shrimp larvae, and a coastal marine origin is generally accepted for limnic palaemonids (Walker 1992, Jalihal et al. 1993, Anger 2001, Murphy & Austin 2005), they may be explained as phylogenetic relics that have persisted from the ancestral clade.

Palaeogeographic and palaeoecological evidence for marine incursions during the early and middle Miocene (Lovejoy et al. 1998, 2006) suggests that the ancestors of *Macrobrachium amazonicum* may first have migrated from the Caribbean coast into western Amazonian inland waters, where brackish conditions prevailed. The invaders subsequently spread over an expanding, interconnected, and increasingly limnic subandine lake system that was formed due to the Andean orogenesis (Räsänen et al. 1990, Hoorn et al. 1995, Wesselingh et al. 2002), gradually adapting to fresh water. Eventually, the southernmost populations were separated from those remaining in the Amazon and Orinoco basins, when the formation of the modern South American drainage system took place and a continental divide became effective between the Amazon and La Plata basins, i.e. during the late Miocene through the Pliocene (Campbell 1990, Lundberg et al. 1998).

This scenario may explain why some developmental traits of estuarine shrimps have persisted in the fully limnic Pantanal population, and why these traits have changed under differential selection pressures and after an interruption of gene flow. These adaptive shifts include a reduced larval tolerance to food limitation (present study), an enhanced tolerance of fresh water (K. Anger et al. unpubl. data), and a complete loss of the physiological function of hypo-osmoregulation at high salt concentrations (G. Charmantier et al. unpubl. data). Although pure freshwater is not an optimal rearing condition, it does allow successful development of Pantanal larvae through metamorphosis, while larvae from the Amazon estuary can survive only through a few early stages (Guest & Durocher 1979, Remetin 2008, Knott 2009). Unlike the larvae of estuarine populations, but similar to those from the Pantanal, larvae produced in central Amazonia must tolerate fresh water, developing in lentic and highly productive shallow inland waters (Magalhães & Walker 1988, Odinetz Collart 1991, Moreira & Odinetz Collart 1993, Odinetz Collart & Magalhães 1994). In spite of continued gene flow among the various northern populations, there is thus also some divergence between coastal and Amazonian inland populations, which should receive further attention in comparative biological and genetic studies.

Besides in the potential for food-independent early larval development as well as in larval salinity tolerance, our study has shown some conspicuous differences in larval biomass and chemical composition (dry mass, CHN) at hatching. These differences include clearly higher average dry mass and CHN values per individual in Pantanal larvae compared to those from the Amazon estuary, but on the other hand a lower percentage C content and a lower C:N mass ratio (Figs. 5 & 6, Table 1; cf. Anger et al. 2009). Furthermore, a biochemical study (A. Urzua & K. Anger unpubl. data) showed significantly lower lipid but higher protein contents in Pantanal larvae, along with consistent differences in fatty acid profiles. In addition to the biomass and biochemical composition at hatching, the patterns of early larval growth also differ between the 2 populations, showing consistently higher biomass in equivalent stages of the Pantanal larvae (see Fig. 4b). Moreover, we have observed significant differences in larval morphology (A. dos Santos et al. unpubl. data) as well as in patterns of adult growth, reproduction, and ecology (Hayd & Nakagaki 2002, L. Hayd & K. Anger unpublished). Although differences in larval characteristics also vary significantly among females from the same population (maternal effects and/or genetic variability) and due to phenotypic plasticity (environmentally induced modifications), there is practically no overlap between the populations from the Pantanal and the Amazon estuary. Altogether, our observations raise the question of whether we are actually dealing with 2 conspecific populations, or rather with 2 separate species. Future studies on various aspects of the biology of the larval, juvenile, and adult life-history stages will therefore further scrutinize this question.

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