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Patterns of larval growth and chemical composition in the Amazon River prawn, *Macrobrachium amazonicum*

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ABSTRACT

The Amazon River prawn, *Macrobrachium amazonicum* (Heller, 1862), is a target species for regional fisheries in Brazil and a candidate for aquaculture. Under controlled laboratory conditions (29 °C, 10‰), the larval phase of this species shows variability in the morphology and number of successive stages (mostly 9–10, occasionally 8 to >12). In the most commonly observed developmental pathway (9 stages, taking approximately 20–22 days from hatching to the first juvenile stage), we studied patterns of larval growth in terms of total body length (TL), carapace length (CL), dry mass (W), and elemental composition (carbon, hydrogen, nitrogen; collectively CHN). At hatching, about 12% of late embryonic W, 15–18% of C and H, but only 7% of N were lost, indicating higher losses of lipids and/or carbohydrates than proteins. Significant variability was observed in the initial biomass and elemental composition of newly hatched larvae from 20 different egg batches. This may cause variation in the endotrophic potential of the early stages, as the zoea I of this species is a non-feeding stage, and also the zoea II may still utilize internal energy stores remaining from the egg yolk. Lacking or low larval feeding activity from hatching through stage II coincided with low initial growth. Concomitantly, the proportions of C and H (in % of W) as well as the C:N ratio decreased from hatching through stage IV, indicating a utilization of stored lipids. The percentage of N showed an opposite pattern, reflecting protein synthesis associated with morphogenesis. Size growth showed maximum increments per moult in the late zoeal stages (III–VI), followed by lower increments in the subsequent decapodid stages (VII–IX). This sigmoidal growth pattern may reflect ontogenetic changes in morphometric relationships. Biomass showed exponential patterns of increase from zoeal stage III throughout later larval development and in the first two juvenile stages. Furthermore, patterns of larval growth in *M. amazonicum* are characterized as linear relationships between larval W in stage n and that in stage $n+1$ (Hiatt diagram), between larval size (CL) and biomass (W, C), and between W and either C or N. Using CHN data, we also provide estimates of the protein and lipid contents of larval biomass (ca. 38–46% and 10–12% of W, respectively). High survival, rapid development, and predictable patterns of larval growth support the assumption that *M. amazonicum* should be a suitable species for production in aquaculture.

1. Introduction

Shrimp cultivation is one of the economically most promising aims in aquaculture (New, 1990; Fast and Lester, 1992; Wickins and Lee, 2002). Besides penaeid prawns, numerous species belonging to the caridean genus *Macrobrachium* (Palaemonidae) are subject of extensive aquaculture research on both basic and commercial aspects (New and Valenti, 2000). While the grow-out to commercial size seems mainly to be a matter of optimizing water quality, health and nutrition (Moraes-Riodades et al., 2006), seed production has remained a major bottleneck. This is also due to poor knowledge of basic developmental traits such as the number, morphology and duration of larval stages, nutritional requirements during the planktonic phase, and larval tolerance of physico-chemical factors. An improvement of larval rearing techniques, however, requires reliable criteria for the comparative evaluation of different cultivation methods, food sources, larval qualities, etc. Hence, we suggest that better knowledge of larval development and growth under controlled and near-to-optimal rearing conditions is a prerequisite for evaluating the success or failure of rearing conditions that must be tested to develop large-scale techniques for an economically feasible seed production.

The present study analyzes larval growth patterns in a species of shrimp that has great potential for aquaculture: the "Amazon River prawn", *Macrobrachium amazonicum* (Heller, 1862). This species shows a very large geographic range of distribution within South America, inhabiting inland waters in the Amazon and Orinoco River

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basins up to 3.400 km away from the Atlantic Ocean (Odinetz Collart and Rabelo, 1996) as well as rivers and estuaries draining to the northern and northeastern coasts of Brazil, the Guayanas, and the southern Caribbean coasts of Venezuela and Colombia (Holthuis, 1952; Rodríguez, 1982; Gamba, 1984; Magalhães and Walker, 1988; Odinetz Collart, 1991a,b; Walker, 1992; Montoya, 2003). Isolated inland populations have recently been reported also from the upper Paraná and Paraguay River systems in southern Brazil, Bolivia, Paraguay and northern Argentina (Pettovello, 1996; Bialetzki et al., 1997; Magalhães, 2000; Hayd and Nakagaki, 2002).

Growing to approximately 12–15 cm body size (females and males, respectively; Holthuis, 1980) and having a high market value, *M. amazonicum* sustains regional fisheries in northern Brazil (Coelho, 1963; Odinetz Collart, 1987, 1993). Since this species has frequently been proposed and, on an experimental scale, been tested as a candidate for aquaculture (e.g., see Coelho et al., 1982, 1989; Romero, 1982; Lobão et al., 1996; Kutty et al., 2000; Valenti and Moraes-Riodades, 2004; Moraes-Riodades et al., 2006), methods for larval rearing and grow-out have been developed (Guest and Durocher, 1979; Lobão et al., 1987; Rojas et al., 1990; Roverso et al., 1990; Moraes-Valenti and Valenti, 2007). Also, field studies of its ecology, reproduction and distribution in limnic inland waters have become available (Magalhães and Walker, 1988; Odinetz Collart, 1991a,b; Walker, 1992; Chaves and Magalhães, 1993; Odinetz Collart and Rabelo, 1996), and descriptions of larval morphology have been provided (Guest, 1979; Vega Pérez, 1984; Magalhães, 1985). Moreover, this species has frequently been used as a model organism for physiological studies of salinity tolerance and osmoregulation (McNamara et al., 1983; Moreira et al., 1986; Zanders and Rodríguez, 1992; Augusto et al., 2007; Santos et al., 2007).

The available information on *M. amazonicum* reveals a great deal of intraspecific variability in reproductive and developmental traits, which may be due to genetic isolation of different populations and, possibly, an incipient speciation within the large range of geographic and ecological distribution (Montoya, 2003). For instance, egg size increases with increasing distance from the sea (Odinetz Collart and Rabelo, 1996), larval morphology may vary between estuarine and fully limnic populations (cf. Vega Pérez, 1984; Magalhães, 1985), developmental changes in larval feeding have remained in contention (cf. Odinetz Collart and Magalhães, 1994; Araujo and Valenti, 2007), and patterns of larval growth and of ontogenetic changes in biomass and chemical composition have remained completely unknown. In summary, our knowledge of the early life history of this species has remained fragmentary.

In the present study, we present a large set of growth data for the larval stages of an estuarine population of *M. amazonicum* from northern Brazil, mainly to describe (1) developmental changes in larval size, dry mass, and elemental composition of successive stages reared under controlled laboratory conditions, and (2) quantitative relationships between various growth parameters. By measuring dry mass and chemical composition of late embryos and newly hatched larvae sampled on the same day from the same egg batch, (3) we analyze biomass variations at hatching. Comparing newly hatched larvae from 20 different egg batches, (4) we quantify intraspecific variability in larval biomass at hatching. Altogether, our data provide comparative basic criteria for evaluations of larval quality, rearing methods, and inter-populational variability in reproductive and developmental traits. This should eventually improve the basis for future aquaculture-related studies on this promising candidate species, towards an optimization of seed production.

2. Materials and methods

2.1. Collection and maintenance of prawns

Male and ovigerous female *M. amazonicum* were obtained from the Crustacean Sector of the Aquaculture Center (CAUNESP, Jaboticabal, SP) of the State University of São Paulo, Brazil (for details of broodstock production and maintenance, see Moraes-Valenti and Valenti, 2007). The broodstock originated from a population living in estuarine tidal creeks and channels near the city of Belém, Brazil, in the Amazon Delta (01°14′30″S 48°19′52″W; Valenti, pers. comm.). In January 2006, fifteen adult shrimps were transported in cooling boxes to the Helgoland Marine Biological Laboratory (BAH), Germany. Here, they were subsequently maintained in individual recirculating aquaria with 30 L freshwater (total ion concentration: 0.2 mg/L; for more details of water quality, see Anger et al., 2006), aeration, constant temperature (29 °C), a 12:12 h light:dark cycle, a gravel filter, and pieces of frozen marine isopods (*Idotea* spp.) provided as food. The females were checked twice daily for the occurrence of freshly hatched larvae.

2.2. Larval rearing, feeding experiments, sampling protocol

Actively swimming newly hatched larvae from one female were pipetted to individual 100 mL Nunc™ plastic bowls filled with unaerated water. A tentatively optimal rearing salinity of 10‰ for shrimp larvae from an estuarine population (Araujo and Valenti, 2007) was obtained by mixing appropriate amounts of tap water with seawater from the North Sea. Salinity was checked to the nearest 0.1‰ using a temperature-compensated electric probe (WTW Cond 330i, Weilheim, Germany). Conditions of temperature and light were the same as in the maintenance of adult prawns. The cultures were checked every 12 h for moults or mortality, and water and food (newly hatched *Artemia franciscana* nauplii provided *ad libitum*, ca. 10–15/ml) were changed every 24 h. No food was given to the zoea I, as this stage is lecithotrophic (Araujo and Valenti, 2007). Since larval feeding in the zoea II has remained unclear (cf. Araujo and Valenti, 2007; Odinetz Collart and Magalhães, 1994), this stage was routinely fed. Approximately in the middle of each moulting cycle, samples of larvae or early juveniles were taken for later determinations of body size, biomass and chemical composition (see below).

In another ovigerous female, samples of eggs in a very late stage of embryonic development were removed when the first larvae hatched. On the same day, also samples of newly hatched zoea I larvae from the same female were taken, so that changes in biomass due to the hatching process and the loss of the egg membrane could be measured.

Throughout 2006–2008, we also sampled newly hatched larvae from different females in order to quantify intraspecific variability in initial larval biomass. The females comprised individuals with different body size, age and history, including those directly transferred from Brazil, while others belonged to the first offspring generation that was produced at Helgoland.

2.3. Measurements of body size, dry mass, and elemental composition

Using a Leica MZ8 stereomicroscope equipped with a calibrated eyepiece micrometer, two dimensions of body size (excluding the rostral spine) were measured to the nearest 0.01 mm: (1) total length (TL) from the anterior margin of the eye orbit to the posterior margin of the telson; (2) carapace length (CL) from the anterior margin of the orbit to the posterior lateral margin of the carapace.

Biomass was measured as dry mass (W) and contents of carbon, hydrogen and nitrogen (CHN), following standard techniques (Anger and 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 analyzed with an Elementar Vario Micro CHN Analyser using acetanilid as a standard. Each set of measurements comprised $n=5$ replicate determinations with 1–6 individuals each (depending on individual dry mass in early or late developmental stages, respectively).