

**UNIVERSIDADE ESTADUAL PAULISTA**

**CENTRO DE AQUICULTURA  
CAMPUS DE JABOTICABAL**

**Ciclo de muda e metabolismo durante o desenvolvimento  
larval do camarão-da-amazônia *Macrobrachium amazonicum*  
(Heller, 1862)**

**Moulting cycle and metabolism during larval development of  
the Amazon River prawn *Macrobrachium amazonicum*  
(Heller, 1862)**

**Liliam de Arruda Hayd  
Bióloga**

**Jaboticabal – São Paulo  
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**Liliam de Arruda Hayd**

**Orientador: Prof. Dr. Wagner Cotroni Valenti**  
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**DEDICO**

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## Resumo

O estudo teve por objetivo descrever o ciclo de muda e estudar o metabolismo nas fases iniciais do desenvolvimento ontogenético de *Macrobrachium amazonicum*. O trabalho está organizado em cinco capítulos. O capítulo 1 apresenta uma introdução geral, apresentando os estudos inerentes à *M. amazonicum* e o programa de tecnologia onde este estudo está inserido. No capítulo 2 estão descritos os estágios do ciclo de muda de *M. amazonicum*. As descrições foram determinadas e documentadas fotograficamente em intervalos diários usando o telson como principal região de referência e aplicando o sistema de classificação de Drach, observando as principais mudanças que ocorrem na epiderme e na cutícula. O desenvolvimento é rápido (1-2 dias ou 2-4 dias por instar larval a 29 e 21°C, respectivamente). Foram descritos os seguintes estágios de muda, A/C (pós-muda/intermuda combinados), D (pré-muda) e E (ecdise). Estima-se que a pós-muda/intermuda (A/C) ocupe cerca de 40-50% do total de duração do instar enquanto que o período da pré-muda (D) requer mais que a metade do tempo nas temperaturas experimentais. O capítulo 3 apresenta a descrição do metabolismo de embriões, larvas e pós-larvas (PL com 1, 7 e 14 dias após a metamorfose) nas fases iniciais do desenvolvimento ontogenético. O peso seco, consumo de oxigênio, excreção de amônia total-N e taxa atômica O:N foram determinados. Os animais em estágio de muda A/C foram separados conforme o estágio de desenvolvimento larval e colocados dentro de câmaras respirométricas (30mL) por 2h para quantificar as taxas metabólicas. Após este período, as amostras foram analisadas pela titulação de Winkler e método de Koroleff para o consumo de oxigênio e nitrogênio amoniacal, respectivamente. As taxas metabólicas foram expressas como taxas individuais e peso-específico. A taxa individual de consumo de oxigênio e excreção de amônia nitrogenada aumentou durante o desenvolvimento larval. Os valores de O:N apresentaram valores baixos, indicando

que em *M. amazonicum* predomina o metabolismo de proteína. O conhecimento das taxas metabólicas irá subsidiar a interpretação de vários experimentos relativos à larvicultura dessa espécie, bem como fornecerá informações importantes para o dimensionamento adequado do sistema de aeração e dos filtros biológicos em sistemas de larvicultura. O capítulo 4 apresenta um estudo do metabolismo e do desenvolvimento das larvas em todos os estágios zoeas, submetidas em diferentes concentrações de nitrito. A sobrevivência, produtividade, ganho de peso e índice de estágio larval (IEL), foram avaliados. As larvas foram cultivadas em água com 0; 0,2; 0,4; 0,8 e 1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N, com 5 repetições para cada tratamento. As larvas foram mantidas em béqueres de vidro de 600mL com 300mL de solução-teste em água salobra (salinidade 10‰), aeração e temperatura constante de 30°C, com fotoperíodo de 12:12h claro:escuro. O consumo de oxigênio e a excreção de amônia foram analisados em zoea I, III, VII e IX expostos a 0; 0,4; 0,8 e 1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N com 5 repetições para cada tratamento. O experimento foi conduzido dentro de câmaras plásticas cilíndricas de 30mL. Sobrevivência, produtividade, ganho de peso e IEL decresceram linearmente com o aumento da concentração de nitrito no ambiente. Entretanto, não houve diferença significativa entre as concentrações 0-0,8mg.L<sup>-1</sup>NO<sub>2</sub>-N em todos os parâmetros avaliados. A concentração de 1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N no ambiente, retarda o desenvolvimento larval, reduz a sobrevivência, a produtividade, o ganho de peso, o IEL e afeta as taxas metabólicas. A conclusão geral do trabalho foi apresentada no capítulo 5. Assim, as informações obtidas nos experimentos fornecem subsídios importantes para o desenvolvimento de sistemas e métodos de produção de pós-larvas de *M. amazonicum*.

## CAPÍTULO 1

### 1. INTRODUÇÃO GERAL

A carcinicultura de água doce é uma forma lucrativa de produção de crustáceos com baixo impacto ambiental (Valenti e Moraes-Riodades, 2004; New, 2005) e atende aos preceitos da aquicultura sustentável (Valenti, 2002; Valenti e Tidwell, 2006). Os camarões de água doce ocupam posição inferior aos marinhos no mercado mundial. No entanto, apresentam maior resistência a doenças, larvicultura mais simples, independência da água salgada na fase de crescimento final e sistema de produção compatível com pequenas propriedades (New, 2005).

As estatísticas de produção são difíceis de serem obtidas porque a maioria desses crustáceos é produzida por pequenos proprietários rurais (Valenti e Moraes-Riodades, 2004). No entanto, sabe-se que a carcinicultura de água doce é um dos setores que mais crescem no mundo (Valenti, 2002; New, 2005). Os dados estatísticos indicam que o volume de *Macrobrachium rosenbergii* aumentou de 19.035 para 193.570 toneladas no período de 1995 a 2004, sendo que a China aparece como principal produtor desta espécie (FAO, 2006). Esses dados indicam que o setor tem apresentado grande desenvolvimento, e somente a produção mundial de *M. rosenbergii* foi estimada em 193.570 toneladas em 2004 (FAO, 2006).

A carcinicultura de água doce no Brasil está baseada na espécie *M. rosenbergii* (Valenti e Moraes-Riodades, 2004). O Brasil apareceu como o 6° produtor mundial em 2004 (FAO, 2006). Essa espécie é de origem asiática e apresenta características biológicas que favorecem o seu cultivo. Porém, não existem estudos referentes ao impacto de sua liberação nos ambientes naturais brasileiros. Isto é preocupante porque além de ser uma espécie alopátrica competidora pode trazer microfauna associada que pode ser disseminada no sistema aquícola brasileiro. Dessa forma, apesar de seu grande

potencial de cultivo em escalas comerciais, estudos com espécies nativas brasileiras tornam-se necessárias.

No Brasil, ocorrem três espécies de camarão de água doce que apresentam grande potencial para o cultivo, *Macrobrachium carcinus*, *Macrobrachium acanthurus* e *Macrobrachium amazonicum* (Valenti, 1993). *M. amazonicum* se destaca por apresentar grande distribuição geográfica, ocorrendo nas bacias do Orinoco, Amazonas, São Francisco, Paraná, Paraguai, áreas costeiras do norte e nordeste da América do Sul, Rio Paraguai (Cáceres e Descalvado) e rios, Miranda, Negro e Taboco (Magalhães, 2001). Devido a esta ampla distribuição, seu cultivo na maior parte do Brasil não oferece riscos de introdução de espécies exóticas na natureza. Essa espécie vem sendo amplamente explorada pela pesca artesanal na região Nordeste (Gurgel e Matos, 1984) e nos estados do Pará e Amapá (Odinetz-Collart e Moreira, 1993). É bem aceita nos mercados consumidores do Norte e Nordeste porque sua carne apresenta textura mais firme e apresenta um sabor mais acentuado quando comparado com *M. rosenbergii* (Moraes-Riodades e Valenti, 2001).

Os estudos existentes sobre *M. amazonicum* estão relacionados aos aspectos ambientais e biologia pesqueira de populações naturais (Odinetz-Collart, 1991a,b; Bialetzki et al., 1997), desenvolvimento larval (Guest, 1979a; Magalhães, 1985; Rojas et al., 1990), crescimento relativo (Moraes-Riodades e Valenti, 2002), morfotipos de machos (Moraes-Riodades e Valenti, 2004), morfofisiologia do hepatopâncreas (Papa et al., 2004; Ribeiro, 2006), desenvolvimento gonadal (Bragagnoli e Grotta, 1995), fecundidade (Odinetz-Collart e Rabelo, 1996; Da Silva, et al., 2004), densidade de estocagem (Lobão et al., 1994; Vetorelli e Valenti, 2004), salinidade (Guest, 1979; McNamara et al., 1983; Araújo, 2005), alimentação (Barreto e Soares, 1982; Araújo e Valenti, 2005), cultivo em laboratório (Guest, 1979a; Hayd et al., 2004), fase de

crescimento (Moraes-Riodades, 2005; Kiyohara, 2006), qualidade de água (Moraes-Riodades et al., 2006), transporte (Sperandio e Valenti, 2006) e viabilidade econômica (Vetorelli, et al., 2006; Hayd et al., submetido).

No início do ano 2000, um programa de pesquisa multiinstitucional e multidisciplinar visando à produção comercial de *M. amazonicum* foi iniciado (Valenti et al., 2003) com o desenvolvimento de vários trabalhos sobre seu cultivo. O presente trabalho está inserido nesse programa. Foram realizados estudos referentes ao ciclo de muda e ao metabolismo das larvas dessa espécie. O trabalho foi dividido em cinco capítulos, no entanto, os capítulos 2, 3 e 4 serão apresentados na forma de artigo científico. No capítulo 2 é apresentada a descrição dos estágios do ciclo de muda de *M. amazonicum*. As descrições são essenciais para padronizar a fase de muda das larvas usadas nos experimentos, reduzindo assim a variabilidade fisiológica e bioquímica dos sucessivos instares larvais. O capítulo 3 apresenta a descrição do metabolismo de embriões, larvas e pós-larvas (PL com 1, 7 e 14 dias após a metamorfose) nas fases iniciais do desenvolvimento ontogenético. O conhecimento das taxas metabólicas em cada estágio de desenvolvimento irá subsidiar a interpretação de vários experimentos relativos à larvicultura dessa espécie, bem como fornecerá informações imprescindíveis para o dimensionamento do sistema de aeração e dos filtros biológicos em sistemas de larvicultura e auxiliará nos cálculos de densidade de estocagem para o transporte de larvas e pós-larvas. O capítulo 4 apresenta um estudo do metabolismo e do desenvolvimento das larvas submetidas a um fator de estresse comum na larvicultura de camarões, o teor de nitrito. Assim, o estudo teve por objetivo descrever o ciclo de muda e estudar o metabolismo nas fases iniciais do desenvolvimento ontogenético de *Macrobrachium amazonicum*, fornecendo informações importantes para o desenvolvimento da tecnologia de produção de pós-larvas do camarão-da-amazônia.



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## CAPÍTULO 2 (submitted in Aquaculture)

**Ciclo de muda dos estágios larvais iniciais do camarão-da-amazônia, *Macrobrachium amazonicum*, cultivado em laboratório**

**The moulting cycle of early life stages of the Amazon River prawn, *Macrobrachium amazonicum*, reared in the laboratory**

### Resumo

O ciclo de muda das larvas do camarão-da-amazônia, *Macrobrachium amazonicum* foi estudado em laboratório. Usando o telson como a principal região de referência e aplicando o sistema de classificação de Drach, foram observadas a epiderme e a cutícula duas vezes ao dia e documentadas as principais mudanças estruturais tais como, a retração dos tecidos da epiderme da cutícula e o desenvolvimento setal. O desenvolvimento é rápido (1-2 dias ou 3-4 dias por instar larval a 29 e 21°C, respectivamente), o tegumento larval é fino, e como as mudanças do tegumento são muito rápidas, permitiram classificar o ciclo de muda em três principais estágios, A/C (estágio de pós-muda/intermuda combinados), D (pré-muda) e E (ecdise). Esses períodos poderiam ser morfológicamente divididos em subestágios; entretanto, não forneceriam um sincronismo definitivo para suas transições. Em pós-muda inicial (estágio A), a cutícula é fina e absorve água, de modo que o corpo da larva se expande rapidamente, alcançando a forma e o tamanho final. Os tecidos epidermais mostram neste estágio uma estrutura esponjosa com numerosas lacunas. Durante a pós-muda final (estágio B) e durante a intermuda (estágio C), a cutícula larval é reforçada, quando a epiderme mostra um condensamento crescente com redução dos espaços lacunares e crescimento conspícuo do tecido. A pré-muda inicial (subestágio D<sub>0</sub>) começa com uma

retração da epiderme da cutícula, denominada de apolise, que é inicialmente visível na base da seta. Durante a pré-muda intermediária (subestágio D<sub>1</sub>) ocorre a invaginação da epiderme, conduzindo a uma ampliação substancial da superfície da epiderme, iniciando a formação de novas setas e apêndices (setogênese, morfogênese). Subseqüentemente, uma nova cutícula fina é secretada na superfície da epiderme (subestágios D<sub>2-4</sub>). A muda (estágio E) é um processo muito rápido, durando somente alguns minutos. Inicia dorsalmente com a ruptura cuticular entre o cefalotórax e o abdome, seguidos por uma retração rápida do abdome do exoesqueleto velho, e eventualmente, retirando as partes anteriores da cutícula velha. O período combinado da pós-muda/intermuda (estágio A/C) ocupou cerca de 40-50% do total de duração do instar nas temperaturas experimentais, e a pré-muda (estágio D) requereu mais da metade do tempo. Como o crescimento larval ocorre predominantemente durante a pós-muda/intermuda, e este último é considerado como a fase mais estável do ciclo de muda, sugerimos que as mensurações fisiológicas e bioquímicas comparando sucessivos instares larvais sejam realizadas perto do fim deste período, isto é, cerca de 30-40% do ciclo de muda.

Palavras-chaves: Ciclo de muda, Larva, *Macrobrachium*, Camarão de água doce



## Abstract

The moulting cycle was studied in laboratory-reared larvae of the Amazon River prawn, *Macrobrachium amazonicum*. Using the telson as main reference region and applying Drach's classification system, we checked twice daily the epidermis and cuticle and documented major structural changes such as the retraction of epidermal tissues from the cuticle and setal development. Rapid development (1-2d or 3-4d per larval instar at 29 and 21°C, respectively), a thin and little structured larval integument, and gradual rather than abrupt integumental changes allowed for only a coarse classification of the moulting cycle with three principal stages, A-C (postmoult and intermoult stages combined), D (premoult), and E (ecdysis). These periods could be morphologically further divided into substages; however, without providing a definite timing for their transitions. At early postmoult (stage A), the cuticle is still thin and water is taken up, so that the larval body expands and rapidly attains its final size and shape. The epidermal tissues reveal at this stage a spongy structure with numerous lacunae. During later postmoult (stage B) and throughout intermoult (stage C), the larvae reinforce the cuticle, while the epidermis shows an increasing condensation with reduced lacunar spaces and conspicuous tissue growth. Early premoult (substage D<sub>0</sub>) begins with a retraction of the epidermis from the cuticle (apolysis), which is first visible at the setal bases. During intermediate premoult (substage D<sub>1</sub>), epidermal invaginations take place, leading to a substantial enlargement of the epidermal surface and initiating the formation of new setae and appendages (setogenesis, morphogenesis). Subsequently, a thin new cuticle is secreted on the epidermal surface (substages D<sub>2-4</sub>). Moulting (stage E) is a very short process, which usually takes only a few minutes. It begins dorsally with a cuticular rupture between the cephalothorax and the pleon, followed by a rapid retraction of the pleon from the old exoskeleton, and eventually,

shedding the anterior parts of the old cuticle. The combined postmoult-intermoult period (stages A-C) took at both experimental temperatures ca. 40-50% of total instar duration, while the premoult period (stage D) required slightly more than one half of the time. As larval growth is known to occur predominantly during postmoult and intermoult, and the latter is generally considered as the metabolically most stable phase within the moulting cycle, we suggest that physiological and biochemical measurements comparing successive larval instars should be carried out near the end of this period, i.e. at ca. 30-40% of the moulting cycle.

Keywords: Moulting cycle, Larvae, *Macrobrachium*, Freshwater prawn

## 1. Introduction

Growth and development of crustaceans appear to be discontinuous processes associated with successive moults (Hartnoll, 2001). Using histological and other morphological methods, Drach (1939) described in great detail moult-related anatomical changes occurring regularly in the integument of adult edible crabs, *Cancer pagurus*. Based on variations in the hardness of the cuticle as well as in epidermal and cuticular structures, he proposed a classification system for the moulting cycle with five principal stages (A-E) and numerous substages. This system was later further elaborated by Skinner (1962) and Drach and Tchernigovtzeff (1967), and it has been used in numerous studies on adult Decapoda and other crustaceans (Charmantier-Daures and Vernet, 2004).

Besides in anatomy and morphology, the moulting cycle implies also changes in behaviour, physiology and biochemistry, including cyclic activities of an antagonistic hormonal control system (for review, see Skinner, 1985; Chang, 1995; Charmantier-Daures and Vernet, 2004). Hence, knowledge of the course of the moulting cycle stage is highly important for the understanding of various aspects of crustacean biology, including physiology and biochemistry (e.g. Spindler-Barth, 1976; Chang, 1995; Ahearn et al., 2004; Gaxiola et al., 2005), behaviour (Thompson and McLay, 2005; Mikami, 2005), food requirements (Mantelatto and Christofolletti, 2001; Giménez et al., 2002; Schmidt et al., 2004), reproduction (Diaz et al., 2003; Tarling and Cuzin-Roudy, 2003; de Lestang and Melville-Smith, 2006), accumulation dynamics of toxic substances (Bondgaard and Bjerregaard, 2005; Norum et al., 2005), as well as fisheries and aquaculture of commercially important species (Ziegler et al., 2004; de Oliveira et al., 2006; Brylawski and Miller, 2006).

In basic scientific investigations as well as in applied research including crustacean aquaculture, all such cyclical changes have therefore implications for the experimental design and for the evaluation of data obtained from either laboratory or field studies. While the moulting cycle has extensively been studied in adult Decapoda and other crustaceans, it is much less known for larval stages, mostly due to practical problems related to small body size, a thin and hardly structured integument, short moulting cycles, and restricted availability of materials with precisely known age within a moulting cycle (for review, see Anger, 2001).

In Brazil, the Amazon River prawn, *Macrobrachium amazonicum* Heller 1862 is commercially fished (Odinetz-Collart, 1993) and has also a high potential for aquaculture (Kutty et al., 2000; Kutty, 2005; New, 2005). Technologies for hatchery and grow-out are presently under development (see New and Valenti, 2000; New, 2005). Populations of this species live in both freshwater and brackish estuarine habitats (Moreira et al., 1986; Magalhães and Walker, 1988; Odinetz-Collart, 1991a, b; 1993; Bialetzki et al., 1997). *M. amazonicum* shows an extended type of larval development with ca. 9-11 free-swimming stages (Guest, 1979; Magalhães, 1985), requiring at optimal rearing conditions (30°C, 10‰ salinity, Valenti, unpubl. data) about 18-21 days from hatching to metamorphosis. The larval physiology of *M. amazonicum* has experimentally been studied by only a few authors. McNamara et al., (1983) and Zanders and Rodriguez (1992) described effects of temperature and salinity on respiration rates of the two earliest zoeal stages, and Moreira et al., (1986) studied effects of salinity on the upper thermal limits for their survival. In these investigations, however, changes during the moulting cycle have not been considered. The present study provides the first information about the course of the moulting cycle in larval Amazon River prawn, *M. amazonicum*.

## 2. Material and methods

Ovigerous females of *M. amazonicum* were obtained from the Aquaculture Center (CAUNESP) broodstocks, at the São Paulo State University, Brazil. They originated from the northeastern Brazilian state of Para (01°13'S 48°17'W). Prawns were transported to the Helgoland Marine Biological Laboratory, Germany, where they were maintained in individual aquaria (30L) with freshwater, aeration, constant temperature 29°C, and artificial 12:12h daylight:darkness regime. The adults were fed daily with fish meat and grated carrots.

After hatching, ca. 500 larvae were mass-reared in gently aerated 1L glass beakers filled with brackish water (10‰ salinity) kept at two constant temperatures (21°C and 29°C), under the same conditions of light. The larvae were fed daily with freshly hatched *Artemia* sp nauplii, the water was changed, and dead individuals were removed. The successive larval stages (in order to avoid confusion with the term “stage” in the context of the moulting cycle, from hereon referred to as larval “instars”, for review of terminology, see Anger, 2001) were microscopically identified using the morphological description provided by Guest (1979). When moults occurred, the larvae were separated according to their instar, so that each rearing beaker contained exclusively individuals being in the same instar and with the same age within a given moulting cycle.

Samples of 3-5 larvae were taken twice daily from the cultures and examined under a BH2-NIC (Olympus) photo-microscope equipped with differential interference contrast. Changes in the epidermal structures were recorded and photographically documented. We used primarily the larval telson as the reference region, and additionally the uropods in advanced stages (from zoea III) (cf. Anger, 1983, 2001), because these body parts are thin and transparent, so that changes occurring in the

epidermis and cuticle could easily be seen. As all larval stages presented in principle the same sequence of anatomical modifications, we describe in this paper the moulting cycles only for the two earliest instars (zoea I-III).

### **3. Results**

The duration of development through successive larval instars depended greatly on temperature. While the average time for each moulting cycle varied from ca. 3-4 days at 21°C, only 1-2 days were required at 29°C. Although late instars (from zoea V) tended to develop at a slightly slower speed than the earliest ones, the duration of the moulting cycle was generally too short to allow for a high temporal resolution of sampling and microscopical examination. As another problem preventing a precise moult-staging, the larval integument was found to be thin and little structured, and morphological changes were often indefinite. Thus, transitions between stages and substages could not be identified with a comparably high accuracy and a precise timing as in Drach's classical system (elaborated for adult crabs with thick and multi-layered structures). We therefore decided to combine the principal stages of postmoult (A-B) and intermoult (C), where gradual rather than abrupt changes occurred. After the combined stages A-C, premoult (stage D) and the moulting process (E) could be identified. Although our description presents also some details including those of the important premoult substages  $D_0$  and  $D_1$ , a precise schedule of their timing cannot be provided.

The course of the moulting cycle was similar in all successive larval instars. We therefore show here only some typical integumental changes, using micrographs taken from the telson and uropods of the first three zoeal instars as examples (see Figs. 1, 2).

The process of ecdysis is documented with photos showing the moult from the zoea I to the zoea II instar.

### 3.1. Stages A-C combined (postmoult-intermoult)

*Stage A (early postmoult).* Immediately after hatching from the egg (zoea I) or moulting (later instars), the cuticle is thin and wrinkled, and the larval body is completely soft (ascertained by probing with a delicate forceps). Microscopical examination revealed a spongy epidermal tissue structure with numerous large and irregularly shaped lacunar spaces. Figures 1A and 2A illustrate this condition for the telson of the zoeal instars I and II, respectively. Within a few minutes during and after ecdysis, the larva takes up water, so that the integument is rapidly stretched, previously invaginated setae and appendages are evaginated, wrinkles disappear, and the body attains its final size and shape (cf. below, Figs. 3D, E).

*Stage B (late postmoult).* The cuticle becomes more rigid, and the epidermal tissues begin to concentrate along the inner surface of the cuticle (as examples, see telson of the zoea II, Fig. 2B, uropod of the zoea III, Fig. 2D).

*Stage C (intermoult).* Both the reinforcement of the cuticle and the condensation of the epidermis tissues continue to a maximum, accompanied by a gradual reduction of lacunar spaces and conspicuous tissue growth (see zoea I, Fig. 1B).

The average duration of stages A-C combined was ca. 2.5d at 21°C and slightly less than 1d at 29°C, representing ca. 40-50% of the total time of the moulting cycle.

### 3.2. Stage D (pre-moult)

*Substage D<sub>0</sub> (early pre-moult).* Substage D<sub>0</sub>, the onset of the pre-moult period, is well characterized by the beginning retraction of the epidermal matrix from the cuticle. This conspicuous process (termed apolysis, Jenkin and Hinton, 1966) is first visible at

the bases of the terminal setae of the telson (Fig. 1C), proceeding only later through other body regions and appendages.

*Substage D<sub>1</sub> (intermediate premoult)*. The beginning of this substage is indicated by the occurrence of epidermal infoldings or invaginations (Figs. 1D, 2C, 2E). This internal enlargement of the tissue surface is a prerequisite for morphological reconstruction processes (morphogenesis) including the lengthening of already existing setae (setal growth), the formation of new setae (setogenesis, Fig. 2F), and the appearance of other completely new organs (organogenesis). During these sub-surface reconstruction processes in the epidermal tissues, the cuticle (i.e. the external shape and size of the larval body) does not change. As an example, Figure 2C shows the formation of uropods. These appendages appear externally only after the moult to the zoea III instar (Fig. 2D), but they can already be seen inside the telson of the zoea II instar (Fig. 2C).

*Substages D<sub>2-4</sub> (late premoult)*. When morphogenesis has been completed, a very thin new cuticle is secreted on the surface of new epidermal structures such as setae and appendages, while the gap between the old and the new cuticle increases (Fig. 2F). No resorption processes or other changes in the cuticular layers, as observed in adult crabs (Drach, 1939), were found during this period, so that no further distinction between substages D<sub>2-4</sub> of Drach's classification system was possible. The average duration of complete Stage D was ca 3-4 days at 21°C and about 1d at 29°C, corresponding to ca. 50-60% of the moult-cycle duration.

### 3.3. Stage E (ecdysis)

The moulting process (ecdysis) takes normally at most a few minutes. It is initiated by larval pumping movements, followed by a dorsal rupture of the cuticle



between the cephalothorax and the pleon. First, the telson is retracted from the old exoskeleton (Figs. 3A-D). Subsequently, the larva sheds also the anterior parts of its exuvia, so that a new larval instar appears, and a new moulting cycle begins (Fig. 3E).

#### 4. Discussion

The course of epidermal and cuticular changes observed during moulting cycles of larval Amazon River prawn, *Macrobrachium amazonicum*, is generally similar to those previously described for other decapod crustacean larvae (Freeman and Costlow, 1980; McNamara et al., 1980; Anger, 1983, 1984). Comparison with adult life-history stages (e.g. Drach, 1939; Peebles, 1977; Dexter, 1981) is difficult, because technical constraints such as very short moult-cycle duration and a thin and little structured larval integument do not allow for a comparably high resolution in the description of larval moulting cycles (for review, see Anger, 2001).

For practical purposes in applied research including crustacean aquaculture, however, the lack of numerous precisely defined substages may not be a serious problem, as an identification of a few major moult stages should be sufficient to allow for a separation of fairly homogeneous materials taken from large cultures, e.g. for subsequent experiments, physiological measurements, or biochemical analyses. Our study may therefore provide a simple and practical guide for the identification of the major moult stages in larval *M. amazonicum*, aiding to the selection of materials for studies of larval metabolism, growth, biochemical composition, or other relevant aspects of crustacean aquaculture.

Various structures can be used for moult-staging in larval decapods. Freeman and Costlow (1980) used for this purpose the antennae of larval mud crab (*Rhithropanopeus harrisi*), while Anger (1983, 1984) used mainly the telson, but additionally also pereopods, antennae, and dorsal spines, to describe changes in the

integumental structures of larval spider crab (*Hyas araneus*). In early juvenile crayfish (*Parastacoides tasmanicus*, *Astacus leptodactylus*) and penaeid prawns (*Penaeus esculentus*), the uropods were most commonly used, at least in addition to the telson (Mills and Lake, 1975; Herp and Bellon-Humbert, 1978; Smith and Dall, 1985). In the present work, the description of the moulting stages was principally based on microscopical examination of the larval telson, because this body part is plane, thin and transparent, so that changes in the epidermal tissues can be easily observed. Similar observations were made in a study by McNamara et al., (1980) with larvae of a congener, the shrimp *Macrobrachium olfersii*. The uropods appear in these palaemonid species only from the zoea III instar, providing an additional reference region for studies of later larval development.

Tayamen and Brown (1999) proposed criteria for the evaluation of larval quality in *Macrobrachium rosenbergii*, based on characteristics of body coloration, setation, muscles, swimming behaviour, etc. An opaque appearance of the muscles in the pleon, for example, along with sluggish behaviour, represent a poor condition. However, the same characteristics occur also during early postmoult, when the larval body is limp and the larvae tend to sink towards the bottom due to weak swimming activity. A “healthy” colour and behaviour, e.g. a positive response to light, are rapidly recovered during later postmoult and intermoult. This shows that the moulting cycle must be taken into consideration when the health condition of shrimp larvae is evaluated.

The same applies to larval feeding and growth, which cease from late premoult through ecdysis and early premoult, re-starting only in late postmoult or intermoult (Anger, 2001). Stage C seems to be a period without dramatic structural and metabolic changes, characterized only by substantial tissue growth. During the premoult period, by contrast, the rates of growth and feeding decrease, while numerous structural

changes occur in the integument, and the mass-specific metabolic rate increases (Anger, 2001). As stage C is the metabolically and morphologically most stable period within the moulting cycle, physiological experiments or biochemical analyses comparing successive larval instars of a species, or equivalent instars of different species, should preferably be conducted during this phase. In the larvae of *M. amazonicum*, the most suitable reference point for such comparisons may thus be found at ca. 30-40% of total moult-cycle duration, i.e. near the end of stage C and shortly before the transition to substage D<sub>0</sub>, when apolysis occurs.

Besides intrinsic hormonal control factors, extrinsic variables such as temperature, food, water chemistry, or photoperiod may affect the moulting cycle in crustaceans (Chang, 1995; Ismael and New, 2000; Kulum and Ku, 2005). Also in the present study, it was accelerated by higher temperature (29° vs. 21°C), shortening the absolute time spans for each stage of the moulting cycle. Preliminary observations suggested, however, that variation in temperature might change also the temporal proportions of individual stages within the moulting cycle, with an apparently increasing duration of stages A-C combined in relation to stage D. However, further studies with a substantially enhanced temporal resolution of sampling and microscopical observation are necessary to evaluate the extent and significance of such an effect. Also, successive larval instars might differ in their response to environmental factors, which requires more detailed studies with various instars exposed to differential experimental conditions.

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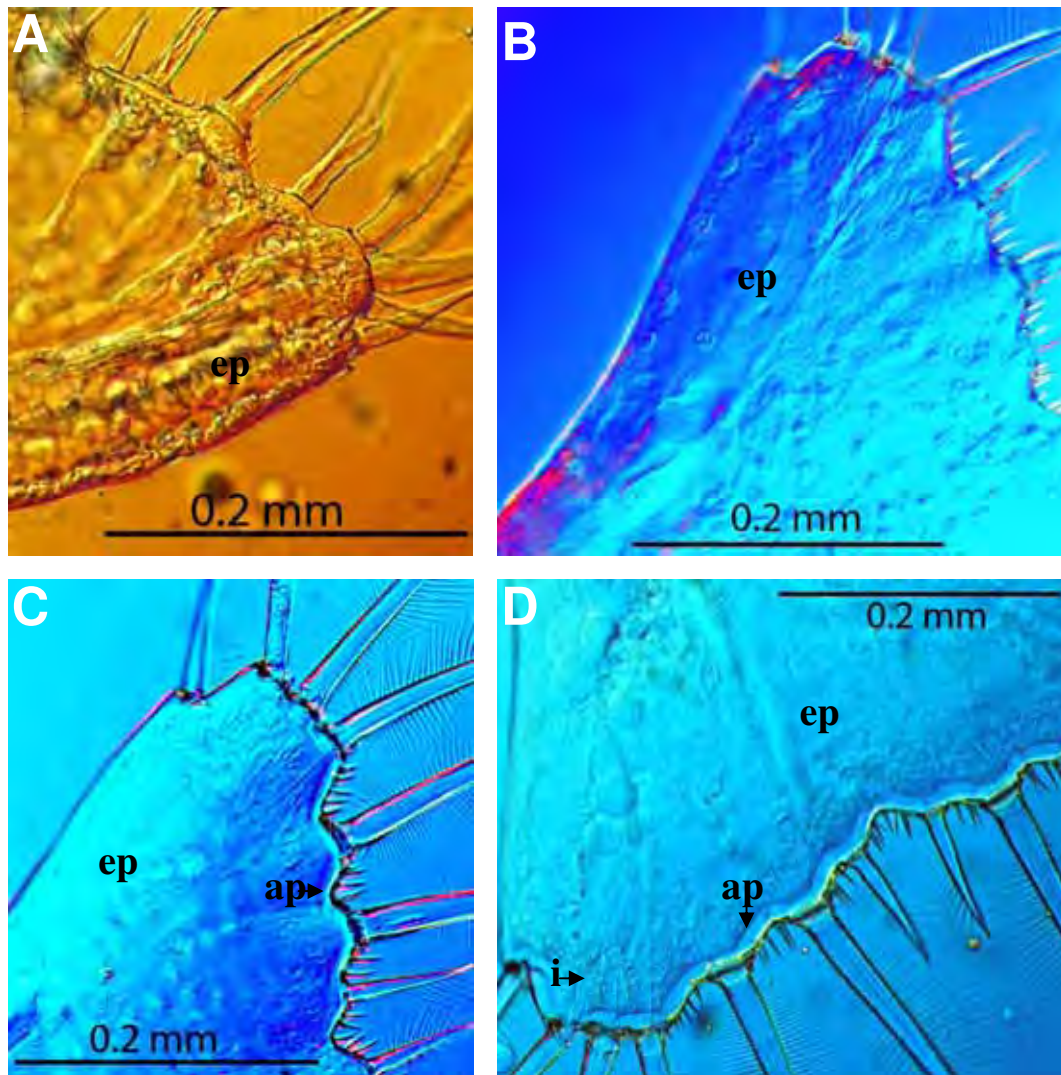
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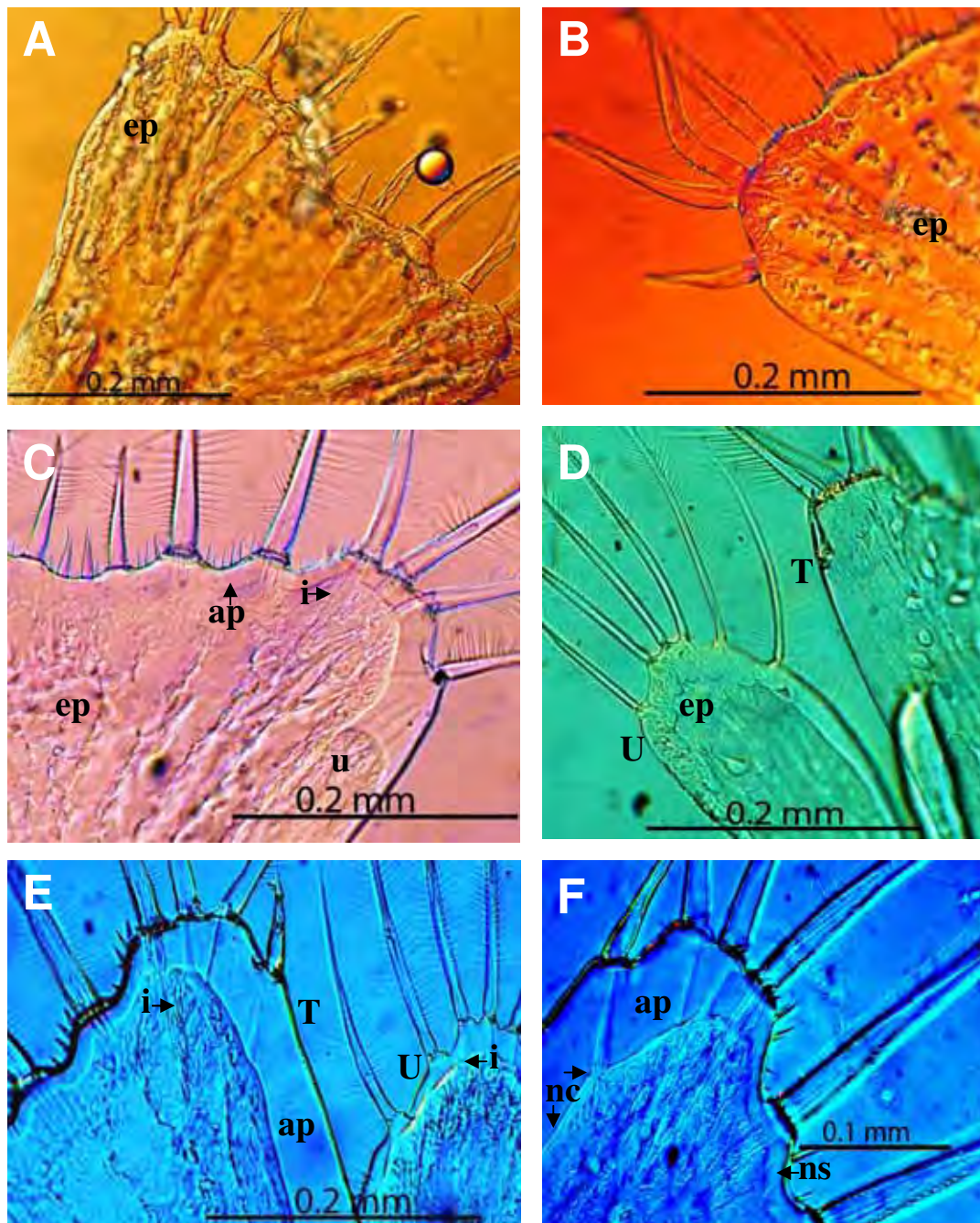


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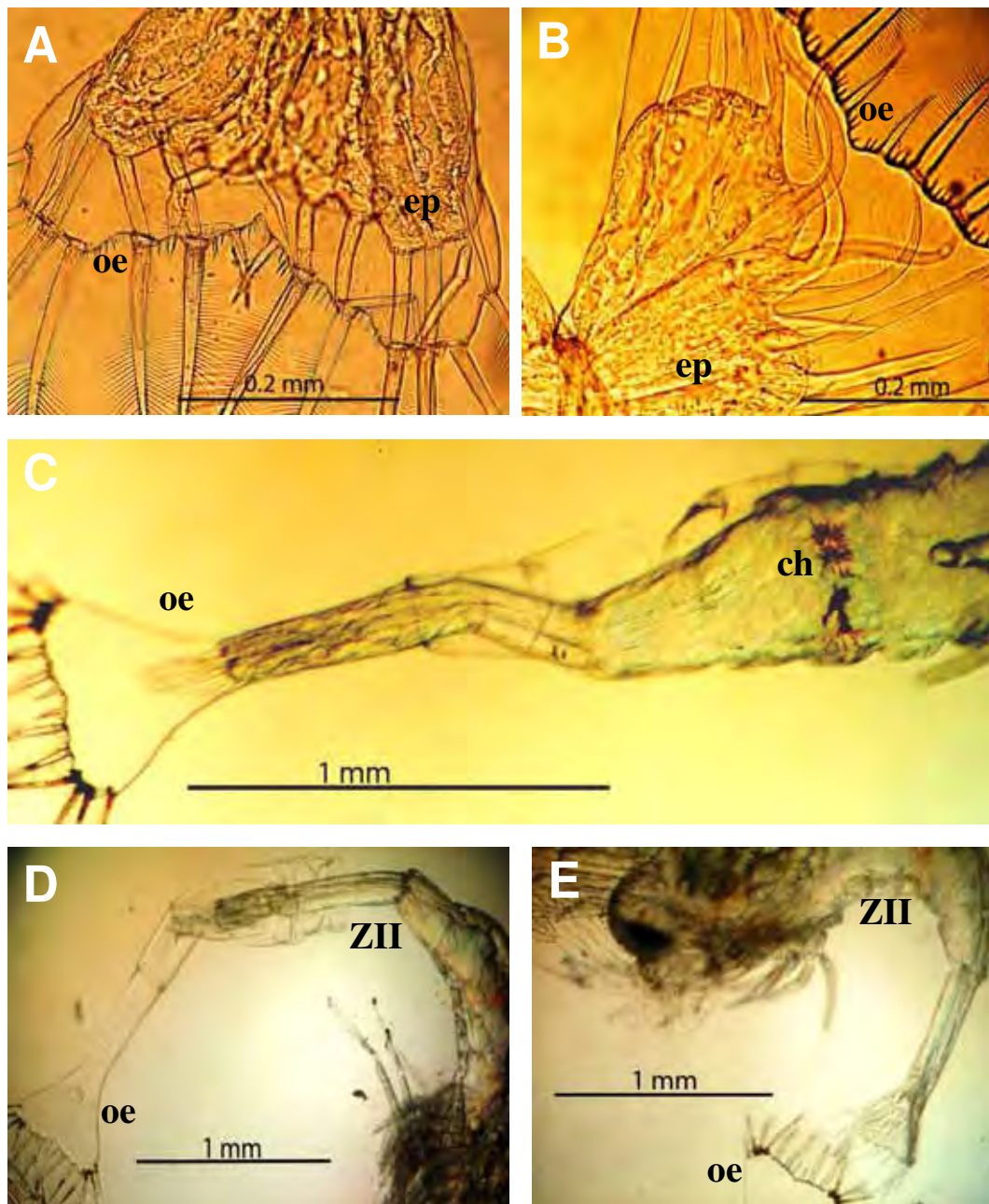
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**Figure 1** – Telson of *Macrobrachium amazonicum*, larval instar zoea I: A. Early postmolt (stage A), with spongy epidermal tissue structure, large hemolymph-filled spaces (lacunae); B. intermolt (stage C), with epidermal growth, tissue concentration along the inner surface of the cuticle, reduced lacunar spaces; C. early premolt (stage D, substage D0), with beginning epidermal retraction from the cuticle (apolysis); D. intermediate premolt (substage D1), with advanced apolysis, beginning epidermal invaginations at the setal bases; ap, apolysis; ep, epidermis; i, invagination.



**Figure 2** - *Macrobrachium amazonicum*, larval instars zoea II (A-C) and zoea III (D-F): A. zoea II, early postmolt (stage A); B. zoea II, late postmolt (stage B), with advancing tissue concentration along the inner surface of the cuticle; C. zoea II, intermediate premolt (substage D1), with uropod formation (u) inside the telson of the zoea II; D. zoea III, postmolt (stage B), with telson (T) and newly appearing uropod (U); E. zoea III, intermediate premolt (substage D1); F. zoea III, late premolt (substages D2-4), with formation of a thin new cuticle (nc); ap, apolysis; ep, epidermis; i, invagination, ns, new setae.



**Figure 3** - *Macrobrachium amazonicum*, moulting (ecdysis) of larval instar zoea I to zoea II: letter A until C., retraction of the telson from the old exoskeleton (oe), epiderme (ep); chromatophore (ch); letter D, shedding of the exuvia, appearance of the zoea II (ZII); letter E, zoea II with shed exuvia; ch, typical dorsal chromatophore on larval pleon.

### CAPÍTULO 3 (Aquaculture Research)

**Varição ontogenética do metabolismo durante os estágios iniciais do camarão-da-amazônia *Macrobrachium amazonicum* (Heller, 1862) (Crustacea, Decapoda, Palaemonidae)**

**Ontogenetic variation in metabolism during the early life stages of the Amazon River prawn *Macrobrachium amazonicum* (Heller, 1862) (Crustacea, Decapoda, Palaemonidae)**

#### **Resumo**

O peso seco, o consumo de oxigênio e a taxa de excreção de amônia-N foram determinados em embriões, larvas (zoea=Z) (ZI a ZIX) e pós-larvas (PL) com 1, 7 e 14 dias após a metamorfose (PL1, PL7 e PL14) em *Macrobrachium amazonicum*. Animais em estágios de pós-muda/intermuda (A/C) foram classificados conforme o estágio de desenvolvimento, e colocados nas câmaras respirométricas (ca. 30mL) por 2h para quantificar as taxas metabólicas. Após esse período, as análises foram realizadas com titulação de Winkler para o oxigênio e método de Koroleff para a determinação da amônia-N. As taxas metabólicas foram expressas como individual e peso-específico. A relação atômica O:N foi calculada. As taxas individuais do consumo de oxigênio e excreção de amônia-N aumentaram durante o desenvolvimento larval. Os valores médios do consumo de oxigênio variaram de  $0,10 \pm 0,02$  a  $4,02 \pm 1,10 \mu\text{g} \cdot \text{ind}^{-1} \cdot \text{h}$  em embrião e PL14, respectivamente e não diferiram de embrião a ZIV e ZV a ZIX ( $P > 0,05$ ). A taxa de consumo de oxigênio foi significativamente maior em zoea e embrião ( $P < 0,05$ ). A taxa de excreção de amônia individual variou de  $0,0090 \pm 0,0039$  em embrião a  $1,0413 \pm 0,2492 \mu\text{gNH}_3\text{-N} \cdot \text{ind}^{-1} \cdot \text{h}$  em PL14 e não diferiram entre embrião-

ZIV e ZV-ZIX ( $P>0,05$ ), mas diferem entre PL1-PL14 ( $P<0,05$ ). Os maiores incrementos na excreção de amônia individual foram observados entre ZIV-ZV, ZIX-PL1 e PL7-PL14. As taxas de excreção peso-específico apresentaram dois grupos, embrião-ZII ( $P>0,05$ ) e ZIII-PL14 ( $P>0,05$ ). O menor valor encontrado foi em embrião ( $0,17\pm 0,07\text{mgNH}_3\text{-N.gPS}^{-1}\text{.h}$ ) e os maiores foram observados em ZV e PL1 ( $0,65\pm 0,25$  e  $0,64\pm 0,27\text{mgNH}_3\text{-N.gPS}^{-1}\text{.h}$ , respectivamente). Foram observados baixos valores de O:N, variando de 3,0 a 10,0, mostrando que em *M. amazonicum* ocorre o predomínio do metabolismo de proteína. O valor de  $b$  obtido da relação consumo individual de oxigênio/peso seco na análise de regressão foi 0,8 e foi significativamente menor que 1 ( $P<0,05$ ) e da excreção individual/peso seco foi 1,1, e não diferiu de 1 ( $P>0,05$ ). A associação com as mudanças morfológicas e comportamentais, tais como a atividade natatória, formação de novos apêndices (urópodos, pleópodos, pereópodos, etc.), o tamanho do corpo, o decréscimo na relação superfície:volume, a estratégia de alimentação e a transição de um estágio de zoea planctônico a pós-larva bentônico é discutida.

Palavras-chaves: Larva, *Macrobrachium amazonicum*, Metabolismo, Excreção de nitrogênio, Consumo de oxigênio, Pós-larva.

## Abstract

Dry weight, oxygen consumption and total ammonia-N excretion rate were determined in embryos, larvae (zoea=Z) (ZI to ZIX) and post-larvae (PL) with 1, 7 and 14 days after metamorphosis (PL1, PL7 and PL14) of *Macrobrachium amazonicum*. Animals in postmolt-intermolt (A-C) stages were sorted according to their developmental stages, and placed into respirometric chambers (*ca.* 30mL) for 2h to quantify metabolic rates. After this period, analyses were carried out in end-point samples by Winkler's titration for oxygen and Koroleff's method for ammonia-N determination. Metabolic rates were expressed both as individual and dry mass-specific oxygen consumption and ammonia-N excretion rates. Atomic O:N ratio was also calculated. Individual rates of oxygen consumption and ammonia-N excretion increased throughout larval development. Average values of oxygen consumption varied from  $0.10 \pm 0.02$  to  $4.02 \pm 1.10 \mu\text{g} \cdot \text{ind}^{-1} \cdot \text{h}$  in embryo and PL14, respectively and did not differ from embryo to ZIV and from ZV to ZIX ( $P > 0.05$ ). Post-larval oxygen consumption rates were significantly higher than in zoea and embryo ( $P < 0.05$ ). Individual ammonia-N excretion rates varied from  $0.0090 \pm 0.0039$  in embryo to  $1.0413 \pm 0.2492 \mu\text{gNH}_3\text{-N} \cdot \text{ind}^{-1} \cdot \text{h}$  in PL14 and did not differ among embryo-ZIV and ZV-ZIX ( $P > 0.05$ ), but differ among PL1-PL14 ( $P < 0.05$ ). The highest increments in individual ammonia-N excretion were observed between ZIV-ZV, ZIX-PL1 and PL7-PL14. Weight-specific excretion rates presented two groups, Embryo-ZII ( $P > 0.05$ ) and ZIII-PL14 ( $P > 0.05$ ). The lowest value was found in embryo ( $0.17 \pm 0.07 \text{mgNH}_3\text{-N} \cdot \text{gDW}^{-1} \cdot \text{h}$ ) and the maximum value was observed in ZV and PL1 ( $0.65 \pm 0.25$  and  $0.64 \pm 0.27 \text{mgNH}_3\text{-N} \cdot \text{gDW}^{-1} \cdot \text{h}$ , respectively). Low O:N values were observed, ranging from 3.0 to 10.0, which showed that *M. amazonicum* has a protein dominated metabolism. The *b* value obtained in the individual oxygen consumption *versus* dry weight regression analysis



was 0.8 and was significantly lower than 1 ( $P < 0.05$ ) and individual excretion *versus* dry weight was 1.1, which did not differ from 1 ( $P > 0.05$ ). The association with morphological and behavioral changes, such as swimming activity, new appendages (uropods, pleopods, pereopods, etc.) formation, body size, a decrease in the surface:volume ratio, feeding strategy and transition from a planktonic zoea stage to a benthic post-larval stage is discussed.

Keywords: Larvae, *Macrobrachium amazonicum*, Metabolism, Nitrogen excretion, Oxygen consumption, Post-larvae.

## 1. Introduction

The Amazon River prawn *Macrobrachium amazonicum* has a large geographic distribution in South America, occurring in Brazil, Colombia, Venezuela, Peru, Ecuador, Bolivia, Paraguay and Argentina (Holthuis, 1952; Pettovelo, 1996; Bialecki *et al.*, 1997). Some populations live in interior areas very far from the sea and complete life cycle in freshwater. On the other hand, the populations that occur close to the coast are dependent of brackish water for larval development (Kutty *et al.*, 2000). The larvae hatch as a typical free-swimming palaemonid zoea and pass through nine zoeal stages (Guest, 1979). They are planktonic, swimming actively, tail first, upside-down within the water column and exhibit positive phototaxy. After metamorphosis, post-larvae are miniature of adult prawns and swim with the dorsal side uppermost assuming a more benthic lifestyle. Both larvae and post-larvae are mainly omnivorous (Araújo & Valenti, 2005).

*M. amazonicum* presents high potential for aquaculture (New, 2005). Some characteristics, which may contribute to the rearing feasibility, are fast larval development (18-21 days) in recirculating systems ( $30\pm 1^\circ\text{C}$  and 10‰ salinity), and high growth and survival rates in hatchery, nursery and grow-out phases (Valenti, unpubl. data). Furthermore, meat has a firmer texture and a more marked taste when compared with *M. rosenbergii*, hence being better accepted by consumers (Moraes-Riodades & Valenti, 2001).

There are some studies on *M. amazonicum* wild populations (Odinetz-Collart, 1991a,b; Bialecki *et al.*, 1997), male morphotypes (Moraes-Riodades & Valenti, 2004), reproduction (Odinetz-Collart & Rabelo, 1996; Da Silva *et al.*, 2004), hatchery systems (Lobão *et al.*, 1994), salinity in culture systems (Guest, 1979; McNamara *et al.*, 1983; Zanders & Rodriguez, 1992), feeding (Barreto & Soares, 1982; Araújo & Valenti,

2005), economic viability as baits (Hayd, *et al.*, submitted-a). However, studies related with metabolic rates in *M. amazonicum* are few reduced. Favaretto *et al.* (1976) studied the oxygen consumption in adults collected from rivers, McNamara *et al.* (1983) described the effects of salinity on respiratory metabolism, survival and moulting in zoea I and II and Zanders & Rodriguez (1992) evaluated the effect of temperature on respiratory metabolism of *M. amazonicum*. None included the evaluation of oxygen consumption and ammonia-N excretion rates from embryo to post-larvae.

Measuring metabolic rates is essential to assess energy requirements for larval development (Lemos & Phan, 2001). Accordingly the authors emphasized that, oxygen consumption and ammonia excretion are probably the main physiological parameters to be evaluated. In addition, information on metabolism and the processes related to the use of energy throughout the ontogenetic development is necessary to understand the ecologic role of the species and to improve culture practices. It allows the establishment of the adequate density for larvae and post-larvae transportation and to dimension the biofilters used in recirculating hatchery systems. Thus, the objective of this study was to evaluate metabolic rates in early life stages of hatchery reared *M. amazonicum* through the simultaneous measurement of oxygen consumption and ammonia-N excretion rates.

## **2. Material and methods**

### **Experimental animals**

Embryo, larvae and post-larvae of *M. amazonicum* were obtained from broodstock formed by wild animals captured in Northeast Para, Brazil (01°13'25"S 48°17'40"W) in 2001. Females carrying eggs in advanced stages of embryonic development (approximately 2h before hatching) were collected. This phase of egg incubation is characterized by slow movements of females and the presence of visible

eyes inside the transparent eggs. Animals were disinfected in formaldehyde solution at 20ppm for 30min. and then kept in a hatching tank at 70 ind.m<sup>-2</sup> and 8‰ salinity. It was provided with aeration, heating system and artificial substrates. Some females had eggs extracted for embryos metabolism and dry weight determination.

After hatching, larvae were stocked at 100 ind.L<sup>-1</sup> in 120 L cylindrical tanks provided with mechanical and biological filter and artificial heater. Water temperature was kept at 30°C and 10‰ salinity until metamorphosis to post-larvae (PL). Larvae was fed on freshly hatched *Artemia* nauplii in zoea (Z) II to ZIV (3-7 nauplii. mL<sup>-1</sup>.day). After this stage, they were fed on inert diet (25µg.mL<sup>-1</sup>.day) (see Mallasen & Valenti, 1998 for diet composition) plus *Artemia* nauplii (8 to 10 nauplii.mL<sup>-1</sup>.day), added to the tanks twice daily until metamorphosis. Post-larvae were transferred to 1000 L tanks at 5 PL.L<sup>-1</sup> and fed on peletized diet twice a day (3–5g.day<sup>-1</sup>.tank Fri-Ribe Fri-Acqua Camarão 35 LD). In this study, PL1 was the stage immediately after metamorphosis while PL7 and PL14 were post-larvae with 7 and 14 days after metamorphosis, reared in freshwater at 30±1°C.

### **Oxygen consumption and ammonia-N excretion determination**

Cylindrical plastic containers with approximately 30mL sealed with silicon tablets (Lemos & Phan, 2001; Lemos *et al.*, 2003) were used as respirometric chambers to incubate animals for oxygen consumption and ammonia-N excretion measurement. Chambers were individually identified and the exact volume was gravimetrically determined. An orifice of 1.5mm in the center of the cover enabled the elimination of air bubbles from inside the chamber during closure. Respirometers were hermetically closed inside 1000mL beakers filled with water (10‰ salinity) to avoid formation of bubbles inside the containers. After being closed, inside water was isolated from air by

a plastic tablet 1.7cm diameter silicon seal so that the tension between orifice water and the silicon seal could block respirometer inside water and air gas exchange.

Only larvae and post-larvae in the postmolt–intermolt (A-C) (Hayd *et al.*, submitted or see chapter 1) and in the same development stage were used. The number of individuals used inside respirometer was determined according to individual dry mass (Table 1). The biomass:volume ratio (B:V) was calculated by dividing the total dry mass of individuals by the chamber volume and varied from 16.9 to 85.6 $\mu\text{g.mL}^{-1}$  (Table 1). Embryos, ZI to ZIX, PL1, PL7 and PL14 were kept in the sealed respirometric chambers samples for 2h. Seawater and Milli-Q (Millipore) fosh water at 10‰ salinity were used as control respirometer without animals. Samples and controls were kept in water bath at  $30\pm 1^\circ\text{C}$ . After incubation, the tablet was removed, and water was sampled through a plastic canula and stored in glass syringes chemically calibrated (syringes plus plastic nozzles).

Variation in oxygen and ammonia-N nitrogen contents was calculated by the difference between values obtained in sample (with animals) and control (no animals) units. Dissolved oxygen in water at the end of every test was determined by Winkler analysis method adapted to small volumes (Fox & Wingfield, 1938) and was never lower than 80% of saturation. For ammonia-N analysis, the method described by Koroleff (1983) in separate water samples was used. Oxygen consumption and ammonia-N excretion were expressed as individual ( $\mu\text{g.ind.}^{-1}\text{.h}$ ) and dry-mass specific ( $\text{mg.g}^{-1}\text{DW.h}$ ) rates. Salinity effect was corrected using the factor 1.06 (Koroleff, 1983). Atomic O:N (oxygen respired to nitrogen excreted) ratios were calculated by dividing the number of gram-atom of consumed oxygen by the number of gram-atom of nitrogen excreted ( $\text{N.O}^{-1}$ ) in each developmental stage (Mayzaud & Conover, 1988).

Individual dry weight (DW) was determined by a groups of 10 (embryo and ZIX) or 6 (PL) individuals gently rinsed with distilled water, dried with filter paper and separated prior to weight determination with eight replicates. After 48h at 70°C, dry samples were weighed on a Mettler Toledo AT21 analytical balance, at the nearest 1µg.

### **Statistical analysis**

The results were subjected to analyses of detection and exclusion of outliers (Statistica software, v. 6.0) with coefficient 1.5. Total excluded data was always lower than 20% of data obtained for each stage (Statistica Software, v.6). The final number of replicates is in Table 2. Normality was tested using the Shapiro-Wilk test and Homocedasticity by Levene's (using SAS 9.0 software). Differences among means were tested by one-way ANOVA followed by Duncan's multicomparison test. Data of the metabolic rates (oxygen consumption and ammonia excretion, in µg.ind.<sup>-1</sup>.h) and dry weight (µg) were logarithmically adjusted by regression analysis (Statistica Software, v.6). Slopes were compared to 1 using a t-test according to Zar (1999). Differences were considered significant at  $P < 0.05$ .

### **3. Results**

Individual dry weight (DW) increased throughout the ontogenetic development (Table 1) and varied significantly in different ages and developmental stages ( $P < 0.05$ ). The highest increases in subsequent stages were verified between the ZIV to ZV (118.03%) and PL1 to PL7 (99.52%), while the lowest value was found between ZIX and PL1 (5.41%).

Individual oxygen consumption rate (VO<sub>2</sub>) showed four groups during the developmental stage, Embryo–ZIV, ZV–ZIX, PL1–PL7 and PL14, and into these groups there was no significant difference ( $P > 0.05$ ) (Table 2). Embryo presented the

lowest value ( $0.10 \pm 0.02 \mu\text{g} \cdot \text{ind}^{-1} \cdot \text{h}$ ) while PL14 presented the highest one ( $4.02 \pm 1.10 \mu\text{g} \cdot \text{ind}^{-1} \cdot \text{h}$ ) and differ to all others stages ( $P < 0.05$ ) (Table 2). The weight-specific oxygen consumption ( $\text{QO}_2$ ) varied during development and showed four groups, Embryo, ZI–ZIV, ZV–ZVI, ZVII–PL14, and into these intervals there was no significant difference ( $P > 0.05$ ), except in PL1 (Fig. 1A). Higher mean values were found among ZI–ZIV and it decreased in subsequent stages, except in PL1 (Fig. 1A).

Individual ammonia-N excretion did not differ among embryo–ZIV and ZV–ZIX ( $P > 0.05$ ), but differ among PL1–PL14 ( $P < 0.05$ ) (Table 2). The minimum observed value was  $0.0090 \pm 0.0039 \mu\text{gNH}_3\text{-N} \cdot \text{ind}^{-1} \cdot \text{h}$  in embryo, and the maximum,  $1.0413 \pm 0.2492 \mu\text{gNH}_3\text{-N} \cdot \text{ind}^{-1} \cdot \text{h}$  in PL14 (Table 2). The highest increments in individual ammonia-N excretion were observed between ZIV–ZV, ZIX–PL1 and PL7–PL14. Weight-specific excretion rates presented two groups, Embryo–ZII ( $P > 0.05$ ), ZIII–PL14 ( $P > 0.05$ ) (Fig. 1B). Stages ZIII, ZV and PL1, presented high standard deviation (SD). Similarly to oxygen consumption, the lowest value was found in embryo ( $0.17 \pm 0.07 \text{mgNH}_3\text{-N} \cdot \text{gDW}^{-1} \cdot \text{h}$ ), however the maximum value was observed in ZV and PL1 ( $0.65 \pm 0.25$  and  $0.64 \pm 0.27 \text{mgNH}_3\text{-N} \cdot \text{gDW}^{-1} \cdot \text{h}$ , respectively) (Fig. 1B). Atomic O:N ratio were generally low, ranging from 3 in ZVII and PL14 to 10 in embryo and ZI (Table 2).

Relationships between metabolic rates and dry weight ( $\mu\text{g}$ ) showed high coefficients of determination ( $r^2 > 0.8$ ). The slope obtained for individual oxygen consumption/dry weight relationship was 0.8 and was significantly lower than 1 ( $P < 0.05$ ), while the  $b$  value for individual excretion/dry weight relationship was 1.1, which did not differ significantly from 1 (Table 3).

#### 4. Discussion

The weight of *M. amazonicum* increases during development stages. This pattern was also observed for *Macrobrachium rosenbergii* larvae (Stephenson & Knight, 1980; Agard, 1999; Ismael *et al.* 2001), and for other caridean larvae, such as *Sesarma rectum* (Anger & Moreira, 2004), *Crangon crangon* and *Crangon allmanni* (Criales & Anger, 1986). The weight increase during larval and later developmental stages clearly influences the weight-metabolic rate (Vernberg *et al.* 1981), since larvae show an increase in individual oxygen consumption rate ( $VO_2$ ) and a decrease in weight-specific oxygen consumption ( $QO_2$ ) (Anger, 2001). The  $VO_2$  values increased significantly in successive developmental stage of *M. amazonicum* ( $P < 0.05$ ). This increase may be related with the different kinds the swimming activities, energy lost and with the gain weight during the course of development.

The  $QO_2$  values varied significantly through *M. amazonicum* ontogenetic development ( $P < 0.05$ ). The highest values occurred in the first stages (ZI–ZIV) and the lowest in the latter ones, except in PL1. High  $QO_2$  at ZI–ZIV may be related to the noticeable natatorium activities due to lower size, which spend more energy for maintenance in water column, and thus, consuming more oxygen. The lower values in the last stages may be due to profound developmental changes (Anger, 2001), a disproportionate increase of metabolically inactive tissues, such as skeletal materials and fat reserves and by overlaying external ecological factors (Anger, 2001) and a decrease in relation surface:volume. Besides, in this period larvae show a bigger size and loose less energy for maintaining in water column during planctonic phase, and after the transition to benthic phase after metamorphosis in post-larvae (PL1). Decreasing in  $QO_2$  during the last larval stages was also observed in *M. rosenbergii* larvae (Agard, 1999) in crabs *Chamagnathus granulate* (Ismael *et al.* 1997) and *Hyas*



*araneus* (Anger & Jacobi, 1985) and in the penaeidae shrimp *Farfantepenaeus paulensis* (Lemos & Phan, 2001). The higher  $QO_2$  rates in PL1 may be due to osmorregulatory response and possible stress due to little time in acclimation at respirometer. This physiological stress may indicate PL1 needs longer acclimation time when transferred from brackish water to freshwater in nursery tanks. According to Agard (1999) *Macrobrachium rosenbergii* larvae were moved by 1‰ salinity per hour and Anger (pers. com.) suggested transferring from 10‰ salinity for 5‰, but staying in this salinity for 4h and then transferring for lower salinity during 2 days to avoid the mortality in subsequent stages.

Individual ammonia-N excretion rates increased during larval development except in ZVI and ZVIII. This fact may be related with the higher larval variability observed in larvae started in ZV. Present results showed three phases in excretion rates, embryo–ZIV, ZV–ZIX and PL1–PL14. In the first phase larvae uses internal energy reserves from yolk (Embryo–ZII) and after ZII a small quantity of the exogenous food. However, there is yolk until ZIV (Araújo and Valenti, submitted). Therefore, larvae present little dependence on exogenous feed. In the second phase (ZV–ZIX) larvae started fed on inert diet plus *Artemia* nauplii and sometimes cannibalistic behavior were observed in the tanks (pers. observ.). Thus, exogenous feed is essential. In the last phase (PL1–PL14) there is an increase in individual ammonia excretion rates due to an increase in larvae weight and consequently in the ingested food. In addition, there are changes in the animal feeding strategy and habitat, assuming a more benthic lifestyle and omnivorous habit. Weight-specific excretion rates also varied throughout developmental stages ( $P < 0.05$ ). However, stages ZIII, ZV and PL1 presented higher standard deviation (SD). In ZIII and ZV this fact may be associated with the beginning of feeding on the *Artemia* nauplii and inert food, respectively. On the other hand, in PL1

may be related with the different life strategies after metamorphosis when larvae changed the planktonic to benthonic habit and the fast transference in ambient of 10‰ salinity to freshwater, described above. Likewise, increasing in weight-specific ammonia-nitrogen excretion was observed in freshwater prawn *M. rosenbergii* larvae when occurred a decreasing in salinity (Agard, 1999). Thus, our results suggested longer time for acclimation when PL1 is transferred from hatchery to the nursery freshwater tanks. Nevertheless, decrease in metabolic rates during the later stages has been reported for crustaceans (Chu & Ovsianico-Koulikowsky, 1994; Agard, 1999; Lemos & Phan, 2001).

Information about metabolic rates is very important to aquaculture. This may help for determination of densities during transportation of larvae and post-larvae and for dimensioning the biofilter in recirculating systems. Our data showed the mean values of the  $0.3\mu\text{g.larva}^{-1}.\text{h}$  for ammonia-N excretion, this represent a load of about  $7.2\mu\text{g.larva}^{-1}.\text{dia}$ , indicating consequently a total ammonia excretion of  $7,200\mu\text{g.dia}^{-1}$  for 1000 larvae. Thus, our results may be used for future calculus of larvae and early post-larvae to transport and biofilter dimensioning of *M. amazonicum*. Biofilter volume of the larval culture tanks has varied from 4 to 20% (Valenti *et al.* 1998) related with size of hatchery tank. This size was based on the daily maximum expected ammonia-nitrogen load in the larval culture system of *M. rosenbergii* and the bacterial carrying capacity of the filter with crushed coral media being used (Valenti & Daniels, 2000). Daniels *et al.*, (1992) showed an example for calculating the amount of crushed coral needed into the biofilters based on empirically data of  $30\mu\text{g.larva}^{-1}.\text{dia}$  (Valenti & Daniels, 2000). Our data indicated that for *M. amazonicum* ammonia load is lower than the recommended for *M. rosenbergii* and therefore biofilters may be lower too.

The O:N ratio is accepted as indicator of the metabolism substrate for energy production (Anger, 2001). The index varies with the N content of the diet, reflecting the biochemical composition of the used energy reserves (Mayzaud & Conover, 1988). O:N ratio in the range 3 and 16 indicates predominant protein catabolism, while higher values show an increasing utilization of lipids and/or carbohydrates. Present results showed O:N values, ranging from 3 to 10 indicating a protein dominated metabolism. The decrease trend in O:N of *M. amazonicum* from ZIII on may be associated with the exhaustion of the lipid reserve (yolk) in the first zoea stages. This pattern was also observed for early larvae of the palaemonid prawn *M. rosenbergii* (Agard, 1999) and penaeidae shrimps *Metapenaeus ensis* (Chu & Ovsianico-Koulikowsky, 1994).

The general relationship between organism size and metabolic rates is described by the simple allometric equation,  $M = a W^b$  (Bertalanffy, 1957), where  $M$  is the metabolic rate per unit time,  $W$  is the body weight, and  $a$  and  $b$  are constants. This equation compares individuals of the same species at different ages or sizes. Bertalanffy's classification showed three metabolic types according with relationship between metabolic rate and body size. In the first, metabolic rate is proportional to the surface or the  $2/3$  power of the body mass ( $b=0.67$ ). In second, metabolic rate is proportional to the body mass ( $b=1$ ) and in the last type, metabolic rate is intermediate between proportionality body mass and surface ( $0.67 < b < 1$ ). Thus, the mass exponent  $b$  generally varies between ca. 0.67 and 1.0, indicating that the metabolic rate is proportional to the animal's body surface area or to its volume. Variation in  $b$  may be caused by changes in the relation between body shape and volume, in the proportions of living protoplasm and metabolically inert components of biomass (Anger, 2001). In the present study, the value of  $b$  in oxygen consumption logarithmic regression analysis was 0.8 below 1 ( $P < 0.05$ ), and the parameter represents the respiration rate of a unit animal

indicating that the increase in oxygen consumption rate was lower than growth in the studied period. This value may indicate favorable conditions to larval development (Lemos & Phan, 2001). On the other hand, the regression for ammonia excretion showed that metabolic rate is proportional to the body mass during the development, because the values are close to 1. The value of  $b < 1$  has been also registered in other decapod, such as 0.904 for *M. rosenbergii* larvae (Stephenson & Knight, 1980) and 0.628 for juveniles (Nelson *et al.* 1977), 0.06 for *Pagurus criniticornis* larvae (Vernberg *et al.* 1981) and 0.668 for adults (Shumway, 1978), 0.67 for *Menippe mercenaria* larvae (Mootz & Epifanio, 1974) and 0.697 for penaeid shrimp *F. paulensis* in early stages (Lemos & Phan, 2001).

Results obtained in the present study may be useful in developing and optimizing rearing techniques of *M. amazonicum*, such as the determination of the proportions biofilter:rearing tank size, aeration volume and stocking density in culture tanks or in transport bags. Further complementary metabolic studies, such as elemental and biochemical composition, activity of digestive enzymes and energy content should be performed to elucidate the adaptative value of the metabolic changes during larval development and to subsidize the formulation of a suitable diet for larvae rearing.

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**Table 1** – Age, dry weight (DW), number of individuals, and biomass:volume ratio in respirometers used to determine oxygen consumption and ammonia-N excretion rates during early life stages of *Macrobrachium amazonicum* at  $30\pm 1^\circ\text{C}$  and 10‰ salinity. Embryo, zoea I-IX=larval stages, PL1=post-larvae, PL7 and PL14=post-larvae with 7 and 14 days after metamorphosis, respectively. Results are expressed as mean values $\pm$ SD. Number of replicates=10.

<b>Stage</b>	<b>Age <sup>1</sup></b>	<b>DW (<math>\mu\text{g}</math>)</b>	<b>Individuals/ respirometer</b>	<b>Biomass:volume (<math>\mu\text{g.mL}^{-1}</math>)</b>
<b>Embryo</b>	-0.1	71.7 $\pm$ 0.6	32	76.5
<b>Zoea I</b>	0.5	58.6 $\pm$ 0.3	12	23.4
<b>Zoea II</b>	2	63.7 $\pm$ 0.6	12	25.5
<b>Zoea III</b>	3	84.3 $\pm$ 1.2	6	16.9
<b>Zoea IV</b>	5	118.3 $\pm$ 2.9	6	23.7
<b>Zoea V</b>	7	258.0 $\pm$ 2.6	4	34.4
<b>Zoea VI</b>	8	301.3 $\pm$ 2.5	4	40.2
<b>Zoea VII</b>	9	433.0 $\pm$ 7.8	3	43.3
<b>Zoea VIII</b>	11	551.0 $\pm$ 24.1	2	36.7
<b>Zoea IX</b>	14	665.7 $\pm$ 16.2	2	44.4
<b>PL1</b>	18	701.7 $\pm$ 66.1	1	23.4
<b>PL7</b>	25	1400.0 $\pm$ 58.6	1	46.7
<b>PL14</b>	32	2569.4 $\pm$ 216.7	1	85.6

<sup>1</sup>-days after hatching

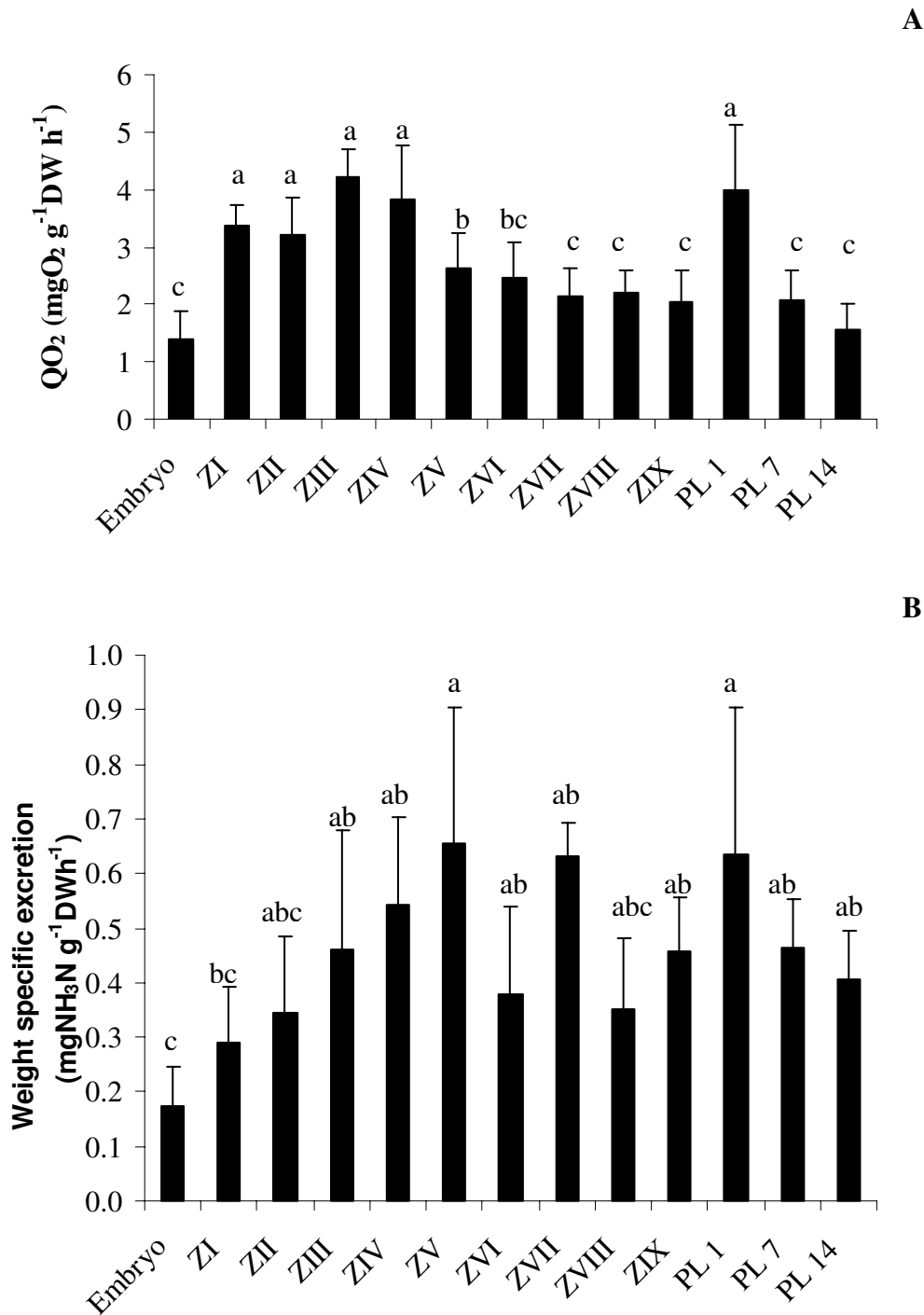
**Table 2** – Individual oxygen consumption and ammonia-N excretion rates and atomic oxygen:nitrogen ratio (O:N) in early life of *Macrobrachium amazonicum* at  $30\pm 1^\circ\text{C}$  and 10‰ salinity. ZI-IX=larval stages, PL1, PL7 and PL14=post-larvae with 1, 7 and 14 days after metamorphosis, respectively. Results are expressed as mean values $\pm$ SD, [number of replicates].

<b>Developmental Stage</b>	<b>O<sub>2</sub> consumption (<math>\mu\text{gO}_2\cdot\text{ind.}^{-1}\cdot\text{h}^{-1}</math>)</b>	<b>NH<sub>3</sub>-N excretion (<math>\mu\text{gNH}_3\text{-N}\cdot\text{ind.}^{-1}\cdot\text{h}^{-1}</math>)</b>	<b>O:N</b>
<b>Embryo</b>	0.10 $\pm$ 0.02 [8] d	0.0090 $\pm$ 0.0039 [6] e	10.0 [6]
<b>Zoea I</b>	0.20 $\pm$ 0.02 [9] d	0.0171 $\pm$ 0.0058 [9] e	10.0 [9]
<b>Zoea II</b>	0.20 $\pm$ 0.04 [12] d	0.0219 $\pm$ 0.0082 [10] e	8.0 [10]
<b>Zoea III</b>	0.36 $\pm$ 0.05 [11] d	0.0387 $\pm$ 0.0185 [6] e	8.0 [6]
<b>Zoea IV</b>	0.45 $\pm$ 0.11 [19] d	0.0644 $\pm$ 0.0255 [21] e	6.0 [21]
<b>Zoea V</b>	0.68 $\pm$ 0.16 [9] c	0.1689 $\pm$ 0.0523 [10] d	4.0 [10]
<b>Zoea VI</b>	0.74 $\pm$ 0.19 [19] c	0.1138 $\pm$ 0.0510 [13] d	6.0 [13]
<b>Zoea VII</b>	0.92 $\pm$ 0.21 [11] c	0.2739 $\pm$ 0.0250 [5] d	3.0 [5]
<b>Zoea VIII</b>	1.21 $\pm$ 0.23 [9] c	0.1931 $\pm$ 0.0408 [11] d	5.0 [11]
<b>Zoea IX</b>	1.37 $\pm$ 0.36 [16] c	0.3036 $\pm$ 0.0667 [4] cd	4.0 [4]
<b>PL1</b>	2.79 $\pm$ 0.81 [14] b	0.4460 $\pm$ 0.1735 [16] c	5.0 [16]
<b>PL7</b>	2.90 $\pm$ 0.72 [12] b	0.6487 $\pm$ 0.1467 [10] b	4.0 [10]
<b>PL14</b>	4.02 $\pm$ 1.10 [11] a	1.0413 $\pm$ 0.2492 [6] a	3.0 [6]

**Table 3** - Regression parameters obtained from relationships between dry weight and oxygen consumption and dry weight and ammonia-N excretion from embryo to PL14 of *Macrobrachium amazonicum*. (temperature=30±1°C, 10‰ salinity from embryo to ZIX and 0‰ for PL1 to PL14).

	Log a	b	r <sup>2</sup>	n
Individual O <sub>2</sub> consumption (μgO <sub>2</sub> .ind <sup>-1</sup> h <sup>-1</sup> )	-2.2	0.8	0.88	143
Individual NH <sub>3</sub> -N excretion (μgNH <sub>3</sub> -N.ind <sup>-1</sup> h <sup>-1</sup> )	-3.7	1.1	0.86	118

The values  $a$  and  $b$  are constants in the equation  $\text{Log } M = a + b \text{Log } DW$ , where,  $M$ =individual metabolic rate and  $DW$ =dry weight (μg).  $r^2$ =determination of coefficient and  $n$ =number of observations.



**Figure 1** – Weight-specific rates oxygen consumption (QO<sub>2</sub>) (A) and ammonia excretion (B) during the early life stages of *Macrobrachium amazonicum* at 30±1°C. Results are expressed as means±SD. (DW=dry weight, Z=zoea stages, PL=post-larvae).

## CAPÍTULO 4 (Aquaculture international)

### **Efeito do nitrito no desenvolvimento e metabolismo das larvas de *Macrobrachium amazonicum***

### **Effects of ambient nitrite on development and metabolism of *Macrobrachium amazonicum* larvae**

#### **Resumo**

O efeito das concentrações de nitrito no desenvolvimento e metabolismo das larvas de *Macrobrachium amazonicum* foi estudado no laboratório. A taxa de sobrevivência, produtividade, ganho de peso e índice de estágio larval (IEL), consumo de oxigênio e excreção de amônia e desenvolvimento larval foram avaliados. As larvas foram submetidas às seguintes concentrações de nitrito na água: 0; 0,2; 0,4; 0,8 e 1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N. Cada tratamento teve 5 repetições. O consumo de oxigênio e a excreção de amônia foram analisados em zoea (Z) I, III, VII e IX expostas a 0; 0,4; 0,8 e 1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N com 5 repetições para cada tratamento. O primeiro experimento foi conduzido em béqueres de vidro de 600mL com 300mL de solução-teste com salinidade 10‰, temperatura constante de 30°C e fotoperíodo 12:12h claro:escuro. O segundo experimento foi conduzido em câmaras cilíndricas plásticas de 30mL. A sobrevivência, a produtividade, o ganho de peso e o índice de estágio larval (IEL) decresceram linearmente com o aumento da concentração de nitrito no ambiente. Entretanto, não houve diferença significativa entre as concentrações de 0 a 0,8mg.L<sup>-1</sup>NO<sub>2</sub>-N em todos os parâmetros avaliados. O consumo individual de oxigênio (VO<sub>2</sub>) aumentou significativamente durante o desenvolvimento larval em todas as concentrações de nitrito ( $P < 0,05$ ). A relação entre a taxa metabólica e a taxa individual peso-específico (QO<sub>2</sub>) variou significativamente durante o desenvolvimento ontogenético ( $P < 0,05$ ).

Altos valores de  $QO_2$  foram encontradas nos estágios iniciais (ZI e ZIII) e decresceram nos estágios subsequentes (ZVII e ZIX). A excreção de amônia individual ( $NH_3-N$ ) mostrou uma tendência de aumento significativo durante a maioria dos estágios de desenvolvimento nas diferentes concentrações de nitrito ( $P<0,05$ ). A taxa de excreção peso–específico de amônia também variou significativamente durante o desenvolvimento larval ( $P<0,05$ ). A tendência de aumento começou nos estágios iniciais e decresceu nos estágios subsequentes. Observou-se baixos valores de O:N, variando de 3,7 a 15,9. As taxas de O:N mostraram que em larvas de *M. amazonicum* predomina o catabolismo de proteínas. A taxa metabólica individual apresentou  $b<1$ , indicando que o aumento da taxa de consumo de oxigênio e excreção de amônia foi menor que o crescimento no período estudado. A concentração de  $1,6mg.L^{-1}NO_2-N$  no ambiente, retarda o desenvolvimento larval, reduz a sobrevivência, a produtividade, o ganho de peso, o IEL e afeta as taxas metabólicas de larvas de *M. amazonicum*. Por outro lado, níveis abaixo de  $0,8mg.L^{-1}NO_2-N$  podem ser considerados seguros para a larvicultura dessa espécie.

Palavras-chaves: Excreção de amônia, Larva, *Macrobrachium*, Nitrito, Consumo de oxigênio.



## Abstract

The effects of ambient nitrite concentration on development and metabolism of *Macrobrachium amazonicum* larvae were studied in laboratory. Survival rate, productivity, weight gain, larval stage index (LSI), oxygen consumption, ammonia excretion and larval development were evaluated. Larvae were reared in water with nitrite concentration of 0, 0.2, 0.4, 0.8 and 1.6mg.L<sup>-1</sup>. Each treatment had five replicates. Oxygen consumption and ammonia excretion were also analyzed in zoea (Z) I, III, VII and IX exposed to 0, 0.4, 0.8 and 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N and each treatment was conducted in five replicates. The first experiment was carried out in 600mL glass beakers filled with 300mL of test solution at 10‰ salinity, constant temperature 30°C and 12:12h daylight:darkness regime. The second experiment was carried out in 30mL cylindrical plastic chambers. Survival, productivity, weight gain and LSI rate decreased linearly with increasing ambient nitrite concentration. However, there was no significant difference among larvae reared at concentration ranging from 0–0.8mg.L<sup>-1</sup>NO<sub>2</sub>-N in all parameter evaluated. Individual respiration rates (VO<sub>2</sub>) increased significantly during larvae development in all ambient nitrite concentration ( $P<0.05$ ). The relation between metabolic rate and individual dry weight (QO<sub>2</sub>) varied significantly through the ontogenetic development ( $P<0.05$ ). Higher means in QO<sub>2</sub> were found in first larval stages (ZI and ZIII) and decreased in subsequent stages. Individual ammonia-N (NH<sub>3</sub>-N) showed a significant increasing tendency during the majority of developmental stages with different nitrite concentration ( $P<0.05$ ). Weight-specific excretion rates of ammonia also varied significantly throughout developmental stages ( $P<0.05$ ). Increase of tendency starting in the first stages and decreased in subsequent stages. It was observed low O:N values, ranging from 3.7 to 15.9. O:N ratio showing that *M. amazonicum* larvae has a protein-dominated metabolism. Individual metabolic rates presented  $b<1$ , indicating that the increase in oxygen consumption rate and ammonia

excretion was lower than the growth in the studied period. In conclusion, increasing ambient nitrite up to  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  delays larval development reduces survival, productivity, weight gain, LSI and affects metabolism rates of the *M. amazonicum* larvae. On the other hand, levels below  $0.8\text{mg.L}^{-1}\text{NO}_2\text{-N}$  and this range may be safe for *M. amazonicum* hatchery.

Keywords: Ammonia excretion, Larvae, *Macrobrachium*, Nitrite, Oxygen consumption.

## 1. Introduction

Freshwater prawn farming has been recognized as one of the aquaculture segments that has shown larger growth (New, 2005; Kutty, 2005). The production of *Macrobrachium rosenbergii* expanded rapidly between 1995 and 2004 from 19.035 to 193.570 tones (FAO, 5 2006). This rapid global development may be probably because freshwater prawn farming is a way of producing crustaceans with low environment impact (New et al. 2000; Moraes-Riodades and Valenti, 2001) and high environmental sustainability (Valenti and Tidwell, 2006).

In Brazil, production of *M. rosenbergii* is about 500mt.year<sup>-1</sup> (New, 2005). 10 Additionally, a large Brazilian group is working on the culture of an indigenous species, the Amazon river prawn *Macrobrachium amazonicum*, which have high potential for small-scale farms in the Amazonia (Valenti and Moraes-Riodades, 2004; New, 2005) and western Pantanal region, Brazil (Hayd et al. submitted-a). This species presents fast larval development, with 9 free-swimming zoea stages (Guest, 1979), and a larval cycle of 18 to 15 21 days in dynamic closed systems (in temperature of 30±1°C and 10‰ salinity). In addition, presents high growth and survival rates in semi-intensive grow-out ponds (Moraes-Riodades and Valenti, 2007).

*M. amazonicum* hatchery is conducted in intensive systems, with high stocking densities (100-140 larvae.L<sup>-1</sup>) (Vetorelli et al. 2006). Thereby, increasing the load of 20 nitrogenous compounds and other toxic metabolites may changes water quality such as increase ammonia and nitrite concentration (Das et al. 2004; Madison and Wang, 2006). These inorganic nitrogen are the most important pollutants in aquaculture systems (Koo et al. 2005). Recirculating water through biological filters lows ammonia and nitrite levels due to nitrification process which convert ammonia to nitrate via nitrite (Valenti and Daniels, 25 2000; Timmons et al. 2002). Nitrite is formed from ammonia oxidation and can be

accumulated in aquatic systems due to imbalances of nitrified bacterial activity (Das et al. 2004; Sowers et al. 2004; Koo et al. 2005; Madison and Wang, 2006). In crustaceans, ambient nitrite reduces their thermal tolerance and induces methaemocyanin formation, causes hypoxia in tissues and diminishes the respiration efficiency (Alcaraz and Carnegas,  
5 1997; Timmons et al. 2002).

In general, high values of nitrite in the tank water is due to imbalance in nitrification process at biological filters (Valenti and Daniels, 2000; Timmons et al. 2002; Jensen, 2003; Madison and Wang, 2006). Several authors recommend levels below  $0.1\text{mg.L}^{-1}$  of the nitrite for *M. rosenbergii* hatchery (Correia et al. 2000; New, 2002). Otherwise, levels around 1–  
10  $2\text{mg.L}^{-1}$  did not stress larvae (Armstrong et al. 1976; Mallasen and Valenti, 2006). Nevertheless, the tolerance limits and mechanisms of nitrite action in caridean development and metabolism are almost unknown (Mallasen and Valenti, 2006). Hence, establishing security levels of ambient nitrite for hatchery systems and knowing its effects on metabolism of *M. amazonicum* is very important. The aim of the present study was to  
15 investigate the effects of ambient nitrite on development and physiological changes of *M. amazonicum* larvae under laboratory conditions.

## 2. Material and methods

### Experimental animals

Larvae of *M. amazonicum* were obtained from broodstock formed by wild animals  
20 captured in Northeast Para, Brazil ( $01^{\circ}13'25''\text{S}$   $48^{\circ}17'40''\text{W}$ ) in 2001. Females carrying eggs in advanced stages of embryonic development (approximately 2h before hatching), were collected. This phase of egg incubation is characterized by slow movements of females and the presence of visible eyes inside the transparent eggs. Animals were disinfected in

formaldehyde solution at 20ppm for 30min. and then kept in a hatching tank at 70 ind.m<sup>-2</sup> and 8‰ salinity. It was provided with aeration, heating system and artificial substrates.

### Larval development test

In this test, the effects of nitrite concentration on survival, productivity, weight gain and larval development were evaluated. Tested concentrations treatments were, 0, 0.2, 0.4, 0.8 and 1.6 mg.L<sup>-1</sup>NO<sub>2</sub>-N; each treatment was conducted in five replicates. All test solutions were prepared by dissolving sodium nitrite in brackish water (10‰ salinity), according to methods presented at APHA (1998). General methodology was adapted from Mallasen and Valenti (2006) and is summarized below.

Larvae were reared in 600mL glass beakers filled with 300mL of test solution gently aerated at 10‰ salinity, constant 30°C, and subjected to 12:12h daylight:darkness regime. Beakers were placed inside black trays to minimize light reflections and prevent larvae crowding around luminous points, due to positive phototactism. Fifteen newly-hatched larvae were carefully washed out with the test solution, acclimated for 2h and transferred to each beaker. Larvae were fed freshly hatched *Artemia* nauplii from ZII to ZIV (4-6 nauplii.mL<sup>-1</sup>). After this stage, they were fed on inert feed (3.3 to 13.2µg.mL<sup>-1</sup>) and *Artemia* nauplii (8 to 12 nauplii.mL<sup>-1</sup>), added to the beakers every day until metamorphosis. Food residues were siphoned 3h after feeding.

Every 24h brackish water was changed for maintaining constant water quality and the tested nitrite concentration. Temperature and salinity were monitored twice a day (measured with a Yellow Springs Instruments, YSI 63) while dissolved oxygen (measured with Yellow Springs Instruments YSI 52 oxymeter) and pH (measured with Yellow Springs Instruments YSI 60 pHmeter) were measured three times a week. Ammonia and nitrite levels were determined twice a week before water replacement, according to methods

presented at APHA (1998). Ammonia and nitrite analyses were performed using a Hach DR 2000 spectrophotometer. Larval stages were identified according to Guest (1979).

At the end of the experiment, larvae and post-larvae were counted, weighed, and observed under a stereomicroscope (Leica MZ6) to determine larval stage. Larval stage index (LSI) was estimated using the equation of Manzi et al. (1977),  $LSI = (\sum Si \times ni) / N$ , where  $Si$  is larval stage ( $i=1-9$ ),  $ni$ =number of larvae in stage  $Si$  and  $N$ =total number of larvae observed.

Larvae were sampled of the hatchery tank to estimate initial dry weight (IDW). At the end of this study, the surviving larvae and post-larvae were used to determine final dry weight (FDW). Weight gain was then given by the difference between FDW and IDW. To determine dry weight, the prawns were briefly rinsed in distilled water, placed in pre-weighed cartridges and dried for 48h at 70°C. Then, they were transferred to a desiccator for 2h and weighed on a Mettler-Toledo Model AT21 analytical balance, at the nearest 1µg. Experiment was finished when around 80% of the larvae of one replicate of any tested treatment metamorphosys to post-larvae. The test followed a completely randomized design with five nitrite concentrations (treatments) and five replicates. Data of the variables (survival, productivity, weight gain and LSI) and ambient nitrite concentration ( $mg.L^{-1}$ ) were described as a linear function,  $Y=a+bX$ , where  $Y$  is the variables and  $X$  is ambient nitrite concentration tested. Constants  $a$  and  $b$  denote elevation and slope, respectively. Mean water temperature was 30°C and did not differ among beakers. Dissolved oxygen was above  $7mg.L^{-1}$  and the pH ranged around 7.8. Ammonia concentration ( $NH_3-N$ ) mean value was  $0.4 \pm 0.1mg.L^{-1}$  and nitrite ( $NO_2-N$ ) was very close to the nominal values of the treatments.

Data were subjected to one-way ANOVA followed by Tukey test and linear regression analysis. All measured values of each variable at each phase were entered into the regression

analysis ( $P < 0.05$ ). Statistics analyses were performed with the Statistical Analysis System (SAS Intiture Inc., version 8.0). Values expressed as percentages were square root arcsine transformed prior to analysis, although they are presented as non-transformed for easier interpretation. Significance level was set at  $P < 0.05$ .

## 5 Oxygen consumption and ammonia-N excretion determination

Oxygen consumption and ammonia-N excretion were determined in larvae at stages I, III, VII and IX subjected to ambient nitrite concentrations of 0, 0.4, 0.8 and  $1.6 \text{ mg.L}^{-1}$  to detect possible alterations in larval metabolism when ambient nitrite increases. Treatments were conducted in six replicates. These selected larval stages and nitrite levels were defined from the results obtained for the larval development test. First, larvae were sampled of the 120L cylindrical tanks provided with mechanical and biological filter and artificial heater, with 10‰ salinity and  $30^\circ\text{C}$  and were acclimated in beaker with water at different nitrite concentration during two hours. After acclimating, larvae at postmolt-intermolt (A-C) stages (Hayd et al., submitted or see chapter 1) were selected and placed into respirometric chambers (*ca.* 30mL) for 2h to quantify metabolic rates.

Cylindrical plastic containers with approximately 30mL sealed with silicon tablets (Lemos and Phan, 2001, Lemos et al., 2003) were used as respirometric chambers to incubate animals for oxygen consumption and ammonia-N excretion measurement. Chambers were individually identified and the exact volume was gravimetrically determined. An orifice of 1.5mm in the center of the cover enabled the elimination of air bubbles from inside the chamber during closure. Respirometers were hermetically closed inside 1000mL beakers filled with water (10‰ salinity) to avoid formation of bubbles inside the containers. After being closed, inside water was isolated from air by a plastic tablet 1.7cm diameter silicon seal so that the tension between orifice water and the silicon seal could block respirometer inside water and air gas exchange.

The number of individuals used inside respirometer was determined according to individual dry mass (Table 1). The biomass:volume ratio (B:V) was calculated by dividing the total dry mass of individuals by the chamber volume and varied from 17.3 to 48.3 $\mu\text{g}\cdot\text{mL}^{-1}$  (Table 1). ZI, III, VII and IX were kept in the sealed respirometric chambers samples for 2h. Seawater and Milli-Q (Millipore) fosh water at 10‰ salinity were used as control respirometer without animals. Samples and controls were kept in water bath at  $30\pm 1^\circ\text{C}$ . After incubation, the tablet was removed, and water was sampled through a plastic canula and stored in glass syringes chemically calibrated (syringes plus plastic nozzles).

Variation in oxygen and ammonia-N nitrogen contents was calculated by the difference between values obtained in sample (with animals) and control (no animals) units. Dissolved oxygen in water at the end of every test was determined by Winkler analysis method adapted to small volumes (Fox and Wingfield, 1938) and was never lower than 80% of saturation. For ammonia-N analysis, the method described by Koroleff (1983) in separate water samples was used. Oxygen consumption and ammonia-N excretion were expressed as individual ( $\mu\text{g}\cdot\text{ind}^{-1}\cdot\text{h}$ ) and dry-mass specific ( $\text{mg}\cdot\text{g}^{-1}\text{DW}\cdot\text{h}$ ) rates. Salinity effect was corrected using the factor 1.06 (Koroleff, 1983). Atomic O:N (oxygen respired to nitrogen excreted) ratios were calculated by dividing the number of gram-atom of consumed oxygen by the number of gram-atom of nitrogen excreted ( $\text{N}\cdot\text{O}^{-1}$ ) in each developmental stage (Mayzaud and Conover, 1988).

Individual dry weight (DW) was determined by groups of 10 larvae at ZI, ZIII, ZVII and ZIX with eight replicates. Individuals were gently rinsed with distilled water, dried with filter paper and separated prior to weight determination. After 48h at  $70^\circ\text{C}$ , dry samples were weighed on a Mettler Toledo AT21 analytical balance, at the nearest  $1\mu\text{g}$ .

The results were subjected to analyses of detection and exclusion of outliers (Statistica software, v. 6.0) with coefficient 1.5. Total excluded data was always lower than



10% of data obtained for each stage (Statistica Software, v.6). The test was a 4x4 factorial design with four nitrite concentrations and four larval stages. Normality was tested using the Shapiro-Wilk test and Homocedasticity by Levene's (using SAS 9.0 software). Differences among means were tested by two-way ANOVA followed by Duncan's multicomparison test.

5 Data of the metabolic rates (oxygen consumption and ammonia excretion, in  $\mu\text{g}\cdot\text{ind}^{-1}\cdot\text{h}^{-1}$ ) and dry weight ( $\mu\text{g}$ ) in 0 and  $1.6\text{mg}\cdot\text{L}^{-1}\text{NO}_2\text{-N}$  were logarithmically adjusted by regression analysis (Statistica Software, v.6). Slopes were compared to 1 using a t-test according to Zar (1999). Differences were considered significant at  $P<0.05$ .

### 3. Results

#### 10 Larval development test

*M. amazonicum* larvae developed until metamorphosis at 0, 0.4, 0.8 and  $1.6\text{mg}\cdot\text{L}^{-1}\text{NO}_2\text{-N}$ . Survival, productivity, weight gain and larval stage index decreased linearly with increase ambient nitrite concentration (Fig. 1). However, there were no significant differences in all parameters at concentration ranging from 0– $0.8\text{mg}\cdot\text{L}^{-1}\text{NO}_2\text{-N}$  ( $P>0.05$ ),  
15 whereas they were significantly lower at  $1.6\text{mg}\cdot\text{L}^{-1}\text{NO}_2\text{-N}$  ( $P<0.05$ ) (Table 2).

#### Oxygen consumption and ammonia excretion determination

Individual dry weight (DW) increased throughout the ontogenetic development (Table 1) and varied significantly in different ages and developmental stages ( $P<0.05$ ). Individual oxygen consumption rates ( $\text{VO}_2$ ) increased significantly during development  
20 larvae ( $P<0.05$ ) in all ambient nitrite concentration (Fig. 2B; Table 3). ZI presented the lowest value and into these stage there was no significant difference in all nitrite concentrations ( $P>0.05$ ), while ZIX presented the highest one ( $P<0.05$ ) (Table 3). The  $\text{VO}_2$  values increasing in ZIII and ZIX at  $1.6\text{mg}\cdot\text{L}^{-1}\text{NO}_2\text{-N}$ , and differ significantly from  $0\text{mg}\cdot\text{L}^{-1}\text{NO}_2\text{-N}$  ( $P<0.05$ ) (Table 3). The weight-specific oxygen consumption ( $\text{QO}_2$ ) in different

ambient nitrite concentration showed significant variation throughout development ( $P<0.05$ ) (Fig. 2A); ZI and ZIII presented the highest rates, contrasting with lower values in the later stages ZVII and ZIX (Fig. 2A). Nitrite concentration at  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  increased the  $\text{QO}_2$  significantly in ZI, ZIII and ZIX.

5 Individual ammonia-N excretion increased while weight-specific ammonia excretion decreased throughout the larval development ( $P<0.05$ ) (Table 3). The stages ZI, ZVII and ZIX showed significant differences in individual and weight-specific ammonia excretion between 0 and  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  ( $P<0.05$ ); excretion increased in ZI and ZIX and decreased in ZVII from 0 to  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  (Table 3).

10 As a general trend, developmental was characterized by low O:N ratio. The highest value ( $18\pm 0.6$ ) was observed in ZI at  $0\text{mg.L}^{-1}\text{NO}_2\text{-N}$ , which strongly decreased at 0.4, 0.8 and  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  (Fig. 2C). The slope obtained for individual oxygen consumption/dry weight relationship at 0 and  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  were 0.63 and 0.60, respectively, and both was significantly lower than 1 ( $P<0.05$ ) (Table 4), while the  $b$  value for individual excretion/ dry weight relationship at 0 and  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$  were 0.71 ( $P<0.05$ ) and 1.23, respectively, and the latter one was significantly higher than 1 (Table 4).

#### 4. Discussion

Complete larval development of *M. amazonicum* occurs in nitrite from 0 to  $1.6\text{mg.L}^{-1}\text{NO}_2\text{-N}$ . However, survival, productivity, weight gain and larval stage index decreased as nitrite concentration increased. Similar results have also been obtained for *M. rosenbergii* larvae (Mallasen and Valenti, 2006) with added nitrite in concentration between 0 to  $16\text{mg.L}^{-1}\text{NO}_2\text{-N}$ . This reduction may be related to physiological processes, such as the changes in haemocyanin provoked by nitrite (Sowers et al. 2004) and may reflect in the oxygen consumption (Harris et al. 1997).

Generally, oxygen consumption increased with increasing the nitrite concentration from 0-0.8 to 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N. Similar results were obtained for ZII of *M. rosenbergii* by Mallasen and Valenti (2006). It suggests that tested nitrite levels did not disrupt significantly oxygen transport and larvae may be allocated energy to adjust physiological mechanisms against the toxic effect when in high nitrite concentration, thus reducing growth and development. Therefore, it may increase culture cycles and decreased productivity and individual size in commercial hatchery. The weight-specific oxygen consumption (QO<sub>2</sub>) varied significantly through the ontogenetic development ( $P<0.05$ ). Higher means in QO<sub>2</sub> were found in first larval stages (ZI and ZIII) and decreased in subsequent stages (ZVII and ZIX), regardless nitrite concentration. Similar result was obtained in chapter 2. Thus, larvae did not changed the metabolic pattern during the ontogenetic development with the addition of nitrite in water. This tendency in QO<sub>2</sub> during growth may be a consequence of a disproportionate increase of metabolically inactive tissues (Anger, 2001) and/or the exponential increases in the weight of the larval exoskeleton during the development of decapods crustaceans (Anger and Ismael, 1997). The same tendency was observed for *M. amazonicum* larvae in a former papers (see chapter 2; Zanders and Rodriguez, 1992), *Macrobrachium rosenbergii* (Agard, 1999), the spider crabs *Hyas coarctatus* larvae (Jacobi and Anger, 1985) and *Hyas araneus* larvae (Anger and Jacobi, 1985) during the larval development.

Individual ammonia excretion showed an increasing tendency during the larval development while weight-specific ammonia excretion rates were high in the first stages and decreased in the latter ones. This is similar to the QO<sub>2</sub> and general patterns find by crustacean larvae. Nitrite effect varied among larval stages. Weight-specific ammonia excretion strongly increased with nitrite concentration in ZI. The mean obtained at 0mg.L<sup>-1</sup>NO<sub>2</sub>-N is compatible with the value obtained in chapter 2 in normal conditions. Therefore,

the higher values obtained for 0.4, 0.8 and 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N may be really due to nitrite effect. This indicate that ambient nitrite increase protein catabolism in lecithotrophic phase. It is corroborated by the great decrease in O:N ratio observed by 0.4, 0.8 and 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N in zoea I.

5           In ZIII, ammonia excretion did not changed with nitrite concentration. However QO<sub>2</sub> significantly increased at 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N. This indicate an increase in catabolism of lipid which is corroborated by the increase in O:N ratio. In ZIII, larvae start exogenous feed and fed on freshly hatched *Artemia* nauplii, which is rich in lipid. Therefore larvae may change metabolic process. Similar results was observed in *Penaeus japonicus* fed with this  
10 microcrustacea (Lemos and Rodrigues, 1998).

          In ZVII, ammonia excretion decreased with nitrite concentration, while QO<sub>2</sub> did not changed. It indicates that protein metabolism decreased, while general metabolism did not change probably due to catabolism of lipid. This stage is characterized by a very large ingestion of *Artemia* nauplii (Maciel, 2007), which may increase of the lipid as source of  
15 energy.

          In Z IX, ammonia excretion significantly increased from 0 to 0.4mg.L<sup>-1</sup>NO<sub>2</sub>-N, while QO<sub>2</sub> only increased at 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N. It indicates that ambient nitrite increased protein catabolism, but the proportion protein:lipid as energy source did not change.

          In this study, the slope in both oxygen consumption/dry weight relationship at 0 and  
20 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N were lower than 1 ( $b < 1$ ). These values suggest a decrease in weight-specific metabolic rates throughout development in the studied period, and that ambient nitrite presents low effect on oxygen consumption. Slope lower than 1 has been already obtained for *M. amazonicum* early development stages (see chapter 2). Like this, the overall patterns of metabolism of *amazonicum* larvae is similar to other larval decapods studied,  
25 such as, *M. rosenbergii* larvae (0.904) (Stephenson and Knight, 1980), and *M. rosenbergii*

juveniles (0.628) (Nelson *et al.*, 1977). On the other hand, the *b* value for individual excretion/ dry weight relationship at 0 and 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N were 0.71 (*P*<0.05) and 1.23, respectively, and the latter one was significantly higher than 1. In several studies with decapod larvae, *b* values above the 1 were usually associated with physiological stress (Anger, 2001). Therefore, the present study indicated that 1.6mg.L<sup>-1</sup> NO<sub>2</sub>-N produce physiological stress, which cause changes in excretion patter. This fact was also showed in caridean shrimp *Palaemon serratus* larvae subjected to stress of temperature and salinity (Yagi *et al.*, 1990), in brachyuran crab *Cancer irroratus* larvae (Johns, 1981) and lobster *Homarus americanus* larvae (Capuzzo and Lacaster, 1979) when exposed to unfavorable temperatures.

Results of the present study indicated that during the lecithotrophic phase of *M. amazonicum*, ambient nitrite concentration decrease the lipid and increase the protein catabolism. On the contrary, when larvae started the exogenous feeding ambient nitrite increases the lipid as source of energy. In addition, growth rate, survival, productivity and weight gain of larvae are negatively affected by 1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N. Therefore, maintaining moderate intensity of the nitrite concentration, lower than 0.8mg.L<sup>-1</sup> in hatchery tanks water is safe for *M. amazonicum* larvae development. However, further physiological studies focus on the effect of the nitrite concentration ranged between 0.8–1.6mg.L<sup>-1</sup>NO<sub>2</sub>-N is necessary to determine the optimum range in hatchery system.

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**Table 1** – Age, dry weight (DW), number of individuals, and biomass:volume ratio in respirometers used to determine oxygen consumption and ammonia-N excretion rate during zoea (Z) I, III, VII and IX stages of *M. amazonicum* at 30±1°C and 10‰ salinity. Results are expressed as mean values±SD. Number of replicates=10.

Larval Stage	Age <sup>1</sup>	DW (µg)	Individuals. respirometer <sup>-1</sup>	Biomass:volume (µg.mL <sup>-1</sup> )
<b>ZI</b>	0.5	60.0 ± 1.2	12	24.0
<b>ZIII</b>	3.0	86.3 ± 1.7	6	17.3
<b>ZVII</b>	9.0	483.0 ± 8.5	3	48.3
<b>ZIX</b>	14.0	700.3 ± 19.2	2	46.7

5 <sup>1</sup>-days after hatching

**Table 2** - Survival rate, productivity, weight gain and larval stage index (LSI) (means±standard deviation) obtained at the end of 17 days of *Macrobrachium amazonicum* culture (30±1°C and 10‰ salinity) in different ambient nitrite concentrations. Mean values

10 in the same column with different letters are significantly different ( $P<0.05$ ). N=25.

Nitrite (mg.L <sup>-1</sup> )	Survival (%)	Productivity (PL.L <sup>-1</sup> )	Weight gain (µg)	LSI
<b>0</b>	88.0 ± 5.6 a	26.8 ± 2.4 a	627.6 ± 4.6 a	8.8 ± 0.1 a
<b>0.2</b>	82.8 ± 3.7 a	20.8 ± 2.3 a	626.7 ± 5.4 a	8.8 ± 0.0 a
<b>0.4</b>	81.4 ± 5.6 a	20.0 ± 2.4 a	620.2 ± 24.8 a	8.8 ± 0.1 a
<b>0.8</b>	80.0 ± 4.9 a	19.2 ± 8.3 a	601.8 ± 16.0 a	8.7 ± 0.1 a
<b>1.6</b>	65.6 ± 3.0 b	9.4 ± 2.8 b	545.0 ± 14.6 b	8.2 ± 0.0 b

**Table 3** – Nitrite concentrations, individual oxygen consumption ( $\text{VO}_2$ ) and ammonia-N excretion rates in zoea (Z) I, III, VII and IX stages of *Macrobrachium amazonicum* in 0, 0.4, 0.8 and  $1.6\text{mg.L}^{-1}$  nitrite concentrations at  $30\pm 1^\circ\text{C}$  and salinity 10‰. Results are expressed as mean values $\pm$ SD. Number of replicates=6.

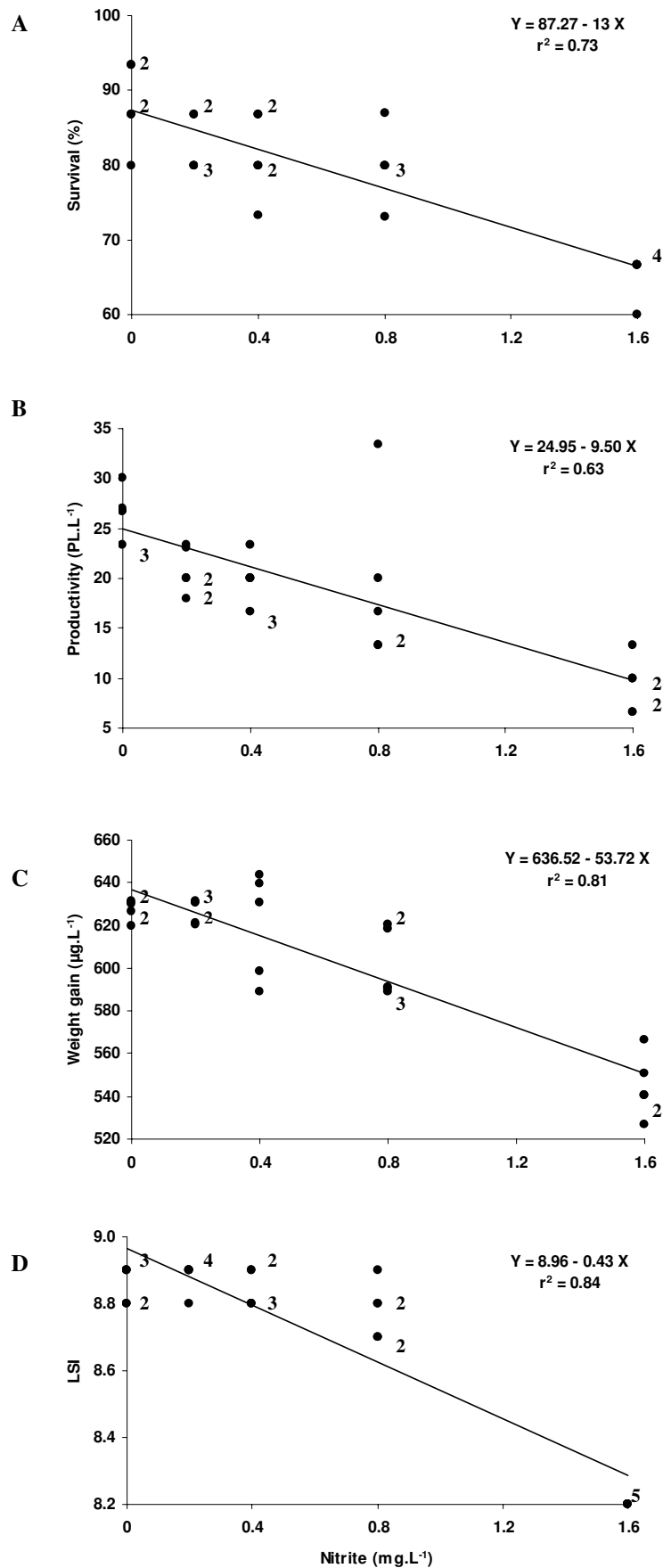
Larval Stage	Nitrite $\text{NO}_2$ ( $\text{mg.L}^{-1}$ )	Oxygen consumption $\mu\text{gO}_2\text{.ind.}^{-1}\text{.h}$	Ammonia-N excretion $\mu\text{g NH}_3\text{-N.ind.}^{-1}\text{.h}$
<b>ZI</b>	0.0	$0.19 \pm 0.02$ a	$0.011 \pm 0.005$ a
	0.4	$0.18 \pm 0.02$ a	$0.032 \pm 0.010$ b
	0.8	$0.18 \pm 0.03$ a	$0.032 \pm 0.007$ b
	1.6	$0.23 \pm 0.05$ ab	$0.041 \pm 0.004$ b
<b>ZIII</b>	0.0	$0.34 \pm 0.02$ bc	$0.035 \pm 0.003$ b
	0.4	$0.36 \pm 0.04$ bc	$0.034 \pm 0.007$ b
	0.8	$0.38 \pm 0.03$ cd	$0.038 \pm 0.006$ b
	1.6	$0.50 \pm 0.06$ d	$0.038 \pm 0.008$ b
<b>ZVII</b>	0.0	$0.83 \pm 0.12$ e	$0.187 \pm 0.028$ d
	0.4	$0.76 \pm 0.08$ e	$0.159 \pm 0.017$ cd
	0.8	$0.81 \pm 0.08$ e	$0.130 \pm 0.013$ c
	1.6	$0.85 \pm 0.17$ e	$0.117 \pm 0.018$ c
<b>ZIX</b>	0.0	$1.08 \pm 0.08$ f	$0.197 \pm 0.050$ d
	0.4	$1.19 \pm 0.12$ f	$0.278 \pm 0.044$ e
	0.8	$1.12 \pm 0.07$ f	$0.258 \pm 0.114$ e
	1.6	$1.53 \pm 0.24$ g	$0.240 \pm 0.059$ e

**Table 4** - Regression between dry weight and oxygen consumption and dry weight and ammonia-N excretion from zoea I, III, VII and IX of *Macrobrachium amazonicum* in different nitrite concentration 0 and 1.6mg.L<sup>-1</sup> NO<sub>2</sub>-N at 30±1°C and 10‰ salinity.

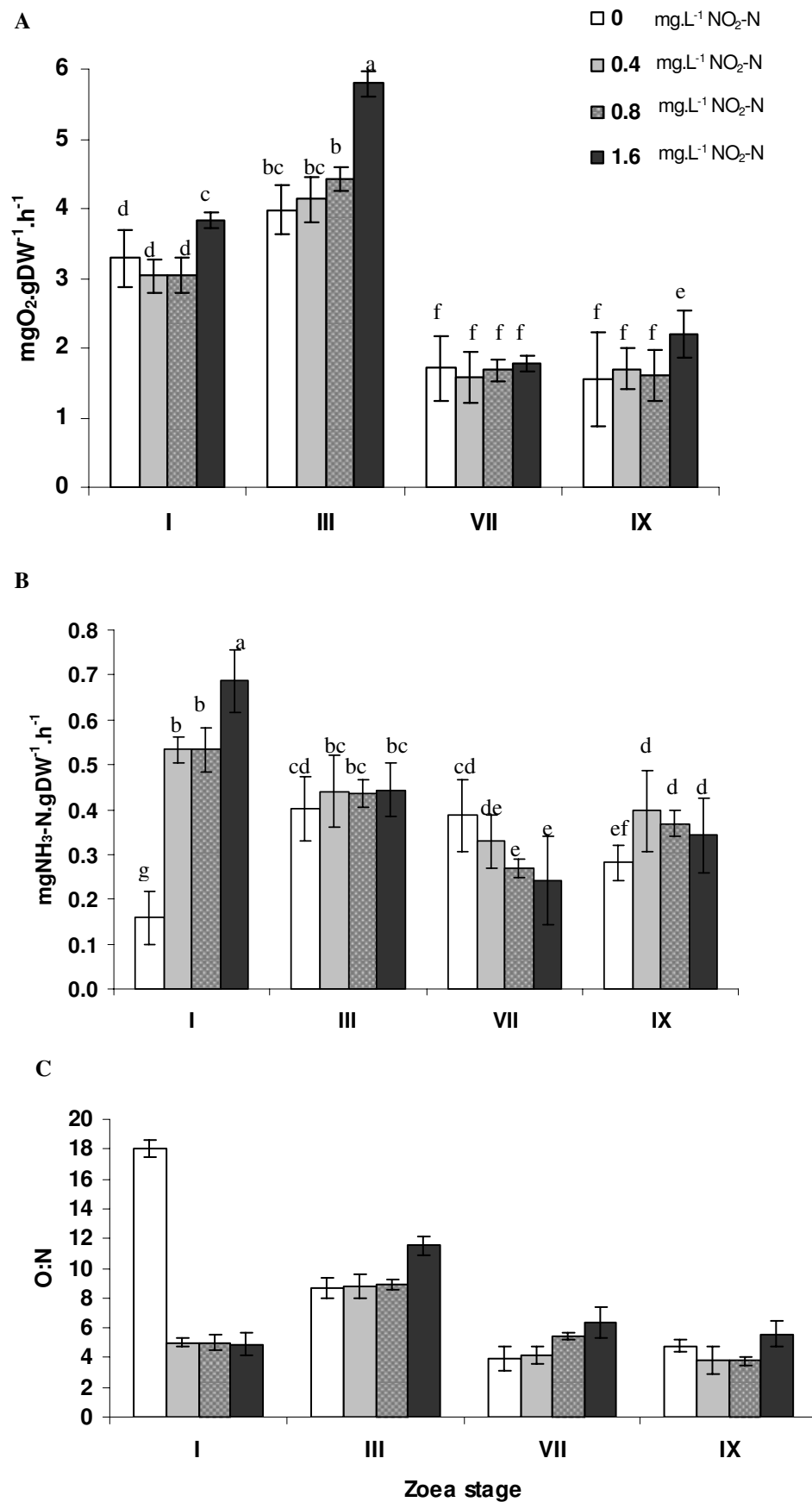
<b>0 mg.L<sup>-1</sup> NO<sub>2</sub>-N</b>	Log a	b	r <sup>2</sup>	n
Individual O <sub>2</sub> consumption (µgO <sub>2</sub> .ind. <sup>-1</sup> .h)	-1.70	0.63	0.95	20
Individual NH <sub>3</sub> -N excretion (µgNH <sub>3</sub> -N.ind. <sup>-1</sup> .h)	-2.70	0.71	0.90	20
<b>1.6 mg.L<sup>-1</sup> NO<sub>2</sub>-N</b>				
Individual O <sub>2</sub> consumption (µgO <sub>2</sub> .ind. <sup>-1</sup> .h)	-1.60	0.60	0.83	20
Individual NH <sub>3</sub> -N excretion (µgNH <sub>3</sub> -N.ind. <sup>-1</sup> .h)	-4.10	1.23	0.90	20

The values *a* and *b* are constants in the equation  $\text{Log } M = a + b \text{Log DW}$ , where, *M*=individual metabolic rate and

5 DW=dry weight (µg). r<sup>2</sup>=coefficient of determination and n=number of observations.



**Figure 1** – Relationship between survival rate (N=25, F=15.59,  $P < 0.05$ ) (A), productivity (N=25, F=10.91,  $P < 0.05$ ) (B), weight gain (N=25, F=26.64,  $P < 0.05$ ) (C) and larval stage index (LSI) (N=25, F=138.87,  $P < 0.05$ ) (D) and nitrite concentration. Figures over data-points indicate the number of identical values.



**Figure 2** – Weight-specific rates oxygen consumption (A), ammonia-N excretion (B) and O:N ratio (C) during the early life stages of *Macrobrachium amazonicum* to different nitrite concentration. Results are expressed as means±SD. (I=zoea I, III=zoea III, VII=zoea VII and IX=zoea IX).



## CAPÍTULO 5

### CONCLUSÕES

1. *Macrobrachium amazonicum* apresentou os estágios de muda, **A/C** (pós-muda/intermuda combinados), **D** (pré-muda) e **E** (ecdise). Esta classificação foi baseada no grau de  
5 homogeneidade do citoplasma, retração da epiderme e no estágio do desenvolvimento das setas do telson.
2. O estágio **A/C** é uma fase muito curta em que a larva absorve água, a cutícula é secretada e ocorre o crescimento e a condensação do tecido da epiderme. O estágio **D** é caracterizado  
10 pelo início da retração da epiderme da nova cutícula, chamada “apolise”. Esta ocorre inicialmente na base da seta do telson em todos os estágios larvais, sendo o período principal da formação da seta (setogênese). O estágio **E** é um processo muito rápido (alguns minutos), e inicia-se com a abertura entre o cefalotórax e o abdome.
3. A pós-muda/intermuda (**A/C**) ocupa 40-50% do total de duração do instar e a pré-muda (**D**) requer mais da metade do tempo nas temperaturas experimentais.
- 15 4. O índice de condição larval (IEL) proposto por Tayamen and Brown, (1999) para avaliar a qualidade larval de *Macrobrachium rosenbergii* não pode ser aplicado às larvas de *M. amazonicum*, porque algumas características negativas atribuídas às larvas de *M. rosenbergii* ocorrem em larvas sadias no período inicial de pós-muda/intermuda (**A/C**) em *M. amazonicum*.
- 20 5. O conhecimento e a identificação dos estágios do ciclo de muda são importantes na seleção das larvas que serão utilizadas em experimentos, reduzindo, assim, a variabilidade das condições fisiológicas do animal e ajudará a entender o padrão de crescimento desta espécie.

6. A taxa individual de consumo de oxigênio e excreção de amônia nitrogenada aumenta com a elevação da massa corporal durante o desenvolvimento larval. A taxa metabólica peso-específico diminui com o aumento da biomassa. As variações metabólicas observadas são decorrentes de um desenvolvimento caracterizado por várias transformações morfológicas externas e comportamentais, durante o desenvolvimento ontogenético de *M. amazonicum*.
7. As taxas atômicas O:N mostraram que durante todos os estágios de desenvolvimento de *M. amazonicum* predomina o catabolismo de proteínas para a produção de energia.
8. Na despesca de tanques de larvicultura, as pós-larvas, precisam de um maior tempo de aclimatação, ao serem transferidas da água de salinidade 10‰ para água doce.
9. As relações entre as taxas metabólicas e o peso seco, indicam que o consumo de oxigênio aumentou em taxa menor que a taxa de crescimento no período estudado e que a excreção de amônia é proporcional à biomassa do corpo.
10. A sobrevivência, a produtividade, o ganho de peso e o IEL decresceu linearmente com o aumento da concentração de nitrito no ambiente. Entretanto, não houve diferença significativa entre as concentrações 0-0,8mg.L<sup>-1</sup>NO<sub>2</sub>-N nos parâmetros avaliados. Assim, a faixa de 0-0,8mg.L<sup>-1</sup>NO<sub>2</sub>-N pode ser considerada segura para o cultivo.
11. A concentração 1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N retardou o desenvolvimento larval em todos os parâmetros avaliados e afetou as taxas metabólicas das larvas de *M. amazonicum*. O nitrito aparentemente causa aumento no metabolismo protéico na fase lecitotrófica e no uso de lipídeos como fonte de energia na fase de alimentação exógena.
12. Estudos precisam ser realizados para avaliar o efeito do nitrito no desenvolvimento larval de *M. amazonicum* no intervalo 0,8-1,6mg.L<sup>-1</sup>NO<sub>2</sub>-N.