Physiological status of *Macrobrachium rosenbergii* larvae reared in artificial brackish water.

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Abstract

The growth pattern during larval development of *Macrobrachium rosenbergii* and the oxygen consumption of selected stages were analyzed in response to different rearing media (natural and artificial). The increment of biomass (dry weight) of larvae reared in both artificial and natural media shows an exponential increase regarding the number of development stages or related to the developmental time. The growth pattern showed by larvae reared in artificial brackish water and in natural brackish water are not statistically different. However, when the dry weight data are plotted in relation to the development time, the growth curves of larvae reared in artificial medium show a displacement to the right. These data indicate that although larvae reared in artificial medium reach the same weight as those reared in natural medium, this fact involves a prolongation in the developmental time. The oxygen consumption data show that the growth rate of larvae reared in artificial medium were kept at the expense of a higher energetic cost.

Key words: artificial medium, growth, *Macrobrachium rosenbergii* larvae, respiration.

Introduction

In their natural environment, the adult and juvenile stages of *Macrobrachium rosenbergii* live in freshwater or in low salinity water. The larval stages, however, are dependent on the estuarine environment as they need brackish water to complete their development. After metamorphosis, the postlarvae remain in the estuaries for some time and then migrate to the environment of the adult (Ling, 1969). While adult specimens of *M. rosenbergii* have developed mechanisms that allow them to face the stress caused by the freshwater environment by their osmoregulatory capability, larvae are highly dependent on the saline medium, as such abilities seem to be functional from metamorphosis on (Harrison *et al.*, 1981). This brings on one of the major problems regarding the rearing of this species, as postlarvae production must take place in regions near the coast. However, grow-out is generally performed in small properties scattered in the inland of developing countries (Valenti, 1995). Therefore, small farmers need to buy postlarvae from large producers, who control the prices; or they have to produce their own postlarvae. In the latter situation, the acquisition of natural seawater is a further complication, once there are costs regarding the collection and transport of seawater.

Aiming to solve this problem, some attempts have been made at rearing larvae of this prawn in artificial seawater (Tansakul, 1983; Prakash, 1988; Valenti, 1993; Weiling *et al.*, 1995). Although all these studies have had positive results, the use of artificial seawater as an alternative to the larvicultures located in the inland, has been restricted due both to the high cost of the formula and the availability of chemicals. Some studies (Silva, 1995; Mallasen and Valenti, 1998a,b) have been carried out to develop a simplified seawater formula, in which only the essential components are employed, allowing its utilization by the farmers at a low cost and with simple technology.

Embryos of *M. rosenbergii* and *M. malcolmsonii* are able to develop in culture medium containing NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and $NaHCO_3$ (Damrongphol *et al.*, 1990; Samuel *et al.*, 1997). However, *M. rosenbergii* larvae reared in the above-mentioned medium show 100% of mortality already in early

development, but when KBr is added to the medium, larvae can develop successfully to postlarval stages (Silva, 1995; Mallasen and Valenti, 1998a,b). Thus, the ion bromide can be characterized as an essential element and therefore must be an indispensable component of the artificial seawater for *M. rosenbergii* larviculture.

The purpose of this study was to evaluate the physiological status of larvae reared in this simplified artificial seawater, analyzing their oxygen consumption and growth, and comparing them with those larvae reared in natural seawater.

Material and Methods

Animals, rearing, water preparation

Ovigerous *M. rosenbergii* were collected from ponds at the Aquaculture Centre of the State São Paulo University (CAUNESP, Jaboticabal, Brazil) and maintained in recirculating tanks until the larvae hatched. After hatching, actively swimming larvae from different females were mass-reared into six 120 L closed-system tanks (3 tanks containing artificial medium and 3 with natural medium) at a density of approximately 60 larvae/L, with water temperature maintained at 30 ± 0.5° C and salinity of 12 ± 0.5 ppt in both media under a 12:12 h light:dark cycle.

The artificial brackish water (ABW) was prepared according to Silva (1995), which was modified after Bidwell and Spott (1985). The formula was prepared with analytical grade salts and deionised water as solvent. The formulation and ionic composition of the studied artificial brackish water is presented in table I. Natural seawater was taken from the Enseada de Ubatuba, SP (23°26'S and 45°02'W) and filtered through a 2μm cellulose cartridge filter. Both artificial and natural media were diluted with deionised water.

Table I: Formula and ionic composition of the simplified artificial seawater.

Salts	g/m^3 of deionized water	Ions	meq/m^3
NaCl	2.76×10^4	Na^+	4.75×10^5
$MgSO_4.7H_2O$	6.90×10^3	Mg^{2+}	1.09×10^5
$MgCl_2.6H_2O$	5.40×10^3	Ca^{2+}	1.90×10^4
$CaCl_2.2H_2O$	1.40×10^3	K^+	8.23×10^3
KCl	6.00×10^2	Cl^-	5.52×10^5
$NaHCO_3$	2.00×10^2	SO_4^{2-}	5.60×10^4
KBr	2.70×10^1	HCO_3^-	2.38×10^3
		Br^-	2.27×10^2

Larvae were fed daily with freshly hatched *Artemia* sp nauplii (ca 5 nauplii/mL), so that from the sixth stage on, the diet was supplied with fresh ration (for more details see Mallasen and Valenti, 1998a). The larval stages were identified using the morphological description by Uno and Kwon (1969).

Water quality parameters including dissolved oxygen, pH, total ammonia and nitrate were monitored three times a week, using a YSI oxygen meter model 55, pH meter model Micronal B374, ammonia and nitrate were analyzed according to Solorzano (1969) and Strickland and Parsons (1972), respectively.

Larval growth

To determine larval growth (as dry weight: DW), samples of each developmental stage (30 zoea I to 10 zoea XI) were taken from each treatment and respective replicates, every two days. After samples were taken (five replicates), larvae were rinsed in distilled water, dried for about 24 hr at 60°C in pre-

weighed cartridges, placed in a desiccator for 2 hr and then weighed on a Mettler-Toledo AT21 balance to the nearest 0.01 mg. Statistical analysis of data followed standard methods (Mead and Curnow, 1983). The test for equality of slopes of the exponential equations was based on a F-test comparison of the two residual mean squares ($p < 0.05$).

Oxygen consumption

The respiratory rate (R) of selected developmental stages (Zoea I - V) was measured using the Winkler method (Grasshoff, 1976). The measurements were carried out during intermoult (stage C) of the moulting cycle. According to Anger (1991), during this phase there is little variability in oxygen consumption. Each measurement comprised of six replicate experiments (bottles with larvae) and four replicate blanks (without larvae). Depending on the larval weight, 25 (zoeae I) to 4 (zoeae V) individuals were confined in each Winkler bottle (ca 60 mL). Oxygen consumption was measured after an incubation period (5 h), using an automatic Mettler-Toledo DL21 titration apparatus. The decrease of oxygen concentration in the experiments was < 10%, which is believed not to affect larval respiration (Dawirs, 1983). Larval respiration was calculated from the difference in O_2 concentrations between experimental and blank bottles, the volume and number of individuals in each bottle, and the time of incubation.

Respiration was expressed per individual (R), as $\mu gO_2 \cdot ind.^{-1} \cdot h^{-1}$ and per dry weight (QO_2), as $\mu gO_2 \cdot mg^{-1} \cdot h^{-1}$.

The effect of different media on respiratory rate (R and QO_2) was analyzed by t-test, with a probability level of $p< 0.05$ and $p< 0.01$.

Results

The water quality parameters remained relatively constant during the duration of our study, so that the percentage of dissolved oxygen ranged from 86.9 ± 1.1% to 87.4 ± 1.4%, pH ranged from 7.7 ± 0.1 to 7.8 ± 0.2, ammonia and nitrate remained below 114.61µg/L and 89.85µg/L, respectively.

The accumulation of biomass (as DW) is positive throughout larval development, except for the stage of zoea II. The increment of biomass of larvae reared in both artificial and natural media shows an exponential increase regarding the number of development stages (fig. 1) or related to the developmental time (fig. 2). Both equation can be described as follow: $W= c \cdot e^{k.t}$ where, "W" is the average weight per individual (in µg), "e" is the base of the natural logarithm, "c" and "k" are constants and "t" the number of stages (related to developmental stages, fig.1) or day (when related to developmental time, fig. 2) . Figure 1, as an example, shows the increase in DW from hatching to the last zoeal stage, for both media (with the fitted regression equations, along with the coefficients of determination, R^2).

The growth pattern (fig. 1) showed by larvae reared in artificial brackish water (ABW) and in natural brackish water (NBW) are very similar; the growth curves are not statistically different ($F_{(2,18)}$ =0.275, not significant). However, when the dry weight data are plotted in relation to the development time (fig. 2), the growth curves of larvae reared in artificial medium show a displacement to the right. Table II shows the regression equations of these curves (fig.2) with fitted parameters and coefficients of determination, R^2.

Respiration (R) increased during development through successive larval stages, except in zoea II (fig. 3). Larvae reared in the artificial medium had an oxygen consumption statistically different ($p<0.05$) in the zoea III and V stages, when compared to those reared in natural medium. The zoea I and II stages showed the inverse tendency (fig. 3).

Weight-specific respiration (QO_2) mean values of larvae reared in ABW show statistical differences ($p<0.01$) in zoeal stages, and whereas the second stages showed a lower oxygen consumption than those reared in NBW, stages III, IV and V showed an enhancement of oxygen consumption (fig. 4).

Table II: Fitted constants (c, k) and coefficients of determination (R^2) of the exponential equations (see fig. 2) describing changes in biomass (dry weight) during time (days) of development in subsequent larval stages (zoea I-XI), in natural and artificial media (3 repetitions).

	Natural			Artificial		
	1	2	3	1	2	3
c	21.05	22.69	22.11	24.65	22.94	22.74
k	0.2165	0.2051	0.2216	0.1631	0.1500	0.1672
R^2	0.9921	0.9887	0.9852	0.9896	0.9943	0.9888

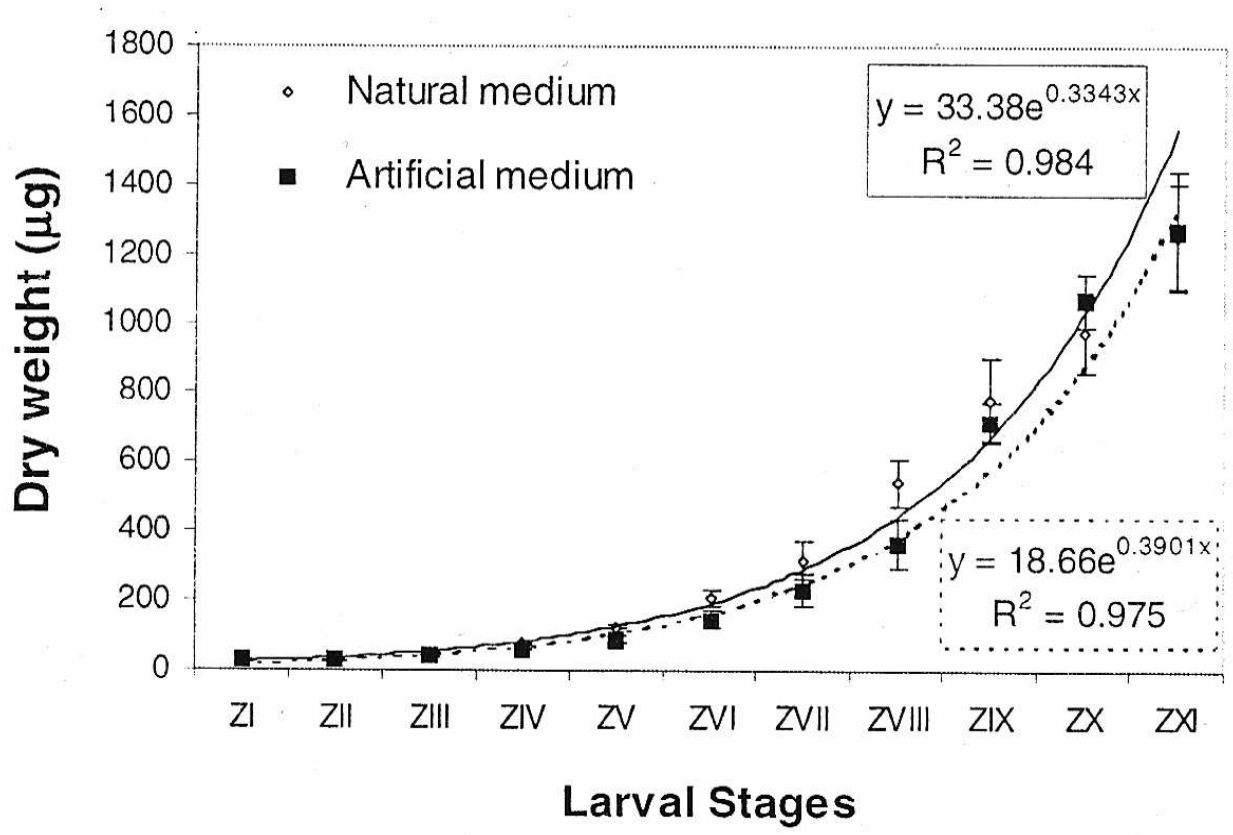

Fig. 1: Growth (dry weight) in successive developmental stages (Zoea I-XI) in artificial and natural brackish water. The regression equations are given with fitted parameters and coefficients of determination, R^2 (equation above: natural medium; below: artificial medium).

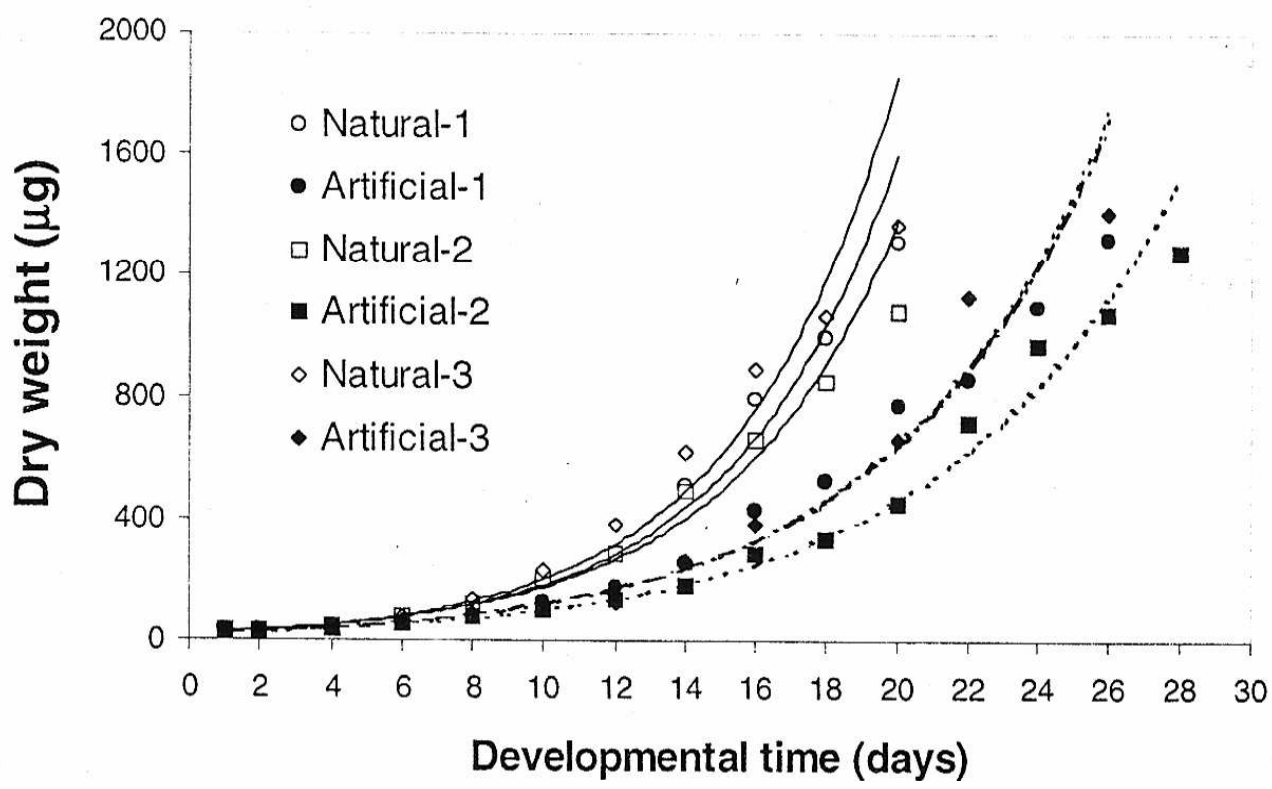

Fig. 2: Larval growth curves (dry weight) in artificial and natural brackish water (3 replicates) during time (days).

Discussion

Water quality parameters in the different media remained within the range recommended for *M. rosenbergii* larval development (Correia *et al.*, 2000; Valenti and Daniel, 2000). The slight changes in salinity and temperature that were observed, occurred homogeneously in the treatments. These were set up simultaneously, being subject to the same influences of the external environment. Based on these facts, it is assumed that the effects observed in this study were due to the rearing media tested, therefore excluding the possible interference of abiotic factors.

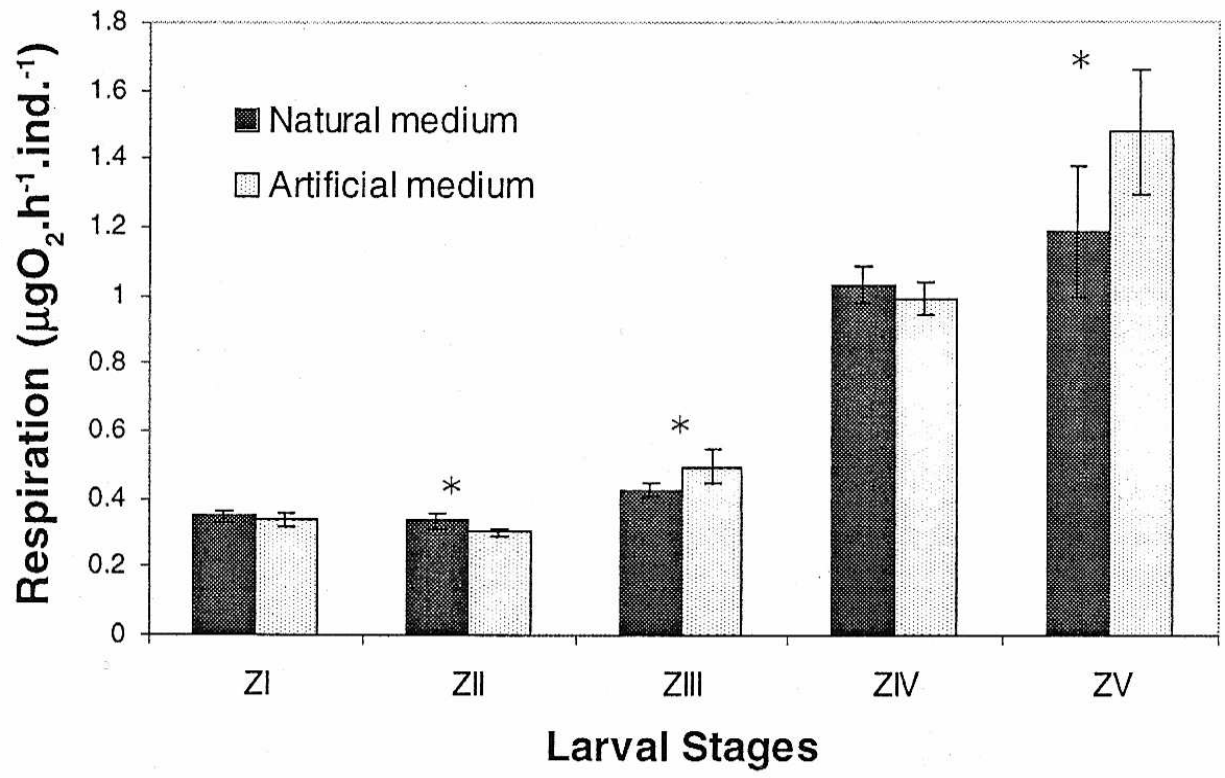

Fig. 3: Effect of different media (artificial and natural) on rates of individual respiration in successive larval stages (Z: zoea I-V). (* indicate significant difference between tested media, $p<0.05$).

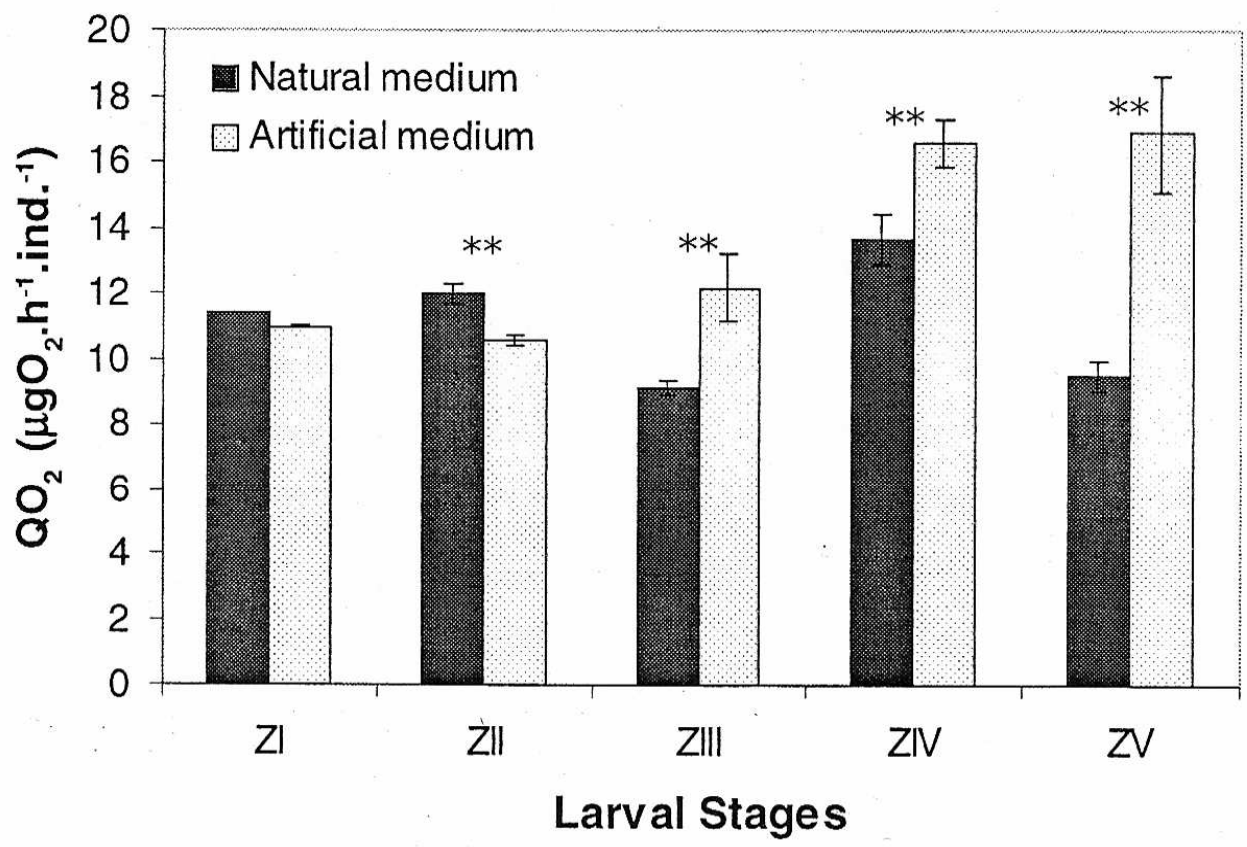

Fig. 4: Effect of different media (artificial and natural) on rates of weight-specific respiration in successive larval stages (Z: zoea I-V). (** indicate significant difference between tested media, $p< 0.01$).

The growth pattern showed by *M. rosenbergii* larvae in the present study, coincides with that obtained by Stephenson, and Knight (1980) and has also been seen in other carideans, such as *Crangon crangon* and *C. allmanni* investigated by Criales and Anger, (1986) and *Pandalus montagui* by Schultze and Anger, (1997, and in other decapods such as *Carcinus maenas* by (Dawirs, 1983), *Liocarcinus holsatus* by (Harms, 1990), *Portunus trituberculatus* Lim and Hirayama (1991), *Menippe mercenaria* by Mootz and Epifanio (1974) and *Ranina ranina* by Minagawa *et al.*, (1993). The biomass of these decapods increases exponentially with the number of development stages (for a detailed review see Anger, 1998).

Our results (fig. 1) show that when the accumulation of biomass of larvae reared in natural and artificial media is related to the number of development stages, the growth pattern showed by larvae reared in natural medium is similar to those reared in artificial medium. However, when the dry weight data are analyzed with the developmental time (fig. 2), we observe that there is a displacement in the growth curve, towards a considerable delay in development. These data indicate that although larvae reared in artificial medium reach the same weight as those reared in natural medium, this fact involves a prolongation in the developmental time.

The processes involved in the growth of crustaceans are very complicated by the partial independence between the occurrence of molts and the growth and morphogenesis (McConaugha, 1985). The energy obtained from food must be used to keep the costs involved in the molting process, growth and morphogenesis, with the first being prioritary (Knowlton, 1974). This is observed in larvae of caridean shrimps, in which the molting frequency remains unchanged under suboptimal conditions, while growth and morphogenesis cease, resulting in a considerable variability in the number of stages and in the

morphology of larval stages. Inadequate conditions of certain environmental parameters are likely to deviate the energy involved in growth to the mechanisms of larvae maintenance. Such occurrence of molts, without the simultaneous differentiation and growth, causes a prolongation of the larval period. The irregularity in larval development was reported by Ngoc-Ho (1976) for several palaemonids, including *M. rosenbergii*. According to Sandifer and Smith (1979), the irregular development comprises a series of repetitive stages as a consequence of environmental and/or genetic factors.

The artificial seawater tested in our study did not affect the accumulation of biomass, however, it was necessary a longer period of time (6-10 days) for larvae to reach the same size as those reared in natural medium. The oxygen consumption data obtained in this study show that the growth rates were kept at the expense of a higher energetic cost, when larvae utilized exogenous food (fig.3 and 4).

The individual respiratory rate (R; in $\mu g\ O_2 \cdot Individual^{-1} \cdot h^{-1}$) during larval development has been studied in some species of decapods. Larval oxygen consumption increases during the development of the freshwater prawn *M. rosenbergii* studed by Stephenson and Knight, (1980), the hermit crab *Pagurus bernhardus* by Anger (1989), the lobster *Homarus americanus* by Sasaki *et al.*, (1986) and the crabs *Hyas araneus* by Anger and Jacobi, (1985), *H. coarctatus* by Jacobi and Anger, (1985), *Libinia ferreirae* by Anger *et al.*, (1989) and *Chasmagnathus granulata* by Ismael *et al.*, (1997). Our data corroborate those cited above, and only the zoea II showed a drop in oxygen consumption. This reduction in the respiratory rate, followed by a negative growth is a tendency found in the development stages that show lecithotrophy (Anger *et al.*, 1990). The first stage of zoea does not depend on food, once it uses internal energy reserves remaining from the egg yolk. Our respirometry experiments were carried out without feeding zoeae II, therefore the results showing a decrease in the respiratory rate are coherent with the tendency found in the lecithotrophic larvae.

Several studies demonstrate a tendency in the decrease of average values of QO_2 along larval development in a series of decapods as *Homarus americanus, Hyas araneus* and *Penaeus monodon,* studied respectively by Logan and Epifanio, (1978); Anger and Jacobi, (1985) and Kurmaly *et al.* (1989). The general decrease in weight specific metabolic rate during growth is considered to be a consequence of a disproporcionate increase of metabolically inactive tissues (Prosser, 1973). Many decapods, however, show a positive relationship between respiratory rate and weight during early larval development (Capuzzo and Lancaster, 1979; Dawirs, 1983). Metabolic rates independent of the larval stages biomass were observed in *Libinia emarginata* by Schatzlein and Costlow, (1978), *Cancer irroratus* by Johns, (1981), *Palaemon serratus* by Yagi *et al.*, (1990) and *Chasmagnathus granulata* by Ismael *et al.*, (1997).

The weight-specific metabolic rate of larvae reared in artificial medium was significantly higher than those reared in natural medium, except for the zoeae I and II stages, which were not fed any exogenous food. The difference in the metabolic rates between larvae reared in natural and artificial media should be reflecting the benefits of the first medium. The zoeae III, IV and V consumed more energy in artificial water, to maintain growth and morphogenesis, but a delay the larval development also occur.

Based on our study, the artificial seawater tested seems to lack important elements in its formula, in spite of presenting the ions that are essential for the development of *M. rosenbergii*. The prolongation of larval rearing causes losses regarding production costs. Another important aspect to be considered is that several physiological studies on crustacean are carried out using simplified artificial seawater. The results could reflect the effect of the medium in the organism, and could be acting as a stress factor, therefore altering several physiological parameters.

Other studies are being carried out at CAUNESP aiming to investigate other elements that should be incorporated to the formula of the artificial seawater in order to consolidate a technology focused on the optimization of production parameters of *M. rosenbergii*.

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