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Short communication

Experimental studies on the effect of food in early larvae of the cleaner shrimp *Lysmata amboinensis* (De Mann, 1888) (Decapoda: Caridea: Hippolytidae)

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Abstract

Identifying appropriate larval husbandry is a key to improve larval quality and shorten duration of larval stages, and culture costs of ornamental cleaner shrimps *Lysmata amboinensis*. Several feeding and zootechnical experiments were conducted under laboratory conditions to test the effect of food during the first day posthatch, the effect of feeding with microalgae on larval digestion after 24 h posthatch, the effect of enriched rotifers upon survival and the combined effect of stocking density and food concentration on growth and survival of young larvae. For the first time, oxygen consumption values, trypsin-like activity and metabolite content (protein and triacylglycerols) were determined for *L. amboinensis* early stage larvae. When starved during the first day of life, *L. amboinensis* larvae had the same oxygen consumption as fed larvae, indicating that food ingestion is not crucial during that period. Nevertheless, energy reserves such as TAG were significantly lower in 24 h starved larvae when compared with fed larvae indicating facultative primary lecitotrophy. Trypsin-like activity of digestive enzymes (U mg prot^{-1}) was low when compared with other decapod larvae. Larvae fed with *Tetraselmis chuii* showed a significant increase in enzyme activity after 24 h. Present results showed that enriched rotifers result in higher larval survival during the first days of life when compared with larvae fed with non-enriched rotifers and that survival is not dependent on the relation between larval density and food concentration. In addition, stocking densities of 10 larvae ml^{-1} showed higher survival compared to that obtained at the stocking density of 20 larvae ml^{-1}.

Keywords: Larval physiology; Larviculture; *Lysmata amboinensis*; Cleaner shrimp; Marine ornamentals

1. Introduction

In contrast to freshwater species, most commercialized marine ornamentals come directly from natural systems, principally from coral reefs (Tlusty, 2002; Wabnitz et al., 2003). The exploitation pressure is more pronounced in Indo-Pacific coral reefs, where countries such as Philippines and Indonesia have the highest rates of exploitation (Wabnitz et al., 2003). Target species are mainly fish and corals, but recently more invertebrates are being commercialized, especially anemones and cleaner shrimps, mostly from the genus *Lysmata*. In order to release some of the exploitation pressure upon natural systems, aquaculture of marine ornamentals has been suggested as a possible solution (Rubec, 1988; Kaiser et al., 1997; Calado, 2006; Lecchini et al., 2006). However, aquaculture of ornamentals can only be profitable through a combination of science and technology as to optimize techniques for culture (Wabnitz et al., 2003). Despite the exponential growth of the marine ornamental market, very few species are presently cultured (Calado et al., 2003; Calado, 2006). Whilst much progress in this field has been made during last years (Penha-Lopes et al., 2005; Rhyne et al., 2005; Espinosa and Allam, 2006; Olivotto et al., 2006; Figueiredo and Narciso, 2006; Penha-Lopes et al., 2006; Watson and Hill, 2006; Calado et al.,

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2007; Palmtag and Holt, 2007), it is still necessary to develop studies on the biology and ecology of potential ornamental species (Calado et al., 2003; Calado, 2006), such as the cleaner shrimp *Lysmata amboinensis*, one of the most valuable and popular ornamental shrimps (Wabnitz et al., 2003).

One of the major constraints of cleaner shrimp's larviculture is the duration and variability of the larval phases, mostly due to mark-time moulting (Gore, 1985). This behavioural and physiological attribute, in turn, helps to explain the broad geographical distribution of some circumtropical species, such as *L. amboinensis*. In fact, the reported larval duration in laboratory studies, which according to Wunsch (1996) is not less than 140 days, could reflect a long planktonic phase in accordance with a strategy of broad larval dispersion. Identifying appropriate larval husbandry is a key to improve larval quality and shorten duration of larval stage, as well as to minimize culture costs. In captivity, larval food quality and quantity (Rhyne and Lin, 2004; Calado et al., 2005), cannibalism (Rufino and Jones, 2001), amiss stocking densities (Simoes et al., 2002) have shown to be important factors determining larval survival of ornamental marine shrimp.

Although the larval stages of decapod crustaceans generally require planktonic food to survive, it is believed that after hatching, *Lysmata* larvae rely on egg yolk reserves (reflecting the degree of parental investment in reproduction), which are assumed to be sufficient for the first 24 h of life (Fletcher et al., 1995; Zhang et al., 1998). This feature is commonly referred to as lecithotrophy, and has been considered as an adaptation to low or unpredictable food availability in the environment where larval development takes place (Anger, 2001; Calcagno et al., 2003). Two different types of lecithotrophy have been described in decapod crustaceans: primary lecithotrophy, where the energy from egg yolk reserves is used post the hatching in order to develop through at least the first larval stage; and secondary lecithotrophy, where the energy from reserves accumulated exogenously by earlier larval stages is used by a late non-feeding larval stage to moult into a juvenile (Anger, 2001).

In agreement with this, rearing of larvae of several ornamental marine shrimps has relied on feeding live prey only 24 h after hatching (Fletcher et al., 1995; Zhang et al., 1998). However, according to Simoes et al. (2002), this commonly adopted practice wrongly assumes that reserves of the newly hatched larvae are enough for larvae to grow and survive through the first 24 h of life. In fact, these authors found that larval survival can be significantly improved if live food is given throughout the first day of life. Their results suggest that newly hatched *L. amboinensis* larvae possibly present facultative lecitotrophic behaviour, equally found in other crustacean larvae (Anger, 2001). Comparative examination of biochemical constituents of tissues such as TAG and soluble-proteins (Fraser, 1989; Anger, 2001) of starved and fed larvae should reveal whether food has a significant effect on their nutritional state at 24 h posthatch.

Although, *Lysmata* species are known to be strict carnivores (Le Vay et al., 2001), Simoes et al. (2002) found that larvae of *L. debelius* ingested microalgae added to culture water, and it improved larval survival. Supply of microalgae may have several beneficial effects on the nutritional state of larvae, one of which is that the presence of microalgae stimulates larval digestive enzyme production and colonization of the digestive tract (Kumlu and Jones, 1995a; Cahu et al., 1998).

The incapacity of crustaceans to produce sufficient amounts of several specific high unsaturated lipids (HUFAs) necessary for larval development drives crustaceans to satisfy their requirements externally (Glencross and Smith, 1999; Gonzalez-Felix et al., 2003) by including such lipids in the diet. One way to ensure inclusion of HUFAs in shrimp culture is through enrichment of live food, such as *Artemia* and rotifers, with commercial products (Rainuzzo et al., 1994; Calado et al., 2005). Despite its high cost, *Artemia* has been the most common enriched live food (Lavens and Sorgeloos, 2000; Sorgeloos et al., 2001). Previous studies with fish larvae have shown that concentrations of enriched rotifers as main food during the first days of life, when adjusted to stocking densities, could be an alternative to *Artemia* (Lubzens et al., 1989). By contrast, similar studies on *L. amboinensis* and *L. debelius* (Fletcher et al., 1995) have shown that by maximising the encounters of larvae to food through appropriate feeding rates, together with adequate water circulation, are sufficient to meet the feeding requirements of these caridean shrimp. Therefore, prey density can have an important effect on larval growth and survival (Minagawa and Murano, 1993).

The present study investigates the effect of zooplankton and microalgae feeds during the first 24 h, and the effect of enriched rotifers and stocking density during the first days of early *L. amboinensis* larvae. In an effort to optimize the current larval rearing protocols, we present, for the first time, biochemical and zootechnical data that will enhance the actual biological and culture knowledge of posthatch *Lysmata* larvae.

2. Materials and methods

Twelve adult *L. amboinensis* were paired and kept at Unidad Multidisciplinaria de Docencia e Investigación (UNAM), Sisal, Yucatán, Mexico during a period of 6 months. Each pair was kept in a 20 L aquaria connected to a 300 L reservoir tank through a recirculation system (total water volume of 420 L) with a continuous filtered and protein-skimmed sea water flow. Shrimp were fed frozen fresh diets (adult *Artemia*, polychaetes, mysids and krill) *ad libitum* three times per day. The recirculation system went through a weekly 20% (84 L) water renewal in order to dilute any potentially toxic substances. General water quality parameters (salinity: 34–35 ppt; temperature: 28±0.5 °C; pH: 8.0–8.5) were checked daily, whilst nitrogen-compounds such as ammonia, nitrites and nitrates were checked weekly to assure that values were always within safe concentrations. Aquaria were kept in a ventilated room with 12 h of light per day, and covered with black plastic sheets to create a shaded environment similar to that found in natural conditions.

By allowing a continuous gentle gravity outflow from the broodstock aquarium into another, smaller aquarium, larvae were gently collected with a sieve (300 μm) and individually counted. Only larvae showing rapid response to light and vigorous swimming activity were selected for use in the experiments. Water was obtained from a continuous recirculation system with aged seawater (continuously UV sterilized and filtered with biofilters and activated carbon) and was again filtered (5 μm) and UV sterilized prior to stocking the 2 L experimental round-bottom glass flasks with larvae. Flasks were maintained in a thermostatically controlled water bath at 28 °C, and gently aerated through a glass pipette.

Survival rate was measured daily from the beginning to the end of each experiment, by gently flowing all the water out of the flasks to a plastic container where surviving larvae were individually counted. Larval development stage

was identified according to criteria defined by Wunsch (1996). *Tetraselmis chuii* and *Chaetoceros gracillis* were used as microalgae, whilst *Artemia franciscana* and rotifers (*Brachionus plicatilis*) were used as zooplankton diets in experiments.

In order to determine initial overall morphometric variation of larvae, the dry weight (dried to constant weight in an oven at 60 °C) and total larval length (tip of rostrum – tip of telson) of a pool ($n=30$) of recently hatched larvae from 3 different spawns were measured. Oxygen consumption (μg O_2 individual^{-1} h^{-1}) was assessed using eleven respirometric chambers (RC-300) connected to a temperature controlled (28 °C) water circulation system (Fisher Scientific Isotherm Refrigerated Circulator 900). Each pair of larvae was placed in a respirometric chamber (1 ml). Oxygen concentration was measured in a closed system with a polarographic electrode connected to a respirometer (Strathkelvin Instruments, Glasgow, UK) during 10 min after a 5 min period of acclimation. A total of 10 replicate pairs of larvae per chamber plus a control chamber without larvae were used.

In order to determine trypsin-like activity (U mg prot^{-1}), and metabolite content (soluble-protein and triacylglycerol concentrations levels; mg mg of dw^{-1}), three replicate samples of 50 larvae per treatment were collected in 1 ml Eppendorf tubes, instantly frozen at −70 °C with liquid nitrogen and then stored until processed in an industrial freezer at −40 °C. Prior to processing, Eppendorf tubes were allowed to defrost in crushed ice (0–4 °C).

Prior to analysis, 350 μl of pyrogen free water (at 4 °C) were added to each tube. Samples were then centrifuged (20 min, 13,200 rpm, 4 °C) and the supernatant removed and added to new labelled Eppendorf tubes. This procedure provided three 10 μl sub-samples per replicate for each test.

Trypsin-like enzyme activity was assessed by measuring the substrate 100 mM Na-benzoyl-DL-arginine *p*-nitroanilide (BAPNA, Sigma B4875) hydrolysis in 0.1 M TRIS buffer pH-8 at 405 nm at 22 °C (Geiger and Fritz, 1988). Changes in absorbance were measured for 2 min at 405 nm. One unit of trypsin-like activity corresponded to 1 μM of 4-nitroaniline liberated in 1 min, based on an extinction coefficient $E_{405}=1.02$ L mmol^{-1} mm^{-1} (Geiger and Fritz, 1988).

A commercial kit was used to determine triacylglycerol concentrations levels (TAG; GPO-PAP, Merck, cat. 14354). Determinations were adapted to a micro-plate using 10 μl of supernatant and 200 μl of enzyme chromogen reagent. Absorbance was recorded in a micro-plate reader (BIORAD model 550) and concentrations were calculated from a standard substrate solution. The supernatant was further diluted 1:10 for protein determination by the Bradford method (1976) using the Sigma Micro Protein Determination Kit (Procedure No. 610). Samples were read in a BIORAD model 550 micro-plate reader at 495 nm.

2.1. Effect of presence versus absence of food

The effect of starvation and food presence in the physiological state of larvae was compared by assessing trypsin-like activity, TAG and soluble-protein content, as well as oxygen consumption in fed and starved larvae at three different moments during the first day of life. Recently hatched larvae, derived from the same female and male parents (collected±3 h after hatching), were maintained in a density of 10 larvae L^{-1} (salinity: 34 ppt; 28 °C; pH: 8.4–8.5) with constant fine bubble gentle aeration in two 4 L aquaria. A set of larvae was let to starve and another was fed a mixture of plankton (*T. chuii* at 25 000 ml^{-1}, *C. gracilis* at 50 000 ml^{-1}, *Artemia* nauplii at 3 ml^{-1}, and rotifers at 10 ml^{-1}) from which samples of larvae were taken at initial time, 12 and 24 h posthatch. A total of 60 observations ($n=10\times6$; 10 pairs of larvae × 6 = 120 larvae) for oxygen consumption, and 18 observations ($n=3\times6$; 3 samples of 50 larvae × 6 = 900 larvae) for metabolite content and trypsin-like activity measurements were made. A factorial ANOVA as well as *post-hoc* test (Newman–Keuls) were used to statistically compare data between treatments using time and diet as factors (Zar, 2007). Cochran's test for homogeneity of variances amongst groups was used prior to analyses to ensure operation at $\alpha=0.05$.

2.2. Effect of microalgae as first food

The effect of microalgae as food on the nutritional state of larvae was assessed by comparing the TAG and soluble-protein of starved larvae and those fed with 2 different diets during the first day of life. Trypsin-like activity was also measured in order to assess if microalgae can stimulate enzyme activity and optimize digestion. Recently hatched larvae were exposed during 24 h to: a) flagellated microalgae, *T. chuii* (TC) at 25 000 cells ml^{-1}, b) diatoms *C. gracilis* (CG) at 50 000 cells ml^{-1}, or c) no food. Larvae were randomly distributed in four 1 L tanks at 10 larvae L^{-1} (salinity: 34 ppt; 28 °C; pH: 8.4–8.5). Metabolite content and trypsin-like activity were measured for larvae in each treatment at the 24th hour. In order to assess the effect of starvation on metabolite content and trypsin-like activity, measurements were also made for a batch of pooled larvae at initial time ($n=3\times4$; 3 samples of 50 larvae × 4 = 600 larvae). Several larvae from treatments with diet were also observed with a light microscope in order to assess the presence of microalgae in the digestive tract. A one way ANOVA as well as *post-hoc* tests (Newman–Keuls) were used to statistically compare the data ($n=12$) between a) 24 h/TC, b) 24 h/CG, c) 24 h/starved, and d) initial time (no food) (Zar, 2007). Cochran's test for homogeneity of variances amongst groups was used prior to analyses to ensure operation at $\alpha=0.05$.

2.3. Effect of enriched versus non-enriched rotifers as food

The effect of enriched rotifers as live food on the survival of recently hatched larvae was assessed by comparing survival of larvae fed with enriched rotifers (*B. plicatilis* with Super Selco (INVE®) and larvae fed with non-enriched rotifers (both at 50 rotifers ml^{-1}). Larvae were distributed in 1 L labelled glass bottom cylindrical flasks in conditions similar to those used in the previous experiment. Recently hatched larvae were randomly distributed in 5 replicate flasks per treatment, and surviving larvae counted after 3 days. Data were transformed (arcsin-transformation) prior to analysis by means of a *t*-test for independent samples ($n=10$; Zar, 2007).

2.4. Effect of initial stocking density and initial food concentration

The combined effect of initial larval stocking density and food concentration was assessed by comparing survival of larvae cultured under 2 different stocking densities (10 and 20 larvae L^{-1}) and fed with 3 different enriched (Super Selco (INVE®)) live food concentrations (20, 35 and 50 rotifers ml^{-1}). Recently hatched larvae were distributed in five 1 L replicate flasks per treatment (6 combinations of stocking density and diet), and surviving larvae counted after 6 days. Data ($n=30$) was transformed (arcsin-transformation) prior to analysis by means of a factorial ANOVA and *post-hoc* tests (Newman–Keuls) (Zar, 2007). Cochran's test for homogeneity of variances amongst groups was used prior to analyses to ensure operation at $\alpha=0.05$.

3. Results

Broodstock of *L. amboinensis* showed a regular and constant reproductive cycle with an average of 575 larvae per spawn per animal, approximately every 14 days. *L. amboinensis* larvae with 24 h of life had an average dry weight of 37±5 μg (±SD) and an average total length (tip of rostrum − tip of telson) of 2.6±0.1 mm (±SD).

3.1. Effect of presence versus absence of food

Results of ANOVA on oxygen consumption showed a non-significant interaction between diet and time, indicating that changes in oxygen uptake through time were similar for both fed and starved larvae ($F=1.15$; $p=0.32$). In addition, no differences were found between fed and starved larvae ($F=1.64$; $p=0.21$). Oxygen consumption of *L. amboinensis* $Zoea_1$ larvae in both groups, however, increased significantly ($F=13.62$; $p<0.001$) from initial time to 12 h and 24 h after hatching, but remained similar from the 12th hour to the 24th hour (Table 1). The interaction between diet and time for soluble-protein content was significant ($F=16.65$; $p<0.001$), indicating that soluble-protein content in fed and starved larvae changed through time in a different manner. *Post-hoc* comparisons showed two disjoint groups of means (Table 1), with soluble-protein content in fed larvae at 12 and 24 h posthatch being significantly higher than in larvae from all other treatments.

Table 1
Mean values (±SE) of oxygen consumption, soluble-protein and TAG content, and trypsin-like activity of fed and starved *L. amboinensis* larvae at three different moments in the first 24 h after hatching (0, 12 and 24 h)

Variable	Time (h)	Starved larvae	Fed larvae
Oxygen consumption	0	0.13±0.02 Aa	0.07±0.02 Aa
(μg O_2 ind^{-1} h^{-1})	12	0.19±0.02 Ab	0.16±0.02 Ab
	24	0.21±0.03 Ab	0.22±0.02 Ab
Soluble-protein	0	0.38±0.01 Aa	0.33±0.01 Aa
(mg mg of dw^{-1})	12	0.30±0.02 Aa	0.45±0.02 Bb
	24	0.30±0.01 Aa	0.47±0.02 Bb
TAG (mg mg of dw^{-1})	0	0.05±0.01 Aa	0.07±0.01 Ba
	12	0.06±0.01 Aa	0.06±0.01 Ba
	24	0.03±0.01 Aa	0.09±0.01 Ba
Trypsin-like activity	0	0.37±0.07 Aa	0.31±0.04 Aa
(U mg $prot^{-1}$)	12	0.4±0.04 Aa	0.39±0.03 Aa
	24	0.24±0.02 Aa	0.3±0.03 Aa

Different capital letters (for treatment effects) and small letters (for larval age effects) denote significant differences ($p<0.05$) between means.

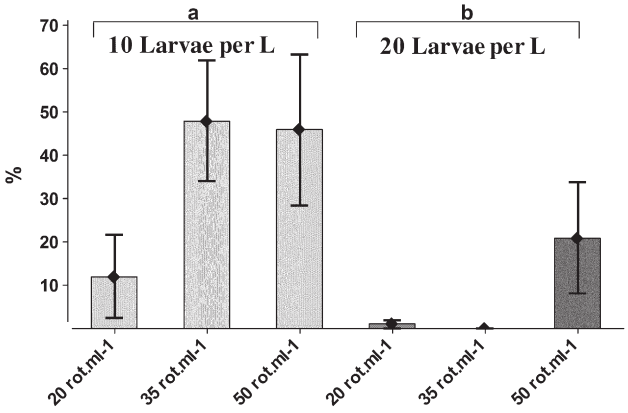

Fig. 1. Mean survival (%±SE) at 6 days after hatching of *L. amboinensis* larvae at two stocking densities (10 and 20 larvae L^{-1}) fed with three different concentrations of enriched rotifers, *Brachionus plicatilis*. Different letters over the bars indicate significant differences at $p<0.05$.

TAG content was similar through time both in fed and unfed larvae (Interaction: $F=3.29$; $p=0.07$; Time: $F=0.04$; $p=0.97$), but fed larvae had significantly higher TAG contents than starved larvae ($F=11.01$; $p<0.01$; Table 1).

Trypsin-like activity did not vary between starved and fed larvae ($F=0.001$; $p=0.97$), through time ($F=3.48$; $p=0.06$) and no interaction was detected ($F=0.86$, $p=0.45$).

3.2. Effect of microalgae as first food

Mean soluble-protein content in larvae from the different treatments varied significantly ($F=7.28$; $p<0.05$). Newman–Keuls *post-hoc* comparisons showed that at 24 h posthatch, soluble-protein content of larvae fed with *Chaetocerus gracilis* was significantly higher than for both starved larvae and that fed with *T. chuii* at the end of a period of 24 h, but was similar to the value obtained at initial time (no food; Table 2).

Mean TAG content was similar amongst all larvae ($F=3.27$; $p=0.08$). Trypsin-like activity varied significantly amongst treatments ($F=6.81$; $p<0.05$), with values for larvae fed with TC being significantly higher than all the other treatments (Table 2).

3.3. Effect of enriched versus non-enriched rotifers as food

Mean survival (±SE) of larvae fed with enriched rotifers was significantly higher (82±8%) then those fed with non-enriched rotifers after three days of life (62±3.7%; $t=2.32$; $p<0.05$; $df=8$). At the end of the experiment all larvae were $Zoea_2$.

3.4. Effect of initial stocking density and initial food concentration

The interaction term in the ANOVA was not significant ($F=1.44$; $p=0.258$), thus, changes in survival between stocking densities were independent of food concentration in larvae during the first six days of life. In addition, there were no significant differences between survival of larvae fed with different concentrations of rotifers ($F=2.73$; $p=0.085$). However, survival of larvae initially stocked at a density of 10 larvae L^{-1} was significantly higher than in those stocked at 20 larvae L^{-1} ($F=10.15$; $p<0.01$) (Fig. 1). The maximum larval survival was 48% and corresponded to larvae stocked at 10 larvae L^{-1}, fed with 35 rotifers ml^{-1}(Fig. 1). At the end of the experiment all larvae were $Zoea_2$.

Table 2
Mean values (±SE) of trypsin-like activity, soluble-protein and TAG content of *L. amboinensis* larvae starved and fed with *Tetraselmis chuii* (TC) and *Chaetocerus gracilis* (CG) during the first 24 h after hatching

Variable	Time (h)	Starved	TC	CG
Soluble-protein	0		0.33±0.01 ab	
(mg mg of dw^{-1})	24	0.27±0.01 a	0.28±0.01 a	0.36±0.01 b
TAG (mg mg of dw^{-1})	0		0.07±0.01 a	
	24	0.04±0.01 a	0.06±<0.01 a	0.08±0.01 a
Trypsin-like activity	0		0.26±0.02 a	
(U mg $prot^{-1}$)	24	0.25±0.06a	0.48±0.03b	0.28±0.03a

Initial time (no food) was used as a treatment. Different letters denote significant differences ($p<0.05$) between means.

4. Discussion

The consistent increase in oxygen consumption during the first 24 h after hatching may indicate physiological changes associated with higher energy demands as a result of ongoing development (Yagi et al., 1990; Agard, 1999). When starved or fed during the first 24 h posthatch, *L. amboinensis* larvae had similar oxygen consumption, indicating that food is not crucial to satisfy energy requirements in this short period. However, changes in TAG and soluble-protein content suggest that feeding occurs during the first 24 h posthatch, and may have a beneficial effect on the nutritional state of larvae. These results are in agreement with the work by Simoes et al. (2002), who found that early larvae of *L. debelius* fed few hours after hatching and this resulted in improved survival. Previous works (Zhang et al., 1998; Fletcher et al., 1995) have shown that newly hatched larvae depend on internal energy reserves, suggesting a lecithotrophic behaviour. Such behaviour can be argued to be facultative in accordance with the occasional planktotrophy revealed in the present study. According to several authors (e.g. Anger, 2001; Gimenez and Anger, 2001; Calcagno et al., 2003; Anger and Schubart, 2005), lecithotrophy is not uncommon among caridean larvae, and is usually related to

environments with low food availability, such as deep ocean waters, fresh water or even in some sub-polar areas. Even in highly productive environments, food resources can be patchy (Omori and Hammer, 1982) and larval feeding strategies are likely to be adapted to the variability in food availability, an important factor determining the nutritional condition and consequent growth and survival of shrimp (Le Vay et al., 2001). Anger and Schubart (2005) suggest that larvae can take advantage of external food in order to compensate pulses of high energy demand as a result of physiological stress. In *Macrobrachium rosenbergii* the transition from dependence upon stored endogenous food material, to the handling of an exogenous food supply is accompanied by physiological changes, in particular, in the hepatopancreas (Agard, 1999). Therefore, in *Lysmata* larvae the energy requirements from endogenous reserves can probably be in part sustained by exogenous food. The lecithotrophic capacity is dependent of the parental energy investment in reproduction (Anger, 2001) and, therefore, adequate husbandry and feed quality in broodstock is crucial for successful cultures.

Trypsin-like activity of digestive enzymes found in larvae of closely related *Lysmata* species is low compared to those reported for a broad range of herbivorous and omnivorous decapod larvae (Jones et al., 1997; Le Vay et al., 2001). Digestive enzymes activity, in particular trypsin-like enzymes, can be used as tools for characterisation of trophic strategies among shrimp (Kamarudin et al., 1994). Carnivorous caridean larvae, present low trypsin-like activity, probably due to the easily digestible proteins in their planktonic prey (Kumlu and Jones, 1995b; Sangha, 1996). In order to increase the digestive capacity and compensate for low trypsin-specific activity, food can stay for a long time in the digestive tract (Jones et al., 1997; Kumlu, 1999; Simoes et al., 2002).

Trypsin-like activity of *L. amboinensis* larvae found in the present study was similar to that reported for carnivorous larvae, such as *L. debelius* (Sangha, 1996).

In spite of the conspicuous carnivorous feeding strategy in *L. amboinensis,* larvae may unintentionally swallow microalgae immediately after hatching. However, intentional predation of microalgae by *L. amboinensis* larvae in the present study can not be ruled out. *T. chuii* is a large, fast moving dinoflagellate ($\approx$300 μm^3), whereas as *C gracilis* ($\approx$80 μm^3) can form long chains of cells, resulting in both microalgae to be easily predated by larvae (Helm, 1990; Renaud et al., 1999). Larvae fed with TC during the first 24 h posthatch showed a significant increase in enzyme activity (Table 2).

This finding agrees with observations that larvae can take in food particles during the first 24 h of life (Simoes et al., 2002), and that microalgae can be a part of the digestion processes by stimulating enzyme production (Kumlu and Jones, 1995a; Jones et al., 1997; Cahu et al., 1998). Apparently contradictory results from experiments 1 and 2 could be explained by the fact that in experiment 2, *T. chuii* (TC) was presented alone at 25 000 cells ml^{-1}, whilst in experiment 1 it was presented within a mixture of plankton (TC at 25 000 ml^{-1}, *C. gracilis* at 50 000 ml^{-1}, *Artemia* nauplii at 3 ml^{-1}, and rotifers at 10 ml^{-1}). Possibly the effect of TC on enzyme production was camouflaged in experiment 1, or more likely, different types, concentrations and combinations of food trigger enzyme production in different ways through complex regulatory mechanisms. According to Jones et al. (1997) enzyme activity is stimulated by food with very low protein content such as phytoplankton, resulting in optimization of protein assimilation. Overall, phytoplankton is known to be a less digestible diet, with lower energy, protein and lipid content than zooplankton. The higher soluble-protein content in larvae fed with CG (Table 2) can be related to possible higher protein content in these microalgae (Renaud et al., 1999), which can also explain why enzyme activity was not increased by the presence of CG.

Although microalgae alone are not enough to support growth or survival of *Lysmata* during larval development (Zhang et al., 1998), the use of TC showed to be valuable for enzyme activity stimulation at least during the first 24 h posthatch.

The quality of live food can have an important role on larval nutrition. However, high quality live food, in general, has a high cost. Indeed, one of the major research themes in aquaculture is directed to finding low cost alternatives to *Artemia*, such as artificial diets or other type of live foods (Thomaz et al., 2004; Martin et al., 2006). Non-enriched rotifers are known to have a low nutritional value (Samocha et al., 1989), but they are commonly used in crustacean larviculture as eventual diet supplements. In order to increase nutritional value it is possible to enrich live food with commercial products rich in lipids (Rhyne and Lin, 2004). In *Macrobrachium* spp. culture, the substitution of relatively highly energetic live food, such as *Artemia* nauplii by rotifers was unsuccessful in increasing survival and larval growth, principally when used as base diet (Thomaz et al., 2004). Results of the present study show that enriched rotifers result in a higher survival of *L. amboinensis* larvae when compared to larvae fed with non-enriched rotifers (Fig. 1), and indicate that enriched rotifers constitute a cost-effective alternative to *Artemia* for *L. amboinensis* culture during the first 3 days of life.

The use of adequate larval stocking densities adjusted to proper rotifer concentrations, provided a low cost alternative to the use of *Artemia* in fish larviculture (Lubzens et al., 1989). The increase of prey density in larviculture of crustaceans is, in general, associated with an increase in feeding rates and consequently in growth and survival (Minagawa and Murano, 1993; Barros and Valenti, 2003; Penha-Lopes et al., 2005; Lima and Souza-Santos, 2007). In the present study, however, survival of larvae after 6 days since hatching was not dependent on the relation between larval density and rotifer concentration (Fig. 1), at least for the relatively low rotifer concentrations tested. Stocking densities of 10 larvae L^{-1} consistently yielded higher survival percentages than those of 20 larvae L^{-1}, irrespective of rotifer concentration. Such observations most probably are a consequence of stress, physical damage or even cannibalism induced by the higher encounter rates between individuals at high stocking densities. However, future studies should involve treatments with rotifer concentrations above 50 rotifers ml^{-1} at higher larval stocking densities. Such studies should also considerer larval age as an important factor, since later larval stages usually show higher feeding rates than early larval stages (Minagawa and Murano, 1993; Barros and Valenti, 2003).

5. Conclusion

For the first time, oxygen consumption values, trypsin-like activity and metabolite content were determined for *L. amboinensis* larvae of different ages. *L. amboinensis* larvae revealed primary facultative lecitotrophic behaviour, which should be considered in current culture protocols. In order to stimulate the enzyme activity and optimize digestion, larvae can be fed with microalgae, such as *T. chuii*, during the first hours posthatch. Due to its low trypsin content, live food should be rich in energy content and highly digestible protein, in order to sustain or increase survival during the first days.

Enriched rotifers are an effective low cost option in *L. amboinensis* larviculture during the first 3 days after hatching, but diets should be reinforced with a variety of nutrient sources, such as *Artemia* or nematodes, thereafter.

Food concentrations should be adjusted to no less than 35 and 50 rotifers ml^{-1} for stocking densities not more than 10 larvae L^{-1}. Applying these recommendations to culture protocols may improve survival of *L. amboinensis* larvae, and should be tested on other closely related species.

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