Lecithotrophic behaviour in zoea and megalopa larvae of the ghost shrimp *Lepidophthalmus siriboia* Felder and Rodrigues, 1993 (Decapoda: Callianassidae)

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ABSTRACT

Food supply is considered critical for a successful culturing of decapod larvae. However, some species may present yolk reserve sufficient to complete their larval development without external food supply (known as lecithotrophic larval development). In the present study, two experiments were carried out in order to verify whether the callianassid *Lepidophthalmus siriboia* have lecithotrophic behaviour or, if they need external food for their larval development: Experiment 1, larvae submitted to an initial feeding period and Experiment 2, larvae submitted to an initial starvation period. High survival rate was observed in both experiments, in which only 2 megalopae and 1 zoea III died. These results strongly suggest that larvae of *L. siriboia* are lecithotrophic as they have sufficiently large yolk reserve to complete their larval development, while the megalopa stage shows facultative lecithotrophy. The larval periods of each stage of the treatments were quite similar and, despite some significant differences in some larval periods, these can be related probably to larval rearing conditions, abiotic factors or, individual variability of larval health, as well as stress caused to the ovigerous females during embryogenesis.

Key words: behaviour, Callianassidae, larval development, lecithotrophy, *Lepidophthalmus siriboia*, starvation.

INTRODUCTION

The family Callianassidae is represented by nine genera with several species in Brazil, but only one belongs to the genus *Lepidophthalmus* (Holmes 1904), the species *L. siriboia*. This species occurs on the Occidental Atlantic-Florida, Gulf of Mexico, West Indies and Brazil (from Pará to Bahia States) inhabiting submerged galleries in the intertidal zones, in very shallow waters (Melo 1999). It lives in association with a small pinnotherid crab *Pinnixa gracilipes* in the northeastern region of Pará (Lima et al. 2006). A recent study has reported that this species has a short larval development consisting of three zoeal and a megalopa stages (Abru-

nhosa et al. 2005). The larvae hatch as prezoea without natatory movements, staying in this phase for approximately 4 hours (Abrunhosa et al. 2005, 2006).

Few studies have been done on the biology of callianassids, mainly those related to their larval biology. The larviculture is recognized as an important instrument in revealing aspects of the crustacean biology. However, for successful larval culture investigations on adequate food and feeding regimes for larvae are indispensable. Thus, the type of food and the favourable period of its addition in the culture are considered critical for the success of larvae and post-larvae development.

On the other hand, some decapod species show lecithotrophic development during their larval development (Anger 1995). They hatch with yolk reserve stored

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in the midgut gland sufficient for the larvae to surpass natural barriers during their development (Anger and Darwirs 1981, Anger et al. 1985, McConaugha 1985, Anger 1995, 1996). In crustacean culture tanks, the addition of live foods may be prejudicial to the larvae in case of lecithotrophy because *Artemia* nauplii, for example, may deteriorate the water quality causing damages to the health of the larva (Abrunhosa and Kittaka 1997a).

The lecithotrophic behaviour of the Callianassidae larvae has been very little studied. Thessalou-Legaki et al. (1999) reported that larvae of *Callianassa tyrrhena* hatch with yolk reserves necessary to complete their larval development until megalopa stage even under complete starvation. But, these authors observed that when food is offered, larvae of this species may consume *Artemia* nauplii during zoeal stages and they concluded that *C. tyrrhena* have a facultative lecithotrophy development. More recently, Abrunhosa et al. (2006), describing the gross morphology of external feeding appendages and foregut of larvae and postlarvae of *L. siriboia*, observed that the mouthparts have a reduced number of setae and the foregut was under-developed. All these structures are fully setose and well-developed in megalopa and juvenile I. These facts strongly suggested a non-feeding behaviour in the zoeal stages although, they are feeding animals in the megalopa and juvenile stages. The present study investigates the effect of starvation on larvae of *L. siriboia*, in order to support the observations reported by Abrunhosa et al. (2006).

MATERIALS AND METHODS

Ovigerous females of *L. siriboia* were collected in the Canela Island (Northeast of Pará State, Brazil) with a hand-operated vacuum suction device (yabby pump). In the laboratory, they were maintained in aquaria (10L) containing filtered seawater with constant aeration and muddy-sand substratum (approximately 500 g was homogeneously added in the bottom) until larval release.

After hatching, the larvae were placed in small recipients (polyethylene, 150 mL) and were individually reared. The temperature, salinity and pH of the cultivation seawater were recorded daily in the recipients. The larvae were reared in salinity 30, temperature 27-29°C and pH 8.1-8.3. The larval development was daily monitored every thirty minutes (day and night) in each experiment. The cultivation seawater was renewed everyday. Two experiments were performed where the *L. siriboia* larvae were individually reared:

Experiment 1 – larvae of *L. siriboia* submitted to an initial feeding period. Four treatments were performed with 10 larvae/treatment, in which they were fed with *Artemia* nauplii for 1 day (treatment 1), 2 days (treatment 2), 3 days (treatment 3) and everyday (control treatment), respectively.

Experiment 2 – larvae of *L. siriboia* submitted to an initial starvation period. Four treatments were performed with 10 larvae/treatment in which, they were starved for 0 day (i.e. they were not fed – control treatment), 1 day (treatment 1), 2 days (treatment 2) and 3 days (treatment 3), respectively. After the respective period of starvation, the larvae were fed with *Artemia* nauplii.

STATISTICAL ANALYSIS

The data analyses of the cumulative larval duration (CLD) of each larval stage (in both, feeding and starvation experiments) were performed in order to verify whether the larval period was affected when the larvae were submitted to different starvation or feeding periods. For the statistical analyses of CLD data the nonparametric *Kruskal-Wallis* (*H-test*) was used, after tests for normality and homogeneity of variances (*Shapiro-Wilk* and *Levene's* tests, respectively). The *Dunn's* test was used for multiple comparisons (*Post hoc* test) among treatments (including control treatment) belonging to the respective experiments. Significant differences were considered when $P < 0.05$. All statistical analysis followed standard techniques (Sokal and Rohlf 1995).

RESULTS

L. siriboia shows a short larval development, consisting of three zoeal and a megalopa stages. No feeding behaviour was observed in the larvae (from zoea I to zoea III) during these whole experiments. But, after metamorphosis to megalopal stage they became feeding crustaceans. High survival rate was observed in both treatments with initial starvation and feeding periods (see below).

EXPERIMENT 1: LARVAE SUBMITTED TO AN INITIAL FEEDING PERIOD

Survival rate and intermoulting periods

In the first experiment (initial feeding period), the percentage of zoea of *L. siriboia* moulting to megalopa stage was 100% in all treatments. However, two megalopae died in this experiment before moulting to juvenile I stage (1 megalopa in the treatment fed during 2 days and 1 in the treatment fed during 3 days, respectively). In the second experiment (initial starvation period), only one larva died (zoea III) in the treatment that larvae have starved during 2 days. No mortality was observed in other zoeal or megalopal stages.

1. Larvae of *L. siriboia* fed everyday (Control): The intermoulting period of zoea I, zoea II, zoea III and megalopa were 17h, 33h, 70h e 189h, respectively (Fig. 1).
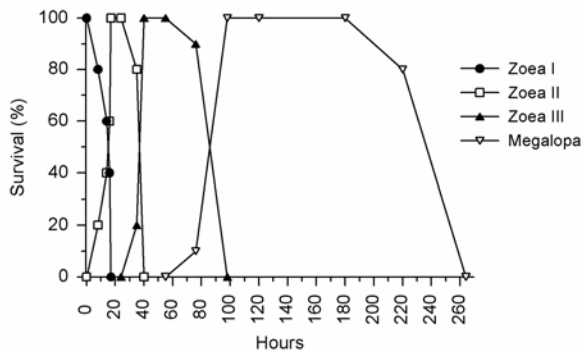

Fig. 1 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial feeding period. In this treatment they were fed everyday (Control treatment).

2. Larvae of *L. siriboia* fed during 1 day: The intermoulting period were 18h, 31h, 77h e 155h for zoea I, zoea II, zoea III and megalopa, respectively (Fig. 2).

3. Larvae of *L. siriboia* fed during 2 days: The intermoulting periods were 15h, 37h, 48h e 197h for zoea I, zoea II, zoea III and megalopa, respectively (Fig. 3).

4. Larvae of *L. siriboia* fed during 3 days: The intermoulting period were 20h, 35h, 90h e 174h for zoea I, zoea II, zoea III and megalopa, respectively (Fig. 4).
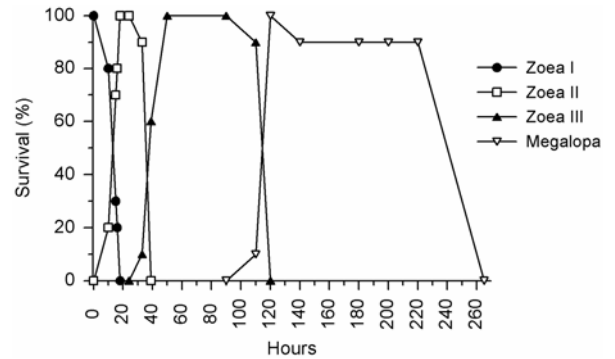

Fig. 2 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial feeding period. In this treatment, they were fed during 1 day only.
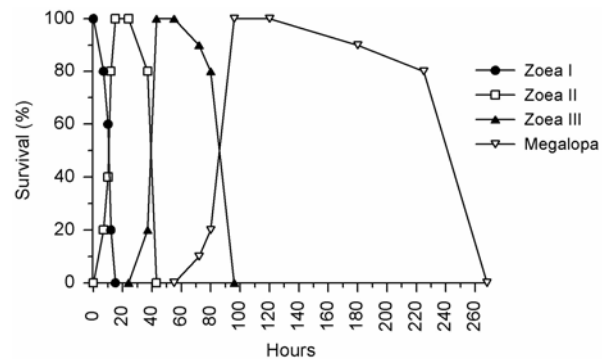

Fig. 3 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial feeding period. In this treatment, they were fed during 2 days only.
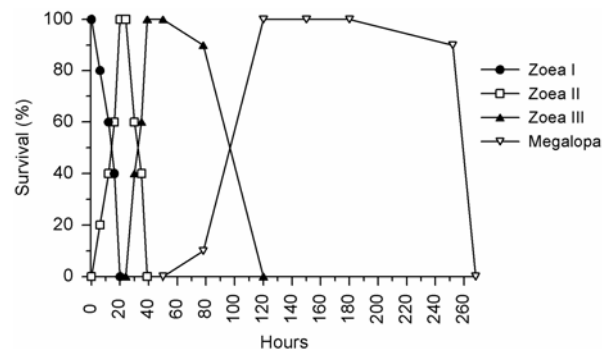

Fig. 4 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial feeding period. In this treatment, they were fed during 3 days.

Cumulative Larval Duration (CLD)

For cumulative CLD of zoea I, a significant difference was observed among respective treatments ($H = 18.2$; $d.f. = 3$; $P < 0.05$; Fig. 5). In this larval stage, the cumulative CLD ranged from 6.0 (± 1.5) hours, observed in treatment where larvae of *L. siriboia* were fed everyday, to 8.9 (± 1.1) hours, recorded in the treatment with larvae fed during 2 days. A significant difference ($P < 0.05$) was observed between treatments 1, 3 and 4, respectively (Fig. 5).
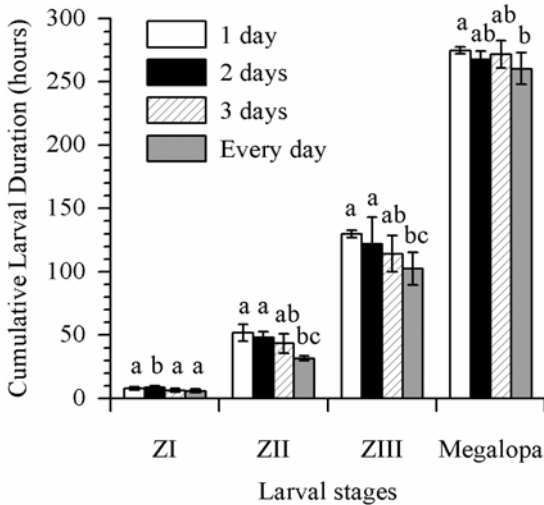

Fig. 5 – Cumulative larval duration in hours (CLD, average $\pm$ standard deviation) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial feeding period. 1 day (treatment 1), 2 days (treatment 2), 3 days (treatment 3) and everyday (control treatment). Significant differences are represented by letters when $P < 0.05$ after *Dunn's* test. Treatments with at least one same letter in common did not differ statistically ($\alpha = 0, 05$). ZI, ZII, ZIII (zoeal stages).

Similar to the previous stage, the cumulative CLD of zoea II, also showed a significant distinction among the treatments ($H = 23.9$; $d.f. = 3$; $P < 0.05$; Fig. 5). The smallest cumulative CLD was recorded in the treatment for those larvae fed everyday (31.7 ± 2.0 hours) and the largest was observed in the treatment in which larvae were fed during one day (52.0 ± 6.6 hours). Significant differences ($P < 0.05$) were observed between treatment 4 versus treatments 1 and 2 (Fig. 5).

For zoea III, the cumulative CLD was significantly distinct among treatments ($H = 12.4$; $d.f. = 3$; $P < 0.05$; Fig. 5). This stage exhibited the smallest cumula-

tive CLD in treatment for larvae fed everyday (102.5 ± 12.8 hours) and the largest in the treatment with larvae fed during one day (129.7 ± 2.8 hours). With zoea II, significant differences ($P < 005$) were also observed between treatment 4 versus treatments 1 and 2 (Fig. 5).

Finally, for megalopa stage, the cumulative CLD also showed a significant distinction among treatments ($H = 8.6$; $d.f. = 3$; $P > 0.05$; Fig. 5) similar to the early zoeal stages. However, significant differences ($P < 0.05$) were observed only between the treatments 1 and 4 (Fig. 5). The smallest cumulative CLD was 260.6 ± 12.8 hours, observed in the treatment with megalopae fed everyday and the largest was 275.0 ± 2.7 hours, recorded in the treatment with megalopae fed during one day (Fig. 5).

EXPERIMENT 2: LARVAE SUBMITTED TO AN INITIAL STARVATION PERIOD

Survival rate and intermoulting periods

Only one larva died (zoea III) in the treatment in which larvae have starved during one day. No mortality was observed in the megalopal stage. Different intermoulting periods were observed in each respective treatment.

1. Larvae of *L. siriboia* submitted to entire starvation (Control): The intermoulting periods for zoea I, zoea II, zoea III and megalopa were 22h, 71h, 82h, 191h, respectively (Fig. 6).
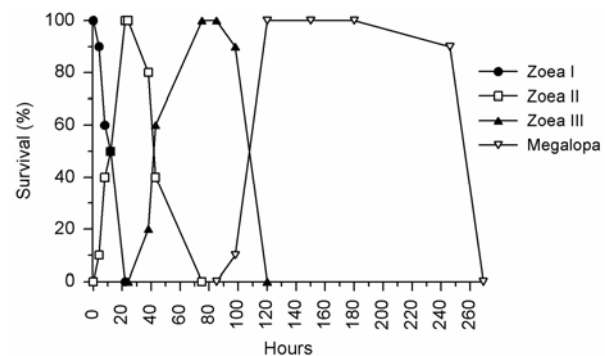

Fig. 6 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial starvation period. In this treatment, they were submitted to entire starvation (Control treatment).

2. Larvae of *L. siriboia* starved during 1 day: The intermoulting periods for zoea I, zoea II, zoea III and

megalopa were 18h, 30h, 60h, 170h, respectively (Fig. 7).

3. Larvae of *L. siriboia* starved during 2 days: The intermoulting periods for zoea I, zoea II, zoea III and megalopa were 16h, 37h, 67h, 176h, respectively (Fig. 8).

4. Larvae of *L. siriboia* starved during 3 days: The intermoulting period for zoea I, zoea II, zoea III and megalopa were19h, 44h, 62h, 183h, respectively (Fig. 9).
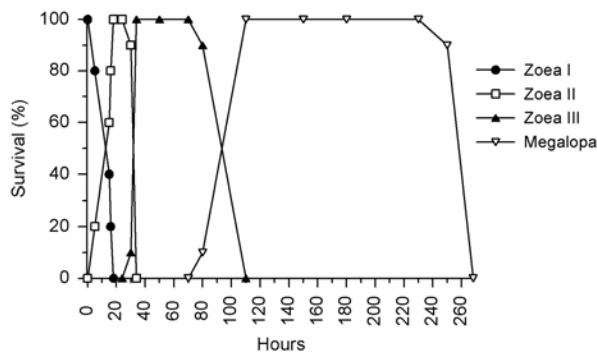

Fig. 7 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial starvation period. In this treatment, they were starved during 1 day.

Fig. 8 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial starvation period. In this treatment, they were starved during 2 days.
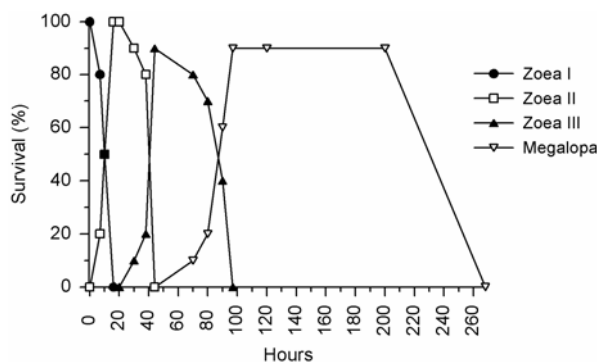
Cumulative Larval Duration (CLD)

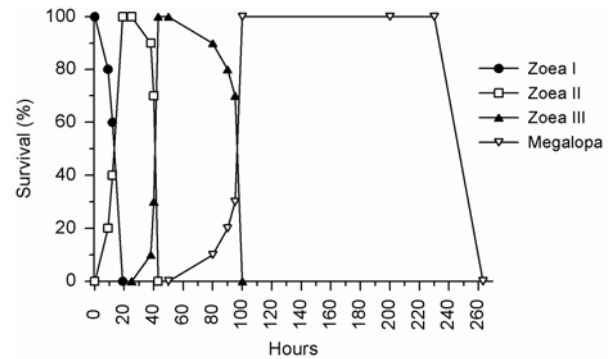The different starvation periods for zoea I did not result in significant differences in the cumulative CLD among

Fig. 9 – Survival (%) and intermoulting period (hours after hatching) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial starvation period. In this treatment, they were starved during 3 days.

treatments ($H = 1.5$; $d.f. = 3$; $P > 0.05$), which showed a cumulative CLD around 7 hours (Fig. 10). In contrast, for zoea II this cumulative CLD presented a significant difference among treatments ($H = 20.4$; $d.f. = 3$; $P < 0.05$; Fig. 10). For this zoeal stage, the smallest cumulative CLD (33.3 ± 8.4 hours) was observed in treatment with larvae of *L. siriboia* starved during 1 day and the largest (53.5 ± 8.8 hours) in treatment with larvae starved during 2 days. Of all treatments, only treatments 1 versus 4 and 2 versus 3 were not significantly distinct ($P > 0.05$) (Fig. 10).

The cumulative CLD of zoea III stage was significantly distinct among treatments ($H = 16.9$; $d.f. = 3$; $P < 0.05$; Fig. 10). The smallest cumulative CLD (111.5 ± 20.1 hours) was recorded in the treatment in which larvae of *L. siriboia* were starved during 1 day and the largest (132.2 ± 2.1 hours) was observed in the treatment with larvae starved during 3 days. For this zoeal stage, significant differences ($P < 0.05$) were only observed between treatments 1 versus 3 (Fig. 10). For cumulative CLD of megalopae stage, no significant differences were observed among the different starvation treatments ($H = 3.9$; $d.f. = 3$; $P > 0.05$), which values ranged from 261 to 274 hours (Fig. 10).

DISCUSSION

In the present study, both larvae of *L. siriboia* submitted to entire feeding and starvation succeeded moult to the megalopa stage. In both, feeding and starvation treatments, the survival rates were quite high. This fact
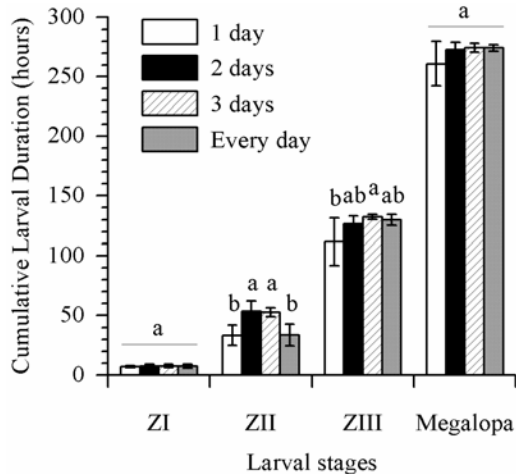
Fig. 10 – Cumulative larval duration in hours (CLD, average ± standard deviation) of *Lepidophthalmus siriboia* larvae reared in the laboratory and submitted to an initial starvation period. 1 day (treatment 1), 2 days (treatment 2), 3 days (treatment 3), no feeding (control treatment). Significant differences are represented by letters when $P < 0.05$ after *Dunn's* test. Treatments with at least one same letter in common did not differ statistically ($\alpha = 0, 05$). ZI, ZII, ZIII (zoeal stages).

strongly suggests a lecithotrophic behaviour in this species. The lecithotrophic behaviour has already been observed for other species of the *Lepidophthalmus* genus. Nates and Mckenney Jr. (2000) have investigated the biochemical composition of the callianassid *L. louisianensis*, and revealed that those larvae are adapted for a lecithotrophic life.

Brachyuran crustaceans with extended larval development have no yolk reserve enough to reach the megalopa stage without external food (Abrunhosa and Kittaka 1997a). The opposite case is when a species has abbreviated larval stages and can complete the whole larval development independently of an external food source (Abrunhosa and Kittaka 1997a, b). Studies accomplished with larvae of the semiterrestrial crab *Sesarma curacaoense* (de Man 1892), in which the larval cycle is short, indicate that they complete their zoeal development without available food. However food is necessary for megalopa to reach juvenile stage (Anger 1995).

Detailed morphological studies on the digestive system of larvae and post-larvae of crustacean decapods have demonstrated a narrow relationship among the morphology of the mouthparts and foregut and feeding behaviour of the individual (Abrunhosa and Kittaka 1997a, Abrunhosa et al. 2003, Abrunhosa et al. 2006). These authors observed that puerulus of the spiny lobster and the glaucothoe of king crabs bear maxillae and foregut poorly developed and uncalcified. Thus, they suggested a non-feeding behaviour in the transitory stage of these crabs. This suggestion has been confirmed in culture experiments in the laboratory (Abrunhosa and Kittaka 1997b). Abrunhosa et al. (2006) described detailed morphological features of the mouthparts and foregut of *L. siriboia* and observed that the inner appendages of maxillule, maxillae and maxillipeds are rudimentary, lacking setae, and the foregut has a reduced filter press. These authors suggested a non functionality of these structures and, consequently, no feeding behaviour during zoeal development of this species. The results of the present study support such hypothesis.

According to Abrunhosa et al. (2006), after the metamorphosis to megalopa stage, a drastic morphological change occurs in the mouthparts and foregut of *L. siriboia*. All these structures become specialized, which indicate that food is necessary during this phase. Thus, *L. siriboia* megalopa may eat a great variety of food (Abrunhosa et al. 2006). However, experiments accomplished in the present study have demonstrated that the *L. siriboia* megalopae were able to succeed in moulting to juvenile stage without external food. This strongly indicates a facultative lecithotrophy for this species during this stage. Some callianassids were described as having a relatively extended larval development. The *Callichirus major*, for instance, has been reported with 5 zoeal and a megalopal stages (Strasser and Felder 1999). These authors observed that the external and internal feeding structures are more specialized in the zoea when compared to *L. siriboia*, indicating that this species is probably unable to surpass the larval period without feeding (Rodrigues 1976). On the contrary, *C. tyrrhena* has presented an optional lecithotrophy if food is not available in the environment or culture (Thessalou-Legaki et al. 1999).

In the first experiment (initial feeding period) of the present study, marked differences were only observed in the cumulative larval duration (CLD) of the zoea II, III and megalopa stages in the treatments with food available for 1 and everyday. The larval periods of each

stage of the treatments were quite similar and, despite some significant differences were observed during the larval period. This probably can be related to larval rearing conditions, abiotic factors or still, individual variability of larval health, as well as stress caused to the ovigerous females during embryogenesis. Further studies are needed to test other factors on the larval development of the *L. siriboia*, in order to verify the effects in the survival rate and CLD.

ACKNOWLEDGMENTS

RESUMO

A alimentação é considerada crítica em cultivo de larvas de decápodes. No entanto, algumas espécies podem apresentar reservas nutritivas suficientes para completar o desenvolvimento larval sem a necessidade de alimentação externa (conhecido como desenvolvimento larval lecitotrófico). No presente estudo, dois experimentos foram realizados para verificar se o callianassídeo *Lepidophthalmus siriboia* tem comportamento lecitotrófico ou se precisa de alimento externo para completar o desenvolvimento larval: Experimento 1, larvas submetidas a um período inicial de alimentação e, Experimento 2, larvas submetidas a um período inicial de inanição. Em ambos os experimentos, observou-se altas taxas de sobrevivência, com apenas 2 megalopas e 1 zoea III mortos. Estes resultados sugerem fortemente que larvas de *L. siriboia* são lecitotróficas, tendo reservas suficientes para completar o desenvolvimento larval, enquanto o estágio de megalopa apresenta lecitotrofia facultativa. Os períodos larvais de cada estágio nos tratamentos foram bastante semelhantes. No entanto, foram observadas algumas diferenças significantes entre alguns períodos, os quais podem estar relacionados às condições de cultivo, provavelmente devido a fatores abióticos, à variabilidade individual da condição larval, ou ainda a fatores como estresse causado às fêmeas ovígeras durante embriogênese.

Palavras-chave: comportamento, Callianassidae, desenvolvimento larval, inanição, lecitotrofia, *Lepidophthalmus siriboia*.

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