

Lecithotrophic development of the pea crab *Orthothenes barbatus* (Desbonne, 1867) (Decapoda: Brachyura: Pinnotheridae).

Galindo, L. A.; Bolaños, J. A.; Hernández, J. E. and Rivero, W.

Laboratorio de Carcinología, Escuela de Ciencias Aplicadas del Mar, Universidad de Oriente, Núcleo Nueva Esparta. Apartado Postal 147, Porlamar, Isla de Margarita, Venezuela.
lgalindo@ne.udo.edu.ve

Abstract

The larval phases of *Orthothenes barbatus* were followed *in vitro*. The species is a symbiont of the gastropod *Cittarium pica* in the Archipelago of Los Roques, Northeastern Venezuela. *O. barbatus* exhibits an abbreviate larval development, expressed by the time necessary to complete it (4 to 5 days from hatching) and by the number of larval stages attained (two zoeas and one megalopa) before complete metamorphosis into the first crab. A total of 180 larvae, released from four different females, were selected and divided in two experimental groups: each larva of the first group (Control) was supplied with 5 to 10 *Artemia* nauplii per ml daily and the larvae of the second group (Essay) were not fed. The larvae were culture individually and water was renewed daily. The experiment lasted 32 to 37 days. By the 5th to 6th day all specimens had molted to first crab. There was no significant difference between treatment groups, which strongly points to a case of lecithotrophy. After 16th day the mortality increased for both groups, reaching 97.22% at 32nd day.

Key words: Lecithotrophy, larval development, starvation, symbiont.

Introduction

The family Pinnotheridae is represented by approximately 275 species, (Cuesta & Felder), unpublished data, of small crabs; most of them live, during their postlarval stage, a symbiotic relationship with other marine invertebrates such as mollusks, echinoderms, annelids and tunicates (cf. Schmitt *et al.*, 1973; Campos, 1990 & 1996; Campos & Griffith, 1990; Stevens, 1992; Manning, 1993; Marques & Pohle, 1995; Manning & Felder, 1996). Throughout the larval development they are planktonic (Silas & Alagaraswami, 1967; Fenucci, 1975), except *Tunicotheres moseri*, whose larvae are not liberated into the water but are retained by the female (Bolaños *et al.*, in prep.). Information concerning the diversity of pinnotherids in Venezuelan marine waters is scarce. Only seven species have been reported: *Dissodactylus crinitichelis*, *Chypeasterophilus stebbingi*, *Pinnixa faxoni*, *Tumidotheres maculatus*, *Zaops ostreum*, *Tunicotheres moseri* and *Orthothenes barbatus* (cf. Rodríguez, 1980; Ramos, 1986; Hernández & Bolaños, 1995; Calderón *et al.*, 1996; and Hernández *et al.*, 1999).

The genus *Orthothenes* is conformed by six species (cf. Campos, 1989; Geiger & Martin, 1999): *O. haliotidis*, *O. turboe*, *O. serrei*, *O. strombi*, *O. unguifalcula* and *O. barbatus*. Of them only *O. barbatus* has been reported in Venezuelan waters, specifically at the Archipelago of Los Roques, where it inhabits the gastropod *Cittarium pica* as a symbiont. As far as we know, *O. barbatus* has been the object of few studies, limited to a faunal report (Ramos, 1986), and the larvae morphology reviewed by Bolaños *et al.* (in prep.). The objective of the present work was to verify whether *O. barbatus* could complete its larval development without capturing food from the field, which can be interpreted as a case of lecithotrophy.

Material and Methods

Ovigerous females of *Orthothenes barbatus* were obtained from pallial cavity of the gastropod *Cittarium pica*. The samples were collected by hand under rocks at the intertidal area of the Archipelago of Los Roques, northwestern Venezuela (11°44'45"-11°58'36" N and 66°32'42"-66°52'27" W) in December 2000. Organisms were transported to the laboratory where they were separated individually in 4 l glass aquaria supplied with aeration, until hatching.

A total of 180 larvae, released from four different females, were selected and divided in two experimental groups: larvae in the first group (Control) were supplied with 5 to 10 *Artemia* nauplii per ml daily, and the second group (Essay) was not fed. The larvae of each group were cultured individually in plastic tissue culture-plates of 2 ml capacity per well. Larvae were checked daily under a stereoscope and percentage of mortality for each stage and percentage of molted specimens were recorded.

The culture was done at ambient temperature (25–28 °C), using flowing sea water (salinity 36–38) previously filtered with a filter sleeve (1 mm-pore) and sterilized with ultraviolet light (UVL). Water was changed and freshly hatched nauplii of *Artemia* were added daily. Proportions for both the Control and the Essay group were analyzed statistically by using a one-way ANOVA ($\alpha=0.05$) (Sokal & Rohlf, 1991).

Results

Larval development of *O. barbatus* includes two zoeal stages (with duration of 1 and 2 days, respectively) and one megalopa (which lasted 1 to 2 days). The first crab was reached by day 4-5 after hatching the eggs.

Figure 1 shows the duration of postembryonic development and the survival of *O. barbatus* for both Control (Fig. 1A) and Essay (Fig. 1B) groups. There were no significant differences ($F_{0.05(1,57)}$) in mortality between groups, demonstrating that larvae may develop without the necessity of capturing food from the environment. This observation is interpreted as evidence of lecithotrophic behaviour in the species.

Between the fifth to the sixth experimenting day, the first crab survival reached its maximum value (78.9 and 72.2% for the groups treated with and without food supply, respectively). The first crab may survive for a long time (25 days) without feeding, though the survival is affected while time passes by. After 15 days the percentage of living crabs was close to 51%. However, the mortality increased, after that date, reaching 97.22 % at day 32.

Discussion

Orthothenes barbatus exhibits abbreviated larval development (Bolaños *et al.*, in prep.), as expressed by the time necessary to complete it (4-5 days) and the number of larval stages required to complete metamorphosis (two zoeas and one megalopa) to molt to first crab. Although in larval culture the major mortality is usually registered during the ecdysis events (Rabalais & Gore, 1985 in Shirley & Zhou, 1997), in the present study, there were no evident relationship between the molt period and mortality, as it has been otherwise reported for other crustacean (Shirley & Zhou, 1997).

The fact that *O. barbatus* can successfully complete the entire larval development with relative high survival values (78.9%) without requiring food from the environment, substantiates that this species possesses lecithotrophic behaviour during these early life cycle stages. It could be a particular adaptation related to the close symbiotic relationship between *O. barbatus* with its host *Cittarium pica*, though this hypothesis has to be demonstrated. It is valid to suppose that once an individual has finished its larval development, will search for an adequate symbiont

where to stay the rest or most part of its life cycle. In this period, feeding is not required yet, however, if during certain amount of time the crab has not found a convenient host, the nutritional reserves will drain off, and the crab eventually will die.

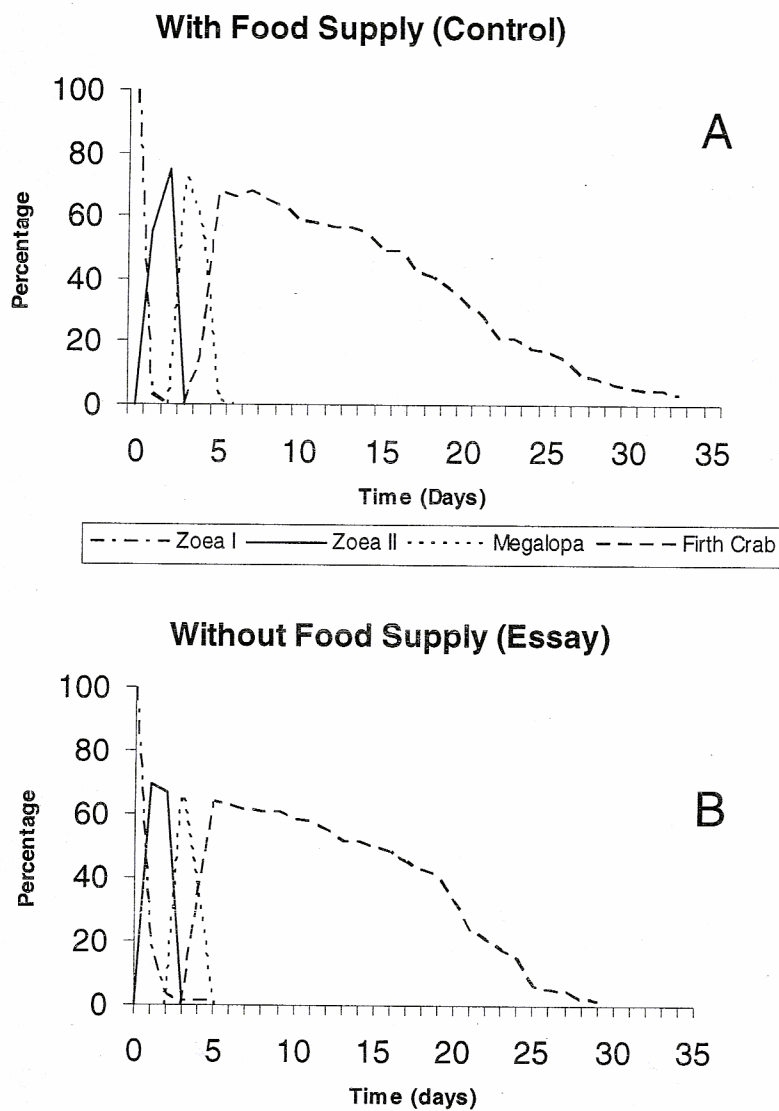


Figure 1: Duration time and survival of the larval stages and the first crab of *Orthothes barbatus*. A: with food supply (Control). B: without food (Essay).

It may be inferred that biochemical characteristics of the egg-yolk of *O. barbatus* should guarantee, within more than one month, the energetic requirements necessary for the organism maintenance. Above all, if it is considered the high metabolism rate that the first crabs should have when it is actively looking for a symbiont to live with, for the rest of its life.

During the present experiment, ecdysis into second crab did not occurred approximately within 25 to 27 days of first crab stage persistence. *O. barbatus* might require an "exogenous agent", probably; some stimulus from the host will induce the process to molt into second crab. Numerous studies point up the influence of external stimuli in the molting patterns of different crustaceans. It has been found that contact with host or adults tissue facilitates settlement or is an obligate requirement to complete development (i.e. Castro, 1978; Pohle & Telford, 1981; Jensen, 1989; O'Connor, 1991). It is necessary to submit some first crabs exemplars of this

crustacean to inputs extracted from the host *Cittarium pica* and/or other gastropod, to confirm the hypothesis of the ecdysis of juveniles of *O. barbatus* is regulated by the symbiont presence and also whether the relation between the two organisms is mono-specific. The demonstrations of these hypotheses are in progress.

In the Control group, *O. barbatus* was not observed ingesting *Artemia*, neither into the digestive track nor actively capturing any pieces of the nauplii. It could be possible that food supply were not propitious, our that larvae might prefer some other special type of food over *Artemia* nauplii. However, as shown by the similarity of both graphics (Fig 1) our data support the hypothesis that *O. barbatus* larvae do not need to be fed at all, at least until the first crab stage. It has been mentioned that *Artemia* nauplii can associate with larval appendices, interfering with swim and breath capacities (see Shirley & Zhou, 1997; Taishaku & Konishi, 2001), giving place to a major mortality rate for the Control than the Essay. Since similar mortalities for both experimental groups were recorded; in the present study, *Artemia* nauplii do not appear to interfere in the regular processes of larval development of *O. barbatus*.

Among crustaceans, it is common to find that the larvae conserve some amount of embryonic yolk after hatching. This yolk will be used as a nutritional complement for the beginning of the larval development. It was observed that *O. barbatus* larvae have complete lecithotrophic habits, so the entire development can be finished without capturing food from the ambient; contrasting to the partial and facultative lecithotrophic behaviour described by Strathmann (1987) for different larvae of other crustacean species. Rice & Provenzano (1965, in Taishaku & Konishi, 2001) mentioned temperature as a determinant factor for development, so the same specie might be lecithotrophic in certain temperature intervals, while in some other temperature range the organisms display a different feeding mode. The present study was done between 25-28 °C, similar to the natural habitat where *O. barbatus* can be found, so temperature should not interfere in our results. Experiment should be planned with different temperature gradients to verify whether there are optimal conditions for lecithotrophy to occur, and/or whether such circumstance is facultative.

This feeding mode can be found in other members of pinnotherids such as *Tunicotheres moseri* (Hernández *et al.*, in prep.). Detailed studies should be done looking for lecithotrophic behaviour as a general strategy among the family Pinnotheridae and to confirm if that feeding mode is related with the symbiotic habits of these organisms.

Starting from the fact that feeding is a every important limitation when using an organism for bioassays; and considering the lecithotrophic feature, the short developmental period and the success of both survival and molting, leads one to suggest that *O. barbatus* could be an excellent model for *in vitro* experiments; as it has also been indicated for the Pinnotherid *Tunicotheres moseri* by López Greco *et al.* (2001).

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