

# Larval cannibalism rates in the mangrove crab *Ucides cordatus* (Decapoda: Ocypodidae) under laboratory conditions

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## Abstract

Predation of zoeas by megalopae of *Ucides cordatus* is frequently observed in the laboratory during larval rearing, a phenomenon that could considerably reduce the output of larviculture. Experiments were carried out in the present study to assess how the survivorship of larvae at the end of the larviculture is influenced by cannibalism by megalopae on the larvae of earlier stages, as well as on other megalopae. In addition, tests were performed to assess whether the adoption of different feeding protocols can decrease cannibalism rates. Experiments were carried out in plastic vials containing ocean water (salinity 25 g L<sup>-1</sup>) under controlled environmental conditions (26 °C and 16:8 h LD photoperiod). An ensemble analysis of all the developmental stages indicated that zoeal mortality rates were significantly higher in the presence of megalopae, a result that is consistent with cannibalism by megalopae. However, separate analysis for each developmental stage indicated that only zoea IV, V and VI show reduced survivorship. No cannibalism was detected among megalopae. Food supplementation using *Artemia* sp. at a density of 6 nauplii mL<sup>-1</sup> proved to be successful in reducing cannibalism rates, whereas supplementation at a lower density (0.3 nauplii mL<sup>-1</sup>) failed to show such an effect. The implications of these results for the larviculture of *U. cordatus* are discussed.

**Keywords:** *Ucides cordatus*, cannibalism, zoea, megalopa

## Introduction

The mangrove crab *Ucides cordatus* (Linnaeus), locally known as 'caranguejo-uçá', has a wide geographical distribution along the western Atlantic, from Florida (USA) to the Brazilian state of Santa Catarina. This crab is considered as an important fishery resource for local populations throughout the Brazilian coast, particularly those surrounding estuarine systems (Glaser 2003).

The combination of overfishing, mangrove habitat degradation and, more recently, an infectious disease (lethargic crab disease, Boeger, Pie, Ostrensky & Patella 2005) has contributed to a drastic decrease in the natural stocks of *U. cordatus* in several parts of Brazil. A possible alternative to help recover these populations is the mass production of megalopae in the laboratory followed by their release in the affected areas.

The technology necessary to achieve this goal is being developed by our research group from 2001. Current efforts have been able to produce more than 1 000 000 megalopae per season during restocking programmes (Silva, Ostrensky, Ventura, Santos & Boeger 2006). Larviculture in the laboratory begins with the capture of ovigerous females in natural mangrove areas. After the eggs are released in rearing tanks, larvae go through all the developmental stages until they are megalopae, when they are finally released into the mangrove areas.

Right after the moult from zoea VI to megalopa, crab larvae experience dramatic morphological and behavioural changes. Megalopae have benthonic

habits, are more agile and cease to use the maxillipeds for locomotion, a function that is taken over by pleopods (Anger 2001). In addition, they possess chelipods that are more strongly developed than in the earlier stages, giving them an enhanced predation capacity in relation to earlier stages (Moksnes, Lipcius, Pihl & Van Montfrans 1997).

During larviculture in the laboratory, particularly after the moulting to megalopa, cannibalism can be observed among the larvae being cultured, particularly in the case of megalopae preying on younger larval stages. Cannibalism is often recognized as a key juvenile mortality factor among crab species (Lovrich & Sainte-Marie 1997; Baylon & Failaman 1999; Moksnes 1999; Aileen, Zulfigar, Fujii, Fukuda & Terazaki 2000). Indeed, according to Zmora, Findiesen, Stubblefield, Frenkel and Zohar (2005), cannibalism among megalopae and juveniles is one of the reasons for failures in the development of larvicultural methods for a variety of crab species.

The aim of the present study was to assess the importance of cannibalism of younger larval stages by megalopae in laboratory larviculture, as well as to determine which larval stages are most susceptible to predation. Finally, we tested whether different feeding protocols reduced mortality rates through cannibalism.

## Materials and methods

### Larval rearing

Larvae for the experiments were obtained from ovigerous females of *U. cordatus* collected in the municipality of Antonina, state of Paraná, southern Brazil (25° 25'08"S, 48° 42'33"W). Collected females were brought to the laboratory, where they were maintained in 1000 L plastic tanks filled with seawater and maintained under controlled environmental conditions (26 °C, pH 8 and salinity 26 g L<sup>-1</sup>) until the larvae hatched, which occurred without any artificial stimulus. The larvae that were used in the experiments were kept in the mass cultivation containers until they reached the desired stage for each test. During larviculture, specific food was provided for each developmental stage, starting from a diet including only algae (*Tetraselmis* sp. and *Chaetoceros* sp.) for the initial stages to the inclusion of nauplii of *Artemia* sp. starting from stage V zoeas.

### Cannibalism of megalopa on zoea

Experiments were carried out in an environmental room under controlled temperature (26 °C) and

photoperiod (16:8 h LD cycle). Each experimental unit was a clear 300 mL plastic container (bottom diameter 5 cm, top diameter 8 cm, height 10.5 cm) kept on a dark surface to facilitate observation of the larvae. The water used in the experiments (salinity 26 g L<sup>-1</sup>) was previously disinfected using sodium hypochlorite (5 mg L<sup>-1</sup>), then neutralized and passed through a 5 µm cellulose filter. The water in each experimental container was replaced and the number of zoea and megalopae (live, dead or cannibalized) was counted every 24 h. Dead individuals were replaced by new larvae of the same developmental stage at the time of the census.

Twenty 300 mL experimental vials were used during the experiments under the same environmental conditions as during larviculture. Each experimental vial received 20 zoeas of different stages and two recently metamorphized megalopae, whereas control vials only received zoeas to provide an indication of background mortality rates in the absence of potential cannibalism by megalopae. A basic diet composed of microalgae (*Chaetoceros muelleri*, 350 000 cells mL<sup>-1</sup>) and rotifers (*Brachionus plicatilis*, 10 individuals mL<sup>-1</sup>) was provided in all containers to serve as food for the zoeas and to prevent deaths unrelated to predation.

### Effect of food supplementation on cannibalism rates

Three experimental treatments were used in addition to the control group, with five replicates each. In the first treatment, larvae did not receive supplementary food in addition to the basic diet described above. In the second treatment, recently hatched *Artemia* sp. nauplii were provided as supplementary food to megalopae at a density of 0.3 nauplii mL<sup>-1</sup>, the same density used in laboratory mass rearing tanks. In the third treatment, food supplementation was increased to a density of 6 nauplii mL<sup>-1</sup>. The control group was identical to the first treatment except for the lack of megalopae. Given that the experiments depended on the availability of crab larvae from mass larviculture in the laboratory, simultaneous tests with larvae of all stages were not feasible. Thus, the experiment was carried out in several stages. Each stage lasted for 2 days and tested the effect of cannibalism of one of the zoeal stages, except for zoea II, given that this stage was not available at the beginning of the experiments.

### Cannibalism among megalopae

The existence of cannibalism among recently metamorphized megalopae was tested by varying their density in the experimental containers. Three experimental treatments were used in which two, three and five megalopae were added to experimental vials containing 200 mL of water, with three replicates for each treatment. A control group was composed of 10 experimental vials, each containing a single megalopa in 100 mL of water. All experimental vials were provided with *Artemia* sp. at a density of 0.3 nauplii mL<sup>-1</sup> (the food density usually provided in the mass rearing tanks). Survivorship was monitored daily for 7 days.

A second set of experiments tested whether the absence of food induced cannibalism among megalopae. Five 200 mL containers with five megalopae each were deprived of food for 5 days, whereas five additional containers were provided with *Artemia* sp. at a density of 0.3 nauplii mL<sup>-1</sup> (control group).

Algae and rotifers were not added in these experiments because previous observations indicated a much stronger preference of megalopae towards *Artemia* than other food items.

### Statistical analysis

Given that a Shapiro–Wilk test indicated that the data from the experiments deviated significantly from normality, all analyses were conducted using non-parametric statistics. First, an ensemble analysis of mortality rates combining all developmental stages and comparing them with their combined control groups was conducted using a Mann–Whitney test, followed by separate tests of each developmental stage with its respective control group. The aim of these tests was to determine if there was a statistically significant effect of the presence of megalopae on the survivorship of zoeas, either in combination or separately for each developmental stage. The possible effect of alternative food sources was tested by comparing the different feeding treatments within each developmental stage using Kruskal–Wallis tests, followed by multiple comparisons of mean ranks for all groups as *post hoc* tests. Finally, survivorship of megalopae under different density groups was tested using a Kruskal–Wallis test, and the test of whether starvation would induce cannibalism among megalopes was tested using a Mann–Whitney test.

## Results

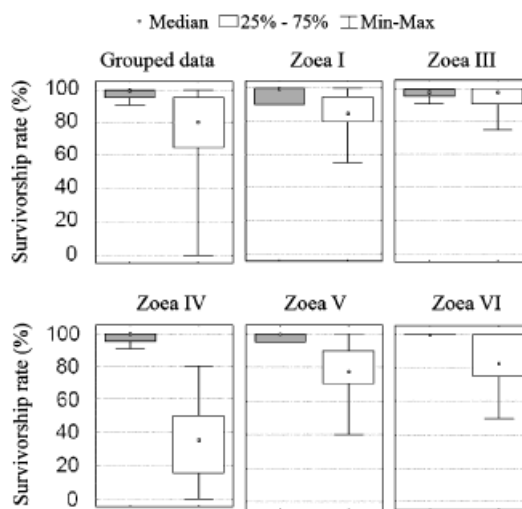
### Cannibalism of zoeas by megalopae

The results of the mortality data were consistent with a marked impact of cannibalistic behaviour of the megalopae of *U. cordatus* on larvae of different stages. When all the developmental stages were combined, there was a statistically significant increase in zoeal mortality in the presence of megalopae in relation to control groups ( $U = 419$ ,  $N = 50$ ,  $P = 0.0001$ ). However, if the analyses were carried out separately for each developmental stage, a statistically significant effect of the presence of megalopae on larval mortality was detected only in zoea I ( $U = 19$ ,  $N = 10$ ,  $P < 0.05$ ), zoea IV ( $U = 0$ ,  $N = 10$ ,  $P < 0.001$ ), zoea V ( $U = 7$ ,  $N = 10$ ,  $P < 0.001$ ) and zoea VI ( $U = 15$ ,  $N = 10$ ,  $P < 0.005$ ), whereas no statistically significant differences were observed in the case of zoea III ( $P > 0.05$ ) (Fig. 1).

Food supplementation at a density of 6 nauplii mL<sup>-1</sup> increased survival in the presence of megalopae in the case of zoea IV ( $H = 10.54$ ,  $N = 30$ ,  $P < 0.01$ ) and zoea VI ( $H = 14.04$ ,  $N = 30$ ,  $P < 0.001$ ). A similar trend was observed in zoea V, although in that case the difference in survival was not statistically significant (Table 1).

### Cannibalism among megalopae

The median survival of megalopae was 100% in all tested densities, with no statistically significant



**Figure 1** Mortality rates of different larval stages of *Ucides cordatus*, either in the presence or in the absence of megalopae. Larvae in the control group (grey bars) were not exposed to the presence of megalopae.

**Table 1** Median survivorship rates of larvae of *Ucides cordatus* in the presence of megalopae under different feeding treatments

Treatments	Zoea I	Zoea III	Zoea IV	Zoea V	Zoea VI
1	85 <sup>a</sup> (55–100)	97.5 <sup>a</sup> (75–100)	35 <sup>b</sup> (0–80)	77.5 <sup>a</sup> (40–100)	82.5 <sup>b</sup> (50–100)
2	97.5 <sup>a</sup> (65–100)	95 <sup>a</sup> (75–100)	32.5 <sup>b</sup> (5–65)	72.5 <sup>a</sup> (30–100)	80 <sup>b</sup> (50–90)
3	100 <sup>a</sup> (85–100)	85 <sup>a</sup> (70–100)	97.5 <sup>a</sup> (15–100)	90 <sup>a</sup> (65–100)	100 <sup>a</sup> (95–100)

Superscript letters indicate homogeneous groups according to multiple comparisons of mean ranks for all groups. Values in parentheses correspond to the observed ranges.

1, control group fed with rotifers and microalgae; 2, diet supplemented with nauplii of *Artemia* sp. at a density of 0.3 nauplii mL<sup>-1</sup>; 3, diet supplemented with metanauplii of *Artemia* sp. at a density of 6 nauplii mL<sup>-1</sup>.

differences among the experimental groups ( $H = 3.98$ ,  $N = 148$ ,  $P > 0.05$ ). Similarly, food deprivation also failed to elicit agonistic behaviour among megalopae, with no statistically significant differences among starved and control groups ( $U = 300$ ,  $N = 25$ ,  $P > 0.05$ ).

## Discussion

Several factors have been suggested as influencing the frequency and intensity of cannibalism among decapod crustaceans, such as refuge availability (Heck Jr & Hambrook 1991; Olmi & Lipcius 1991; Moksnes *et al.* 1997) and the level of satiation (Luppi, Spivak & Anger 2001). In the present study, although the survival of larvae of *U. cordatus* in the presence of megalopae was indeed substantially reduced because of cannibalism, such an effect was only evident in the case of the last three zoeal stages, a pattern that might indicate some degree of preference towards larger prey (but see Fernandez 1999). However, prey size is probably not the only determinant of the probability of cannibalism, given that the experiments showed that megalopae of *U. cordatus* did not prey on one another, regardless of the experimental conditions.

The availability of *Artemia* nauplii as an alternative food source was efficient in reducing the cannibalism rates of zoeas by megalopae of *U. cordatus* during larviculture. However, such an effect was only evident at the highest density of *Artemia* sp. (6 nauplii L<sup>-1</sup>), even though the lowest density (0.3 nauplii mL<sup>-1</sup>) is usually used during their larviculture. A similar result has been shown in the case of the crab *Chasmagnathus granulata* (Dana), where availability of alternative food items (*Artemia* nauplii) significantly decreased the cannibalism rates of zoeas by megalopae (Luppi *et al.* 2001).

The results of the present study have important implications, both for larviculture of *U. cordatus* and to understand its larval biology. In particular, providing

supplementary food sources was shown to be an efficient strategy to minimize larval cannibalism, either in isolation or combined with other strategies such as decreasing larval density, as has been shown in the case of the mud crab, *Scylla serrata* (Forsk.) (Quinitio & Parado-Esteva 2000). In fact, it is possible in principle that even lower densities of *Artemia* (e.g. <6 nauplii mL<sup>-1</sup>) could still elicit the desired result, and this possibility should be investigated further. Despite the relatively small-sized containers used in the experiments, the conditions were very similar to the actual mass larviculture tanks used for the species in terms of both larval density and food items. However, future studies using larger containers are necessary to corroborate the extent to which such extrapolation is warranted.

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