### Intraspecific interactions in the mangrove crab *Ucides cordatus* (Decapoda: Ocypodidae) during the metamorphosis and post-metamorphosis periods under laboratory conditions

### Robson Ventura<sup>1, 5</sup>; Ubiratã A. T. da Silva<sup>2</sup>; Antonio Ostrensky<sup>3</sup> & Kelly Cottens<sup>4</sup>

<sup>1</sup> Centro de Desenvolvimento em Aqüicultura e Pesca – CEDAP/EPAGRI. Rodovia Admar Gonzaga 1118, Itacorubi, Caixa Postal 502, 88034-901 Florianópolis, SC, Brazil.

<sup>2</sup> Centro de Estudos do Mar, Universidade Federal do Paraná. Avenida Beira Mar, Caixa Postal 50002, 83255-000 Pontal do Paraná, PR, Brazil.

<sup>3</sup> Departamento de Zootecnia, Universidade Federal do Paraná. Rua dos Funcionários 1540, Juvevê, 80035-050 Curitiba, PR, Brazil.

<sup>4</sup> Instituto Chico Mendes de Conservação da Biodiversidade. Avenida Conselheiro Furtado 1303, Batista Campos, 66035-350 Belém, PA, Brazil.

<sup>5</sup> Corresponding author. E-mail: robson.ventura@gmail.com

ABSTRACT. Current efforts for restocking natural populations of the mangrove crab *Ucides cordatus* (Linnaeus, 1763) in Brazil have focused on developing a methodology for stimulating the metamorphosis of *U. cordatus* larvae at a large scale. The aim of the present study was to compare the mortality rates both in individual and mass conditions, during the induction of metamorphosis of megalopa to juvenile in *U. cordatus*, without the use of mangrove sediment as substrate. Furthermore, the importance of intraspecific antagonistic behavior on survivorship rates during early post-metamorphosis period was investigated. Metamorphosis was induced by the use of water conditioned with conspecific adults (30 indiv. 100 L<sup>-1</sup> for 24 hours). In the first assay, megalopae were stimulated into metamorphosis in experimental vials, both under individual and mass rearing conditions. The second assay assessed the interactions between megalopae and first instar juveniles, which have metamorphosed for more than 24 hours. In the third assay, the existence of cannibalistic behavior among first instar juveniles under different experimental densities was investigated. Significant differences between survivorship rates of individuals that metamorphosed under individual and mass rearing conditions. Were no cannibalistic behavior between juveniles and megalopae was observed in the second assay. Juveniles reared at a density of 200 indiv.m<sup>-2</sup> showed survivorship rates similar to those obtained under individual conditions. Yet 500 juveniles.m<sup>-2</sup> treatments showed significantly lower survivorship rates. Intraspecific interactions appear to be an important problem in *U. cordatus* specifically during the metamorphosis, but not during larval and post-larval rearing.

KEY WORDS. Cannibalism; juvenile; megalopa; nursery.

Several species of fish and invertebrates are currently showing signs of overexploitation, even while apparently coherent governmental management strategies are in place (MASUDA & TSUKAMOTO 1998). The mass production of young forms in laboratory, followed by their release directly into areas with depleted stocks, is one of the alternative strategies being developed in different parts of world (BELL *et al.* 2008).

Populations of edible crab species, such as *Callinectes sapidus* Rathbun, 1896 and *Portunus trituberculatus* Miers, 1876, are in decline in recent decades. Such declines have been tentatively circumvented by experimental restocking efforts. Postrelease *in loco* analysis have shown that success strongly depends, among other factors, on the age of young forms at the moment of release into the wild (SECOR *et al.* 2002).

The restocking technology for the Brazilian mangrove crab *Ucides cordatus* (Linnaeus, 1763) is being developed by our research group since 2001. Currently, the annual production is over 1,000,000 megalopae per season (SILVA *et al.* 2006, 2009). So far, the young forms produced are released during the megalopa phase. Recent studies have indicated that releasing individuals during juvenile phase could be more effective, due to their naturally lower susceptibility to predation by fish, as well as a higher potential to compete with crabs of other species (VENTURA *et al.* 2010a).

However, in order to release more ontogenically advanced recruits, the first key process to be developed is a methodology for stimulating metamorphosis of *U. cordatus* larvae, at a large scale.

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Mangrove sediments have naturally occurring cues known to stimulate the metamorphosis of different species of mangrove crabs (O'Connor & Gregg 1998, O'Connor 2007). Although effective, the amount of mangrove required to induce metamorphosis indoors, and at a large scale, would be so large that problems such as widespread contamination and logistics would make management impracticable.

SIMITH & DIELE (2008) have shown that the induction of metamorphosis in *U. cordatus* can be obtained using only water, conditioned with chemical stimulus from conspecific adults, without the utilization of sediment. Although the assays conducted by this researcher were developed in a small scale, using individualized experimental units, it is possible that, under mass rearing conditions, a variation of this methodology could achieve satisfactory results, permitting the maintenance of larvae during metamorphosis and during early juvenile phase under laboratory conditions, inside nursery structures. Different kinds of nursery structures are widely used for other crab species: tanks with volumes ranging from 8L (BAYLON & FAILAMAN 1999) to 8,000L (DAT 1999), hapa net-cages (RODRÍGUEZ *et al.* 2007), or even earth bottomed ponds (MARASIGAN 1999).

In general, growth rates achieved by juveniles in ponds are the highest. However, rearing using indoor tanks and hapa net-cages facilitates the prevention of young forms mortality. The food supplied to crabs in nurseries may include minced fish (HAMASAKI *et al.* 2002), diets for penaeid shrimp (ZMORA *et al.* 2005) and *Artemia* sp (nauplii or adults) (BAYLON & FAILAMAN 1999, MANN *et al.* 2001, QUINITIO & PARADO-ESTEPA 2000).

Rearing densities also vary enormously. In nurseries of *Scylla* crabs, stock densities of 1 to 150 indiv.L<sup>-1</sup> were tested using indoor tanks (BAYLON & FAILAMAN 1999) and 0.1 to 125 indiv.m<sup>-2</sup> in ponds (MARASIGAN 1999, RODRIGUEZ *et al.* 2007). Juveniles of the blue crab *C. sapidus* were also experimentally cultivated in nurseries using indoor tanks at densities of 2.5 to 40 indiv.L<sup>-1</sup> (ZMORA *et al.* 2005). Higher densities were related to lower rates of survivorship, mainly due to cannibalism.

The cannibalistic behavior is considered the most important factor during the nursery period. This problem is especially limiting when the crabs reared, under controlled conditions, suffer asynchronous metamorphosis (QUINITIO & PARADO-ESTEPA 2000, ZMORA *et al.* 2005). Technologies to decrease mortality rates, related to this behavior, have already been developed involving food supplementation (DUTIL *et al.* 1997, LUPPI *et al.* 2001) and the use of artificial substrates (CHEN 1990, MARICHAMY & RAJAPACKIAM 2001, HAMASAKI *et al.* 2002, BAYLON & FAILAMAN 1999, AILEEN *et al.* 2000, ZMORA *et al.* 2005).

The goal of the present study was to compare the mortality rates, both under individual and mass rearing conditions, which occur during the induction of metamorphosis from megalopa to juvenile in *U. cordatus*, without the use of a mangrove sediment as a substrate. Additionally, the effect of intraspecific antagonistic behavior on survivorship rates during the early post-metamorphosis period was investigated.

#### MATERIAL AND METHODS

#### Larval rearing

Larvae for the experiments were obtained from *U. cordatus* ovigerous females collected in the mangroves in Santo Amaro, Bahia state, Northestern Brazil (12°40'29"S 38°44'09"W). Collected females were brought to the laboratory, where they were maintained in 1,000 L plastic tanks filled with sea water under controlled environmental conditions (at 26°C, pH 8, and 30 psu) until hatching, which occurred without any artificial stimulus. During the larval rearing period, a specific food protocol was provided at each developmental stage, consisting only of microalgae (*Thalassiosira* sp. and *Chaetoceros* sp.) for the initial stages and *Artemia* sp. nauplii from stage zoeae V to the end of the larviculture. The larvae used in the experiments were kept in mass cultivation containers until they reached the desired stage for each assay.

#### **Experimental setup**

The experiments were conducted in an environmental room under controlled temperature (26°C) and photoperiod (16:8 h L/D cycle). Each experimental unit consisted of a clear plastic container (100 mL vial - diameter 6.5 cm, height 7 cm or 500 mL vial - diameter 11.3 cm, height 5 cm), which was kept on a dark surface, to facilitate the observation of the larvae. The water used in all of our experiments was adjusted to a salinity of 30 psu, filtrated through a 5 micrometer cellulose filter and disinfected using ultraviolet light. Metamorphosis induction procedures followed SIMITH & DIELE (2008), using water, conditioned with chemical stimulus from conspecific adults, as an inducer. This conditioned water was prepared through the 24 h immersion of 30 U. cordatus adults (males and females in the same proportion) for each1,000 L of seawater, followed by its filtration through a sieve (100 microns) into a sealed container. The water in each experimental container was replaced every 24 hours.

The individuals were placed in the experimental containers either as megalopae or as juveniles, following the different protocol established for each assay. The proportion at which the developmental phases were placed in the containers varied according to the objective of the assays described below. The basic diet used in all experiments was composed of newly hatched *Artemia* nauplii (0.3 indiv.mL<sup>-1</sup>). As juvenile *U. cordatus* cannot swim (VENTURA *et al.* 2008), it was anticipated they would have difficulties preying on live nauplii. Therefore, depending upon the assay, dead *Artemia* nauplii (killed by freezing) were additionally provided, at the same density.

### Metamorphosis under individual and mass rearing conditions

In the first assay, megalopae were stimulated to metamorphose in experimental vials, both under individual and mass rearing conditions. Three sets of experimental vials were used in this assay. In all sets the megalopae/saltwater proportion was one individual/100 mL. The first set was composed of 100 mL vials containing only one megalopa each. This setup was used to establish the survivorship and metamorphosis rates in the absence of effects exerted by any other individual. The second set used 500 mL experimental vials which contained five megalopae each (10 megalopae.L<sup>-1</sup>). Assuming that intraspecific antagonistic behavior could be related to insufficient feeding, the last set also used 500 mL experimental vials, the same number of megalopae as the second set but also received dead *Artemia* nauplii in addition to the basic diet (live *Artemia* nauplii) at a density of 0.3 indiv.mL<sup>-1</sup>. After 24 hours, dead young forms and metamorphosed megalopae were counted to determine metamorphosis and survivorship rates.

The individual rearing set was performed with 25 replicates and the other two sets with five replicates each. This assay was performed eight times in consecutive days, for a total of 200 repetitions (25 replicates x 8 assays) for the first set and 40 repetitions (5 replicates x 8 assays) for each of the other two sets, for a total sample size of 280 vials.

# Interactions between *U. cordatus* juveniles and megalopae

The aim of this second assay was to investigate the interactions between first instar juveniles and megalopae. Juveniles used in this assay were maintained in a 20 L container, at a density of 100 indiv.m<sup>-2</sup>, for 24 hours, before they were transferred to experimental vials. This procedure was adopted to ensure that their exoskeleton has completely hardened and that they were able to display antagonistic behavior to the megalopae.

The assay was conducted with two sets as the control group and two sets as treatment groups. All of them used 500 mL experimental vials and were fed with the same basic live *Artemia* nauplii (0.3 indiv.mL<sup>-1</sup>) diet. The megalopae control group was composed of only five megalopae and the juveniles control group of only two juveniles. Control groups were aimed to provide an indication of background mortality rates in the absence of potential cannibalism between juveniles and megalopae. In the treatment group, both sets were composed of five megalopae and two first instar juveniles in the same vial. The difference between the treatment sets was that one of them received dead *Artemia* nauplii as supplementary food.

All sets were performed with five replicates. After 24 hours, dead young forms were counted to determine survivorship rates. The assay was performed 5 times in consecutive days, for a total of 25 repetitions (5 replicates x 5 assays) for each set, for an overall sample size of 100 vials.

#### Interactions among first instar U. cordatus juveniles

In this third assay, the existence of cannibalistic behavior among first instar juveniles was investigated by varying their density in the experimental containers. The control group was composed of one set of 500 mL experimental vials, each containing a single juvenile in 250 mL of water. Two different densities were tested. In the first set, two juveniles were placed in experimental vials containing 500 mL of water (corresponding to 200 indiv.m<sup>-</sup><sup>2</sup>), same proportion of larvae/saltwater of the control group (one individual/250 mL). In the second, 5 first instar juveniles were added to vials with the same water volume (at a density equivalent to 500 indiv.m<sup>-2</sup>). In this assay, only dead *Artemia* nauplii were provided as food, at a density of 0.3 indiv.mL<sup>-1</sup>.

All treatments were performed with five replicates. After 24 hours dead young forms were counted to infer survivorship rates. The assay was performed seven times in consecutive days, for a total of 35 (5 replicates x 7 assays) repetitions for each treatment and an overall sample size of 105 tested vials. For this assay it was not possible to perform the control group with 25 replicates, as in the first experiment, given that the experimental availability of first instar juveniles for the tests was limited.

#### Statistical analysis

All data were analyzed using the program Statsoft Statistica® 7.0, at a 5% level of significance. Given that the first experiment compared treatments with different number of repetitions and that the Shapiro-Wilk test indicated that the data from the other experiments departed significantly from normality, all analyses were conducted using nonparametric statistics. Survivorship and metamorphosis rates were compared using Kruskal-Wallis tests, followed by multiple comparisons of groups using the Bonferroni Correction, as *post hoc* tests.

#### RESULTS

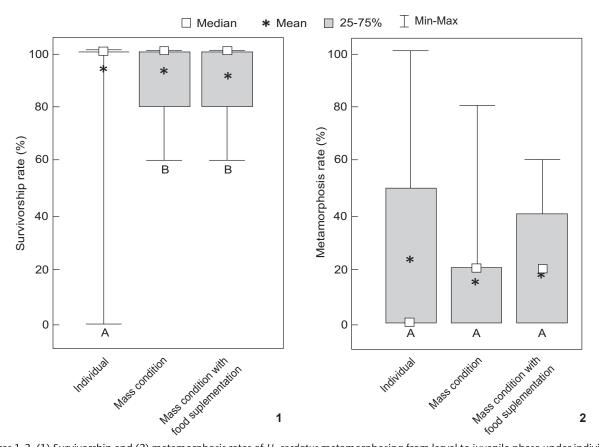
# Metamorphosis under individual and mass rearing conditions

Survivorship rates of individuals maintained individually were higher than those submitted to mass rearing conditions (N = 280; H = 29.44; p <0.01). However, significant differences were not detected between survivorship rates in the group in which dead *Artemia* nauplii were used as supplementary food and those observed in the group under mass rearing conditions. No significant differences were detected between metamorphosis rates obtained in all treatments (N = 280, H = 7.22, p = 0.27) (Figs 1 and 2).

### Interactions between *U. cordatus* juveniles and megalopae

No significant differences were observed between survivorship rates of megalopae (N =  $75^*$ , H = 0 p = 1.00) and juveniles (N =  $75^*$ , H = 0, p = 1.00) obtained in control groups and

<sup>\*</sup> The N of this analysis is 75 because it compared exclusively the megalopae survivorship rates: 25 replicates of Megalopae control, 25 of Megalopae+juveniles group and 25 of Megalopae+juveniles with food supplementation group. Megalopae survivorship is an absent data for the Juveniles control group. The same logic can be used for the comparison among juvenile survivorship rates.



Figures 1-2. (1) Survivorship and (2) metamorphosis rates of *U. cordatus* metamorphosing from larval to juvenile phase under individual and mass rearing conditions, in the presence or absence of supplementary food. Letters under boxes indicate homogeneous groups according to the statistical analysis.

in treatments in which megalopae and juveniles were maintained together, neither in the presence or absence of supplementary food (Fig. 3).

#### Interactions among first instar U. cordatus juveniles

Survivorship rates observed in control group were similar to those observed in the treatment with a density of 200 juveniles m<sup>-2</sup>. However, treatment in which density was 500 juveniles m<sup>-2</sup> showed significantly lower survivorship rates (N = 105, H = 8.20, p = 0.01) than those observed in the control group, although, in all cases, at least 40% of the juveniles survived during the assays (Fig. 4).

#### DISCUSSION

The results of the first assay showed significant differences between survivorship rates of *U. cordatus* megalopae metamorphosing under individual and mass rearing conditions. However, the results of the second assay point in another direction, in that juveniles that had metamorphosed for more than 24 hours, thus showing a completely hardened exoskel-

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eton, do not appear to prey on megalopae. This can be an indication that intraspecific interactions are important sources of mortality of *U. cordatus* specifically during the molting period in which larvae metamorphose to the juvenile phase.

Studies on the importance of intraspecific relations during the settlement process carried out with other crab species have reported that intraspecific interactions play an important role in modulating the survivorship of young forms. In assays conducted with C. sapidus, MOKSNESS et al. (1997) observed that juveniles (instars 3, 5 and 9) prey on megalopae and cause significant mortality, regardless of density and type of substrate (sand or seagrass, in that case). FERNANDEZ (1999) also observed cannibalistic behavior of juveniles (instars 3 and 4) on megalopae of the crab Cancer magister Dana, 1852, reporting that the mean number of individuals consumed by juveniles was affected by both predator size and megalopae density. It is important to note that even the highest predator and prey densities tested by MOKSNESS et al. (1997) (0.05 and 0.34 indiv.L<sup>-1</sup>, respectively) and by FERNANDEZ (1999) (0.1 and 0.4 indiv.L<sup>-1</sup>, respectively) were still lower than those used in our assays. That

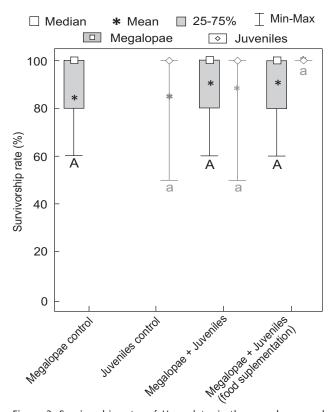


Figure 3. Survivorship rates of *U. cordatus* in the megalopae and juvenile phase reared separately and together, in the presence or absence of supplementary food. Letters under boxes indicate homogeneous groups according to the statistical analysis.

could be an indication that these species are characteristically more aggressive than *U. cordatus* at the same age. However, these studies tested more ontogenically advanced young forms than only first instar juveniles. Therefore, we cannot disregard the fact that more developed *U. cordatus* juveniles may prey on megalopae. For this reason, our findings should not be readily extrapolated to what would occur in rearing tanks containing larvae with highly heterogeneous growth rates, although the coexistence of several ontogenetic stages in the same rearing tank is not common.

The low level of aggressiveness of *U. cordatus*, in relation to other species, was previously reported in the specific case of the megalopa phase. Experiments on larval cannibalism and transport simulation tests, both conducted with *U. cordatus*, showed that survivorship rates of megalopae, maintained at high densities and in stressful situations, are not affected by cannibalism (VENTURA *et. al.* 2008, 2010b). On the other hand, studies about transport under high densities conducted with *C. sapidus* indicate that megalopae prey on each other when maintained in confined conditions (QUINITIO & PARADO-ESTEPA 2000).

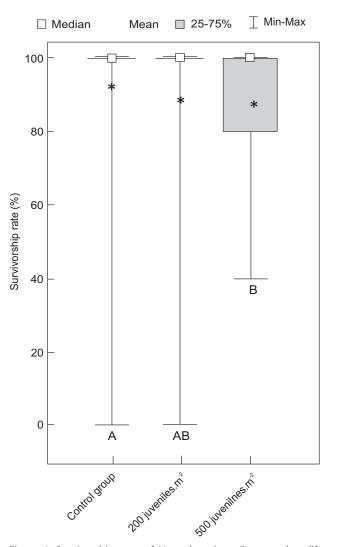


Figure 4. Survivorship rates of *U. cordatus* juveniles reared at different densities. In the control group, juveniles were reared individually. Letters under boxes indicate homogeneous groups according to the statistical analysis.

In the present study, the use of dead *Artemia* nauplii was tested as a tentative way of avoiding antagonistic intraspecific behavior of newly metamorphosed juveniles on metamorphosing larvae. However, this protocol, in our first assay, does not appear to have a positive effect on survivorship rates. In other crab species, cannibalistic behavior among juveniles is frequently reported, especially by the more developed individuals on younger ones (AILEEN *et al.* 2000, BAYLON & FAILAMAN 1999, LUPPI *et al.* 2001, ZMORA *et al.* 2005).

The results of the last assay indicate that first instar juveniles of *U. cordatus* tolerate densities up to 200 indiv.m<sup>-2</sup>, with survivorship rates similar to those obtained under individual

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rearing conditions. Even considering that, at a density of 500 indiv.m<sup>-2</sup>, a significantly lower survivorship than the control group was observed, the results indicate that the use of higher densities of megalopae and juveniles, than those used in other crab species cultures, is possible.

On the other hand, by taking into account that intracohort cannibalism among juveniles is normally related to the molting process (Moksness *et al.* 1997), it is conceivable that the mortality observed could be exacerbated by maintaining juveniles in such conditions during longer periods of time, involving more molting events. Therefore, further studies are needed to determine the most suitable densities, allowing for the maintenance of juveniles in nurseries during periods involving numerous molting events.

Nevertheless, important clues for the development of laboratory nurseries were obtained in this study. Intraspecific antagonistic behavior appears to be a problem during the metamorphosis to the juvenile phase of *U. cordatus*. Studies should be developed to investigate different ways of avoiding mortality during this phase, such as the use of other feeding protocols or substrates. Not withstanding, it seems possible to maintain megalopae and first instar juveniles in the same tanks free of mangrove sediment without significant mortalities. Further studies are needed to investigate if laboratory-produced juveniles can be cultivated until they reach a more suitable age for release into the environment.

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