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Survival of *Ucides cordatus* (Decapoda: Ocypodidae) megalopae during transport under different conditions of density and duration

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ABSTRACT. Target areas for *Ucides cordatus* (Linnaeus, 1763) restocking programs are often located far from the laboratory where larval rearing is developed. During translocation, the larvae are submitted to highly stressful conditions due to handling, packing, and transport activities. The aim of the present study was to assess the mortality rates of *U. cordatus* megalopae caused by different transportation procedures. Megalopae at loading densities of 50, 150, and 300 ind.L^{-1} were packed in double polyethylene 12 x 25 cm plastic bags with 200 ml of marine water at salinity 30. The bags were filled with oxygen at a proportion of 1:2 parts of water and sealed tightly. The trepidations during transport were simulated by the use of a shaker device (800 vibrations/minute) over periods of three and six hours inside a dark container. The survivorship rates of larvae after simulation were compared to those obtained in control groups, which consisted of plastic vials with megalopae at a loading density of 50 ind.L^{-1} maintained at rest. Immediately after the two transport simulations, there was no significant difference in survivorship between the treatments and the control. However, 24 hours after simulation some of the tested densities resulted in significantly lower survivorships. The results demonstrated that *U. cordatus* megalopae can tolerate six hours of shaking during transportation, at high densities with minimal mortality.

KEY WORDS. Cannibalism; larvae; mangrove crab; stress; transport.

The mangrove crab *Ucides cordatus* (Linnaeus, 1763), known in Brazil as "caranguejo-uçá", has a wide geographical distribution along the western Atlantic shores, from Florida (USA) to the Brazilian state of Santa Catarina. This crab is considered 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, the Lethargic Crab Disease (LCD) has contributed to a drastic reduction of natural stocks of *U. cordatus* in several regions of Brazil (Boeger *et al*. 2005). Among more classical measures, such as minimum carapace size policies and the prohibition of capture during reproductive periods, a possible alternative to help recover these populations is the mass production of megalopae in laboratory, followed by their release directly into the affected areas.

Different aspects of the necessary technology to achieve this goal have been developed by our research group since 2001, when the restocking efforts began. Currently, the annual production is over 1,000,000 megalopae per season (Silva *et al*. 2006). In the laboratory, ovigerous females captured from nearby mangrove areas are kept until larvae hatch. The larvae are cultivated in rearing tanks using live planktonic organisms and the females are returned to the original environment. The larvae go through five or six developmental stages until they reach the megalopa phase (Rodriges & Hebling 1989). At this stage, they are released into specific mangrove target areas of the stock-enhancement program. Usually, these areas are distant from the laboratory where the larviculture is being developed. Therefore, the larvae must be transported to the releasing sites and are submitted to stress due to handling, packing, and shaking during transport.

Transport stress may cause mortality of larvae and juveniles, as was shown for other crab and shrimp species. In their transport simulation study, Quinitio & Parado-Estepa (2000) observed lower survivorship rates for *Scylla serrata* (Forskål, 1775) megalopae when compared to the results obtained by Hamid & Mardjono (1979) or, even, those obtained by Smith & Ribelin (1984) in their work with shrimp species under similar

conditions. Quinitio & Parado-Estepa (2000) attributed the observed pattern to the cannibalistic behavior of *S. serrata* larvae. However, Ventura *et al.* (2008) observed that, under experimental conditions, cannibalistic behavior of *U. cordatus* megalopae is negligible, even in conditions of food deprivation.

The aim of the present study was to assess the mortality rates of *U. cordatus* megalopae during transportation.

MATERIAL AND METHODS

Larvae for the experiments were obtained from ovigerous females of *U. cordatus* collected in the municipality of Santo Amaro, state of Bahia, Northeastern Brazilian region (12°40'29"S, 38°44'09"W). In the laboratory, collected females were maintained in 1,000-L plastic tanks filled with filtered (0,5 µm mesh cellulose filter) sea water.

The hatchings occurred in tanks without any artificial stimulus. Resulting larvae were moved to large containers (20,000 L) where they were cultivated until they reached the megalopa phase. Larvae were cultivated following the method of Silva *et al.* (2006), with specific food items being provided at each developmental stage, including microalgae-only diets (*Thalassiosira* sp. and *Chaetoceros* sp.) for the initial stages, until the inclusion of *Artemia* sp. nauplii from stage of zoea V to the end of the larval cycle.

The general methodology (including densities and simulation times) used in the experiments followed Quinitio & Parado-Estepa (2000) with the aim of comparing the generated data to the results obtained by those researchers. Megalopae at loading densities of 50, 150, and 300 ind.L^{-1} were packed in 12 x 25 cm double polyethylene plastic bags, filled with 200 mL of marine water at salinity 30. Bags were inflated with 100 mL of oxygen, at a proportion of 1:2 (part of water: parts of oxygen), and sealed tightly. No food was supplied. Ten bags were used for each tested density: five of them were submitted to three hours of transport simulation and the remaining to six hours. The transport trepidations were simulated by the use of a shaker device positioned inside a dark container. The device was constructed using an engine (1/20 hp) eccentrically connected to a round plastic platform whose diameter was 40 cm. The platform, suspended by flexible cables, vibrated 800 times per minute with the force of the running engine. Ten plastic vials filled with 200 ml of marine water containing megalopae at loading density of 50 ind.L^{-1} were used as control groups. Five of them remained in resting conditions during three hours and the others rested during six hours while the bags were submitted to transport simulation. The larval survival rates were analyzed for each bag and plastic vials (control groups) immediately after transport simulation. Then, the experimental larvae of each container were transferred to separate rearing vials where the proportion of larvae/water was adjusted to 20 ind.L^{-1}. Marine water was added gradually to reduce larval densities inside the vials: those with megalopae at loading densities of 50 ind.L^{-1} received 300mL of water; vials with 150 ind.L^{-1} received 1.3 L; and those with 300 ind.L^{-1} received 2.8 L. Neither aeration nor food was supplied. The megalopae were maintained in these vials for 24 hours (post-transport simulation) and survivorship rates were re-analyzed after this period. The entire procedure was performed in a climate controlled room at a temperature of 26ºC. The experiment was conducted with five replicates for each treatment.

One way ANOVA was used to detect the differences between survivorship rates obtained in treatments with different densities. This test was conducted separately for each transport simulation time and for results obtained immediately and after 24 hours of simulation. The homogeneous groups were determined by using Fisher LSD tests with a 5% significance.

To evaluate if there was any interaction between tested densities and transport times a Factorial ANOVA was performed.

RESULTS

Survivorship rates in all tested groups were high, ranging between 70 and 100%. Immediately after the transport simulation, survival in the treatments in which larvae were submitted to three hours of vibration did not differ significantly from the control group ($F = 1$, $p = 0.41$). However, 24 hours after the transport simulations significantly more larvae were alive in the treatment with low stocking densities (50 indiv.L^{-1}) than in the treatments with higher larval densities (150 and 300 indiv.L^{-1}) (Fig. 1).

No significant differences were detected between survivorship rates of larvae submitted to six hours of transport simulation in any tested density, including control groups, both immediately ($F = 5.15$ $p = 0.11$) and 24 hours after simulation ($F = 0.9$ $p = 0.46$) (Fig. 1).

Factorial ANOVA did not detect interaction between density and duration of transport, either immediately ($F = 2$, $p = 0.21$) or 24 hours after transport ($F = 36$, $p = 0.69$).

DISCUSSION

Survivorship rates observed in our assay were similar to those obtained by experiments using shrimp species. According to Hamid & Mardjono (1979), survivorship rates of up to 95% are attainable in experiments with *Penaeus monodon* Fabricius, 1798, at 500 to 600 postlarvae.l^{-1}, over 8 hours, at temperatures of 22°C. *Litopenaeus vannamei* (Boone, 1931) postlarvae can be packed and shipped at 190 ind.l^{-1} for 18 hours, at 18-20°C, with negligible mortalities (Smith & Ribelin 1984).

However, when comparing our results with the survivorship rates obtained in experiments conducted with other crab species, it is notable that *U. cordatus* megalopas show higher resistance to transport. Quinitio & Parado-Estepa (2000), studying the transport of *S. serrata* megalopae, have observed mean survivorship rates of 78% and 58% in two transport assays,

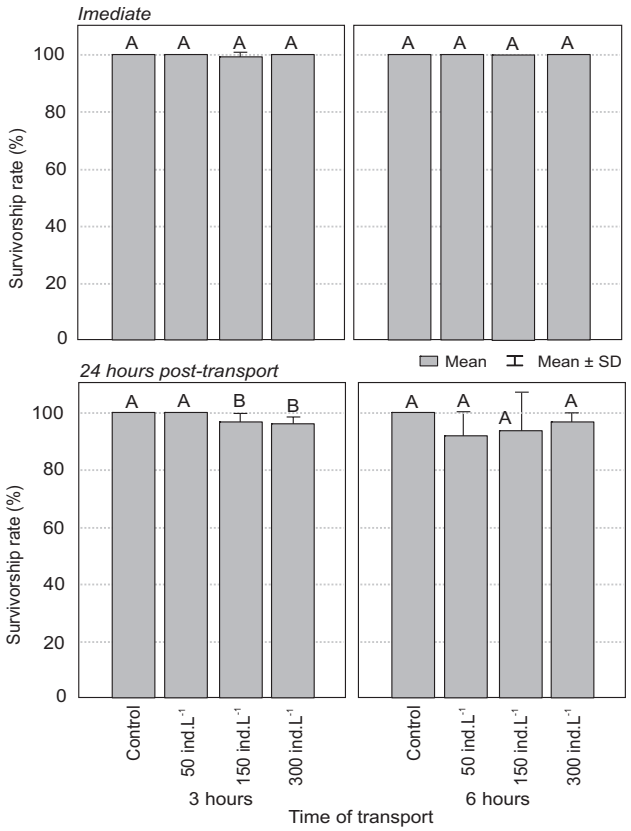

Figure 1. Survivorship rates of *U. cordatus* megalopae submitted to transport simulations at different loading densities and durations, immediately and after 24 hours of simulation. Letters over bars indicate homogeneous groups according to Fisher LSD Test with a 5% significance.

conducted under density of 150 ind.L^{-1}, temperature of 22-24°C, during six hours of transport simulation. Survivorship rates after transport were significantly affected by larval density and by the duration of transport simulation, especially 15 hours after the simulation. Considering the significant mortality rates observed, these authors suggest that the optimal loading density of transport of *S. serrata* megalopae is 50 ind.L^{-1}.

The high survivorship observed in the experiments corroborates the results of Ventura *et al.* (2008), who report that *U. cordatus* megalopa only show cannibalistic behavior on conspecific younger stages, with negligible mortalities related to cannibalism among megalopae, even in conditions of food deprivation.

After a 24 hour resting period, significant differences between the survivorship of megalopae maintained at different densities were detected only when the simulations lasted three hours. However, when the simulation time was six hours such differences were not detected. The explanation for this result was unclear.

Based on our results, it is possible to suggest that *U. cordatus* megalopae can be transported at loading densities of 300 individuals.L^{-1} during periods of six hours with minimal mortality. This information will help the establishment of a transport strategy for *U. cordatus* stock restoration programs. More experiments are needed to show how much longer megalopae can endure under transport conditions.

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