Notas Científicas

Morphometric comparison between hatchery-reared and wild-caught megalopae of the mangrove crab

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Abstract – The objective of this work was to compare the morphometry of hatchery-reared and wild-caught mangrove crab (*Ucides cordatus*) megalopae. Ten *U. cordatus* megalopae of each group (hatchery-reared and wild-caught) were individually analyzed using a stereoscopic microscope equipped with an ocular micrometer. Length, width, and height of all megalopae were measured, and the size of body appendices was determined. The results indicate that the hatchery-reared megalopae are more robust than the wild ones. Furthermore, some significant differences in the size of certain appendices can be cues of the kind of alterations that hatchery-reared individuals experience.

Index terms: *Ucides cordatus*, body appendices, larviculture, rearing-environment, restocking programs.

Comparação morfométrica entre megalopas do caranguejo do mangue produzidas em laboratório e capturadas na natureza

Resumo – O objetivo deste trabalho foi comparar a morfometria de megalopas de caranguejo do mangue (*Ucides cordatus*) produzidas em laboratório e capturadas na natureza. Dez megalopas de *U. cordatus* de cada grupo (produzidas em laboratório e capturadas na natureza) foram analisadas individualmente utilizando microscópio estereoscópico equipado com ocular micrométrica. Comprimento, largura e altura de todas as megalopas foram medidos, e o tamanho dos apêndices corporais foi determinado. Os resultados são indicativos de que as megalopas produzidas em laboratório são mais robustas que as selvagens. Além disso, algumas diferenças significativas no tamanho de certos apêndices podem ser pistas do tipo de alterações que os indivíduos produzidos em laboratório experimentam.

Termos para indexação: *Ucides cordatus*, apêndices corporais, larvicultura, ambiente de cultivo, programas de repovoamento.

In Brazil, from 2001 to 2009, the Grupo Integrado de Aquicultura e Estudos Ambientais (GIA) developed techniques for laboratory production and restocking of the mangrove crab *Ucides cordatus* (Crustacea, Ocypodidae). The resulting techniques produced more than 1 million young forms in the megalopae phase per reproductive season (Silva et al., 2009). During this period, several issues related to this technology were investigated, such as settlement behavior (Ventura et al., 2008) and predation of megalopae by fishes (Costa et al., 2009) and by other crab species after release into the environment (Ventura et al., 2010).

In general, the study of post-release events indicates that the recruits of hatchery-reared *U. cordatus* experience high mortality rates while interacting with natural predators and competitors, which could lead to low restocking efficiency. According to Ventura et al. (2010), this result could be related to the age of the recruits when they are released, as suggested for other species (Shikenjō, 2000; Secor et al., 2002).

However, it is also feasible to consider the existence of differences between hatchery-reared recruits and the wild-caught ones, in terms of behavior, physiology or even of morphology. Therefore, the observed fragility of *U. cordatus* recruits could be inherent to the hatchery-reared animals.

Several researches have reported differences between wild and hatchery-reared younglings, apparently linked to survival after release. Most of these studies are on salmonid fish species. However, works are also available on crustaceans (Bannister, 1998; Davis et al., 2004; Young et al., 2008). Besides morphological differences between hatchery-raised and wild recruits, other problems regarding the restocking process were reported, including antagonistic ecological interaction of hatchery-reared recruits on natural stocks (Jonsson & Jonsson, 2006), alterations in natural reproductive patterns (Hard et al., 2000), or even hereditability of undesirable characteristics (Wessel et al., 2006). In addition, after the identification of those differences, some studies showed that it is possible to circumvent the effects of rearing under captivity using techniques such as conditioning of larvae (Brown & Smith, 1998; Davis et al., 2005).

The objective of this work was to compare the morphometry of hatchery-reared and wild-caught mangrove crab (*U. cordatus*) megalopae.

Both wild-caught and hatchery-reared larvae used in this investigation were obtained during the breeding season of *U. cordatus* (summer of the Southern hemisphere), in January and February of 2005. Wild larvae were collected from the Guaratuba Bay (25º49'52"S, 48º34'44"W), in South Brazil. An electric pump (½ HP) connected to a 50 mm-section hose was used to pump water that was filtered using a plankton net (100 µm). Samples were fixed using 4% buffered formalin and stored in labeled plastic vials. In the laboratory, the zooplankton samples were transferred to a plastic tray and homogenized. All of the brachyuran megalopae were transferred to new labeled vials filled with a mixture of 70% alcohol and liquid glycerin, in the proportion of 1:1. Megalopae of *U. cordatus* were then identified according to Rodrigues & Hebbling (1989), isolated, and stored. Hatchery-reared larvae were obtained from laboratory larviculture. For the larviculture, ovigerous females were collected from mangroves in the state of Paraná, South Brazil, and brought to the laboratory. The females were maintained in 1,000 L plastic tanks filled with sea water, under controlled environmental conditions (26ºC, pH 8, and 30 psu) until hatching, which occurred without any artificial stimulus. During the period of larval rearing, a specific food protocol was provided for each developmental stage, consisting only of microalgae (*Tetraselmis* sp.) and *Brachionus plicatilis* rotifers for the initial stages, and of *Artemia* sp. nauplii from the stage V of the zoeae phase to the end of the larviculture period. The larvae, hatched from several females, were kept in massive cultivation containers until they reached the phase of megalopae. Larval samples of several larvicultural batches of this breeding season were stored in 4% buffered formalin.

Ten wild-caught *U. cordatus* megalopae were compared with ten hatchery-reared ones. Megalopae were chosen from each group based on the integrity of the individuals. The megalopae initially fixed in 4% formalin were transferred to 70% alcohol for the investigation. The analysis was carried out using a stereoscopic microscope equipped with an ocular micrometer. Length, width, and height of all megalopae were measured. The megalopae of both groups were dissected subsequently using entomologic needles, and a morphometric comparison of the dissected appendixes was performed. The following body appendices were evaluated: first maxilliped, second maxilliped, third maxilliped, chelipod, second pereiopod, third pereiopod, fourth pereiopod, fifth pereiopod, first pleopod, second pleopod, third pleopod, fourth pleopod, and fifth pleopod.

Measurements of body and appendices observed in wild and hatchery-reared megalopae were compared by the t test. It was not possible to measure the maxillule, first maxilliped, and fifth pleopod of some specimens due to problems with the conservation of the samples, and the number of observations differed between the two groups (Table 1). Therefore, the measurements were also compared by the Mann-Whitney U test. Comparative analysis of the fourth pereiopod was also not possible, since only a single wild megalopae had this apparently more fragile appendix in suitable conditions of preservation.

Significant morphometric differences between wild-caught and hatchery-reared megalopae were detected for body length and width (no difference was observed for height) and for five of the 18 appendices evaluated (Table 1). Body length and width of

hatchery-reared megalopae were higher than that of the wild ones. This same pattern was found for the antennule and the third pereiopod. The opposite was observed for the antenna, maxilla, and the second pleopod, which were larger in the wild megalopae. Chelipeds also showed a tendency to be larger in wild megalopae (p=0.054).

The results consistently indicate that the hatchery-reared megalopae of *U. cordatus* are more robust than the wild ones. Increased growth rates of hatchery-reared individuals may be genetic in origin because individuals with the highest growth rates can be selected as candidates for broodstock (Barber et al., 1998). However, this cannot be applied to the present study, since ovigerous females were captured directly from natural environments, with no selection for growth rates or size. Moreover, studies on the genetic structure of populations failed to show significant geographical structure in the pattern of genetic variation, indicating that populations of *U. cordatus* are capable of extensive gene flow among estuaries (Oliveira-Neto et al., 2007). For *U. cordatus*, the observed differences are more likely related to feeding conditions. Hatchery organisms are usually fed to satiation, unlike wild organisms which must actively forage for prey under risk of predation. Therefore, hatchery-reared individuals may be released in better physiological condition than wild ones (Young et al., 2008).

The differences observed in the size of appendixes with sensory and feeding functions, such as antennule, maxilla, and cheliped, could be cues of the kind of alterations that hatchery-reared individuals experience. According to Davis et al. (2004), the hatchery environment may impact body shape by failing to provide the necessary cues or stimuli for proper development of predator defenses and can also have influence on animal feeding behavior. Further investigations are needed to evaluate if such differences are merely morphological or if they can have significant impact for the success of recruits after their release.

Table 1. Morphological measurements (in micrometers) of hatchery-reared and wild-caught *Ucides cordatus* megalopae.

Morphological measurement	Hatchery-raised			Wild-caught			p-value[(1)]
	N	Mean	Standard deviation	N	Mean	Standard deviation	
Body length	10	60.4a	1.58	10	55.4b	2.59	>0.001
Body width	10	38.8a	1.32	10	35.8b	2.44	0.003
Body height	10	29.7	1.49	10	29.9	1.29	0.752
Antenna	10	89.7b	2.75	10	96.3a	2.91	>0.001
Antennule	10	46.3a	4.32	10	37.5b	5.64	0.001
Mandible	10	38.7	3.09	10	39.0	1.41	0.783
Maxillule	7	38.4	1.81	10	36.8	2.35	0.145 (0.113)
Maxilla	10	52.9b	1.29	10	55.1a	2.18	0.013
1st maxilliped	8	101.3	5.20	10	103.5	19.29	0.754 (0.304)
2nd maxilliped	10	80.3	7.59	10	76.2	4.21	0.152
3rd maxilliped	10	141.9	6.47	10	144.6	10.76	0.505
Cheliped	10	150.0	5.29	10	156.0	7.51	0.054
2nd pereiopod	10	233.7	5.33	10	229.2	6.60	0.111
3rd pereiopod	10	236.3a	4.55	10	225.3b	15.25	0.042
4th pereiopod	10	213.7	7.01	1	205.0[(2)]	-	-
5th pereiopod	10	166.8	13.44	10	162.0	11.35	0.399
1st pleopod	10	82.9	3.31	10	83.5	1.58	0.612
2nd pleopod	10	78.3b	2.50	10	81.3a	1.42	0.004
3rd pleopod	10	76.1	4.28	10	77.7	2.63	0.327
4th pleopod	10	67.3	2.67	10	67.3	2.45	1.000
5th pleopod	8	60.6	3.54	10	64.2	8.08	0.263 (0.221)

[(1)]p-value of t tests comparing the two groups. Values in parentheses refer to the p value of Man Whitney's U test performed exclusively for parameters comparing samples with different number of observations (N). [(2)]In this case, only one wild-caught megalopae had the fourth pereiopod in suitable conditions of preservation.

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