EXPERIMENTAL CULTURE OF THE FRESHWATER CRAB *Dilocarcinus pagei*: EFFECT OF DENSITY ON THE GROWTH*

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ABSTRACT

This study investigated the effect of culture density on the juvenile development of the freshwater crab *Dilocarcinus pagei*, analyzing the mortality, size increment and mutilation. Juvenile crabs were cultured for 90 days in three treatments with three replicates: T1 - 2 animals L^{-1}; T2 - 3 animals L^{-1}; and T3 - 4 animals L^{-1}. Mortality was high and increased with density: T1 = 70.0 ± 10.0%, T2 = 73.3 ± 3.2% and T3 = 81.6 ± 2.9%. The size increment (mm) did not differ significantly among the treatments, and the means were 7.86 ± 1.76 (T1), 9.33 ± 1.21 (T2) and 8.56 ± 0.53 (T3). However, the specific growth rate was higher in T2, that had a tendency for more growth, as demonstrated by linear regression (ANCOVA, F =10.04; DF = 2; P<0.001). Crabs cultured in the same period grew to different sizes over the course of the experiment. The mutilation was not density dependent, and the size of the mutilated individuals was significantly smaller than that of the non-mutilated crabs (Z = 2.4565; P = 0.0140). Among crab densities tested, the most suitable density for aquaculture was 3 animals L^{-1} because it had the highest specific growth rate, despite the high mortality.

Keywords: Trichodactylidae; Brachyura; Decapoda; aquaculture

CULTIVO EXPERIMENTAL DO CARANGUEJO DE ÁGUA DOCE *Dilocarcinus pagei*: EFEITO DA DENSIDADE NO CRESCIMENTO

RESUMO

O presente estudo investigou o efeito da densidade no cultivo e desenvolvimento do caranguejo de água doce *Dilocarcinus pagei*, analisando a mortalidade, incremento de tamanho e mutilação. Caranguejos juvenis foram cultivados por 90 dias em três tratamentos com três repetições: T1 - 2 animais L^{-1}; T2 – 3 animais L^{-1}; e T3 – 4 animais L^{-1}. A mortalidade foi elevada e aumentou com a densidade: T1 = 70 ± 10%, T2 = 73,3 ± 3,2% e T3 = 81,6 ± 2,9%. O incremento de tamanho (mm) não diferiu entre os tratamentos e a média foi de 7,86 ± 1,76 (T1); 9,33 ± 1,21 (T2) e 8,56 ± 0,53 (T3). Entretanto, a taxa de crescimento específico foi maior em T2, apresentando uma maior tendência de crescimento, como demonstrado pela regressão linear (ANCOVA, F = 10,04; gl = 2; P<0,001). Caranguejos cultivados no mesmo período atingiram tamanhos diferentes durante o desenvolvimento. A mutilação não foi dependente da densidade e o tamanho de indivíduos mutilados foi significantemente menor do que em caranguejos não mutilados (Z = 2,4565; P = 0,0140). Dentre as densidades testadas, a mais adequada para aquicultura é 3 animais L^{-1}, que apresentou a maior taxa de crescimento específico, apesar da alta mortalidade.

Palavras chave: Trichodactylidae; Brachyura; Decapoda; aquicultura

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INTRODUCTION

Decapod crustaceans are a diversified group, occurring in all depths of marine and freshwater environments (MELO, 1996; MAGALHÃES, 2003). Biology and ecology studies of marine brachyurans such as the mangrove crab *Ucides cordatus* (Linnaeus, 1763) and the blue crabs *Callinectes sapidus* Rathbun, 1896 and *Callinectes danae* Smith, 1869 are common (BRANCO and MASUNARI, 2000; HINES *et al.*, 2003; DIELE *et al.*, 2005), principally because of the economic importance and abundance of many marine species. However, knowledge of the biology and ecology of freshwater crabs is relatively limited.

The global diversity of freshwater crabs is estimated at about 1,476 species in 14 families (YEO *et al.*, 2008). The Trichodactylidae family is composed of 50 species, among them the crab *Dilocarcinus pagei* Stimpson, 1861, which occurs in many regions of Brazil including the Amazon basin (MAGALHÃES, 2003) and is the most studied species of this family. Studies of the population biology of *D. pagei* have focused on the relative growth, condition factor, reproduction, population growth, and structure (MANSUR and HEBLING, 2002; MANSUR *et al.*, 2005; PINHEIRO and TADDEI, 2005 a, b; TADDEI and HERRERA, 2010; DAVANSO *et al.*, 2013) and osmoregulation (ONKEN and McNAMARA; AUGUSTO *et al.*, 2007).

Although *D. pagei* is economically valuable as bait for sport fishing (TADDEI and HERRERA, 2010), investigations of culturing methods are nonexistent. Around the world, crabs are important economic resources, and investigations with a view toward culturing them have been conducted to help to supply the demand. In East Asia, the Chinese mitten crab *Eriocheir sinensis* (H. Milne Edwards, 1853) is a commercially important freshwater crab with a high value, and studies of culture performance and profitability of wild-caught and captive, pond-reared crabs of this species have been recently done (HE *et al.*, 2014). In Japan, *Portunus trituberculatus* (Miers, 1876) is one of the most important fish resources, and culture techniques for mass-seedling production have been studied since 1963 for restocking purposes (NOGAMI *et al.*, 1997).

Species of the genus *Scylla* have been investigated a lot for aquaculture. Because of mortality due to cannibalism, the total production was low in the 1990s (KEENAN, 1999). Currently, studies with mud crabs and other species have intensified. MIRERA and MOKSNES (2015) compared the performance of wild juveniles in different culture systems evaluating the effect of shelter, crab size and stocking density. JIANG *et al.* (2014) studied the amino acid composition of the edible parts of mud crabs, swimming crabs and Chinese mitten crabs. This data is very important for monitoring the food properties of crabs.

In Brazil, the crab that is most exploited in nature is *U. cordatus*, and some efforts to culture it have been made (VENTURA *et al.*, 2008, 2011). One of the most limiting factors in rearing larvae of this crab in the laboratory is the period of high mortality that usually occurs late in larval development, specifically during the metamorphosis from the zoea to the megalops phase (SILVA *et al.*, 2012).

In the culture of crustaceans, cannibalism that mutilates the crabs is common, due to agonistic behavior. To avoid mutilation or to reduce the mutilation rate, investigations on the use of refuges contribute to the survival of crabs, especially for juveniles that are most vulnerable (MARSHALL *et al.*, 2005). The stoking density can also negatively influence the crab larvae survival (LI *et al.*, 2007) and mutilation rate (DALY *et al.*, 2009), due to cannibalism.

In Brazil, there are environmental rules for the capture, transport, storage, trade and culture of live bait, specifically for the State of Mato Grosso do Sul (Brazil). The Resolution Nº 22, of August 25, 2011, from the Secretaria do Estado de Meio Ambiente, Planejamento, da Ciência e Tecnologia, Mato Grosso do Sul, Brazil (SEMAC, 2011), determines the minimum legal size (3 cm of carapace width) to capture the red crab *D. pagei* for use as bait. Thus the red crab culture should contribute to the conservation of natural populations, and this study investigated the effect of density on the juvenile development of *D. pagei*, analyzing the size increment, mortality and mutilation experimentally.

MATERIAL AND METHODS

Crab sample and culture experiment

In January 2012, one ovigerous female *D. pagei* was collected in the Amazon River (03º08'13.7"S; 58º27'46.8"W) at Itacoatiara, Amazonas, Brazil. This ovigerous female had no eggs attached to the pleopods, but many small juvenile crabs were born (Figure 1). This female was kept in an aquarium (37x28x12 cm) with aerated water and ambient conditions of light and temperature. The crab was fed daily with fish meat. After 20 days, these juvenile crabs moved out of the female's abdomen and were used in the experiment. To avoid manipulation and damage of the juveniles, the crabs were not sexed.

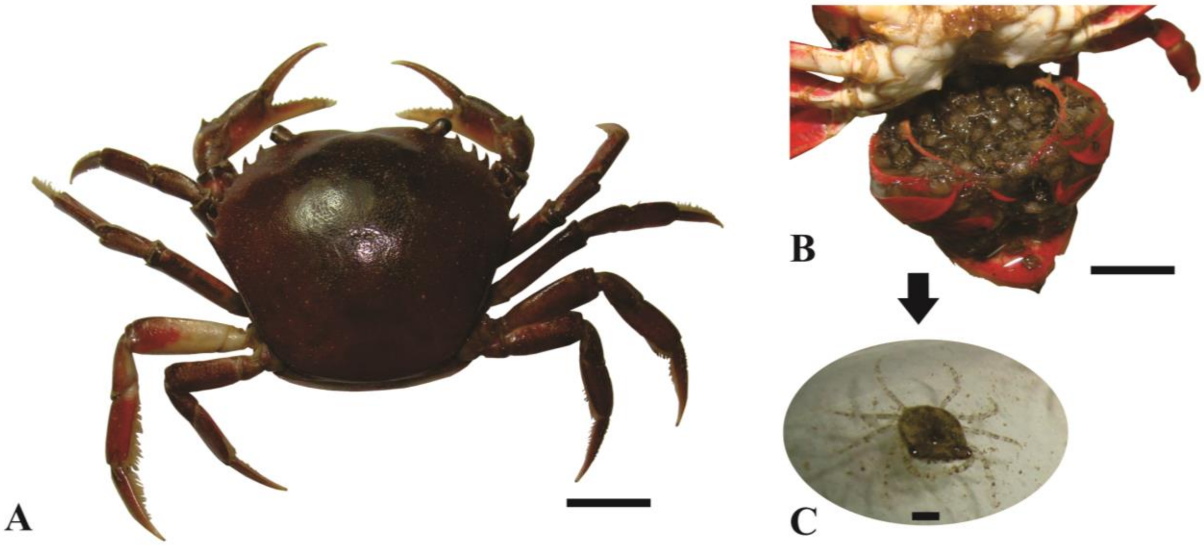

Figure 1. A) Ovigerous female of *Dilocarcinus pagei* (carapace width = 37.1 mm). B) abdomen opened with juvenile crabs, C) juvenile crab removed from abdomen of female. Scale bars: A and B = 10 mm; C = 1 mm.

A total of 135 juvenile crabs with a mean carapace width (CW, measured with a 0.05 mm caliper) of 2.63 ± 0.18 mm were kept in nine aquariums (experimental units) with dimensions of 37x28x12 cm (filled with 5 L of water and 1 cm of fine sand), with aerated water for 90 days (from February to April 2012), under ambient conditions of light and temperature. The experiment was developed with three treatments (densities): Treatment 1 (T1), 10 crabs with a density of 2 crabs L^{-1} (96 m^{-2}); Treatment 2 (T2), 15 crabs with a density of 3 crabs L^{-1} (145/m^{-2}); and Treatment 3 (T3), 20 crabs with a density of 4 crabs L^{-1} (193 m^{-2}). All treatments were performed in three replicates. The water in the aquariums was changed every 48 hours. The temperature (°C) and pH (using a portable digital Hanna pH-meter, model: HI8314) of the water were measured daily. The crabs were fed with an abundant supply of pieces of fish (with food to spare) after each water change. The size (CW in millimeters) of five crabs of all replicates of each treatment was measured, using a stereomicroscope, every 5 days. The presence of mutilated crabs, with lost appendages, was also recorded at these times. The mean temperature recorded during the three months of the experiment was 28.01 ± 2.13 ºC and the mean pH was 8.36 ± 0.65.

The total mortality was estimated by the formula:

$$NDC \times 100/T,$$

where: NDC = number of dead crabs at the end of the experiment and T = total number of crabs in the treatment.

The specific growth rate (G) was determined for each treatment at the end of the experiment:

$$G = \ln s_2 - \ln s_1 / t_2 - t_1,$$

where: S_2 = size at the end of the experiment (90th day), S_1 = size at the start of the experiment (first measurement on day 5), t_2 = end of the experiment on day 90, and t_1 = first day that the crabs were measured, day 5.

Statistical analysis

The normality of the data was tested by the Kolmogorov–Smirnov (KS) test, and the size of individuals was compared by the nonparametric Kruskal–Wallis test, between treatments on each day when the crabs were measured. The nonparametric Mann-Whitney test was used to compare the size of mutilated and non-mutilated crabs, and the chi-square test (χ^2) was used to compare the proportion of mutilated and non-mutilated crabs. The crab size during the experiment was analyzed by linear regression and the slopes and intercept of different regression lines were compared with an analysis of covariance (ANCOVA). A significance level of 5% was adopted for all statistical analyses (SOKAL and ROHLF, 1995).

RESULTS

The mortality increased according to the increase in the density of the culture. At the end of the experiment, the total mortality was 70.0 ± 10.0% in Treatment 1, 73.3 ± 3.2% in Treatment 2 and 81.6 ± 2.9% in Treatment 3.

The size of the crabs during the experiment differed significantly from normality in all treatments (T1, KS = 0.2750; P<0.01; T2, KS = 0.1649; P<0.01; and T3, KS = 0.1579; P<0.01). The mean crab size remained similar during the course of the experiment. However, in Treatment 2, the maximum size was larger than the crabs of Treatments 1 and 3 on average at the end of experiment (Figure 2). However, the mean size did not differ significantly among Treatments 1, 2 and 3 with different densities (Table 1), in the days of measurement of the experimental period. The specific growth rates were G = 0.0131 ± 0.0009 (T1), G = 0.0155 ± 0.0006 (T2), and G = 0.014 ± 0.001 (T3). The linear regression between days of experiment and crab size differed significantly among treatments (ANCOVA, F = 10.04; DF = 2; P<0.001) (Figure 3).

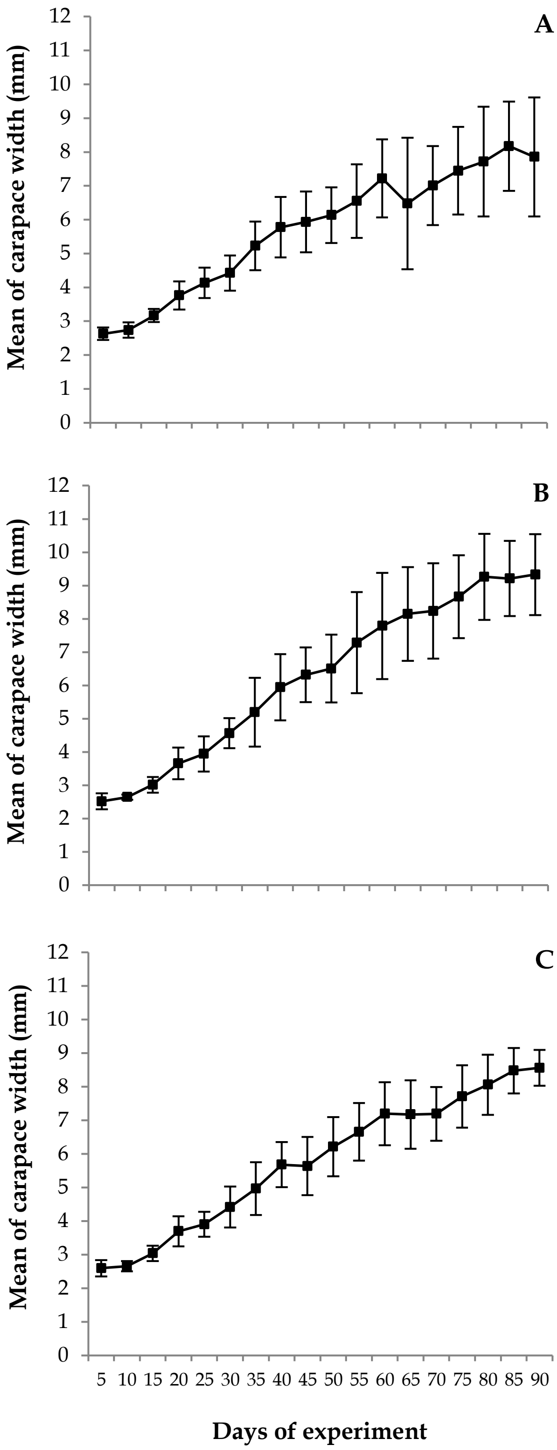

Figure 2. Size (mean and standard deviation) of crabs, *Dilocarcinus pagei*, during the three months of experiment in Treatment 1 (A), Treatment 2 (B), and Treatment 3 (C), at intervals of five days. Density of treatments: Treatment 1 (T1) = 2 crabs L^{-1} (96 m^{-2}), Treatment 2 (T2) = 3 crabs L^{-1} (145 m^{-2}) and Treatment 3 (T3) = 4 crabs L^{-1} (193 m^{-2}).

Table 1. Comparison of the size of freshwater crab *Dilocarcinus pagei* among treatments (analysis of variance, Kruskal-Wallis test) during the three months of the experiment.

Days of experiment	H	DF	*P*
5	3.4327	2	0.1797 ns
10	2.636	2	0.2677 ns
15	5.5252	2	0.0631 ns
20	0.5172	2	0.7721 ns
25	1.9434	2	0.3784 ns
30	0.4842	2	0.7850 ns
35	0.6423	2	0.7253 ns
40	1.6193	2	0.4450 ns
45	3.3808	2	0.1844 ns
50	1.5279	2	0.4658 ns
55	2.4923	2	0.2876 ns
60	1.3749	2	0.5029 ns
65	4.6475	2	0.0979 ns
70	4.467	2	0.1072 ns
75	4.6229	2	0.0991 ns
80	5.4155	2	0.0667 ns
85	3.148	2	0.2072 ns
90	4.7948	2	0.0910 ns

ns = not significant (P>0.05).

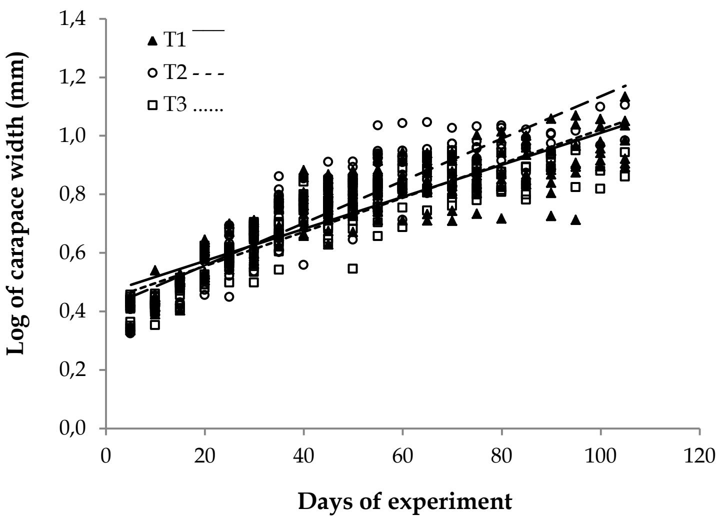

Figure 3. Days of experiment *versus* crab size (mm), *Dilocarcinus pagei*, during the three months of the experiment in Treatment 1 (T1), Treatment 2 (T2), and Treatment 3 (T3) at intervals of five days. The regression lines differed significantly (ANCOVA, F = 10.04; DF = 2; *P*<0.001). Treatment densities: Treatment 1 (T1) = 2 crabs L^{-1} (96 m^{-2}), Treatment 2 (T2) = 3 crabs L^{-1} (145 m^{-2}) and Treatment 3 (T3) = 4 crabs L^{-1} (193 m^{-2}).

The number of mutilated crabs did not increase with the increases in the culture density (χ^2 = 0.6225; DF = 2; *P*>0.05) and was higher in the first days of culture (Figure 4). The size of the mutilated individuals was significantly smaller than that of the non-mutilated crabs (Z = 2.4565; *P* = 0.0140).

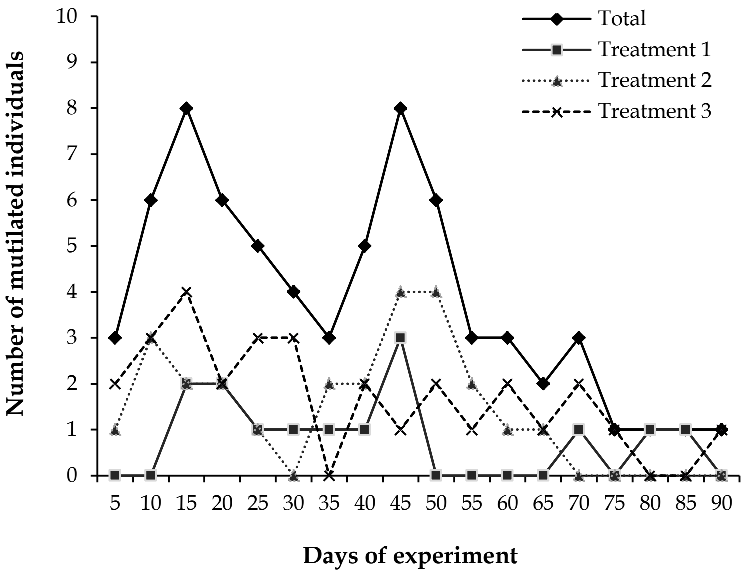

Figure 4. Mutilated individuals of freshwater crab *Dilocarcinus pagei* during the experimental period in all treatments. Treatment densities: Treatment 1 (T1) = 2 crabs L^{-1} (96 m^{-2}), Treatment 2 (T2) = 3 crabs L^{-1} (145 m^{-2}) and Treatment 3 (T3) = 4 crabs L^{-1} (193 m^{-2}).

DISCUSSION

The population of crabs, such as the red king crab, *Paralithodes camtschaticus* (Tilesius, 1815), and the blue king crab *Paralithodes platypus* Bandt, 1850, have supported extensive commercial fishing in Alaskan waters. However, red king crab stocks have declined precipitously in recent decades (PERSSELIN, 2006). An alternative to minimize the pressure of commercial fishing or population decline by other causes is to development crab aquaculture, an initiative that should be employed before the natural populations are reduced by overfishing. In this study, the culture of *D. pagei* showed the lack of any difference in size increment between the different densities, and high mortality and mutilation at all densities.

The mortality was higher than 70% in all treatments, and it increased with crab density. MORAES-VALENTI *et al.* (2010), in their study on *Macrobrachium amazonicum* (Heller, 1862), observed that the population structure was density dependent, but this was not related to the mortality. The high mortality of *D. pagei* observed in this study was associated with their cannibalistic behavior, as also observed by KOVATCHEVA (2006) in rearing the red king crab *P. camtschaticus*, and by KEENAN (1999) in the aquaculture of the mud crab of the genus *Scylla*, with low production caused by mortality due to cannibalism. This conclusion is supported by the high proportion of mutilated crabs, which did not increase with density and was higher for smaller *D. pagei* individuals. Similarly, MARSHALL *et al.* (2005) found that small *Portunus pelagicus* individuals (Linnaeus, 1766) were more vulnerable than larger individuals. The crab mutilation found in *Scylla serrata* (Forskäl, 1775) in the juvenile phase shows important effects in specific growth rates, mainly in the juvenile phase (QUINITIO *et al.*, 2011). Those same authors suggest the chelotomy and trimming of dactyls and pollex have been shown to reduce the competitive ability of conspecifics. Trimming during the intermolt or postmolt stage seems to be an effective method in reducing the degree of cannibalism in the juvenile phase. The process might be difficult to apply, but economic gain is increased through improved survival (QUINITIO *et al.*, 2011).

The high mortality due to cannibalism might be reduced by use of other technique, with grading crabs by size, as shown by DALY *et al.* (2012) in the culture of *P. camtschaticus*, which this management improved the biomass produced per rearing area compared to ungraded populations, adding structure (e.g. DALY *et al.*, 2009) or by rearing crabs individually that eliminates losses due to cannibalism and results in a substantial increase in yield (SWINEY *et al.*, 2013). The use of transparent walls to isolate juveniles of the swimming crab *Portunus sanguinolentus* (Herbst, 1783) proposed by NICHOLSON *et al.* (2008), showed no difference in survival, but a slight gain in growth weight. The culture of *D. pagei*, with the use of shelters or individual culture should be tested to try to reduce the high mortality observed due to cannibalism.

However in this study, the sex was not considered in evaluating the growth. The growth of *D. pagei* was evaluated in natural populations (PINHEIRO and TADDEI, 2005a; TADDEI and HERRERA, 2010), using the indirect method of size-class distribution followed by the Bhattacharya decomposition method. In both studies, females showed faster growth rates than males, which is related to their reproductive strategy.

The stocking density did not influence the growth of the animals. However, in a similar study of the growth of the prawn *M. amazonicum*, MORAES-VALENTI *et al.* (2010) found a different result: the individual growth rate decreased in high densities, and the authors suggested that these results were associated with intrinsic regulatory mechanisms of the species and/or intraspecific competition. In this study, the similarity in the specific growth rates of *D. pagei* in the three treatments was associated with the great variability of crabs of different sizes, occasioned by their different size increment under the same treatment and at the same time of culture. Although on average the size did not differ between treatments during the experiment, the size of crabs and specific growth rate in Treatment 2 was larger than in the other treatments on average and linear regression demonstrates a tendency to grow more than the other treatments.

Dilocarcinus pagei is a freshwater crab with wide geographical distribution, including the Amazon basin (MAGALHÃES, 2003). In addition to playing an ecological role in the trophic chain, this species is used as bait in sport fishing in some areas of Brazil (TADDEI and HERRERA, 2010). The results of this study are the first contribution to the culture of this crab, which can provide supporting information to aid fishing communities in developing an alternative source of income during the fishing off-season.

CONCLUSIONS

Among crab densities tested, the most suitable density for aquaculture is 3 animals L^{-1} because it had the highest specific growth rate, despite the high mortality.

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