

Effect of stock density of cultured Amazon Apple Snail *Pomacea dolioides* (Gastropoda: Ampullariidae) in Brazil

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ABSTRACT - This study evaluated the biomass gain, specific growth rate, absolute weight gain, and survival rate of the gastropod *Pomacea dolioides* cultivated in different stocking densities. To perform the experiment, samples were collected in March and June 2015. A total of 40 adult specimens were collected in the Jauary neighborhood (03°08'19.9"S, 58°27'32.5"W), municipality of Itacoatiara, AM, Brazil. They were kept in the laboratory for breeding to obtain the young *P. dolioides*, which were distributed into tanks containing 4 L of water and an aeration system, in three densities: 2.5 animals/L, 5 animals/L, and 10 animals/L, and were fed fish ration (34% gross protein). The specimens were measured for shell length and weight every fifteen days. The survival rate did not differ between treatments 2.5 and 5 animals/L (100%) and 10 animals/L (95.83%). The density with the best cultivation results was 2.5 animals/L, being most effective for the parameters of growth, weight gain, specific growth rate, and percentage weight gain, when compared with the other treatments after 225 days of the experiment. For future cultivation of this species, a density of 2.5 animals/L should be used to improve handling performance.

Keywords: aquaculture, growth, mollusk, stock enhancement

Introduction

Mollusk cultivation in Brazil has developed since 1990 in Santa Catarina and then in other regions. In the last decade, most coastal states have mollusk research or production activity (Cavalli and Ferreira, 2010). The cultivation is evidenced in various parts of the world, such as in Japan (Itami et al., 1963), Chile (Campalans and Lohrmann, 2009), Taiwan (Chen, 1984), French Polynesia (Coeroli et al., 1984), Mexico (Cardenas, 1984), United Kingdom (Laing and Spencer, 2006), France and Spain (Magdalena et al., 2003), China (Yan et al., 2006), and in other places, as an important economic and social activity. In Trinidad, *Pomacea urceus* (Müller, 1774), is captured from nature and sold in local markets (Lum-Kong and Kenny, 1989). In the Brazilian countryside, freshwater mollusks are consumed by the poorer populations (Barboza and Romanelli, 2005).

There are several factors such as temperature (Seuffert and Martín, 2010), food availability (Tamburi and Martín, 2016), and others that are not taken into consideration as much, such as lighting, genetic variability, stocking density, dimorphism, and gender ratio (Estebenet and Martín, 2002), which directly influence the development of the apple snails species. However, the stocking density affects

growth, breeding, and survival. This negative dependency caused by the density usually contributes to the instability of a population (Yoshida et al., 2013). Such instability has been reported in a study with *Pomacea paludosa* (Say, 1829) in Florida USA, in which the presence of adult individuals causes a reduction in the growth of young ones, and increased mortality (Conner et al., 2008) due to competition for food and mating (Souza Júnior et al., 2013).

Tanaka et al. (1999), studying *Pomacea canaliculata* (Lamarck, 1819) in rice fields, found that the population size was relatively density-dependent, causing reductions in oviposition frequency and size of the egg masses. The same situation was described by Posch et al. (2012), in a study with *P. paludosa*, whose lower density promoted higher egg production and more young individuals per female. The effect of density was also observed with the snail *Helix aspersa* (Muller, 1774). Higher densities cause a reduction of the final adult size and increase mortality (Lazaridou-Dimitriadou et al., 1998). Regarding biology and culture of *Pomacea dolioides* (Reeve, 1856), only the recent study of Melo et al. (2017) investigated the reproduction, including fecundity, embryonic development, and substrate selection for oviposition, and Pires-Júnior et al. (2017) addressed aspects associated with growth and weight gain, analyzing different diets and feeding frequency.

Although *P. dolioides* does not have economic interest, several species of apple snails of the similar size are used for human or animal feeding (Santos, 1982; Ruiz-Ramírez et al., 2005; Vázquez-Silva et al., 2011; Chimsung and Tantikitti, 2014), with the development of research on meat yield (Barbosa and Romanelli, 2005), nutritive compound (Luo et al., 2012), time of ensiling (Phonekhampheng et al. 2009), among other applied research. Thus, the objective of this study was to analyze the culture of *P. dolioides* in different stocking densities.

Material and Methods

To develop the cultivation experiment, the gastropods were collected between March and June 2015. Forty adult gastropods were collected in the Jauary neighborhood (03°08'19.9" S, 58°27'32.5" W). To perform the collection, hand sieves and dip nets were used. The animals were placed in plastic boxes with water and aeration and later transported to the laboratory, where they were identified according to Simone (2006) (Figure 1) and kept in a circular 310-L capacity PVC tank with a closed recirculation system with 180 L/h water renewal and external biological filter. The animals were kept under these conditions until the cultivation experiment.

The sampled gastropods were kept alive in the laboratory for twenty days. They were acclimated and underwent a river ebb and flood simulation in the tank to induce breeding. After mating and oviposition, three egg masses from different females were collected and transported to floating surfaces in PVC containers (5 L capacity) with water and aeration, until hatching, which occurred after eighteen days of embryonic development.



Bar = 1 cm.

Figure 1 - Morphology of *Pomacea dolioides* shell.

The young individuals were cultivated for twenty days in plastic boxes (26 × 36 × 11 cm) with 4 L of water and an aeration system and were fed *ad libitum* fish ration (34% gross protein), with the following compounds: ground whole corn, wheat bran, soybean meal, protenosis, fish meal, meat and bone meal, vitamins (A, D3, E, K3, B1, B2, B6, B12, folic acid, niacin, pantothenic acid, choline chlorate), copper sulphate, iron sulphate, manganese monoxide, zinc oxide, iodate calcium, selenite sodium, cobalt sulfate, DL-methionine, and ethoxyquin. After the 20-day period, the total shell length was measured using a digital caliper (0.01 mm) and the snails were weighed using an analytical scale (0.0001 g). They were then transferred to plastic boxes (26 × 36 × 11 cm) and fed 10% of biomass per day, with the food amount adjusted every fifteen days according to the biometric data and mortality.

Each day, 25% of the tank water was siphoned off to remove excreta and food leftovers. Replenishment was performed from a clean water reservoir with aeration and a biological filtration system. The photoperiod during the experiment was kept constant with 12 h of light and 12 h of darkness.

The experiment lasted 225 days, and three stocking density treatments were tested: 2.5 animals/L, 5 animals/L, and 10 animals/L. Each treatment was triplicated, resulting in a total of nine boxes, considering the young individuals coming from different females as a treatment replica.

The water and air temperature (°C) and relative humidity (%) were measured with a digital thermometer and a thermohygrometer, respectively. The dissolved oxygen content (mg/L) in water was recorded with an oximeter and pH, with a portable pH meter. The contents of nitrite and ammonia (mg/L) were measured with colorimetric tests from samples taken from the treatments (Table 1). To maintain water calcium levels, 0.3 g of calcium carbonate (CaCO₃) was added twice a week for each replica. Voucher specimens were deposited in the Malacological Collection of Instituto Oswaldo Cruz (CMIOC 10552) and identified by Prof. Dr. Silvana Carvalho Thiengo.

The biomass gain (BG) was evaluated according to the following formula: $BG = (Fw \times Fn) - (Iw \times In)$, in which Fw = final weight, Fn = final number of gastropods, In = initial number of gastropods, and Iw = initial weight. For the analysis of growth, the specific growth rate (SGR) was calculated using the following formula: $SGR = (\text{Log } Fw - \text{Log } Iw) \times 100 / T$, in which T = time elapsed. The absolute weight gain (AWG) was calculated from the equation: $AWG = Fw - Iw$. Percentage weight gain (PWG) was calculated from the equation: $PWG = (Fw - Iw) \times 100 / Iw$. The survival rate (SR) was evaluated according to the equation: $SR = (Fn \times 100) / In$.

Shell length, weight, BG, SGR, AWG, and PWG data of individuals were subjected to the Shapiro-Wilk test for normality. The normal distribution data were subjected to one-way analysis of variance (ANOVA), complemented by the Tukey test for means comparison. For non-normal data, the Kruskal-Wallis test was used, complemented by Dunn's test for treatment comparison. The chi-square test was used to analyze survival data in contingency table format. The size and weight gain of gastropods in relation to growth time were investigated by analysis of covariance and F test. For all statistical analyses used in this study, we adopted a significance level of $P < 0.05$.

Table 1 - Minimum, maximum, mean, and standard deviation values of water quality parameters during the experiment

Treatment	Water Temperature (°C)	pH Min/Max		Nitrite (mg/L)	Ammonia (mg/L)	Dissolved oxygen (mg/L)
2.5 animals/L	28.06±0.37	7.13	7.73	0.04±0.10	0.006±0.002	5.26±0.17
5 animals/L	28.09±0.32	6.93	7.66	0.08±0.12	0.007±0.001	5.31±0.21
10 animals/L	28.07±0.36	6.87	7.59	0.21±0.10	0.009±0.001	5.28±0.18

Results

The treatment with the lowest culture density (2.5 animals/L) showed significantly higher gain for growth ($F = 103.8464$; $DF = 2$; $P < 0.0001$), weight ($H = 60.0887$; $DF = 2$; $P < 0.0001$), absolute weight ($H = 60.0887$; $DF = 2$; $P < 0.0001$), specific growth rate ($F = 109.5193$; $DF = 2$; $P < 0.0001$), and percentage weight gain ($H = 60.0887$; $DF = 2$; $P < 0.0001$), in relation to the other treatments (5 and 10 animals/L) after 225 days of cultivation (Table 2). As for biomass gain, there was no significant difference between densities 2.5 and 5 animals/L, differing only for 10 animals/L ($H = 49.2701$; $DF = 2$; $P < 0.0001$). The recorded survival rate showed no significant difference between treatments ($\chi^2 = 3.841$; $DF = 2$; $P = 0.1465$) (Table 2).

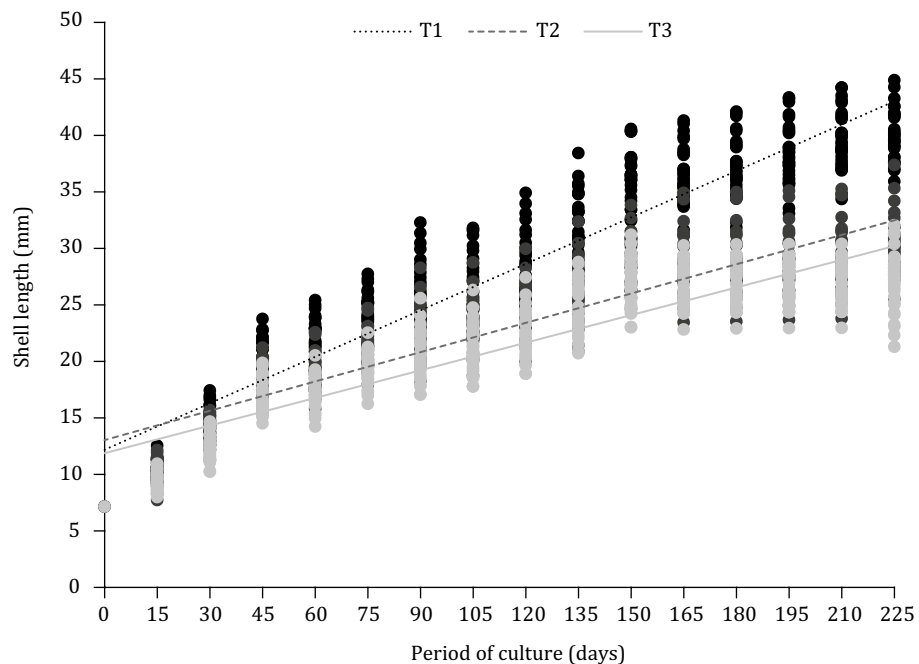
In the comparison between the lines of the shell length and days of cultivation ratio treatment with 2.5 animals/L was significantly different among the tested densities (ANCOVA; $F = 68.75$; $DF = 2$; $P < 0.0001$) (Figure 2), showing increased gastropod growth. The slope of the lines for

Table 2 - Performance parameters of the culture of *Pomacea dolioides* under three stock density treatments

Variable	Treatment		
	2.5 animals/L	5 animals/L	10 animals/L
SL (mm)	40.05±2.25a	30.28±2.56b	26.98±2.70c
Weight (g)	16.10±3.86a	7.04±1.78b	5.05±1.39c
BG (g)	480.80±115.88a	314.24±72.94b	521.53±111.22a
SGR (%/day)	1.54±0.07a	1.31±0.07b	1.21±0.08c
AWG (g)	16.02±3.86a	6.96±1.78b	4.97±1.39c
WGP (%)	21,199.60±5109.6a	9,218.70±2354.80b	6,580.70±1840.90c
SR (%)	100±0.00	100±0.00	95.83±3.82

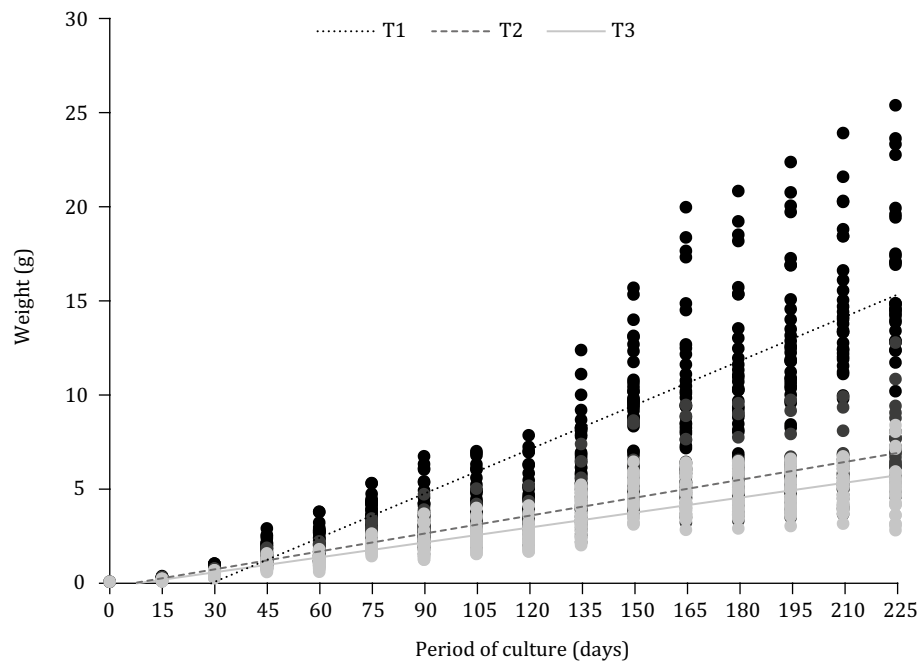
SL - shell length; BG - biomass gain; SGR - specific growth rate; AWG - absolute weight gain; WGP - percentage weight gain; SR - survival rate.

Means followed by different letters in the same row are significantly different ($P < 0.05$). Kruskal-Wallis test and one-way analysis of variance complemented by Dunn and Tukey, respectively, and SR analyzed by chi-square.



T1: 2.5 animals/L; T2: 5 animals/L; T3: 10 animals/L.

Figure 2 - Relationship between the period of culture (days) and shell length (mm) of *P. dolioides* in three different stocking densities.



T1: 2.5 animals/L; T2: 5 animals/L; T3: 10 animals/L.

Figure 3 - Relationship between period of culture (days) and weight gain (g) of *P. dolioides* subjected to three different stocking densities.

the relationship of period of culture to weight gain differed significantly between treatments (ANCOVA; $F = 170.8$; $DF = 2$; $P < 0.0001$). Treatment with 2.5 animals/L provided greater weight gain for gastropods (Figure 3).

Discussion

In this study, the survival rate was not affected by stocking density at the tested levels, as reported for other species of gastropods by Souza Júnior et al. (2013), for *Pomacea lineata* (Spix, 1827), *P. bridgesii* (Reeve, 1856), and *P. paludosa* by Garr et al. (2011). However, for *P. canaliculata*, the density effect caused a decrease in the survival of individuals (Tanaka et al., 1999), as also observed for other gastropod species of other genera such as the *Littorina irrorata* (Say, 1822) (Stiven and Kuenzler, 1979).

The treatment with 2.5 animals/L was superior for size and weight gain of *P. dolioides* when compared with the highest densities, which agrees with Conner et al. (2008), in which lower densities had higher performance results, and the higher the density, the lower growth of young *P. paludosa* individuals. The density also negatively influenced the size of *P. canaliculata* (Yoshida et al., 2013) and other species of gastropods such as *L. irrorata* (Stiven and Kuenzler, 1979), and pulmonary species such as *Helix aspersa* (Muller, 1774) and *Bulimulus tenuissimus* (d'Orbigny, 1835), with an inversely proportional relationship between density and growth (Mayoral et al., 2004; Meireles et al., 2010). Almeida and Bessa (2001a) pointed out that, for *Leptinaria unilamellata* (d'Orbigny, 1835), individuals that are kept isolated reach larger shell length than individuals in a group, but in this species, grouped individuals become sexually mature earlier, as observed for *Bradybaena similis* (Férussac, 1821) (Almeida and Bessa, 2001b). As for individuals of *Balea perversa* (Linnaeus, 1758) held at high densities, their size is reduced and, consequently, their sexual maturity is delayed; however, their reproductive rate does not differ among different densities (Baur and Baur, 1994). For *Pomacea dolioides*, within the same treatment, there was great variation in size, even though in treatments with higher density, individuals had less growth. Pires-Júnior et al. (2017), who cultured *P. dolioides* in different conditions of food and feeding frequency, observed the same result.

Gastropods at densities of 5 and 10 animals/L had lower SGR. Garr et al. (2011) reported that a monospecific diet and high densities over time are detrimental to the development of young *Pomacea paludosa*, causing low growth rates, which can be minimized with the use of a wider variety of feeds at lower densities, so that young individuals reach larger sizes and sexual maturity faster. Alves et al. (2006) noted that, for *P. lineata* and *P. bridgesii*, SGR did not differ between species in a 16-week period, but, at a lower density than in our study, the gains were higher than for *P. dolioides*.

The biomass gain was highest in treatment 10 animals/L, probably due to the larger number of individuals in the treatment. As for the absolute weight gain, treatment with 2.5 animals/L had the highest increases, reaching more than 43% than the other two treatments, corroborating Souza Júnior et al. (2013), who found that the increase in density reduced the weight gain of individuals of the two investigated *Pomacea* species, probably due to social factors. Hence, it can be inferred that intraspecific competition may have aspects such as territoriality or hierarchy, which affect the development of individuals. However, for freshwater snails, it has not been established whether these individual differences are caused by competitive interactions such as competition for exploration, environmental factors, or other factors such as genetics. They do not grow uniformly, which implies that factors such as interaction between individuals partially affect their development (Kawata and Inaba, 1992).

Based on studies on the effect of density on the growth of different species of the *Pomacea* genus, Conner et al. (2008) stated that this gastropod seems to be quite sensitive to initial density increases compared to other species, confirming our findings with *P. dolioides*. Increasing the number of individuals per area tends to make the performance drop, even under similar conditions of water and food. Species of the genus *Pomacea* is not yet commercially explored in the Amazon region, but it can be considered an alternative protein source to supplement human food and feeding of other animals of husbandry interest. Thus, in laboratory conditions, *P. dolioides* showed higher gains with lower density (2.5 animals/L) treatments, and the mentioned densities are recommended in association with a rate of food at least three times a day, as suggested by Pires-Júnior et al. (2017), when seeking to adapt other techniques, to explore the full potential of still unknown cultivations.

Conclusions

The density with the best cultivation results is 2.5 animals/L, being most effective for the parameters of growth, weight gain, specific growth rate, and percentage weight gain, when compared with other treatments after 225 days of the experiment. For future cultivation of this species, a density of 2.5 animals/L should be used to improve handling performance.

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