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Growth and survival of the native oyster *Crassostrea gasar* cultured under different stocking densities in two grow-out systems in tropical climate

[*Crescimento e sobrevivência da ostra nativa Crassostrea gasar cultivada em diferentes densidades em dois sistemas em clima tropical*]

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ABSTRACT

Survival and growth of the native oyster *Crassostrea gasar* along the juvenile and adult phases were evaluated in three different stocking densities [low (D), medium (2D) and high (3D)] and in two grow-out systems (fixed and floating system). The fixed system consisted of a rack made with PVC, fixed from the bottom with wood sticks. The floating system consisted of floating bags suspended by a rack made with PVC and maintained submerged from the seawater surface by eight floats. Survival and shell height of oysters cultured after 30, 60 and 90 days were registered in each phase and in each grow-out system. Results showed that the grow-out system did not affect survival and growth of *C. gasar* in the juvenile and adult phases. The tested densities affected the survival of oysters cultured over time in both phases but did not affect oyster growth. At times analyzed, it was observed positive growth in juvenile oysters grow after 90 days of culture. However, in the adult phase, no growth was observed after 90 days of culture. Oyster yield was higher in the density 3D, in both juvenile and adult phases. These findings contributed to the development of the oyster *C. gasar* culture.

Keywords: floating system; fixed system, native oyster; stocking density

RESUMO

A sobrevivência e o crescimento da ostra nativa Crassostrea gasar nas fases juvenil e adulta foram avaliados sob três diferentes densidades de estocagem [baixa (D), média (2D) e alta (3D)] e dois sistemas de engorda (fixo e flutuante). O sistema fixo consistiu em uma mesa de PVC, fixada na parte inferior com varas de madeira. O sistema flutuante consistiu em travesseiros flutuantes suspensos por uma mesa de PVC e mantidas submersas da superfície da água do mar por oito flutuadores. Registraram-se sobrevivência e altura da concha de ostras cultivadas após 30, 60 e 90 dias, em cada fase (juvenil e adulta) e em cada sistema (fixo e flutuante). Os resultados mostraram que o sistema de engorda não afetou a sobrevivência e o crescimento de C. gasar nas fases juvenil e adulta. As densidades testadas afetaram a sobrevivência das ostras ao longo do tempo, em ambas as fases, mas não afetaram o crescimento em altura. Nos tempos analisados, ostras juvenis apresentaram crescimento após 90 dias de cultivo. Porém, na fase adulta, não foi observado crescimento após 90 dias de cultivo. A produção de ostras, foi maior na densidade 3D, nas fases juvenil e adulta. Os presentes achados contribuíram para o desenvolvimento do cultivo da ostra C. gasar.

Palavras-chave: sistema flutuante, sistema fixo, ostra nativa, densidade de estocagem.

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INTRODUCTION

Oyster farming stood out in the world marine aquaculture with an approximated production of 5.9 million tonnes in 2018 (FAO, 2020a). Within oyster species, the genus *Crassostrea* is the most cultured worldwide, and represents 99.8% of total oyster production (FAO, 2020a). Brazil occupies the second position in the ranking of oyster production in Latin America (FAO, 2020b). The major produced species in Brazil is the Pacific oyster, *Crassostrea gigas*, with 2,759.6 tonnes in 2019 (EPAGRI, 2020). The native oyster, *Crassostrea gasar* (syn. *Crassostrea tulipa*; syn. *Crassostrea brasiliana*) is the second most cultured species in Brazil. In 2019 the production of *C. gasar* reached 96,7 tonnes in Santa Catarina State (EPAGRI, 2020) and 86.6 tonnes in Pará State (IBGE, 2020). In other Brazilian States, as Maranhão, Rio Grande do Norte, Alagoas, Sergipe, Bahia, Espírito Santo, São Paulo and Paraná, available total production data do not discriminate *C. gasar* from other cultured oyster species.

Crassostrea gasar has specific characteristics that make it attractive for culture, such as resistance to varied temperatures and salinity (Horodesky *et al*., 2019), easy implementation (Sampaio *et al*, 2019), high market value and acceptance (Lameira-Silva *et al*., 2020). Studies on *C. gasar* oyster culture in estuarine waters have been developed to evaluate different stocking densities (Pereira *et al*., 2001), growth performance in different environments (Lopes *et al*., 2013; Legat *et al*., 2017; Oliveira *et al*., 2018) and yield of hatchery-produced seeds (Tureck *et al*., 2014).

Stocking density is an important factor that can influence oyster's growth performance, as observed by Brunetto et al. (2020) for *C. gasar* culture in subtropical climate, and for other species as observed by Treviño *et al*. (2019) for the pearl oyster *Pteria sterna* and by Capelle *et al*. (2020) for *C. gigas* in intertidal off-bottom culture. Lower stocking densities may cause the underutilization of space generating economic loss (Liu *et al*., 2019), or higher densities could affect feeding process (Honkoop and Bayne, 2002; Gosling, 2015) and consequently growth of oysters. Thus, determining the adequate density provides an optimization of space without affecting growth performance and final productivity (Treviño *et al*., 2019).

Despite the importance of stocking density for oysterculture, there is still missing scientific information about the relationship between this factor and grow-out systems for *C. gasar* culture along the Brazilian coast. In this context, we evaluated the effect of different stocking densities on the growth and survival of *C. gasar* cultured in two different grow-out systems (fixed and floating) in Northern Brazil, tropical climate.

MATERIAL AND METHODS

Our experiment was performed in the Emboraí Velho river (01°03'16,7''S 46°26'49,4''W), Nova Olinda community, Augusto Corrêa, Northeast of Pará, Brazil (Figure 1), between November 2018 and May 2019. Oyster used in the study, *C. gasar*, were purchased from local growers at Lauro Sodré community, Curuçá, Pará, Brazil. Oysters cultured in Nova Olinda, which seeds come from Lauro Sodré community, have been identified as *Crassostrea gasar* species by Melo *et al.* (2010). After oyster selection by height, oysters were transported to the experimental area in boxes without water, only with moistened sponges to avoid dryness. *Crassostrea gasar* size (height) was classified according to Sühnel *et al.* (2017): seed: height between 1.5 and 5 mm; juvenile: height between 5 and 30 mm; and adult: height bigger than 30 mm.

We conducted a 2x3 factorial design with two grow-out systems (fixed and floating) and three different stocking densities in the juvenile and adult phases. Low (D), medium (2D) and high (3D) stocking densities were tested at juvenile phase and at adult phase with different initial animal quantities (see Table 1). For juvenile phase, densities were equivalent to the oyster number per bag (bag-area = 0.5 m^2) used by farmers. For adult phase, D was equivalent to approximately 50% bag-area coverage, according to Honkoop and Bayne (2002), 2D 100% and for 3D 150% of bag-area coverage.

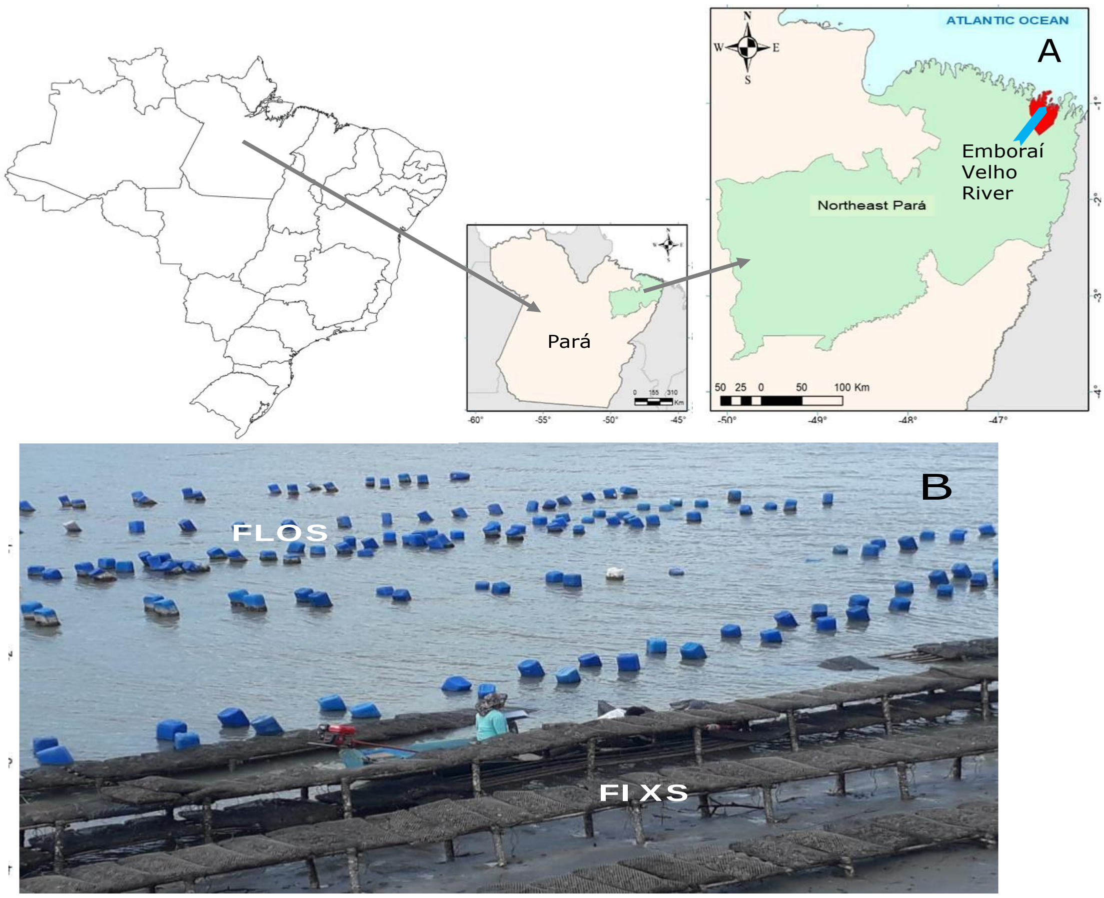

Figure 1. Location of the mangrove oyster (*Crassostrea gasar*) culture field in the municipality of Augusto Corrêa (01°03'16.7''S; 46°26'49.4''W), State of Pará, Brazil (A). Details of floating (FLOS) and fixed (FIXS) grow-out system used in the experiment (B). Cartographic base: IBGE and MMA, data accessed by Romão E.P (2019).

Table 1. Oyster number per bag (0.5 m^{-2}) in each stocking densities (D, 2D and 3D), mesh size of the bag (between opposite knots) and culture time used in the juvenile and adult phases to evaluate growth and survival of *Crassostrea gasar* cultured in fixed and floating grow-out systems, in State of Pará, Brazil

Oyster life phase	Total experiment time (days)	Mesh size of bag (mm)	Oyster number per bag		
			D	2D	3D
Juvenile	90	9	500	1000	1500
Adult	90	14	216	462	648

The fixed system consisted of a rack (6 x 0.8 m; length x width, respectively) made with PVC (40 mm), fixed at 0.9 m from the bottom with ten wood sticks. Oyster in the fixed system were exposed naturally to air for 3-4 hours during low tide. The floating system consisted of floating bags suspended by a rack (6 x 0.8 m, length x width respectively) made with PVC (40 mm) and maintained 0.5 m submerged from the seawater surface by eight floats (20L). To anchor the rack, two concrete blocks (0.8 x 0.8 x 0.6 m, length, width and height, respectively) were used. For both tested grow-out systems, oysters were cultured in plastic bags (1 x 0.5 m), each plastic bag was an experimental unit (EU).

For initial planting in the juvenile phase, a total of 24,000 oysters (height 22.6±1.1 mm; n=100) were used to test the first three stocking densities (Table 1) in fixed and floating systems. For planting in the adult phase, oysters from each stocking density (D, 2D and 3D) from juvenile phase, were planted in the new tested stocking density (D, 2D and 3D for adult phase) in each EU per grow-out system (Table 1). Each studied oyster life phase (juvenile and adult) was carried out for 90 days.

Monthly, we cleaned the plastic bags to remove fouling and registered the number of dead oysters per EU and the height of 30 oysters from each EU. Survival was calculated subtracting the number of dead oysters from the total live oysters after 30, 60 and 90 days of culture (T30, T60 and T90, respectively). Shell height was measured according to Galtsoff (1964), with a digital caliper (0.01 mm). Total oyster number per EU, in each treatment over time, was used to analyze "oyster yield" for the factors: grow-out system (fixed and floating), densities (D, 2D and 3D) and times (T30, T60 and T90).

Temperatures (measured monthly with digital thermometermV-363) ranged from 27.8 to 29.9ºC with average of 28.7±0.8ºC in the seed phase. In the juvenile phase, temperature ranged from 26.0 to 27.8ºC with average of 27.1±0.7ºC. Salinity (measured monthly with refractometer vodex VX100SG) ranged from 4 to 33, with average of 20.2±10.6 in the seed phase. In the juvenile phase, salinity ranged from 4 to 9, with average of 6.3 ± 1.9. Standard deviation of salinity in the seed phase was high, because the rainy season started in February (salinity 4). Precipitation (rainfall) data were obtained from INMET (available at: http://portal.inmet.gov.br/, for station "Bragança A226"). In the seed phase, precipitation ranged from 13.6 to 654.5mm with average of 366.3±167.8 mm. In the juvenile phase, precipitation ranged from 439.8 to 654.5 mm with average of 549.5±68.6mm. Standard deviation of precipitation in the seed phase was high, because a high rainfall (654.5 mm) was registered in February (T90).

Shell height and survival values were submitted to the normality (Shapiro-Wilk) and homoscedasticity of variance (Levene) tests. Non-parametric Kruskal-Wallis's Test was applied using significance level of 5%. Statistical analysis was performed at R software.

RESULTS

Survival of juvenile oysters (Figure 2) in the fixed grow-out system ranged from 75.1± .6% (density 3D) at T90 to 95.1±2.4% (density D) at T30. In the floating system, survival ranged from 82.6±0.8% (Density 3D) at T90 to 97.3±0.2% (density D) at T30. Survival in juvenile phase was affected significantly ($p<0.05$) by density and by culture time, but not by grow-out system (Table 2). Mean survival of juveniles in the density D was significantly ($p<0.05$) higher than in the treatments 2D and 3D. The densities 2D and 3D were not statistically different between them. In relation to time, mean survival of juveniles after 30 days of culture was significantly ($p<0.05$) higher than after 60 and 90 days. Mean survival of juveniles after 60 and 90 days of culture were not significantly different.

Shell height of juvenile oysters (Figure 3) in the fixed grow-out system ranged from 24.8±2.4mm (density 2D) at T30 to 43.6±2.0mm (density D) at T90. In the floating system, juvenile oyster sizes ranged from 27.6±0.9 mm (density 3D) at T30 to 45.8±1.6 mm (Density D) at T90. Shell height in the juvenile phase was affected significantly ($p<0.05$) by culture time, but not by density nor by grow-out system (Table 2). Mean height of juvenile oysters after 90 days of culture was significantly ($p<0.05$) higher than at T0 and after 30 and 60 days. Mean height at T0 was significantly ($p<0.05$) lower than after 30, 60 and 90 days of culture. Mean height of juveniles at T60 and T30 were not statistically different between them.

Oyster yield in the juvenile phase was affected significantly ($p<0.05$) by density, but not by culture system and time (Table 2). Mean oyster yield was significantly ($p<0.050$) higher in the density 3D compared to D and 2D. Also, mean oyster yield in the density 2D was significantly ($p<0.050$) higher than in D. Oyster yield in density 3D represents 2.7 and 1.5 times more oyster number than in D and 2D, respectively.

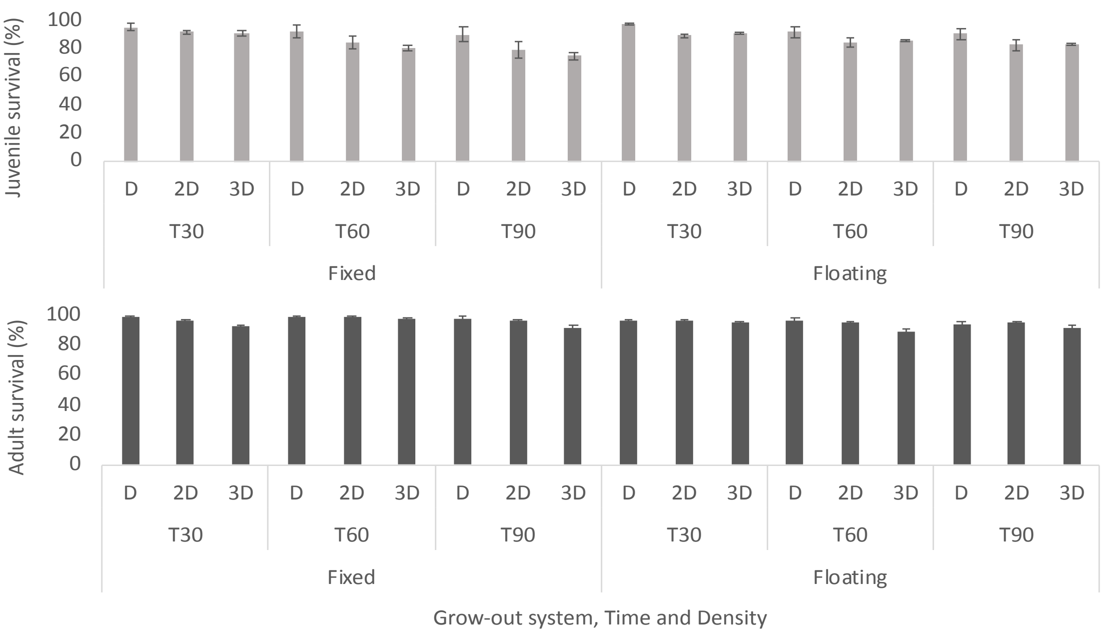

Figure 2. Survival (%; mean ± standard deviation) of juvenile and adult oysters cultured in fixed and floating grow-out systems, under stocking densities D, D2 and D3 (D=500, 2D=1000 and 3D=1500 animals per bag in the juvenile phase; and D=216, 2D=462 and 3D=648 animals per bag in the adult phase), after 30 (T30), 60 (T60) and 90 (T90) days (time).

Table 2. Oyster survival (%; mean ± standard deviation), shell height (mm; mean ± standard deviation) and oyster yield (number of oysters; mean ± standard deviation) in each analyzed factor: grow-out systems (fixed and floating), densities (D=500, 2D=1000 and 3D=1500 animals per bag in the juvenile phase; and D=216, 2D=462 and 3D=648 animals per bag in the adult phase) and times: at the beginning of the experiment (T0) and after 30, 60 and 90 days of culture (T0, T30, T60, T90, respectively). Lower letter shows Kruskal Wallis's Test results

Phase	Data	Analyzed factor			
Juvenile		Fixed	Floating		
	Survival (%)	86.6±7.5[a]	88.3±5.2[a]		
	Height (mm)	35.1±7.0[a]	37.8±7.4[a]		
	Oyster yield (number)	849.4±323.4[a]	872.1±341.8[a]		
		D	2D	3D	
	Survival (%)	92.8±4.3[a]	85.3±5.5[b]	84.4±6.0[b]	
	Height (mm)	37.9±7.5[a]	35.5±7.1[a]	35.8±7.2[a]	
	Oyster yield (number)	464.1±21.5[a]	852.9±55.0[b]	1265.3±89.3[c]	
		T0	T30	T60	T90
	Survival (%)	-	92.6±3.1[a]	86.4±5.4[b]	83.4±6.7[b]
	Height (mm)	22.5±0.1[a]	27.5±2.1[b]	37.9±3.9[b]	43.8±2.2[c]
	Oyster yield (number)	-	917.7±362.6[a]	849.3±322.5[a]	815.3±302.5[a]
Adult		Fixed	Floating		
	Survival (%)	94.8±3.0[a]	95.9±2.4[a]		
	Height (mm)	41.0±3.2[a]	42.5±2.9[a]		
	Oyster yield (number)	414.6±156.8[a]	422.8±166.9[a]		
		D	2D	3D	
	Survival (%)	96.9±2.2[a]	96.2±1.5[a]	93.0±2.8[b]	
	Height (mm)	42.9±2.5[a]	41.1±2.5[a]	41.3±3.9[a]	
	Oyster yield (number)	209.3±4.7[a]	444.3±7.1[b]	602.6±17.8[c]	
		T0	T30	T60	T90
	Survival (%)	-	97.2±2.4[a]	95.3±2.1[b]	93.6±2.6[b]
	Height (mm)	43.8±2.3[a]	40.4±2.9[a]	40.9±3.6[a]	41.9±2.5[a]
	Oyster yield (number)		426.7±165.5[a]	419.1±162.8[a]	410.4±157.2[a]

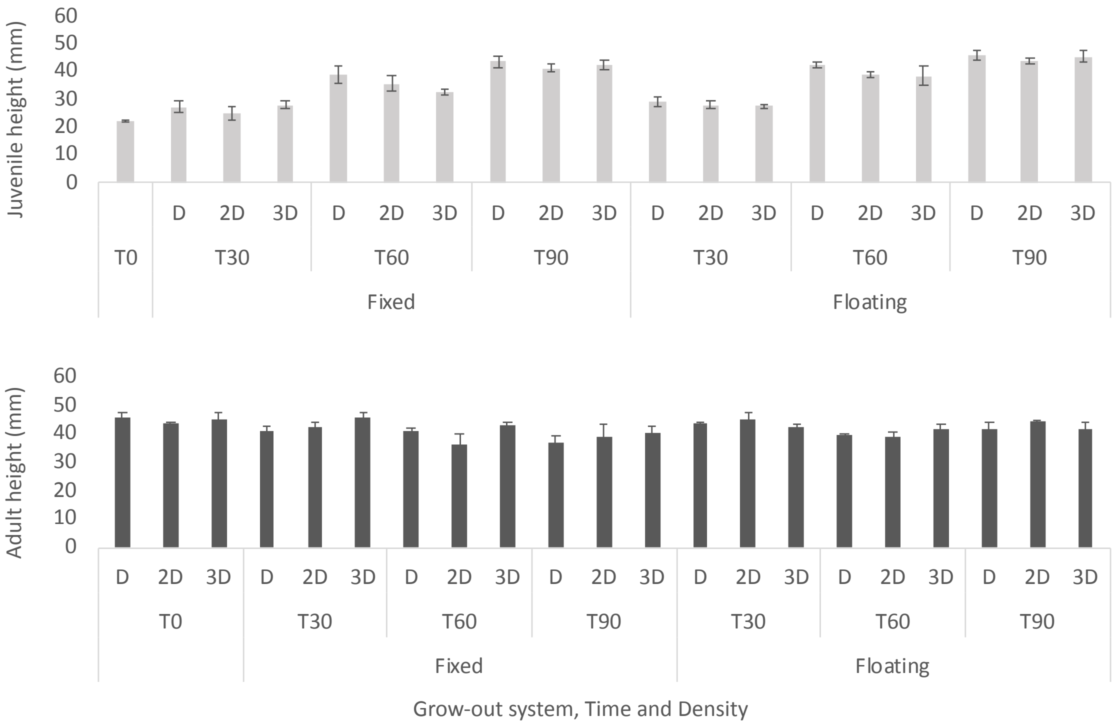

Figure 3. Shell height (mm; mean ± standard deviation) of juvenile and adult oysters cultured in fixed and floating grow-out systems, under stocking densities D, D2 and D3 (D=500, 2D=1000 and 3D=1500 animals per bag in the juvenile phase; and D=216, 2D=462 and 3D=648 animals per bag in the adult phase), after 30 (T30), 60 (T60) and 90 (T90) days (time).

Survival of adult oysters (Figure 2) in the fixed grow-out system ranged from 91.5±1.2% (density 3D) at T90 to 99.2±0.4% (density D) at T60. In the floating system, survival ranged from 89.5±1.3% (Density 3D) to 96.4±1.9% (density D), both at T60. Survival in adult phase was affected significantly ($p<0.05$) by density and culture time, but not by the grow-out system (Table 2). Mean survival of adult oysters in the density D and 2D were not significantly different between them, but they were higher ($p<0.05$) than in the treatment 3D. In relation to time, mean survival of adult oysters after 30 days of culture was significantly ($p<0.05$) higher than after 60 and 90 days. Mean survival of adult oysters after 60 and 90 days of culture were not significantly different.

Shell height of adult oysters (Figure 2) in the fixed system ranged from 36.5±3.8mm (density 2D) at T60 to 45.8±.6mm (density 3D) at T30. In the floating system, oyster sizes ranged from 39.4±1.7 (density 2D) at T60 to 45.5±2.2mm (Density 2D) at T30. Shell height of oysters cultured in the adult phase was not affected by grow-out system, density and time (Table 2). Oyster sizes after 30, 60 and 90 days of culture were not significantly different from the beginning of the experiment (T0).

Oyster yield in the adult phase was affected significantly ($p<0.05$) by density, but not by culture systems and times (Table 2). Mean oyster yield was significantly ($p<0.050$) higher in the density 3D compared to D and 2D. Also, mean oyster yield in the density 2D was significantly ($p<0.050$) higher than in D. Oyster yield in density 3D represents 2.9 and 1.4 times more oyster number than in D and 2D, respectively.

DISCUSSION

Juveniles and adults of the native oyster *Crassostrea gasar* showed good performance in the two grow-out systems and in the three densities tested in this study (State of Pará, North of Brazil). Oyster survival in juvenile and adult phases was not affected by fixed nor by floating culture systems tested. Mean survivals were high after 90 days of culture, up to 75% in the juvenile phase and up 91% in the adult phase. High survival rates of *C. gasar* in the juvenile and adult phases were also observed by Brunetto *et al*. (2020), but in a subtropical climate region (South of Brazil) and using seeds from hatchery production. In a similar climate region (Southeastern of Brazil), but in an estuarine environment and using capture-based seeds, Galvão *et al*. (2009) registered lower survival rates of oysters (*Crassostrea* sp.) in the juvenile phase, after 90 days of culture.

Despite better survival rates were observed under low densities (D: 500 oysters.0.5.m^{-2}) in the juvenile phase and under low and medium densities (D: 216 oysters.0.5.m^{-2}; 2D: 462 oysters.0.5.m^{-2}) in the adult phase, survival rates under high densities (3D) were also satisfactory for the juvenile and adult phases of *C. gasar* cultured in Pará. Small differences in the survival rates could be related to the density range used in this study. Brunetto *et al*. (2020) tested similar densities for *C. gasar* in juvenile phase, and also observed minimal differences in survival rates.

Changes in shell length or height, in weight or energy content are parameters to measure growth in bivalves (Bayne, 2017). Growth in bivalves is affected by many factors, as location of culture system, water temperature and salinity, quantity and quality of food and others (Gosling, 2015).

Our studied area (tropical climate) showed to be adequate for growth of *C. gasar* in the juvenile phase. Shell height of juvenile oysters increased approximately 26 mm after 90 days, independent of the culture system. In subtropical climate (South of Brazil), Legat *et al*. (2017) registered approximately 20 mm of growth after 30 days in the juvenile phase of *C. gasar*. Also, Brunetto *et al*. (2020) observed juveniles of *C. gasar* with shell height ranging approximately from 50 to 52 mm after 48 days of culture.

Nevertheless, no growth of *C. gasar* in the adult phase was observed after 90 days of culture in both grow-out systems (fixed and floating) tested in our study. This fact could be related to the salinity recorded in this phase.

Salinity is an important environmental factor for mollusks (Berger and Kharazova, 1997). Responses to changes in external salinity involve closing of bivalve shell valves, adjust of the intracellular concentrations of ions (Gosling, 2015) and decrease of functional activity (Berger and Kharazova, 1997). Oysters are osmoconformers (Shumway, 1977; Fuhrmann *et al*., 2018) and demand energy to maintain tissue hydration level (Shumway, 1977), an energy that may be reallocated from growth or other physiological function. Salinity can also affect calcium concentration in the water, an important constituent in the bivalve shell formation (Zhao *et al*., 2016). According to Riley and Chester (1971), calcium concentration in freshwater are low (1.5 x10^3 µg.L^{-1}) compared to seawater with salinity 35 (422.0 x10^3 µg.L^{-1}).

Our studied area in State of Pará is known as *Am* climate following Köppen criteria (Alvares *et al*., 2014), with basically two seasons, one dry and other rainy. During the studied period, salinity fluctuated seasonally while oysters grew from the juvenile to adult phase. The juvenile phase was performed during the end of dry season, and the adult phase in the beginning of the rainy season. Consequently, in the adult phase, when average salinity was low (6.3±1.9), a ceased growth was registered. This fact was not observed in the juvenile phase, when average salinity was higher (20.3±10.6). Oliveira *et al*. (2018) evaluated growth performance of *C. gasar* in Pará using fixed culture system, and also registered lower growth rates in the rainy season.

Oyster growth in adult phase seemed to be more affected by environmental conditions than by grow-out system. This effect was not observed for survivals rates in the adult phase. *Crassostrea gasar* is a euryhaline species that can survive in salinities between 4 and 40 for a long period (Horodesky *et al*., 2019). Differences in growth of *C. gasar* were also observed using floating system in two different marine and estuarine areas, in the South of Brazil (Legat *et al*., 2017). Legat *et al*. (2017) also observed the effect of salinity on growth of *C. gasar* in tropical estuarine waters of Maranhão State. After 390 days of culture, these authors registered an average shell height of 48.90 ± 8.65 mm in salinities between 5 and 32 and of 36.20±12.40mm in salinities between 25 to 37. To better understand growth performance in the adult phase of *C. gasar* cultured in the studied area of Pará State, the effects of grow-out system and densities should also be tested in the dry season.

Independent of the phase, grow-out system did not affect growth of *C. gasar* in this study. Despite the floating system maintained oysters constantly submerged, with greater availability of phytoplankton, there was no differential growth in the end of the adult phase, compared to the fixed system. Three to four hours of air exposition, registered in the fixed system, seemed to not affect oyster growth. This can be an interesting result, especially for control of some types of fouling in the shells and in the culture equipment. Fouling can affect feeding mechanisms and cause stress to bivalves (Fitridge *et al*., 2012). Desiccate periods from 4 to 24 hours are listed by Hood *et al*. (2020) as a control strategy against organisms like macroalgae, bryozoan and barnacles in the oyster culture.

Total yield or total oyster number was not affected by culture system and time. Yet, the higher oyster yield in the density 3D, in both juvenile and adult phases, provided relevant information for oyster culture. Using high stocking densities (3D or more) in juvenile phase and in the beginning of adult phase (until 90 days of culture) could increase oyster production per area, without expanding farming area, once production in estuarine environments is limited to the available area to install culture systems.

CONCLUSION

Fixed and floating grow-out systems did not affect survival and growth of *C. gasar* in juvenile and adult phases. Densities D, 2D and 3D tested in juvenile and adult phases of *C. gasar* did not affect growth but affected survival. High survival rates were observed in low (D) densities for juvenile oysters and in low (D) and medium (2D) densities for adult oysters. Final total oyster yield was higher in the density 3D, in both juvenile and adult, phases. Growth (in shell height) after 90 days of culture was observed only in juvenile phase.

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