SEEDLING AND GROWTH OF *Rhizophora mangle* L. PROPAGULES AT A RESTORATION SITE (APA DE GUAPI-MIRIM - RJ)

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ABSTRACT

Capinam, V. S., De Oliveira, A. A. & Finotti, R. (2023). Seedling and Growth of *Rhizophora mangle* L. Propagules at a Restoration Site (APA de Guapi-Mirim - RJ). Braz. J. Aquat. Sci. Technol. 27(1). ISSN 1983-9057. DOI: 10.14210/bjast.v27n1.17187. Studying plantations and other restoration strategies for mangrove forests is important to understand the dynamics of this ecosystem and enhance such strategies. Size can be one of the most important characteristics for propagules success. Some studies state that larger seedlings can resist more and have greater survival than smaller seedlings. Therefore, this study aims to analyze the germination and development of *Rhizophora mangle* L. propagules at an *in situ* plantation in the mangroves of the Guapi-Mirim Environmental Protection Area. A total of nine plots were delimited with spacing of 4 meters by 1 meter each in which *Rhizophora mangle* propagules were separated into small and large groups and planted. Their germination and development were subsequently monitored for a period of 6 months. The diameter (cm), height (m) and initial weight (g) of the propagules and the increase in height (m) and diameter (cm) at 76, 93 and 154 days after planting were measured. Periodic observations were made approximately every 20 days in order to quantify the rate and type of mortality (predation, water current carriage and drying) and to monitor time (in days) of pairs of leaves opening. There were no differences between the propagules according to the size for the opening time of leaf pairs, nor for the mortality rate. The increments in diameter and height were larger for the small propagules in the first development periods, but not in the last ones. The large and small propagules did not show significant differences in diameter or height at 154 days. There was high mortality of small propagules due to predation and/or water current carriage (47.3%) after 76 days, while the mortality for large propagules was higher due to desiccation (22.2%) at the same period. The results, in general, suggest there was no difference in the survival and development of the propagules related to size, as they were more affected by local ecological conditions.

Key Words: Mangroves. Restoration. Management. Guapi-Mirim APA.

INTRODUCTION

Rio de Janeiro mangrove ecosystems occupy nearly 160 km^2 (Kjerfve & Lacerda, 1993), and its largest mangrove areas are located at the Paraíba do Sul river mouth and at Guanabara, Sepetiba and Ilha Grande bays (Araujo & Maciel, 1979; Feema, 1980; Menezes, 2000). Nowadays, a great part of the ecosystem in Guanabara Bay has been suppressed and only 1/3 of its 261.90 km² of original vegetation can be observed (Amador, 1997; Kjerfve et al., 1997; Pires, 2010). About 61.80 km² of these remnants are in the Guapi-Mirim Environmental Protection Area (APA Guapi-Mirim) within the municipalities of São Gonçalo, Itaboraí, Guapimirim and Magé (Pellens et al., 2001).

Guanabara Bay mangroves already presented much damage by the end of the 1970’s caused by deforestation, grounding for industrial area construction sites, lining for water courses, irregular occupation of permanent preservation areas, domestic sewage, garbage and industrial pollution (Amador, 1997). These areas are presently protected by many Environmental laws (4771/1965 and 12.651/2012 Brazilian Federal Forestry Codes, 9965/2000 Federal Conservation Units System, 369/2006 and 303/2002 CONAMA’s resolutions), but still suffer from irregular occupation and illegal exploitation due to the lack of inspection and the scrapping of environmental agencies, as well as political attempts to bend environmental protection regulations and abolish CONAMA Resolutions, such as those mentioned above. Thus, these ecosystems need increased protection and restoration policies and strategies.

According to Soares et al. (2003), three of the six typical Brazilian mangrove plant species are present on the Guanabara Bay coast: *Rhizophora mangle* L., *Avicennia schaueriana* Stapf & Leechman and *Laguncularia racemosa* (L.) C.F. Gaertn. There are only a few studies analyzing the success of *in situ* restoration sites for these species (Menezes et al., 2005). The survival rates in the case of *Rhizophora mangle* L. seem to be quite variable. Pádron (1997) showed survival rates above 85% in studying *Rhizophora mangle* propagule plantings in Cuba. Menezes et al. (2005), in Baixada Santista (SP), and Moscatelli & Almeida (1994), in Angra dos Reis (RJ), found a survival rate higher than 70% for seedling and propagule planting in some areas, but a high mortality rate (0 - 10% survival rate) for other sites in the same region.

Seedling and propagule survival depends on several factors; for example, the degree of site protection from tidal waves and currents (Goforth & Thomas, 1979; Menezes et al., 2005), difficulties in fixation, predation, desiccation and other mechanical damage (Hamilton & Snedaker, 1984), Mckee, 1995; Ball, 2002, Doropoulos et al., 2016), variations on salinity and irradiance and vegetal cover (Ball 2002, Kraus et al. 2008). Many authors agree that nursery propagules have higher survival rates than *in situ* planted propagules (Vanegas, 2013; Kinder et al., 2019); however, as shown above, survival may differ at *in situ* plantings, and their factors should be analyzed.

Propagule mass and size can be important factors for the establishment and development success. Davis (1940) says that there is a connection between the seedling mass and its survival, and Kinder et al. (2019) found that longer propagules generate larger seedlings. Studies of *Rhizophora mangle* nursery plantings in the city of Veracruz, Mexico, also point out that propagules which are larger than 20 cm, without spots and perforation points are of higher quality for germination (Vanegas, 2013). Based on the study of *Rhizophora mangle* species in Panama's mangrove forest, Rabinowitz (1978) found that seedlings generated from small propagules have high mortality because of the tidal flooding which generates difficulty in photosynthesis and respiration, can lead to exhaustion of the propagules' nutritive reserves. However, in analyzing direct planting of *Rhizophora mangle* propagules at sites in Baixada Santista, Fruehauf (2005) found no correlations between total initial height and seedling rate and mortality. This author says that the seedling choice for planting must be based on propagules which do not present damage regarding its dimension. Ball (2002) also found that propagule initial mass did not influenced survivorship but larger propagules resulted in larger individuals after 1 year growth.

Thus, studying propagule growth and development characteristics can present important information to be considered in restoration plantations. Therefore, the objective of this study is to monitor and analyze germination and development of *Rhizophora mangle* L. propagules *in situ* plantations at the Guapi-Mirim Environmental Protection Area, and to compare large and small propagules in relation to the propagule development parameters such as survival and mortality rates, time for leaf pair emission and height and diameter increase. The reasons why we choose *Rhizophora mangle* propagules for study are explained at Materials and Methods.

The study of restoration strategies for this biome is an important tool to understand ecosystems dynamics and to optimize such strategies. One of these studies is to analyze the factors which influence seedling and propagule *in situ* development (Menezes et al., 2005).

MATERIALS AND METHODS

Study area

The study area is located in the Guapi-Mirim Environmental Protection Area in the micro river bay of the Guaraí-Mirim River (Figure 1). The locality was accessed by a boat provided by the *Associação Manguezal Fluminense*.

The experiments were conducted under the Uçá Project, supported by Petrobras, via the Petrobras Socio-Environmental Program and developed by the Non-Governmental Organization (NGO) Guardiões do Mar. One of the actions of this project was to restore degraded mangrove areas. We made a partnership with the NGO in which one of the restoration areas

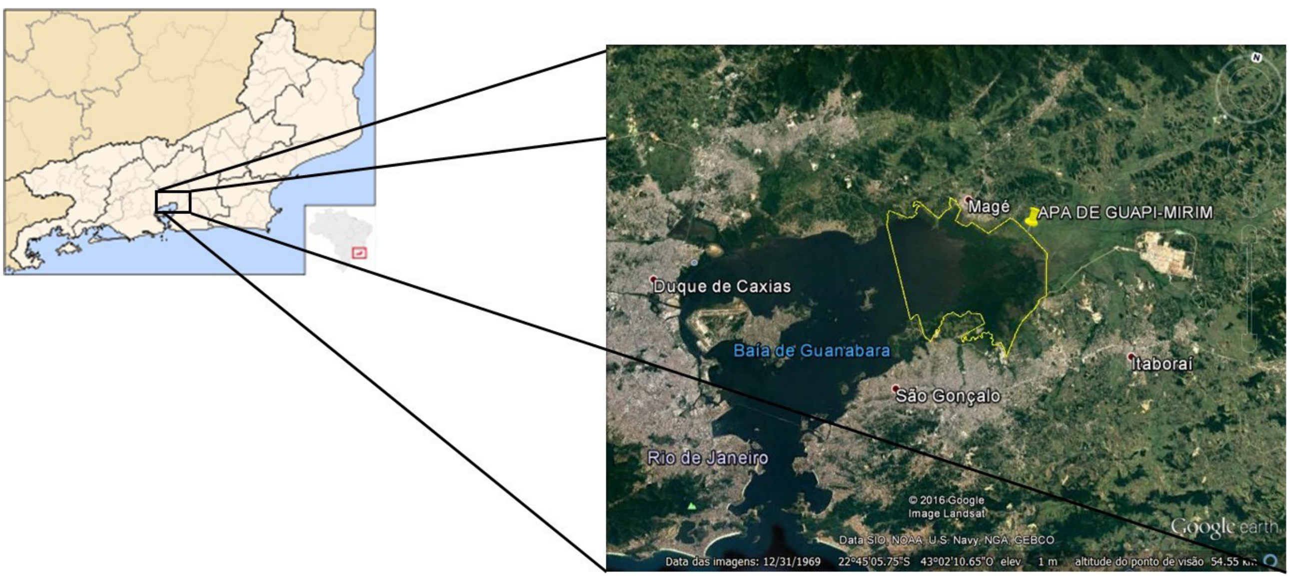

Figure 1 - Location of Guapimirim APA at Guanabara Bay and surrounding municipalities, Rio de Janeiro State (coordinates: -22º70'85"S/-42º99'70"W, Datum WGS 84).

were used six months before they started the planting actions. The area was cleared for plantation, in order to avoid competition with others species and habitat for possible predators as *Ucides cordatus* (Linnaeus, 1973). We started by analyzing the propagules of three plant species: *Rhizophora mangle* L., *Avicennia schaueriana* Stapf & Leechman and *Laguncularia racemosa* (L.) C.F. Gaertn. However, we were only able to continue the experiments using *Rhizophora mangle* due to predation problems, propagules being carried away by the water current and difficulties in finding the planted propagules for *Avicennia schaueriana* Stapf & Leechman and *Laguncularia racemosa* (L.) C.F. Gaertn.

Sampling design and data collection

The experiments were performed from January to July 2016. According to the Guanabara Ecological Station Management Plan, this is the period of highest propagule production for the three mangrove species. Soares et al. (2006) and Chaves (2007) show the same period for other Guanabara Bay regions. Other authors have reported that the highest production and recruitment of propagules occurs in rainfall periods (Ponte et al., 1984; Jimenez, 1988; Duke, 1990; Menezes, 1994; Lamparelli, 1995; Fernandes, 1997). Thus, this period was chosen to begin the experiment in order to provide a greater number of propagules.

We randomly collected propagules on the water and on the substrate by using a boat at different localities of the Environmental Protection Area. The collections were done on the way to the experiment area to guarantee genetic diversity of the plantations. The collected propagules were selected according to the recommendations of Goforth & Thomas (1979), namely: undamaged apical gem, no signs of dehydration, and no signs of insect attacks, which are considered characteristics of a newly released propagule.

Before planting propagules, they were visually separated between 2 groups: (1) big propagules and (2) small propagules. They were weighted with a precision balance until 0.01 g and total length measured until 0.01 cm. Differences were tested between the two classes using Mann-Whitney U test and were significative (Table 1). So, these classes were adopted.

A total of nine rectangular 4 m x 1m plots were used. These plots were circulated with shading fixed with zip ties to PVC pipes. The plots were installed in two lines starting 20 meters from the riverside with 5 meters lateral spacing between them (Figure 2).

Table 1 - Mean ± standard deviation (SD) for the characteristics of the measured variables between small (1) and large (2) propagules. N = sample number, DGH = Diameter at Ground Height (cm), Height (cm) and mass (g), and U = Mann-Whitney test parameter.

Group	n	Height ± SD	DGH ± SD	Mass ± SD
1	180	22.56 ± 3.37	1.03 ± 0.19	12.54 ± 3.45
2	180	30.81 ± 3.72	1.3 ± 0.15	25.36 ± 5.32
U/p		1442.5/0.01	5046.5/0.001	349.5/0.04

Tidal variations in the first three months of 2016 were extremely high compared to the usual regional ones because of an atypical precipitation rate recorded for the months of January and March 2016 (Brazilian Navy, 2016). According to the information from Rio de Janeiro meteorological station (INMET), the accumulated precipitation registered in the year 2016 for the months of January, February and March was 494.9 mm, versus 403.3 mm obtained for the same months in 1961 to 1990 (Figure 3).

They were subsequently seeded directly into the substrate after measurement at about 6 cm depth inside the plots. A total of 40 *Rhizophora mangle* propagules were planted in each plot, separated into two groups of 20 according to their mass and size. Thus, 180 small and 180 large propagules were analyzed, totaling 360 propagules.

The analyzed variables were: propagule height (cm), mass (g) and DGH (cm) before planting (day 0), height (cm) and DGH (cm) at 76 days, 93 days and 154 days. Height measurements were taken using a measuring tape and DGH was measured using a pachymeter, both with millimeter accuracy. DGH measurements after propagule planting were taken from the base at the substrate to the apical bud. Allen & Duke (2006) say that seedlings in a nursery can be considered good for plantations when they present the third pair of developed leaves, usually taking nearly 6 months to achieve this. Thus, a period of 154 days can be considered the time necessary for propagules to reach a development stage which is “mature” enough to continue to growth in a natural situation.

Data analysis

All variables were tested for normality using Kolmogorov-Smirnov (K) test, non-parametric comparisons were chosen. The height, mass and diameter (DGH) values before planting (day 0) were compared between the large and small classified propagules to evaluate whether these groups were really different according to the observed variables. We correlated height, mass and diameter using simple linear regression (r). The differences in height and diameter between the measuring time periods were calculated to determine relative growth. Total and relative growth were then compared between the groups. Total growth was the total length, height or DGH measures taken at a time period and relative growth was calculated as the difference between the final and initial values of two times periods. Graphical analyses with mean and standard deviation were used for all comparisons, and the Kruskal-Wallis (H) test for

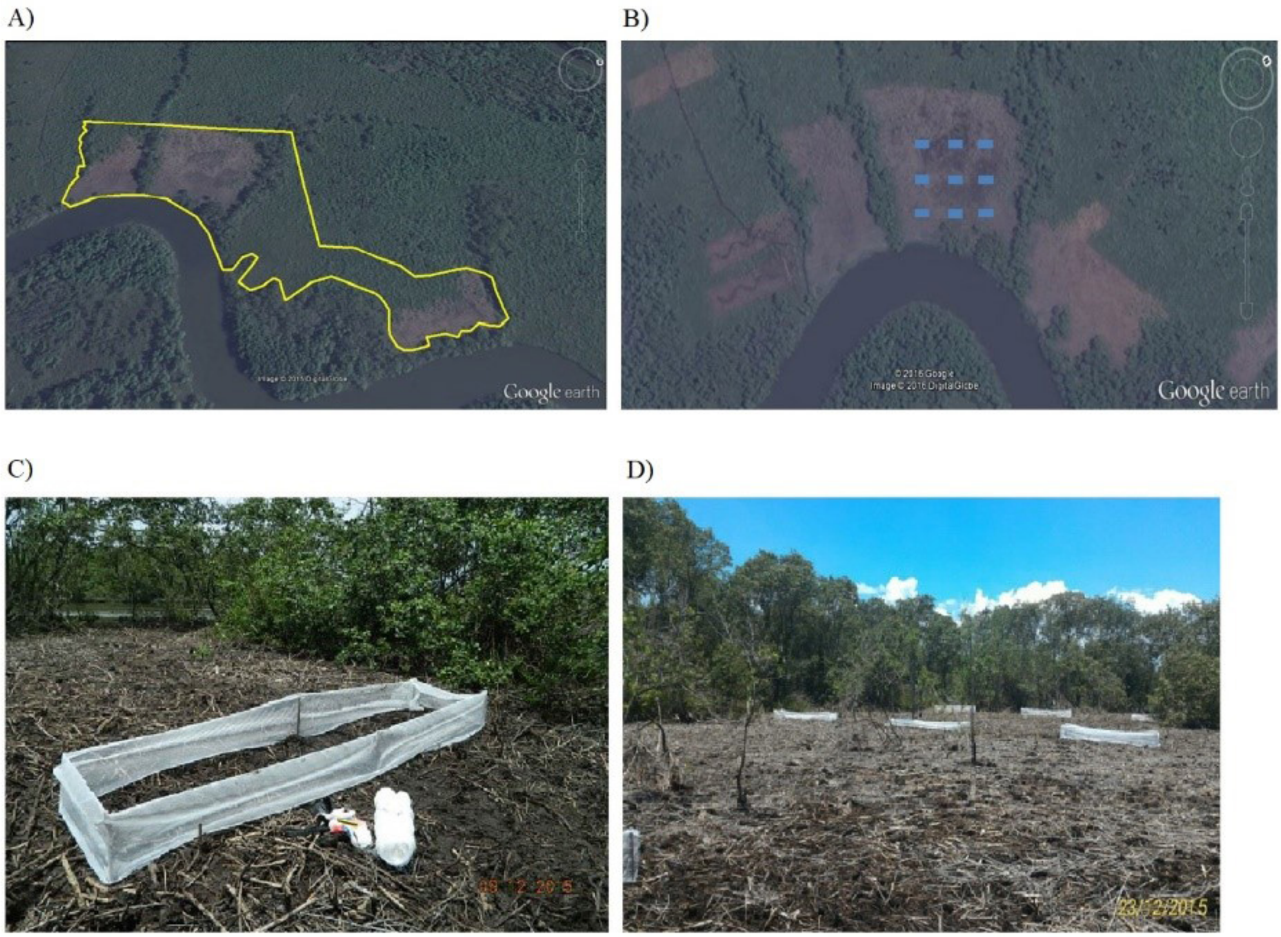

Figure 2 - (A) Reforestation area; (B) Study area, (C) Plot details and (D) Plot disposition.

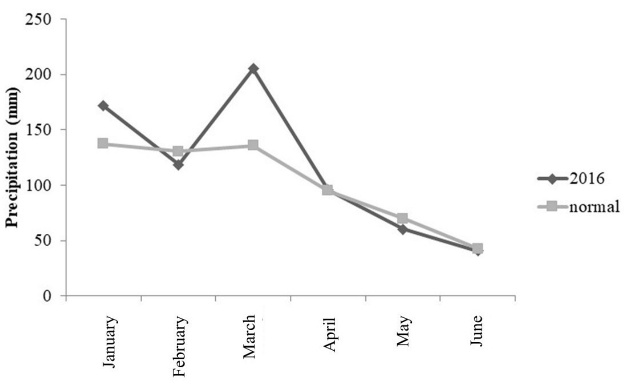

Figure 2 - Monthly accumulated precipitation January-June 2016 and Climatological Means (1961 to 1990) for the same climate station. Source: National Institute of Meteorology (INMET).

multi-parametric comparisons and the Mann-Whitney (U) test were used as a posteriori test and for paired comparisons with a significant level of 5% using Bonferroni correction. For all the analyzes we used Past 3.0 (Hammer et al. 2001).

We also monitored propagule development in periods of approximately 20 days, and registered the emission of leaf pairs and mortality. We considered leaf pair emission when leaf pairs were near 180°, and the emission date was registered and the emission time in days was calculated. The propagules and seedlings were counted and observed in relation to their aspect and health state, as well as signs of injury or predation. Were considered injury perforations, decomposition marks or other damage to the propagule structure that could not be identified as a bite. Those which were dry, clearly dehydrated or having severe predation damage were classified as dead standing. Those which were absent were considered predated or carried away by the tide. Propagule mortality was analyzed using the dead percentage value in relation to the total number of individuals planted.

RESULTS

The two established groups (small (1) and large (2) propagules) showed significant differences for height, diameter and mass (Table 1), showing that these variables are good characteristics for the established groups above.

Regression analyzes between these variables shows a significant correlation for both the small and large groups for mass and height (Figure 4, 1c and 2c) (r^2 = 0.62, p > 0.01 and r^2 = 0.69, p > 0.01; respectively) and for mass and DGH (Figure 4, 1b and 2b) (r^2 = 0.32, p > 0.01 and r^2 = 0.69, p > 0.01; respectively), but not for DGH and height (Figure 4, 1a and 2a) (r^2 = 0.03, p = 0.12 and r^2 = 0.07, p = 0.92; respectively) (Figure 4).

Comparing growth rates between small and large propagules, DGH for small propagules were significantly higher for the first time periods (a and b) (0 - 76 days and 76 - 93 days, U = 17, p = 0.000001 and U = 198, p = 0.006, respectively), but not for the last time period (c) (93 - 154 days, U = 226, p = 0.34). Height growth rates were significantly higher for small propagules in the first time periods (a and b) (0 - 76 days, U = 0, p = 0.000001 and 93 - 154 days, U = 153, p = 0.001, respectively) and significantly higher for large propagules in the last time period (c) (76 - 93 days, U = 5, p = 0.00002) (Figure 5). No significant differences were found for height and DGH when the difference between day 0 and day 154 were compared (U = 1.87, p = 0.12 and U = 0.91, p = 0.34, respectively) (Figure 6). Negative growths are the result of propagules that are probably dying and losing mass.

The growth differences resulted in a similar final height between the two groups at day 154 (U = 493.5, p > 0.01). The differences in final height became smaller from day 0 to day 93, until no significant differences were found at day 154. Significant differences between propagule groups were only found for the first period for DGH (Figure 7, Table 2).

Propagule survival was low for both groups, reaching nearly 10 to 15% on day 154 (32 small and 17 large propagules). There were no significant differences in mortality between the plots (N = 9, U = 29, p = 0.32) nor in the number of live propagules on day 154 (N = 9, U = 50.5, p = 0.11). The mortality causes differed significantly for the two groups; 55% of the small propagules were considered dead after 76 days, with 7.27% considered dead standing and 47.73% by predation or carried away by the tide.

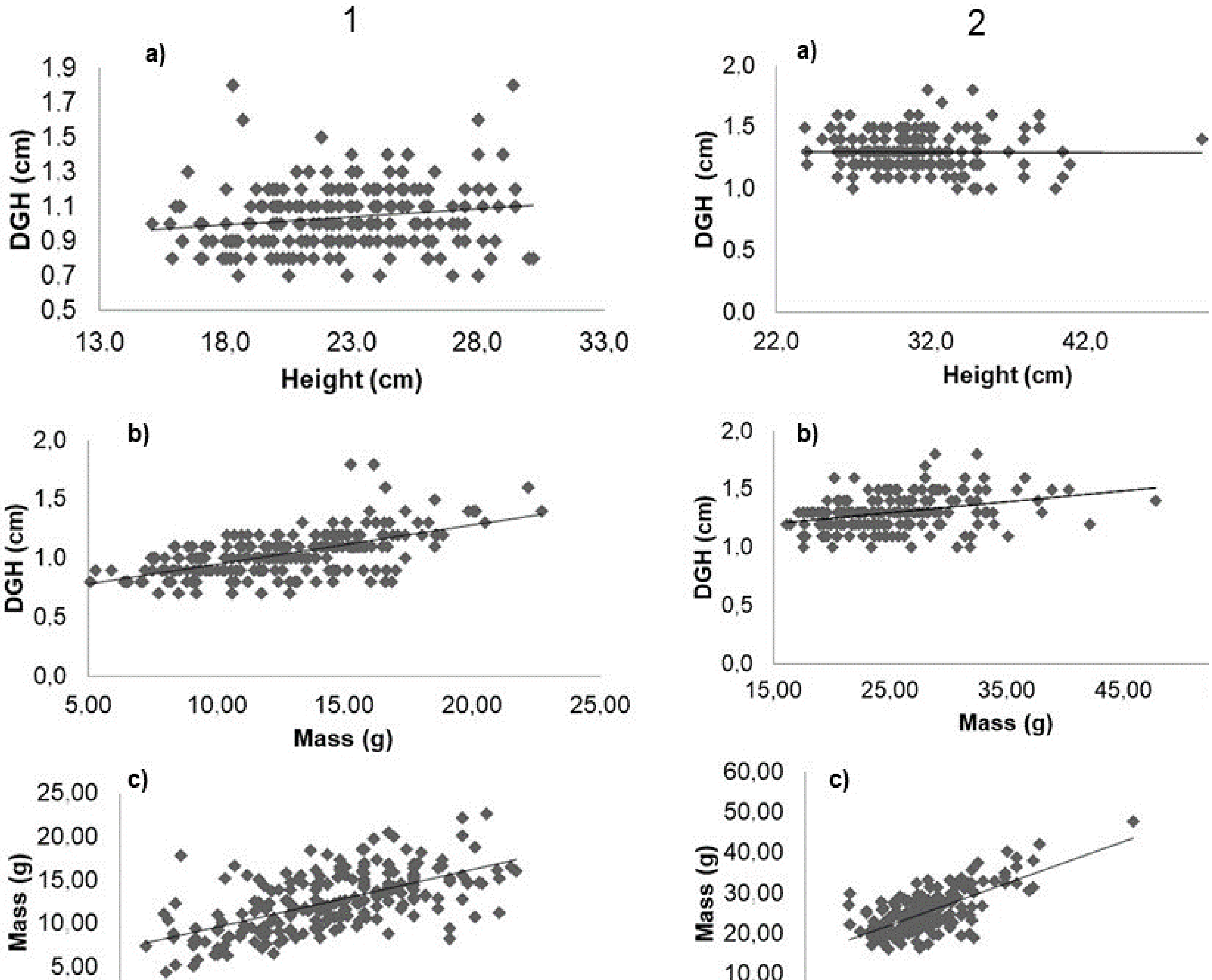

Figure 4 - Scatter plots and regression lines between the variables measured for both groups (1 - small and 2 - large) for variables Height (cm), Diameter at Ground Height (DGH) (cm) and mass (g).

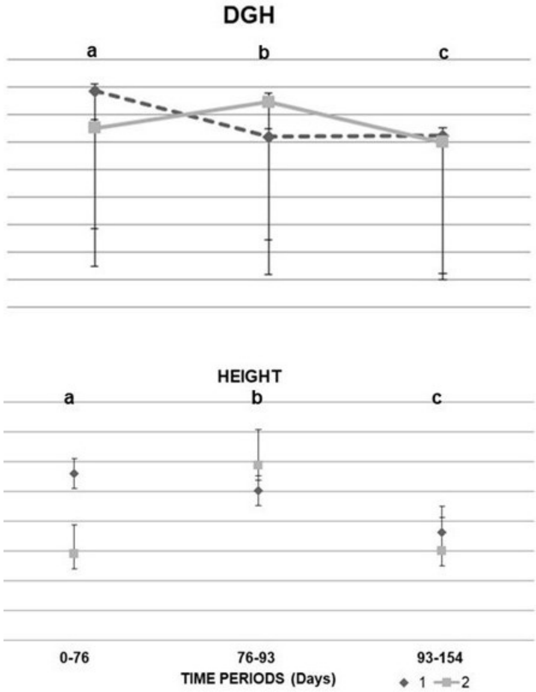

Figure 5 - Means (squares and diamonds) and standard deviation (bar) for the diameter (A) and height (B) growth (DGH) (cm) for the 3 time periods in days.

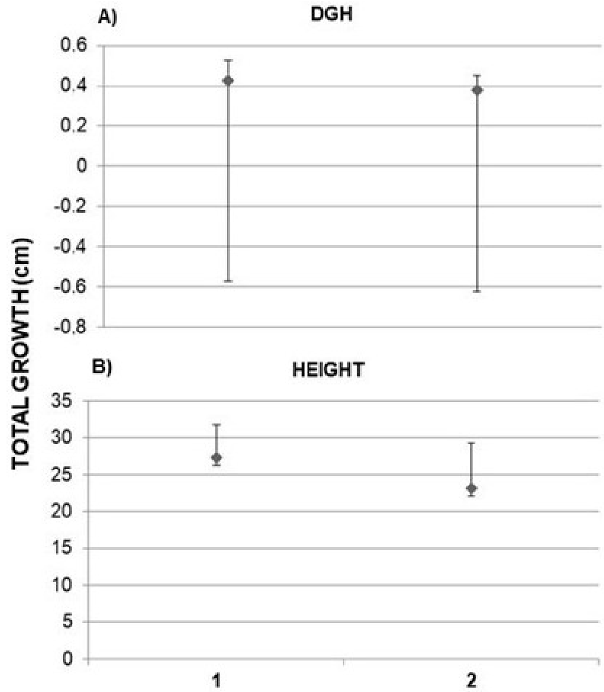

Figure 6 - Total Growth between day 0 and 154 for the groups for Diameter at Ground Height (DGH) (A) and Height (B). All measures are in centimeters (cm).

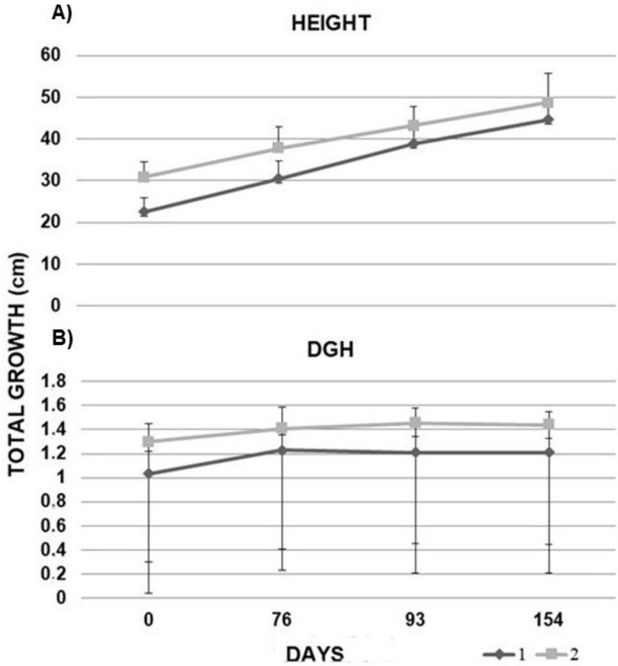

Figure 7 - Total growth of height (A) and DGH (B) comparisons for both groups at each measure day periods. (1) Small propagules and (2) Large propagules.

Figure 6 - Significance levels for Mann-Whitney U-test with Bonferroni correction for Height and DGH measures for the studied time periods. (1) Small propagules and (2) Large propagules.

HEIGHT					
		(1) Small propagules			
(2) Large propagules	Days	0	76	93	154
	0	> 0.001	1	> 0.001	> 0.001
	76		> 0.001	1	> 0.001
	93			0.15	1
	154				0.60

DGH					
		(1) Small propagules			
(2) Large propagules	Days	0	76	93	154
	0	> 0.001	0.11	0.02	0.02
	76		> 0.001	> 0.001	> 0.001
	93			> 0.001	> 0.001
	154				> 0.001

For large propagules in the same period, 48.33% were considered dead, with 22.22% dead standing and 26.11% by predation or carried away by the tide (Figure 8).

The two groups presented very similar times for the leaf pair emission (Figure 9). The majority of the propagules (80 small and 87 large propagules) emitted their first pair of leaves around day 51, the second by day 70, and the third between day 93 and 114.

DISCUSSION

The results for *Rhizophora mangle* propagule growth and development are generally in accordance with that found at other studies. It seems to be a general characteristic to achieve heights of nearly

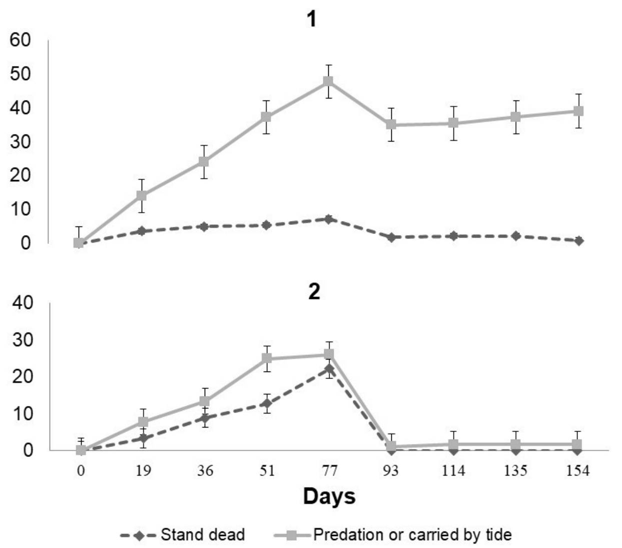

Figure 8 - Mean (squares and diamonds) and standard deviation (lines) of propagule mortality proportions on each observation day. (1) Small and (2) large.

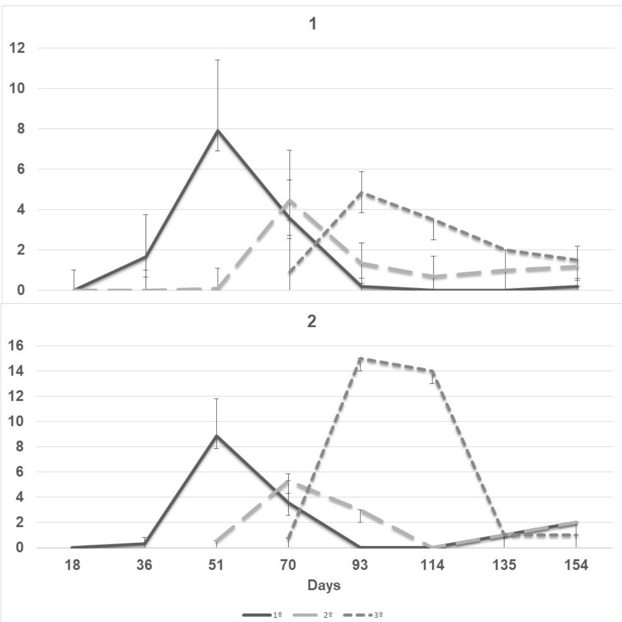

Figure 9 - Means and standard deviation (vertical bars) of the propagule proportion which emitted the 1st, 2nd and 3rd leaf pairs at days 18, 36, 51, 70, 93, 114, 135 and 154. (1) Small and (2) large.

40 - 50 cm at about 150 days of growth, with the emission of the first leaf pair between 40 to 60 days, and then having more than one leaf pair at nearly 120 days (Banus & Kolemmainen, 1975; Menezes et al., 2005; Kinder et al., 2019). Thus, these seems to be a general pattern in seedling and development of *R. mangle* propagules. However, important differences on propagules size were found in survivorship and seedling growth and its relations to the variables studied here.

Lin and Sternberg (1995), examining *R. mangle* propagules from different adult plant sizes at mangrove forests of Southern Florida, found that scrub forms (small) have significant lower seedling mass and size when compared to tall forms, and that larger propagules can result in larger individuals after six months in laboratory experiments with controlled conditions. Kinder et al. (2019) also found that *R. mangle* larger propagules resulted in larger seedlings after six months and at greenhouse and in controlled conditions also, and Ball (2002) also found that propagules with higher mass had higher average higher shoot mass after one year growth, depending on conditions of salinity and irradiance. Different to that found by these authors, there were no significant differences for growth in height and diameter relating to the initial propagule size after 154 days. The higher initial total increments in height and DGH for smaller propagules at the first days of growth induced a reduction in height and diameter differences in relation to larger propagules, by which smaller propagules achieve similar values to larger propagules at nearly 93 to 154 days. Therefore, larger sizes which could be indicative of more energy available for establishment and growth at the initial phases (Coleman et al., 2020) did not result in any advantage for larger propagules in relation to survival and growth up to 154 days. Size adjustment for diameter occurs at the very initial phases of propagule development (0 and 76 days), without any great detectable differences in the following periods. Height seems to be a more accurate measure and a better parameter to evaluate growth at this phase, as vertical growth seems to be more intense and may be important for this stage due to the needs of light capture for photosynthesis. The mean growth of nearly 30cm in height at 150 days is in accordance with that found by other studies (Banus & Kolemmainen, 1975; Menezes et al., 2005; Kinder et al., 2019).

Mortality rates above 80% after 154 days are also in accordance with that found for some *in situ* plantation studies (Fruehauf, 2005; Menezes et al., 2005) and do not agree with Davis (1940), who found that there is an increase in mortality of smaller propagules regarding *Rhizophora mangle*. The factors which cause high mortality rates are very variable and seem to be related to specific ecological local conditions such as irradiation, salinity, nutrient availability and biotic interactions (Krauss et al. 2008, Vanderklift et al., 2020), initial propagule mass seems not to be an important factor for survivorship in field conditions (Ball 2002). Menezes et al. (2005) found extremely variable survival rates (from 0 to 85%) between sites of the same locality, with predation and the water current being considered the main factors affecting them. These factors are related and are not mutually excluding. Sousa et al. (2003) and Devlin (2004) shows that predation intensity by the *Coccotrypes rhizophorae* beetle is higher at sites inside areas of vegetal cover which are more protected from water current compared with open sites and is probably the result of a higher beetle and propagule density at these sites. Other studies show that predation rates differ between these microhabitats and are influenced by predator specific preferences (Sousa and Mitchell, 1999). In studying mangrove forests in Índia, Praven et al. (2017) found that the *Neosarmatium malabaricum* crab had a preference for propagules of other species than those of the *Rhizophora* genus, and this was directly related to propagule density and inversely related to the chemical composition of the propagules. Ball (2002) found intense grazing pressure on *Rhizophora stylosa* seedlings in sites where salinity was high. Thus, predation intensity can be related to the propagule density or quality and site characteristics, such salinity and vegetal cover. By personal observations at the study site, we noted intense predation by *Neohelice granulata* (Dana, 1851) crabs which were frequently found carrying R. mangle propagules and eating them. It is possible that these crabs prefer smaller propagules. Neohelice granulata feed on bentonic invertebrates, debris, marismas and propagules (Alberti et al., 2007; Daleo et al., 2009; Barutot et al., 2011). It is possible that the propagule size is an important factor which influences the crab's carrying capacity and/or predation. Crab predation not only caused mechanical leaf damage, but also favors fungi and other pathogen infections (Costa et al., 2003; Alberti et al., 2007). Many predated propagules which were not carried by crabs or by the water current presented these characteristics. Thus, it is possible that smaller propagules are more susceptible to predation due to their quality.

However, another possible reason for the differences found for the two groups is that smaller propagules are more susceptible to being carried by the water current, as more frequent flooding increases the chances for propagules to dragged (Clarke and Kerrigan, 2002; Lovelock et al., 2015). At our study site, higher tidal variations in the study period (pages 8 and 9) could have been responsible for higher *in situ* mortality of small propagules.

In conclusion, the mortality causes varying between different sized propagules, despite this, local ecological conditions do not seem to favor *in situ* propagule planting at the studied period. Propagule development and growth up to 154 days shows that the ecological conditions are adequate for plant development as far as it can survive against the ecological filters (tidal variations and predation) discussed here. Studies that analyzes propagules establishment and growth and under different tidal conditions, vegetal cover, and predation levels can be important evaluate the influence of these factors on propagules of different sizes on the studied site.

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