Trees (2026) 40:40
https://doi.org/10.1007/s00468-026-02740-3

ORIGINAL ARTICLE

Ex situ experiments with *Rhizophora mangle*: what is the ideal sample and plot size?

Ully Depolo Barcelos[1] · Edilson Romais Schmildt[2] · Andreia Barcelos Passos Lima Gontijo[2] · Mônica Maria Pereira Tognella[1,2]

Received: 6 March 2025 / Accepted: 2 February 2026 / Published online: 24 February 2026
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Abstract
Sample and plot size are two pertinent questions in experiments with *Rhizophora mangle* L. propagules that this study aimed to address. Our analysis was performed with 1,724 propagules collected in six different years in the same mangrove. Individual fresh mass (g), length (cm) and apical, median, and basal diameter (mm) were recorded for each propagule. After descriptive statistics, associated with linear correlation analyses and normality tests, sample and sizing methodologies were defined. For non-experimental designs with *R. mangle* propagules, we used interval bootstrapping to determine the required propagule amount, providing an abacus and equations for decision-makers to dimension their samples according to errors tolerated around means, for example employing a random collection sample with coefficient of variation of 25% and selection of 10% error, 25 propagules are needed. In designed experiments, plot size was defined as the number of propagules per experimental unit based on the objective. The Hatheway method was used to determine the appropriate number per plot for both fully randomized and randomized block designs. Our results provide a clear ideal range: for a common experimental setup (e.g., 5 treatments, 5 replications and 15% precision), the suitable plot size is 29 propagules per plot for a fully randomized and 30 for a randomized design. The study provides practical guidance for experimental planning in restoration and ecology studies. The developed methodology underscores the importance of a rigorous statistical approach in ensuring replicability and validity of *ex situ* studies with *R. mangle* propagules.

Key message
This study establishes sample and plot sizes for *Rhizophora mangle ex situ* experiments, highlighting statistical precision, morphological variability, and climate influences to improve experimental planning and ensure reliable, replicable ecological research outcomes.

Keywords Experimental planning · Experimental design · Experimental precision variability · Mangrove

Communicated by RD Guy

✉ Ully Depolo Barcelos
ullydbarcelos@gmail.com

Edilson Romais Schmildt
e.romais.s@gmail.com

Andreia Barcelos Passos Lima Gontijo
albarcelos@hotmail.com

Mônica Maria Pereira Tognella
monica.tognella@gmail.com

[1] Department of Oceanography and Ecology, Federal University of Espírito Santo, Vitória, ES, Brazil

[2] Department of Agrarian and Biological Sciences, Federal University of Espírito Santo, São Mateus, ES, Brazil

Introduction

Rhizophora mangle is a widely distributed species in new world mangroves. It plays an important ecological and economic role. *In* and *ex situ* experiments widely use the species due to its wide distribution and continued propagule production. *R. mangle* propagules have a low genetic variability among individuals in the same estuary (Hogarth 2007) and along their latitudinal gradient (Pil et al. 2011). However, propagules show a great morphological variability with varying length, diameter, and weight (McKee 1995; Simpson et al. 2017; Smith and Snedaker 1995). This high variability may increase uncontrolled error in experimental analyses since mature *R. mangle* propagules range between

10 and 50 cm (Deyoe et al. 2020). Variability of length, diameter, and mass has several causes. We highlight studies evaluating responses to insect damage (Baena et al. 2020; McKee 1995), crab herbivory (Dangremond 2015), abiotic influences, such as climatic variations during embryo ontogenesis (Peel et al. 2019), and endogenous factors like the physiological age of propagules (Smith and Snedaker 2000).

Scientific evidence shows that propagule size (Dangremond 2015; Sousa et al. 2003) and mass (Lin and Sternberg 1995; Smith and Snedaker 2000) significantly affect the initial growth of *R. mangle*. Sousa et al. (2003) concluded that larger propagules provide plants with greater length, number of leaves, and dry mass. For Sousa et al. (2003)., natural propagule length variation reflects significant differences in seedling establishment and/or initial growth, which could influence forest dynamics.

Recommendations to minimize experimental error involve using propagules of the same physiological age (Smith and Snedaker 2000), free of apparent damage (Boehm et al. 2016; McKee 1995), and with the same length and weight (Krauss and Allen 2003; Simpson et al. 2017; Smith and Snedaker 2000). Smith and Snedaker (2000), after separating propagules for treatment and by experiment, took additional care of analyzing variance in length, diameter, and mass via a fully randomized design (FRD) variance analysis to assess whether they could accept their null hypothesis. In fact, adopting an FRD model assumes a uniform experimental material since it is unable to control local effects (Gotelli and Ellison 2013).

Depending on the type of experiment and the required propagule amount, it is often impossible to standardize propagules by length and weight (Dangremond 2015; Luna et al. 2019). Parametric statistical analyses permit using propagules without prior size or mass standardization since sampling may employ experimental blocks – in the case of experiments with randomized blocks (Pimentel-Gomes 2009; Zar 2010) – or rows and columns – in Latin Square experiments, although ecologists use this type of experiment less often (Gotelli and Ellison 2013).

Among the experimental studies involving the use of *R. mangle* propagules regardless of length and mass uniformity, research employs different plot sizes, with five (Koch and Snedaker 1997; Luna et al. 2019; Zhou et al. 2021), eight (Ellison and Farnsworth 1997), 16 (Ellison and Farnsworth 1993; Dangremond et al. 2015), and up to 18 propagules per plot (Simpson et al. 2017). Thus, studies lack a standard plot size, defining it arbitrarily, although most researchers seem to use 10 propagules in their experimental plots.

If research has no statistical design (especially if it only has two treatments), rather than researchers focusing on plot size, they define sample size for each treatment when installing their experiments. Therefore, statistical analyses may include the parametric Student's *t*-test for independent samples or paired data and the Mann-Whitney test for non-parametric ones.

In general, non-experimental studies lack an assertive approach to define sample size. Studies with *R. mangle* propagules without design show several sample sizes. They include 10 (Bompy et al. 2014; Farnsworth et al. 1996; Krauss and Allen 2003), 16 (Ellison and Farnsworth 1993), 17 (Smith and Snedaker 2000), 30 Febles-Patrón et al. 2007), 40 (Walsh et al.1979), 50, 60 (Dangremond 2015), and 80 propagules per sample group (Rabinowitz 1978).

Statistical recommendations for sample size establish it is directly proportional to data variability and the desired reliability of the estimates, which is inversely (though not linearly) related to the estimation error defined a *priori* by the researcher (Cochran 1977; Zar 2010). Likewise, plot size is also related to data variability, and this relationship is also non-linear (Khan et al. 2017). Blank tests have shown that the smaller the plot size, the higher its coefficient of variation (CV). As size increases, CV decreases, a relation describing a potentially nonlinear model and indicating plot size via statistical analyses (Meier and Lessman 1971).

The statistical approach used to determine the significant plot sizes of experimental designs are different (Lúcio et al. 2012). The literature has several methodologies to determine plot size, of which Hatheway (1961) is a widely used one. Other analyses include the modified maximum curvature (Meier and Lessman 1971) and the maximum curvature of the coefficient of variation (Paranaíba et al. 2009). Of these three, Hatheway's (1961) has the advantage of contemplating plot size as a function of repetition and treatment number, desired experimental precision, and the used experimental design. Celanti et al. (2016a) includes cases in which it is impossible to establish the neighborhood of blank tests, as with *Rhizophora mangle* propagules since they fail to identify a mother tree-propagule relation.

Regarding sample sizing, research has used Cochran's (1977) methodology. It is based on the confidence interval semi-amplitude as a rule or on percentile bootstrap simulations if sample data show abnormal distribution (Schmildt et al. 2017; Chirara and Hesterberg 2019). Thus, this study aims to determine the sample and plot sizes needed to evaluate experiments with *Rhizophora mangle* L. and one or more variables.

In this study, the term "plot size" (X_0) specifically refers to the number of propagules or seedlings grouped within a single ex situ experimental unit (e.g., a pot, a tank, or a defined section within a growth facility) for treatment application and data collection. It is crucial to distinguish this from the physical area occupied by the plot, as the primary concern is establishing the suitable number of biological replicates per unit to control experimental error.

Materials and methods

Mangrove propagules were randomly collected at the mouth of an estuarine system in a median latitude (18°S) across several months and years, as per Amorim (2015) and Barcelos (2017). This estuary is protected by law as a conservation unit under State Jurisdiction – The Itaúnas State Park (ES). The local mangrove climate is classified, according to Koppen, as tropical with well-defined seasons. Its humid period ranges from October to March and its autumn and winter season, ranging from April to September, is dry. Annual rainfall ranges between 1,000 and 1,400 mm (Alvares et al. 2013).

In a laboratory, propagules were disinfected with distilled water and neutral detergent and their length, fresh mass, and diameter, measured via a digital caliper and a calibrated measuring tape (Tognella et al. 2019). The basal, middle, and upper diameter of the propagule hypocotyl were assessed. Fresh mass, length, and diameter of each propagule collected on various dates were evaluated.

Biometric data of propagules were subjected to statistical analysis to characterize the samples in terms of central position (mean), dispersion (standard deviation, coefficient of variation), and range (minimum and maximum values), as well to define the confidence interval of our dataset. Data were tested for normality via Shapiro-Wilk analysis. Correlation estimates between the evaluated traits (length and diameter and length and fresh mass) were determined by Pearson's linear correlation. These statistics were used to characterize our database and assess its adequacy for deterministic sample sizing or the need for bootstrap simulation (Schmildt et al. 2017; Chirara and Hesterberg 2019).

Sample sizing

Whereas most propagule samples showed abnormal data distribution, fresh mass, length, and diameter were used in a bootstrap simulation, the most appropriate statistical technique (Chirara and Hesterberg 2019). Its initial sample size was considered adequate according to Good's (2013) criterion, in which the author establishes that the sample size for reliable studies applying bootstrap simulation ought to include at least 100 observations.

Interval estimation via bootstrap was used by percentiles intervals (Good 2013; Qian 2017; Chirara and Hesterberg 2019) for simulations with each propagule characteristic and collection time. In total, 40 sample sizes were planned for each characteristic and collection date, and our initial sample size was estimated for 10 propagules. From this value, increments of 10 were performed until 400 propagules were reached.

Overall, 4,000 simulations were performed for each planned sample size and characteristic via resampling (Rosner 2016). Means for each simulated sample were estimated. Thus, 4,000 mean estimates were obtained. Next, the amplitude of confidence intervals was calculated at 95% (95% CI), determined by the difference between the 97.5% and 2.5% percentiles for each sample size. Results were graphically plotted. Then, sample size (propagule number) was determined to estimate the mean of each characteristic and collection time. For this simulation — and using our initial size (10 propagules), the number of propagules in which means remained within the 95% confidence interval was considered as our sample size (Qian 2017).

Finally, covariance analysis was performed for linearized data in an equation with a potential model of the relationship between sample size and the v coefficient of variation (CV) to assess whether the traits used as covariates failed to alter the curve intercept and slope. Covariates failed to change both equation parameters. Thus, CVs and sample sizes were used to build an abacus so we could determine our sample size.

Statistical analyses and graphs were performed via R, version 4.2.0 (R Development Core Team, 2022).

Suitable plot size

Hatheway's method (1961) was used to determine suitable plot size (X_0) according to the following equation, only using fresh propagule mass.

$$X_0 = \sqrt[b]{2(t_1 + t_2)^2 CV^2 / Jd^2} \quad (1)$$

In Eq. 1, Smith's heterogeneity coefficient (**b**) (1938) and the coefficient of variation (**CV**) were obtained by bootstrap simulation with 4,000 resamplings, as per Celanti et al. (2016a). In Eq. 1, **d** refers to experimental precision and comprises the difference between significant treatment means at 5% probability, expressed as percentages of the general mean; $\mathbf{t_1}$, to the tabled *t* value of significance tests (bilateral test at 5%), with *gl* degrees of freedom; $\mathbf{t_2}$, to the tabled bilateral *t* value corresponding to an error of 2 (1- *p*) with *gl* degrees of freedom; and a $p = 0.80$ to the probability of obtaining significant results (Schmildt et al. 2016). We obtained our tabled *t* distribution values via residue *gl* degrees of freedom, depending on I treatments and J replicates, in which $gl = (I)(J-1)$ for fully randomized design (FRD) experiments and $gl = (I-1)(J-1)$ for those with randomized block design (RBD).

In our simulations, combination criteria were determined as the lowest number of treatments equal to three ($I=3$). This minimum was chosen since we can detect the difference

between two means by variance analysis or Student's *t*-test during sample sizing. The smallest number of repetitions must equal to 2 ($r=2$), given the minimum to detect experimental errors. Moreover, I treatments and J replicates were combined to provide at least 20 plots per experiment, as per Pimentel Gomes (2009). Following these assumptions, the first simulation was composed of I = 3 treatments and J = 7 replicates, providing 21 experimental plots.

Some experimental situations require the evaluation of more than one factor. To address this, researchers employ factorial experimental designs, most arranged in a in fully randomized design (FRD) or a randomized block design (RBD). In these designs, the suitable plot size remains the same whether the experiment involves one or multiple factors. This is because the tabled *t* values used in Hatheway's method (1961) depend solely on the residual degrees of freedom (*df*) which will be the same for one or multiple factors (Pimentel-Gomes 2009; Zar 2010). However, the reasoning applied to factorial designs does not extend to split-plot designs, such the latter involve multiple experimental residuals, increasing their complexity (not addressed here).

When analyzing experiments, researchers may assess several destructive and/or non-destructive traits, which could demand different plot sizes (*Xo*) (Boyhan 2013; Celanti et al. 2016a, b; Schmildt et al. 2017). Consequently, evaluating multiple traits would typically require varying plot sizes. However, when designing experiments, researchers must establish a single plot size. In this study, the plot size was determined based on the coefficient of variation (CV) of the propagule sample. *Xo* was calculated using the trait with the highest CV. This approach is justified because, under Hatheway's method (1961) with bootstrap simulations, CV is the sole variable in Eq. 1, while precision (*d*) and the number of replications (*J*) are predetermined by the researchers, and *t* values are fixed based on the number of treatments and replications. Furthermore, the heterogeneity index (*b*) is considered equal to one, as specified by Celanti et al. (2016a), reflecting that it corresponds to a single propagule.

Data were analyzed using R, version 4.2.0 (R Development Core Team 2022). By using simulations, the *set.seed* command was applied to ensure that the same "seed" was used to calculate the FRD and RBD sample size. Since it is a discrete random variable, the result was rounded to the nearest upper integer (Celanti et al. 2016b).

Results

Morphological variability and descriptive statistics

The results describe measures of central tendency and variability, as well the Shapiro-Wilk normality test, for five traits of mature *Rhizophora mangle* L. propagules collected over different periods, revealing significant morphological variability (Table 1). They showed an average length of about 26 cm for all evaluation times, with a minimum of 11.7 cm and a maximum of 44.0 cm. We found these values in February 2019, which also showed the highest CV variability (around 20%).

February 2019 collections also showed the greatest variability in fresh mass, with a 43.85% CV (Table 1). Average fresh mass varied depending on the collection time, with a minimum mean of 13.57 g (November 2018) and maximum of 22.24 g (April 2016). Concerning diameter measurements, the propagules showed the highest mean values at their base, approximately 1.4 cm, with CVs being lowest for this trait.

With respect to descriptive analyses, the Shapiro-Wilk normality test showed that only basal diameter had a normal distribution ($p>0.05$) across all collection times. The distribution of the other traits (length, fresh mass, middle and upper diameter) was variable, showing in some periods but not in others (Table 1).

Correlation between morphological traits

Figure 1 shows a significant linear correlation (Pearson correlation, p value <0.01) between the measured characteristics, and $r>0.70$ registered between diameters in different positions of the propagules (median, basal, and apical). The correlation observed between propagule mass and its length was also significant ($r=0.823$, $p<0.01$).

Sample size determination for non-designed experiments

We used fresh mass data to define sample size as a function of simulated sample details. Sample size included 75 propagules selected by fresh mass of propagules and collected in February 2019, assuming a 10% error around the average (Fig. 2). The 95% confidence interval amplitude gradually decreased as sample size increased (number of propagules), raising mean estimation precision Note that the estimated bootstrap average for each sample size remains constant accrediting graphic analysis to determine the sample size of each characteristic for different sampling errors assumed around the mean.

Table 1 Summary of field sample size (n) for *Rhizophora mangle* L. mature propagules, including minimum (Min), maximum (Max), mean (X), standard deviations (s), coefficients of variation (CV), and results from the Shapiro-Wilk normality test (SW). Samples were collected at various times at the mouth of the Itaúnas river to evaluate five distinct characteristics

Collection Period	n	Min	Max	X	s	CV (%)	SW[1/]
Length (cm)							
Apr-14	132	16.5	34.6	25.77	3.5	13.58	0.3673
Mar-15	551	12.3	41.5	25.04	4.81	19.19	<0.05
Apr-15	248	13	42	26.31	4.85	18.44	<0.05
Apr-16	200	15.5	39	27.15	3.89	14.32	<0.05
Nov-18	96	20	35.05	25.88	3.35	12.95	<0.05
Feb-19	497	11.7	44	25.18	4.91	19.51	0.1974
Fresh Weight (g)							
Apr-14	132	7.5	29.5	18.14	4.45	24.51	0.7515
Mar-15	551	5.5	46.9	18.17	6.4	35.24	<0.05
Apr-15	248	6	49	19.99	6.6	33.02	<0.05
Apr-16	200	8.31	39.5	22.24	5.98	26.87	<0.05
Nov-18	96	7.85	23.79	13.57	4.01	29.54	<0.05
Feb-19	497	3.04	39.5	16.01	7.02	43.85	<0.05
Base Diameter (mm)							
Apr-16	200	10.4	18	14.29	1.21	8.44	0.1414
Feb-19	497	5.3	18.2	10.64	1.84	17.31	0.1956
Middle Diameter (mm)							
Apr-16	200	9.8	16.2	12.82	1.35	10.54	0.444
Feb-19	497	6.1	14.3	9.57	1.72	18.01	<0.05
Upper Diameter (mm)							
Apr-16	200	5.6	10.6	7.27	1.37	15.64	<0.05
Feb-19	497	3.5	8.5	6.01	0.76	12.65	<0.05

[1/]*p*-value of the Shapiro-Wilk normality test

Table 2 shows the sample sizes from the percentile bootstrap confidence interval of 4,000 average estimates for several errors around the mean. Sample size decreases with reduced precision increasing errors around the mean (Figure 2). Thus, evaluating the propagules collected in February 2019 by increasing the acceptable margin of error from 10% to 15%, the required sample size decreases from 75 to 33 propagules.

By comparing the evaluated characteristics, the required sample size is larger for fresh mass than length and diameter within the same range of the sampling error.

Development of a general model for sample size Estimation

Based on the 18 CV values (Table 1), and the corresponding sample size for each margin of error (Table 2), we assessed the relation using a potential model. Figure 3 presents the resulting abacus to determine the sample size of *R. mangle* propagules from the coefficient of variation of propagule fresh weight, considering different estimation errors around the mean. The equations derived from model are presented in Table 3. All equations showed a high coefficient of determination (R^2 >0,98), indicating a very strong fit of the model to the data.

Suitable plot size for designed experiments

We used Hatheway's method (1961) to elaborate suitable plot size, i.e., the ideal number of propagules per experimental unit (X_0), as presented in Table 4. The results are structures for experiments using fully randomized (FRD) and randomized block (RBD) designs. Exemplifying the use of Table 4, we assumed that the FRD experiment has 10 treatments (I=10), six repetitions (J=6), and high precision (d=5%) around the mean. In this case, each experimental unit (plot) should contain 201 propagules, and the entire experiment would require collecting 12,060 propagules, as a result of (I)*(J)*(X_0). Note that an experiment with this formulation is practically unfeasible. If researchers decrease the precision, they will also have to decrease the sample size. If they choose a 20% precision the suitable size will contain 13 propagules per plot and the whole experiment will consume 780 propagules.

By comparing FRD and RBD, for the same I, J, and d the RBD will show the highest plot size for experiments of smaller size. Thus, if I=4 treatments, J=5 replicates, and a precision of d=5% within the FRD design, this experiment will contain 262 propagules. While the same decision is made for the RBD method, it will result in 274 propagules per plot. However, as the experiment size increases, the

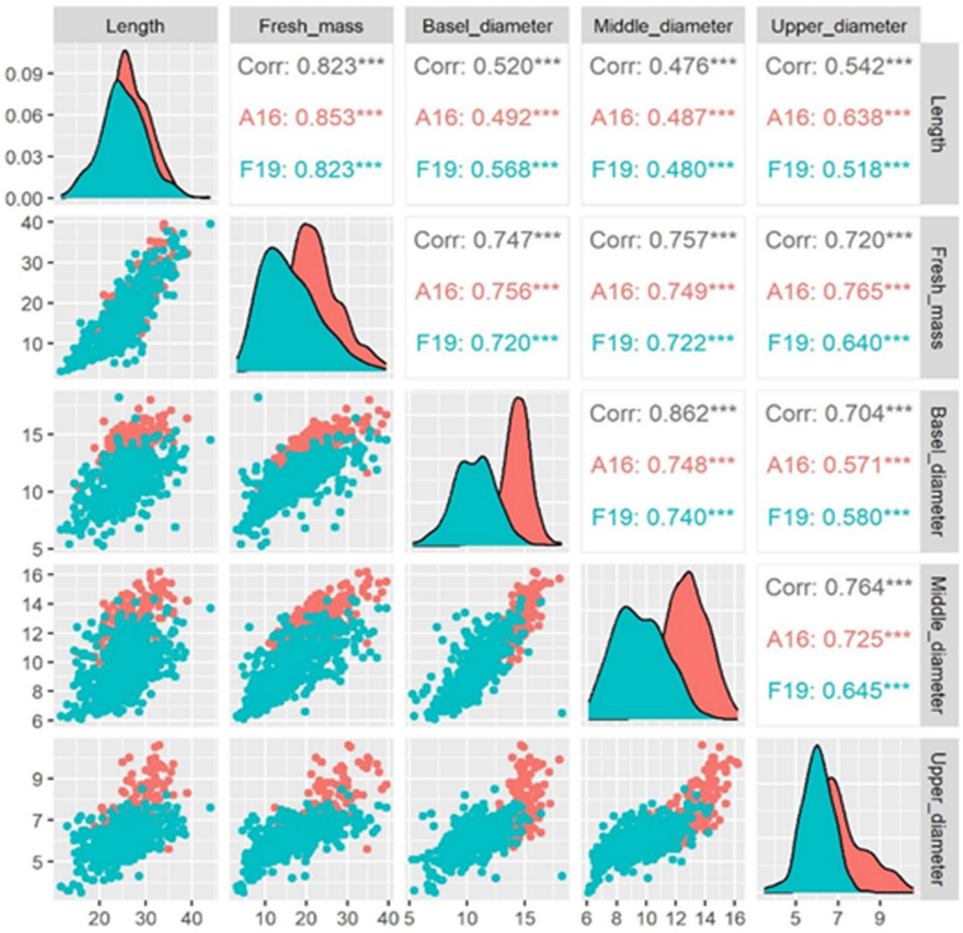


Fig. 1 Pearson correlation coefficient (upper diagonal) and dispersion diagram (lower diagonal) of *Rhizophora mangle* L. propagule length, fresh mass, and diameters. Red circles correspond to data from 200 propagules collected in April 2016 and blue ones, to 497 propagules collected in February 2019

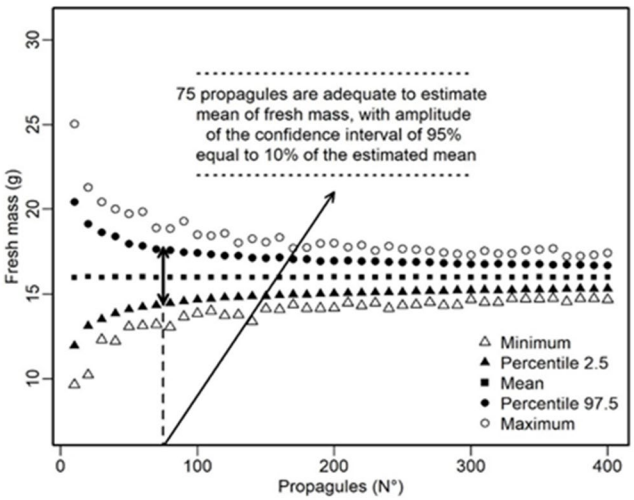


Fig. 2 Description of the simulation behavior of propagule fresh mass (g) according to △Minimum, ▲ Percentile 2.5%, ■Average, ●Percentile 97.5%, and a Maximum of the average fresh mass of 4,000 propagules obtained by sampling in different sample sizes (10, 20, 30, … 400 propagules) from 497 *Rhizophora mangle* L. propagules.

difference between plot size for FRD and RBD decreases or even disappears. Considering the previous FRD example with I = 10 treatments, J = 6 repetitions, d precision = 5% and, thus, 60 experiment plots, the plot size will be the same for FRD and RBD in any d precision, i.e., from 5% to 25% (Table 4).

Discussion

Although mean length remained constant throughout our collections, variability differed, as other studies have also found. Simpson et al. (2017) observed a CV around 10.0% for Florida mangroves, unlike Smith and Snedaker (1995), who found a 15.40% variation in that region. On the other hand, Rabinowitz (1978), in his studies in Panama, recorded a higher coefficient of variation, i.e., 24.28%. This country has one of the best environmental conditions in the Americas for mangrove development and these aspects could explain the observed variation.

The variability in fresh mass observed in our study (e.g., 43.85% CV) resembles that found by Rabinowitz (1978). Although discrepant, our mean values fell within the

Table 2 Experimental sample size (number of propagules) to estimate the average of five traits of *Rhizophora mangle* L. mature propagules for the amplitude of 95% confidence intervals, considering 5, 10, 15, 20, and 25% error margins of the average estimate from propagules collected in six seasons at the mouth of the Itaúnas river

Collection Period	Error (%)				
	5	10	15	20	25
Length (cm)					
April 2014	29	8	4	2	2
March 2015	57	15	7	4	3
April 2015	53	14	6	4	3
April 2016	32	8	4	2	2
November 2018	27	7	3	2	2
February 2019	59	15	7	4	3
Fresh Weight (g)					
April 2014	95	24	11	6	4
March 2015	192	48	22	12	8
April 2015	170	43	19	11	7
April 2016	113	28	13	8	5
November 2018	138	35	16	9	6
February 2019	297	75	33	19	12
Base Diameter (mm)					
April 2016	12	3	2	1	1
February 2019	47	12	6	3	2
Middle Diameter (mm)					
April 2016	18	5	2	2	1
February 2019	50	13	6	4	2
Upper Diameter (mm)					
April 2016	56	14	7	4	3
February 2019	25	7	3	2	1

expected for the population dynamics of *R. mangle* as its average mass may fluctuate according to the time of year (Tyagi 2003) and by year. Rabinowitz (1978) also found an average mass of around 14 g for propagules harvested in Panama between April and November, a result obtained during its rainy season. Most authors, however, have found an average fresh weight around 20 g (McKee 1995; Smith and Snedaker 1995; Simpson et al. 2017), similar to what we found in most of our collection times. Concerning our diameter measures, the propagules showed the highest mean values at their base similar to that found by Smith and Snedaker (1995) and Proffitt and Travis (2010).

Practical implications and recommendations for researchers

The strong correlation between diameters means researchers may omit measuring the diameter in three sections when characterizing their samples, demanding only one of these three measurements. We recommend the median diameter. The strong mass-length relationship ($r=0.823$) is similar to that obtained in Sousa et al. (2003), who found a 0.97 significant correlation ($p<0.01$), confirming that length is a good proxy for mass.

From our analysis, three questions arise which must be considered to make decisions. The first is: which feature will define sample size? In this situation, we advise that the characteristic which has the greatest variability should be

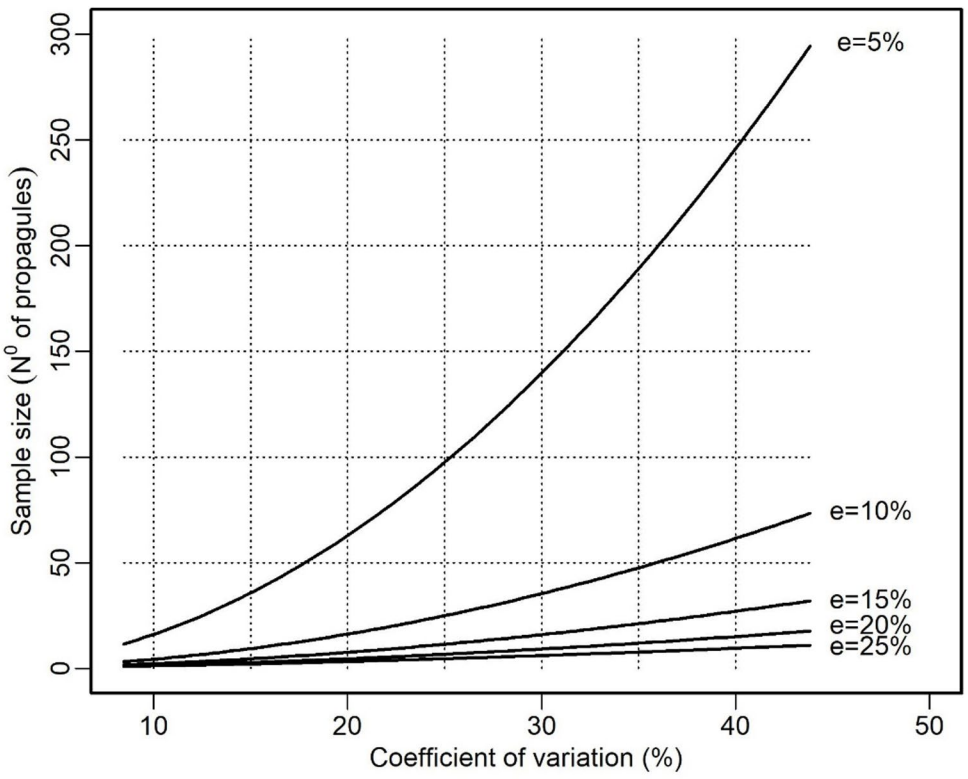


Fig. 3 Abacus to determine the sample size of *Rhizophora mangle* L. propagules to install experiments from the coefficient of variation of propagule fresh weight, considering a 5, 10, 15, 20, and 25% estimation error around the mean

Table 3 Estimated equations to determine the sample size (n_{exp}) of *Rhizophora mangle* L. propagules to install experiments from the coefficient of variation of propagule fresh weight, considering a 5, 10, 15, 20, and 25% Estimation error around the mean

Error (%)	Equation	R^2
5	$n_{exp(5\%)} = 0{,}17284CV^{1{,}96819}$	0,9999
10	$n_{exp(10\%)} = 0{,}05067CV^{1{,}92502}$	0,9950
15	$n_{exp(15\%)} = 0{,}03057CV^{1{,}83868}$	0,9988
20	$n_{exp(20\%)} = 0{,}02309CV^{1{,}75585}$	0,9945
25	$n_{exp(25\%)} = 0{,}02632CV^{1{,}59407}$	0,9860

used to define the sample size. In this study, this characteristic was fresh mass, as recommended by other studies with different kinds of species (Cargnelutti Filho et al. 2012, 2018; Schmildt et al. 2017). Given the distinction among reproductive structures (shape and size) in all studies, we can claim that fresh mass is a good one, being an assertive estimator for sample size. From an experimental point of view, we see no problem in relying on biomass since it is highly linearly correlated with the other morphological aspects evaluated in propagules (Fig. 1).

The second approach concerns the precision of defining the sample size. In this case, the answer lies in the researchers' desire, which will define the precision according to their interest (Schabarum et al. 2017). This may involve ethical decisions regarding species vulnerability, experimental costs, and operational difficulties. As pointed out, the more precise the experiments, the greater the need to expand the sample size. However, this also increases the probability of significant differences between treatments.

Influence of climate

The third question concerns the climate. Our results show collections under different precipitation regimes (end of the dry period, beginning of the wet period, among others) and indicate that the precipitation regime during propagule ontogenesis implies distinct variability in the same morphological characters (Table 1), resulting in different sample sizes for each period (Table 2). Studies show that the phenological development of propagules in tropical regions is mainly controlled by precipitation and the availability of water in the soil (Couralet et al. 2013; Singh and Kushwaha 2005). Van der Stocken et al. (2017) evaluated the abscission of mangrove propagules in the world. Their results show the significant relation between precipitation and temperature, as propagules are especially released during the rainy season (Lima et al. 2023). Fernandes (1999), also reported this relation between propagule abscission and precipitation in Brazil. The literature has suggested that propagules are released during periods in which environmental factors are optimal for seedling growth and development (Duke et al. 1984).This release may be specific to species and sites. We could consider the preferential propagule production in the rainy season an advantageous strategy for the species as the way of dispersion is by hydrochory (Fernandes et al. 2007). Since anthropogenic activities will increasingly influence these characteristics, we recommend that the climate be considered to define sample size. This aspect will imply the unpredictability that the researchers will have with their collected sample.

After these decisions, if the morphological traits show variability different from our research, the statistical modeling we provide (Fig. 3; Table 3) may configure a solution (Gotelli and Ellison 2013). Based on the 18 CV values (Table 1), we developed an abacus and equations to assist the decision-making. To exemplify its use, employing a randomly collected sample with a CV of 35% and assuming a 10% error, the sample size will be 50 individuals. Thus, in an experiment with only two treatments, if it ignores an experimental design, consumption will include 100 propagules.

In addition to the abacus, results enabled us to elaborate equations which would assist the decision-making if researchers faced difficulties to estimate the size of their experimental sample via our abacus (Fig. 3; Table 3). Again, in an example collection of propagules that has a 32% coefficient of variation, the experimental sample would consume 159 propagules per treatment being highly precise (i.e., with 5% error around the mean). If researchers have collected only 200 propagules and the experiment will include two treatments, this number will be insufficient (as the study would require 318 propagules), thus needing another collection or lower precision. In this previous assumption, the change of the margin of error to 10% around the average, would require 40 propagules per treatment, totaling 80 for the experiment. Sousa et al. (2003) reported a similar situation. They collected 33 or 34 propagules per collection site and concluded that this sample size was insufficient to significantly analyze variation in propagule size, opting for a supplementary collection.

The results defined for the suitable plot size (Table 4) imply decision-making for experiments using randomly collected propagules without prior size and weight selection, selected only eliminating damaged propagules, as recommended by research standardization (Sousa et al.2003).

Table 4 Suitable plot size by the Hatheway method (1961) for experiments with *Rhizophora mangle* L. propagules in a fully randomized design (FRD) and in randomized blocks (RBD) considering different numbers of treatments (I) and repetitions (J), and precisions (d) around the mean

I	J	FRD					RDB				
		d=5%	d=10%	d=15%	d=20%	d=25%	d=5%	d=10%	d=15%	d=20%	d=25%
3	7	185	47	21	12	8	196	49	22	13	8
4	5	262	66	30	17	11	274	69	31	18	11
4	6	213	54	24	14	9	221	56	25	14	9
4	7	180	45	20	12	8	185	47	21	12	8
5	4	331	83	37	21	14	343	86	39	22	14
5	5	256	64	29	16	11	262	66	30	17	11
5	6	209	53	24	14	9	213	54	24	14	9
5	7	177	45	20	12	8	180	45	20	12	8
6	4	323	81	36	21	13	331	83	37	21	14
6	5	251	63	28	16	11	256	64	29	16	11
6	6	206	52	23	13	9	209	53	24	14	9
6	7	175	44	20	11	7	177	45	20	12	8
7	3	445	112	50	28	18	457	115	51	29	19
7	4	318	80	36	20	13	323	81	36	21	13
7	5	248	62	28	16	10	251	63	28	16	11
7	6	204	51	23	13	9	206	52	23	13	9
7	7	173	44	20	11	7	175	44	20	11	7
8	3	437	110	49	28	18	445	112	50	28	18
8	4	314	79	35	20	13	318	80	36	20	13
8	5	246	62	28	16	10	248	62	28	16	10
8	6	203	51	23	13	9	204	51	23	13	9
8	7	172	43	20	11	7	173	44	20	11	7
9	3	431	108	48	27	18	437	110	49	28	18
9	4	311	78	35	20	13	314	79	35	20	13
9	5	244	61	28	16	10	246	62	28	16	10
9	6	201	51	23	13	9	203	51	23	13	9
9	7	171	43	19	11	7	172	43	20	11	7
10	2	710	178	79	45	29	728	182	81	46	30
10	3	426	107	48	27	18	431	108	48	27	18
10	4	309	78	35	20	13	311	78	35	20	13
10	5	243	61	27	16	10	244	61	28	16	10
10	6	201	51	23	13	9	201	51	23	13	9
10	7	171	43	19	11	7	171	43	19	11	7
15	2	661	166	74	42	27	668	167	75	42	27
15	3	412	103	46	26	17	413	104	46	26	17
15	4	302	76	34	19	13	303	76	34	19	13
15	5	239	60	27	15	10	240	60	27	15	10
15	6	198	50	22	13	8	198	50	22	13	8
15	7	169	43	19	11	7	169	43	19	11	7
20	2	638	160	71	40	26	642	161	72	41	26
20	3	405	102	45	26	17	406	102	46	26	17
20	4	299	75	34	19	12	299	75	34	19	12
20	5	229	58	26	15	10	229	58	26	15	10
20	6	190	48	22	12	8	190	48	22	12	8
20	7	162	41	18	11	7	162	41	18	11	7
25	2	625	157	70	40	25	628	157	70	40	26
25	3	401	101	45	26	17	401	101	45	26	17
25	4	297	75	33	19	12	297	75	33	19	12
25	5	236	59	27	15	10	236	59	27	15	10
25	6	196	49	22	13	8	196	49	22	13	8
25	7	168	42	19	11	7	168	42	19	11	7
30	2	617	155	69	39	25	618	155	69	39	25
30	3	398	100	45	25	16	398	100	45	25	16

Table 4 (continued)

I	J	FRD					RDB				
		d = 5%	d = 10%	d = 15%	d = 20%	d = 25%	d = 5%	d = 10%	d = 15%	d = 20%	d = 25%
30	4	295	74	33	19	12	296	74	33	19	12
30	5	235	59	27	15	10	235	59	27	15	10
30	6	195	49	22	13	8	195	49	22	13	8
30	7	167	42	19	11	7	167	42	19	11	7
35	2	611	153	68	39	25	612	153	68	39	25
35	3	396	99	44	25	16	396	99	44	25	16
35	4	294	74	33	19	12	295	74	33	19	12
35	5	235	59	27	15	10	235	59	27	15	10
35	6	195	49	22	13	8	195	49	22	13	8
35	7	167	42	19	11	7	167	42	19	11	7

Table 4 speeds up the experiment execution since it lacks morphological determination and maintains decision precision. We recommend its use in large-scale experiments that would require greater effort to determine biometrics.

On the other hand, in cases of experiments that require propagule selection by size or weight, the plot size will always be smaller than those presented in Table 4, although it can still be used as a reference. In a factorial experiment under an FRD, with $I = 10$ treatments (Factor A = 5 levels and factor B = 2 levels) and J = 6 repetitions, Simpson et al. (2017) used 18 propagules per plot each. In the context of our study, this plot size corresponds to a precision between 15 and 20% around the mean. However, because Simpson et al. (2017) standardized propagules by weight, the experimental precision was substantially higher, with d < 15%.

The analyses of this study, based on the set of samples collected, contribute to more accurate decision-making for *ex situ* experiments with *R. mangle*. Given the morphological similarity between the propagules of the genus (Tomlinson 1986), we encourage the application of our tables, abacuses, and models for other *Rhizophoraceae* species.

Beyond controlled experiments, our results may also provide a valuable foundation for informing propagule density (e.g., number per m^2) in restoration planting schemes, where success depends on the interaction of multiple environmental stressors characteristics of mangrove ecosystems, such as salinity, inundation and solar irradiance. For example, mangrove sites with elevated salinity may require higher initial planting densities to offset natural mortality and ensure adequate seedling establishment. Thus, the ability to calculate a precise baseline sample size, tailored to propagule variability, could allow researchers to strategically adjust planting efforts based on the site-specific intensity of these key environmental tensors, moving restoration planning towards a more nuanced and efficient framework.

Final considerations

This study provides a detailed methodology for determining the appropriate sample and plot sizes for *ex situ* experiments with *Rhizophora mangle* L. propagules. By addressing the critical questions of sample and plot size, we provide clear and practical guidelines that enhance the reliability, efficiency, and replicability of ecological research on this species. Our analysis confirms that the high morphological variability in *R. mangle* propagules, particularly in fresh mass, is a major source of experimental error sustained by CV and that must be actively managed. For researchers conducting non-designed experiments or comparative studies, we recommend using fresh mass as it exhibited the highest variability among morphological traits and was strongly correlated with them, making it the most robust and comprehensive metric for planning. Our bootstrapping approach and the resulting abacus (Fig. 3) and equations (Table 3) offer a flexible tool to determine the necessary sample size for a desired precision level.

For designed experiments (FRD and RBD), the application of Hatheway's method (1961) yielded definitive recommendations for plot size (X_0). The plot size and propagule amount of experiments will depend on the objective of the research. This precision-based approach prevents the underpowering of experiments or the wasteful use of propagules, which is crucial for conservation and restoration efforts where material may be limited.

The results highlight that the fresh mass of propagules is a reliable estimator for determining sample size due to its high correlation with other morphological aspects. Moreover, the variability in fresh mass was the characteristic with the greatest variation, justifying its selection for sample size determination. The bootstrap analysis reinforced the importance of adjusting sample size according to variability and the desired precision, providing a flexible and adaptable approach to the specific conditions of each experiment.

The application of statistical models, including covariance analyses, confirmed that the relationships between

morphological variables and propagule variability can be effectively modeled, aiding decision-making in defining sample and plot sizes more precisely and efficiently. The strong correlation between propagule length and mass ($r = 0.82$) provides a valuable non-destructive proxy for rapid field assessments, allowing for efficient screening and selection. Furthermore, the influence of climatic conditions on propagule variability underscores the importance of considering collection timing during experimental planning.

Ultimately, the methodologies, tables and models presented in this study are designed to be directly applied by researchers. They serve as source to assist in experimental planning with *Rhizophora mangle* L. propagules and support the application of these tools for other species within the *Rhizophoraceae* family.

Author contributions All authors contributed to the study's conception and design. Edilson Romais Schmildt and Ully Depolo Barcelos wrote the initial draft of the manuscript, and all authors provided critical feedback and revisions. All authors read and approved the final manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). The first author was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) during her doctorate.

Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare there is no conflict of interest.

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