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Variation in the growth parameters and biomass of Rhizophora mangle seedlings with distances from Playa Estrella, Bocas del Toro, Panama

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SIT Study Abroad

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Abstract

Mangrove is a salt-tolerant, intertidal, tropical tree or shrub and make up a rich community of various organism. On the Caribbean coast of Panama, in Bocas del Toro, mangrove forests cover 28 km^2 and are dominated by *R. mangle*, followed by *L. racemosa* and *A. germinans.* Simultaneously, Isla Colón, the most populated and developed among all islands in the Bocas del Toro Archipelago, is a tourist center. Unfortunately, tourism comes at the price of environmental degradation via alteration of natural habitats, solid and wastewater pollution. Therefore, this study aims to gain a preliminary understanding on how the growth parameters of *R. mangle* seedlings and dry biomass vary with distances from a tourism site, Playa Estrella, Bocas del Toro, Panama. Three sites with different linear distances from Playa Estrella were studied. Results showed that stem diameter and number of nodes were larger in Site 3, the furthest from Playa Estrella. Larger diameter and node count in Site 3 may indicate less impact by pollution created by human on the beach. However, no significant differences were found between total and stem heights across study sites. Thus, the lack of differences in total and stem height in this study may be accounted for the similarity in environmental condition between the sites, and the heights were not as sensitive as the stem diameter and node count. The first two to four internodal heights were larger in Site 1 and Site 2. It's unclear why this was the case, but these differences may be accounted by variation in the availability of nutrients in the hypocotyl reserve, or the ability to of the seedlings to absorb nutrients to allocate to the stem. Furthermore, dry biomass analysis showed high allocation towards the hypocotyls and low leaf mass ratio (LMR). The result found is potentially a preliminary insight on the impact of human activities on Playa Estrella on the growth parameters of *R. mangle* seedlings. However, it is not sufficient to draw a holistic conclusion about human impact from Playa Estrella definitively. Future studies should consider not just environmental factors that may impact *R. mangle* seedlings growth, but also factors that are clearer proxy of human impact such as presence of trash, and nutrient enrichment from waste.

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Acknowledgement

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Introduction

Mangroves globally

Mangrove is a salt-tolerant, intertidal, tropical tree or shrub and makes up a rich community of various organisms (Odum et al., 1984; Spalding et al., 2010) The ecology and resilience to environmental stress of mangroves are dependent on temperature, salinity, and tide (Odum et al., 1984). Nonetheless, they are incredibly resilient due to their ability adapt to harsh environmental conditions such as variations in salinity, fluctuation of tides, low oxygen and high temperatures (Hogarth, 2015). True mangroves are consisted of around 70 species and found in 112 countries. Global mangrove forests cover ranges from 110,000 km^2 and 240,000 km^2, with best the estimate being 152,308 km^2 (Hogarth, 2015). They offer abundant ecosystem services such as buffering marine ecosystems, preventing erosion by stabilizing soil, habitats for aquatic and terrestrial organisms, being a primary producer, and sequestering carbon (Hogarth, 2015). Unfortunately, mangroves have been threatened in the last few decades. Mangrove forest extent has decreased worldwide due to both anthropogenic and natural causes, where about 40% can be attributed to anthropogenic drivers such as agriculture and deforestation (Thomas et al., 2017).

Mangroves on Bocas del Toro Archipelago

Bocas del Toro Archipelago, on the Caribbean coast of Panama, is home to a plethora of ecosystems one of which is mangrove forest. The archipelago encompasses six islands: Islas Colón, Bastimentos, Solarte, Cristóbal, Popa, and Cayo Aqua (Collin, 2005). The islands are influenced by the Bahía Almirante and Laguna de Chiriquí which give rise to diverse ecosystems of mangroves, seagrass beds, and coral reefs (Collin, 2005). In Bocas del Toro, mangrove forests cover 28 km^2 and are dominated by *Rhizophora mangle*—due to its ability to tolerate inundation—with some presence of *Laguncularia racemosa* and *Avicennia germinans* (Collin, 2005; Lovelock et al., 2005). There is also a high variation in forest structure (e.g. tree heights) and soil condition (e.g. salinity, phosphorous, and sulfide concentration). These variables are interconnected and dynamic; for instance, the gradient in mangrove tree heights between ecosystems adjacent to the ocean and land can be influenced by multiple biotic and abiotic factors including tides, and the chemical and physical condition of sediments (Lovelock et al., 2005).

Reproduction of Rhizophora mangle

The reproduction of *R. mangle* is viviparous and parent trees produce seeds that are called propagules (Smith & Snedaker, 2000). They are up to 40 cm long and weigh from 20 g while they are on their parent trees. They eventually drop and float upright until they find an ideal substrate to station on. This movement is driven by water current and tide (Smith & Snedaker, 2000). *Rhizophora* seedlings in the Caribbean coast of Panama grow in height as well as 3.8 to 7 leaf scar nodes per year in closed and open canopy, respectively (Duke & Pinzon, 1992). The introduction of seedlings into an ecosystem is a critical stage for forest recovery and regeneration (Cardona-Olarte et al., 2006).

Additionally, the health of mangrove ecosystems can be studied from the characteristics of their seedlings because they are influenced by many environmental, physical, and biological factors.

For example, increase in the stem growth and leaf area of *R. mangle* in the south Florida Everglades can be attributed to elevating phosphorous level (Koch & Snedaker, 1997). Simultaneously, light and nutrient availability influence the growth and productivity of seedlings and the degree of impact varied interspecifically (McKee, 1995). In high nutrient and light availability, the growth of *R. mangle* exceeds that of *A. germinans* and *L. racemosa*. On the other hand, at low nutrient and light availability, there is little interspecific difference because all three species allocate resources to root biomass and leaf area to maximize nutrient uptake (McKee, 1995). In contrast, when tide and salinity are taken into account, the growth of seedlings of *R. mangle* and *L. racemosa* varied more at low salinity with constant flooding (Cardona-Olarte et al., 2006). Moreover, an experiment where photosynthesis was inhibited and light was limited found seedlings were still able to be developed (Smith & Snedaker, 2000). This indicates that roots, stem, and leaves develop from the hypocotyl reserves and photosynthesis is not required during initial growth.

Tourism and environmental impact on Isla Colón, Panama

Isla Colón is a tourist center, most populated and developed among all islands in the Bocas del Toro Archipelago (Moody, 2005). Both natural and cultural attractions brought tourists to the island. Isla Colón consists of diverse ecosystem ranging from tropical rainforests to coral reefs to mangrove forests, which are home to a diverse array of organisms. (Moody, 2005). As a result, it has potential for ecotourism and has become a popular destination since the 1990s (Montero, 2011; Moody, 2005). After World War II, the economies of many Caribbean islands, including Isla Colón have shifted from agriculture-based to tourism-based (Montero, 2011). Additionally, the Panamanian government has enacted laws that created tax-deferred development incentives that made building property for tourism easier (McKee, 2013). As a result of attractions and increase in restaurants and hotels, the tourism industry in Bocas del Toro increased by 70% in the past decade (Sellier, 2009). Unfortunately, tourism comes at the price of environmental degradation via alteration of natural habitats, solid and wastewater pollution (Rhodes, 2018; McKee, 2013; Sellier, 2009; Gochfeld et al., 2007). For instance, Rhodes 2018 found that 25.125 grams per meter squared of solid waste were present along beachside and roadside in Bocas del Drago and Playa Bluff on Isla Colón. The island also suffers from runoff of sediments and other land-based pollution, while most places lack sewage treatment plants (Gochfeld et al., 2007).

Ultimately, studying seedlings of mangroves and the health of mangrove ecosystems is important to evaluate risk and assess conservation efforts. While petroleum pollution from spills is the biggest threat to mangrove ecosystems in the Caribbean, chemical, industrial, and urban wastes also threaten the quality of the ecosystem (Ellison & Farnsworth, 1996). These pollution sources increase heavy metal concentration in seedlings and decrease species richness. Additionally, mangroves are lost for urbanization and tourism, which is particularly relevant for areas like Bocas del Toro. The area also has the potential for mangrove ecotourism; however, the impact of tourism on mangroves should be studied, and rehabilitation strategies need to be put into place (Yaeni et al., 2022).

Research Objective

This study aims to gain a preliminary understanding on how the growth parameters of *R. mangle* seedlings and dry biomass vary with distance from a tourism site, Playa Estrella, Bocas del Toro, Panama. Playa Estrella (9°24'18.9936"N, 82°19'30.3024"W) is a beach located in Bocas de Drago. It is adjacent to a tropical rainforest, seagrass beds and mangrove forests dominated by *R. mangle* as well as *L. racemosa*. Being a cheap tourist destination and home to abundant starfish make the beach a popular site for tourists on Isla Colón. Due to the beach's proximity to various important habitats, it is crucial to understand what impact tourism on Playa Estrella has on those habitats such as mangrove forests. Therefore, distance from Playa Estrella, in this study, acts as a proxy for human impact and the findings can be an estimation of how the quality of *R. mangle* seedlings varied with different degrees of human activities.

Research Question: Is there a variation in the stem diameter, total and stem heights, nodes and dry biomass of seedlings of *Rhizophora mangle* with distance from Playa Estrella, Bocas del Toro, Panama?

Methods

Study Site

The study was conducted near Playa Estrella on Isla Colon, Bocas del Toro, on the Caribbean coast of Panama. To test potential impact of human activities on Playa Estrella on the mangrove forests, three different mangrove sites at different linear distances to the beach were sampled (*Figure 1*). All sites were linearly about 600 m away from each other. Site 1 (9°24'19.6482"N, 82°19'25.2732"W) acted as the experimental site and was approximately 150 m from the beach. Site 2 (9°24'13.2444"N, 82°19'7.0926" W) acted as an intermediate site and Site 3 (9°24'3.6102" N, 82°18'51.8004"W) was the control site.

The mangrove forests studied could be classified as fringing mangroves because they were bordering the sea, as opposed to a river or estuary. The sites were selected because they were dominated by *R. mangle,* which are distinguished by their prop roots (Lovelock et al., 2005). However, there was at least one *L. racemosa* in Site 1 and 2. Canopy height weren't measured; however, based on observation and estimation, most trees range approximately from 5 – 6 m. In Site 3, the height of mangrove trees decreased further from the sea and many trees were no more than 3 m.

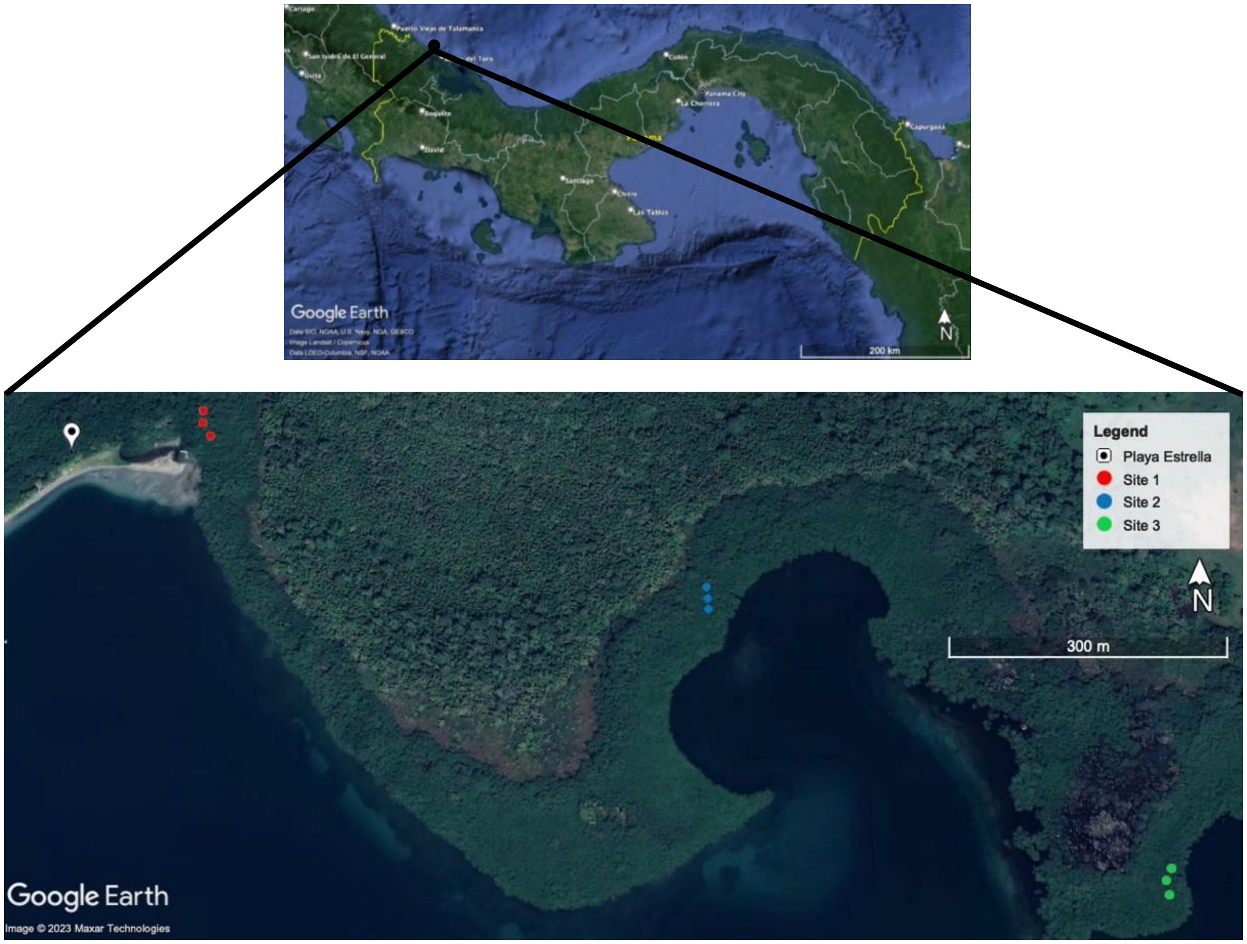

Figure 1: Map of Panama and the three study sites near Playa Estrella, Bocas del Toro, Panama. Each dot represents a 3-m radial plot.

Field Data Collection

In each site, seedlings in three radial plots with 3 m radii were sampled. They were approximately 15 m apart from each other and stretched from South to North (*Figure 1*). Seedlings were classified as less than 1 m in height and 25 mm in stem diameter (Duke & Pinzon M., 1992). Data collected in the field included stem diameter, total and stem heights, number of nodes and internodal heights.

Measurements of seedlings were done in reference to Duke and Pinzon M. 1992 (*Figure 2*). Stem diameter was measured using a caliper at the hypocotyl node or node 0. Total height was the distance between the substratum level to the apical shoot and stem height was the distance from the hypocotyl node to the apical shoot. Nodes were identified by the presence of scars and the internodal height was measured from a stipular scar to the next one. Notes were taken in addition to the measurements if the stems were broken or absent. In Site 1, 10 seedlings were randomly obtained from each plot for analysis of dry biomass.

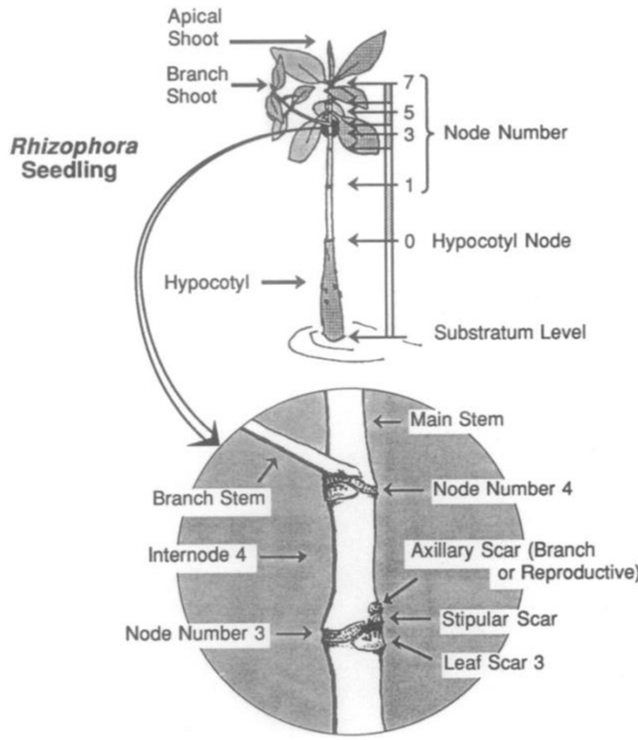

Figure 2: A diagram of the structure of Rhizophora seedlings (Duke and Pinzon M., 1992, p. 174)

Dry Biomass

In Site 1, 10 seedlings per radial plots were removed for dry biomass analysis. The leaves, stems and hypocotyls were separated. Because of the scale's inability to detect mass under 1 g, all leaves, stems, and hypocotyls were combined by circular plots. Previous methods dried mangrove seedlings at 80ºC for 4 days to obtain the dry mass (Lopez-Hoffman et al., 2006). However, due to the lack of a lab furnace to dry samples for such a long period of time, the seedlings obtained from the field were dried outdoor for 12 days under indirect sunlight. To ensure the seedlings were completely dried, they were put in a kitchen oven for two hours at about 150 ºC. The remaining masses were the approximation of the dry biomass of the leaves, stems, and hypocotyls of *R. mangle* seedlings. Dry biomass can inform the energy allocation to different parts of the seedlings, and their photosynthetic rate (Lopez-Hoffman et al., 2006).

Data Analysis

All data was analyzed in RStudio. Shapiro Wilk test results displayed that 22 out of 30 data sets were not normal ($P < 0.05$). Therefore, a non-parametric test was opted. Kruskal–Wallis analyses were conducted to compare the medians of stem diameter, total and stem heights, number of nodes and internodal heights across the three sites, and Dunn tests were conducted for results with statistical significance for pairwise analyses. The distribution of each parameter was visualized in the form of boxplots. To take into account the different time the seedlings are established, seedlings were grouped into total height categories: short (≤ 20 cm), medium (> 20 cm and ≤ 40 cm) and tall (> 40 cm). Kruskal-Wallis and Dunn test analyses were also run to compare the median stem diameter, total and stem heights, number of nodes and internodal heights across the three sites in each height categories. Significant results are reported in addition to the results of the data that were not categorized by height. The mean dry biomass of each seedling part was visualized in a bar plot and compared by Kruskal-Wallis analysis. Leaf mass ratio (LMR) was calculated by taking ratio the dry mass of leaves to total dry mass (Lopez-Hoffman et al., 2006).

Ethics

Because field work was done in mangrove forests, damage was inevitably done to the forest and habitats of mangrove-dwelling organisms. This damage was minimized by carefully navigating in the forest (i.e., avoiding stepping on mangrove roots when possible, and not putting entire body weight when stepping on the roots). Damage was also done when seedlings were removed from the Site 1 for dry biomass analysis. To avoid causing further destruction, no seedlings were removed from Site 2 and 3. This research was approved by the SIT Institutional Review Board (IRB), although no further review was needed since the research did not require human subjects.

Result

A total of 202 seedlings were measured across all nine transects in three sites near Playa Estrella. The median of stem diameter, total and stem heights, number of the nodes and internodal heights are displayed in *Table 1*. Generally, the internodal heights decreased as they were further from the hypocotyl in all sites (*Table 1*).

Table 1. Median (IQR) of 10 parameters of R. mangle seedlings in three sites near Playa Estrella, Bocas del Toro, Panama. Parameters are stem diameter, total height, stem height, number nodes, and six internodal heights.

Parameters	Site 1	Site 2	Site 3	All site
Stem Diameter (mm)	3.5 (1.3)	3.4 (1.2)	3.7 (1.1)	3.6 (1.4)
Total Height (cm)	34.0 (17.7)	32.5 (15.9)	31.6 (11.6)	32.0 (14.2)
Stem Height (cm)	16.2 (18.0)	17.1 (11.7)	16.8 (10.6)	16.7 (13.4)
Number of Nodes	3.0 (2.0)	2.0 (3.0)	4.5 (3.8)	4.0 (3.0)
Internodal Height 1 (cm)	8.2 (7.1)	10.1 (10.0)	6 (5.8)	7.2 (7.2)
Internodal Height 2 (cm)	4.7 (5.6)	4.4 (4.2)	3.4 (2.6)	4.0 (4.2)
Internodal Height 3 (cm)	2.5 (1.7)	2.2 (0.9)	1.8 (1.8)	2.1 (1.8)
Internodal Height 4 (cm)	1.6 (1.5)	1.2 (0.9)	1.2 (0.8)	1.2 (1.0)
Internodal Height 5 (cm)	1.1 (0.8)	1.0 (0.3)	0.8 (0.6)	0.8 (0.6)
Internodal Height 6 (cm)	0.7 (0.2)	0.8 (0)	0.6 (0.2)	0.8 (0.2)

Stem diameter

The distributions of stem diameters in all three sites are illustrated in *Figure 3*. Several seedlings were neglected in the analysis of stem diameter due to broken or absence of stem: one in Site 1, two in Site 2 and four in Site 3. Site 1 had a minimum stem diameter of 1.4 mm, Q1 of 2.9 mm, median of 3.5 mm, Q3 of 4.2, and maximum of 5.5 mm. In Site 2, the minimum stem diameter was also 1.4 mm, Q1 2.8, median 3.4 mm, Q3 4.0 mm, and maximum 11.2 mm. In Site 3, there was a minimum stem diameter of 1.5 mm, Q1 of 3.3 mm, median of 3.7 mm, Q3 of 4.4 mm, and maximum of 8.2 mm.

A Kruskal-Wallis analysis of the stem diameters of *R. mangle* found no statistically significant difference in the medians across the three sites ($H = 3.4484$, $df = 2$, $P = 0.1783$). Nonetheless, there were seedlings with outliers on the higher side in Site 2 and 3, but not Site 1. However, a closer examination at different height categories showed that the stem diameter of seedlings with medium height varied across sites (Kruskal-Wallis, $H = 8.6947$, $df = 2$, $P = 0.0129$; *Figure 4*). There are statistically significant differences between the median stem diameter of medium-height seedlings in Site 1 and 3 ($P = 0.04112$) and Site 2 and 3 ($P = 0.0341$).

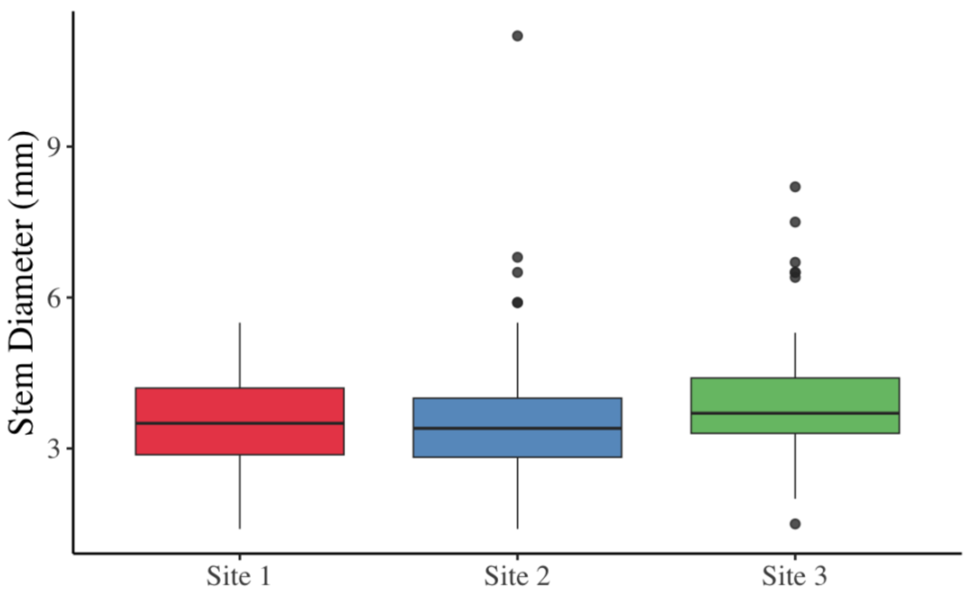

Figure 3: Boxplot comparing stem diameters (mm) of R. mangle seedlings in three sites near Playa Estrella, Bocas del Toro, Panama

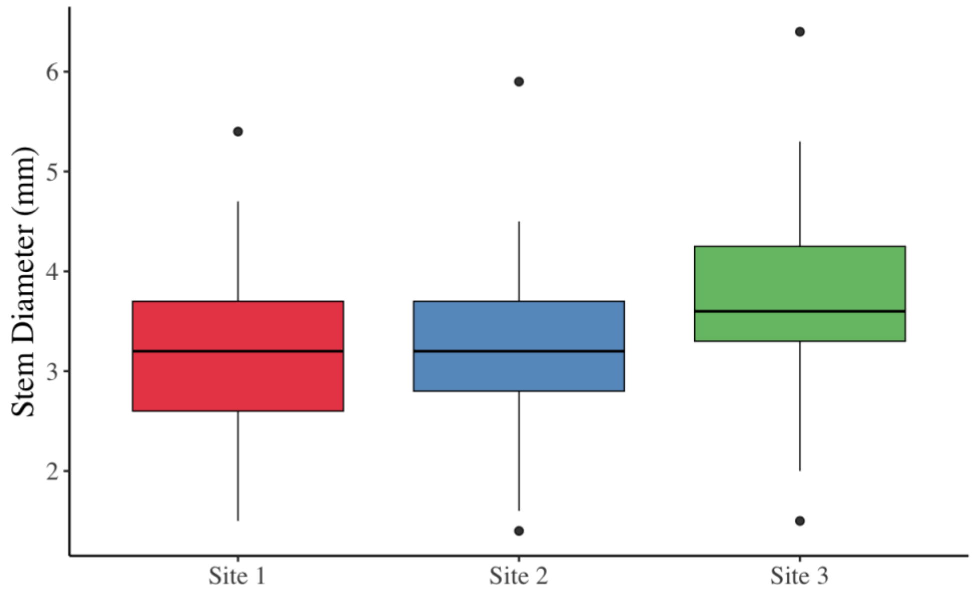

Figure 4: Boxplot comparing stem diameters (mm) of R. mangle seedlings with medium height in three sites near Playa Estrella, Bocas del Toro, Panama

Total and stem height

Figures 5 and *6* shows the distributions of total and stem heights of *R. mangle* seedlings in the three sites, respectively. Because of broken or absence of stem, one seedling from Site 1, two from Site 2 and four from Site 3 were neglected in the analysis of stem heights of *R. mangle* seedlings.

Site 1 had a minimum total height of 8.8 cm, Q1 of 23.9 cm, median of 34.0 cm, Q3 of 41.6 cm, and maximum of 52.6 cm. Additionally, Site 2 had a minimum total height of 12.2 cm, Q1 of 23.4 cm, median of 32.5, Q3 of 39.3 cm, and maximum of 77.2 cm. Finally, Site 3 had a minimum total height of 11.0 cm, Q1 of 26.2, median of 31.6 cm. Q3 of 37.8, and maximum of 52.2 cm.

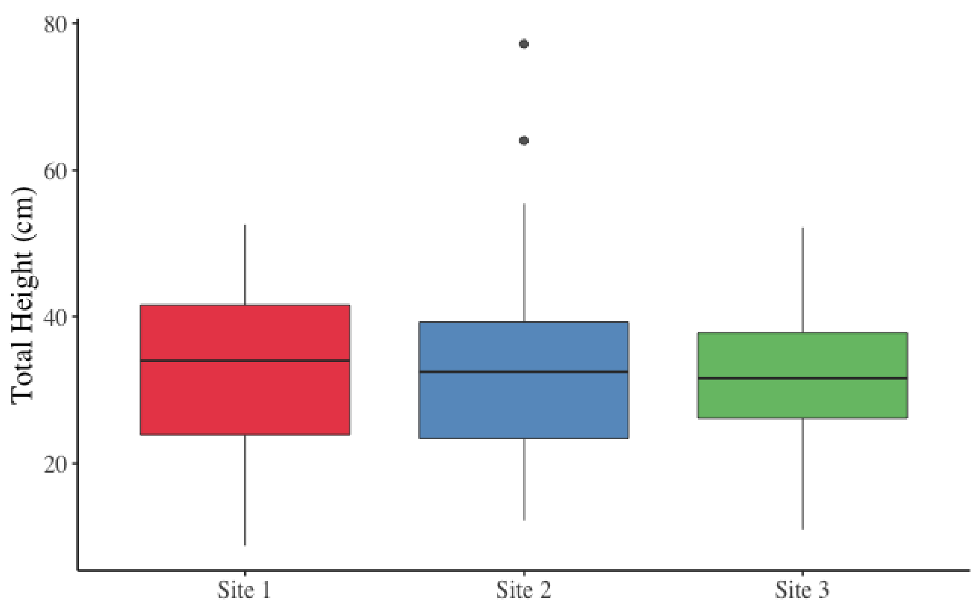

Figure 5: Boxplot comparing total height (cm) of R. mangle seedlings in three sites near Playa Estrella, Bocas del Toro, Panama

Site 1 had a minimum stem height of 0.5 cm, Q1 of 9 cm, median of 16.2 cm, Q3 of 27.0 cm, and maximum of 37.5 cm. In Site 2, the minimum stem height was 2.4 cm, Q1 11.7 cm, median 17.1 cm, Q3 23.4 cm, and maximum 69.6. Site 3 had a minimum stem height of 0.7 cm, Q1 of 10.6 cm, median of 16.8 cm, Q3 of 21.2 cm, and maximum of 35.6 cm.

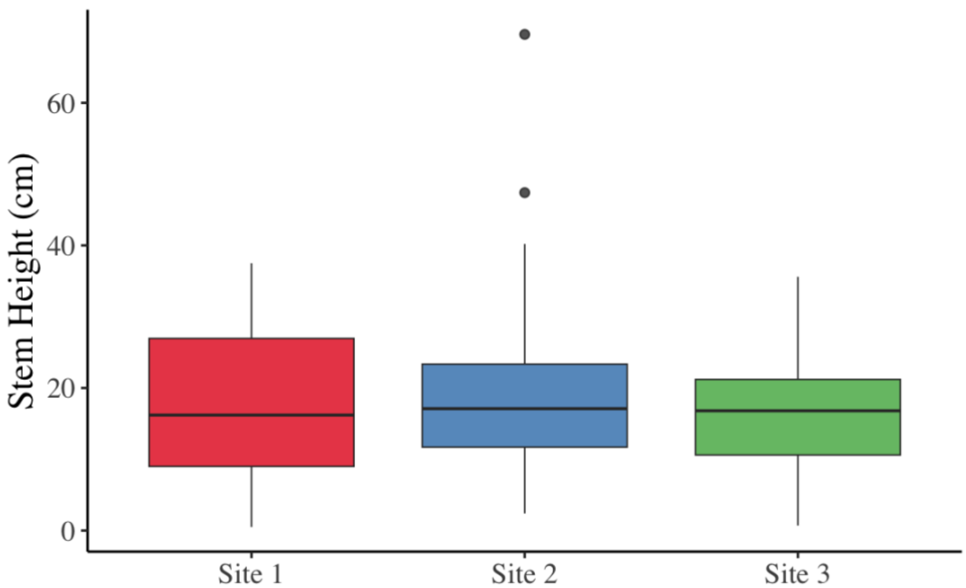

Figure 6: Boxplot comparing stem height (cm) of R. mangle seedlings in three sites near Playa Estrella, Bocas del Toro, Panama

A Kruskal-Wallis analysis found no statical significance in the difference between the median total height of *R. mangle* seedlings in all three sites (H = 0.8720, df = 2, P = 0.6466). The same result was found for stem height: a Kruskal-Wallis analysis found no statistically significant difference between the median stem height of *R. mangle* seedlings in the tree sites (H = 0.3233, df = 2, P = 0.8508). However, it's also worth noting that Site 2 had two outliers on the high side.

Number of nodes

The distribution of the number of nodes of *R. mangle* seedlings in the three sites is shown in *Figure 7.* Three seedlings from Site 1, six from Site 2 and 11 from Site 3 were neglected in the analysis of the number of nodes on seedings due to absence of nodes (new buds developing), broken or absence of stem. In all sites, the minimum number of nodes was 1. In Site 1, Q1 was 2, median 3, Q3 4, and maximum 6. In Site 2, Q1 was 1, median was 2, Q3 4, and maximum 11. Site 3 had a Q1 of 3, median of 6, Q3 of 9.8, maximum of 31.

Site 3 had the highest number of nodes with many outliers on the higher side. A Kruskal-Wallis analysis of the number of nodes on *R. mangle* seedlings supported this observation. The analysis showed a statically significant difference in medians between the three sites (H = 23.737, df =2, P = 7.009×10^{-6}). The Dunn test showed there are statistically significant differences between the median number of nodes of seedlings in Site 1 and Site 3 (P = 3.6×10^{-4}), and Site 2 and Site 3 (P = 1.8×10^{-4}). The same analysis for different height categories also revealed statistically significant difference in the number of nodes for medium (H = 32.093, df = 2, P = 1.074×10^{-7})

and tall (H = 12.77, df. 2, P = 0.0017) height categories. For medium seedlings, the node counts varied between Site 1 and 3 (3.3131×10^{-6}) and Site 2 and 3 ($P = 1.2662 \times 10^{-5}$). For tall seedlings, the node counts were different between Site 1 and 3 (P = 0.0011)

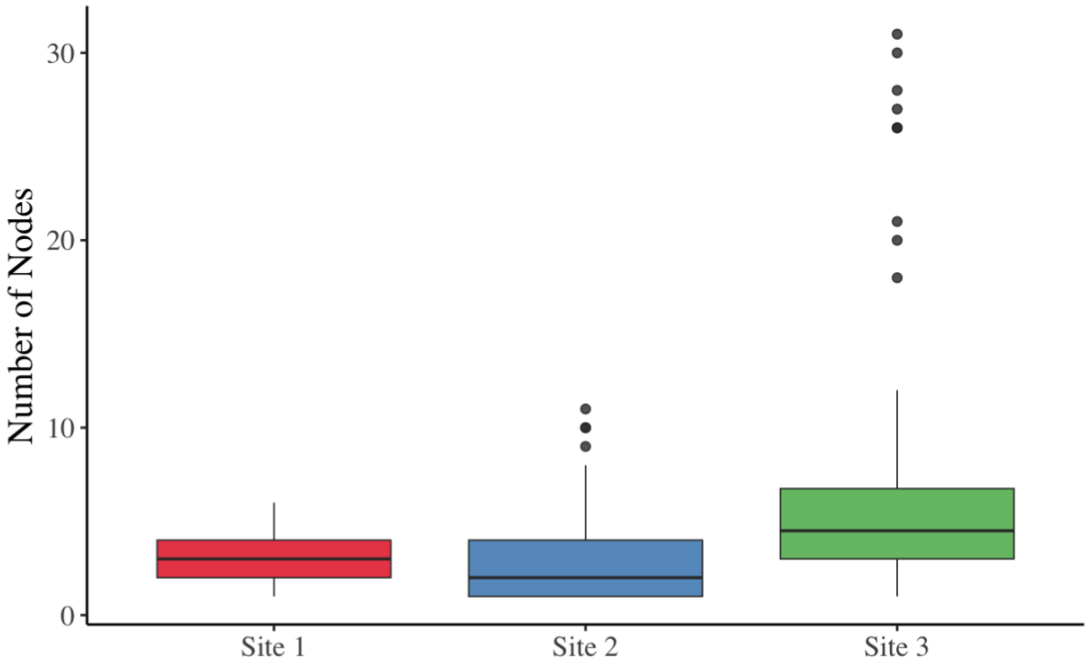

Figure 7: Boxplot comparing the number of nodes on R. mangle seedlings in three sites near Playa Estrella, Bocas del Toro, Panama

Internodal heights

The internodal heights decreased as they were further from the hypocotyl node (*Table 1; Figure 8*). Sites 1 and 2 generally had taller internodal heights than Site 3 (*Table 1, Figure 7*). Kruskal-Wallis analyses showed that the differences in medians across sites also decreased as they were further from the hypocotyl node. There were significant differences between the three sites in the median first internodal height (H = 7.3017, df = 2, P = 0.0260), second internodal height (H = 8.404, df = 2, P = 0.0150), third internodal height (H = 8.1079, df = 2, P = 0.0174), and fourth internodal height (H = 6.5624, df =2, P = 0.0376). The Dunn test showed that there were statically significant differences in the median first internodal height between Site 2 and 3 (P = 0.024), the second internodal height between Site 1 and 3 (P = 0.0385), and Site 2 and 3 (P = 0.0409). The same tests also showed statically significant differences in the median third internodal height between Site 1 and 3 (P = 0.0191). No statistically significant differences in the median fifth internodal height (H = 5.9525, df = 2, P = 0.0510) and sixth internodal height (H = 1.8134, df = 2, P = 0.4038). The same tests for different height categories showed few significant differences and only tall seedlings had significant differences in the first internodal height (Kruskal-Wallis, H = 13.097, df = 2, P = 0.0014) and the second (H = 8.0768, df = 2, P = 0.0176).

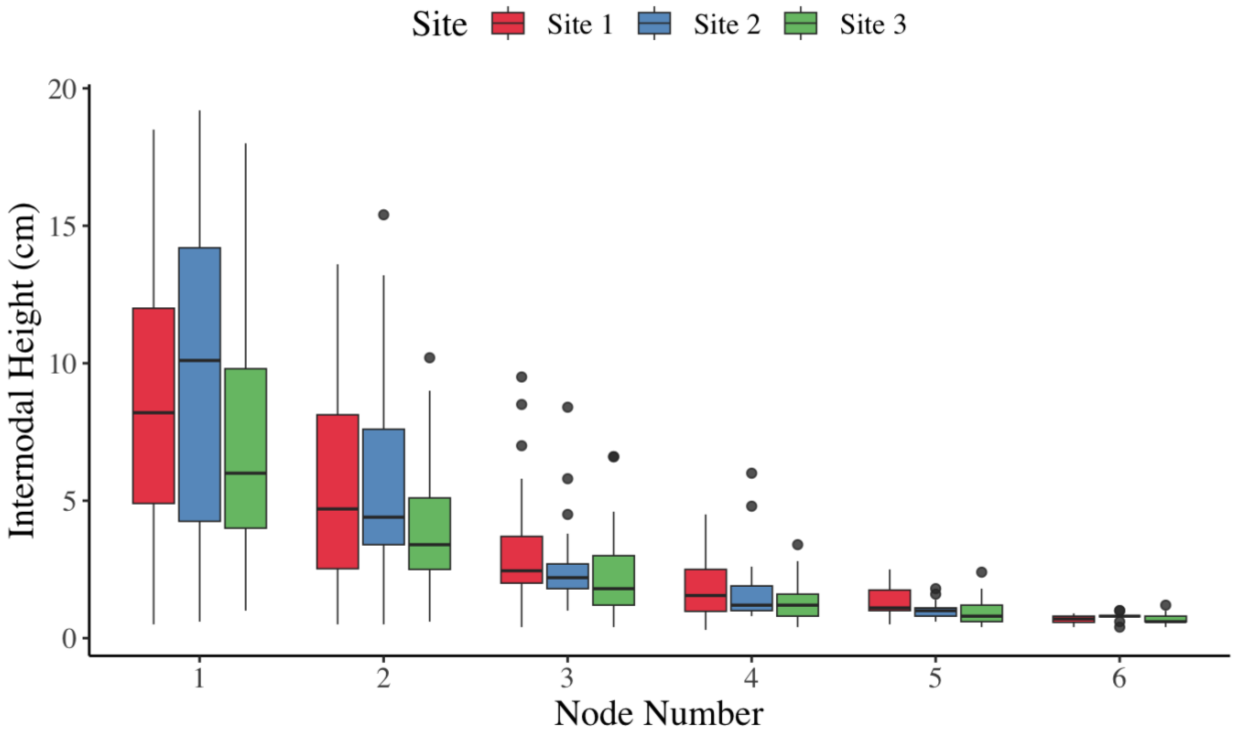

Figure 8: Comparison of the distributions of internodal heights of the first six nodes on R. mangle seedlings in three sites near Playa Estrella, Bocas del Toro, Panama

Dry biomass

The dry biomass of the leaves, stems, hypocotyls and LMR of *R. mangle* seedlings in Site 1 is listed in *Table 2*. The median and IQR are illustrated in *Figure 9*.

Kruskal-Wallis analysis showed a statistically significant difference in the median dry biomass between the leaves, stem, and hypocotyl of *R. mangle* seedlings in Site 1 ($H = 6.0565$, $df = 2$, $P = 0.0484$). The Dunn test resulted in a statistically significant difference in the median dry biomass of the hypocotyl and leaves ($P = 0.0485$).

Table 2: Dry biomass of the leaves, stems, hypocotyls in grams and leaf mass ratio (LMR) in gram/gram of 10 R. mangle seedlings from each plot in Site 1 near Playa Estrella, Bocas del Toro, Panama.

	Leaf (g)	**Stem (g)**	**Hypocotyl (g)**	**LMR (g/g)**
Plot 1	3	5	32	0.075
Plot 2	1	2	47	0.02
Plot 3	1	2	37	0.025

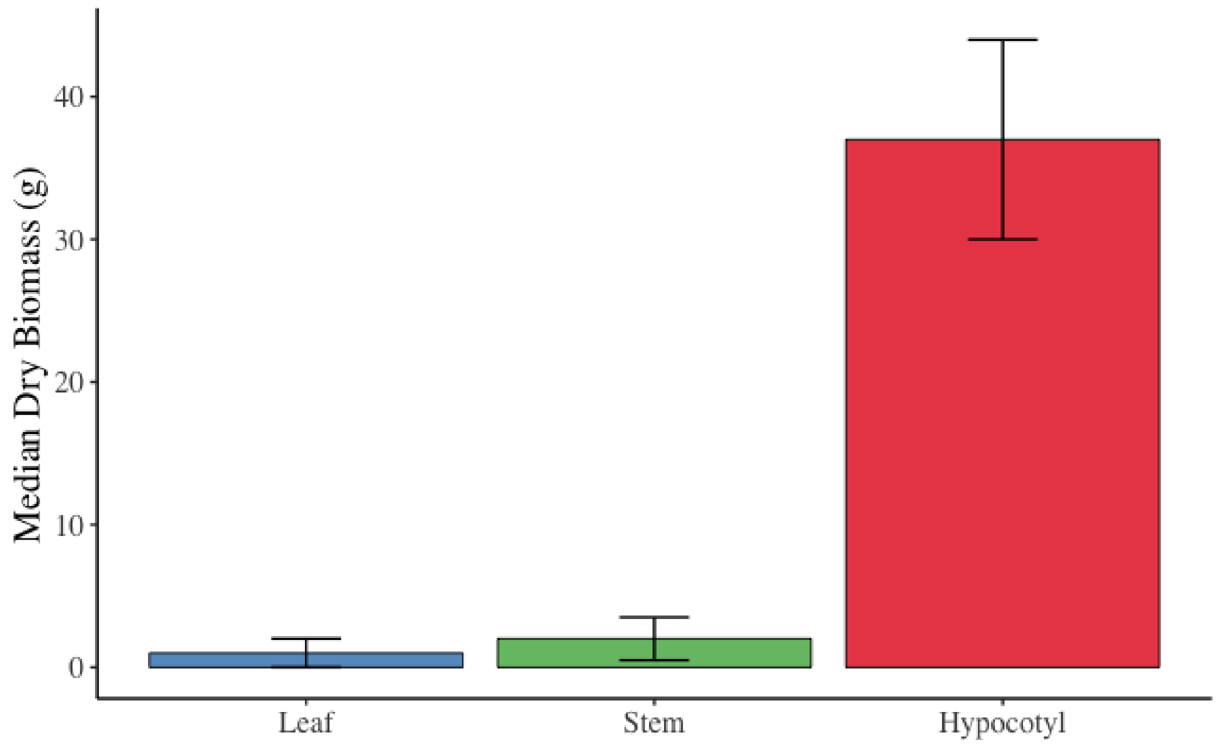

Figure 9: Median and IQR of estimate dry biomass of three parts (leaf, stem, hypocotyl) of 30 R. mangle seedlings from Site 1 near Playa Estrella, Bocas del Toro, Panama

Discussion

Stem diameter, total and stem heights

A statistically significant difference was found in the stem diameter, while no significant differences were found in the total and stem height of *R. mangle* across all three sites near Playa Estrella. Stem diameter appeared to be larger in Site 3 (the control site) than Site 1 and 2 (experimental sites) when grouped by height categories. The median stem diameter of medium-height seedlings was larger in Site 3 than Site 1 and 2. Agraz-Hernández et al., 2018 tested the growth in diameter and height of *R. mangle* seedlings in environments treated and non-treated with wastewater. Seedlings in control group consistently had bigger diameter and taller height then the ones that were treated. Because Site 3 is the furthest away from Playa Estrella, the variation in diameter in this site may indicate less impact by pollution created by human, potentially wastewater pollution, on the beach. On the contrary, total and stem height were close to uniform for all sites, whether grouped by height or not. Previous studies showed that the growth of these seedling parameters can be controlled by their age, salinity, nutrient and light availability (Agraz-Hernández et al., 2018; Lopez-Hoffman et al., 2006). Thus, the lack of differences in total and stem height in this study may be accounted for the similarity in environmental condition between the sites, and the heights were not as sensitive as the stem diameter. Potentially, if the sites were further apart, differences may be more pronounced. Ultimately, more parameters, such as light availability, and nutrient content (e.g., by porewater analysis of nitrogen, and phosphorous), should be measured to determine factors that may impact stem diameter, total and stem heights in the study sites.

Number of nodes

The number of nodes on the stems of *R. mangle* seedlings in Site 3 was drastically higher than in Site 1 and 2. Medium and tall seedlings also exhibited the same pattern, while no differences were found in short seedlings because each seedling had no more than two nodes. Site 3 also had saplings (those with stem diameter greater than 2.5 cm as defined by Duke & Pinzon M., 1992) that were not observed in the other sites. The number of nodes is an indication of age as older seedlings have higher node counts (Duke & Pinzon M., 1992). Therefore, the combination of bigger seedlings and presence of saplings potentially means that seedlings in Site 3 are older because they had the right condition and minimal pollution disturbance to survive due to a further distance from Playa Estrella. However, light availability also plays a role on the number of nodes that are established per year; *R. mangle* seedlings in shaded area have smaller node counts than those in areas with exposed sunlight when they are the same age (Duke & Pinzon M., 1992). Duke & Pinzon M. 1992 concluded that *R. mangle* seedlings produce about 4 to 7 seedlings per year in closed and open canopy respectively. This means depending on canopy cover, the age of seedlings measured in this study could be anywhere from less one year old to about 4 years old. As such, it is possible that the high node counts on seedlings in Site 3 can be attributed to a more open forest and shorter forest. To be more certain, light availability parameters (e.g., canopy cover and height) need to be measured.

Internodal heights

The first six internodal heights on the stems of *R. mangle* seedlings decrease further away from the hypocotyls. This pattern matches the result found in Duke & Pinzon M., 1992, where there was a rapid growth in the first four nodes. This fast initial growth may be explained by the availability of resources in the hypocotyl reserve (Duke & Pinzon M., 1992). Furthermore, growth rates of plants do not remain constant, and the initial growth is faster due to the law of diminishing returns (Agraz-Hernández et al., 2018). On the other hand, differences across sites were observed: the internodal heights of the first four nodes were higher in Site 1 and 2 than Site 3. For tall seedlings, differences were found in the first two nodes where the internodal heights were higher for seedlings in Site 1 and 2 than Site 3. Seedlings efficiency to remove nutrients from the water and utilize it can be slowed down by nutrients enrichment by wastewater (Agraz-Hernández et al., 2018). Subsequently, we would expect the Site 3 to have the fastest initial growth, although that was not the case. Therefore, the differences in internodal heights between sites may be accounted by differences in the availability of nutrients in the hypocotyl reserve, or the ability of the seedlings to absorb nutrients to allocate to the stems. It can also be because of variations in the parent trees or different environmental conditions that impact the efficiency of the hypocotyls. Moreover, internodal extension is a function of rainfall thus rainfall should not be factor for the deviation of internodal heights since the sites are impacted by the same amount of rainfall. Unlike the previous parameters, there’s a low likelihood that light availability has an influence on the first few nodes development because photosynthesis is not required (Smith & Snedaker, 2000).

Dry biomass

The biomass of each section of the seedlings speak to their resource allocation and productivity. *R. mangle* seedlings in Site 1 seemed to prioritize the development of the hypocotyls. On top of that, comparison between the LMR found in this study and Lopez-Hoffman et al., 2006 showed that the former LMR is smaller. The total biomass and LMR of *R. mangle* is impacted by light availability, salinity and floatation duration of propagules (Agraz-Hernández et al., 2018; Simpson et al., 2017). Therefore, a reason for this can be a response to environmental stress. For instance, high root to leaf biomass ratio at high salinity can be the result of low nutrient or water availability, which limits the seedlings ability to respond to light availability (Lopez-Hoffman et al., 2006).

Limitations and potential sources of errors

A limitation to this study is the lack of control for confounding variables. As mentioned earlier, environmental factors such as salinity, light and nutrient availability play a role in the growth of *R. mangle* seedlings. These variables were not considered in this study, so it is difficult to draw a definitive conclusion about why there were and were not differences in the growth parameters. Potentially, future studies can take environmental factors into account. Another limitation is the precision of equipment such as the field measuring tape and scale to measure small heights and weights, respectively, and the oven to estimate dry biomass. With more precise equipment such as a lab scale and lab furnace, a more accurate estimate can be obtained. Finally, due to a limited time, actual growth over time could not be measured. The conclusion drawn in this study is from a cross section of *R. mangle* seedlings growth that was captured.

Conclusion

The study objective was to investigate how the growth parameters of *R. mangle* seedlings and dry biomass change with distance from Playa Estrella, Bocas del Toro, Panama. The results found in this study act as a preliminary investigation on the potential impact of human activities on Playa Estrella on the growth parameters of *R. mangle* seedlings. The parameters—stem diameter, total and stem heights, nodes count, internodal heights, and biomass—are proxies to the understanding of the health of seedlings. Stem diameter of medium height seedlings were the largest in Site 3, while the number of nodes was also highest in Site 3. Yet, internodal heights of the first few nodes showed the opposite trend where taller heights appeared in Site 1 and 2. Dry biomass analysis showed low allocation of resources to leaves production in Site 1. These results offer a glimpse of how human activities on Playa Estrella may inversely impact nearby mangrove forests through changing the development of *R. mangle* seedlings. However, the results found is not sufficient to draw a holistic conclusion about human impact from Playa Estrella.

With more resources and time, more sites can be studied, particularly sites that potentially have less human impact, and sites with evident human impact such as Bocas Town, Bocas del Toro, Panama. Dry biomass should be compared among sites, ideally using a non-destructive method. Additionally, more time could be designated to finding possible point source pollution in Playa Estrella. Fundamentally, future studies should consider not just environmental factors that may impact *R. mangle* seedlings growth, but also factors that could be caused by human such as presence of trash, and nutrient enrichment from waste.

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Appendices

Appendix 1: Results of Shapiro-Wilk test for normality for 10 parameters of R. mangle seedlings across three sites. Most results yielded a non-normal distribution, except for eight.

Site	Parameter	Statistic	Sig.
Site 1	Stem diameter	0.9819	0.5177
	Total height	0.9514	0.0168
	Stem height	0.9577	0.0388
	Number of nodes	0.9057	0.0003
	Internodal height 1	0.9590	0.0510
	Internodal height 2	0.9402	0.0198
	Internodal height 3	0.8615	0.0004
	Internodal height 4	0.9033	0.0253
	Internodal height 5	0.9290	0.2952
	Internodal height 6	0.9158	0.3970
Site 2	Stem diameter	0.8237	1.568×10^{-6}
	Total height	0.9499	0.0213
	Stem height	0.8807	6.525×10^{-5}
	Number of nodes	0.8124	1.709×10^{-6}
	Internodal height 1	0.9401	0.0313
	Internodal height 2	0.9317	0.02814
	Internodal height 3	0.7676	0.0001
	Internodal height 4	0.6632	2.088×10^{-5}
	Internodal height 5	0.8783	0.0833
	Internodal height 6	0.8463	0.0679
Site 3	Stem diameter	0.9299	0.0003
	Total height	0.9878	0.6129
	Stem height	0.9849	0.4605
	Number of nodes	0.6339	2.717×10^{-12}
	Internodal height 1	0.9619	0.0267
	Internodal height 2	0.9357	0.0018
	Internodal height 3	0.8875	3.637×10^{-5}
	Internodal height 4	0.8831	0.0001
	Internodal height 5	0.8323	7.627×10^{-5}
	Internodal height 6	0.9011	0.0193

Appendix 2: Histograms of the distribution of 10 parameters of R. mangle seedlings across three sites near Playa Estrella, Bocas del Toro, Panama