

GROWTH RATE AND SURVIVORSHIP OF *RHIZOPHORA MUCRONATA*, *AVICENNIA MARINA*, AND *CERIOPS TAGAL* SEEDLINGS WITH FRESHWATER AND SEAWATER TREATMENT FOR MANGROVE PROPAGATION IN NURSERIES

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Abstract. Mangroves play an important role in coastal ecosystems worldwide, performing vital functions like protecting coastlines, seagrass, and coral reefs, purification of water, trapping sediments, and providing nursery grounds for many terrestrial and aquatic organisms. Despite their importance, mangroves are threatened worldwide due to increasing human development in coastal areas, and therefore, efforts to restore degraded mangrove ecosystems have gained traction. Mangrove restoration requires specialized knowledge and skills, ranging from selecting seeds, to the planting of seedlings in nurseries and along degraded coastlines. The following study explores the survivorship and growth rate of seedlings of three mangrove species – namely *Avicennia marina*, *Rhizophora mucronata*, and *Ceriops tagal* – in freshwater and seawater treatments. The experiment was conducted in a nursery-based environment to inform mangrove rehabilitation programs. Root count and length, leaf count and length, and the length of the entire seedling were measured for each sample species every week for 12 weeks. The data were analyzed using two-way ANOVA to identify significant differences in the measured variables between each species in the freshwater and seawater treatments. Seedlings grown in freshwater revealed a more rapid growth rate and lower mortality relative to those in seawater. Mangrove seedlings can be raised in nursery-based environments. They can be irrigated using freshwater with species like *A. marina*, less constrained by freshwater and hence display higher growth rates. Therefore, *A. marina* should be considered a priority species for mangrove restoration, given its relatively higher growth rate than the other species in the experiment.

Keywords: coastal protection, mangrove rehabilitation, survival rate, climate change mitigation, zonation, Seychelles

Introduction

The importance of mangroves to human well-being and a variety of coastal ecosystems has been well documented (Ellison, 2008). Mangrove forests comprise of unique plant species that form a critical interface between terrestrial, estuarine, and nearshore marine

ecosystems in tropical and subtropical regions. Mangroves are known for stabilizing coastlines by controlling erosion and facilitating sediment deposition (Dahdouh-Guebas et al., 2005; Das, 2020). In addition, they provide critical habitats for various terrestrial, estuarine, and marine species, as well as a source of and sink for nutrients and sediments, benefitting other inshore marine habitats like seagrass beds and coral reefs (Kathiresan and Bingham, 2001; Duke et al., 2007). Mangrove ecosystems protect coastlines through the protection of coastal and nearshore habitats during extreme weather events (e.g., storm surges and severe winds) (McIvor et al., 2012), they also ensure the safety of coastal assets such as agricultural areas by suppressing wave activity, as well as the stabilization of coastlines and dunes by reducing erosion through wave reduction and sediment accretion (Das, 2020). Furthermore, mangrove forests provide a range of services to coastal communities, such as food, timber, non-timber forest products, and traditional medicines, as well as shelter for indigenous people from physical disturbances like coastal erosion (Osti et al., 2009).

In addition to mangrove forests protecting coastal communities and Small Island Developing States (SIDS) from floods and erosion, they provide diverse employment and income opportunities to local communities (Field et al., 1998). Although mangrove forests only make up 0.2% of the world's total land area, they provide a host of essential ecosystem services which can be difficult to quantify (Dodd and Ong, 2008). According to Costanza et al. (1997), global mangrove forests provide ecosystem services valued at ~US\$1.6 billion per year. An estimated 80% of global fish catches are directly or indirectly dependent on mangroves (Ellison, 2008). Additionally, mangroves sequester up to 25.5 million tonnes of carbon annually and provide more than 10% of essential organic carbon to seawater worldwide (Dittmar et al., 2006). Mangrove forests are highly productive, producing carbon at rates equivalent to tropical forests. Specifically, mangrove tree species allocate relatively more carbon belowground than tropical forests. They have higher below-to-above-ground carbon mass ratios than terrestrial trees – which is an important aspect considering climate change mitigation potential of mangrove forests (Alongi, 2012).

For a country like the Republic of Seychelles, the economic benefits derived from mangrove forests are an essential service considering the country's dependence on ocean resources (as stipulated within Seychelles' Blue Economy Strategic Policy Framework and Roadmap) (Government of Seychelles, 2018). The vision of Seychelles' Blue Economy Strategic Policy Framework and Roadmap is to “develop a blue economy as a means of realizing the nation's development potential through innovation, knowledge-led approaches, and being mindful of the need to conserve the integrity of the Seychelles marine environment and heritage for present and future generations.” As such, healthy mangrove ecosystems can contribute positively to achieving this vision. Two previous studies on mangrove ecosystems revealed its importance as nursery grounds for juvenile reef fishes while concomitantly providing habitat for numerous animals ranging from various invertebrates to seabirds (El-Regal and Ibrahim, 2014; Rog et al., 2017). Therefore, the loss of mangrove forests will reduce coastal water quality and biodiversity, eliminate fish and crustacean nurseries, and adversely affect adjacent coastal habitats and ecosystem services and resources relied on by coastal communities (Walters et al., 2008).

The populated nature of coastlines around the world has exacerbated the clearing of mangrove forests to make way for development, aquaculture, resource extraction, and urbanization. At least 40% of animal species associated with mangrove habitats and previously assessed by the International Union for Conservation of Nature's (IUCN)

Categories and Criteria are at an elevated risk of extinction due to extensive habitat loss (Luther and Greenburg, 2009). Approximately 26% of mangrove forests worldwide are degraded due to over-harvesting fuelwood and excessive timber production (Valiela et al., 2001). Similarly, clearing mangroves for shrimp farming have contributed to 38% of mangrove forest loss globally, with mariculture, algaculture, and integrated multitrophic aquaculture accounting for another 14% (Ellison, 2008).

Mangrove ecosystems are becoming increasingly degraded and threatened due to numerous human activities (Thomas et al., 2017). This degradation trend is evident in Seychelles, which has lost most of its original mangrove forests that were once in abundance around the coast of Mahé Island (Palacios et al., 2021). There has been a growing recognition among scientists of the importance of mangroves and the need for mangrove rehabilitation since 2006 - when the IUCN and the United Nations Development Programme (UNDP) developed the mangrove for the future (MFF) initiative (Erwin, 2009; Sandilyan and Kathiresan, 2015; Das, 2020). Mass production of mangrove seedlings and successful germination of propagules are essential in rehabilitating degraded forests and coastlines to ensure the provisioning of valuable ecosystem goods and services. However, a greater understanding of the underlying factors leading to successful seedling growth and propagation for rehabilitation programs is needed. It is therefore essential to sustain the functions of mangrove ecosystems through restoration, as conditions are not always suitable for mangroves to naturally self-repair without human intervention.

There are several mangrove restoration projects occurring around the world. Various countries, like Bangladesh, Cambodia, China, India, Lao PDR, Maldives, Myanmar, and Nepal, are affiliated with the IUCN and UNDP in the global MFF project (IUCN, 2018). The project emphasizes coastal ecosystems' restoration, conservation, and sustainable management. Mangrove restoration in certain countries aimed to restore mangrove forests to subsequently harvest timber products (Lewis, 2005). In Seychelles, the Ecosystem-based Adaptation (EbA) project, through South-South cooperation, was implemented - leading to various coastal ecosystems being restored to enhance resilience to climate change. This project established several sites for mangrove restoration, in which mangrove seedlings raised in nurseries were planted in degraded wetlands around three islands in Seychelles, Mahé, Praslin, and Curieuse (Henriette, 2016).

However, Mangrove restoration is not easy, and numerous challenges exist. These challenges are evident based on experience gathered from six years of continuous monitoring at the EbA project site at Anse Royale by the University of Seychelles. These challenges include a limited understanding of mangrove ecology, an absence of long-term monitoring of restored mangrove forests, data gaps, and a lack of baseline studies to enhance learning and best practices. This study assesses the survivorship and growth rates of seedlings of three mangrove species in Seychelles, *Rhizophora mucronata*, *Avicennia marina*, and *Ceriops tagal*, when irrigated by freshwater and seawater in an experimental setup. To achieve the objectives of this study, the following research questions are critical:

- I. Does the type of water treatment (i.e., seawater or freshwater) influence the growth rate of the species during propagation in a controlled environment/nursery?
- II. Which species has the highest growth rate in seawater or freshwater treatments in mangrove nurseries?
- III. Which factors affect mangroves' growth and survival rate in controlled environments/nurseries?

During the research phase of the study, the researchers noted that no previous study had addressed this topic in the context of Seychelles. This paper addresses the research and knowledge gap by gathering data on the propagation of mangrove seedlings that can lead to a better understanding of mangrove species and improve the restoration of degraded coastal ecosystems. Therefore, before initiating restoration interventions, research is needed to improve the planning, implementation, and management of mangrove restoration projects in Seychelles.

Materials and Methods

Site description

The Anse Royale wetland covers an area of ~3 ha. It consists of densely vegetated riparian and mangrove habitats drained by two rivers; the Rivière Au Berlin and Rivière Anse Royale (Fig. 1). Three of the seven mangrove species found in Seychelles (*Rhizophora mucronata*, *Avicennia marina*, *Sonneratia alba*, *Bruguiera gymnorhiza*, *Ceriops tagal*, *Lumnitzera racemosa*, and *Xylocarpus granatum*) are present in Anse Royale, namely *Avicennia marina*, *Rhizophora mucronata*, and *Bruguiera gymnorhiza*. These three species show differences in salinity tolerance which influences their establishment in the wetland. In addition, according to a baseline survey done for the EbA South project (Senterre et al., 2015), *R. mucronata* and *C. tagal* dominate the low water mark and are considered the front shore tidal estuarine mangrove species, while *A. marina* dominating the high water mark is the backshore tidal estuarine mangrove species. The zonation is as follows: i) *A. marina* was located on the seaward side; ii) *R. mucronata* dominates at the forest's center but is widespread; and iii) *B. gymnorhiza* dominates the south-west side, further inland. The mangrove trees, however, are remarkably more abundant further inland. There are diverse species of fauna found in mangrove ecosystems, including species of fish, birds, invertebrates, reptiles, and mammals.

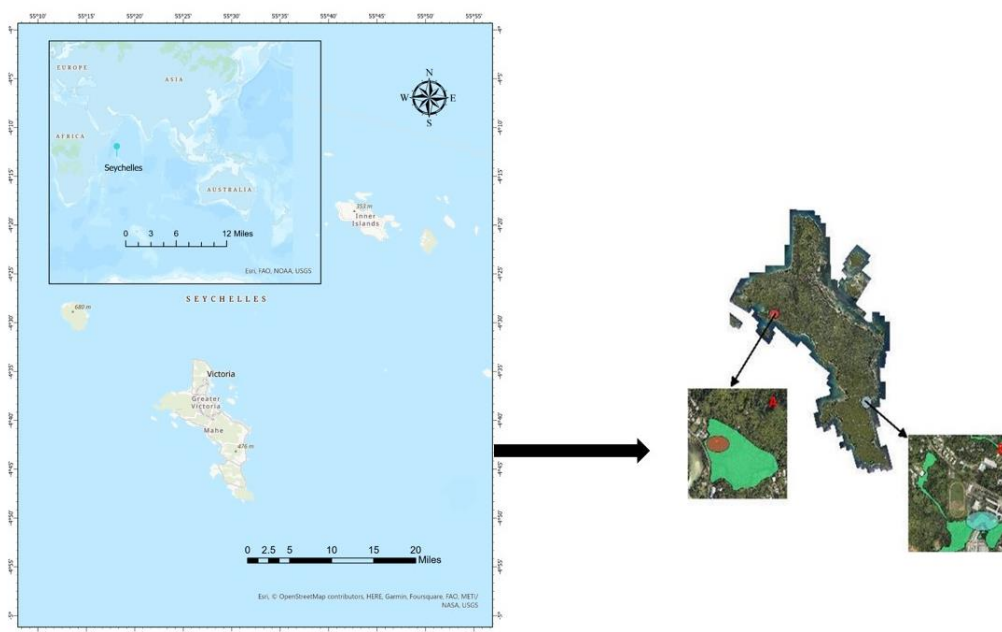


Figure 1. Map of Mahé Island with study sites at Port Launay (A) and Anse Royale (B)

In contrast, the Port Launay wetland is located on the northwest coast of Mahé (Fig. 1). It was declared a Ramsar site in 2004, and ~29 ha of the 124 ha area is covered by mangrove forest (Henriette, 2016). The mangrove forest starts at the seaward intertidal zone, stretching to the upper zone and meeting the foot of the hill that connects to the riverine system. The Port Launay mangrove comprises of all seven native mangrove species of the Seychelles (*Rhizophora mucronata*, *Bruguiera gymnorhiza*, *Ceriops tagal*, *Sonneratia alba*, *Lumnitzara racemose*, *Avicennia marina* and *Xylocarpus granatum*). Numerous bird, invertebrate, fish, reptile, and mammal species are found in the Port Launay mangrove forest.

The Anse Royale mangrove area was selected to collect substrate and seawater, as it was near the experimental site, which made transportation easy. At the time of the propagule collection, *A. marina* individuals were fruiting and were ripe for collection. Propagules of *R. mucronata* and *C. tagal* were sampled at Port Launay because *R. mucronata*, in terms of occurrence, were relatively few at this site, while *C. tagal* was not present at Anse Royale.

Role of zonation in mangrove establishment

Mangrove forests often exhibit zonation patterns due to intertidal gradients along the coastline. It is essential to understand the zonation of mangrove species (ZMS), which often contributes to the responses of individual species to variations in different abiotic and biotic factors (Bullock et al., 2017). Some mangrove species tolerate longer durations of tidal inundation than other species, while some have a relatively higher salinity tolerance (Lewis, 2006). Furthermore, different parameters should be accounted for in a particular species' zone. These parameters include depth, duration, and frequency of tidal inundation, soil salinity, and the amount of freshwater available. Thus, according to Hogarth (2015), mangroves are not distributed randomly but are established at specific sites with suitable abiotic and biotic conditions that are optimal for each species. Additional physical attributes that influence the ZMS are the establishment of mangrove propagules by water movement, the distance seedlings travel, and the time taken to establish. This explains why in some instances, certain species of mangrove trees have a bimodal form of distribution where they can be present inland and along the coastline. Mangrove seedlings vary in size and shape, influencing their buoyancy and determining which propagules are carried further inland with tidal movement.

Experimental design and data collection

Preparation of planting pots and collection of mud substrate

Five-liter bottles were cut at 19 cm heights (Appendix 1a) and used as pots for the experiment. A total of 60 five-liter bottles were designed with the exact height specification, and each was labeled with the relevant species name. An identification number and reference number referring to the freshwater and seawater treatments were printed, laminated, and stapled to the respective bottles (Appendix 1b & c).

Regarding the collection of substrates, different parameters were considered in the zones in which certain species occurred. The substrate was collected at the Anse Royale mangrove forest according to the mangroves' zonation: i) silty clay mud was used for species *R. mucronata* and *C. tagal*, and ii) sandy mud was collected further towards the coastline for *A. marina*. *Avicennia marina* is a pioneer species on newly formed mud habitats with high proportions of sand. However, it does not grow in pure mud (Duke et

al., 2010). The choice of sandy mud substrate for *A. marina* in the experimental design was guided by the conditions best suited for the species to thrive.

Silty clay mud substrate was collected in buckets at low tide for easy access. Any debris, such as broken glass, large rocks, and plastic, was removed. This was to prevent the obstruction of root growth, given the small size of the pots. After removing debris, 20 bottles containing seedlings of *R. mucronata* and *C. tagal* were filled with mud using a trowel shovel.

The same method was employed to fill up 20 pots for *A. marina*. All the pots were placed indoors close to windows in a laboratory at the University of Seychelles, Anse Royale Campus. They were all exposed to the direct effects of sunlight. Given that light intensity and shading can affect seed development (Ball, 2002), all the pots during the experiment at the window which allowed for sufficient sunlight. The laboratory was preferred to a nursery to control better the salinity levels in the rainy season, which coincided with the timing of the experiment - given that rainwater is likely to dilute the seawater in the pots and therefore influence the results. Also, additional illumination wasn't necessary since there was no shading effect across the windows. Therefore, the need for the pots to be randomised periodically was not needed.

Collection of propagule seedlings

Avicennia marina, *Rhizophora mucronata*, and *Ceriops tagal* (Appendix 2) were chosen for this experiment because they are either viviparous or crypto-viviparous. This means their propagules germinate and gain nutrients while still attached to the parent tree (Lewis, 2006). Before collecting mangrove propagules, it is crucial to have knowledge of the fruiting season and how to identify mature propagules. *Rhizophora mucronata* propagules are best collected when the cotyledon is yellow and the hypocotyl is green (Appendix 2b). In *Ceriops tagal*, a propagule is ready for collection when the cotyledon is yellow, and the hypocotyl is brownish-green in color (Appendix 2c). As for the *Avicennia marina*, it is optimal to collect the propagule when the pericarp (fruit skin) is yellowish (Appendix 2a), but even better to collect the ones that have already shed their pericarp, exposing the cotyledon.

Twenty ripe propagule seedlings of *R. mucronata* and *C. tagal* were handpicked from their mangrove parent tree along the Port Launay mangrove boardwalk. This is to ensure seedlings had no contact with seawater before the experiment. Deducing whether a propagule was mature and ripe in *R. mucronata* and *C. tagal* was done by identifying the yellow cotyledon with a brownish-green hypocotyl. *A. marina* seedlings were collected at the Anse Royale mangrove area as they were readily available. Additionally, the 20 ripe propagules collected from the ground had already shed their pericarp, exposing the cotyledon, making it ideal for planting. *A. marina* seedlings with sheath were present. Still, they were not picked, as these would have required a soaking period for the sheath to split (Sukendi, 2018). Only propagules that were in good condition, with regards to their shape and color, were collected. Therefore, propagules that had significant visible physical damages, like withering, desiccation, holes, missing parts, and those with noticeable damage from crabs or larvae, were not collected. These are conditions that, according to Sukendi (2018), will result in low germination rates and a relatively lower chance of establishing.

Planting of propagule seedlings

The propagules were potted on the collection day, and initial height and weight measurements were taken. Hypocotyls were measured using a wooden folding ruler for the elongated propagule of *R. mucronata* and *C. tagal*. In contrast, the plumules of *C. tagal* propagules were measured using a dial caliper - as their plumules are smaller than those of *R. mucronata*. After taking measurements, the seedlings were planted in their respective pots. Ten *A. marina* propagules were placed on the surface in freshwater pots, and the other ten were placed in seawater pots containing sandy mud substrate. Ten *R. mucronata* and *C. tagal* propagules were potted in their pots in freshwater containing silty clay mud, and the other 20 *R. mucronata* and *C. tagal* propagules were potted in the seawater pots in the silty clay mud. Each propagule was gently inserted into the soil, up to a third of its length.

After the seedlings were planted in their pots, water was added. Freshwater from the rainwater harvesting tanks at the University of Seychelles was used to irrigate the seedlings under the freshwater treatment. Rainwater was used instead of tap water to prevent traces of chlorine or other desalination chemicals from affecting the treatments. Seawater was collected from the Anse Royale Beach at high tide. Salinity was measured with a YSI-556 MPS probe and reached 27.3 parts per thousand (ppt).

Thirty pots were irrigated with 850 ml of freshwater, while the remaining 30 with 850 ml of seawater per week. To compensate for evaporation in the pots which was relatively higher for freshwater (El-Dessouky et al., 2002), 400 ml of freshwater was added weekly. All salinity levels were tested upon collection. Since evaporation rates were relatively low especially for seawater when compared to freshwater (El-Dessouky et al., 2002), the water remained stagnant and became green in some pots. In such cases, water was drained by ensuring no substrate was removed and replaced with new batches of seawater which was collected from the Anse Royale Beach.

Root, leaf, and stem measurements

The establishment of propagules in each pot was evaluated weekly by recording root initiation, root length, first leaf initiation, and length measurements of the longest leaf and stem. Details on the measurement procedure applied in this experiment are explained in the following sections on the root, leaf, and stem measurements.

Root measurements

The roots for *R. mucronata* and *C. tagal* were measured using a dial caliper, while for *A. marina*, roots were measured with a 30 cm ruler. However, the roots of *R. mucronata* and *C. tagal* were too small to be measured with the 30 cm ruler. Only the longest were measured upon initiation of the first measurable roots, but all noticeable roots were counted and recorded. Measurements of *A. marina* roots were as follows: the longest root was placed in an elongated position on the 30 cm ruler, and the length was recorded (*Appendix 3a*). For species *R. mucronata* and *C. tagal*, the roots were measured using a dial caliper. The jaws of the dial caliper were opened, and the longest roots were placed between the jaws, partially closing the lower jaw of the caliper until the tip of the longest root rested on the lower jaw. The length of the root was measured based on the calibration.

At the early stages of root measurements in *A. marina*, propagules were handled with care, and the number of roots was counted and measured. As the roots grew more prolonged and deeper into the substrate, extra care was given when removing the

seedlings from the pots. The seedlings were pulled out slowly, requiring the substrate's removal around the roots to prevent damage. In some cases, the tips of the roots were further down and covered by mud, which made them difficult to remove, while in other cases, causing root damage. When the recording of measurements was completed, a hole in the mud was created, and the seedling was then carefully re-inserted into their pots. The same steps were followed for *R. mucronata* and *C. tagal*. Data on root count and root length were recorded for all three species in both fresh- and seawater treatments during the first six weeks of the experiment.

Leaf and stem measurements

After the shooting of the first measurable leaves, the longest was measured, and all noticeable leaves were counted and recorded. The leaf measurements were conducted using a 30 cm ruler, as the leaf could be placed flat on the ruler, and the length was easily measured (*Appendix 3b*). Leaf count was also done by counting the number of visible leaves. The stems of *A. marina* propagules were measured as soon as the stem completely emerged from the cotyledon. A wooden folding ruler was used to measure the stem by placing the *A. marina* seedling on the ruler and measuring from the base of the stem (above the roots) to the end of the stem (*Appendix 3c*). Each seedling's stem and leaf length were measured again, starting from the base of the stem above the roots to the tip of the highest leaf. This was to get more detailed measurements of the entire seedling.

Data analysis

The seedling growth measurements were analyzed using the Rcmdr package (Fox and Bouchet-Valat, 2018) in R Software Version 2.4-1 (R Core Team, 2017). Statistical tests, such as two-way ANOVA and one-way ANOVA, were used to test for significant differences in the data. A two-way analysis of variance was used to detect differences in: i) the mean number of days for root and leaf initiation between all three species and between freshwater and seawater treatments, as well as the interaction of species and water treatments; and ii) the mean root length and mean leaf length at week 12 (end of the experiment) between all three species in freshwater and seawater treatments, as well as the interaction of species and water treatments. A one-way ANOVA and a pairwise comparison test was used for differences between species. The data were then plotted to show the average length of the roots and leaf. The average numbers of roots and leaves were plotted along with the associated standard error. Average growth measurements from weeks 1–12 (the duration of the experiment) were also calculated.

Results

Root and leaf initiation

The first indication of growth was measured by observing the average number of days taken for root and leaf initiation of mangrove seedlings in freshwater and seawater. Similar rates of root initiation were observed in the two water treatments for all mangrove species (*Fig. 2*). *A. marina* took an average of seven days in both freshwater and seawater for the first root to initiate. For *R. mucronata*, the average root initiation time was ten and nine days in freshwater and seawater, respectively, while *C. tagal* seedlings took an average of eight days in freshwater and seven days in seawater for the first root to initiate. There was a significant difference in the mean number of days until first root initiation

between mangrove species, but not between water treatments, nor for the interaction between species and water treatments (*Table 1*).

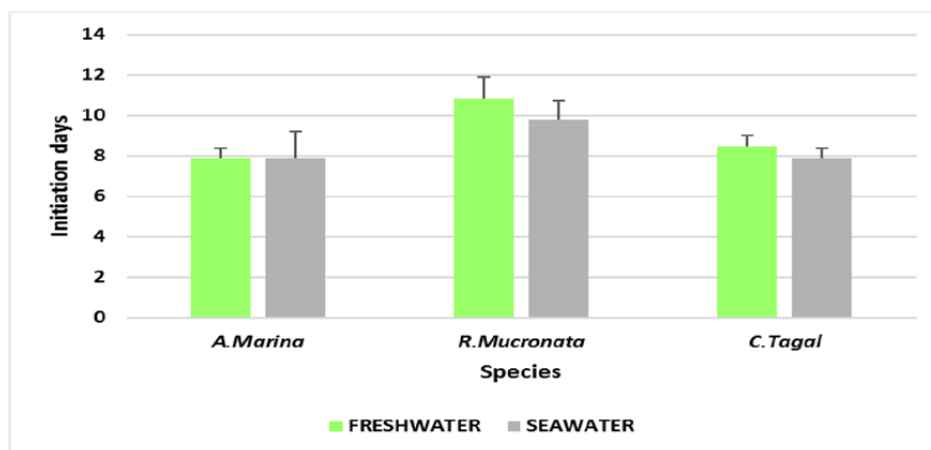


Figure 2. Average root initiation time (number of days) for the three mangrove species in fresh and seawater treatments

Table 1. Comparison of mean root initiation time (number of days) between species, water treatments, and their interaction. Results from multiple comparison tests between mangrove species are also shown

	Df	F-value	P-value
Species	2	4.5668	0.01471
Water	1	0.5697	0.45364
Species: Water	2	0.1691	0.84484
Species: Substrate	0		
<i>C. Tagal</i> – <i>A. Marina</i>			0.9336
<i>R. Mucronata</i> – <i>A. Marina</i>			0.0175
<i>R. Mucronata</i> – <i>C. Tagal</i>			0.0427

The time taken for root initiation amongst propagules of *A. marina* grown in freshwater showed slight variation, while the propagules in seawater had a more significant variation (see error bars in *Fig. 2*). The root initiation time was consistent across the two water treatments for both *C. tagal* and *R. mucronata*, as displayed by the small standard error bars in *Fig. 2*. There was, however, a significant difference in the mean root initiation days between seedlings of the three species ($p=0.015$; *Table 1*). According to the multiple comparisons test, this significant difference occurs between *R. mucronata* and *A. marina* ($p=0.018$) and between *R. mucronata* and *C. tagal* ($p=0.043$), but there is no real difference in root initiation period between *A. marina* and *C. tagal* ($p=0.935$).

The number of days until first leaf initiation was the second measurement recorded to monitor growth (*Fig. 3*). *A. marina* seedlings spent an average of 17 days in freshwater and 15 days in seawater before the first leaf germinated. *C. tagal* propagules took an average of 24 days in freshwater and 22 days in seawater, while *R. mucronata* individuals took on average 48 days in freshwater and 52 days in seawater for the first leaf to germinate. There was a significant difference in mean leaf initiation days between mangrove species and the interaction of species and water treatments (*Table 2*).

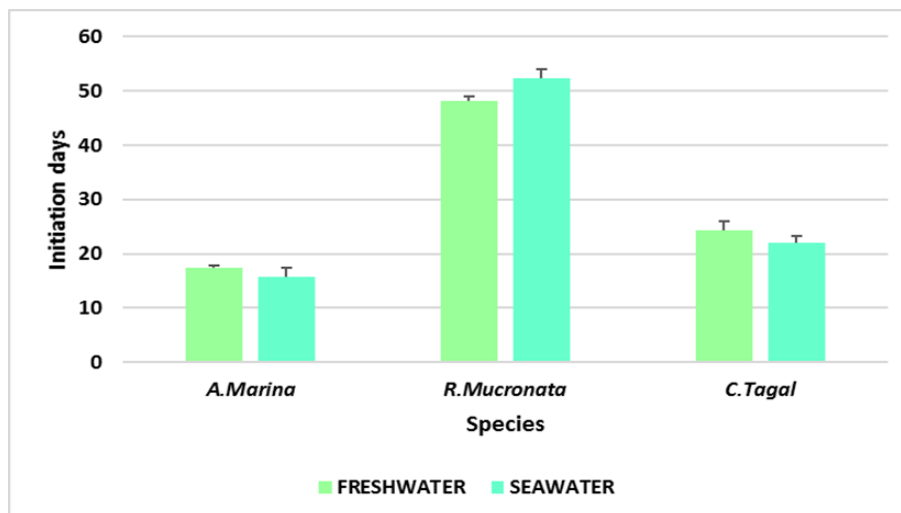


Figure 3. Average leaf initiation time (number of days) for the three mangrove species in fresh and seawater treatments

Table 2. Comparison of mean leaf initiation time (number of days) between species, water treatments, and their interaction. Results from multiple comparison tests between mangrove species are also shown

	Df	F-value	P-value
Species	2	352.119	<2e-16
Water	1	0.0037	0.95183
Species: Water	2	3.3884	0.04109
Species: Substrate	0		
<i>C. Tagal</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>C. Tagal</i>			<0.0001

Of the three mangrove species, *R. mucronata* seedlings took the highest number of days for the first leaf to unfurl, with leaf initiation taking longer in seawater than in freshwater. On average, the leaves of *A. marina* individuals unfurled the fastest. Given that the ANOVA test does not provide information on where those differences lie, a Tukey Honest Significant Difference test was performed to determine which specific species' means differed. The multiple comparisons test showed that the mean number of days for leaf initiation was significantly different between all three species ($p < 0.0001$), and its results are presented in Table 2. The interaction of species and water treatment also had a significant F-value ($F = 3.388$, $p = 0.041$; Table 2), meaning the water treatment affected leaf initiation time for at least one species.

Root count and length

Root count

Appendix 4 displays the average number of roots counted for all species in freshwater and seawater treatments during the first six weeks of the observation before the roots became entangled. Weekly root counts of the different species indicated a sharp increase in week two, which stayed relatively high for all three species in both treatments until

week six. *A. marina* had a gradual increase in root number in both treatments from week one to week six and had similar root counts for the two water treatments in weeks two and five. *C. tagal* propagules, on the other hand, showed an increase in root count in the freshwater treatment from week four, with a limited increase in root count in the seawater treatment over the same period. *R. mucronata* had minimal numbers of roots during the first two weeks and experienced a rapid increase in root count towards the last four weeks of the experiment.

Focusing on week six (Fig. 4), there is a clear difference in root count between *R. mucronata* and the other two species. The average number of roots for *A. marina* seedlings was nine and eight in freshwater and seawater treatments, respectively. *C. tagal* had an average of eight roots in the freshwater treatment and seven roots in the seawater treatment. *R. mucronata*, meanwhile, had an average of 15 roots in the freshwater treatment and 16 in the seawater treatment – representing the more significant number of roots in both treatments for the entire experiment. There was a significant difference in the number of roots between mangrove species, but not between water treatments nor the interaction of species and water treatment (Table 3). A significant difference in the number of roots amongst mangrove species was observed between *R. mucronata* and the two other mangrove species ($p < 0.0001$, as seen in Table 3). However, no significant difference was found between the number of roots of *A. marina* and *C. tagal* ($p = 0.849$; Table 3).

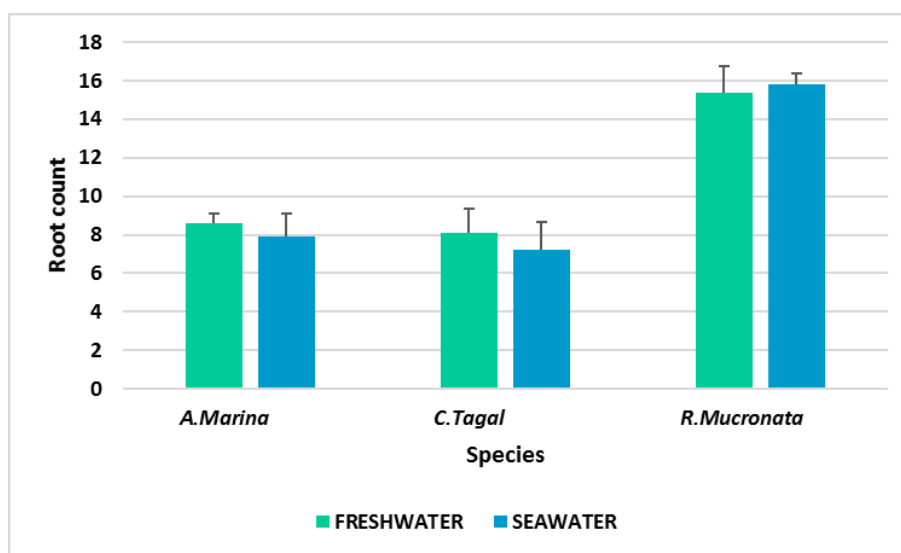


Figure 4. Root count (+SE) for each of the three mangrove species in fresh and seawater treatments for six weeks

Root length

A. marina had the faster growth in root length during the first week of measurements corresponding to 2.2 cm and 1.2 cm in freshwater and seawater treatments, respectively. The root length stood at 13.2 cm in the freshwater treatment and 13.4 cm in the seawater treatment at the end of six weeks (Appendix 5). For *C. tagal* and *R. mucronata*, maximum root lengths of 4.2 cm and 7.8 cm were recorded, respectively. Therefore, *A. marina* had the highest root length growth for freshwater and seawater treatments at the end of week six of the experiment. It was not until week four that variations in growth rate between

water treatments were observed in the case of *C. tagal*. For *R. mucronata*, the root growth in seawater showed a slight increase compared to those in the freshwater treatment between weeks 3 to 6 of the experiment. However, *A. marina* demonstrated a different pattern of root growth with relatively higher rates in freshwater treatments during weeks 1, 2, 3, and 5 of the experiment, while root growth in seawater treatments exceeded those in freshwater by <2 cm during weeks 4 and 6, respectively (*Appendix 5*).

Table 3. Summary statistics of ANOVA comparing differences in mean root count between species, water treatment, and their interaction. Results from a multiple comparisons test between mangrove species are also shown

	Df	F-value	P-value
Species	2	30.9525	1.106e-09
Water	1	0.1895	0.665
Species: Water	2	0.1935	0.8247
Species: Substrate	0		
<i>C. Tagal</i> – <i>A. Marina</i>			0.849
<i>R. Mucronata</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>C. Tagal</i>			<0.0001

A two-way ANOVA showed a significant difference in mean root length between mangrove species, but not between water treatments nor the interaction of species with water treatment (*Table 4*). The average root length at week six was 9.4 cm in freshwater and 8.6 cm in seawater for *A. marina*. *C. tagal* had an average root length of 1.6 cm in the freshwater treatment and 1.8 cm in the seawater treatment. In comparison, *R. mucronata* had an average root length of 3.2 cm and 3.6 cm in the freshwater and seawater treatments, respectively (*Fig. 5*). All three species differed significantly from each other in their mean root length at week six (*Table 4*).

Table 4. Summary statistics of ANOVA comparing differences in mean root length between species, water treatment, and their interaction. Results from a multiple comparisons test between mangrove species are also shown

	Df	F-value	P-value
Species	2	135.804	<2e-16
Water	1	0.0093	0.9234
Species: Water	2	0.9503	0.3876
Species: Substrate	0		
<i>C. Tagal</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>C. Tagal</i>			0.000581

Leaf count and length

Leaf count

Other indications of growth used were the leaf count and leaf length. Leaves were counted and measured over 12 weeks, as opposed to the roots only observed over the first six weeks because of root entanglement after that. Leaf count did vary throughout the observation for the different species in the two water treatments (*Appendix 6*). For

example, *A. marina* had a much higher number of leaves in the freshwater treatment throughout the experiment, which is evident in the steep slopes of the graph, compared to the seawater treatment, where there was initially a rapid increase, followed by a plateau from weeks seven through 12 (Appendix 6a). *C. tagal* showed a gradual increase in the number of leaves, with a rapid increase during weeks 11 and 12 (Appendix 6b). *R. mucronata* had few leaves at the start of week six but displayed a rapid increase in the number of leaves in week seven, particularly in the freshwater treatment, followed by a plateau until a gradual increase in weeks 11 and 12 (Appendix 6c).

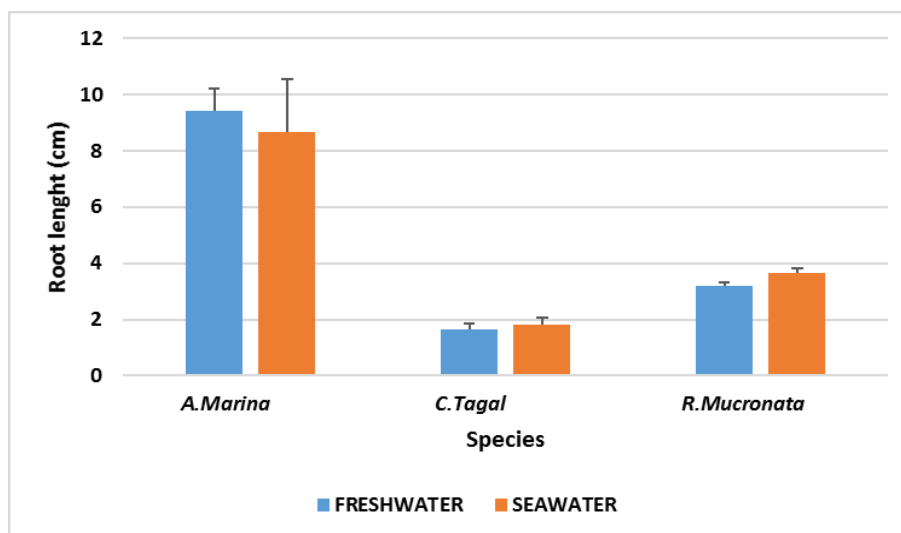


Figure 5. Average root length for *A. marina*, *C. tagal*, and *R. mucronata* during the six weeks of observation

Additional statistical results on leaf counts in week 12 showed that there was a significant difference in the mean number of leaves between mangrove species, between water treatments alone, and between the interactions of species with water treatment (Table 5). *A. marina* had an average of eight leaves in the freshwater treatment and six leaves in the seawater treatment, whereas *R. mucronata* and *C. tagal* had three leaves in the freshwater treatment and three leaves in the seawater treatment, thus having no significant difference in the number of leaves between water treatments or species (Fig. 6). Leaf count was significantly different between *A. marina* and *C. tagal* ($p < 0.0001$), and between *A. marina* and *R. mucronata* ($p < 0.0001$), but not between *C. tagal* and *R. mucronata* ($p = 0.751$). These results are presented in Table 5.

Table 5. Summary statistics of ANOVA comparing differences in mean root length between species, water treatment, and their interaction. Results from a multiple comparisons test between mangrove species are also shown

	Df	F-value	P-value
Species	2	125.8046	<2.2e-16
Water	1	5.6685	0.020899
Species: Water	2	7.1543	0.001776
Species: Substrate	0		
<i>C. Tagal</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>C. Tagal</i>			0.751

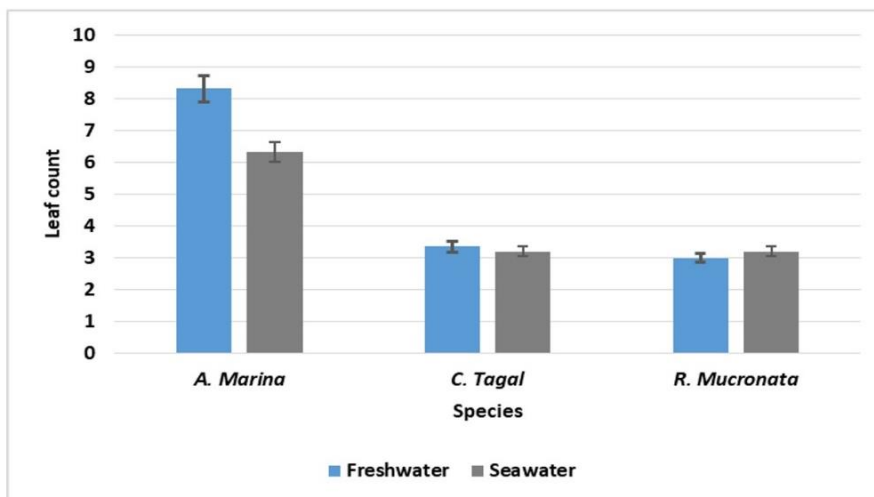


Figure 6. Average leaf count (+SE) for each of the three mangrove species in fresh and seawater treatment for six weeks

Leaf length

The *A. marina* species showed an initial rapid and gradual increase in leaf length in both water treatments throughout the weeks of observation, with much longer leaf length in the freshwater treatment (Appendix 7a). For *R. mucronata*, the first leaf was measured in week six with an average of 0.6 cm in the freshwater treatment and 1.2 cm in the seawater treatment (Appendix 7b). A sudden increase in leaf length began during week five until week twelve for *A. marina* in the freshwater treatment. This increase resulted in a maximum leaf length of 12 cm recorded during the last three weeks of the experiment compared to *A. marina* in the seawater treatment, which had a full leaf length of 9 cm. *C. tagal* had consistent leaf growth as of week four with no abrupt changes throughout the weeks of observation (Appendix 7c).

During week 12, *A. marina* had an average leaf length of 11.8 cm in freshwater and 10.4 cm in seawater. *C. tagal* had an average length of 7.23 cm in freshwater and 7.14 cm in seawater, and *R. mucronata* 10 cm in freshwater and 9.53 cm in seawater (Fig. 7). The statistical analysis revealed a significant difference in leaf length between the three mangrove species and between the two water treatments, but not between the interactions of the species with water treatment (Table 6).

Table 6. Summary statistics of ANOVA comparing differences in mean leaf length between species, water treatment, and their interaction. Results from a multiple comparisons test between mangrove species are also shown

	Df	F-value	P-value
Species	2	90.9111	<2.2e-16
Water	1	7.6859	0.007663
Species: Water	2	2.6694	0.078601
Species: Substrate	0		
<i>C. Tagal</i> – <i>A. Marina</i>			<0.0001
<i>R. Mucronata</i> – <i>A. Marina</i>			0.00022
<i>R. Mucronata</i> – <i>C. Tagal</i>			<0.0001

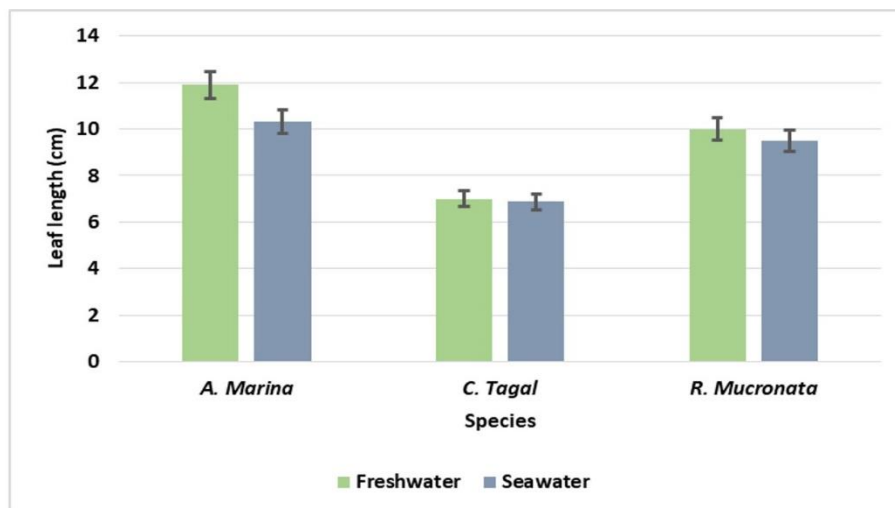


Figure 7. Average leaf length for *A. marina*, *C. tagal*, and *R. mucronata* in fresh and seawater during the six weeks of observation

Discussion

Effect of fresh and seawater during propagation of mangrove species

Our results show significant variation in growth rates between species and between the water treatments for specific growth metrics. Regarding root initiation days, the significant difference amongst species is unsurprising as the species differ in physical structure and genetic makeup. Given that there was no significant difference in root counts and root lengths between species in freshwater and seawater treatments, it is evident that water treatment did not influence the amount of days species took for root initiation. This implies that water treatment did not affect the root growth rate of the mangrove seedlings. Since propagules were planted in individual pots, the chance of seedlings of one species affecting the other species was reduced. Due to this controlled environment, the interaction amongst species in the experiment cannot be compared with mangroves in the wild.

Data collection on root growth was halted during week six of the experiment because, at this point, the roots became entangled. Extracting continuous measurements resulted in the breakage of some of the roots, which could have affected the propagules' growth rate in both treatments. The mean root initiation days between *R. mucronata* and *A. marina*, and between *R. mucronata* and *C. tagal* did not differ when compared, while mean root initiation days did differ between *A. marina* and *C. tagal*. Regarding root count, *R. mucronata* recorded the highest number in freshwater and seawater treatments, while *A. marina* dominated regarding root length in both treatment groups. Thus, freshwater and seawater influence the growth rate of mangrove species, but it depends entirely on the type of mangrove species – a view supported by Ye et al. (2005).

Growth response of mangrove species in fresh and seawater treatments

There were apparent differences in *A. marina* species compared to the other two species in freshwater and seawater treatments. *A. marina* is the mangrove species that grows much better in freshwater in nurseries. This can be due to *A. marina* being a pioneer species with the ability to cope and adapt to changes in its environment and develop more

rapidly in harsh conditions (Osborne and Berjak, 1997; Nguyen et al., 2017) compared to the other two mangrove species. This is, however, an exciting result as *A. marina* demonstrated to be the species more adaptable to relatively more saline conditions – a view supported by Li et al. (2016). They observed that these mangrove species are widely distributed along complex salinity and aridity gradients. Li et al. (2016) further indicated that even under unfavorable conditions such as fluctuation in salinity, *A. marina* could exploit the situation and grow faster than other species. *A. marina* individuals revealed a pattern of rapid growth rate in the freshwater treatment where they seemed to reach their full growth potential, with lower growth rates in seawater treatments.

Suitability of fresh and seawater for irrigation of mangrove nurseries

Our results showed that freshwater, as opposed to seawater, is more suitable for irrigating mangrove seedlings in nurseries for specific species. This was the case with *A. marina*, for which compelling evidence from the experiment showed that it grew much better in the freshwater treatments. These findings can be beneficial in the design of terrestrial nurseries, as freshwater irrigation requires less time and manual labor than seawater irrigation. Rain harvesting systems can be set up for irrigation purposes instead of constructing nurseries in a mangrove area and pumping seawater for irrigation. There are no other documented studies conducted in Seychelles to compare the findings from this experiment. An earlier study, however, used different salinity gradients and analyzed the effects of salinity on mangrove species (Ye et al., 2005). This study can be further amplified on a larger scale, as the sample size was relatively small because of time constraints.

Our findings show that mangrove seedlings have a slower growth rate in seawater than in freshwater, which is especially evident in *A. marina* species. Nevertheless, given that species were constantly being removed and re-planted each time measurements were recorded, the growth rate of the seedlings in this experiment might have been influenced by the methods followed. From observations made during the investigation, additional seedlings that were potted at the same time in polystyrene boxes without disturbance had grown considerably in length, and this was noted through visual observation.

Other factors influencing the growth and survival rate of mangroves

Throughout the experiment, only one mortality was recorded: an *A. marina* seedling in seawater. The cause of the mortality could not be attributed to any specific factor, but likely, the seedling may not have been in a healthy state before the experiment. Therefore, the timing of collecting the seedlings and propagules and their health influence the survival rate of mangrove seedlings in nurseries. Seed predation by insects affects the viability of mangrove seedlings, with a lot of uncertainty on survivorship when such seeds are sowed in nurseries (Kathiresan and Bingham, 2001). The chances of seeds affected by insect predation surviving are relatively low. Additionally, small animals such as snails are likely to affect the successful establishment of mangrove propagules. Tiny snails were seen in the substrate feeding on the pericarp, another possible cause of death that cannot be disregarded. Other factors influencing seedling growth may include exposure to sunlight, irrigation frequency, water availability, and unidentified debris in the mud, such as plastics. These can all harm the growth and survivorship of mangrove seedlings in nurseries under experimental conditions. If the experiment were to be conducted in a nursery outside a laboratory, additional factors such as pests, diseases, and physical damage might negatively affect growth and survival rates.

Limitations and recommendations

This study has a number of limitations that needs to be highlighted to ensure improvement for future studies. Removing the seedlings from the substrate for root measurement could influence the overall growth of the seedlings. Although marked differences were not observed for the root length of the undisturbed seedlings at the end of the observation period, this could be a problem for experiments over longer timeframe for at least six months. Other challenges were also encountered during this experiment and the following recommendations have the potential to improve best practices for mangrove seedling growth. In repeating this experiment, it is suggested to have a more long-term monitoring process of the growth rate of different species irrigated by freshwater or seawater. Using the entire seedling growth parameter will likely provide a better understanding of survival rates, as it depicts a more precise visualization of species growth patterns compared to root and leaf length. In addition, more species should be included to gain information in a broader pool of species. In terms of measurements, roots should be measured when they are at shorter lengths in the earliest stage possible, and the removal of seedlings from pots when measuring roots should be revised to ensure that roots are not damaged. The methodology used for root measurements should also be improved to accommodate root measurements even when entangled. A good example will be to use softwares such as WinRHIZO or RootSnap that are very precise for detecting the total root length and other parameters of the roots. Potting seedlings in much larger containers will provide more allowable space for much better observation of interactions between species, a better understanding of the species' behavior in the wild, and whether there are competing factors such as space, nutrients, and water.

Conclusions

Some of the findings in this study relate to root and leaf initiation, root and leaf counts, and the growth rates of roots and leaves of three different mangrove species in freshwater and seawater treatments. Regarding root initiation, *A. marina* recorded the least number of days in freshwater and seawater for the first root to initiate, followed by *C. tagal* and then *R. mucronata*. This implies differences in root initiation rate among the three mangrove species. Concerning leaf initiation, the pattern was consistent with root initiation, with *R. mucronata* individuals requiring more than double the number of days *A. marina* and *C. tagal* individuals needed for their first leaves to germinate. Regarding root count, *A. marina* mangroves recorded an increase in root count for freshwater and seawater treatments from week one to week six. However, the number of roots was slightly more remarkable in the freshwater treatment, except for week five, where both treatment groups had the exact root count. *C. tagal* propagules, on the other hand, showed an increase in root count in the freshwater treatment from week four, with a limited increase in root count in the seawater treatment. *R. mucronata* mangroves had minimal numbers of roots during the first few weeks and experienced a rapid increase in root count towards the last three weeks of the experiment.

A. marina recorded the highest root length growth for freshwater and seawater treatments at the end of week six of the experiment. This same species had a much higher number of leaves in the freshwater treatment. In contrast, *R. mucronata*, which had few leaves at the start of week six, experienced a rapid increase in the number of leaves in week seven in the freshwater treatment, followed by a plateau and a gradual increase in week 11. The *A. marina* species showed a rapid, then a gradual, increase in leaf length in

both treatments throughout the observation, with much longer leaf length in the freshwater treatment. For *R. mucronata*, a sudden increase in leaf length occurred in week eight for species in the freshwater treatment. *C. tagal* did not record any leaf growth until week four; the growth rate increased from 1 cm to 7 cm between weeks four and twelve. Important to note is that *A. marina* propagules showed much better growth rates in the freshwater treatments. These findings can be beneficial in designing terrestrial nurseries for mangrove restoration programs, as freshwater irrigation requires less time and manual labor than seawater irrigation.

Mangroves provide multiple benefits in terms of ecosystem goods and services, primarily for tropical coastal countries and Small Island Developing States (SIDS). They provide breeding, feeding, and nursery grounds for many estuarine and marine organisms, including commercial fish and crustaceans. Thus, their importance in sustaining the local abundance of fish and shellfish populations is described as follows: “Mangroves are like kindergarten, seagrass is the secondary schools, and coral reefs are the high schools and colleges for fishes! And once the fishes graduate from university, they return to kindergarten to spawn.” (Khun Pisit, cofounder of Thailand’s Yad Fon mangrove preservation project). Such a statement underscores the importance of mangroves and the need for their rehabilitation, especially in the Seychelles, where over 70% of the original mangrove forests have been degraded (Henriette, 2016). In this regard, this study provides information on the propagation of mangrove seedlings in nurseries that can be useful to many stakeholders in Seychelles for creating nurseries that will ensure a higher success rate for mangrove restoration projects and interventions. The experiment proved that mangrove seedlings can be irrigated with fresh water and that some species like *A. marina* are less constrained by freshwater; hence, the same species grow faster even in seawater.

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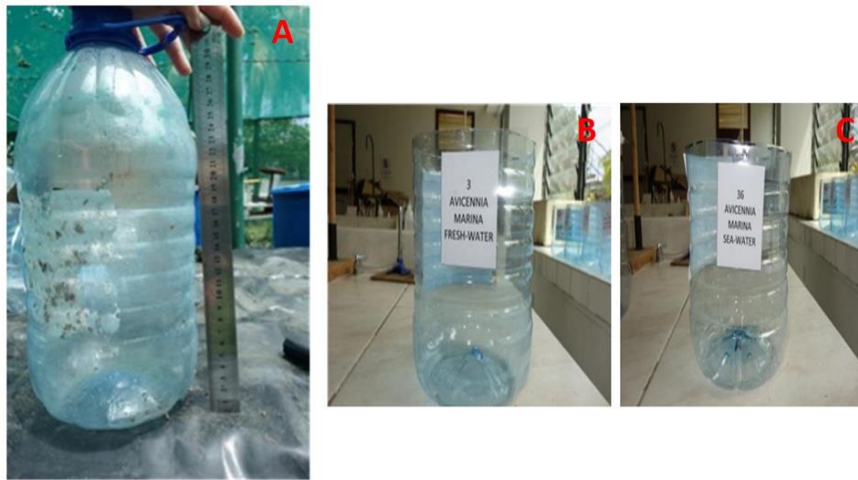
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APPENDIX



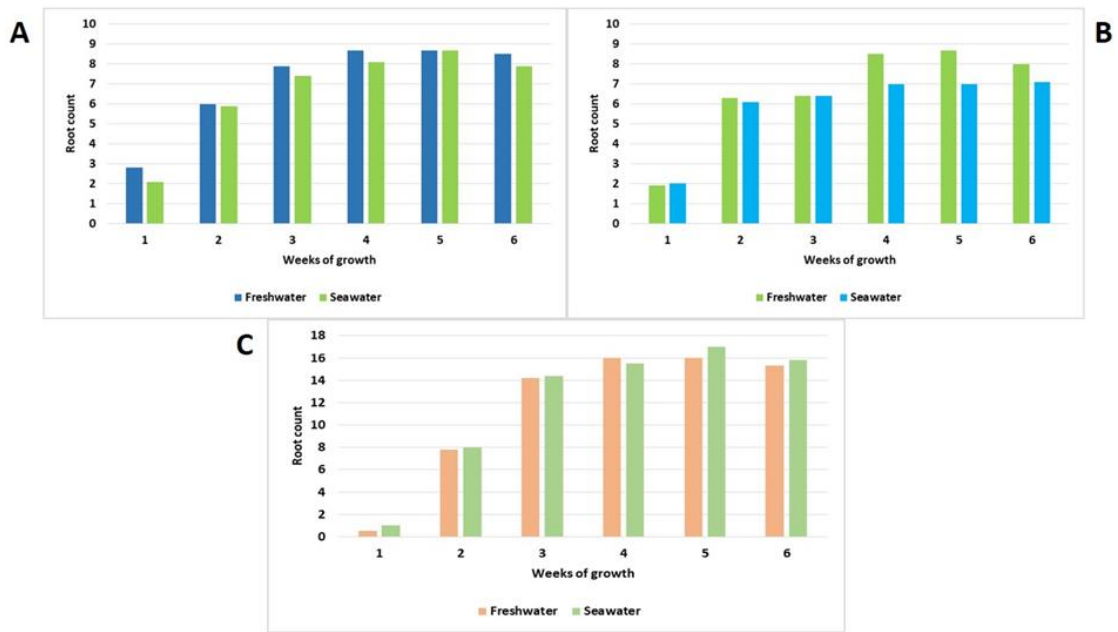
Appendix 1. A five-liter container cut at 19 cm (a) to be filled with freshwater (b) and seawater (c)



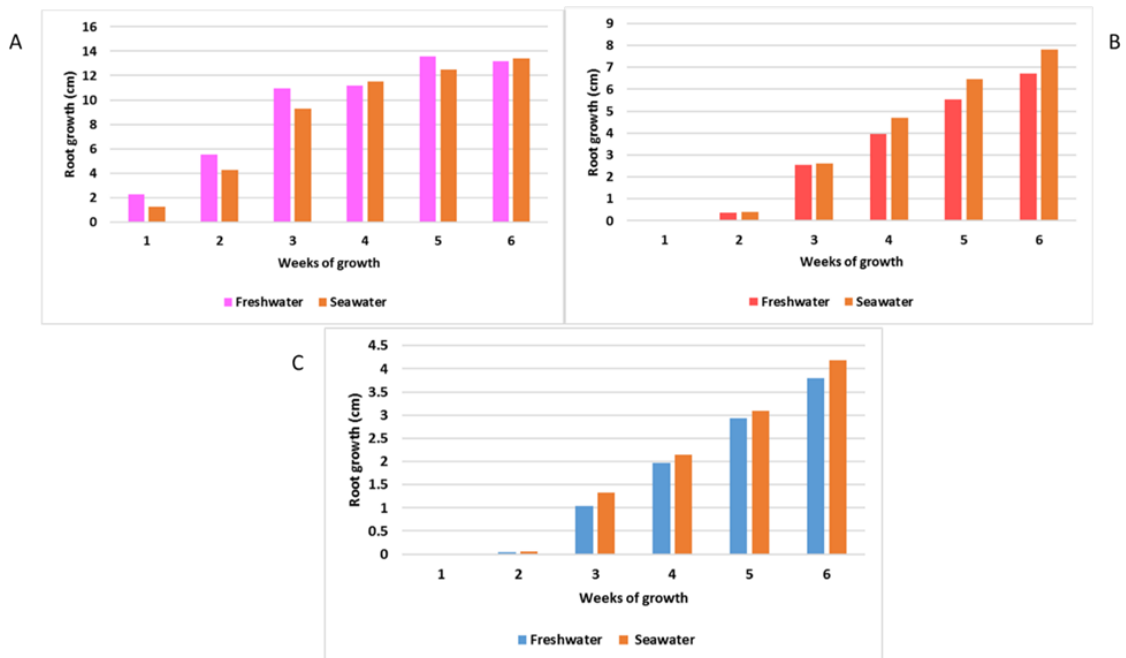
Appendix 2. Seedlings of three mangrove species collected for the experiment – *Avicennia marina* (a), *Rhizophora mucronata* (b) and *Ceriops tagal* (c)



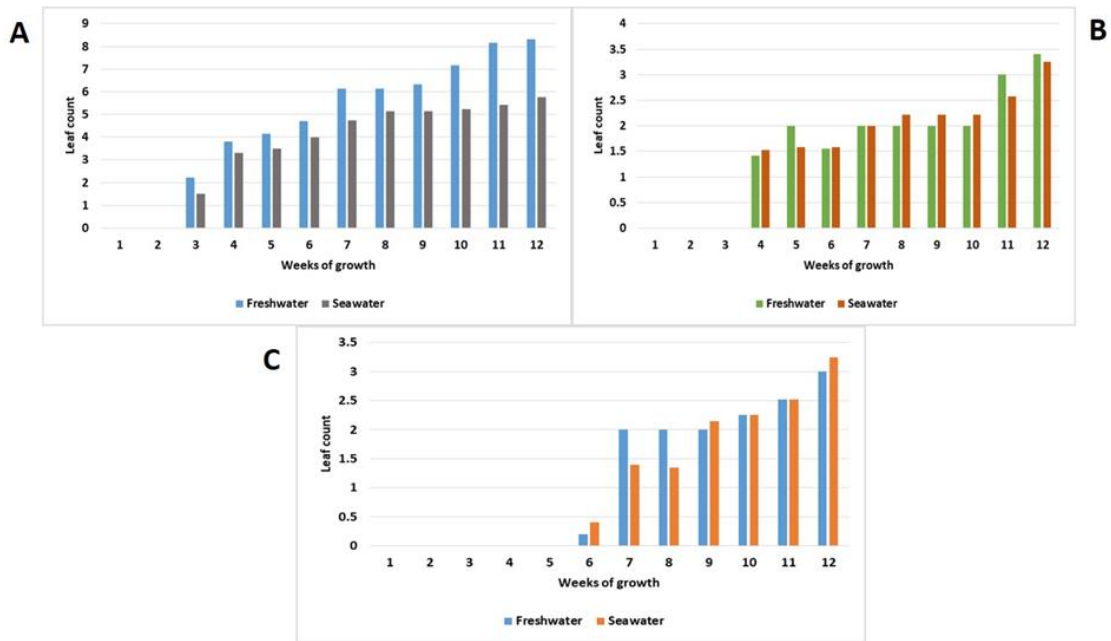
Appendix 3. Measuring the root (a), leaf (b) and stem (c) of *Avicennia marina* propagules



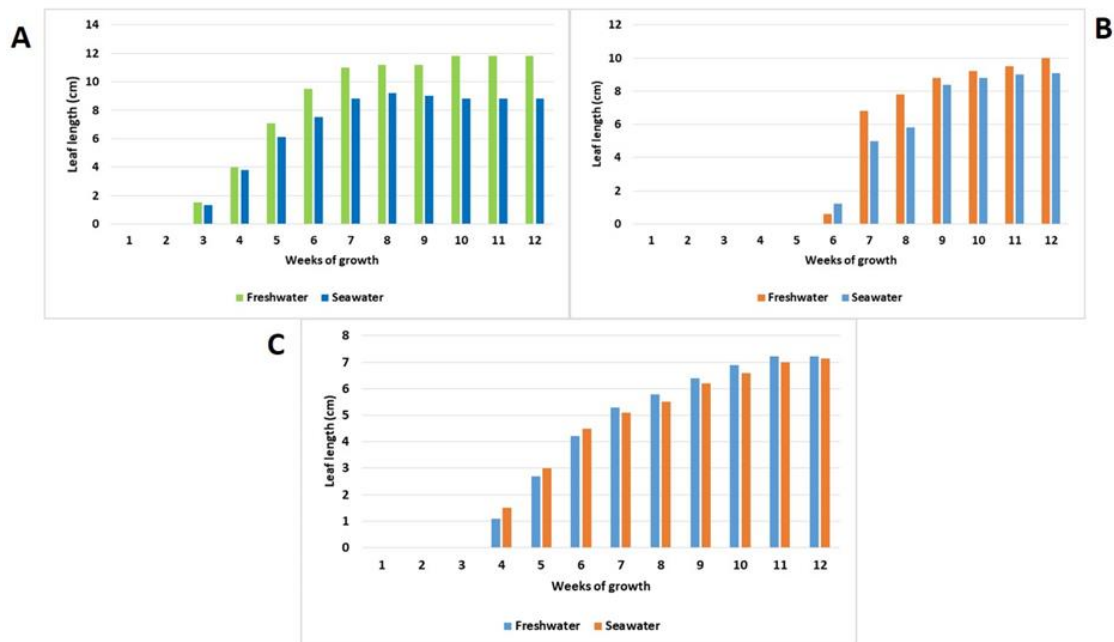
Appendix 4. Average number of roots for *A. marina* (a), *C. tagal* (b) and *R. mucronata* (c) during the six weeks of observation



Appendix 5. Average root growth for *A. marina* (a), *C. tagal* (b) and *R. mucronata* (c) during the six weeks of observation



Appendix 6. Average leaf count for *A. marina* (a), *C. tagal* (b) and *R. mucronata* (c) during the six weeks of observation



Appendix 7. Average leaf length for *A. marina* (a), *R. mucronata* (b) and *C. tagal* (c) during the 12 weeks of observation