

## Viviparous mangrove propagules of *Ceriops tagal* and *Rhizophora mucronata*, where both Rhizophoraceae show different dispersal and establishment strategies



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### ABSTRACT

For plants to reproduce successfully, it is crucial that their seeds be adapted to survive the environmental conditions in which they disperse and establish. The buoyant dispersal units (propagules) of viviparous mangrove species seem perfect adaptations for dispersal and establishment within the mangrove environment. However, much remains unknown about the structural changes mangrove propagules undergo between abscission from the parent tree and establishment. Mature propagules of two viviparous mangrove species (*Rhizophora mucronata* and *Ceriops tagal*) were submitted to experimental conditions in order to test: (i) how substrate influences propagule mass and volume during dispersal; (ii) if stranding of propagules on solid soil triggers root development and thus establishment. Our results showed that dehydration stimulates root formation and propagule establishment and that the establishment phase is faster at lower rather than higher salinity. Furthermore, it was found that the larger propagules of *R. mucronata* were less vulnerable to dehydration than those of *C. tagal*, that their root growth started later and that, once initiated, their roots grew faster. This indicated that *Rhizophora* propagules are better suited for long distance dispersal than those of *Ceriops* and that *Rhizophora* has an advantage for establishment in the lower part of the intertidal zone, where inundation is more frequent and propagules need to anchor more rapidly. This study therefore points out that two co-living species of the same family have different dispersal and establishment strategies, thereby contributing to the understanding of their local and global distribution and of the species-specific dynamics within mangrove forests.

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### 1. Introduction

Reproductive success in plants is highly dependent on the survival of their seeds during dispersal. The conditions in which seeds reside between leaving the parent tree and establishment, and the adaptations of seeds for surviving these conditions are crucial. In the various and phylogenetically distant mangrove tree genera (Spalding et al., 2010; Stevens, 2001), vivipary is common (Spalding et al., 2010; Tomlinson, 1994) and has been found to have multiple evolutionary origins (Shi et al., 2005), suggesting it

to be an adaptive trait to the mangrove habitat. In littoral plant populations, where conditions are frequently unfavourable for germination, vivipary is advantageous as it avoids germination in a saline environment (Joshi et al., 1972). Having germinated before abscission, the hydrochorous mangrove propagules (i.e., dispersal units, Fig. 1) leave the parent tree as seedlings that can either plant directly into the substrate or disperse to nearby locations and thereby replenish the forest. In some cases, these structures may disperse over long distances and if still viable, can colonize remote suitable areas (Dodd et al., 2002).

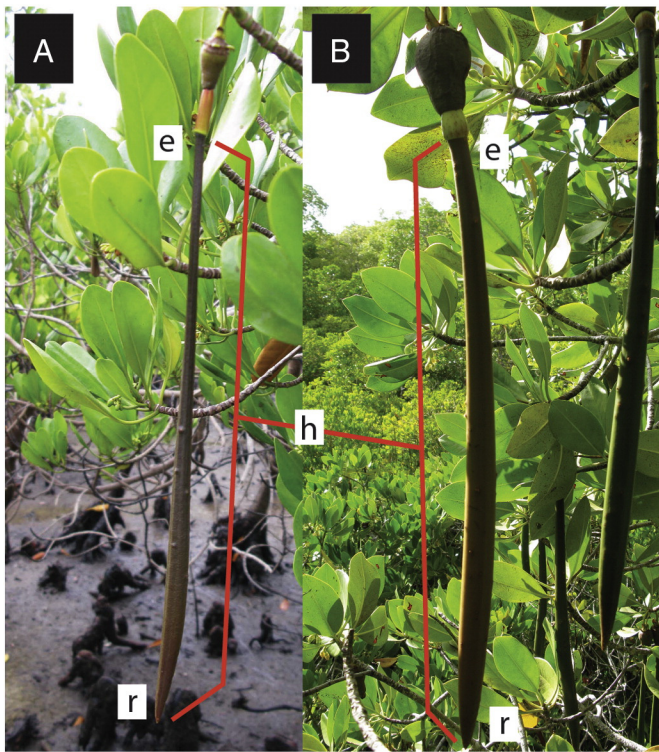
During dispersal, the buoyancy of mangrove propagules can decrease (Allen and Krauss, 2006; Drexler, 2001), whilst the floating orientation can move between being horizontal and vertical (Clarke and Kerrigan, 2002; Rabinowitz, 1978), which indicates changes in propagule density over this life stage. The loss of buoyancy can be advantageous for stranded or establishing propagules, as it may decrease the chance of being flushed away again by the tides. The exact physiological and morphological changes that mangrove propagules undergo during

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**Fig. 1.** Pictures of a mature *Ceriops tagal* (A,  $\pm 25$  cm long) and *Rhizophora mucronata* (B,  $\pm 45$  cm long) propagule showing (e) the epicotyl side, (h) the hypocotyl and (r) the radicle side of a propagule. Pictures: Dennis J.R. De Ryck.

dispersal and in particular how these changes are influenced by environmental conditions remains unclear. Abscised propagules that are taken away by the tides survive in saline water, whilst stranded propagules are subjected to conditions that are often dynamic and/or harsh. Propagules can, for example, not only wash ashore on a muddy substrate at the seaward side of the mangrove forest, but also strand in the landward side of the forest where salinity in the sandy mangrove soils can reach over 100 (Robert et al., 2009) due to evaporation and low fresh water or seawater input. It is sensible to assume that these conditions and the period that propagules are subjected to these conditions can affect their physiology and morphology, and hence their buoyancy and chances for successful establishment during available windows of opportunity, i.e., periods during which hydrodynamic and wind forcing are limited (Balke et al., 2011). As such, the period of exposure to these environmental stressors might determine their potential for dispersal, as well as the species distribution in the mangrove forest.

In the highly dynamic environment of a mangrove ecosystem, it is important for successful establishment that a propagule is able to anchor itself in the sediment rapidly during a period with limited hydrodynamic and wind forcing, which during neap tide in the lower intertidal sometimes just last only a few days (Balke et al., 2011). Herein, root development is crucial, both in terms of its extent and its initiation timing. An establishing seedling has to withstand wave action, water currents and other disturbing influences that might thwart further development. Furthermore, roots are needed for taking up water and the nutrients necessary for the development of leaves and length growth. It has already been shown that *Ceriops tagal* has faster root initiation than *Rhizophora mucronata* upon establishment in the Kenyan mangrove forest, whilst the speed of root growth thereafter is higher in the latter (De Ryck et al., 2012). Furthermore, Smith et al. (1996) showed that root growth in *Rhizophora mangle* is dependent on salinity and light conditions at the moment of establishment. However, the way in which these processes are influenced by the life history of the propagules and the

type of substrate they establish on has not yet been tested for viviparous mangroves.

In this paper, the viviparous mangrove propagules of *C. tagal* (Perr.) C.B. Robinson and *R. mucronata* Lamk. were studied during the period between abscission from the parent tree and establishment. Our first hypothesis states that during the period between abscission and establishment, the substrate influences change in propagule mass and volume, and this occurs in a species specific way: the large volume and high water reserves of *R. mucronata* propagules results in a long floating and viability period; this is in contrast to short floating and viability period of the small *C. tagal* propagules (Fig. 2). Secondly, it is hypothesized that the trigger for root formation and thus propagule establishment, is linked to the stranding of propagules on solid ground, whether moist mud or dry sand (Fig. 2). It is expected that propagules floating in seawater have delayed root development compared to propagules stranded on solid ground. To test these hypotheses, morphological changes and the root initiation of propagules were studied under different experimental conditions – in seawater, on moist mud and on dry sand – each simulating the potential natural conditions for an abscised propagule (Fig. 3). Thereafter, propagules were put in a vertical position in a hydroponic setup with different salt concentrations and atmospheric relative humidity (Fig. 3) in order to test: (i) the effect on root formation according to the substrate a propagule has lied on between abscission and establishment; and (ii) the effect of rainfall on root growth during propagule establishment.

## 2. Materials and methods

### 2.1. Study sites and species

This study was conducted in Gazi, a village at an open estuary located about 50 km south of Mombasa, Kenya (Matthijs et al., 1999). Gazi Bay is surrounded by a mangrove forest of approximately 600 ha (UNEP, 1998), where three species have a prominent share: *Avicennia marina*, *C. tagal* and *R. mucronata*. In this paper, *C. tagal* and *R. mucronata* (Rhizophoraceae), tree species that reproduce via oblong viviparous propagules (Fig. 1), were studied. *C. tagal* trees grow in two elevation zones in the bay, differing in terms of soil variables, with most *C. tagal* trees growing higher up in the intertidal zone (Dahdouh-Guebas et al., 2004; Matthijs et al., 1999), whilst most *R. mucronata* trees grow close to the water's edge, in the lower elevation zone of the forest (Dahdouh-Guebas et al., 2004; Gallin et al., 1989). For all experiments and for both species, propagules were collected from at least two sites differing in terms of inundation frequency (*C. tagal* – seaward site: 4° 25' 15.58" S, 39° 30' 44.59" E; landward sites: 4° 25' 5.51" S, 39° 30' 26.37" E and 4° 25' 27.57" S, 39° 30' 44.54" E; *R. mucronata* – seaward sites: 4° 25' 15.58" S, 39° 30' 44.59" E and 4° 25' 33.87" S, 39° 30' 43.78" E; landward site: 4° 25' 27.57" S, 39° 30' 44.54" E). Sites with low or high inundation frequency are hereafter referred to as the seaward or landward side of the forest, respectively. Due to the sloping topography and high tidal amplitude (approximately 4 m) at Gazi Bay, the difference in inundation frequency between the landward and the seaward side of the mangrove forest is high (Matthijs et al., 1999; Robert et al., 2009). The inundation frequency ranges from only once per month at the most landward side of the forest to twice per day at the seaward side (Robert et al., 2009). There is evidence that landward and seaward populations within the mangrove forest of Gazi Bay may show slight genetic divergence (Dahdouh-Guebas et al., 2004).

### 2.2. Propagule sampling method

For each species, 138 propagules were collected, 69 from the landward side of the forest and 69 from the seaward side (Supplementary Fig. S1). To ensure that the studied propagules were mature, propagules that were released upon lightly shaking trees were collected. Where trees were too rigid to shake, propagules were gently pulled at and

| Hypotheses   | Results - observed patterns  | Results - differences between species  | Conclusion   |
|--|--|--|--|
| <p><b>H1:</b> Substrate influences changes in propagule mass and volume during the period between abscission and establishment in a species specific way. (<i>Rhizophora</i>: high water reserves &gt; long floating and viability period &lt;&gt; <i>Ceriops</i>)</p> | <p>&gt; The mass and volume of propagules is decreasing fastest on a dry sand substrate, second on moist mud and slowest in seawater.</p> <p>&gt; The density of propagules in a horizontal position is increasing with time.</p>                        | <p>&gt; The mass, volume and density changes over time are larger in <i>Ceriops</i> than in <i>Rhizophora</i> propagules</p>   | <p><b>H1</b> - Confirmed<br/><b>H2</b> - Confirmed</p> <p>&gt; Dehydration stimulates root formation and establishment of mangrove propagules.</p> <p>&gt; The establishment phase of mangrove propagules is faster in a less saline environment than in more saline conditions.</p> <p>&gt; For successful regeneration, the establishment of <i>Ceriops</i> propagules needs to happen faster than the one of <i>Rhizophora</i> propagules. This influences the species distribution within the mangrove forest.</p> |
| <p><b>H2:</b> The trigger for activation of root development is linked to the stranding of propagules on solid ground, whether it is moist mud or dry sand.</p>  | <p>&gt; Root formation is initiated when propagules are stranded and delayed when propagules stay in seawater.</p> <p>&gt; Propagules growing in water with low salinity show longer roots as compared to propagules growing in seawater of 34-35 ‰.</p> | <p>&gt; Root formation in floating propagules of <i>Ceriops</i> is faster than in <i>Rhizophora</i>.</p> <p>&gt; After 24 days in the hydroponic setup, the length of newly formed roots shows a maximum in <i>Ceriops</i> propagules that stayed for 12 days in an horizontal position.</p> |  |

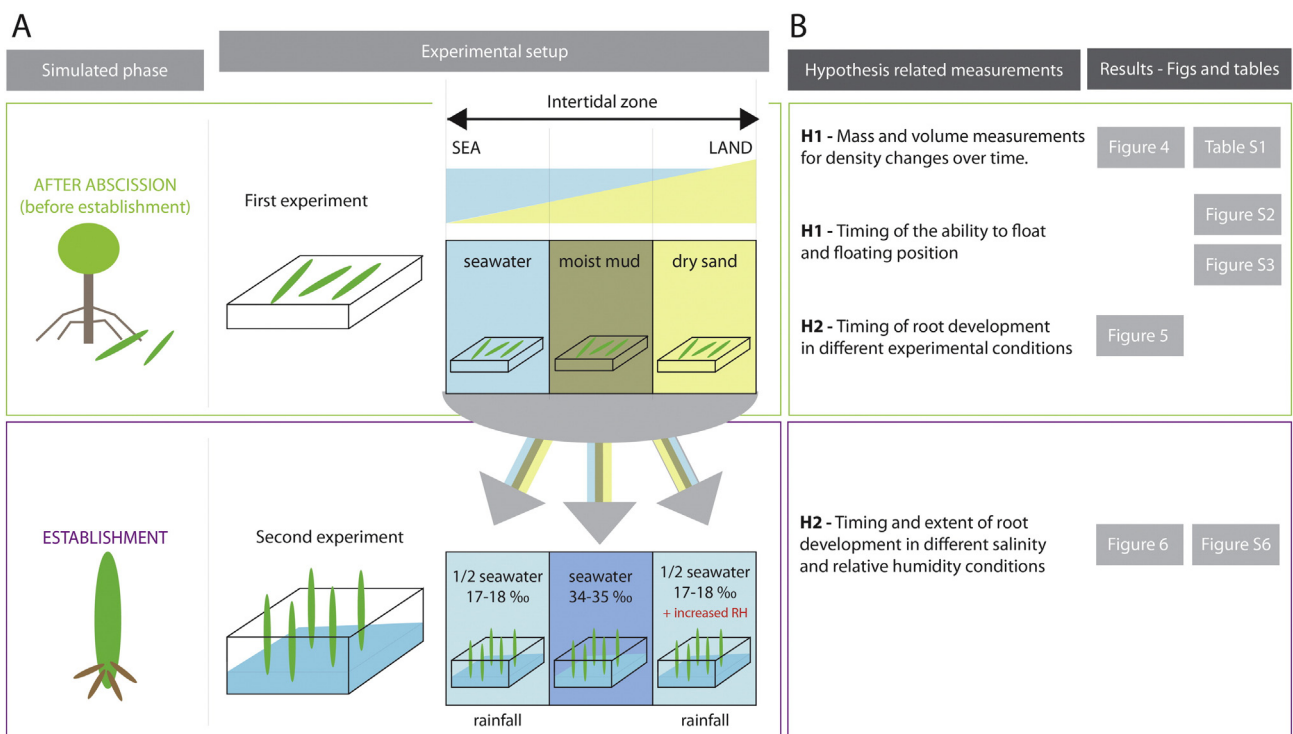
**Fig. 2.** Schematic overview of the hypotheses, results and conclusions of the study. Results are split up in general patterns observed for both studied species and the discovered differences between both species.

only collected when they detached easily and at once. The time between propagule collection and the start of the experiments varied between 12 and 48 h. During this time, propagules were stored in a shaded place within the laboratory without the application of specific conservation measures.

In addition to the above, samples of the two species were collected as both fresh propagules collected from trees and propagules air-dried in the field; these were stored in 50% ethanol and transported to the laboratory. After embedding samples in Polyethylene Glycol 1500, transverse sections of 25 µm thickness were made with a sliding microtome and sections were double stained with safranin–alcian blue (Schmitz et al., 2011). The internal anatomy of fresh and air-dried propagules was studied and visually compared on the basis of these sections.

2.3. Experimental setup

The experimental setup was done ex situ, in Gazi village, but in the vicinity of the mangrove forest in almost identical environmental conditions (unless experimentally changed), particularly in terms of light, day length and temperature. Characteristics of the propagules – hypocotyl length, diameter at mid-length, weight, volume and buoyancy behaviour – were measured for the first time between 12 and 48 h after collection in the field, before the propagules were placed in the experimental setup. For both species, all of the 138 collected propagules were used in two consecutive experiments. Details regarding the number of propagules used in each experiment can be found in Supplementary Fig. 1. For the first experiment, all but nine propagules of each species and each side of the forest (seaward and landward) were placed



**Fig. 3.** (A) Schematic representation of the experimental setup simulating the period between propagule abscission and establishment (first experiment – light green box) and directly following establishment (second experiment – purple box). Propagules of *Rhizophora mucronata* and *Ceriops tagal* were placed in horizontal position in three different experimental conditions that they can experience during dispersal (in seawater, on moist mud and on dry sand). Afterwards the propagules were put in a vertical position in water with different salt concentrations to simulate the potential effect of position in the intertidal zone, and in an environment with or without increased atmospheric relative humidity to simulate the potential effect of rainfall. (B) The conducted measurements per experiment and per hypothesis (Fig. 2) together with the figures and tables in which the results of the experiments are shown.

in a horizontal position in one of the following experimental conditions: in seawater (collected in the bay of Gazi), on moist mud (collected at one of the seaward sites where *R. mucronata* propagules were collected) and on dry sand (collected at one of the landward sites where *C. tagal* propagules were collected) (Fig. 3, Supplementary Fig. S1) in open air conditions near the sampling sites. For propagules floating in seawater, physical contact between propagules or with the container was not prevented.

Every six days, five propagules of each species and collected on each side of the forest were taken from each experimental condition. The propagule characteristics – hypocotyl length, diameter at mid-length, weight, volume and buoyancy behaviour – were determined for a second time and root growth was measured. Three out of the five propagules were then transferred to the second experiment. They were placed in a vertical position in a hydroponic setup with one of the following hydroponic treatments: seawater with a salinity of 17–18, seawater with a salinity of 34–35 or seawater with a salinity of 17–18, with increased (20%, from ca. 70 to ca. 90%) atmospheric relative humidity (Fig. 3, Supplementary Fig. S1). Seawater with a salinity of 17–18 was obtained by diluting seawater with tap water. In this hydroponic setup, the part of the propagules that was under the water surface (3–7 cm for *C. tagal* and 5–15 cm for *R. mucronata*) was also in the dark, due to the opaque nature of the lower part of the boxes. The propagules were put through slits in a piece of polyether foam spaced with a regular distance of 5–7 cm to keep them in a vertical position and were not in contact with the box or with other propagules. For each species, the nine propagules from each side of the forest that were left out of the first experiment were placed directly in a vertical position in the hydroponics of the second experiment; three propagules of each species and from each side of the forest were put in each hydroponic treatment (Supplementary Fig. S1). After 24 days in the hydroponic treatments, propagules were taken out of the treatment and the final lengths of their roots were measured.

#### 2.4. Measurement of propagule characteristics

Propagule length, defined as the length of the hypocotyl without the plumule (Fig. 1), was measured with a measuring tape (1 mm resolution) measured along the outer side of curved propagules. Propagule diameter was measured at the mid-length of the propagule using digital callipers (0.01 mm resolution). Propagule mass was measured with an analytical balance (0.001 g resolution). To calculate propagule tissue density, propagule volume was measured using the water-displacement method (Hughes, 2005), which is based on the principle of Archimedes. The mass and volume data were then used to calculate the density of each propagule. Propagule buoyancy was determined by putting the propagules one by one in a basin filled with seawater under open air conditions (for *C. tagal*) or in the bay (for *R. mucronata*) at ambient temperatures. The water in the basin and in the bay was deep enough to allow the propagules to move freely in all directions without touching the edges or the bottom. A score was given to the level of buoyancy (floating at the water surface (A), floating between the water surface and the bottom (B) or sunk to the bottom (C)) and the orientation of the propagule relative to the water surface was defined (Supplementary Figs. S2 and 3).

Root length was measured by determining the straight distance from the base to tip of the longest root using digital callipers (0.01 mm resolution), as roots were not or only slightly curved. If the roots were too short to be measured precisely with digital callipers, i.e., <2 mm, root growth was scored using three different stages: (1) root bumps that could be felt or seen; (2) roots appearing through little cracks in the root bumps with a length of up to 1.5 mm; and (3) roots with a length between 1.5 and 2 mm.

#### 2.5. Statistical analysis

The initial propagule length, diameter, volume, mass and density measured before the start of the experiments had non-Gaussian distribution; therefore, differences between propagules collected at the seaward and the landward sides of the forest were tested using the nonparametric Mann-Whitney U test. For changes in propagule length, diameter, volume, mass and density, relative values were used. These were calculated for each individual propagule as the difference between the initial value and the value measured after 6, 12, 18 or 24 days, divided by the initial value. The data for relative change in propagule diameter, volume, mass and density also had non-Gaussian distribution and were therefore ranked. Then, a full factorial ANOVA, testing primary and second degree interaction effects was applied to the data for relative changes in propagule length, diameter, volume, mass, density and root length which was measured at the end of the second experiment. The effects of the following factors were tested: species, the side of the forest from which propagules were collected, experimental conditions in the first experiment and the period for which the propagules were subjected to these conditions. For root length measured at the end of the second experiment, the effect of the hydroponic treatment was also tested using similar full factorial ANOVA models. Including all interaction terms for a full factorial model with five factors resulted in too few residual degrees of freedom for conducting the model. Therefore, we made separate models for each species and dropped species as a factor from the models. However, we did explore interspecific differences in separate, reduced models in terms of the side of the forest from which propagules were collected or concerning the experimental conditions in the first experiment.

All the above-mentioned statistical analyses were conducted using Statistica 8 (StatSoft Inc., Tulsa, USA). All results were corrected for multiple testing with the Holm correction, using MS Office Excel 2007 (Microsoft, Redmond, USA).

### 3. Results

Propagules of *C. tagal* and *R. mucronata*, collected at the seaward and landward sides of the forest, were placed in two consecutive experiments. In the first experiment, propagules were placed in a horizontal position under one of three experimental conditions (in seawater, on moist mud or on dry sand). After four different time intervals (6, 12, 18 and 24 days), part of the propagules were transferred to the second experiment and placed upright in a hydroponic setup with one of three hydroponic treatments (seawater with a salinity of 17–18, seawater with a salinity of 34–35 or seawater with a salinity of 17–18 with increased atmospheric relative humidity).

In both studied species, there was a small but significant difference in the length of propagules collected from the seaward and the landward sides of the forest at the time of collection (*C. tagal*: longer landward propagules, *R. mucronata*: longer seaward propagules; see Supplementary Table S1). For both species, the relative change in propagule length during the first experiment, simulating the period between release from the parent tree and establishment, depended on the experimental conditions (in seawater, on moist mud and on dry sand) ( $F_{2, 215} = 48.02$ ,  $p < 0.0001$ ,  $n = 40$ ; see Supplementary Fig. S4A) and differed between both species ( $F_{1, 215} = 158.82$ ,  $p < 0.0001$ ,  $n = 120$ ). *C. tagal* propagules showed a small increase in length and small differences between experimental conditions, whilst the length of *R. mucronata* propagules decreased when on solid substrates (moist mud or dry sand), but showed a small increase when floating in seawater. The diameter of the propagules of both species measured at mid-length decreased during the time ( $F_{3, 215} = 8.49$ ,  $p < 0.01$ ,  $n = 60$ ) that the propagules were placed under one of the three experimental conditions (in seawater, on moist mud, on dry sand) ( $F_{2, 215} = 63.54$ ,  $p < 0.0001$ ,  $n = 40$ ; see Supplementary Fig. S3B). Propagules shrank

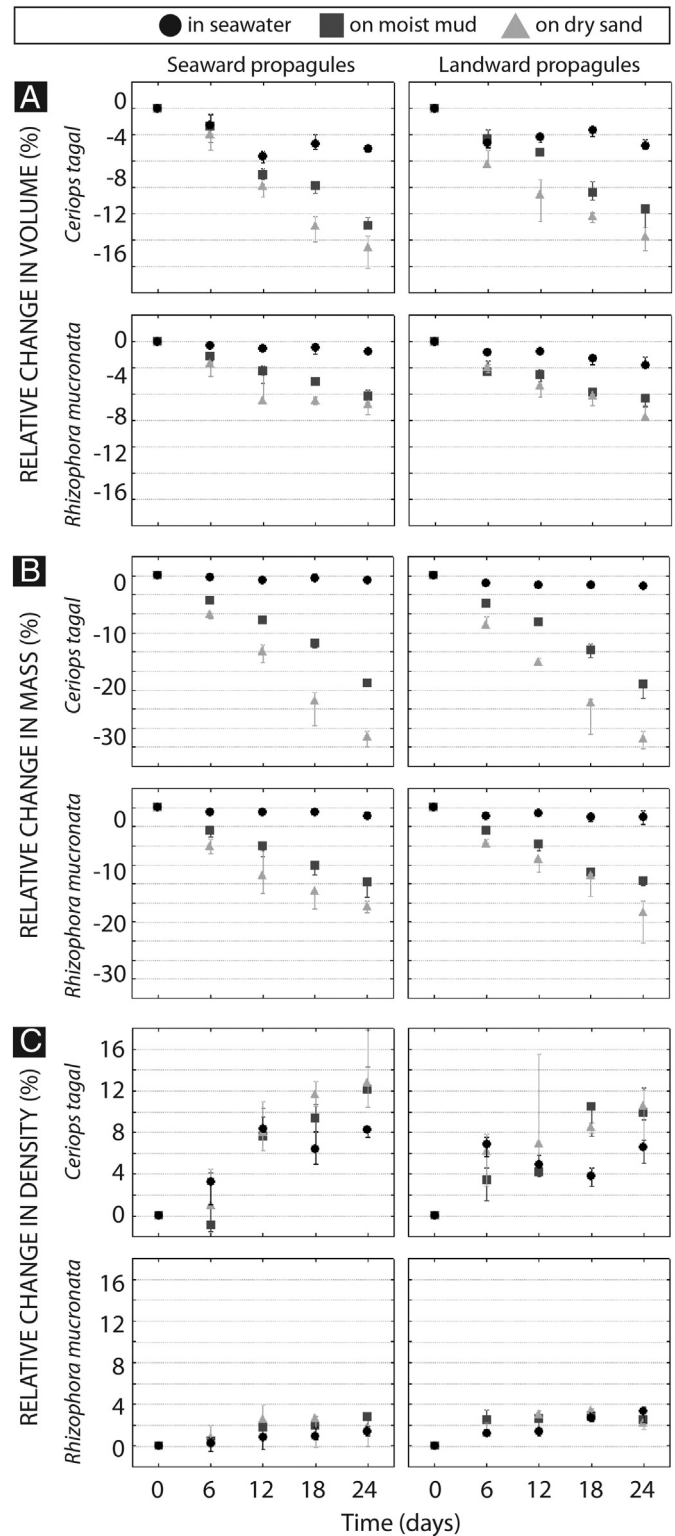
more over time when lying on dry sand or moist mud than when in seawater ( $F_{6, 215} = 5.37$ ,  $p < 0.01$ ,  $n = 20$ ), although this effect was less clear during the first 12 days of the experiment.

As a result of the length and diameter changes of the propagules, the relative volume decreased over time ( $F_{3, 215} = 84.45$ ,  $p < 0.0001$ ,  $n = 60$ ); this was more pronounced for *C. tagal* propagules than for *R. mucronata* ( $F_{1, 215} = 173.61$ ,  $p < 0.0001$ ,  $n = 30$ ; see Fig. 4A). The relative volume decrease was more prominent for propagules that had been lying on dry sand than for those that had been lying on moist mud, with the smallest change observed for propagules left in seawater ( $F_{2, 215} = 243.21$ ,  $p > 0.0001$ ,  $n = 80$ ). The effect of the period during which the propagules had been exposed to these experimental conditions differed between conditions ( $F_{6, 215} = 12.96$ ,  $p < 0.0001$ ,  $n = 20$ ) and depended on the side of the forest (seaward or landward) from which the propagules originated ( $F_{3, 215} = 7.08$ ,  $p < 0.05$ ,  $n = 30$ ). Over time, volume loss was largest for propagules placed on dry sand, followed by those placed on moist mud and was smallest for those placed in seawater. *C. tagal* propagules collected on the landward side of the forest lost more volume over time, whilst for *R. mucronata*, propagules from the seaward side did. These volume changes can be linked to changes concerning the internal propagule anatomy (Supplementary Fig. S5). The internal air spaces of air-dried propagules had shrunk and the thick-walled parenchyma cells had collapsed.

The relative mass of propagules decreased over time ( $F_{3, 215} = 110.79$ ,  $p < 0.0001$ ,  $n = 60$ , Fig. 4B), with a larger decrease for *C. tagal* propagules than for *R. mucronata* ( $F_{1, 215} = 16.40$ ,  $p < 0.01$ ,  $n = 120$ ). The strongest decrease was observed for propagules lying on dry sand, followed by moist mud and seawater ( $F_{2, 215} = 666.06$ ,  $p < 0.0001$ ,  $n = 80$ ). The effect of the period for which the propagules were left under one of the three experimental conditions differed between the different conditions ( $F_{6, 215} = 18.65$ ,  $p < 0.0001$ ,  $n = 20$ ). The strongest decrease of mass over time was observed for propagules placed on dry sand, followed by propagules on moist mud, whilst the effect of time was much smaller for propagules in seawater.

The density of *C. tagal* propagules prior to the start of the experiments was lower than the density of *R. mucronata* propagules (Supplementary Table S1). Furthermore, for *C. tagal* the density of propagules collected on the seaward side of the forest was lower than for those collected on the landward side, whilst the opposite was true for *R. mucronata* propagules. For both species, relative propagule density increased over time ( $F_{3, 215} = 16.29$ ,  $p < 0.0001$ ,  $n = 60$ ; see Fig. 4C), since the change in volume (Fig. 4A) was stronger than the change in mass (Fig. 4B). The density increase was more pronounced for *C. tagal* than for *R. mucronata* ( $F_{1, 215} = 234.51$ ,  $p < 0.0001$ ,  $n = 120$ ). For *C. tagal*, the density of propagules collected on the landward side of the forest increased more than for propagules collected on the seaward side, whilst for *R. mucronata* this effect was smaller and inverse ( $F_{1, 215} = 15.75$ ,  $p < 0.01$ ,  $n = 60$ ). In general, the strongest increase was observed for propagules that were put on dry sand or on moist mud, whilst a smaller increase was observed for propagules left in seawater, although these differences were not significant ( $F_{2, 215} = 6.13$ ,  $p = \text{ns}$ ,  $n = 80$ ).

One to two days after collection of the propagules, before they had been in contact with any of the selected experimental conditions (0 days), the majority of the propagules were floated (Supplementary Figs. S2 and 3). As a result of the increasing density (Fig. 4C), the floating capacity of *C. tagal* propagules decreased over time. This loss in buoyancy was faster for propagules collected at the landward than at the seaward side of the forest (Supplementary Fig. S2). None of the propagules from the seaward side of the forest that were left in seawater sank during the 24 days of the experiment. Propagules that had been lying on dry sand for six to 24 days sank faster than those that were put on moist mud or in seawater. Only propagules lying on dry sand sank completely until lying horizontally at the bottom (Supplementary Fig. S2). In agreement with the slighter increase in density, no pattern was observed in the buoyancy of *R. mucronata* propagules during time spent in one of the three experimental conditions (in seawater, on



**Fig. 4.** Relative change in volume (A), mass (B) and density (C) of *Ceriops tagal* and *Rhizophora mucronata* propagules during the first experiment simulating the period between abscission and establishment (Fig. 3). Propagules were collected from seaward (left) and landward (right) sites in the mangrove forest and spread out horizontally on one of three experimental conditions: in seawater (black circles), on moist mud (dark grey squares) and on dry sand (light grey triangles) for different periods of time (0, 6, 12, 18 and 24 days). Plotted values are medians and 25–75 percentiles with  $n = 3$  (time = 0 days) or  $n = 5$  (time = 6–24 days).

moist mud and on dry sand). However, of the smaller share of the propagules that lost buoyancy, the majority originated from the seaward side of the forest (Supplementary Fig. S3).

Root formation was initiated faster in *C. tagal* than in *R. mucronata* for propagules lying horizontally in seawater or on dry sand (Fig. 5, 12 days). On moist mud, the rate of root initiation was similar for both species (Fig. 5, 12 days). Once initiated, however, root formation for propagules in a horizontal position was faster in *R. mucronata* than in *C. tagal*, except for propagules in seawater (Fig. 5, 24 days). For *C. tagal* propagules, root formation started faster for propagules collected from the landward side of the forest than for those collected from the seaward side. For *R. mucronata*, no such effect was observed.

After 24 days in an upright position in water of different salt concentrations and with or without increased atmospheric relative humidity, most propagules had formed roots of at least 2 cm in length (Supplementary Fig. S6). Root length differed significantly between species (reduced models: excluding the effect pertaining to side of the forest:  $F_{1,141} = 114.39$ ,  $p < 0.0001$ ; excluding the effect of the period for which propagules were in the first experiment:  $F_{1,159} = 95.81$ ;  $n = 89$  (*C. tagal*),  $n = 90$  (*R. mucronata*)) but was not influenced by the origin location of the propagules within the intertidal zone (*C. tagal*:  $F_{1,51} = 0.92$ ,  $p = ns$ ,  $n = 44$  (seaward side),  $n = 45$  (landward side); *R. mucronata*:  $F_{1,52} = 8.0$ ,  $p = ns$ ,  $n = 45$ ). The experimental conditions that propagules resided in prior to being transferred to the hydroponic setup had an influence on root growth; however, this influence was significant only for *R. mucronata* (*C. tagal*:  $F_{2,51} = 0.55$ ,  $p = ns$ ,  $n = 30$  (in seawater, on moist mud),  $n = 29$  (on dry sand); *R. mucronata*:  $F_{2,52} = 18.14$ ,  $p < 0.0001$ ,  $n = 30$ ). In general, propagules that had been left horizontally on moist mud before being placed in an upright position in the hydroponic setup had the longest roots after 24 days. *R. mucronata* propagules left on moist mud or dry sand in a horizontal position formed roots faster when positioned upright than did propagules left in seawater. The time for which propagules were left in a horizontal position affected both species differently (Fig. 6), although this effect was not statistically significant (*C. tagal*:  $F_{4,51} = 4.25$ ,  $p < ns$ ,  $n = 18$ , except for propagules that spent 12 days in a horizontal position  $n = 17$ ; *R. mucronata*:  $F_{4,52} = 2.37$ ,  $p = ns$ ,  $n = 18$ ), i.e., for *R. mucronata* propagules there was no clear

pattern, whilst for *C. tagal* propagules an optimum was observed after 12 days. Root growth was positively influenced by atmospheric relative humidity and negatively influenced by water salinity (*C. tagal*:  $F_{2,51} = 8.81$ ,  $p < 0.05$ ,  $n = 30$ , except for propagules in seawater with a salinity of 34–35  $n = 29$ ; *R. mucronata*:  $F_{2,52} = 19.97$ ,  $p < 0.00001$ ,  $n = 30$ ).

#### 4. Discussion

Both main hypotheses tested in this study were confirmed by the results: (i) *C. tagal* and *R. mucronata* propagule mass and volume were influenced by the substrate in the period between abscission and establishment, and in a species manner; (ii) root formation in both species was initiated upon stranding on solid ground (Fig. 2). The results contribute to explaining the distribution of both species within the mangrove forest, since *Cerriops* propagules are more dehydration-sensitive and thus depend on faster establishment than *Rhizophora* propagules, making them less suitable for long dispersal periods and occurring more frequently at the most seaward side of the mangrove forest.

##### 4.1. Do *Rhizophoraceae* propagules have a dormancy phase after germination?

During the first experiment, when simulating the period between abscission and establishment, the relative volume decline of propagules among both species was largest for propagules placed on dry sand and smallest for propagules placed in seawater (Fig. 4A). The propagule diameter decreased rather than the propagule length and the volume of the propagules declined faster than their mass (Fig. 4A, B). The volume loss can be attributed to a combination of a decrease in intercellular air spaces and collapsing cells when losing turgor (Supplementary Fig. S4). The changes in volume and mass were most likely caused by propagule dehydration, which happened faster on dry sand, slower on moist mud and slowest in seawater. This resulted in a density increase over time (Fig. 4C). Once propagule density became higher than the density of

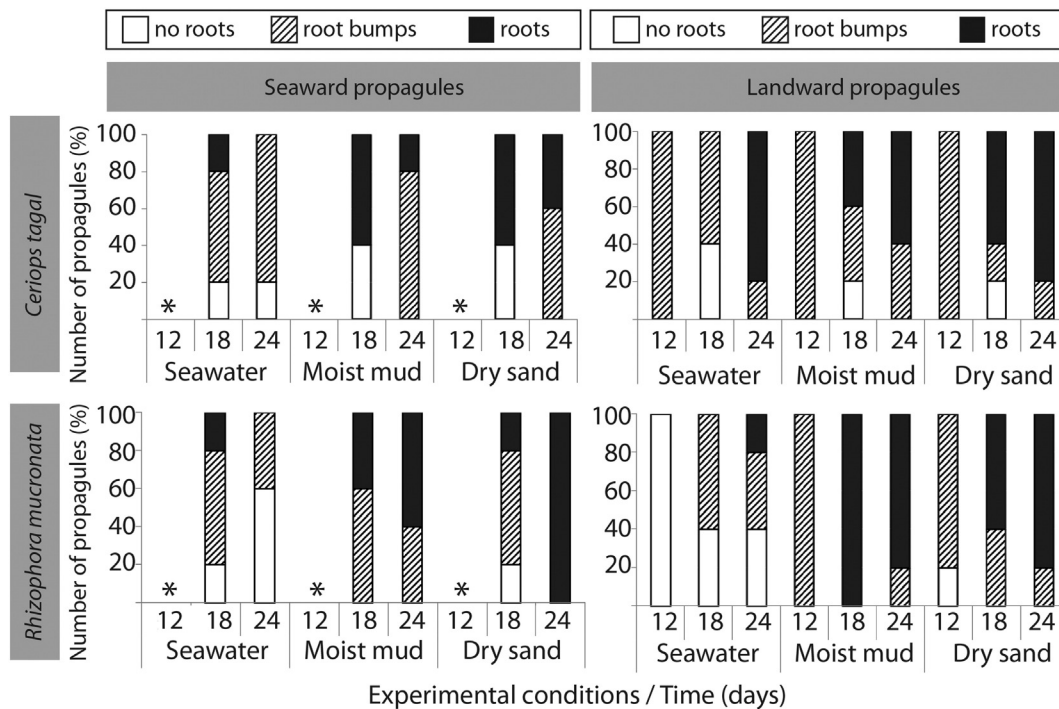
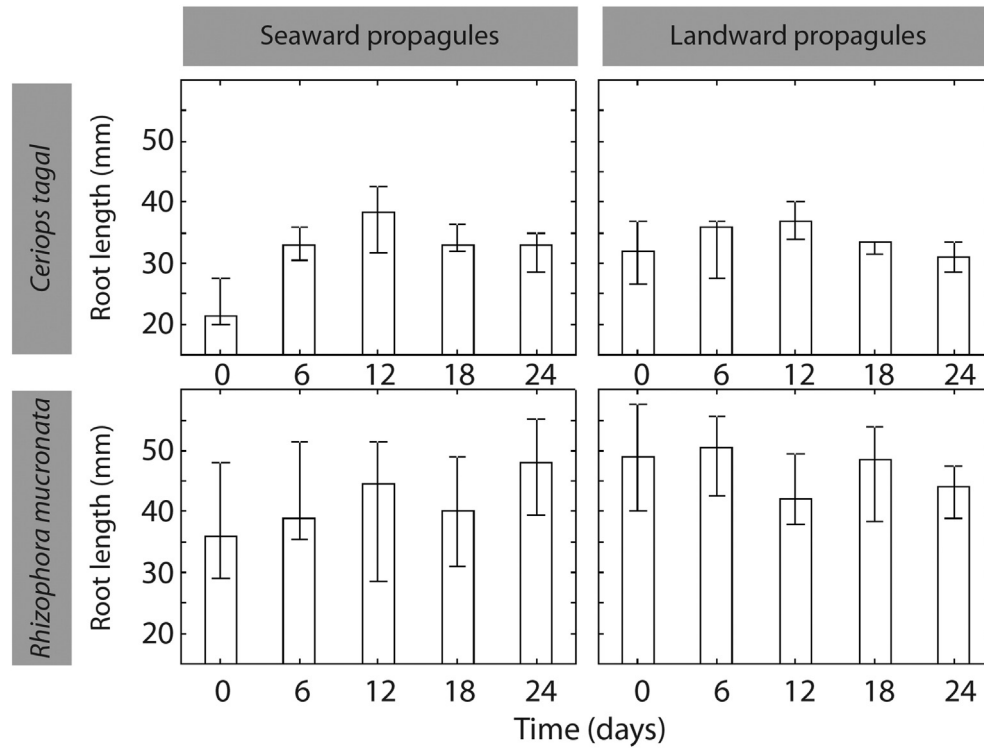


Fig. 5. Root initiation in propagules of *Cerriops tagal* and *Rhizophora mucronata* lying in horizontal position in different experimental conditions (in seawater, on moist mud and on dry sand). Three stages could be observed in root formation for both species in the period between abscission and 24 days thereafter: no external sign of root development (white), bumps visible at the basal end of the propagule (striped) and roots shorter than 1.5 mm in length (black). No bumps were visible before day 12 after abscission.  $N = 5$ , \* = missing data.



**Fig. 6.** Root length of *Ceriops tagal* and *Rhizophora mucronata* propagules after 24 days in vertical position in hydroponic setup (during the second experiment) after different periods in horizontal position (during the first experiment) (Fig. 3). Propagules subjected to different experimental conditions during the first experiment and to different hydroponic treatments during the second experiment were pooled to create five groups per species and per side of the forest based on the time spent in a horizontal position during the first experiment. Bars represent median root lengths and whiskers represent the 25th and 75th percentiles.

seawater, propagules lost buoyancy. An important consequence of this buoyancy loss is that propagules are less likely to be carried away when inundated by seawater, hence increasing the chances for successful establishment.

The time needed for propagules to initiate root formation and form root bumps depended on the experimental conditions in which the propagules resided when in a horizontal position during the first experiment (Fig. 5). For both species, root formation was delayed when propagules were floating in seawater (even though thigmomorphogenesis might have fastened root formation under the chosen experimental set-up, compared to propagules floating in the open sea). This indicates that root formation is delayed during hydrochorous dispersal and that dehydration after stranding on a solid substrate might trigger the start of root formation. Only when stranded do propagules need to form roots for anchorage, in order to withstand tidal inundation, currents and waves (Balke et al., 2011).

Under natural conditions, propagules of both *R. mucronata* and *C. tagal* formed root bumps prior to abscission; however, when wrapped in aluminium foil for 28 days, they formed roots of about 1 cm long when still attached to the parent tree (Jorien Oste, personal observation). This indicates that the necessary machinery for root formation is already present already prior to abscission; however, actual root formation is delayed during hydrochorous dispersal until propagules are stranded.

Although the propagules of *C. tagal* and *R. mucronata* are viviparous – defined as the continuous growth of an embryo without any dormancy period whilst still attached to the parent plant (Elmqvist and Cox, 1996; Tomlinson, 1994) – this study shows that the initiation of root formation and the growth of the propagules ceases, or at least slows down significantly during hydrochorous dispersal. Since our results also suggest that dehydration stimulates root initiation and that the speed and level of dehydration are influenced by environmental conditions, the process seems very similar to that of a dormant seed being triggered to

germinate when environmental conditions become favourable. However, in the case of viviparous mangrove propagules, the “dormancy” period takes place after germination. Nevertheless, more research on the physiological processes taking place within the propagule during this period of slow or ceased growth is required to establish whether this period can be called a true dormancy period.

After being placed in a vertical position in hydroponic setups for 24 days, propagules of both studied species that were growing in low salinity had the longest roots (Supplementary Fig. S6). *C. tagal* propagules were also positively influenced by the increased relative humidity in the air. These results suggest that rain positively influences root growth and therefore, root growth might be fastest during the rainy season, when soil salinity is reduced by rainwater intrusion and air humidity is higher (Robert et al., 2014). Other studies have shown that *Ceriops australis* and *Ceriops decandra* propagules grow optimally in 50 and 25% seawater, respectively, by making better use of available light, than propagules of the same species growing under higher salinity conditions (Ball, 2002).

#### 4.2. Species-specific buoyancy and root initiation due to distinct dehydration sensitivity

During the first experiment, the relative volume and mass decline was larger for *C. tagal* than for *R. mucronata*, regardless of the experimental conditions (in seawater, on moist mud, on dry sand). In addition, a gradual change from floating to sinking was observed for *C. tagal* propagules (Supplementary Fig. S2), whilst most *R. mucronata* propagules remained buoyant during the 24 days of the first experiment (Supplementary Fig. S3). *Ceriops*' small and thin propagules undergo fast volume and mass changes and are hence dehydration sensitive, whilst *R. mucronata* propagules are better able to retain stored water.

When floating in seawater, the higher surface to volume ratio of the slender *C. tagal* propagules, combined with their lower initial density

(Supplementary Table S1) results in a larger above-water volume. This makes them more susceptible to dehydration through evaporation as it concerns the part of the propagule that sticks out above the water surface and that is exposed to irradiation. The part of the propagule below the water surface can moreover experience dehydration due to a difference in water potential between propagule tissues and the surrounding seawater.

*R. mucronata* propagules have a more voluminous shape and a smooth surface compared to the ribbed surface of *C. tagal* propagules, making them less vulnerable to dehydration and buoyancy loss. Thus, the propagules of *R. mucronata* seem to be better at retaining water when placed in seawater than *C. tagal* propagules and have therefore a better chance to survive long periods of floating in seawater and dispersing over long distances. A similar difference in buoyancy between small and large propagules was found by Drexler (2001), who observed that *R. mucronata* propagules can remain buoyant for an average period of 53 days, compared to an average period of only 15 days for the smaller *Rhizophora apiculata* propagules.

The low initial density and high vulnerability to dehydration of *C. tagal* propagules when compared to *R. mucronata* propagules in Gazi Bay were also observed by De Ryck et al. (2012). However, they did not link propagule dehydration with an increase in density, but considered the lower initial density of *C. tagal* propagules to be a trait that favours long distance dispersal (LDD – here defined as propagule movement over oceanic expanses). Although after abscission, most propagules are dispersed over short distances from their parent tree (e.g., Komiyama et al., 1992; McGuinness, 1997; Sousa et al., 2007), studies regarding genetic variation within and between different populations have pointed out the existence of LDD. Examples are trans-Atlantic dispersal of *Avicennia germinans* propagules (Dodd et al., 2002; Nettel and Dodd, 2007) and dispersal across the northern South China Sea and East China Sea of *Kandelia candel* propagules (Chiang et al., 2001). For *C. tagal*, genetic studies have shown that LDD is possible, despite it rarely happening (Huang et al., 2008; Liao et al., 2009).

During the first experiment, the delay of root formation of propagules floating in seawater was more pronounced for *R. mucronata* propagules. When these propagules were placed in an upright position in the hydroponic setups, root growth was strongly slowed down compared to propagules that had lain on moist mud or dry sand before being placed upright. A similar delay in root formation for propagules in seawater or on solid ground was found for *Rhizophora harrisonii* over a period of 40 days and less than 20 days was needed for firm rooting, respectively (Rabinowitz, 1978). On the other hand, root growth in *C. tagal* propagules in a vertical position was not influenced by the experimental conditions in which they were placed prior to being put upright; however, root growth was influenced by the time elapsed between abscission and being put upright. The longest roots were observed for propagules that spent 12 days in a horizontal position before being transferred to the hydroponic setup (Fig. 6). The observed difference in the timing of root growth initiation can be explained by the difference in dehydration sensitivity between the propagules of both studied species. *C. tagal* propagules dehydrate faster than those of *R. mucronata* and reach the level of dehydration that indicates propagule stranding faster, triggering root formation earlier after abscission. Again, this makes *R. mucronata* propagules better suited for successful LDD when compared to *C. tagal* propagules.

#### 4.3. Landward versus seaward populations

The initial density of *C. tagal* propagules collected on the landward side of the forest was lower than for propagules collected on the seaward side. Furthermore, propagules from the landward side of the forest dehydrated faster. Propagule volume and density decreased faster and therefore, buoyancy was lost earlier. This indicates that the propagules of *C. tagal* trees growing on the landward side of the forest had lower water reserves to start with than their seaward side counterparts or

that these propagules were more prone to dehydration, or a combination of both. In addition, root formation started faster for *C. tagal* propagules collected from the landward side of the forest (Fig. 5). Again, this suggests that a certain degree of dehydration is needed to stimulate root formation, acting as a cue for indicating the stranding of the propagule. For *R. mucronata* propagules, the opposite pattern was observed: propagules collected on the seaward side of the forest had a higher initial density and experienced larger volume losses and larger density increases; therefore, more of them lost buoyancy than propagules collected from the landward side. However, the difference in initial density between propagules from the landward and seaward sides of the forest was smaller than for *C. tagal* propagules; additionally, no such difference was observed regarding root formation.

For both species, root length after 24 days in the hydroponic setups did not differ between propagules from the seaward and landward side of the forest (Supplementary Fig. S6). In general, a necessity for propagules to grow roots quickly once stranded can be supposed for both sides of the intertidal zone. At the landward side of the forest, the risk for dehydrating to the point of not surviving is higher, whilst at the seaward side, frequent inundation forces propagules to grow long enough roots for anchoring themselves quickly in order to withstand tidal inundation and waves (Balke et al., 2011).

#### 4.4. Species distribution in the mangrove forest of Gazi Bay (Kenya)

In Gazi Bay, *R. mucronata* mostly grows on the seaward side of the mangrove forest, where inundation frequency is high, increasing dispersal chances and lowering the risk of dehydration. This, combined with the higher resistance to dehydration shown in this study, allows *R. mucronata* propagules to postpone root formation during dispersal at sea, starting root formation only when washed ashore, with propagule dehydration as a cue. *C. tagal* grows at the landward side of the *R. mucronata* zone and its distribution is extended further landward, higher up the intertidal zone. Propagules of the more seaward and dense growing *C. tagal* trees have lower chances for seaborne dispersal due to a higher risk of retention by aerial roots (De Ryck et al., 2012). The more landward growing *C. tagal* trees form a less dense forest with a more open canopy. Here, dispersal chances are limited due to a low inundation frequency and dehydration risk is higher (De Ryck et al., 2012). In addition, *C. tagal* propagules are also more vulnerable to dehydration than *R. mucronata*, also when afloat in seawater. This explains their fast root formation, preventing excessive dehydration prior to establishment. Furthermore, herbivory is an important factor in mangrove propagule survival (e.g., Cannicci et al., 2008; Dahdouh-Guebas et al., 1998; Farnsworth and Ellison, 1997; Minchinton, 2006; Minchinton and Dalby-Ball, 2001; Sousa et al., 2003). *C. tagal* propagules are found to be more vulnerable to predation by crabs than the larger propagules of *R. mucronata* (De Ryck et al., 2012), contributing to the importance of fast establishment for *C. tagal*. Fast self-erection, starting shortly after root formation (Cheeseman, 2012) and further development into a plant with a wooden stem may thus prevent predation.

Our results are supported by those of De Ryck et al. (2012), who found that for propagules planted in Gazi Bay in field conditions, the initiation of root formation was faster for *C. tagal* propagules; however, once initiated, the roots of *R. mucronata* gained length more rapidly. Fast root growth during establishment is particularly important for anchoring the seedling sufficiently so as to withstand inundation, wave and wind action (Balke et al., 2011), especially in the low intertidal zone. A similar result is expected for propagules in other mangrove forests where both species occur, since *C. tagal* typically grows more landward than *R. mucronata* (e.g., Lambs and Saenger, 2011; Matsui et al., 2010; Saifullah and Rasool, 2002; Wakushima et al., 1994). This zonation pattern is due to the fact that *C. tagal* is adapted to dry conditions



and high soil salinity (Spalding et al., 2010), whilst *R. mucronata* is able to cope with a high inundation frequency (Kitaya et al., 2002).

#### 4.5. Conclusion

In conclusion, it can be stated that for the viviparous propagules of both *C. tagal* and *R. mucronata*, after abscission, a certain degree of dehydration stimulates the initiation of root formation as a cue for indicating propagules stranding. Consequently, root formation is postponed when propagules float at sea during dispersal. Furthermore, the establishment phase of mangrove propagules is faster in less saline conditions than in more saline conditions, indicating that during the rainy season, the conditions for propagule establishment are more favourable. Nevertheless, the two species studied in this paper follow different strategies for dispersing and establishment, and these findings contribute to the explanation of the different species distributions. *Ceriops* grows more landward in the intertidal zone, where dehydration risk is higher and dispersal chances are lower. Additionally, *Ceriops* propagules are more vulnerable to dehydration, thus reaching the dehydration level that stimulates root initiation faster. In this way, *Ceriops* propagules reduce the chance of excessive dehydration prior to establishment. On the other hand, *Ceriops* propagules also dehydrate faster when floating in seawater, thus decreasing their chances for successful LDD. *Rhizophora* propagules are better able to retain water. Root growth and establishment can thus be postponed longer, especially when propagules float in seawater during hydrochorous dispersal, increasing the chances for successful LDD.

Mangrove species must strike a balance between local rejuvenation through immediate establishment in the dynamic environment and long distance voyages for establishing extended viability. As shown for *C. tagal* and *R. mucronata*, representative for the most characteristic family of mangrove systems worldwide, this balance can be influenced by the local distribution of a species, by propagule characteristics and by local environmental conditions. The survival strategies of the propagules after abscission differ substantially between both species, influencing their chances to be dispersed over longer distances. It will be useful for the further development of mangrove propagule dispersal models and for understanding mangrove vegetation dynamics to discover whether other mangrove species follow one of the strategies found in this study, and whether the species studied here follow the same strategies at other locations.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jembe.2015.03.014>.

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