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Growth and structural changes of viviparous mangrove propagules: the effect of environment on dispersal and establishment

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ABBREVIATIONS

ACC	1-aminocyclopropane-1-carboxylic acid
C	*Ceriops tagal*
D	dry sand
E	energy
etc.	*etcetera*
i.e.	*id est*
L	landward site
M	moist mud
ns	not significant
R	*Rhizophora mucronata*
RH	relative humidity
S	seaward site
SD	standard deviation
W	sea water

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ABSTRACT

Most true mangrove species are viviparous, meaning that their seeds germinate while still attached to the mother tree. The dispersal unit of viviparous species is a small seedling, also called propagule. Viviparous reproduction of plants has been linked to wet habitats, such as mangroves, however, it is not yet fully understood what the precise adaptive advantages and disadvantages of vivipary are that make that not all mangrove species are viviparous.

We hypothesize that: (1) the disadvantage of vivipary, the mother tree having to support the seedlings, is counteracted by autonomous growth of seedlings that takes in addition a higher percentage of total growth in species with larger propagules (like *Rhizophora mucronata*) than seedlings of species with smaller propagules (like *Ceriops tagal*), (2) the advantage of vivipary is a delayed dormancy period during the (most dangerous) period for the plant between abscission and establishment, as a seedling that is less sensitive than a seed, (3) this delayed dormancy is broken by environmental cues that indicate favourable conditions to trigger root growth and establishment, (4) during establishment, longitudinal growth of the propagule, root growth and leaf development are lower in high than in low salinity conditions and higher when relative air humidity is increased.

This study was conducted in Gazi Bay, Kenya, where two viviparous mangrove species were studied: *Ceriops tagal* (Perr.) C. B Robinson and *Rhizophora mucronata* Lamk. To test hypothesis 1, propagules that were still attached to the mother tree were deprived of light to inhibit autonomous growth (experiment A). The growth of covered and uncovered propagules was followed and compared. However, because the used propagules were not young enough, many of them abscised before the end of the experiment and hypothesis 1 could not be tested. Nevertheless, it was observed that propagules that were still attached to the mother tree and covered in aluminium foil developed roots, while this does not occur under natural conditions. We hypothesize that ethylene produced by the seedling had built up in the aluminium foil bags, due to a lack of air circulation, and induced root growth.

To test hypotheses 2-4, mature propagules of both species were collected and placed horizontally on three different substrates, simulating different environmental conditions during the period between abscission and establishment (experiment B). Every six days, five propagules were taken of the substrates and three of them were placed vertically in a hydroponic set-up with different salinity and air humidity conditions, simulating different environmental conditions during establishment (experiment C). For both species, no length growth was observed during experiment B, and root growth was not initiated before the 13th

day for which the propagules were left on one of the substrates. This indicates a period of delayed dormancy and therefore supports hypothesis 2. During experiment C, *R. mucronata* propagules that had been placed on the drier substrates during experiment B, and thus were dehydrated, started to form roots earlier than propagules from sea water that were not dehydrated. Therefore we hypothesize that dehydration is the environmental cue that triggers establishment of *R. mucronata* propagules. In contrast, *C. tagal* propagules that had experienced least dehydration during experiment B grew longer roots during experiment C. Therefore, we hypothesize that humidity stimulates root growth of *C. tagal* propagules. This supports hypothesis 3 as drier conditions are favourable to establish for *R. mucronata* propagules that come from wetter areas and do thus have enough water stored. For *C. tagal* propagules, wetter conditions indicate favourable conditions for establishment as they come from drier areas and thus need some water to be able to establish. As was hypothesized (hypothesis 4), more length (*C. tagal*) or length and root growth (*R. mucronata*) was observed for propagules experiencing low salinity and increased air humidity. Leaf growth was only observed for *C. tagal* propagules, of which most were treated with 50 % sea water.

SAMENVATTING

Een groot aantal mangrovesoorten plant zich voort via viviparie, d.w.z. dat de zaden ontkiemen en de jonge zaailingen zich ontwikkelen terwijl ze nog steeds aan de ouderlijke boom hangen. Vivipare mangrovesoorten verspreiden dus zaailingen, propagules genaamd, in plaats van zaden. Hoewel de vivipare reproductie van planten wordt gelinkt aan ecosystemen in waterrijke gebieden, zijn de adaptieve voordelen en nadelen ervan, die ervoor zorgen dat niet alle mangrovesoorten vivipaar zijn, nog steeds niet helemaal duidelijk.

Onze hypotheses zijn: (1) het nadeel van viviparie, de ondersteuning van de groei van de propagule door de ouderlijke boom, wordt gecompenseerd autonome groei van de propagule, waarvan bovendien het percentage hoger is voor soorten met grotere propagules (zoal *Rhizophora mucronata*) dan voor soorten met kleinere propagules (zoals *Ceriops tagal*), (2) het voordeel van viviparie is een uitgestelde dormantie, tijdens de (meest gevaarlijke) periode voor de plant tussen abscissie en vestiging als een zaailing, die bovendien minder gevoelig is dat een zaad, (3) deze uitgestelde dormantie wordt verbroken door bepaalde omgevingsfactoren die duiden op gunstige omstandigheden en die de groei van wortels en dus vestiging induceren, (4) terwijl een propagule zich vestigt, is de lengtegroei, de groei van wortels en de ontwikkeling van bladeren hoger wanneer de saliniteit laag is en de luchtvochtigheid hoog.

Dit onderzoek werd uitgevoerd in Gazi Bay, Kenia, waar twee vivipare mangrovesoorten, *R. mucronata* en *C. tagal*, werden bestudeerd. Hypothese 1 werd getest d.m.v. een experiment op propagules die zich ontwikkelden aan de ouderlijke boom. Propagules werden van het licht afgedekt met aluminiumfolie om autonome groei te verhinderen (Experiment A). De groei van afgedekte en niet afgedekte propagules werd gevolgd en vergeleken. Omdat de propagules niet jong genoeg waren, vielen echter veel propagules van de boom voor het einde van experiment, waardoor hypothese 1 niet kon worden getoetst. Desondanks werd wortelgroei vastgesteld voor propagules bedekt met aluminiumfolie, die niet waren afgevallen. Wortelgroei komt bij deze propagules onder natuurlijke omstandigheden enkel voor na abscissie. Volgende hypothese werd geformuleerd om deze observatie te verklaren: Omdat het aluminiumfolie rond de propagules weinig tot geen luchtcirculatie toelaat, kon ethyleen, geproduceerd door de propagule, zich opstapelen en de verhoogde ethyleenconcentratie induceerde de groei van wortels.

Hypotheses 2-4 werden getest door volgend experiment: rijpe propagules van beide soorten werden verzameld en op een van drie verschillende substraten geplaatst (horizontaal),

die verschillende omgevingsomstandigheden tijdens periode tussen abscissie en vestiging van de propagules simuleerden (experiment B). Na telkens zes dagen werden vijf propagules van ieder substraat genomen en werden drie ervan in hydroponische opstellingen geplaatst (verticaal), met verschillende salinitieit en luchtvochtigheid, wat verschillende mogelijke omgevingsomstandigheden tijdens propagule-vestiging simuleerde (experiment C). Voor geen van beide soorten werd lengtegroei vastgesteld tijdens experiment B en wortelgroei werd pas waargenomen na minstens 13 dagen. Deze resultaten stemmen overeen met de uitgestelde dormantie periode die werd voorgesteld in hypothesis 2. Voor *R. mucronata* propagules die op drogere substraten hadden gelegen tijdens experiment B, en dus blootgesteld waren aan dehydratatie, werd sneller wortelgroei waargenomen dan voor propagules uit zeewater, die niet gedehydrateerd waren. Dus, de omgevingsfactor die wortelgroei induceerde, was dehydratatie. Voor *C. tagal* propagules werd meer wortelgroei waargenomen voor propagules die niet of minder waren gedehydrateerd. Daarom suggereren we dat bij *C. tagal* propagules wortelgroei werd gestimuleerd door vochtigheid. Deze resultaten steunen hypothese 3, omdat drogere omstandigheden gunstig zijn voor *R. mucronata* propagules die afkomstig zijn uit een nattere omgeving en daardoor genoeg water konden opslaan. Voor *C. tagal* propagules zijn nattere omstandigheden net gunstiger, omdat ze afkomstig zijn uit een drogere omgeving en dus water nodig hebben om zicht te kunnen vestigen. Tijdens experiment C werd de meeste lengtegroei (*C. tagal*) en lengte- en wortelgroei (*R. mucronata*) waargenomen voor propagules die werden behandeld met lage saliniteit en hoge luchtvochtigheid. De ontwikkeling van bladeren werd enkel waargenomen voor *C. tagal* propagules, waarvan de meesten waren behandeld met lage saliniteit. Deze resultaten steunen hypothese 4.

INTRODUCTION

1. The mangrove ecosystem

1.1 Definitions

The term ‘mangrove’ is used for the tree as well as for the ecosystem in which the trees grow. The mangrove ecosystem is sometimes also called ‘mangal’. The mangrove trees are the major elements forming the mangrove ecosystem; without these trees it would not be a mangrove. A distinction can be made between true mangroves, or major elements of mangal, minor elements of mangal and mangrove associates. True mangroves occur exclusively in mangrove ecosystems, where they fulfil a major structuring role. They also are very well adapted to their environment in the form of aerial roots, in most cases viviparous reproduction and a salt regulating mechanism (all three discussed later). Further, true mangroves have no terrestrial congenerics (Tomlinson, 1999). Minor mangrove elements lack the ability to form pure stands and to fulfil the same structural role in the ecosystem as the true mangroves do (Tomlinson, 1999). There are 55 species of mangroves (major and minor mangal elements), belonging to 20 genera and 16 families (Hogarth, 2007). Mangrove associates are species that are frequently but not exclusively found in the mangrove ecosystem (Tomlinson, 1999).

1.2 Biogeography

Mangroves occur mainly in the tropics and always at places that are influenced by the tides, such as coastal zones, but also river banks (Tomlinson, 1999; Spalding *et al.*, 2010). The southernmost mangroves grow in Australia at 38° 45’ S (Hogarth, 2007). The global distribution of mangroves is related to sea surface and air temperatures and to coastal aridity. Different mangrove species respond differently to combinations of these climatic characteristics. Therefore, the global mangrove distribution cannot be described by any isotherm (Quisthoudt *et al.*, submitted). However, generally, more and richer mangals are found at coasts that receive more water through rainfall or run-off (Tomlinson, 1999). Further, mangroves do not tolerate frost and grow mainly in regions where the minimum temperature of the coldest month is at least 20°C (Hogarth, 2007). Global mangrove distribution is, however, not limited by this minimum winter temperature isotherm (Quisthoudt *et al.*, submitted).

The global distribution of the true mangrove species can be divided in two biogeographical regions: (1) the Indo West Pacific region, including all mangroves from East Africa to the

West-Pacific Ocean and containing 40 true mangrove species and (2) the Atlantic East Pacific region, including all mangroves of West-Africa, the Caribbean and America and containing only eight true mangrove species (Tomlinson, 1999; Spalding *et al.*, 2010).

Both *Ceriops tagal* (Perr.) C. B. Robinson and *Rhizophora mucronata* Lamk. can be found along most of the coasts of East Africa, Madagascar, the Seychelles, the Maldives, the Solomon islands and tropical Asia and Australia. The global distribution of both species differs mainly in the northwest of the Indo-Pacific Ocean, where only *R. mucronata* can be found at the coasts of the northern half of Somalia, the west of the Arabic peninsula and the South of Iran. In the northeast of the Indo-Pacific Ocean, *R. mucronata* grows at the coasts of Japan, while *C. tagal* does not (Duke, 2006; Spalding *et al.*, 2010).

1.3 Value of mangroves and their threats

The mangrove ecosystem is important, in different ways, for a variety of species (reviewed in Nagelkerken *et al.*, 2008): diverse sponge species live on the aerial roots of mangrove trees and a variety of meiofauna species can be found in mangrove sediments. Many prawn and fish species can avoid predation in the turbid water of the mangal, while this ecosystem also serves as nursery and feeding grounds. Different elasmobranch species, among which sharks and rays, nurse their young in mangroves. Further, a variety of insects that feed on parts of mangrove trees and associates or on animal species living there can be found in the mangroves. Also vertebrates are present in the mangal, some species of frogs, turtles, crocodilians, lizards and snakes live and/or feed there. Lots of bird species can be found in mangroves, although only a few are mangrove specialists (Nagelkerken *et al.*, 2008).

Often mangroves, seagrass beds and coral reefs are found in close vicinity of each other, therefore it is very likely that these ecosystems are connected in some way. It has been shown that there are fluxes of carbon and other elements from the mangrove ecosystem to seagrass beds, where carbon can be assimilated. Mangroves can also protect coral reefs from sedimentation and eutrophication by trapping riverine sediments and nutrients before these can reach the coral reef (Hogarth, 2007). These three ecosystems are also connected by species that spend a part of their life in one of these ecosystems and then migrate to one of the other two. Coral reefs are the habitat for several commercial fish species of Bonaire, Netherlands Antilles (Nagelkerken *et al.*, 2000). The juveniles of these fish are nursed in the shallow waters of mangroves, seagrass beds and shallow coral reefs.

Mangrove associated fishery is of great importance for local communities. Fish can be caught for human consumption, but also as broodstock and forage for aquaculture (Walters *et*

al., 2008). Mangroves provide various other goods and services for local communities (reviewed in Walters *et al.*, 2008): one of the most important goods is wood, used as fuel and in construction, but also bark and leaves of trees are used for diverse purposes. The wood of different mangrove species has different characteristics. For example, the hard and dense wood of *Rhizophora*, *Ceriops* and *Bruguiera* is mostly used for construction purposes. Mangrove associated plants are used as fodder, food and traditional medicines. Further, mangroves serve as biofilters for untreated waste water in urban regions and as natural protection against storms, flooding, tsunamis, *etc.* (Walters *et al.*, 2008).

The survey of 38 sites in 16 nations by Farnsworth and Ellison (1997) revealed the anthropogenic activities threatening mangrove ecosystems on a global scale: the most occurring threats to mangrove ecosystems, which also have the largest impact, are clear cutting and reclamation for agriculture, aquaculture, urban expansion, resort development and wood chips used for industrial production of rayon (such as viscose). Other threats, for which the impact is more difficult to estimate, are pollution by sewage, agricultural run-off, industry, oil and also the dumping of garbage. In addition, some mangroves are threatened by hydrological changes in the landscape that cause the diversion of fresh water away from the mangrove (Farnsworth and Ellison, 1997).

Another very important threat, that was already mentioned by Farnsworth and Ellison (1997), but gained more and more attention during the last decade, is sea level rise (Soares, 2009). The influence of sea level rise on mangroves is highly dependent on different characteristics of the region where the mangroves grow and on the rate of sea level rise. The local topography will determine the land cover as well as the possibility for the mangal to migrate landward. Sediment input and deposition rate are also important factors that can positively influence the survival chances by compensating the sea level rise. The regional oceanography can influence the above described factors differently for mangroves at different locations (Soares, 2009).

According to the results of Di Nitto *et al.* (2008) both anthropogenic activities, such as clear cutting, and sea level rise negatively influence the distribution patterns and establishment rates of propagules of *R. mucronata* and *C. tagal* in the mangroves of Gazi Bay, Kenya. For example, clear cutting results in naked areas where no aerial roots are that can trap dispersing propagules. At the Kenyan coast, propagules of both species establish most successful in areas where mature trees of the same species grow. This is part of the positive development cycle proposed by Di Nitto *et al.* (2008). Changes in the distribution pattern and establishment rate of propagules due to human activities can negatively affect the forest

structure. In turn, this changed forest structure can negatively affect the distribution pattern and establishment of propagules. This vicious circle can thus lead to degradation of the mangrove forest (Di Nitto *et al.*, 2008).

To cope with these threats towards mangroves, ecosystem management is needed. The management of mangrove ecosystem is particularly difficult because the ecosystem is located at the interface between land and sea, causing ambiguity about which governmental institution it should manage. In addition, most mangroves are seen as common property by local communities who make use of the different goods and services provided by the ecosystem (Walters *et al.*, 2008).

2. Adaptations of mangrove trees to their environment

2.1 Vivipary

Three growth phases have been described for the viviparous embryos and seedlings, mostly based on studies on *Rhizophora mangle* L. (Figure 1), but assumed to be the same for all mangrove Rhizophoraceae:

1. Development and growth of the embryo and extension of the cotyledon: during this stage endosperm enlargement causes the embryo to protrude the seed coat, but not yet the fruit (Juncosa, 1982; Tomlinson and Cox, 2000), in *R. mangle* this happens after about 70 days (Sussex, 1975). In *Rhizophora* and *Ceriops*, the cotyledons are almost completely fused and form a cylinder around the developing epicotyls (Juncosa, 1982; Das and Ghose, 2003). Three pairs epicotyledonary leaf primordia are formed before abscission. The first pair already starts to develop leaves and stipules, but the growth of the leaves ceases rapidly after it started. Therefore, only the stipules are developed well and these will protect the plumule after abscission (Juncosa, 1982). Stipules are small outgrows of the leaf-base and mainly have a protective function, in most cases to protect buds (Goebel, 1905). *R. mucronata* propagules have one pair of stipules that protects the plumule, while *C. tagal* propagules have two pairs (Tomlinson, 1999).
2. Germination: the hypocotyl extends due to intercalary growth and protrudes the fruit (Juncosa, 1982; Tomlinson and Cox, 2000), in *R. mangle* germination occurs about 100 days after fertilisation (Sussex, 1975). After germination, the hypocotyl continues to grow in length and also lateral root development starts at the radicle

tip. Before abscission, root bumps can become visible, but roots do not protrude the cortex yet (Juncosa, 1982).

3. Further extension of the cotyledon causes the cotyledonary collar to become visible outside the fruit. Therefore, the abscission zone, at the node between the cotyledonary collar and the hypocotyl, is moved outside the fruit. The cotyledonary collar stays attached to the fruit at the time of abscission (Juncosa, 1982; Tomlinson and Cox, 2000).

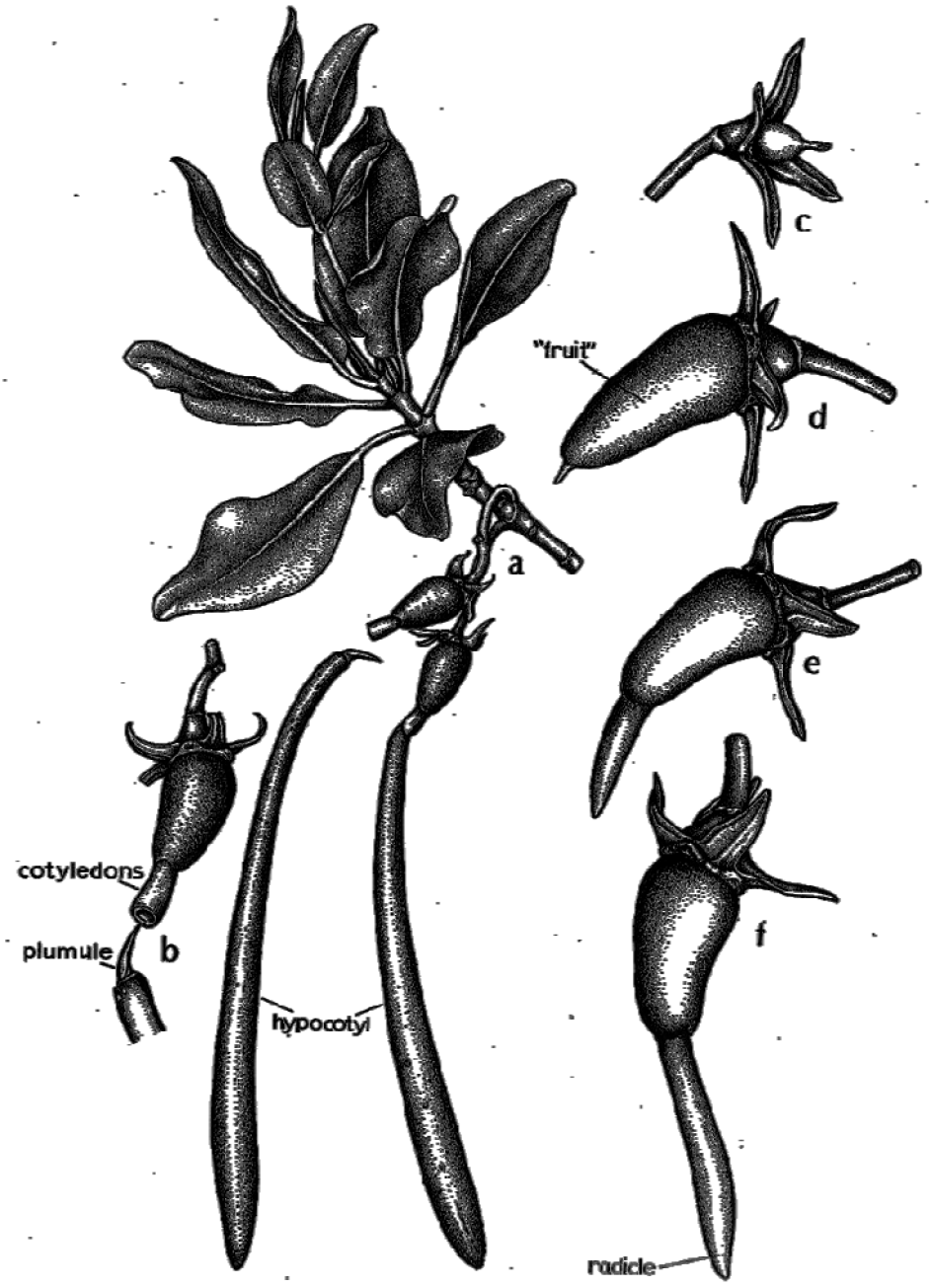

Figure 1. Development and growth stages of a Rhizophora mangle propagule. (Tomlinson, 1999)

2.1.1 Absence of seed dormancy

Thus, embryos of viviparous mangroves do not have a dormancy period. Dormancy, under natural conditions, is defined as the lack of germination due to certain characteristics of the seed (Baskin and Baskin, 2000). Or, more precise, seeds are dormant when there is a delay between the shedding and germination of the seed (Finkelstein *et al.*, 2008). In some cases it are characteristics of the embryo itself that prevent germination, this is called endogenous dormancy. In other cases the structures surrounding the embryo prevent germination, then it is called exogenous dormancy. Dormancy can be physiological, morphological, morphophysiological, physical, chemical or mechanical (Baskin and Baskin, 2000). After the dormancy period, germination starts with water imbibition and ends when the embryo becomes visible outside the surrounding tissues, in most species with the radicle end first (Finkelstein *et al.*, 2008; Nonogaki *et al.*, 2010). To break dormancy, dormant seeds need to undergo enzymatic and biochemical changes to allow germination, this is called after-ripening. After-ripening can be induced by environmental signals, so that the seed will germinate when conditions are favourable. For example, in temperate regions, after-ripening of dormant seeds can be induced by cold temperatures during winter, so the seed will be ready to germinate in warmer spring period (Raven *et al.*, 2005). This illustrates the most important advantage of seed dormancy: the ability to survive periods during which conditions are unfavourable for germination. Viviparous species often grow in tropical regions with no seasonality, where conditions are always favourable for germination, or, when they grow in regions with seasonality, their offspring develops on the parent plant during the unfavourable season and is released when conditions are favourable for dispersal and establishment.

2.1.2 Seed recalcitrance

The majority of spermatophyte seeds tolerate drying to low water levels (less than 7%) and are called desiccation tolerant or orthodox. Desiccation tolerant seeds almost all undergo a period of dormancy (Tweddle *et al.*, 2003). Seeds that do not tolerate desiccation are called recalcitrant seeds (Tweddle *et al.*, 2003). Not all seeds belong to one of these two categories. Some seeds tolerate drying to 10-12 % of their initial water content and are called intermediate (Hong *et al.*, 1998 in Tweddle *et al.*, 2003). Desiccation tolerant species can survive their low water content by achieving a glassy state of their cytoplasm (reviewed in Buitink and Leprince, 2008). Most of their water is lost and the concentration of sugars and late embryonic abundant (LEA) proteins becomes very high. These two components, and

possibly also other molecules interact with each other and form a very viscous cytoplasm in which almost all metabolic activity stops, or happens very slowly due to low diffusion rates.

Recalcitrance of seeds, as in viviparous plant species, has ecological advantages. The absence of a dormancy period during which metabolism is extremely low enables seeds to continue building up reserves while it is attached to the parent plant (Juncosa, 1982; Farnsworth, 2000).

2.1.3 Viviparous reproduction

When there is no dormancy at all and seeds even germinate before abscission, this is called true vivipary. In viviparous plants the offspring grows continuously while still attached to the mother tree (Goebel, 1905). Vivipary includes different forms of plant reproduction. First, there is the division between true vivipary, cryptovivipary and pseudovivipary. True vivipary and cryptovivipary on the one hand and pseudovivipary on the other differ substantially: true vivipary and cryptovivipary are forms of sexual reproduction, while pseudovivipary is a form of asexual reproduction (Elmqvist and Cox, 1996). In this work, only the former two are of interest. In plants exhibiting true vivipary, the seed germinates while it is still attached to the mother tree. Germination is generally defined as the moment at which the embryo protrudes the surrounding structures, in most cases with the radicle first, so that the embryo becomes visible (Nonogaki *et al.*, 2010). In some species, the embryo only grows through the seed coat and not true the surrounding fruit while it is still attached to the parent tree, this is called cryptovivipary (Tomlinson, 1999).

In the case of mangroves, true vivipary exists in all species of the following four genera: *Rhizophora*, *Kandelia*, *Bruguiera* and *Ceriops*, all belonging to the tribe Rhizophorae of the family Rhizophoraceae and all major mangal elements. Cryptovivipary occurs in *Avicennia* (Avicenniaceae) and *Nypa* (Palmae), both major mangal elements and *Aegiceras* (Myrsinaceae), *Pelliciera* (Pellicieraceae) and *Aegialitis* (Plumbaginaceae), that are minor mangal elements (Tomlinson, 1999). Therefore, vivipary is well represented in different taxonomic groups in the mangrove ecosystem. Molecular phylogenetic analysis of 26 genera of mangroves and mangrove associates, belonging to 17 different families, revealed that vivipary originated multiple times during evolution (Shi *et al.*, 2005). This implies that vivipary is an adaptive trait, but what are the ecological advantages of vivipary in the mangrove ecosystem? It has been suggested that vivipary is another way for mangroves to avoid germination in the saline environment where the adult trees grow. The seedlings can develop while attached to the mother tree and can gradually adapt to higher salinity (Joshi,

1933; Joshi *et al.*, 1972; Smith and Snedaker, 1995). Nonviviparous mangrove species would germinate in high salinity conditions by dispersing their seeds at the beginning of the wet season, when salinity decreases (Joshi *et al.*, 1972). Nevertheless, other studies revealed that during the development of the hypocotyl, after germination of viviparous mangrove seeds, the concentration of ions like chlorine, sodium, potassium, calcium and magnesium decreases with time. The ion concentrations in fruit and pericarp (i.e. the part of the fruit that is developed from the ripened wall of the ovary and that encloses the seed) are high and therefore the embryo develops and the seed germinates in an environment with high salinity. During seedling growth on the mother tree, the seedling gradually excludes most of these ions from its tissues and so the seedling develops the ability to exclude salt and thus to cope with high salinity (Zheng *et al.*, 1999). However, Wang *et al.* (2002) concluded, from their study on *Kandelia candel* (L.) Druce, that ion concentrations do not change during hypocotyl development. Therefore, they did not find a significant contribution of changing ion concentration in the developing hypocotyl to salt tolerance (Wang *et al.*, 2002).

Another possible advantage of vivipary is the size of the dispersal units. Propagules of *R. mucronata* often reach lengths of more than 70 cm (Tomlinson, 1999; Duke, 2006). This allows the propagules to establish in zones with high water levels while avoiding inundation of their developing shoot (Rabinowitz, 1978; McKee, 1995; Sousa *et al.*, 2007). Propagule size is also linked with rapid establishment through the formation of roots to anchor in the substrate. Large propagules can contain high amounts of reserves, which can provide energy during root formation (Smith *et al.*, 1996; Patel *et al.*, 2010). It has also been suggested that larger propagules can remain buoyant for a longer time and thus can disperse over longer distances (Rabinowitz, 1978). Nevertheless, larger propagules also have higher chances to be prevented from dispersing away from their parent tree due to physical barriers such as prop roots (Sousa *et al.*, 2007). However, in general, most mangrove species, viviparous as well as non-viviparous, have large dispersal units, so this trait cannot be exclusively linked to vivipary, but is rather a general adaptation to the condition of the tropical intertidal swamp (Clarke *et al.*, 2001).

Of all angiosperms, only about 100 species are viviparous, of which 50 % exhibit true vivipary. Most of these latter species grow in tropical shallow marine habitats, more specifically mangroves and seagrass beds (Elmqvist and Cox, 1996). However, certainly not all seagrasses and also not all mangroves are viviparous. Farnsworth (2000) reviewed ecological, morphological and physiological characteristics of 195 angiosperm species of which the seeds lack dormancy. She concluded that all these species share certain hormonal

characteristics and in addition all grow in wet habitats. Therefore, she hypothesises that the lack of dormancy, and thus recalcitrance and vivipary are selected for in wetland habitats like the one of mangroves. This hypothesis explains the high percentage of viviparous mangrove species.

2.2 Aerial roots

The substrate in mangrove swamps is regularly waterlogged due to tidal inundation. To allow respiration, the roots have to be ventilated, but this is hindered by the waterlogged substrate. Most mangrove species overcome this problem by developing roots that grow partly above the substrate, these are called aerial roots. Another function of these roots is to support the tree in the often unstable substrate (Tomlinson, 1999). Different forms of aerial roots, occurring in different species, have been described by Tomlinson (1999) and are shown in figure 2:

- Stilt roots grow from the stem or lower branches above the substrate and form a supporting loop to grow further under the substrate (Figure 2A).
- Pneumatophores are vertical outgrowths from subterranean roots. They grow from roots under the substrate to protrude the substrate at some distance from the stem (Figure 2B).
- Knee roots are formed by roots that grow under the substrate and bend to make knee-like curves above the substrate (Figure 2C-D).
- Plank roots are vertically growing roots of which the upper part sticks out above the substrate (Figure 2E).

R. mucronata has well developed stilt roots, while *C. tagal* has stilt roots that only grow close to the stem and knee roots (Tomlinson, 1999).

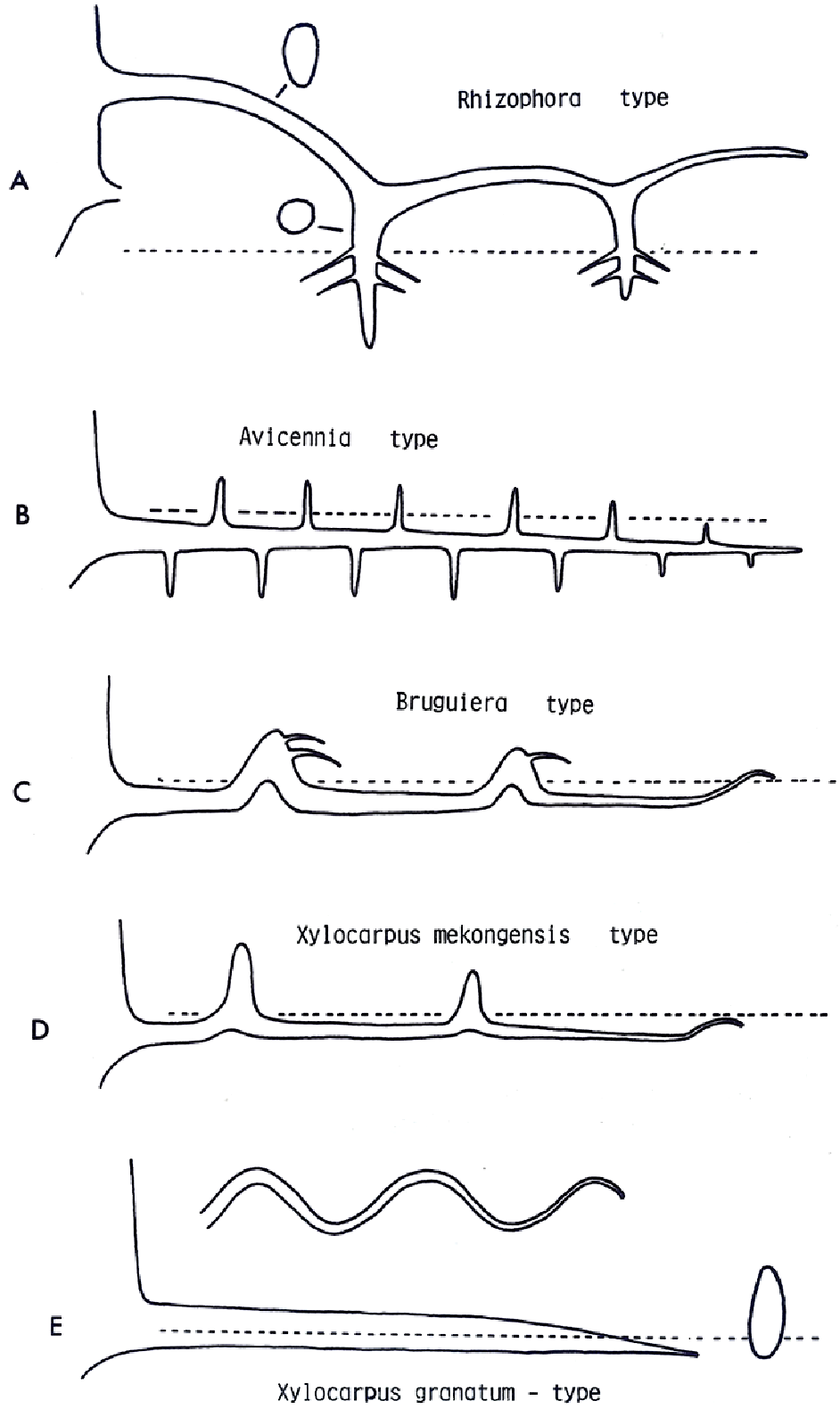

Figure 2. Types of aerial roots in mangroves. (Tomlinson, 1999)

2.3 Mechanisms to cope with salty water

Growing in soil water of high salinity causes difficulties for plants to take up water, because salt makes the osmotic potential more negative (Hogarth, 2007). To be able to take up enough water for living, mangroves developed mechanisms to cope with the high salinity conditions in which they live. To regulate their salt uptake, three mechanisms exist in mangrove species: salt exclusion, salt excretion and salt accumulation. Salt exclusion takes place at the membranes of root epidermal or cortical cells in the form of ultrafiltration of excess salts. Salt excretion is performed by salt glands in leaves which excrete excess salts. Some species can accumulate high concentrations of salt in the vacuoles of their leaf cells. Excess salts are then lost by leaf shedding or translocation of the salt outside the leaf (reviewed in Parida and Jha, 2010).

Both species studied here, *C. tagal* and *R. mucronata* are salt excluders (Parida and Jha, 2010), but do not have salt glands (Tomlinson, 1999). *R. mucronata* is also known to accumulate salt in its leaves (Tomlinson, 1999).

AIMS AND HYPOTHESES

The aim of this work is to study the growth and structural changes of the viviparous mangrove propagules of *Ceriops tagal* and *Rhizophora mucronata* before and after abscission from the mother tree. We want to gain more insight in the alleged dormancy period and the trigger for establishment (= to become firmly rooted), or in other words, how do propagules "know" that they have stranded after dispersal by sea water? We hypothesize that:

1. The percentage autonomous growth (compared to growth supported by the mother tree) is higher for *R. mucronata* propagules than for *C. tagal* propagules, because *R. mucronata* propagules are much bigger and therefore production costs are higher.

2. Between abscission from the mother tree and establishment, there is a period during which propagules do not grow or form roots, a delayed (see Introduction section 2.1.1) dormancy period. The dormancy is said to be delayed as we want to refer to a quiescent period between propagule dispersal and establishment, while dormancy is defined by a quiescent period between seed dispersal and germination.

3. Environmental cues break this delayed dormancy period so that root growth and therefore establishment is triggered.

4. Longitudinal growth of the propagule, root growth and leaf development during establishment are lower in high than in low salinity conditions and higher when relative air humidity is increased.

We test these hypotheses in the following experiments (Figure 3):

A. **Shading experiment**: propagules that are still attached to the mother tree are deprived of light to inhibit **autonomous growth**. When growth of these propagules is compared to the growth of uncovered propagules, the percentage autonomous growth can be estimated.
   → **Hypothesis 1**

B. **Growth** experiment with mature propagules collected from trees, placed **in a horizontal position** on different substrates: this experiment simulates the period between abscission and establishment, during which we expect dormancy, but tests the potential growth and structural changes of the propagules on different substrates.

   → **Hypotheses 2 and 3**

C. **Growth** experiment with the propagules of experiment B, placed **in a vertical position** in hydroponic set-ups with different salinity and air humidity treatments. This experiment simulates establishment and tests the effects on longitudinal growth and root formation of (1) the different substrates in used experiment B and (2) different salinity and air humidity treatments.

   → **Hypotheses 3 and 4**

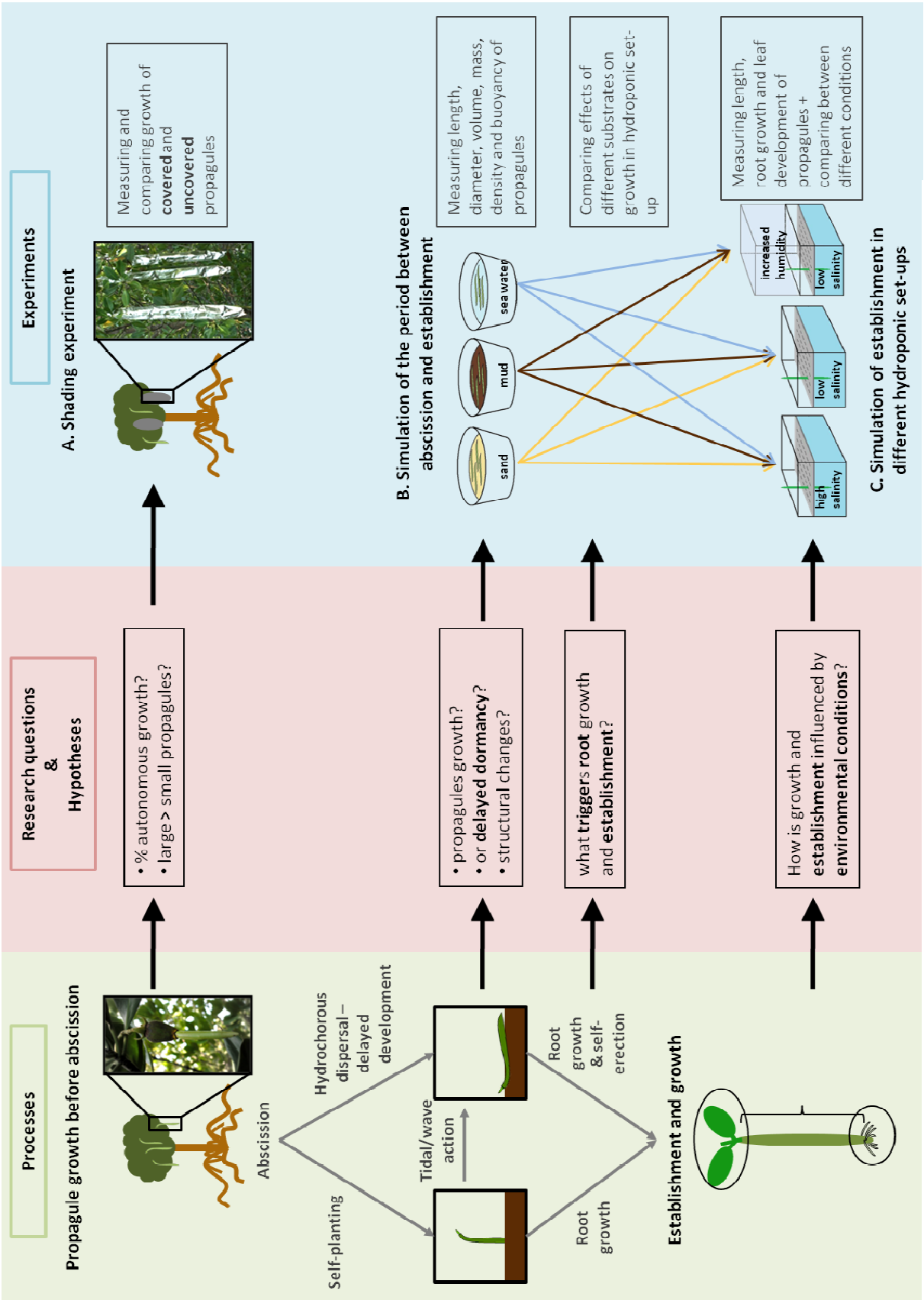

Figure 3. Schematic overview of the processes, the research questions and hypotheses and the experiments of this study.

MATERIAL AND METHODS

1. Description of study sites

This study was conducted in Gazi (Maftaha) Bay (39° 30’ E, 4° 22’ S), an open estuary located at the south coast of Kenya (East Africa) about 50 km south of Mombasa (Figure 4). The bay is surrounded by a mangrove forest of approximately 600 ha (UNEP, 1998). In this forest the ten East African mangrove species are represented: *Avicennia marina* (Forsk.) Vierh., *Bruguiera gymnorrhiza* (L.) Lam., *C. tagal*, *Heritiera littoralis* Dryand., *Lumnitzera racemosa* Willd, *Pemphis acidula* Forst., *R. mucronata*, *Sonneratia* alba Sm., *Xylocarpus granatum* Koen and *Xylocarpus moluccensis* (Lamk.) Roem. The dominant species in this mangal are *A. marina*, *C. tagal* and *R. mucronata* (Dahdouh-Guebas *et al.*, 2004).

The total area of the bay, excluding mangroves, is about 10 km². In the south, a shallow entrance of approximately 3500 m wide connects the bay to the Indian Ocean. The whole bay is relatively shallow with a mean depth of less than 5 m. The water circulation in the bay is dominated by the influence of the semi-diurnal tide regime (Kitheka, 1997). The spring tidal amplitude in Kenya is 3,5 m (Mobile Geographics, 2011). The strong tidal currents carry organic material and nutrients from the mangroves to the seagrass beds in the bay and are therefore responsible for a tight connection between these two ecosystems (Kitheka, 1997).

During the wet seasons, two seasonal rivers, Kidogoweni River and Mkurumuji River, are responsible for an influx of fresh water and nutrients in the bay (Kitheka, 1997).

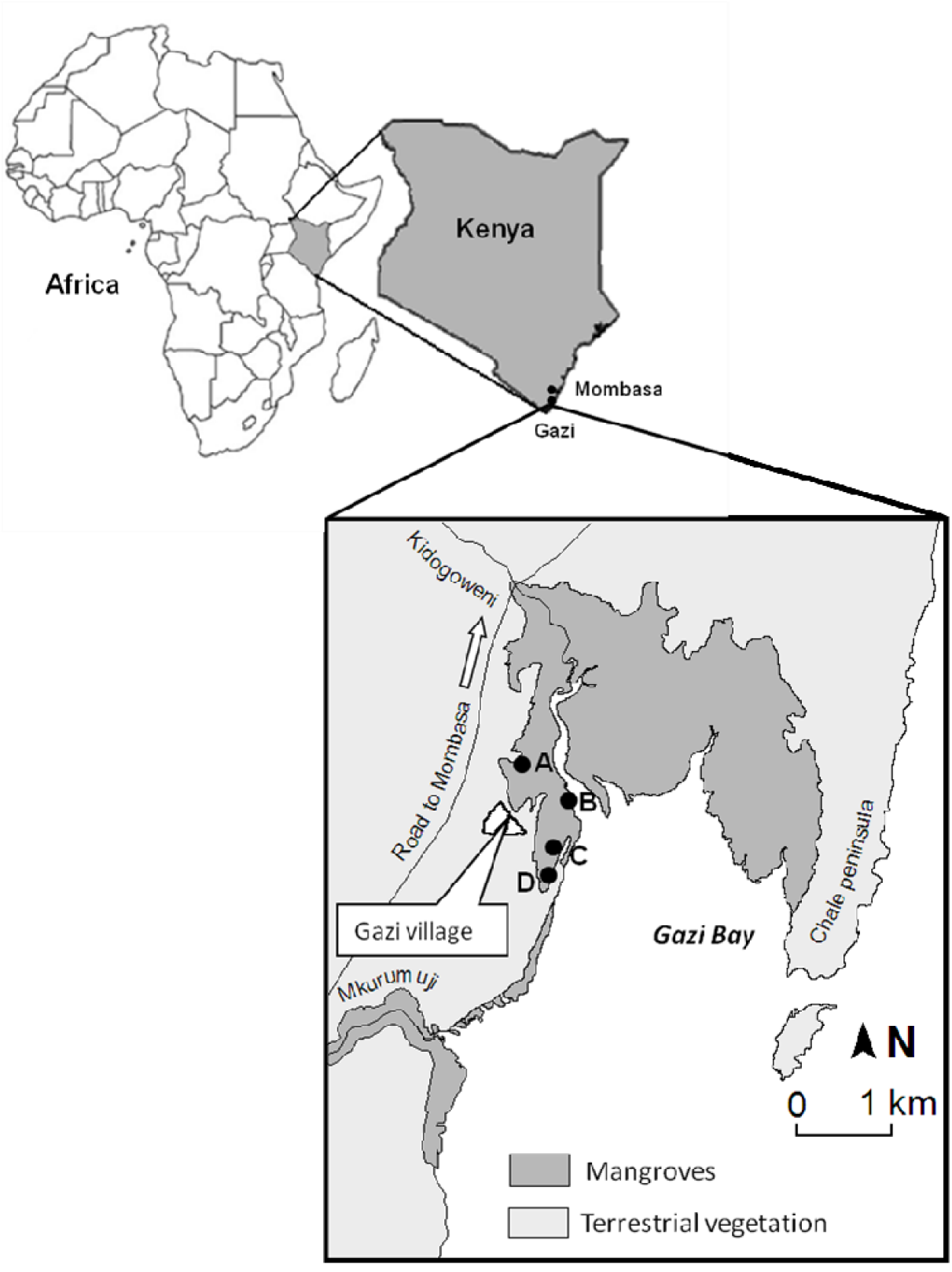

Figure 4. Map of the study sites in Gazi Bay, Kenya. Black dots and letters indicates the following study sites: A = landward site where *Ceriops tagal* propagules were collected, B = seaward site where *C. tagal* and *Rhizophora mucronata* propagules were collected and where the shading experiment for *R. mucronata* was set up, C = landward site where *C. tagal* and *R. mucronata* propagules were collected and where the shading experiment for *R. mucronata* was set up, D = seaward site where *R. mucronata* propagules were collected and where the shading experiment for *R. mucronata* was set up.

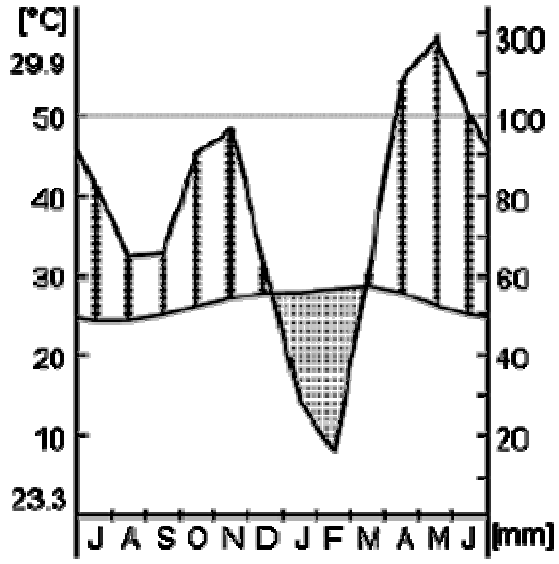

Figure 5. Climate diagram for Mombasa, Kenya (39° 36' O, 4° 0' Z). Dry season: December-March, dotted area. Short dry period: August-September. Long wet season (April-July) and short wet season (October-November); striped area. The mean daily minimum and maximum temperature of the coldest and warmest month are 23.3°C and 29.9°, respectively. The rainfall scale is lowered by 1/10 above 100 mm rainfall. (Lieth *et al.*, 1999)

The annual rainfall of the Kenyan coast is characterized by a bimodal pattern, every year two wet (April-July and November-December) and two dry (January-March and August-October) seasons occur (Camberlin and Planchon, 1997, Figure 5). This rainfall pattern is mainly formed by the southeast monsoon (March-October) and the northeast monsoon (October-March) (McClanahan, 1988). In the coastal regions of Kenya, the highest monthly rainfall occurs in May, instead of April as in the more inland regions (Camberlin and Planchon, 1997). All our experiments were conducted during the months February and March, during the dry season of the northeast monsoon.

For all experiments two sites were chosen, one which was frequently inundated and one that was less frequently inundated. Sites with high inundation frequency were close to sea or close to a tidal channel or pool, while sites with low inundation frequency are more landward and/or on higher elevations. Sites with low or high inundation frequency are further referred to as landward or seaward sites, respectively.

2. Studied species

Propagules of *Rhizophora mucronata* Lamk. and *C. tagal* (Perr.) C. B. Robinson were used in this study. Both species belong to the family of the Rhizophoraceae. The propagules of

these species differ in size and shape. *R. mucronata* has big propagules that have a smooth surface and can reach a length up to 80 cm, while those of *C. tagal* are small and ribbed and can reach a length of 35 cm (Duke, 2006). Nevertheless they share sufficient anatomical characteristics to compare both species in this study.

Mature propagules of both species that are still attached to the mother tree can be recognised by their yellow cotyledonary collar that becomes visible just under the fruit. At this point propagules of both species have a long hypocotyl, dark green to brown coloured for *C. tagal* and lighter green coloured for *R. mucronata*. For both species, the propagules are released from the mother tree by detaching the yellow cotyledonary collar from the hypocotyl, so that the stipules become visible.

Both species were selected for this study because of their viviparous reproduction and because they are dominant species in the mangrove forest in Gazi Bay. In addition, the distribution of these two species in Gazi Bay allows for comparison between trees that grow in zones with contrasting inundation frequencies. *C. tagal* trees grow in two elevation zones in the bay, one zone close to the sea and one zone far from the sea, more inland. However, more *C. tagal* trees grow in the inland zone. *R. mucronata* trees do not show this disjunct distribution, but grow in a wide range of elevations, but most trees grow close to sea.

3. Propagule sampling method

- To ensure that the collected propagules were mature, and of approximately the same age, propagules were collected by:
    - shaking branches or whole trees and collecting the propagules that were released,
    - or, when branches or trees were too stiff to shake, gently pulling propagules from a branch and only collecting those that detached easily.
- All propagules were numbered with a permanent marker.
- Time between propagule collection and start of the experiments varied between 12 and 48 hours. During this time propagules were stored in the shadow in the lab without specific conservation.

4. Measuring methods for general propagule characteristics

Propagule length, defined as the length of the hypocotyl without the plumule, was measured with a measuring tape.

Propagule diameter was measured at three fixed points on every propagule: at 1/8, 1/2 and 7/8 of the length, with digital callipers (with a precision of 0.01 mm).

Propagule mass was measured with an electronic balance (with a precision of 0.001 g).

To calculate the **propagule density** propagule volume was measured using the water-displacement-method. A plastic graduated cylinder with a volume of 2 litres was filled with water and placed on the balance. Using long forceps, a propagule was held in place in the cylinder without touching the cylinder itself. The plumule of the propagule was kept above the water surface and the length of the forceps that was under the water surface to keep the propagule in place was approximately the same during all measurements. The different forces acting on this propagule are: the gravitational force, the upward buoyancy and the force applied by the forceps to keep the propagule under the water surface. When all these forces are in balance with one another, the mass that is weighed by the electronic balance is equal to the mass of the water that is displaced by the submerged propagule. This is in agreement with the principle of Archimedes (Hughes, 2005). Because the density of water is very close to 1 g/ml, the mass displayed in grams on the electronic balance is a very close approximation of the volume of the propagule expressed in mm^3 or ml. The mass and volume data were then used to calculate the density of each propagule.

Propagule buoyancy was measured by putting the propagules one by one in a basin filled with seawater (for *C. tagal*) or in the bay (for *R. mucronata*). The water in the basin and 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 buoyancy and the orientation of the propagule relative to the water surface, as is shown in figure 6.

Root length was measured by measuring the longest root with digital callipers (with a precision of 0.01 mm). As long as the roots were too short to be measured precisely with digital callipers, root growth was scored using three different stages: (1) root bumps that can be felt and/or seen, (2) roots appearing through little cracks in the root bumps with a length up to 1.5 mm, and (3) roots with a length between 1.5 and 2 mm. These stages are presented in the results as 0.5, 1.0 and 1.5 mm, respectively.

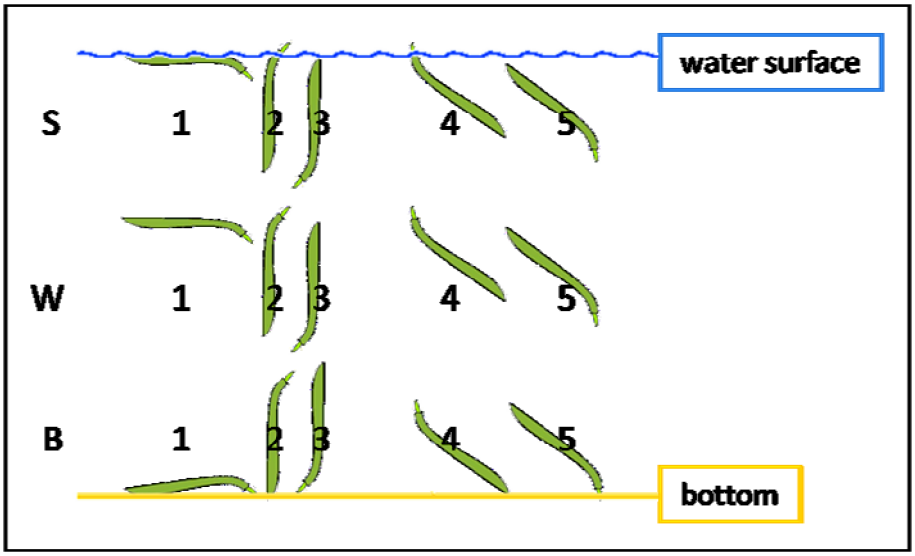

Figure 6. Key to scoring-method for the floating behaviour of propagules (Floating power: S= touching water surface, W = between water surface and bottom, not touching either, B = touching bottom and orientation: 1 = horizontal, 2 = vertical with plumule pointing up, 3 = vertical with plumule pointing down, 4 = diagonal with plumule pointing up, 5 = diagonal with plumule pointing down).

5. Experimental set-up

Three experiments were performed to study the growth and development of *C. tagal* and *R. mucronata* propagules:

A. before abscission from the mother tree
   - ➤ shading experiment on the tree

B. during the period between release from the mother tree and establishment
   - ➤ growth experiment in horizontal position on different substrates

C. during and after establishment
   - ➤ growth experiment in vertical position in different treatments

In addition, three experiments were performed to study the potential interfering effect of:

1. the type of shading material on the results of experiment A
2. changing air humidity on the results of experiment B
3. the hydroponic conditions on root growth results of experiment C

5.1 Shading experiment on the tree (experiment A)

To study the autonomous growth of propagules before abscission versus the growth supported by nutrients from the mother tree, a shading experiment was set up for *C. tagal* and *R. mucronata*. The experiment was carried out on landward and seaward sites to study the potential interfering effect of salinity and water availability.

Wherever available, propagules growing in pairs at the same branch were selected. The propagule pairs were supplemented with a set of solitarily growing propagules of approximately the same length and growing under comparable light conditions. In total 186 *C. tagal* propagules and 114 *R. mucronata* propagules were tagged. To follow their growth, each individual propagule was tagged and its length was measured. One propagule of each pair and half of the solitary propagules per tree were covered with aluminium foil. After 25-28 days, the lengths of all propagules were measured a second time.

Unexpected root development was observed and measured.

5.2 Control experiment: potential influence of shading material

A control experiment was arbitrarily set up on the landward site for *C. tagal* and on the seaward site for *R. mucronata*. The goal of this experiment was to find out if the lack of air circulation around propagules wrapped in aluminium foil caused premature abscission. Therefore, bags of about the same size as the aluminium bags were made out of two layers of fabric. The inner layer was a black fabric that existed of 50 % cotton and 50 % polyester, this layer was meant to inhibit as much light as possible. The outer layer was made of white, 100 % cotton fabric, its function was to avoid heat accumulation in the bag. The lengths of these propagules were measured before they were wrapped and at the end of the experiment. The time between the two measurements was 14 days for *C. tagal* and 16 days for *R. mucronata*.

5.3 Growth experiment in horizontal position on different substrates (experiment B)

For both *R. mucronata* and *C. tagal* 69 propagules were collected on a landward site and 69 propagules on a seaward site. Landward sites are sites with low inundation frequency and seaward sites are sites with high inundation frequency. Propagules from seaward sites were collected on the 12th and the 13th of February 2011 and propagules from landward sites were collected on the 13th of February 2011.

On day 1 of the experiment (13th of February for seaward sites, 15th of February for landward sites), all propagules were subjected to one of three treatments. These treatments existed of plastic basins filled with sea water, dry sand, or moist mud, simulating the different environments a propagule can encounter after abscission. The dry sand and moist mud were collected on the same sites as the propagules. The mud was kept moist using fresh water to avoid an increase in salinity during the experiment. Sea water was collected in the bay and

replaced every five to seven days. For each site (landward and seaward) there were 25 propagules of each species in every treatment. The basins were placed under a canopy, shielded from sun, except for one to two hours in the morning. Every five to seven days, the basins were replaced randomly.

For each site and species, every six days, starting on day 1, five propagules were taken out of each treatment to start experiment C (see below). Exceptionally, on day 1 itself, only three propagules were taken out of each treatment to start experiment C. For these propagules length, diameter, mass, volume and buoyancy were measured and compared with the initial values, measured within two days after propagule collection.

5.4 Control experiment: potential influence of changing air humidity

To test if propagules of *C. tagal* are able to take up water from the atmosphere after dehydration during dispersal, five propagules were collected from a landward site. These propagules were weighed and then left to dry on dry sand, in moderate shade for one day and one night and weighed again afterwards. Then the propagules were put in a plastic box with a wet sponge, not touching the propagules. To make sure the plastic box was airtight it was wrapped in plastic foil. The propagules were left in this box for approximately 24 hours and were then weighed again.

5.5 Growth experiment in vertical position in different treatments (experiment C)

Three of the five propagules that were taken out of each of the three substrates of experiment B (see above) were subsequently subjected to one of three treatments simulating the different environmental conditions that can be encountered during and after establishment. The propagules were put vertically in three different hydroponic set-ups: a basin with 100 % sea water, 50% seawater, or a basin filled with 50 % seawater and placed in a small greenhouse containing a wet sponge to increase the relative humidity. The propagules were put in the basins through slits in a piece of polyether foam at regular distances (5-7 cm) from one another so that the lower part of the propagule, 3 to 7 cm for *C. tagal* and 5 to 15 cm for *R. mucronata*, was under water. These lower parts of the propagules were shut from light by the polyether foam on top and by aluminium foil wrapped around the sides of the basins. The basins were placed under the same canopy as the basins of experiment B, shielded from sun, except for one to two hours in the morning. Every five to seven days, the basins were replaced randomly.

The length of the propagules, root growth and leaf development were measured every two days, from the 19th of February onward.

5.6 Control experiment: potential influence of hydroponic conditions on root growth

To test the possible influence of the hydroponic set-up on the rate of root development and growth, a small control experiment was set up at two places in the mangrove forest. For both species six abscised propagules were collected from the beach and six mature propagules were collected from the trees. For *C. tagal*, propagules were collected from trees on a site with low inundation frequency and for *R. mucronata*, propagules were collected on a site with high inundation frequency. The propagules of each species were then planted on the same site as where propagules were collected from trees: a site with low inundation frequency for *C. tagal* and a site with high inundation frequency for *R. mucronata*. Five and seven days after planting these propagules, the root growth was checked by carefully taking the propagules out of the sediment. The root growth was scored in the same way as for the root growth of propagules in the hydroponic set-ups.

6. Statistical analyses

All data about the change in propagule length, diameter, mass, volume, density and root length over a certain time span were first tested for normality and homogeneity of variances, using Statistica 8. To test these two assumptions the Shapiro-Wilk test and Levene's test were used, respectively, but none of the data sets fitted the assumptions. Further, logarithm, square root and inverse transformations were tried, but this did not improve the normality of the data.

Therefore non-parametrical methods were used to test the significance of our data. We used the Aligned Rank Transform (ART) for nonparametric factorial data analysis (Wobbrock *et al.*, 2011). A free available web-based program, ARTweb 1.1.4, was used to perform the rank transformation. Then, factorial ANOVA was used to analyze the transformed data, this was done using Statistca 8.

To test the significance of the correlation between length growth and root length after 24 days in a hydroponic set-up, parametric methods could be used because the plotted data were distributed normally and the variances were homogenous. This was also tested with the Shapiro-Wilk test and Levene's test, using Statistica 8.

RESULTS

1. Growth and development of *C. tagal* and *R. mucronata* propagules before abscission from the mother tree (experiment A)

56 of the *C. tagal* propagules and 58 of the *R. mucronata* propagules abscised before the end of the experiment and could therefore not be measured for a second time to follow up growth. Of the 130 remaining *C. tagal* propagules, 72 were covered with aluminium foil, while for *R. mucronata* 33 of the 56 remaining propagules were covered.

However, different reasons rendered the length measurement data for the remaining propagules unfit for use in this study. First, the propagules that were used were already too old. Therefore, not enough length growth could be measured and it is probably also the reason why so many propagules abscised before the end of the experiment. Second, due to difficulties to reach the propagules and their unregular shape, measurement errors were too large.

However, other very interesting results appeared during this experiment. Surprisingly, root growth was observed on part of the covered propagules of both species (Figure 7). 25 of the 72 measured and shaded *C. tagal* propagules (34.7 %) showed noticable root growth. Of these propagules showing root growth 44.0 % had root bumps and 56. % had visible roots with a length up to 2 mm. For *R. mucronata* 23 of the 33 measured and shaded propagules (69.7 %) showed root growth. 13.0 % of these propagules had root bumps, 69.6 % had visible roots with a length up to 2 mm and 17.4 % had roots with a length of 2 to 15 mm. None of the uncovered propagules of either species showed any visible signs of root growth.

2. Control experiment: influence of shading material

For *R. mucronata*, none of the 16 propagules wrapped in foil or fabric abscised from the mother tree during the 16 days of the experiment. Three of the eight propagules wrapped in aluminium foil had roots with a length up to 2 mm and one propagule had roots of 3-5 mm. None of the propagules wrapped in fabric showed visible root growth.

None of the *C. tagal* propagules wrapped in aluminium foil were released from the mother tree before the end of the 14-16 day long experiment, and also none of the propagules wrapped in fabric abscised. Root bumps, but no visible roots were observed for one of the propagules covered with aluminium foil.

Figure 7. Root development of a *Rhizophora mucronata* propagule, that had been wrapped in aluminium foil for 28 days, before abscission from the mother tree.

3. Growth and development of *C. tagal* and *R. mucronata* propagules during the period between release from the mother tree and establishment (experiment B)

Table 1 gives the mean and standard deviation of the propagule length, diameter, volume and mass, per species and per site, measured within 48 hours after propagule collection.

Table 1. Average and standard deviation of propagule length, diameter, volume and mass, measured within 48 hours after collection.

	Length (cm)		**Diameter** (mm)		**Volume** (mm^3)		**Mass** (g)	
	mean	SD	mean	SD	mean	SD	mean	SD
C. tagal								
landward site	26.6	2.7	7.44	0.76	9.4	1.7	9.023	1.661
seaward site	23.9	3.4	7.64	0.69	9.3	1.8	8.994	1.852
R. mucronata								
landward site	39.5	4.8	15.54	1.94	62.6	17.4	62.436	17.573
seaward site	43.5	5.3	15.20	1.63	59.9	15.68	60.837	16.845

During experiment B, simulating the period between release from the mother tree and establishment, change in propagule length with time was measured. A clear effect of time was shown on the length of most *R. mucronata* propagules, but not of *C. tagal* propagules (Figure 8, Table 2). The different substrates on which the propagules were left for different time periods did have a significant effect (Table 2). The median changes in length of *C. tagal* propagules were all below 5 mm and did not show a clear trend (Figure 8A-B). *R. mucronata* propagules collected on the seaward site and left in sea water gained up to 6 mm on average after 24 days (Figure 8D). In contrast, *R. mucronata* propagules from both sites left on moist mud or dry sand had shrunk and propagules from the landward site left in sea water showed almost no length change.

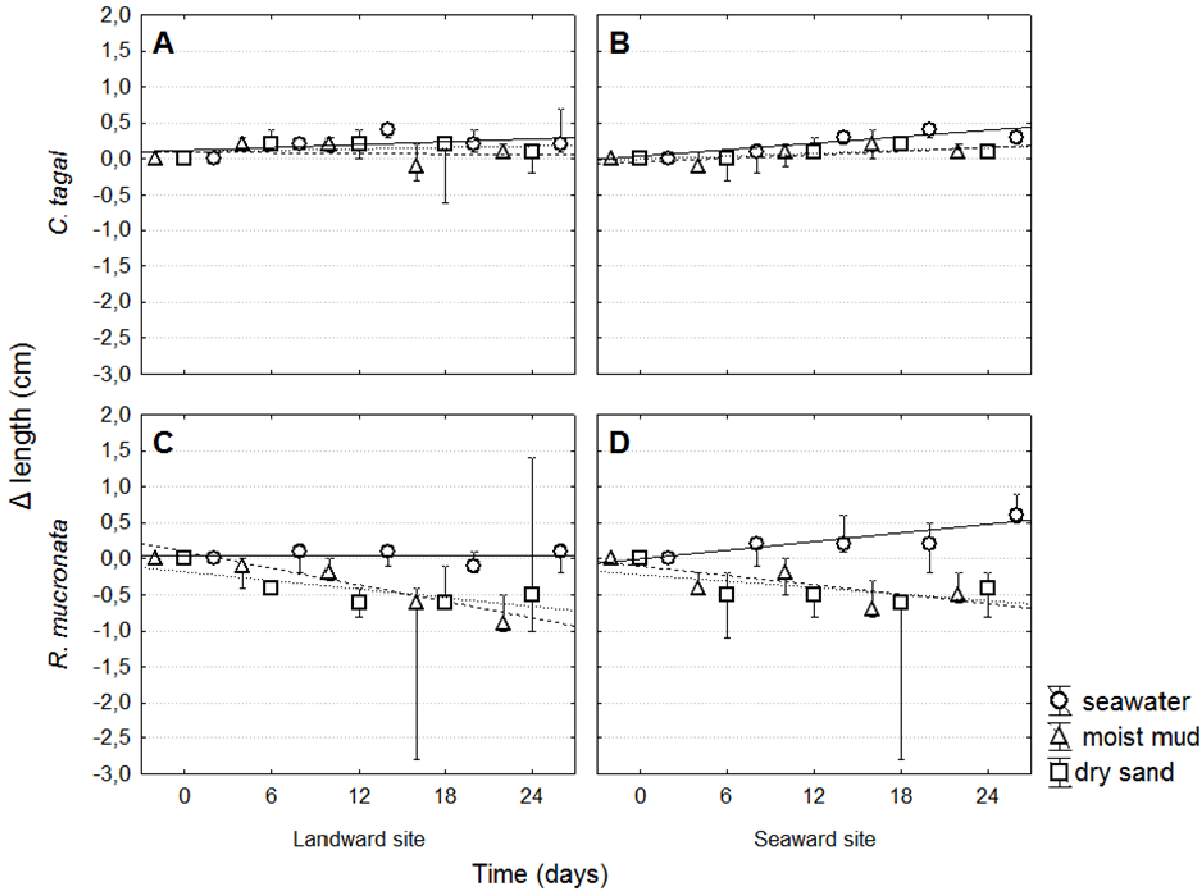

Figure 8. Change in length with time of *Ceriops tagal* propagules (A, B) and *Rhizophora mucronata* propagules (C, D) spread out horizontally in basins filled with three different substrates. They simulate the different environmental conditions possible in the period between release from the the mother tree and establishment (experiment B). Propagules were collected from landward (A, C) and seaward (B, D) sites. Plotted values are medians with 25 and 75 percentiles, N = 3-5.

Table 2. F-values and probabilities for all main effects and significant interaction effects for the change in length of *Ceriops tagal* and *Rhizophora mucronata* propagules during experiment B, simulating the period between release from the mother tree and establishment. Sample sizes differed for different time periods, numbers between brackets after sample sizes refer to time (days).

Variable	F-value	P-value	N
Species	172.69	<0.0001	138
Site	3.73	ns	138
Time	2.04	ns	36 (0), 60 (6-24)
Substrate	65.75	<0.0001	92
Species*Substrate	31.39	<0.0001	46
Species*Time*Substrate	4.16	<0.001	6 (0), 10 (6-24)

Table 3. F-values and probabilities for all main effects and significant interaction effects for the change in diameter at ½ of the length of *Ceriops tagal* and *Rhizophora mucronata* propagules during the experiment simulating the period between release from the mother tree and establishment (experiment B). Sample sizes differed for different time periods, numbers between brackets after sample sizes refer to time (days).

Variable	F-value	P-value	N
Species	11.44	0.001	138
Site	1.83	ns	138
Time	19.65	<0.0001	36 (0), 60 (6-24)
Substrate	70.37	<0.0001	92
Species*Time*Substrate	2.99	0.01	6 (0), 10 (6-24)

The diameter at 1/2 of the length of the hypocotyl also changed during this experiment (Figure 9). The length of the period for which propagules were placed on one of the three substrates had a significant, mainly negative effect on the diameter change (Table 3). The different substrates additionally influenced the change in diameter considerably. For both species, the diameters of propagules decreased more when placed on dry sand, than on moist mud, than in seawater. For propagules of *R. mucronata* the difference in diameter change with time between sea water on one hand and dry sand and moist mud on the other hand was larger than the difference between moist mud and dry sand. This was not the case for propagules of *C. tagal* resulting in a significant interaction between species, time and substrate effects (Table 3). The diameter change differed significantly between both species, but not between the landward and the seaward site (Table 3).

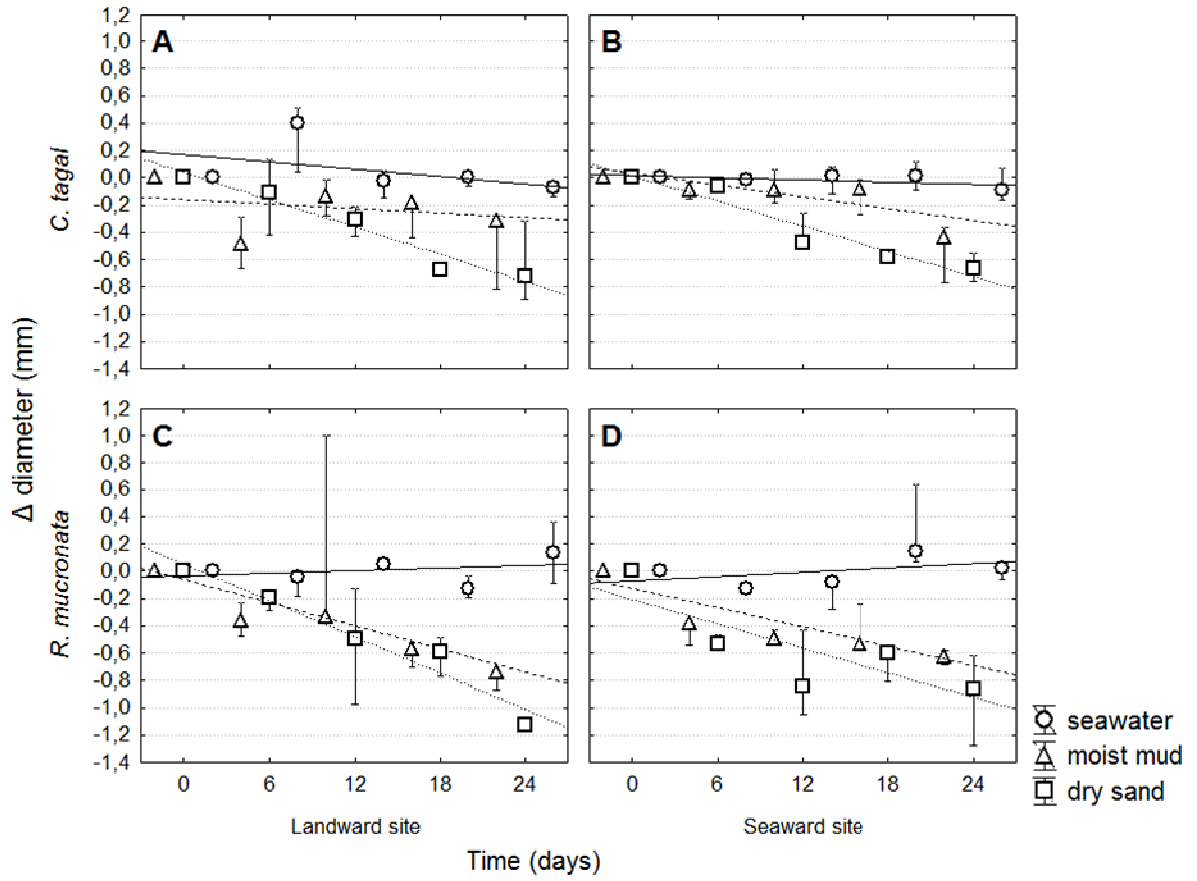

Figure 9. Change in propagule diameter at ½ of the length of *Ceriops tagal* propagules (A, B) and *Rhizophora mucronata* propagules (C, D) during the experiment simulating the period between release from the mother tree and establishment (experiment B). Propagules were collected from landward (A, C) and seaward (B, D) sites and spread out horizontally on one of three substrates. Plotted values are medians and 25 and 75 percentiles, N = 3-5.

In accordance with the propagule length and diameter, the volume of propagules decreased during experiment B (Figure 10). The time for which the propagules were left on the substrate had a significant negative effect, that was stronger for *R. mucronata* than for *C. tagal* (Table 4). All substrates, including sea water, had a significant (Table 4) reducing effect on the volume of propagules of both species. But the volume decreased most on dry sand, less on moist mud and least in sea water. For *R. mucronata*, there was a larger volume change with time between sea water on the one side and moist mud and dry sand on the other side. For *R. mucronata* propagules, the sites where the propagules were collected also significantly influenced the change in volume (Table 4). *R. mucronata* propagules collected on the landward site exhibited a larger volume loss. The proportion of the volume lost, relative to the initial volume measured at the time of collection, was about 10 % for *C. tagal* propagules, while the relative volume loss was only about 5 % for *R. mucronata* propagules.

Table 4. F-values and probabilities for all main effects and significant interaction effects for the change in volume of *Ceriops tagal* and *Rhizophora mucronata* propagules during the experiment simulating the period between release from the mother tree and establishment (experiment B). Sample sizes differed for groups with different species, sites, time and substrates. The variables defining the groups are indicated between brackets after the sample size (C = *C. tagal*, R = *R. mucronata*; L = landward site, S = seaward site; W= sea water, M = moist mud, D = dry sand; numbers refer to time (days)).

Variable	F-value	P-value	N
Species	367.47	<0.0001	138 (C), 136 (R)
Site	4.55	<0.05	138 (L), 136 (S)
Time	88.09	<0.0001	34 (0), 60 (6-24)
Substrate	180.99	<0.0001	91 (M, W), 92 (D)
Species*Time*Substrate	8.85	<0.0001	5 (R; 0; M, W), 6 (0), 10 (6-24)

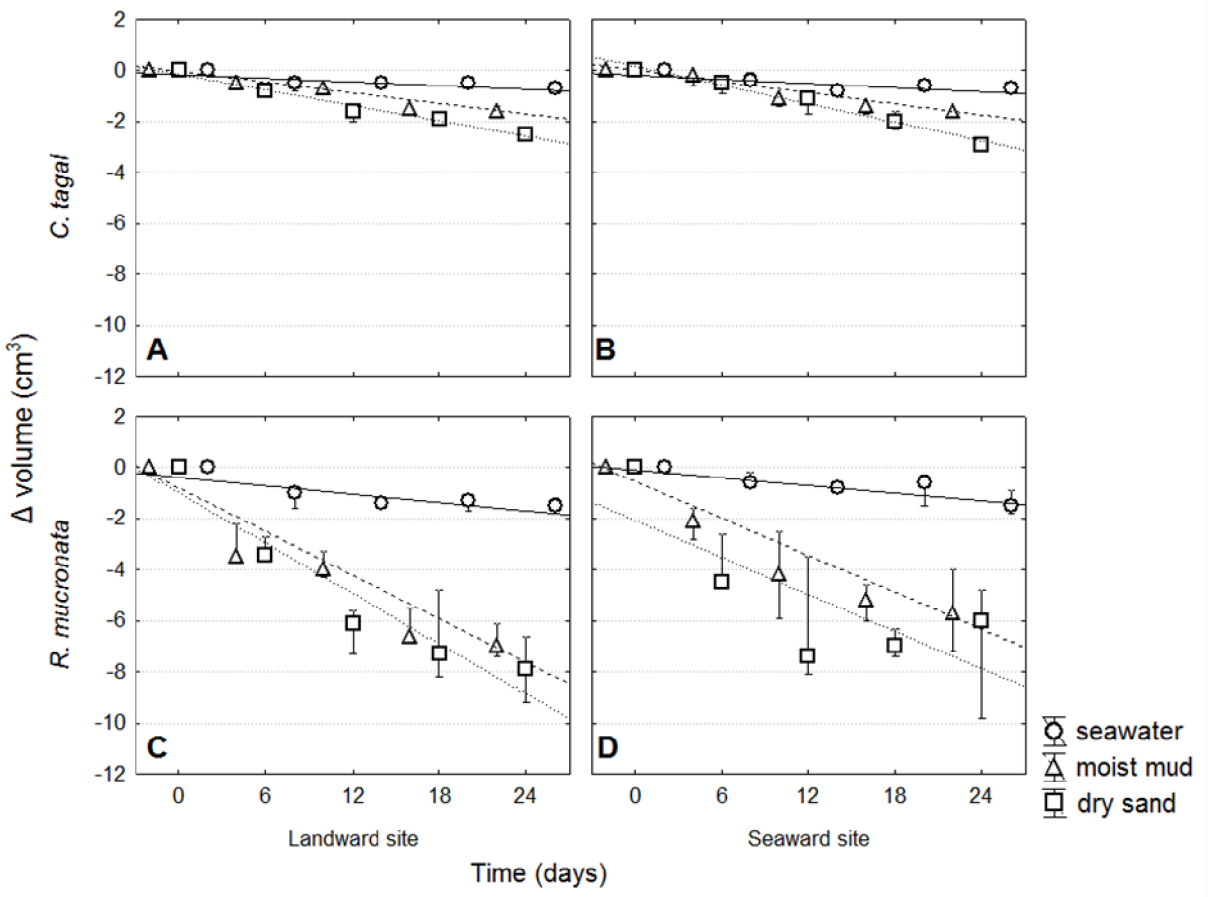

Figure 10. Change in volume of C*eriops tagal* propagules (A, B) and *Rhizophora mucronata* propagules (C, D) during the experiment simulating the period between release from the mother tree and establishment (experiment B). Propagules were collected from landward (A, C) and seaward (B, D) sites and spread out hozirontally on one of three substrates. Plotted values are medians and 25 and 75 percentiles, N = 3-5.

Figure 11 shows the change in mass during experiment B, simulating the period between abscission and establishment. The length of the period that propagules were left on one of the three substrates had a significant influence on the mass of the propagules of both species (Table 5, Figure 11). The longer the propagules were left on a substrate, the more mass was

lost. However, the effect of the three substrates differed significantly (Table 5). Propagules on dry sand lost more mass than propagules on moist mud, while propagules left in sea water showed little (propagules of *R. mucronata*) or almost no (propagules of *C. tagal*) decrease in mass. For *R. mucronata* propagules, the difference in mass change between propagules left in sea water and propagules left on moist mud was larger than the difference between propagules left on moist mud and propagules left on dry sand. Changes in mass were significantly (Table 5) smaller for *C. tagal* propagules than for *R. mucronata* propagules. However, proportionoal to the initial mass measured at the time of collection, the mean mass change for both species was 4-5 %. As was seen for volume change, also for change in mass the site-effect was the weakest, but still statistically significant (Table 5) and most pronounced in *R. mucronata* propagules. Propagules collected on the landward site showed significantly (Table 5) more mass loss than propagules collected on the seaward site and the variation in mass change was larger for propagules from the seaward site.

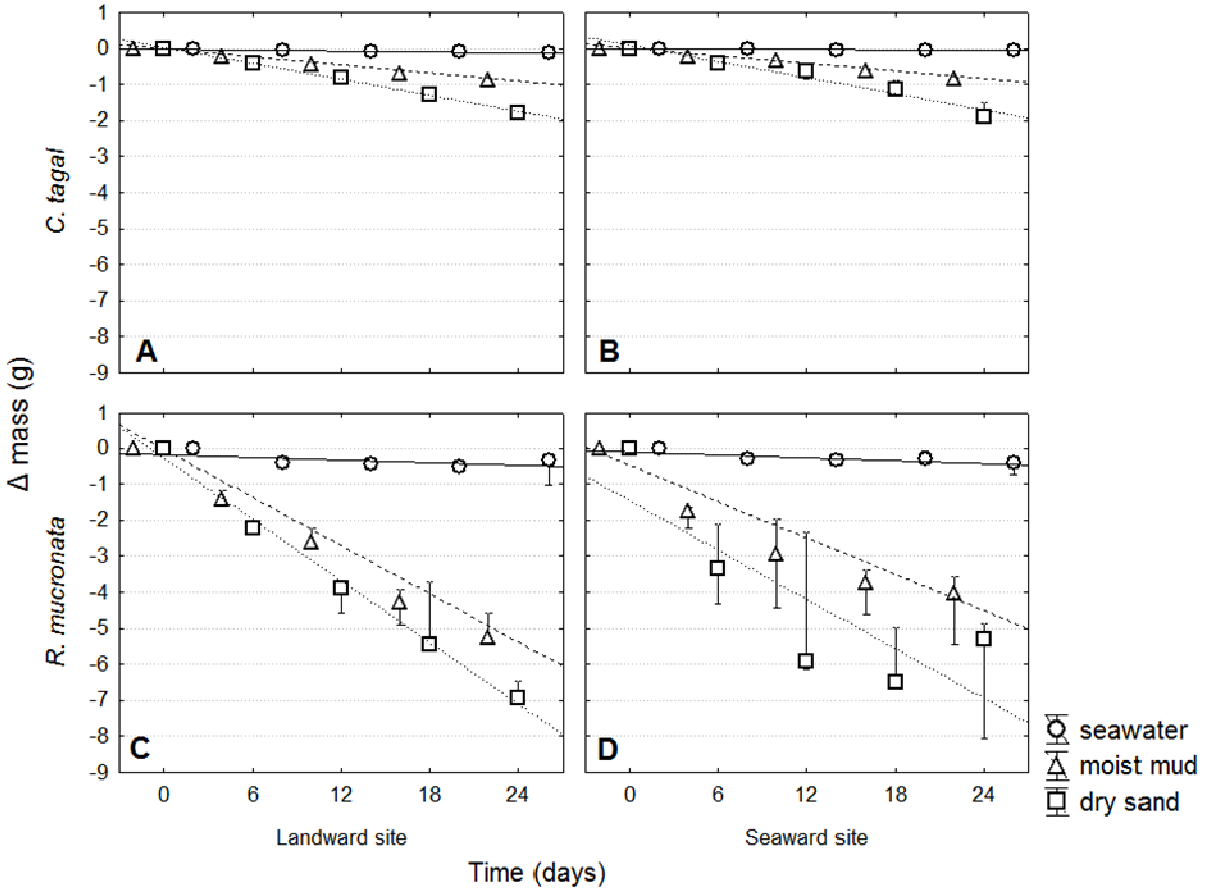

Figure 11. Change in mass of *Ceriops tagal* propagules (A, B) and *Rhizophora mucronata* propagules (C, D) during the experiment simulating the period between release from the mother tree and establishment (experiment B). Propagules were collected from landward (A, C) and seaward (B, D) sites and spread out on one of three substrates. Plotted values are medians and 25 and 75 percentiles, N = 3-5.

Table 5. F-values and probabilities for all main effects and significant interaction effects for the change in mass of *Ceriops tagal* and *Rhizophora mucronata* propagules during the experiment simulating the period between release from the mother tree and establishment (experiment B). Sample sizes differed for different time periods, numbers between brackets after sample sizes refer to time (days).

Variable	F-value	P-value	N
Species	400.56	<0.0001	138
Site	4.65	<0.05	138
Time	100.49	<0.0001	36 (0), 60 (6-24)
Substrate	234.69	<0.0001	92
Species*Site*Time*Substrate	3.35	<0.01	3 (0), 5 (6-24)

Table 6. F-values and probabilities for all main effects and significant interaction effects for the change in density of *Ceriops tagal* and *Rhizophora mucronata* propagules during the experiment simulating the period between release from the mother tree and establishment (experiment B). Sample sizes differed for groups with different species, sites, time and substrates. The variables defining the groups are indicated between brackets after the sample size (C = *C. tagal*, R = *R. mucronata*, L = landward site, S = seaward site, W= sea water, M = moist mud, D = dry sand, numbers refer to time).

Variable	F-value	P-value	N
Species	263.93	<0.0001	138 (C), 136 (R)
Site	0.44	ns	138 (L), 136 (S)
Time	49.85	<0.0001	34 (0), 60 (6-24)
Substrate	17.53	<0.0001	91 (M, W), 92 (D)
Time*Substrate*Species	5.48	<0.0001	5 (R; M, W; 0), 6 (R; D; 0), 6 (C; M, W, D; 0), 10 (R, C; M, W, D; 6-24)

Propagule density is per definition related to mass and volume, but, as was observed here, does not necessarily follow the same trend (Figure 12). The length of the period for which propagules were placed on one of the three substrates had a significant positive influence on propagule density (Table 6). For *C. tagal* there was a clear difference between the effects of the different substrates on density change. However, for *R. mucronata* propagules, each of the three substrates had a very similar effect on density change. The density of *C. tagal* propagules left in sea water increased more slowly than for propagules left on moist mud or dry sand. Comparing propagules of both species shows that the density change for *C. tagal* propagules is significantly larger than for *R. mucronata* propagules (Table 6) and the variation in density change is larger for *C. tagal* than for *R. mucronata*.

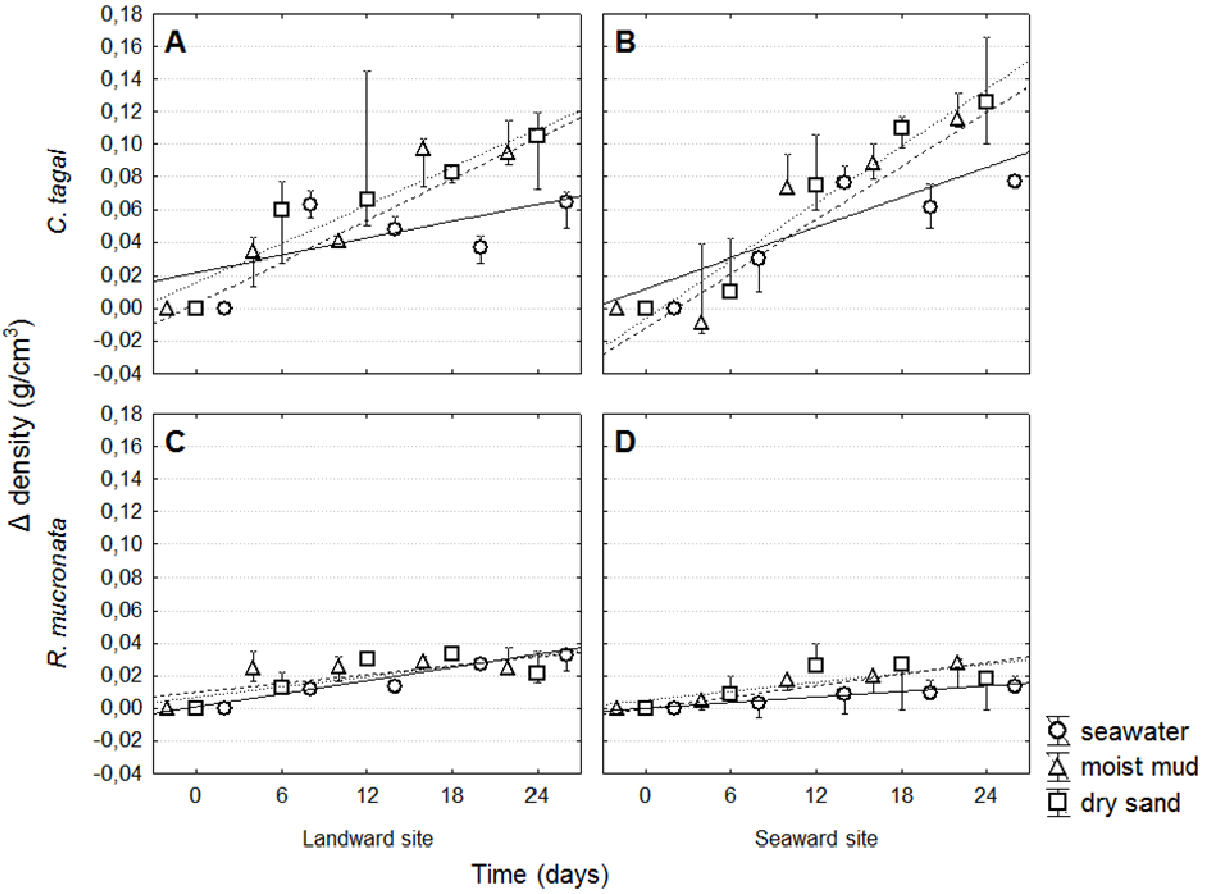

Figure 12. Change in density of *Ceriops tagal* propagules (A, B) and *Rhizophora mucronata* propagules (C, D) during the experiment simulating the period between release from the mother tree and establishment (experiment B). Propagules were collected from landward (A, C) and seaward (B, D) sites and spread out on one of three substrates. Plotted values are medians and 25 and 75 percentiles, N = 3-5.

As a result of the change in propagule density, propagule buoyancy changed as well (Figure 13, Figure 14). For *C. tagal* propagules there was a clear effect of substrate on floating power with sea water > moist mud > dry sand (Figure 13). One to two days after collection of the propagules, before they had been in contact with any of the substrates (0 days), the majority of the propagules were floating. After 12 days, most of the propagules placed on dry sand sank. Only propagules lying on dry sand sank to lie flat on the bottom (B1) within the 24 days of our experiment. Propagules collected from the seaward site and left in sea water did not sink at all within 24 days.

With respect to substrate, *R. mucronata* propagules behaved much more randomly, than propagules of *C. tagal* (Figure 14). Comparing sites, more propagules from the seaward site than from the landward site sunk to the bottom, irrespective of the time they had been in contact with one of the substrates. In contrast to *C. tagal*, none of the *R. mucronata* propagules was seen lying flat on the bottom.

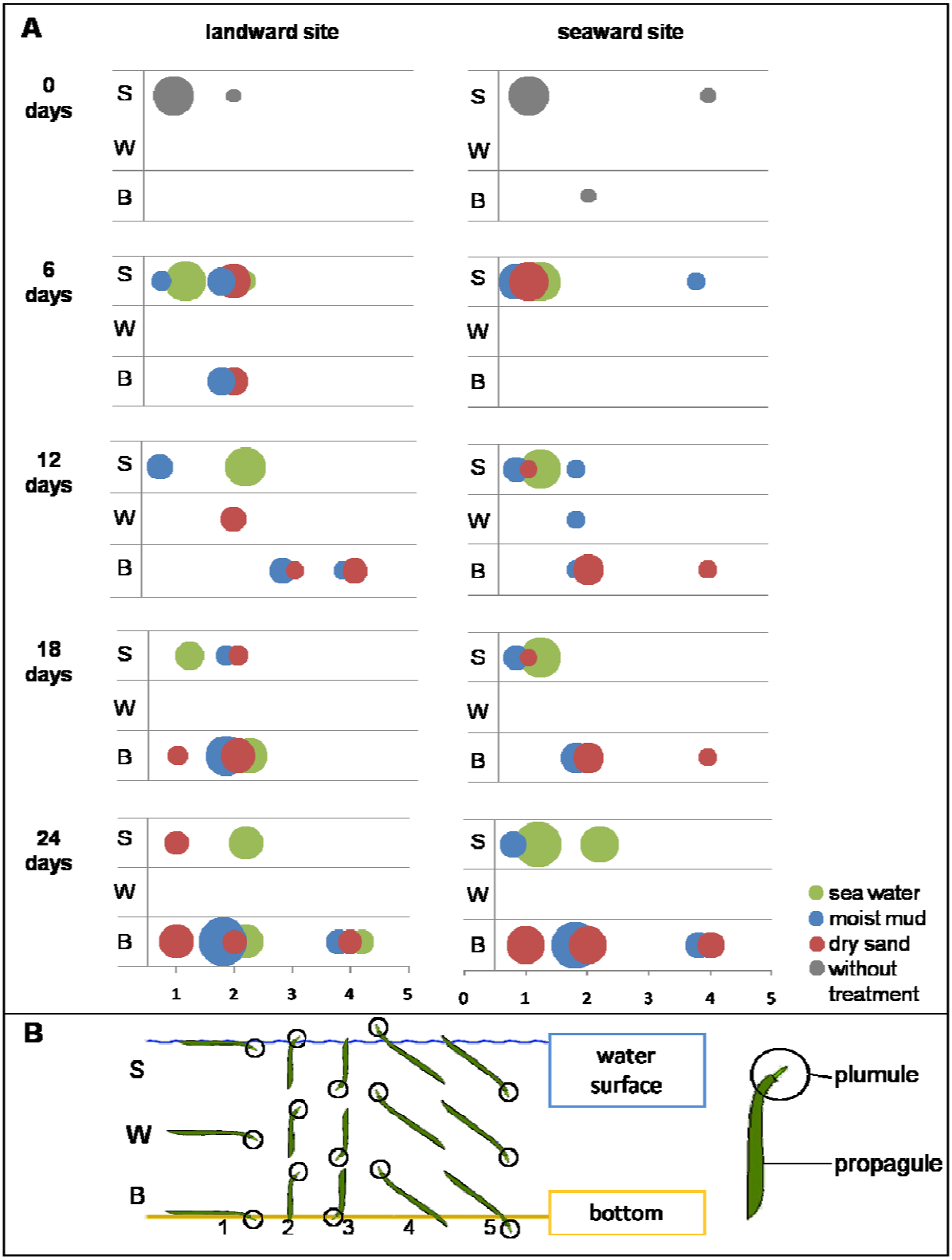

Figure 13. (A) Floating power (y-axis: S = touching water surface, W = between water surface and bottom, not touching either, B = touching bottom, as depicted in B) and orientation (x-axis, 1 = horizontal, 2 = vertical with plumule pointing up, 3 = vertical with plumule pointing down, 4 = diagonal with plumule pointing up, 5 = diagonal with plumule pointing down, as depicted in B) for 26 sets of 5-9 *Ceriops tagal* propagules left on one of three substrates for 5 periods of different length during the experiment simulating the period between release of the mother tree and establishment (experiment B). Size of bubbles indicates percentage of propagules with the same floating behaviour. (B) Code explanation.

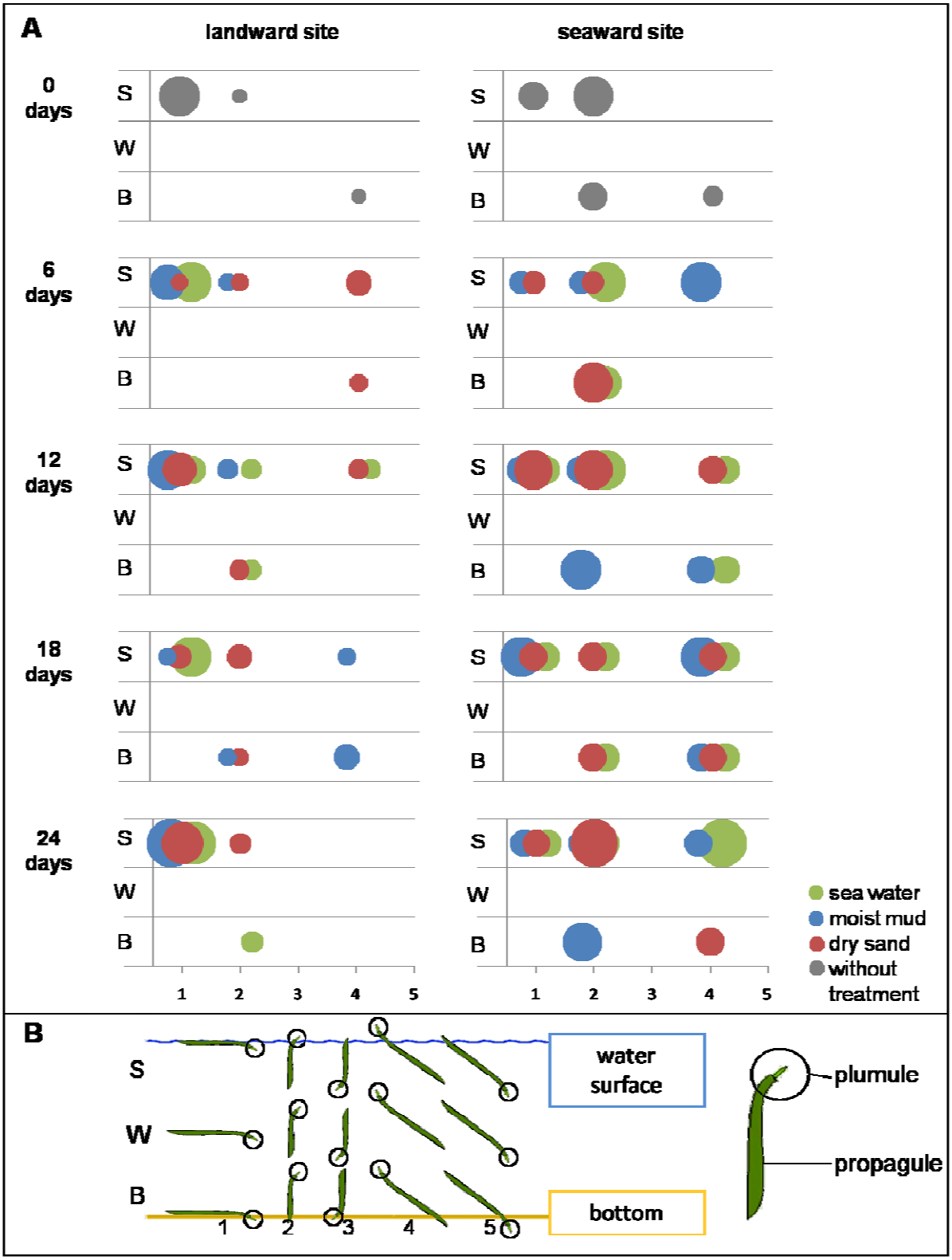

Figure 14. (A) Floating power (y-axis: S= touching water surface, W = between water surface and bottom, not touching either, B = touching bottom, as depicted in B) and orientation (x-axis, 1 = horizontal, 2 = vertical with plumule pointing up, 3 = vertical with plumule pointing down, 4 = diagonal with plumule pointing up, 5 = diagonal with plumule pointing down, as depicted in B) for 26 sets of 5-9 *Rhizophora mucronata* propagules left on one of three substrates for 5 periods of different length during the experiment simulating the period between release of the mother tree and establishment (experiment B). Size of bubbles indicates percentage of propagules with the same floating behaviour. (B) Code explanation.

The buoyancy power and orientation of seperate pieces of propagules, which themselves exhibited different floating behaviour, is shown in figure 15. The same pattern of floating and sunken pieces was observed for *R. mucronata* propagules that floated at the surface and for propagules that stood vertically on the bottom with their plumule pointing up (Figure 15A, C). For the two propagules of *R. mucronata* floating vertically with their plumule at the water surface the middle pieces showed different behaviour, for one propagule it floated while for the other it sank. Similarly, for the 18 *C. tagal* propagules standing diagonally on the bottom, the middle piece sank for sixteen propagules, while in the other two cases the middle piece floated.

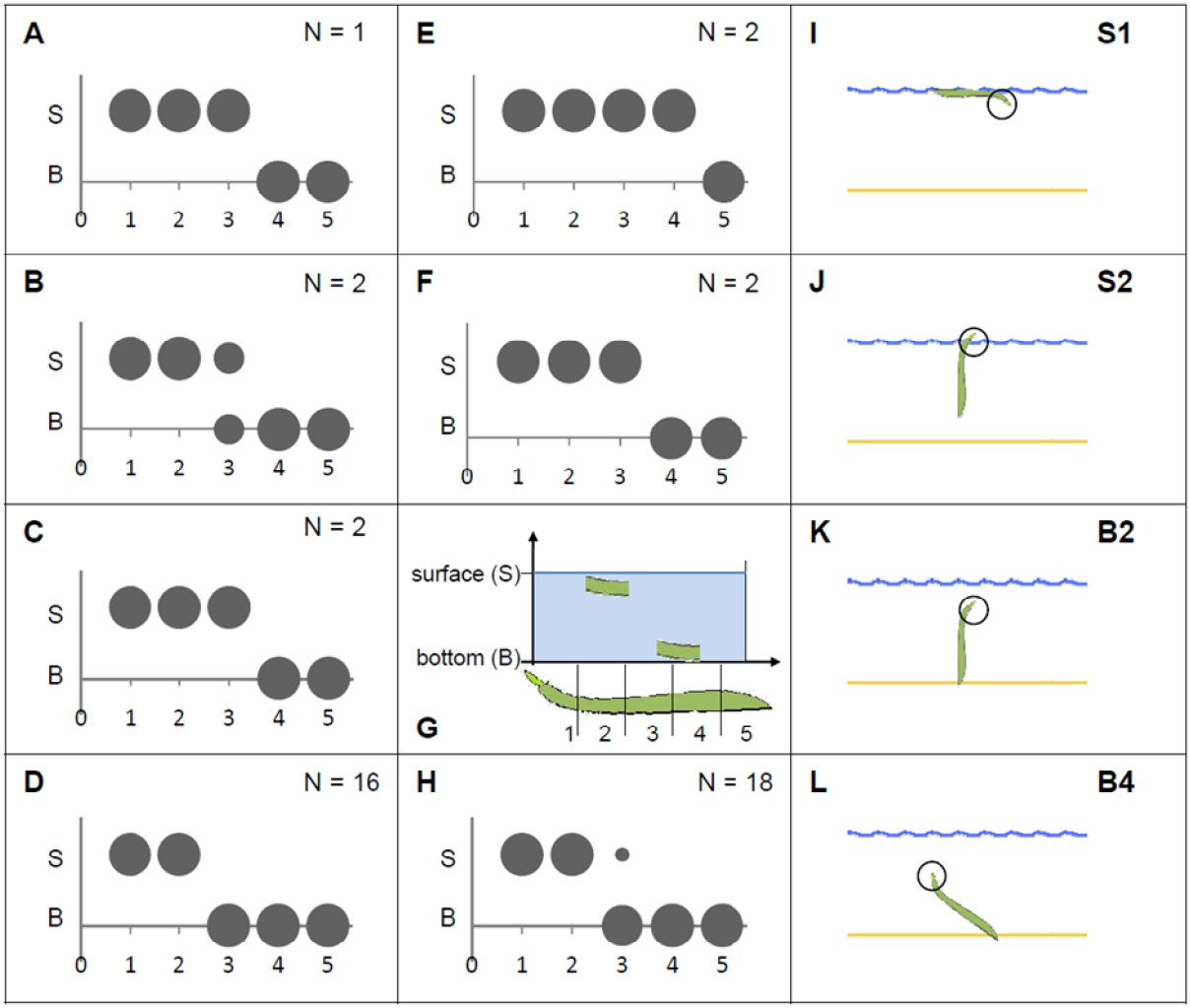

Figure 15. (A-F, H) Floating behaviour of pieces of propagules, 1/5 of the length each, for propagules of *Rhizophora mucronata* (A-D) and *Ceriops tagal* (E, F, H) with different floating behaviour (I-L). In upper right corner, sample sizes are indicated or codes that are used in Figure 13-14. (G) Interpretation key for graphs A-F and H. (I-L). Size of bubbles indicates percentage of propagules pieces with the same floating behaviour.

For some propagules of both species, root growth started already during this experiment. However, this was only the early beginning of root growth: some cracks in cortex of root bumps became visible. 11 of the 30 *R. mucronata* and 12 of the 30 *C. tagal* propagules that were left on dry sand or moist mud for 18 days, before being transferred to the hydroponic set-ups, showed the early beginning of root growth on the 18th day. This was also seen for only one of the *C. tagal* and none of the *R. mucronata* propagules left in sea water. For the propagules that were left 24 days on dry sand or moist mud, 19 of the 30 *R. mucronata* propagules and 14 of the 30 *C. tagal* propagules showed cracks in the cortex of their root bumps and all of the other propagules had at least developed root bumps. For propagules left in sea water for 24 days, two of the *R. mucronata* propagules and four of the *C. tagal* propagules also showed the early beginning of root growth, while some of the remaining propagules did not yet have clear root bumps.

4. Control experiment: influence of changing air humidity on root growth and development of propagules

After dehydrating for about 24 hours on dry sand in moderate shade, the five *C. tagal* propagules collected on the landward site had lost 0.09 g mass on average. During the rehydration period of about 24 hours the propagules did not lose nor gain any mass, their mass was exactly the same as before the rehydration period.

5. Growth and development of *C. tagal* and *R. mucronata* propagules during and after establishment (experiment C)

The propagules from experiment B, simulating the period between abscission and establishment (propagules in horizontal position), were transferred to hydroponic set-ups, which simulated establishment conditions (propagules in vertical position) in experiment C. The change in propagule length with time was much smaller to non-existent for *C. tagal* than for *R. mucronata* propagules, that did show length growth (Table 7, Figure 16). Although there was also much more variation in length change for *R. mucronata*, propagules left in sea water during experiment B clearly showed less growth compared to the other two substrates. An exception were the propagules collected on the landward site and placed in 50 % sea water (Figure 16C). In general, there was a positive trend in length change of *R. mucronata* propagules with changing conditions of the hydroponics from 100 % sea water < 50 % sea

water < 50 % sea water and increased relative humidity. Site did not have a significant effect on changes in propagule length (Table 7).

Table 7. F-values and probabilities for all main effects for the change in length of *Ceriops tagal* and *Rhizophora mucronata* propagules in a hydroponic set-up with three different treatments. Sample sizes differed for different species, sites, substrates and treatments in hydroponic set-up. The variables defining the groups are indicated between brackets after the sample size (C = *C. tagal*, R = *R. mucronata*, L = landward site, S = seaward site, W= sea water, M = moist mud, D = dry sand, 100 % = hydroponic set-up with 100 % sea water, 50 % = hydroponic set-up with 50 % sea water, 50 % + RH= hydroponic set-up with 50 % sea water and increased relative humidity).

Variable	F-value	P-value	N
Species	71.88	<0.0001	89 (C), 90 (R)
Site	2.50	ns	89 (S), 90 (L)
Substrate	12.93	<0.0001	59 (D), 60 (W, M)
Treatment in hydroponic set-up	13.76	<0.0001	59 (100 %), 60 (50 %, 50 % + RH)

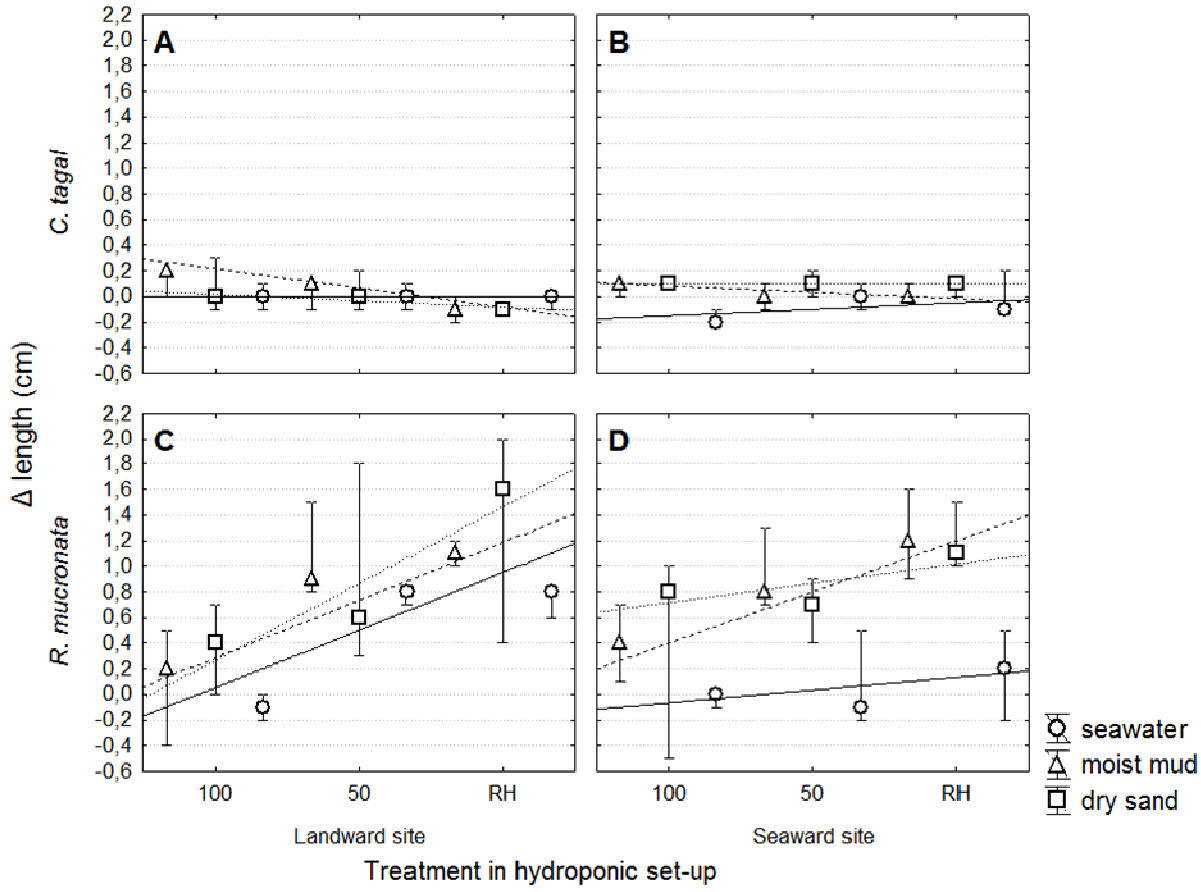

Figure 16. Change in length over time for *Ceriops tagal* propagules (A,B) and *Rhizophora mucronata* propagules (C,D) standing vertically in a hydroponic set-up with three different treatments (50 = 50 % seawater, 100= 100 % sea water, RH = 50 % sea water and increased relative humidity). Propagules were collected from landward (A,C) and seaward (B,D) sites and spread out on one of three substrates before being placed in the hydroponic set-up. Plotted values are medians and 25 and 75 percentiles, N = 5.

Table 8. F-values and probabilities for all main effects for the root length of *Ceriops tagal* and *Rhizophora mucronata* propagules after 24 days in hydroponic set-up with different treatments. Sample size differed with species, site, substrate and treatment in hydroponic set-up. The variables defining the groups are indicated between brackets after the sample size (C = *C. tagal*, R = *R. mucronata*, L = landward site, S = seaward site, W= sea water, M = moist mud, D = dry sand, 100 % = hydroponic set-up with 100 % sea water, 50 % = hydroponic set-up with 50 % sea water, 50 % + RH= hydroponic set-up with 50 % sea water and increased relative humidity).

Variable	F-value	P-value	N
Species	115.21	<0.0001	89 (C), 90 (R)
Site	3.32	ns	89 (S), 90 (L)
Substrate	10.34	<0.0001	59 (D), 60 (M, W)
Treatment in hydroponic set-up	29.25	<0.0001	59 (100 %), 60 (50 %, 50 % + RH)

Root growth was also measured during experiment C. For both species most propagules had roots of at least 2 cm long after 24 days in one of the three hydroponic set-ups. Nevertheless, there was again a significant difference between both species (Table 8). This difference was partly due to differences in the effects of substrate during experiment B and in hydroponic treatment during experiment C. For *C. tagal* propagules, there were only small differences between propagules left on different substrates during experiment B. Although the effect of different substrates was smaller compared to *R. mucronata* propagules, propagules that were left on moist mud had the longest roots. The shortest roots, after 24 days in the hydroponic set-up, were found for propagules left on dry sand for *C. tagal* (Figure 17A-B) but for propagules left in sea water for *R. mucronata* (Figure 17C-D). Root growth started later for *R. mucronata* propagules that were left in sea water than for propagules placed on dry sand or moist mud (Figure 18). At both sites and for both species root length after 24 days in hydroponics increased from a treatment with 100 % sea water < 50 % sea water < 50% sea water with increased relative humidity.

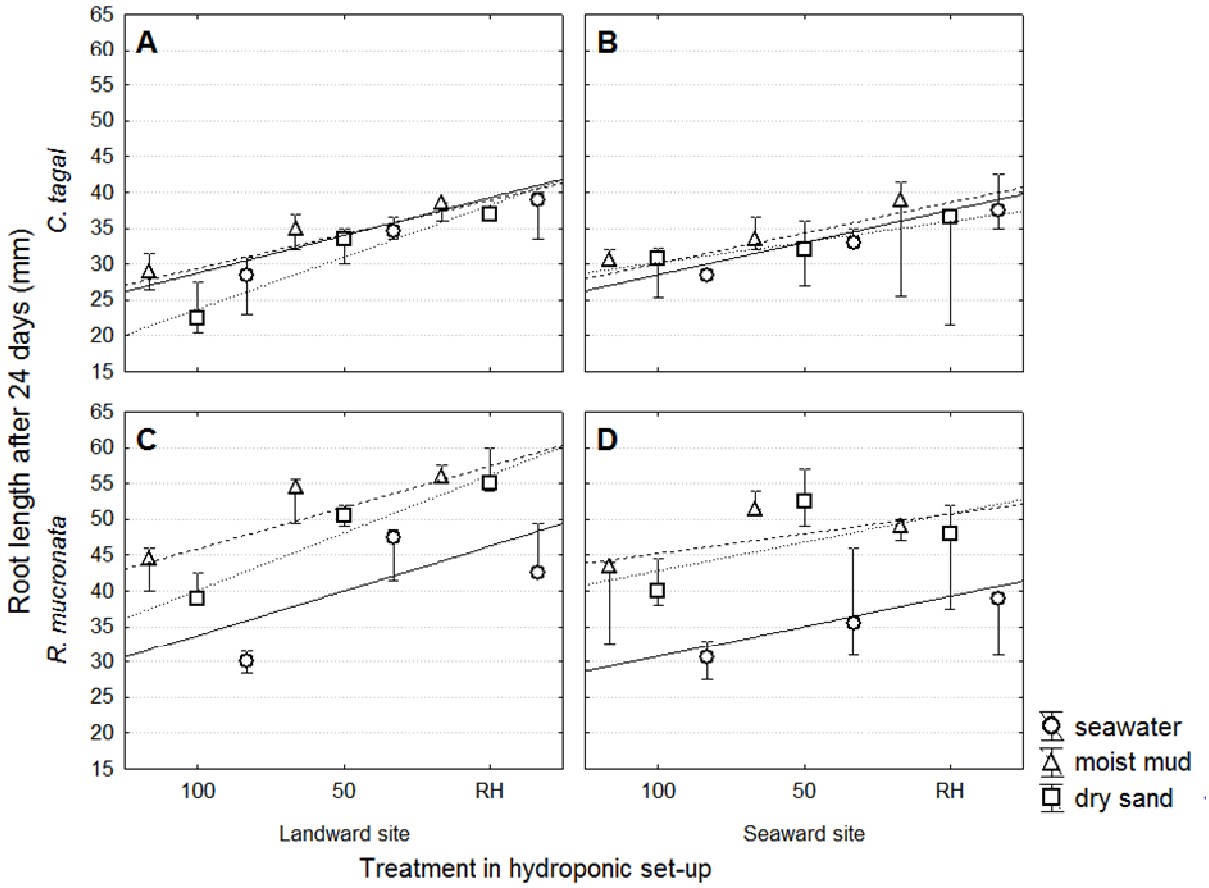

Figure 17. Root length after 24 days for *Ceriops tagal* propagules (A,B) and *Rhizophora mucronata* propagules (C,D) in a hydroponic set-up with different treatments (50 = 50 % seawater, 100= 100 % sea water, RH = 50 % sea water and increased relative humidity). Propagules were collected from landward (A,C) and seaward (B,D) sites and spread out on one of three substrates before being placed in the hydroponic set-up. Plotted values are medians and 25 and 75 percentiles, N = 5.

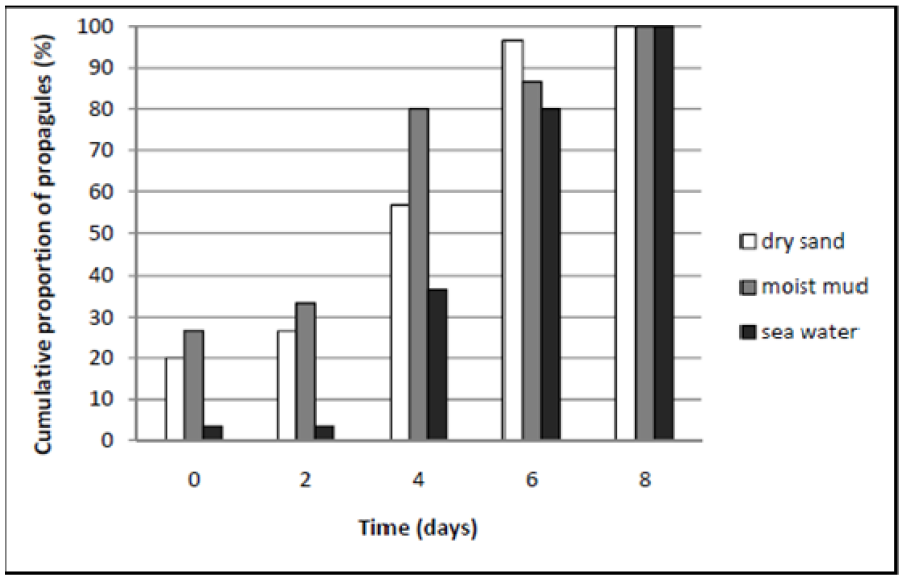

Figure 18. Timing of the initiation of root growth (observed as cracks in the cortex of root bumps) for *Rhizophora mucronata* propagules that were left on different substrates and subsequently were placed in a hydroponic set-up. The x-axis shows the number of days for which propagules had been placed in the hydroponic set-up before the initiation of root growth was visible. The y-axis represents the cumulative proportion of propagules with initiated root growth.

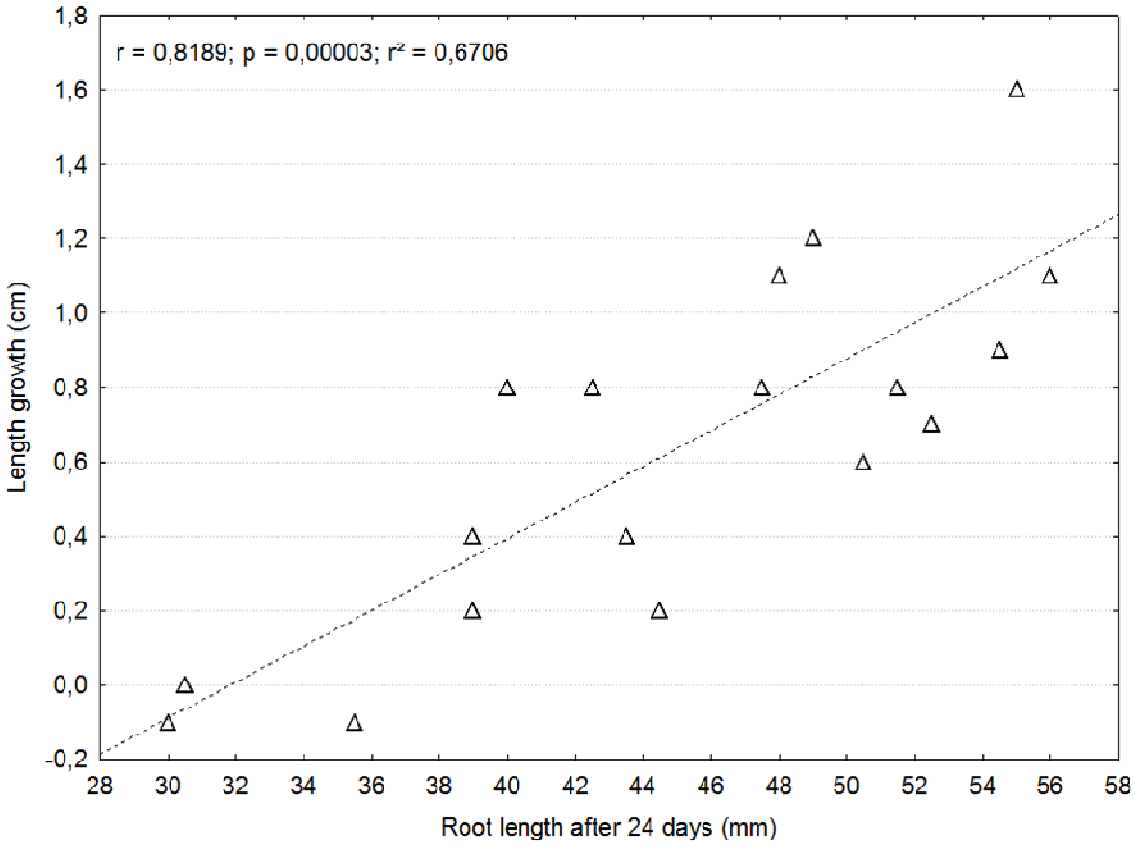

Figure 19. Correlation between length growth and root length after 24 days in a hydroponic set-up for *Rhizophora mucronata* propagules. Plotted values are medians per site, substrate and treatment in hydroponic set-up.

For *R. mucronata*, the propagule length growth and the length growth of the roots was significantly, positively correlated ($r = 0.82$ and $p < 0.0001$, Figure 19). Because *C. tagal* showed almost no length growth, there was no significant correlation between the propagule length growth and the length growth of the roots.

Five different stages could be described for developing *C. tagal* leaves. The first stage, as was observed at the time of collection of the propagules, is shown in figure 20A. At this stage only the four stipules of the first pair of leaves were visible. The growth of the leaves themselves aborted very early, before abscission, as was decribed for *R. mangle* (Juncosa, 1982). The four stipules were joining each other over their whole length and coming together in one point at the top. In a second stage the tips of the four stipules started to separate (Figure 20B). This was followed by the third stage where the four stipules separated completely from one another and the next pair of leaves, folded together, became visible (Figure 20C). During the fourth stage this pair of leaves grew longer than the four stipules, but were still folded together (Figure 20D). Finally in the fifth stage, the pair of leaves opened. The first unfolded leaves were observed for two propagules that had been for 20 and 22 days in the hydroponic

set-up, in total 34 and 35 days after collection. In total, eight propagules with opened leaves were observed during our experiment. Six of these propagules with unfolded leaves grew in 50 % sea water and two of them in 50 % sea water with increased air humidity. None of the propagules growing in 100 % sea water opened their leaves during experiment C.

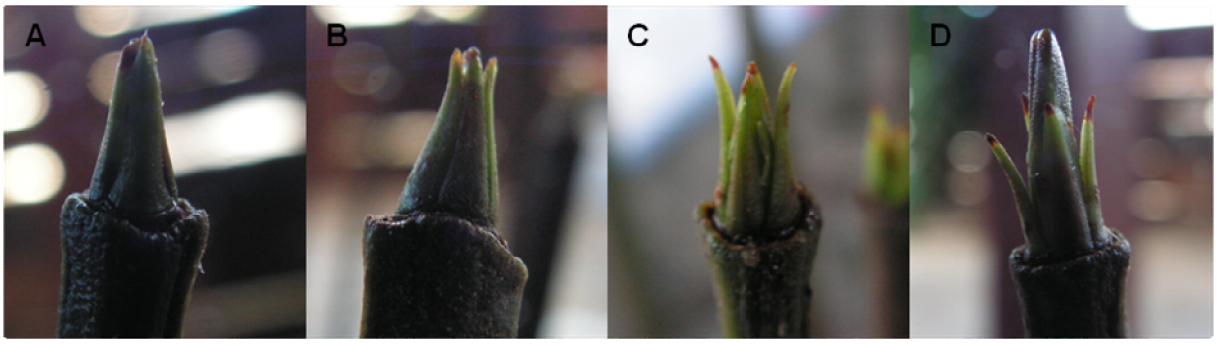

Figure 20. Developing leaves of *Ceriops tagal* propagules. (A) Stage 1: first stipules leaves folded together (B) Stage 2: opened tips of first four stipules (C) Stage 3: first four stipules completely separated (D) stage 4: growing pair of leaves, grown longer than first four stipules, but still folded together.

6. Control experiment: influence of hydroponic conditions on root growth

Root growth under hydroponic conditions on one the hand and natural conditions on the other hand were compared to check for possible artefacts in the establishment-simulating-experiment (experiment C). Figure 21A shows that roots grew faster for *C. tagal* propagules placed in a hydroponic set-up than for *C. tagal* propagules planted in sand, on the place where they had been collected. The roots of *R. mucronata* propagules placed in a hydroponic set-up also grew quicker than the roots of propagules collected on the seaward site and planted there in the mud (Figure 21B).

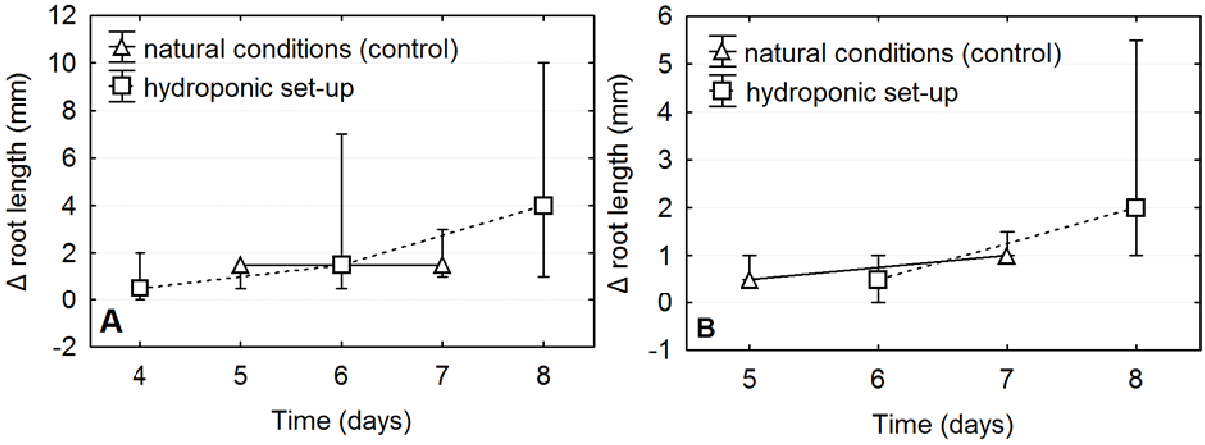

Figure 21. Root growth of *Ceriops tagal* (A) and *Rhizophora mucronata* (B) propagules under natural (in the soil) and experimental (hydroponics) conditions. Plotted values are medians and 25 and 75 percentiles, N = 6-9.

DISCUSSION

During the period between abscission and establishment, dehydration (water loss) of *R. mucronata* and *C. tagal* propagules caused a decrease in volume and an increase in density. This influenced the propagule's buoyancy. However, dehydration did not hinder root growth or leaf development, on the contrary, for dehydrated *R. mucronata* length and root growth was initiated earlier.

Further, this study revealed the possibility for *R. mucronata* and *C. tagal* propagules to grow roots while still attached to the mother tree, as a response to the combination of a reduction of light and air circulation, or any of these.

1. Growth and development of *C. tagal* and *R. mucronata* propagules before abscission from the mother tree

As no young propagules were available, the experiment was tried after all with the older propagules available. However, for both *C. tagal* and *R. mucronata*, 30 % and 50 % of the propagules, respectively, abscised from the mother tree before the end of our shading experiment. This was most likely not caused by the shading material used, but by the almost mature age of the propagules used. Slightly more than half of the remaining propagules belonged to the group that was shaded with aluminium foil (55 % and 59 % for *C. tagal* and *R. mucronata,* respectively). During our control experiment, when comparing two different shading materials, fabric and aluminium foil, none of the propagules abscised before the end of the 14-16 day long experiment. During the main experiment of 25-28 days, at least five *R. mucronata* and three *C. tagal* propagules were released from the mother tree after 12 days or less. All of these propagules had been wrapped in aluminium foil. However, almost all propagules that were released before the end of the experiment and that were found on the forest floor were wrapped in aluminium foil. The reason for this is that unwrapped propagules were only tagged with a number and, once they had abscised, were difficult to recognize as propagules that were part of the experiment. However, since no propagules abscised during the control experiment, no difference in influence on propagule abscission between the two materials was shown. Hence, the early release of propagules from their mother tree was due to the maturity of propagules and not to the shading material.

A very interesting and unexpected result of the shading of propagules was the growth of roots on the shaded propagules, still attached to the mother tree. Under natural conditions, lateral roots start to develop but not to grow before abscission. Already shortly after propagule elongation on the mother tree, when only a few centimetres of the radicle end of the hypocotyl are visible outside the fruit, root development starts with cell divisions in the pericycle opposite the protoxylem points (Juncosa, 1982). However, roots do normally not protrude through all of the cortex of the propagule, so that only root bumps can become visible on the outside (Juncosa, 1982, personal observations 2011). We hypothesize that the early and unexpected root development and growth observed during our shading experiment is the result of an increased amount of the gaseous phytohormone ethylene, built up in the aluminium foil wrapped around the propagules. The control experiment revealed that only propagules covered with aluminium foil, and not propagules covered with fabric, showed increased root growth while still attached to the mother tree. The main difference between these two methods is the air circulation around the propagule, which was very low to possibly unexistent when aluminium foil was used. Therefore gaseous substances, like ethylene, produced by the propagule in the aluminium bag can easily build up to reach a higher concentration than occurring under natural conditions. Ethylene is formed from its precursor methionine, in this process 1-aminocyclopropane-1-carboxylic acid (ACC) serves as an intermediate. For the conversion of ACC to ethylene, O_2 is needed (Taiz and Zeiger, 2006). On the other hand, ethylene can also be deactivated by O_2, through oxidation to ethylene oxide (Taiz and Zeiger, 2006). Ethylene production by the hypocotyl and/or other plant parts has been repeatedly reported to increase when seedlings are shut from light, or are under the influence of far-red light, indicating a controlling effect of phytochromes on ethylene biosynthesis and/or activity (Samimy, 1978; Vangronsveld *et al.*, 1988; Finlayson *et al.*, 1999; Walton *et al.*, 2010). According to the "lateral root initiation hypothesis" of Aloni *et al.* (2006), ethylene is produced in the hypocotyl by differentiating protoxylem vessel cells. Ethylene locally inhibits auxin transport from the shoot to the radicle end of hypocotyl through the hypocotyl pericycle. This results in a build-up of auxin at the radicle end of the hypocotyl, which induces lateral root development (Aloni *et al.*, 2006). Ethylene-induced lateral root formation has been reported for various species (reviewed in Aloni *et al.*, 2006), for example in the common ice plant (*Mesembryanthemum crystallinum* L.) (Konieczny *et al.*, 2009). Ethylene does not only influence the initiation of lateral root development, but also the growth of lateral roots. Although this influence of ethylene on root growth has often been described as a negative influence – an inhibition (for example by Ruzicka *et al.*, 2007), a

positive influence is not out of the question. It is thought to be impossible to predict the effect of ethylene on plant development for several reasons (reviewed in Dugardeyn and Van der Straeten, 2008). The response to ethylene differs between species, developmental stages and even tissues. Further, the function of this phytohormone depends on internal and environmental factors, such as the interaction with pathways of other phytohormones. Moreover, also the concentration of ethylene is important regarding its function (Dugardeyn and Van der Straeten, 2008). Nevertheless, from our observations, we suggest that darkness caused an increased ethylene concentration in the aluminium foil wrapped around the propagules and that this increased ethylene concentration increased the growth rate of the lateral roots of these propagules.

In summary, the propagules used for the shading experiment were already too old and therefore 30 % of the *C. tagal* propagules and 50 % of the *R. mucronata* propagules abscised before the end of the experiment. The control experiment comparing aluminium foil and fabric and the fact that the proportions of shaded and unshaded abscised propagules were similar, confirmed that the early abscission was probably not due to the shading material. Further, the remaining shaded propagules showed root growth, which does normally not occur under natural conditions. We hypothesize that increased levels of ethylene, built up in the aluminium foil bag around the propagules, stimulated lateral root growth, because: (1) several other studies have shown that ethylene production increases in plant parts that are shut from light, (2) the control experiment showed that this root growth was only stimulated in propagules covered with aluminium foil, which inhibits air circulation, and not in propagules covered with fabric, which allows air circulation and (3) it is possible that lateral root growth is stimulated by increased ethylene concentrations.

2. Growth and development of *C. tagal* and *R. mucronata* propagules during the period between release from the mother tree and establishment

After propagules were taken from *R. mucronata* and *C. tagal* trees and placed on different substrates to simulate the period between abscission and establishment (experiment B), their mass and volume decreased with time. However, the decrease in mass and volume was much more pronounced in propagules that were placed on dry sand or moist mud than in sea water. This indicates that dehydration caused the decrease in volume. Also the seeds of some tropical

rainforest species have been found to be dehydration-insensitive or to be able to tolerate certain degrees of dehydration (Rodriguez *et al.*, 2000; Yu *et al.*, 2008).

For *R. mucronata* propagules the volume decrease was due to a decrease in length as well as a decrease in diameter. *C. tagal* propagules only reduced in diameter resulting in a small reduction in volume. The absence of a length reduction in *C. tagal* propagules can be related to the presence of long fibre cells, or sclereids, in the cortex of *R. mucronata* propagules that are not present in *C. tagal* propagules (De Ryck, 2009). As fibres are the only longitudinally stretched cells in propagules, their shrinkage could be a driving power to length reduction. Nevertheless, relative to the initial volume measured at the time of collection, the volume loss of *C. tagal* propagules was twice that of *R. mucronata* propagules, which can be linked to their smaller size. Also for neotropical rainforest species in Mexico, it was seen that smaller seeds dehydrated faster (Rodriguez *et al.*, 2000). The reduced volume by dehydration of the mangrove propagules has been shown by De Ryck (2009) to be due to squeezed parenchyma cells and deflated air pockets between the cells. This causes the ribs of the *C. tagal* propagules to become more pronounced during dehydration (the grooves between the ribs become deeper), indicating a decrease in volume. *R. mucronata* propagules do not have such ribs.

For *R. mucronata* propagules there was a small but significant difference in mass and volume change between propagules collected on the landward and seaward site. First, at the time of collection, *R. mucronata* propagules collected on the seaward site were lighter and longer, but thinner and therefore of smaller volume. Second, when left on dry sand or moist mud, propagules from the landward site lost most mass and volume. This indicates that *R. mucronata* propagules from the seaward site contained less water at the time of collection. The higher water content of *R. mucronata* propagules from the landward site can be explained in two ways. First, propagules collected from trees on the landward site may have stored more water as an adaptation to their dry environment. Another possible cause may be an artefact caused by collecting propagules of different ages. On the landward site, fewer trees were available to collect propagules from. Therefore it is possible that, unintentionally, younger propagules were collected on the landward site by shaking trees harder or pulling propagules with more force. Younger propagules will contain more water that can be lost by dehydration, according to the results of Sussex (1975). For *R. mangle* the embryo contains a high amount of water during the whole period of embryogenesis and loses water afterwards, during germination, and also during further growth on the mother tree (Sussex, 1975).

Thus, for both species, loss of mass during the period between abscission and establishment is most likely due to dehydration. Because the changes in mass relative to the

initial mass at the time of collection did not differ between both species (4-5 % reduction for both species), we suggest that the dehydration rate is the same for both species. Nevertheless, the relative change in volume was larger for *C. tagal* than for *R. mucronata*. Although this hypothesis was not tested, this can be due to a larger amount of aerenchyma in *C. tagal* than *R. mucronata* propagules. Further, also the initial water content determines how much mass and volume can be lost and this depends on the species, the environment in which the mother tree grows and the environment in which the propagule resides after abscission.

The relative volume loss of *C. tagal* was much larger than for *R. mucronata*, while the relative mass change was about the same for both species. This resulted in a much larger changes in density for *C. tagal* propagules compared to *R. mucronata* propagules. The slower change in density of *R. mucronata* propagules resulted in a longer buoyancy. In general, changes in propagule density resulted in changes in buoyancy power and orientation. When the density increased, propagules started changing their orientation and eventually sank. In general, propagules floated horizontally at the water surface just after abscission. The buoyancy of separate pieces of propagules revealed that the density started to increase in the radicle end first. Therefore horizontally floating propagules progressively changed their position to a diagonal one, followed by a vertically hanging position, still at the water surface. This was also observed in other studies on *R. mucronata* (Davis, 1940 in Rabinowitz, 1978a) *C. tagal* and other viviparous species with elongated straight propagules such as *Bruguiera exaristata* Ding Hou, *B. gymnorrhiza* (L.) Lamk., *Ceriops australi*s (White) Ballment, Smith & Stoddart, *Ceriops decandra* (Griff.) Ding Hou and *Rhizophora stylosa* Griff. (Clarke *et al.*, 2001). Later, when the density of the whole propagule increased more, it sank towards the bottom where its position changed from vertically hanging to diagonal to horizontal. This pattern was seen for *C. tagal* propagules, but not for *R. mucronata* propagules, which behaved much more randomly. Because the propagules of *R. mucronata* were bigger, contained more water and did not have ribs, their density and therefore also their buoyancy changed at a lower rate and varied more in time (individual propagules were not followed for the entire study period but results were pooled of different sets of propagules that were left on one of the three substrates for periods of different length). This causes *R. mucronata* propagules to be able to retain their floating capacity longer, even when they were left on moist mud or dry sand. In addition, *R. mucronata* propagules were longer than *C. tagal* propagules and therefore density differences could exist over a longer distance in the propagule. This also resulted in more varying buoyancy patterns. Propagules of *Rhizophora harrisonii* Leechman that were kept in

an aquarium filled with sea water and then sunk after 28 days have been observed regaining buoyancy after 30 days (Rabinowitz, 1978a). Also *R. mucronata* propagules, kept in aquaria with different salinity conditions for 59 days, have been observed to regain buoyancy (De Caluwé *et al.*, 2008). However, regaining buoyancy has also been observed in *C. tagal* propagules kept in aquaria with different salinity conditions for 30 days (De Ryck *et al.*, 2007).

The longer floating period of *R. mucronata* propagules compared to *C. tagal* propagules translates in a longer period to be dispersed by water. The longer a propagule floats, the further it can be carried away from its mother tree. However, not only propagule buoyancy defines the dispersal capacity of a propagule. A study comparing dispersal distances between three different mangrove species with floating propagules, *R. mangle*, *Avicennia germinans* (L.) Stearn and *Laguncularia racemosa* (L.) Gaertn.f., revealed that smaller propagules are dispersed over longer distances than larger propagules (Sousa *et al.*, 2007). This was explained by the greater ability of small propagules to pass physical barriers like prop roots. Despite the shorter *C. tagal* propagules, compared to *R. mucronata*, very limited dispersal distances were observed, however, by different research groups (McGuinness, 1997; Raju and Karyamsetty, 2008; De Ryck, 2009). Nevertheless, the study of the De Ryck (2009), also conducted in Gazi Bay, showed that *C. tagal* propagules dispersed further and faster than *R. mucronata* propagules. The longer dispersal distance was explained by faster dispersal capacity of *C. tagal* propagules, due to their shape, density and rough propagule surface (De Ryck, 2009). We suggest that in the cases of lower dispersal capacity of *C. tagal* propagules, the dispersal capacity might depend more on the fast increase in density after release from the mother tree, which has a negative effect on dispersal capacity, because it lowers the propagule buoyancy. The shape, smaller size and rough propagule surface, which could have a positive effect on dispersal capacity, would be less important in these cases.

During this experiment, root growth started for some propagules that were placed on dry sand or moist mud for 18 days, but not for the propagules that were placed on dry sand or moist mud for 12 days or less. For propagules left in sea water, root growth started later, but mostly not at all. According to Rabinowitz (1978a), this means that the obligate dispersal period for both *C. tagal* and *R. mucronata* lies between 13 and 20 days (including the 1 to 2 days between the collection of propagules and the start of the experiment). Because also no growth, not in length nor in diameter, was observed, the propagules seem to be ‘dormant’ during this period. Nevertheless, seeds of viviparous plants have no dormancy period

(reviewed in Farnsworth, 2000). Indeed, the embryo of viviparous mangrove propagules is never dormant (Sussex, 1975; Juncosa, 1982; Tomlinson and Cox, 2000; and reviewed in Farnsworth, 2000). Dormancy in orthodox seeds is associated with a high concentration in abscisic acid and low concentrations in gibberellins (reviewed in Finkelstein *et al.*, 2008). In viviparous mangroves, embryos have low abscisic acid levels (Farnsworth and Farrant, 1998; Farnsworth, 2004), while gibberellins are important for stem elongation (Smith *et al.*, 1996). Nevertheless, this study reveals a 'dormant-like' state of the seedling during the early phase of dispersal, when the hypocotyl does not grow nor gains weight, the roots do not grow and no leaves are developed. Moreover, the seedlings dehydrate during this period, as do dormant seeds after they abscised from the mother tree, although the degree of dehydration in dormant seeds is most likely much higher.

In addition, dehydrin-like proteins have been found in *R. mucronata* embryos (Ismail *et al.*, 2010). Dehydrins are proteins that are produced as a response to increased abscisic acid levels, dehydration, cold temperatures, salinity and wound stress. They protect the cells against damaging effects of these stressors, for example during seed dormancy. Normally, these proteins are found in older embryos and mature plants, but not in young embryos nor in young seedlings (reviewed in Allagulova *et al.*, 2003). Also, mature *C. tagal* and *R. mucronata* propagules that had not been exposed to dehydration, do not contain dehydrins (Farrant *et al.*, 1996). Mature propagules of *Barringtonia racemosa* (L.) Spreng. and *A. marina* that were not dehydrated did also not contain dehydrin, after dehydration dehydrins were produced by *B. racemosa*, but not by *A. marina*. This shows that some tropical wetland species, like *B. racemosa* are able to produce dehydrins, while others, like *A. marina* are not (Farrant *et al.*, 1996). Therefore we suggest that, during the period between abscission and establishment, when *C. tagal* and *R. mucronata* propagules do not show any growth in length or diameter but shrink due to dehydration, both species also produce dehydrins to protect cells from damage caused by this dehydration. We especially expect this for *R. mucronata* propagules, because the propagules of this species required dehydration to trigger root growth in experiment C.

In summary, for both species there was no growth in length or in diameter during the period between abscission and establishment. Therefore, we hypothesize that for the viviparous propagules of *C. tagal* and *R. mucronata* the period between abscission and establishment is a delayed dormancy period, during which there is no growth and metabolic

activity is most likely low. This dormancy period is called delayed, because it occurs at the seedling stage instead of the seed stage as is seen for plants with dormant seeds. During this period of delayed dormancy, the volume and mass of the propagules of both species decreased due to dehydration and/or anatomical changes. Most dormant seeds also have a period of dehydration, during which their cells are protected by, among others, dehydrins (Allagulova *et al.*, 2003). The mass and volume changes resulted in density changes that influenced propagule buoyancy. The volume of *C. tagal* propagules decreased faster relative to the mass, probably due to shrinking air pockets and parenchyma cells. Therefore, the density of *C. tagal* propagules increased faster than the density of *R. mucronata* propagules that remained buoyant for a longer time and could potentially disperse over a longer distance.

3. Growth and development of *C. tagal* and *R. mucronata* propagules during and after establishment

When put in a vertical position in different hydroponic treatments (experiment C), *C. tagal* propagules did not show longitudinal growth. In contrast, propagules of *R. mucronata* did show such growth. Growth was influenced by the different substrates on which propagules resided between release from the mother tree and the simulated establishment. In contrast to propagules that were left in sea water, propagules that had experienced dehydration before establishment showed more longitudinal growth. The same effect of dehydration, in the period between release from the mother tree and establishment, was seen for root development of *R. mucronata* propagules. For *R. mucronata* propagules that did not experience dehydration, root development started later and was possibly also slower. Unfortunately we cannot say anything about the timing of longitudinal growth, because length could only be measured accurately at the start and the termination of experiment C, but we assume that, similar to the timing of root growth, longitudinal growth also started later for propagules that were not dehydrated. Although *C. tagal* propagules did not grow in length, root growth was observed. In contrast to what was observed for *R. mucronata*, root growth was slightly faster in propagules that had resided on more humid substrates than on dry sand.

These results lead to the following hypothesis: *R. mucronata* propagules need some degree of dehydration to be able to start growing in length and to grow roots, i.e. as a trigger for establishment. *C. tagal* propagules, on the contrary, need humidity to stimulate root growth. Both patterns have been observed for the seeds of different tropical rainforest species (Rodriguez *et al.*, 2000; Yu *et al.*, 2008). Even for some species with recalcitrant seeds it has

been shown that their seeds need some degree of dehydration for optimal germination (Yu *et al.*, 2008). The species difference observed in our study can be related to the different distribution of both species in the mangrove forest. *R. mucronata* grows closer to the sea in sites that are more regularly flooded by the tides. Dehydration could thus be a sign for the propagule that it has stranded and is not floating on the water anymore. *C. tagal* grows higher in the intertidal and hence drier sites. Here soil water is the limiting factor for establishment and for not being flushed away by the tide. Experiments on *R. mangle* propagules have shown that root growth of floating propagules, which are totally exposed to light, is inhibited by solar irradiance (Smith *et al.*, 1996). This could explain for both species why root growth does not start while propagules float in open sea. For both species, stranded propagules can form roots and subsequently erect themselves with tension wood fibres (Tomlinson and Cox, 2000; Fisher and Tomlinson, 2002). Nevertheless, propagules of both species can plant themselves vertical in the substrate when released from the mother tree during low tide (Tomlinson and Cox, 2000; Fisher and Tomlinson, 2002; Raju and Karyamsetty, 2008), when the substrate permits. Propagules are most likely able to detect their orientation relative to gravity. Hence, when they are placed upright in the substrate by self-planting, they can start growing roots as quickly as possible with fast establishment as a result. Higher plants are able to sense gravity, and therefore their orientation relative to the Earth's surface, through amyloplasts in statocytes, or gravity sensing cells (reviewed in Morita, 2010). In roots, columella cells are statocytes and in shoots epidermal cells fulfill this function. In responses to gravity signals, auxin together with several auxin influx and efflux facilitator proteins play an important role (reviewed in Palme *et al.*, 2006).

Thus, we hypothesise that: Propagules of both species are able to detect their upright position after self-planting, so establishment can start right away. When propagules abscise during high tide, or are carried away by the sea water, light inhibits them from forming roots (Smith *et al.*, 1996). When *R. mucronata* propagules are floating in sea water, at open sea or in the forest, they will normally not form roots and postpone root formation. Dehydration acts as a signal that they have stranded. For *C. tagal*, in contrast, water availability will accelerate root growth of stranded propagules.

Root growth of *C. tagal* propagules and longitudinal and root growth of *R. mucronata* propagules were highest in hydroponic treatments with 50 % sea water and increased air humidity. In agreement with this finding, growth of *R. mucronata* seedlings in Pakistan was found to be optimal in 50 % sea water (Aziz and Khan, 2001) and in Sri Lanka in 76 % sea

water (Jayatissa *et al.*, 2008), but both studies only included seedlings that already had established. Rabinowitz (1978a) found that propagules of *R. harrisonii* that were placed in sea water (free floating) formed roots as quickly as propagules placed in fresh water. This agrees with the dumbbell curve of growth characteristics of *R. mangle* and *R. stylosa* with low salinity being the optimal condition and both fresh water conditions and high salinity being suboptimal (Clough, 1984; Lin and Sternberg, 1992; Lin and Sternberg, 1993; Biber, 2006). For *C. tagal* seedlings from India, establishment rates decreased with increasing salinity, but optimal growth for six months after establishment was observed in 40 % sea water (Patel *et al.*, 2010), which resembles our results. However, none of the studies discussed above tested the influence of increased air humidity. In our study, both species showed optimal growth in the lowest salinity combined with the highest air humidity. The control experiment for the influence of changing air humidity showed that increased relative humidity prevented *C. tagal* propagules from dehydrating. We assume this would also be true for *R. mucronata* propagules. However, not only the relative humidity but also the temperature had increased in the greenhouse where the experiment was set up and we were not able to distinguish the possible effect of either factor. It is possible that growth rates increased, compared to treatments with 50 % sea water but without increased relative humidity, because excessive water loss was prevented. But, then, less growth would be expected for *R. mucronata* propagules that were placed in sea water during experiment B, because these propagules would not have had the opportunity to dehydrate sufficiently to trigger the start of length and root growth. However, also the increased temperature can have had a positive effect on the growth of both species.

In the field, salinity differs between zones with different elevation, because the influence of the tides differs between higher and lower elevations. When there is no fresh water input, the salinity at low elevation zones, close to sea, is very similar to the salinity of sea water, while at higher elevations, the salinity is higher due to higher evaporation rates. Mangroves have species specific adaptations to different zones. Kitaya *et al.* (2002) tested for seven mangrove species in which elevation zones their propagules established most successfully. Two of the tested species were *R. mucronata* and *C. tagal*. *R. mucronata* was one of the species that grew best on low elevations with high tidal influence, while *C. tagal* did not tolerate these conditions (Kitaya *et al.*, 2002). In addition, different studies showed that *R. mangle* propagules were better able to establish and grow at low elevation sites than *A. germinans* and *L. racemosa*, partly because of their longer shape and good floating capacity (Rabinowitz, 1978a; McKee, 1995; Sousa *et al.*, 2007). The results of these studies accord to

the distribution pattern of *C. tagal* and *R. mucronata* in Gazi Bay, where *R. mucronata* grows closest to the sea, at the lowest elevations where tidal influences are strongest. In our study, which only focussed on the influence of salinity and air humidity and not on other tidal influences, comparable responses to the different treatments were seen for the longitudinal growth of *C. tagal* and the longitudinal and root growth of *R. mucronata*.

Decreased salinity combined with increased air humidity are two conditions occurring in the mangroves of Gazi Bay during the wet season, when there is fresh water input from the inland region via the seasonal rivers, Kidogoweni River and Mkurumuji River (Kitheka, 1997; UNEP, 1998). Therefore the wet season provides the best conditions for *R. mucronata* and *C. tagal* propagules to grow, although *R. mucronata* propagules need some dehydration to trigger establishment first.

During the 24 days of our experiment, no leaf development for *R. mucronata* propagules was observed. Also Sousa *et al*. (2007) did not see any leaf development during their 28 day field experiment on establishing *R. mucronata* propagules in Panama. For *C. tagal* propagules leaf development was observed during our study and some propagules opened their first pair of leaves within the 24 days they were growing in the hydroponic set-ups. Mature *C. tagal* propagules collected in India, planted in soil and watered with fresh water, opened their first leaves as early as 9 days after planting and most leaves opened 28 days after planting, but under higher salinity conditions it took longer for propagules to develop leaves (Patel *et al.*, 2010). Also, during our experiments, most propagules with opened leaves were observed in the low salinity treatments.

For the longitudinal growth, root formation and leaf development discussed above, propagules need carbohydrates, which can come from several possible sources: reserves stored in the hypocotyl, photosynthates of the hypocotyl, or a combination of both, as was shown for *R. mangle* propagules (Patel *et al.*, 2010). For propagules of the same species, the larger propagules are seen to develop into larger seedlings, this illustrates the importance of hypocotyl reserves for early growth (Smith and Snedaker, 2000). The possibility to use two carbohydrate sources, reserves and photosynthates, can be a very important advantage for seedlings that strand at a place with non-favourable conditions, like a shady environment where there is not enough light for adequate photosynthesis. While reserves can be crucial for survival, photosynthesis is needed for optimal growth. Newly originating light patches can therefore be of great importance for young seedlings (Ball, 2002). *R. mangle* seedlings

established in a shady environment show no or only little growth. This observation of Rabinowitz (1978b) supports a suggestion made by Smith and Snedaker (2000) that photosynthetic activity in the hypocotyl tissue may be needed for more efficient use of the carbohydrate reserves in the hypocotyl.

In summary, *R. mucronata* propagules grew in length and formed roots but did not show any leaf development during our experiment. *C. tagal* propagules, on the other hand, did not show longitudinal growth, but did show leaf development and root growth, although less than for *R. mucronata*. Therefore, we can conclude that the energy reserves, stored in large hypocotyls, are mobilized and used in different ways by both species. These results, combined with the previously described findings of other researches about the function and importance of hypocotyl carbohydrate reserves, lead to the following hypothesis: The size difference between *C. tagal* and *R. mucronata* propagules entails a difference in the amount of carbohydrate reserves. Because *C. tagal* propagules did not show longitudinal growth, these propagules used their carbohydrate reserves first for root and shoot growth, so water and nutrients can be taken up by the roots and photosynthesis can be performed by the leaves, early after establishment. On the contrary, *R. mucronata* propagules showed length growth short after establishment. This matches the environment in which both species grow in Gazi Bay. *C. tagal* grows in open forest with much light, where water is often scarce, while *R. mucronata* grows in forest assemblages with a more closed canopy where light is a limiting factor, but which are flooded more frequently. Therefore, for *R. mucronata* early longitudinal growth is important for light competition. Leaves will develop later, when the seedling has grown longer and there is more chance to capture sufficient light to perform photosynthesis. *C. tagal* seedlings mostly do not have to compete for light in the open forest, therefore length growth is less important than root and leaf growth, which allows them to take up water and perform photosynthesis, early after establishment. Nevertheless, we observed that *C. tagal* propagules formed shorter roots than *R. mucronata* propagules. However, *C. tagal* propagules are shorter and thus less root length will be needed to ensure the vertical position of an established propagule compared to the longer and heavier *R. mucronata* propagules.

CONCLUSION

Hypothesis 1: The percentage autonomous growth (compared to growth supported by the mother tree) is higher for *R. mucronata* propagules than for *C. tagal* propagules, because *R. mucronata* propagules are much bigger and therefore production costs are higher.

Because propagules that were too old (mature) already were used, many of them abscised before the end of the experiment. In addition, length measurements were not precise enough. Therefore we were not able to test our first hypothesis.

Nevertheless, while propagules were still attached to the mother tree, root formation was observed for propagules that were wrapped in aluminium foil, but not for propagules that were wrapped in fabric. Therefore, we suggest that darkness stimulated the ethylene production and, due to a lack of air circulation, the concentration of ethylene increased inside the aluminium foil wrapped around the propagules. This increased ethylene levels induced the growth of lateral roots.

Hypothesis 2: Between abscission from the mother tree and establishment, there is a period during which propagules do not grow or form roots, a delayed dormancy period.

No length nor diameter growth was observed during the experiment simulating the period between abscission and establishment (horizontal position), while root growth was observed after at least 13 days. Therefore, we assume that metabolic activity was low, at least during the first 12 days after abscission. This **supports our second hypothesis** that the propagules of viviparous species have a delayed dormancy period between abscission and establishment.

However, for both species **mass and volume decreased** during this experiment simulating the period between abscission and establishment. For *C. tagal* propagules, the volume decrease relative to the initial volume and relative to the mass decrease was larger than for *R. mucronata* propagules. This resulted in a higher rate of **density increase** for *C. tagal* propagules. Because the density increased **slower for *R. mucronata* propagules**, after abscission from the mother tree, they remained **buoyant for a longer time**, resulting in a higher potential to **disperse over a longer distance**.

Hypothesis 3: Environmental cues break this delayed dormancy period so that root growth and therefore establishment is triggered.

During the second experiment simulating establishment (vertical position), *R. mucronata* propagules that were placed on dry sand or moist mud, during the experiment simulating the period between abscission and establishment (horizontal position), started to form roots earlier than propagules that were placed in sea water. This indicates that ***R. mucronata*** propagules have to **dehydrate** to a certain degree to **trigger root growth**. This matches with the distribution pattern of *R. mucronata* in Gazi Bay: close to the sea at sites that are frequently inundated. Water is thus not a limitation, but a site that is not flooded for a period long enough to establish is. Dehydration informs the propagule of being stranded.

In contrast, *C. tagal* propagules that were placed in sea water or on moist mud during the first experiment formed longer roots than propagules that had been placed on dry sand. This indicates that ***C. tagal*** **propagules** need **humidity** to **accelerate root growth**. This also accords to the distribution pattern of *C. tagal* in Gazi Bay: *C. tagal* grows mainly landward in more open forests, where water availability is low. An unflooded are to establish is thus not a limitation here, but water is. An increased humidity is thus the only factor missing for establishment.

Next to differences in environmental cues triggering establishment, both species were found to use their energy reserves, stored in their large hypocotyls, in a different way. ***R. mucronata*** propagules, which often grow under dense canopies, seemed to **first invest energy in length growth** to compete for light and root growth to anchor themselves, before developing leaves. In contrast, *C. tagal* propagules, which often grow in more open forests that are less frequently inundated, seemed to **first use their stored energy to grow roots** to search for water and to develop leaves, before growing in length. Roots were shorter for *C. tagal* than for *R. mucronata* propagules, which corresponds to their size and weight.

Hypothesis 4: Longitudinal growth, root growth and leaf development during establishment are lower in high than in low salinity conditions and higher when relative air humidity is increased.

Propagules of both species showed most longitudinal (*C. tagal*) or longitudinal and root growth (*R. mucronata*) when treated with low salinity (50 % sea water) and high relative humidity. Least length and/or root growth was observed for propagules that were treated with 100 % sea water. For *C. tagal* propagules, leaf development was observed some propagules,

of which most were treated with 50 % sea water. These results **support our fourth hypothesis**.

A schematic overview of the processes and conclusions of this study is given in figure 22.

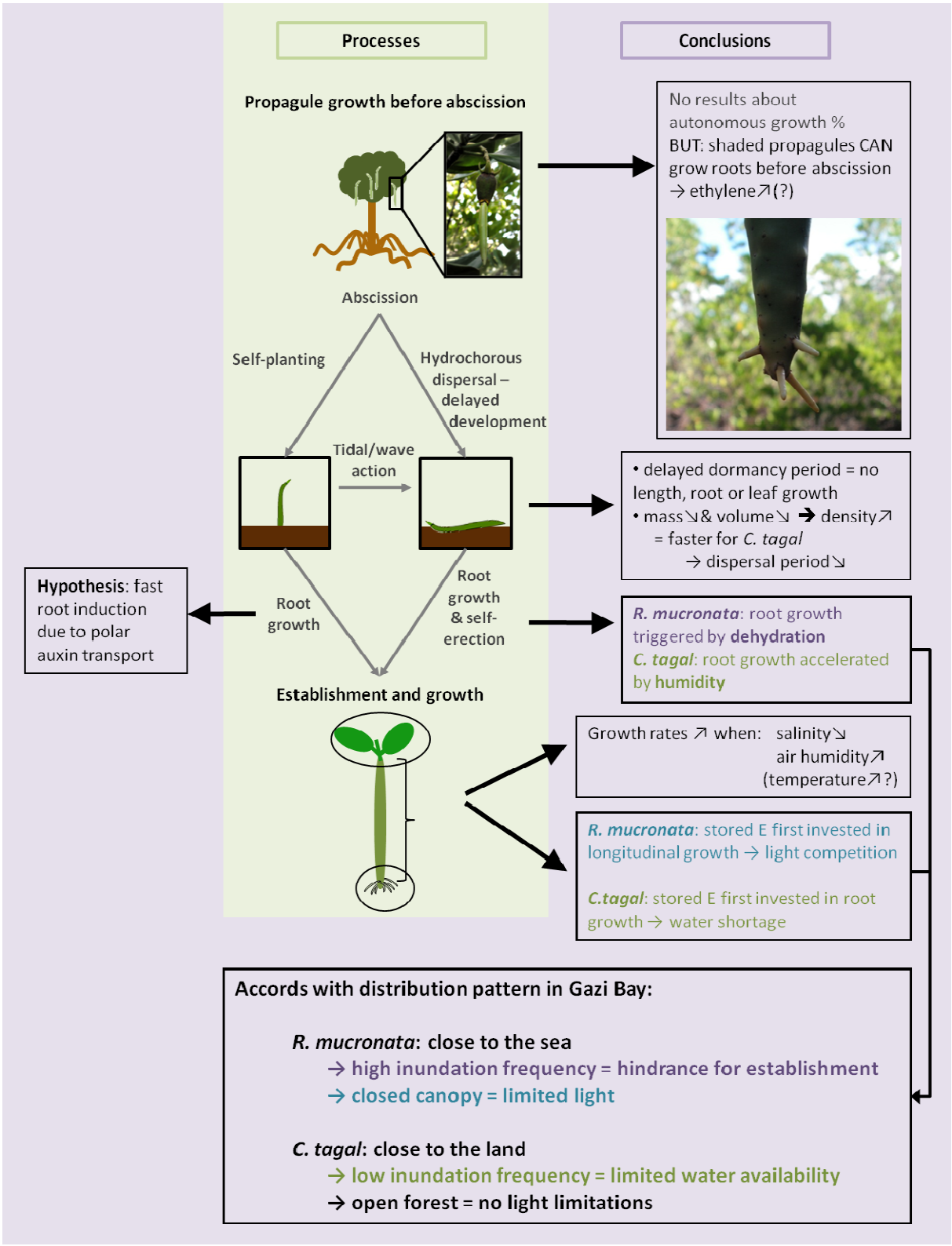

Figure 22. Schematic overview of the processes and conclusions of this study.

FUTURE PERSPECTIVES

This work contributes to the knowledge about the biology of viviparous mangrove propagules, which can be very useful for mangrove regeneration by plantations. However, still many questions are to be answered by further research:

- The shading experiment showed that possibly increased ethylene concentrations can induce lateral root growth of propagules, before they are released from the mother tree. First of all, it should be confirmed that it is ethylene that had built up in the aluminium foil bags and induced the root growth. Second, if the role of ethylene is confirmed, the role of ethylene in root growth under natural conditions (after abscission) should be studied.

- Because we could not test our first hypothesis, that the percentage autonomous growth is higher for larger propagules, this experiment should be repeated with younger propagules and breathable shading material.

- During the suggested delayed dormancy period of propagules that are released from the mother tree, we observed no growth. However, it should be tested if there is metabolic activity to confirm it really as a dormancy period. In addition, it should be checked if other viviparous species also exhibit a delayed dormancy period. This will contribute to the full understanding of the adaptive advantage of this delayed dormancy period and of vivipary in general.

- In this study, two different environmental cues were found to trigger establishment in two different species. These results suggest that for other species, establishment might be triggered by still other environmental cues. This knowledge about other species would also contribute to the revealing of the adaptive advantages of vivipary.

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