Journal of the Department of Wildlife Conservation 2014-2:143-153

PRELIMINARY STUDY ON THE PROPAGULE DEPENDENCY OF *Rhizophora* SEEDLINGS

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ABSTRACT

Early life of viviparous mangroves merely depends on the propagule and it can be assumed that the period and the degree of dependency could depend on the size and the intrinsic factors of the propagule as well as on the edaphic and environmental factors in which the seedlings are growing. However scientific studies on the propagule dependency of mangrove seedlings is poorly studied, irrespective to the fact that such information is vital particularly in mangrove restoration programs. However, propagule dependency of mangrove seedlings is poorly studied scientifically, irrespective to the fact that such information is vital particularly in mangrove restoration programs.

This study was carried out to investigate the growth performances of seedlings and the variations in the content of carbohydrate foods (starch content) in the propagule during the first 20 weeks period of the seedling growth of two viviparous species, *Rhizophora apiculata* and *Rhizophora mucronata*, which are having larger propagules and commonly used in replanting programs. The experiment was conducted, under three salinity regimes (i.e. 5psu; 15psu; 30psu) in a planthouse. A separate set of propagules was planted within the mangrove forest of Pambala lagoon under natural conditions and subjected to the same investigation as above.

Growth performances of both species grown under high salinity regime were significantly lower than those grown in low and moderate salinity regimes. Total leaf area of the seedlings of *R. mucronata* increased in higher order compared to that of the *R. apiculata* during the study period. After an initial drop in the content of starch in the propagules of both species, it started to increase slowly in the propagule of *R. mucronata* seedlings whilst the reduction was continued in *R. apiculata* propagules during the study period. However, the initial starch concentration of *R. apiculata* was remarkably higher than that of *R. mucronata* and hence, the starch content in *R. apiculata,* even after continued decreasing, was higher at the end of the study period. It can be hypothesized that the higher concentration of the stored food in smaller propagule of *R. apiculate,* compared to lower concentration of the stored food in propagules of *R. mucronata,* might lead to a similar longevity of viviparous mangrove seedlings of the two species allowing more or less the same chance to survive and establish in the same habitat as observed in many mangrove ecosystems.

KEYWORDS: Mangroves, Restoration, Starch content, Propagule dependency, *Rhizophora*, Sri Lanka

INTRODUCTION

Vivipary, i.e. seed germinating within the fruit before shedding them at the maturity producing a seedling attached to the parent plant (Elmqvist and Cox, 1996), is an adaptive feature in mangroves. Furthermore the propagule absorbs foods from the mother plant and accumulates within it, becoming a food storage organ. Initial anatomical and chemical analysis of *Rhizophora* propagules verified that propagules store foods mainly as starch. The stored foods in the propagule are spent for the longer survival of itself and to produce a seedling from it. There are evidences that higher the content of stored food larger the size of the *Rhizophora* propagule, and longer the distance it can travel during the dispersion through water (Drexler, 2001). In the same way it can be assumed that the content of the stored food can be a critical factor on the survival of seedlings in mangrove restoration fields as it is obvious that the seedlings at the early stage depend on the propagule. Thereby, the survival potential of planted propagules or seedlings in restoration fields could be higher even under hostile environmental conditions if the content of the stored food in the propagule is higher and the rate of consumption is lower.

Mangrove restoration has received a lot of attention worldwide in recent decades firstly as prominent life support systems, since the coastal livelihood highly depends on products and services provided by mangroves; Secondly, the unprecedented losses of mangroves on account of reclaiming mangrove areas for other land uses such as human settlement, industries, urban development, shrimp farms, high ways and dump sites (Dahdouh-Guebas *et al.*, 2002; Alongi, 2002; Kasawani *et al.*, 2006); Thirdly, the buffering function of mangroves that showed against the tsunami, 2004 that hit South East Asia. Government, international and national NGOs and coastal communities have shown great interest in mangrove-restoration activities, though in spite of the success in Kalpitiya and Pambala, most attempts showed higher failure rate in Sri Lanka (unpublished, Kodikara KAS). In determining the major factors for the failures, ignorance of major ecological drivers like salinity, hydrology etc., inappropriate species and site selection, intervention of unskilled or untrained people, no or little involvement of expertise, lack of preliminary research (Filed, 1996; Chapman and Underwood, 2000; Elster, 2000; Erftemeijer and Lewis III, 2000; Lewis III, 2005; Primavera and Esteban, 2008) or lack of scientific understanding in the field of restoration have been identified.

In addressing these factors, it is really useful to study the strength that a propagule has, to overcome these unfavorable conditions. Factors play simultaneously in reality rather than on one's own. Mangrove propagules or seedlings used for restoration are then subjected to several stresses due to above mentioned poor restoration practices. As a result plants have to maintain higher metabolic energy to overcome these stresses (De Block *et al.*, 2005, Rizhsky *et al.*, 2002, Tiwari *et al* 2002). Therefore, initial food storage, *i.e. starch is the common form in this study* (Bewley, 1994) *or food use efficiency,* directly affects survival of propagules or seedlings. The importance of investigating starch content of mangrove propagules and its variation over time in restoration attempts is clear. Also, little is known in terms of physiology that accounts for the failures as the studies on the physiological basis of restoration failures are scarce. Therefore, propagule dependency should then be one of the top considering factors in mangrove restoration.

Therefore, the main objective of this study is to investigate starch content and its variations over time in response to different salinity levels *i.e. major stressor in mangrove fields* (Parida and Das, 2005), which were selected based on naturally occurring salinity ranges in lagoons and estuaries in Sri Lanka and to add more knowledge to the field of physiology and thereby, this knowledge can be applied to field of mangrove restoration in Sri Lanka.

METERIALS AND METHODS

2.1 Species selection

Seven species of true viviparous mangroves belong to three genera, *i.e. Rhizophora apiculata, R. mucronata, Bruguiera cylindrica, B. gymnorhiza* and *B. sexanguila, Ceriops decandra* and *C. tagal* are reported from Sri Lanka (Jayatissa *et al*, 2002, and pers. communication).

Among the true viviparous genera, *Rhizophora* is the most frequently used genus in mangrove restoration programs, probably due to the fact that they bear the largest propagules in mangroves. Accordingly, the two *Rhizophora* species, *Rhizophora apiculata* and *Rhizophora mucronata*, were selected for the study. Propagules about to detach from trees were collected in August, 2013 in Pambala lagoon, Sri Lanka (7°34'N, 79°48'E) to use in the experiment.

2.2 Green house preparation and experimental design

Initially, the planted propagules were kept in a nursery irrigated with low saline (i.e. 5psu) water. This was done for a period of one month until first two leaves were unfurled. A soil mixture was prepared by mixing garden top soil, mangrove top soil, sand and compost in 1:1:1:1 (v/v) proportion. The collected propagules were planted in plastic pots with 20 cm diameter and 40 cm height, filled with the previously prepared soil mixture. For the green house experiment, three salinity regimes were selected to conduct the experiment as low saline (i.e. 5psu), moderate saline (i.e. 15psu) and high saline (i.e. above 30psu), each consisting of three replicate tanks. Water with those salinities were prepared by mixing sea water and aged tap water (Jayatissa *et al.*, 2008) and stored accordingly in separate nine replicate tanks which were assigned randomly under greenhouse conditions. Pots containing established seedlings with two unfurled leaves were transferred to above mentioned replicate tanks under three salinity regimes according to completely randomized design. Each pot was irrigated twice a day by the water with the salinity assigned to each pot. The salinity of the water in tanks was checked once in three days by a hand refractometer (ATAGO S/Mill-E, Japan) and adjusted when necessary. Similar amount of commercially available fertilizer was also applied once a month to each plant. All the plants were maintained under greenhouse conditions till the end of experiment.

A separate set of propagules from the above collection were planted at three sites within the mangrove forest of Pambala lagoon, Puttalama, Sri Lanka, where the average salinity is 12-14psu, having 10 replicates of each species in each site and sacrificed for the analysis in the same way described below .

2.3 Data collection

Three propagules were harvested from each replicate from each species including propagules planted in the field and used to get measurements in growth performances and for the starch content at the ages of 7, 9, 14 and 20 weeks. Cumulative shoot height and total leaf area of each harvested seedling were measured. Growth rate, *i.e. cumulative height increment per week,* was calculated for each treatment.

Ten grams (10g) from each harvested propagule was taken for the experiment representing top, middle and bottom of the propagule. Five milliliters (5 mL) of 1.0M Thiourea solution was added in order to minimize the browning effect due to tannins. Each sample of propagule was blended separately giving similar blending time (5 minutes) and extracted using 10 mL of distilled water and 20 mL of ethanol followed by heating to 70°C. Filtered extracts were then homogenized using a magnetic stirrer for 30 seconds. Ten milliliters (10 mL) of the homogenous extract was titrated against 0.02M KI/I_2 solution. Concentration of starch per gram of propagule was determined by using the reacted volume of KI/I_2 solution.

2.4 Data analysis

Means &standard deviations of cumulative shoot height, and total leaf area, were calculated separately for each species grown under different salinity levels. One-way ANOVA was used to test for significant differences in cumulative shoot height, total leaf area and starch content between the two mangroves in different salinity regimes. Tukey's post hoc was used to check for pair-wise differences on the mean values in relation to above parameters.

RESULTS AND DISCUSSION

Growth performances

3.1 Cumulative shoot height

The shoot height of both species in all three salinities did not show a noteworthy increase until the 8th week, but increases after the 8th week were clearly visible (Fig 1.). The shoot height of seedlings of both species grown under high salinity level (i.e. > 30psu) increased at a lower rate throughout the study period; nevertheless, those grown under medium salinity and low salinity levels increased at a higher rate. This trend remained same during the study period.

3.2 Growth rate

In each species, the mean growth rate of seedlings grown under the high salinity regime remained lowest throughout the study period as compared to that of the seedlings grown under moderate and low salinity regimes. In *R. apiculata*, the mean growth rate had increased gradually from 7th week giving a peak at the 15th week and then started to reduce gradually during the rest of the study period with a sharp decline until the 14th week. The variations in the growth rate of *R. mucronata* also showed the same pattern except that the peak at the 14th week was less prominent.

3.3 Total leaf area

The mean total leaf area of seedlings under different salinity levels over the study period are shown in figure 2. During the first 9 weeks period, there was no significant difference in the total leaf area between the two species or among the three salinity levels in either species. However, at the 14th week and after, the seedlings under high salinity level showed significantly lower total leaf area compared to that in the other two salinity levels and this pattern was common to both species. Furthermore, at the end of the study period it was revealed that the total leaf area of *R. apiculata* under any salinity level was significantly lower than that of *R. mucronata*.

3.4 Starch concentration in propagule extractions

The starch content in the propagule of *R. apiculata* under all the salinity levels remained at a higher level throughout the study period compared to those of *R. mucronata*. However the starch content in the propagule of both species reduced sharply until 9th week and then the reduction continued in a lower rate in *R. apiculata* during the rest of the study period whilst it was increasing slowly in *R. mucronata*. When the starch content of propagules under different salinity levels are compared, in both species the starch content was well-maintained in moderate salinity regime throughout the study period, whilst in those under the high salinity level it remained lowest. The variation pattern and the level of starch content in propagules of seedlings grown in the natural environment were similar to that of the propagules grown in the plant house under the medium salinity (Figure 3).

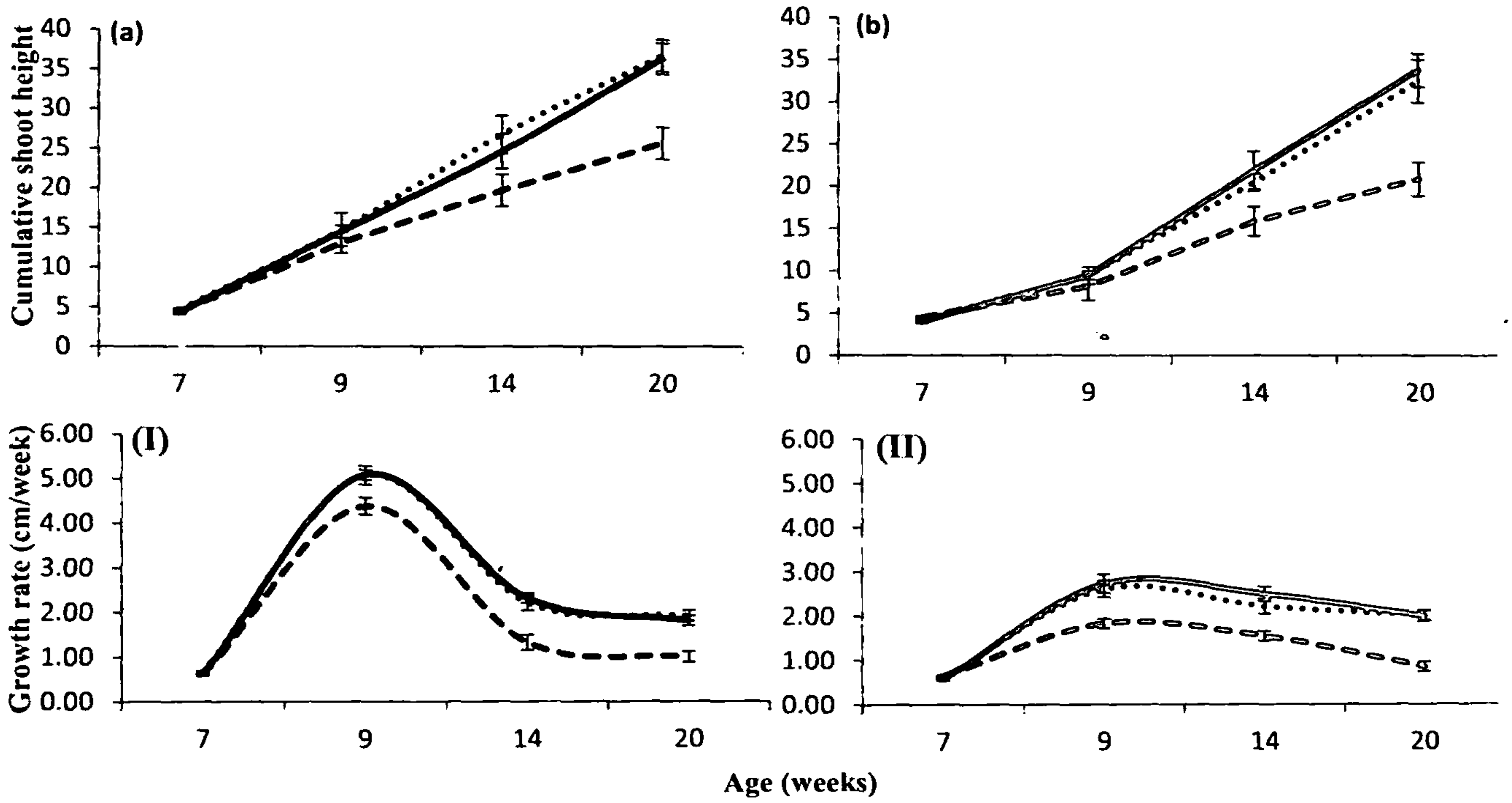

Figure 1. The cumulative shoot height (I), and growth rates (II), of seedlings of *Rhizophora apiculata* (a), and *R. mucronata* (b), under three different salinity levels over the study period

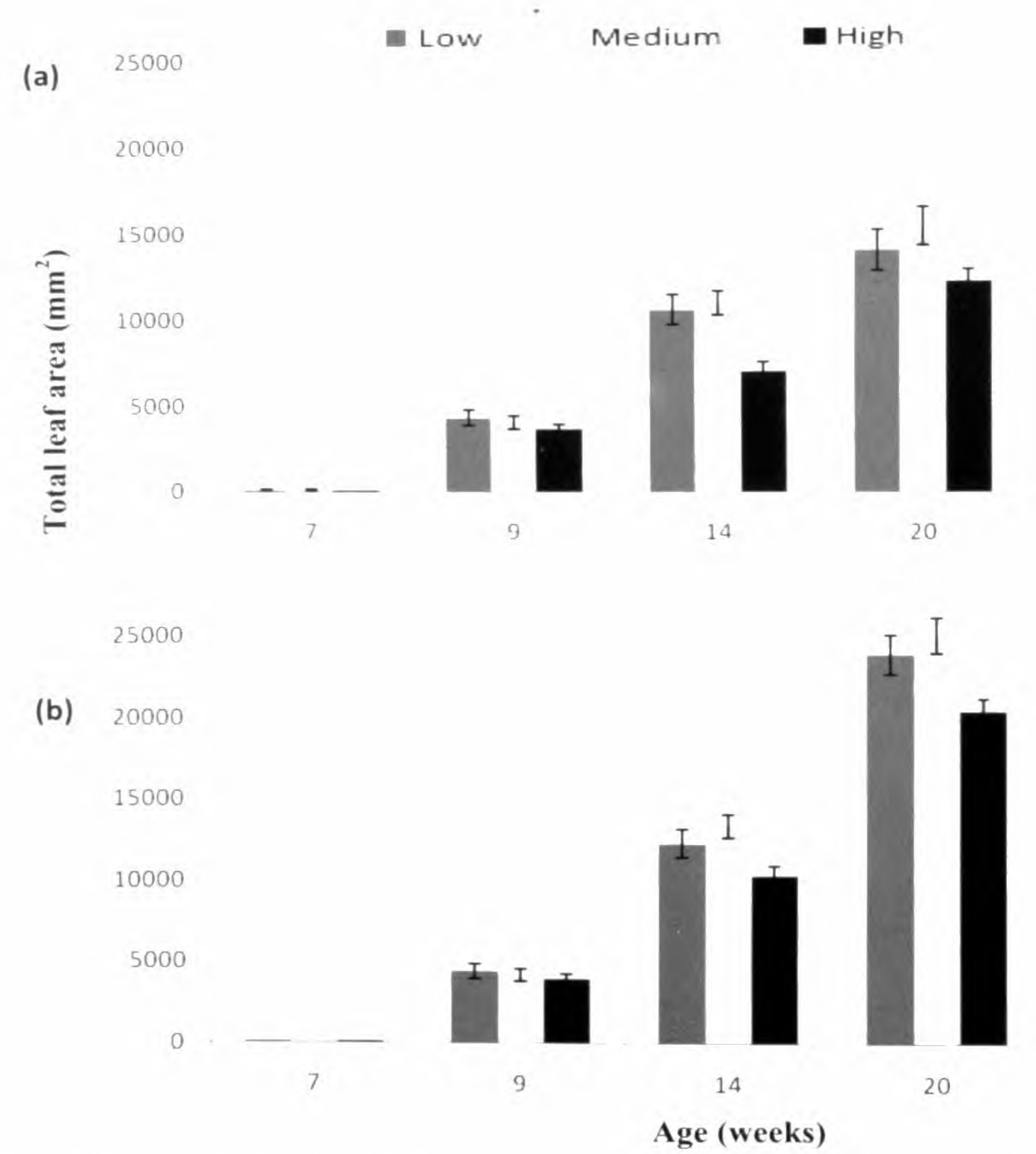

Figure2. The variation in total leaf area of (a) *R. apiculata*, and (b) *R. mucronata*, over the 20 weeks study period.

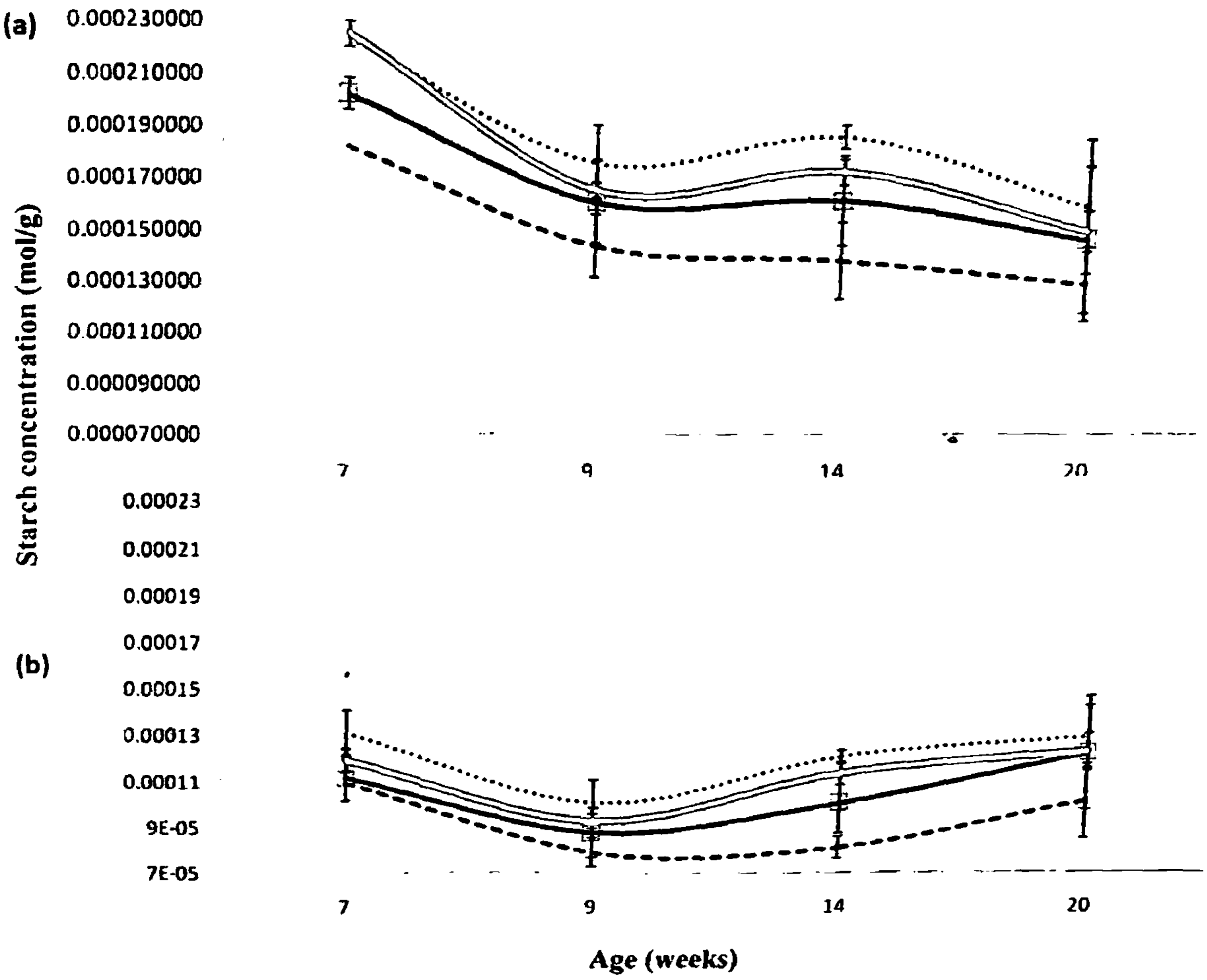

Figure 3. Variations of the starch content over the study period, in the propagule of (a) *R. apiculata* and (b) *R. mucronata* seedlings grown under different salinity levels. (10 g of the propagule was extracted in 30 ml of solvent)

Mangrove restoration attempts throughout the world including Sri Lanka have shown higher failure rates. Therefore, many research attempts have been carried out to address the major failures and to investigate the potential solutions (Parida and Das 2005; Ye *et al.*, 2005; Hogarth, 2007; Jayatissa *et al.*, 2008).Knowledge on the physiological basis of the propagule dependency of mangrove seedlings could be vital in such an attempt, however, studies on that aspect are scarce and this study can be considered as a preliminary attempt on the same aspect.

Rhizophora species were selected for this study as a species most commonly used in restoration programs throughout the world including Sri Lanka. The three different salinity levels were selected, based on naturally occurring salinity ranges in lagoons and estuaries along the coastline of Sri Lanka and naming the salinity levels [i.e. low saline (i.e. 5psu), moderate saline (i.e. 15psu) and high saline (i.e. above 30psu)] was also carried out to fit with the Sri Lankan scenario. In general salinities higher than 40psu are quite rare in coastal water bodies of Sri

Lanka, Evidently, restoration efforts in Sri Lanka have been made under all these salinity levels selected for this study. (Unpublished, Kodikara KAS). In most of the studies, it clearly stated that early growth or the initial establishment of seedling is crucial in deciding the success of mangrove restoration (Farnsworth, 2004). Therefore, it is indeed essential to investigate the behavior of energy sources in the early growth seedlings as it mainly decides the resistance or tolerance of that particular plant to the stresses and other disturbances. Further, 20 weeks period is a rather longer period to study such behaviors of plants and longer enough to study even slowly developing responses of mangrove seedlings (Jayatissa *et al.*, 2008). Field plantation in Pambala, from where the propagules for experiments were collected, was used to compare the variation of starch content with those found in greenhouse experiments.

The observed reduction of growth performances, i.e. shoot height and total leaf area, of mangrove seedlings grown under high salinity regime in this experiment is consistent with other studies (Aziz and Khan, 2001; Parida and Das, 2005). However, the performances of seedlings grown under low and medium salinity regimes did not show a significant difference. This is consistent with many other studies which have demonstrated that optimal growth rates can occur at concentrations ranging from 5 to 75% seawater (Smith and Snedaker, 1995; Aziz and Khan, 2001).

Starch concentration of the propagules of both species under all three salinity levels reduced up to 9th week implying that stored foods are used for the initial development of the seedlings (Wang, 1993). This reduction was common for both species. As expected, the initial sharp increment of the growth rate of both species overlapped with the initial sharp decline of the starch content in the propagules. After the 9th week, the starch content in the propagule of *R. mucronata*, started to increase gradually whilst that in *R. apiculata* continued the decrease of starch concentration until end of the study period. This indicates that the photosynthetic capacity of *R. apiculata* is still not high enough for the self-sustenance. In contrast, the increasing trend of the starch content in propagules of *R. mucronata* could imply that the photosynthetic capacity of *R.mucronata* seedlings is higher at this stage, when compared with that of *R. apiculata*. This is compatible with the variations in the total leaf area of the seedlings of the two species, where *R. mucronata* showed a higher total leaf area since 14th week to onward. However, the initial starch concentration of *R. apiculata* propagules which are smaller in size is remarkably higher when compared with that of *R. mucronata*. Hence, even though the starch content in *R. Apiculata* has been reduced continuously over the study period, the remaining level of the starch content even at the end of the study period is still higher than that of *R. mucronata*. It can be predicted that the starch content of the propagules of *R. apiculata* also will start to increase after reaching a critical level and when the total leaf area gets increased beyond a certain level or a breakeven point. However, this indicates that the smaller size of the propagule of *R. apiculata* appeared to be compensated by the higher content of stored food in the propagule when compared with the larger size propagules of *R. mucronata*. This intrinsic feature may help to maintain more or less same longevity of viviparous mangrove seedlings of the two species, facilitating the growth of both species together in the same habitat as observed in many mangrove ecosystems.

ACKNOWLEDGEMENT

This study was carried out under the GREEN DYKE Project ZEIN2008PR347 supported by the Vlaamse Inter-Universitaire Raad (VLIR), Belgium. We are very grateful to Douglas Thisera for his practical help in the field and to Mr. Anuradha Wickramasinghe of the Small Fishers Federation of Lanka (SFFL) in Pambala–Kakkapalliya for his logistical support.

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