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Effects of Seasons and Zonation on Biochemical Contents of Two Seagrass Species along Dar es Salaam Coast, Tanzania

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Abstract

Accumulation and allocation of biochemicals in plants are affected by various factors. The influence of seasons and vertical zonation on biochemical contents of seagrasses *Cymodocea rotundata* and *Thalassia hemprichii* from Mjimwema seagrass meadow along the coast of Dar es Salaam were assessed. Samples were collected in 2016 during wet/rainy (April–May) and dry (June–July) seasons. Concentrations of crude protein, soluble carbohydrates, phosphorus, lipid, and proline were determined using standard methods. Significant higher levels of phosphorus, lipid, protein and proline were observed during the dry season than in wet season ($p < 0.05$). Likewise, seagrass population occurring at the shore zone expressed higher levels of biochemical contents than those found at the subtidal zones. The highest concentration of protein was recorded in *Thalassia hemprichii* (5.8 mg/g dw)), while those of lipid and carbohydrates (27.1 and 53.1 mg/g dw, respectively) were recorded in *Cymodocea rotundata*. The two species studied did not vary significantly in their biochemical concentrations in both seasons. Generally it was observed that the concentrations of studied biochemicals were up-regulated during the dry season and varied depending on the locations where the seagrass grow. Thus, both season and zonation played roles in biochemical accumulation in the seagrasses understudy, however not interactively.

Keywords: Biochemical composition, *Cymodocea rotundata*, *Thallasia hemprichii*, Seagrass meadows

Introduction

Biochemical constituents in plants play vital roles in maintenance of growth, development and reproduction (Lambers et al. 2008). Their accumulation and allocation vary depending on many prevailing factors inherent to but not limited to individual plants, the influence from the environment where they grow, as well as the stage of growth (Alcoverro et al. 1999, Lambers et al. 2008, McLaughlin and Shriner 2012). In the course of development, seagrasses like any other plants continually accumulate and partition acquired resources in different organs as well as in different metabolic activities (Alcoverro et al. 1999, Heldt 2005). They acquire and allocate resources via both below and above ground tissues (Short and Coles 2001) and the process can be affected by complex interactions by both abiotic and biotic factors that contribute to the balance between plant production and utilization of resources (Zimmerman et al. 1995, Lambers et al. 2008). Influence of seasonal variation (Moore and Wetzel 2000, Duarte et al. 2006) and zonation patterns (Fonseca et al. 2002) in seagrass performance in their natural habitats is appreciated.

Intertidal areas are often exposed to strong light intensity and high temperatures especially during low tides (Rasheed and Unsworth 2011). Thus submerged vegetations are also exposed to varying levels of light and temperature regimes; as a consequence, variation in the biochemical composition and allocation between seagrass population located at different zones of the intertidal areas (i.e., shore/upper zone and those at the subtidal zone) is likely. The influence of environmental conditions on acquisition and allocation patterns of biochemicals has been extensively studied in terrestrial plants, but little has been done on the submerged aquatic vegetation (Madsen 1991, Bazzaz and Grace 1997). In seagrasses, relatively few studies on variation due to seasons as well as vertical zonation have been documented (e.g., Huong et al. 2003, Fonseca et al. 2002). Along the coast of Dar es Salaam, little has been done (see for example, Mvungi and Mamboya (2012), Kajela and Mvungi (2018)), thus, there is paucity in information regarding patterns of biochemical composition in seagrasses in response to seasonality as well as vertical zonation especially along the Western Indian Ocean in Dar es Salaam coast. Therefore the present study was set out to assess concentrations of different biochemicals, namely soluble carbohydrates, crude protein, phosphorus, lipids and proline in two seagrass species (*Cymodocea rotundata* and *Thalassia hemprichii*) with respect to their vertical zonation (from the shore to the subtidal zones) and seasonal variation (wet and dry). We hypothesized that allocation of biochemicals in seagrasses vary significantly with season as well as with zones. The findings of this study foster our understanding on the physiological status of seagrasses subjected to various environmental conditions in their natural habitats.

Materials and Methods

Description of study site

Samples were collected from Mjimwema seagrass meadows along the Indian Ocean coast in Dar es Salaam between 6°50´S, and 39°21´E; about 7 km south of Dar es Salaam city centre (Figure 1). The area is regarded as the least polluted as it lacks key sewerage pipes from the city centre (Mvungi and Mamboya 2012).

Sampling procedures

Sampling was conducted during spring low tides, where intact shoots (with roots) of both *Cymodocea rotundata* and *Thalassia hemprichii* were collected from the shore and subtidal zones in two different seasons; rainy (April – May 2016) and dry (June – July 2016) seasons. During each sampling occasion, two transects of about 200 m apart were established perpendicular to the shore extending seaward from the upper seagrass zone to the subtidal zone. At each zone along each transect line, 10 sampling points were established. At each sampling points, ten to fifteen intact seagrass shoots were randomly selected and uprooted, cleaned off sediments, stored in a cool box and taken to the laboratory at the department of Botany of the University of Dar es Salaam for further processing. In the laboratory, the collected seagrass materials were cleaned of epiphytes, apportioned differently into two sub-samples with about 5 seagrass shoots each and further separated into above (leaves) and below ground (rhizomes + roots) parts. The first sub-samples were oven dried at 60 °C until constant weight, and was used for analysis of crude protein, carbohydrates, phosphorus and lipids. On the other hand, the second sub- samples were frozen at –20 °C until extraction, for proline analysis which was done within 48 hours after sampling.

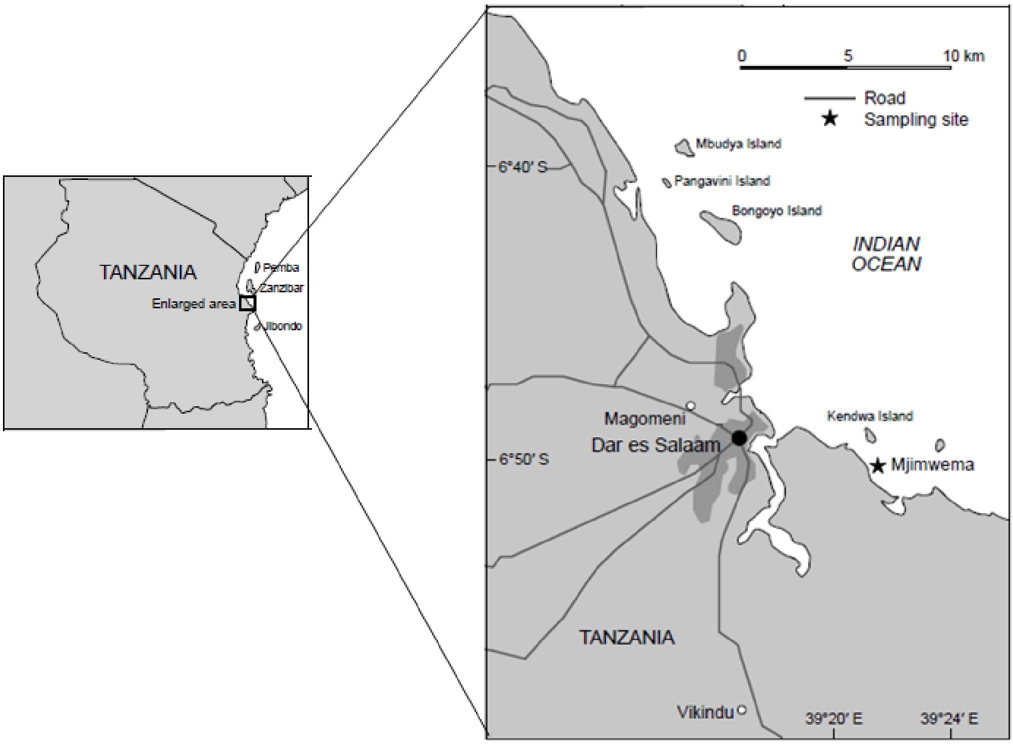

Figure 1: Map of Dar es Salaam coast showing the study site, Mjimwema. Modified from Mvungi and Mamboya (2012).

Biochemical analyses

Determination of crude protein, lipids, phosphorus and carbohydrates were done on both below and above ground parts of the seagrasses, whereas determination of proline was done only on above ground parts. Crude protein was determined from total nitrogen extracted from 0.1 g of powdered samples using micro-Kjeldahl digestion methods followed by indophenols-blue colorimetric procedures detailed in Allen (1989). Thereafter the concentration of crude protein was calculated using a nitrogen–to–protein conversion factor of 6.25 (Jones 1931). Total soluble carbohydrates were analyzed using Anthrone method described by Maness (2010) and Allen (1989). Briefly, 1 g of dried powdered samples were mixed with 30 ml distilled water, boiled for one hour, then allowed to cool and filtered through number 44 Whatman filter paper into a 50 ml volumetric flask. 2 ml of the extract were transferred into new tubes and 10 ml of anthrone reagent was added and mixed thoroughly with the tubes immersed into cold water. Later on the tubes were placed in beaker of boiling water in dark for about 10 minutes then allowed to cool. The absorbance of the solution was read at 625 nm. The same procedures were followed for the standard solution (glucose) which was used to generate calibration graph. Concentrations of phosphorus were determined using dry oxidation method according to the procedures outlined in Allen (1989). Lipid content was determined according to the method described by Atkinson et al. (1972) as explained in Allen (1989). In summary, 1 g of powdered dried sample was extracted with 20 ml ether using Soxhlet extractor for 4 hours on a heating mantle. To remove residual solvent, the flask containing extract was allowed dry (evaporate) for 30 minutes, thereafter allowed to cool in the desiccator and weighed. The weight of lipid extract was calculated as:

$$\text{Lipid} = \frac{\text{W (g)}}{\text{Sample weight (g)}} \times 100$$

where W = weight of residue (g).

Furthermore, determination of proline content was done on fresh seagrass leaves by using acid ninhydrin method (Ábrahám et al. 2010), with minor modifications. Briefly, 0.5 g of fresh leaves were homogenized with 5 ml of 95% ethanol, and centrifuged at 3500 rpm for 10 minutes at 4 °C. Then 5 ml of ninhydrin (composed of 0.125 g ninhydrin, 2 ml of 6 mM NH_3PO_4, 3 ml of glacial acetic acid) was added to 1 ml of the sample. The mixture was brought to boiling at 100 °C using water bath for 45 minutes, allowed to cool on ice and thereafter 10 ml of toluene was added. The absorbance was read immediately at 515 nm wavelength using a UV-visible spectrophotometer (Jen way 6305 S UK). The concentration of proline was calculated from the calibration curve drawn from known concentrations of D-proline (Sigma-aldrich) and is expressed as µmol/g FW.

Statistical analysis

Differences in biochemical compositions in the two seagrasses with respect to seasons and zonation were analyzed using two-way Analysis of Variance (ANOVA) in R version 3.3.3. Before analysis, the data were subjected to normality check using Shapiro-Wilk test, when the assumption was rejected, the data were log-transformed. The statistically significant results were set at the level of $p = 0.05$.

Results

Crude protein

Seasonal changes had significant effects on the concentrations of crude protein in both of the seagrass species ($p < 0.05$, Figure 2, Table 1), being higher during the dry season than in wet season. Likewise, significant effect of zonation on concentrations of crude protein was evident where the highest concentration was recorded at the upper zone compared to subtidal zone ($p < 0.05$, Figure 2). In addition, there was no significant effect in allocation of crude protein between the above and below ground parts of both seagrass species under study ($p > 0.05$). Albeit their insignificant differences when compared, higher concentrations were frequently observed in leaves with mean values ranging from 2.3 to 4.5 mg/g dw and from 2.7 to 5.4 mg/g dw in *C. rotundata* and *T. hemprichii*, respectively, compared to the underground tissues, with few exceptions (Figure 2).

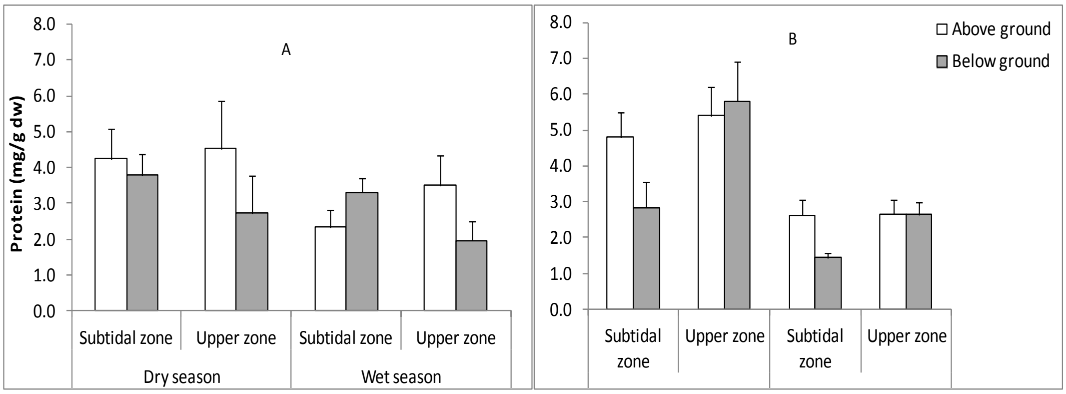

Figure 2: Crude protein in the leaves and roots of *C. rotundata* (A) and *T. hemprichii* (B) from Mjimwema seagrass meadow assessed from the upper zone to subtidal zone during dry and wet seasons. Values are mean ± SE, n = 10.

Soluble carbohydrates

The concentrations of soluble carbohydrates in seagrass species did not vary significantly with either season or zonation in both above and below ground parts of both seagrass species (p > 0.05, Figure 3). Although not statistically different, the *T. hemprichii* persistently allocated more carbohydrates on the below ground tissues in both seasons as well as in both zones with mean values ranging from 33.9 ± 4.9 to 53.9 ± 4.5 mg/g dw (Figure 3B). On the other hand, soluble carbohydrates for *C. rotundata* were slightly higher in the above ground parts except for the wet season at the upper zone where the allocation was higher in roots than in the leaves (Figure 3A).

Table 1: ANOVA table showing effects of seasonal variation and vertical zonation on accumulation of biochemicals in *Cymodocea rotundata* and *Thalassia hemprichii*

Response variable	Source of variation	Df	MS	F value	P value
Phosphorus	Season	1	0.0239	17.774	0.0012
	Zone	1	0.0009	0.663	0.4313
	Residuals	13	0.00135		
Lipid	Season	1	2.5295	44.165	$2.38e^{-05}$
	Zone	1	0.3134	5.471	0.0374
	Residuals	13	0.0573		
Protein	Season	1	0.13859	29.872	0.0001
	Zone	1	0.03794	8.177	0.0144
	Residuals	13	0.00464		
Soluble carbohydrates	Season	1	1.4426	2.602	0.133
	Zone	1	1.0353	1.867	0.197
	Residuals	13	0.5544		
Proline	Season	1	3379	56.357	0.00168
	Zone	1	18	0.307	0.60881
	Residuals	5	60		

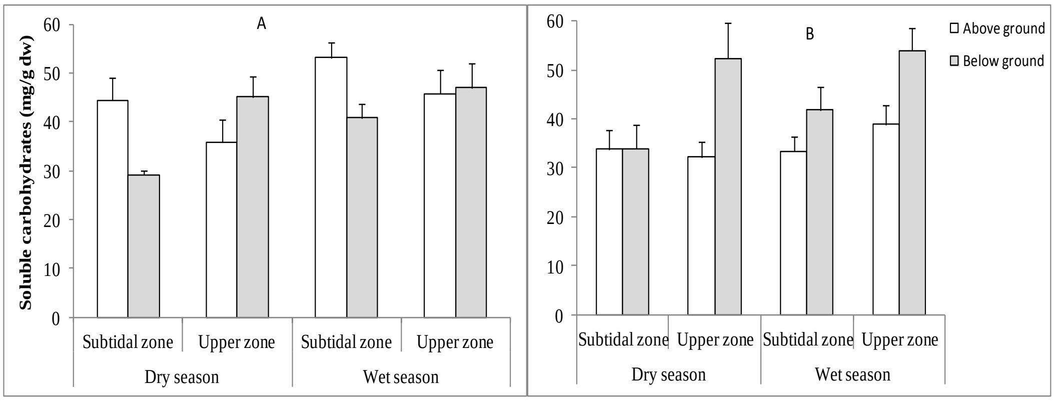

Figure 3: Concentration of soluble carbohydrates in the leaves and roots of *C. rotundata* (A) and *T. hemprichii* (B) from Mjimwema seagrass meadow assessed from the subtidal and upper zones during dry and wet seasons. Values are mean ± SE, n = 10.

Phosphorus

Seasonal variations had significant effects on the concentrations of phosphorus in seagrass species (*p* > 0.05, Figure 4, Table 1). The highest concentrations of phosphorus for both species were recorded in samples collected during the wet season from both zones (Figure 4). Furthermore, the allocation of phosphorus between above and below ground parts did not vary significantly in both species ($p > 0.05$). When comparison was made between zones, both species accumulated relatively more levels of phosphorus at the upper zone even though the differences were not statistically significant ($p > 0.05$, Figure 4).

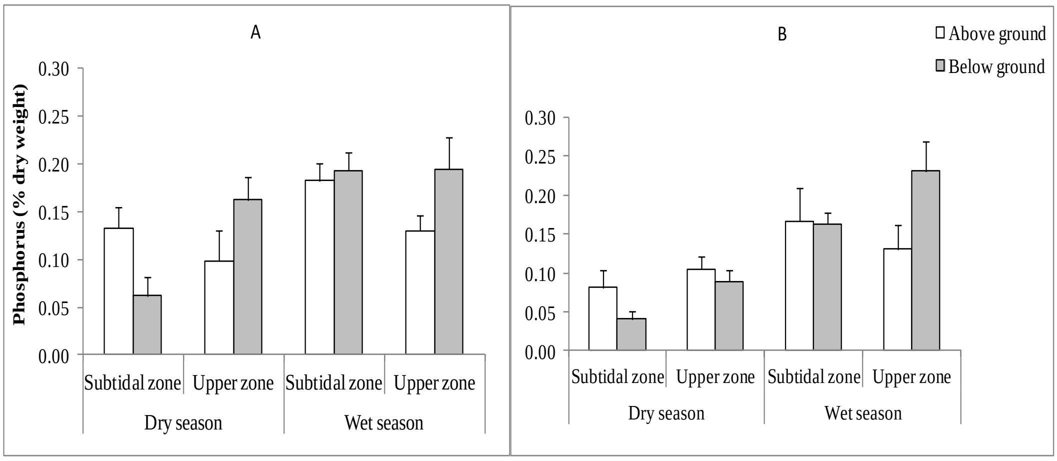

Figure 4: Percentage phosphorus in the leaves and roots of *C. rotundata* (A) and *T. hemprichii* (B) from Mjimwema seagrass meadow assessed from the subtidal and upper zones during dry and wet seasons. Values are mean ± SE, n = 10.

Lipid

The composition of lipid varied significantly between the seasons whereby the highest level was recorded during the dry season in both species ($p > 0.0001$, Table 1, Figure 5). Similarly, when compared between zones, there was significantly higher ($p = 0.03$) lipid concentrations in the dry upper zone than in the subtidal zone. In addition, there were significant differences in lipid concentrations between the studied species ($p = 0.044$, Table 1, Figure 5). The highest mean concentration of lipid was 27.1 ± 1.19 mg/g dw found in the leaves of *C. rotundata* in dry season whereas the lowest was 10.4 ± 2.88 mg/g dw recorded in the roots of *T. hemprichii* in wet season. Furthermore, it was observed that *C. rotundata* accumulated slightly higher lipid percentages compared to *T. hemprichii* (Figure 5), although did not vary significantly.

Proline

The concentrations of proline in seagrass species varied significantly between seasons ($p < 0.001$, Table 1 and 2) whereby they were higher in dry season than in wet season for both species (Table 2). In *C. rotundata*, proline concentrations in wet season ranged from 1.58 to 3.95 µmol g^{-1} and from 2.10 to 9.21 µmol/g in subtidal and upper zone, respectively. In the dry season, the ranges were from 5.5 to 112.8 µmol g^{-1} and 8.4 to 143.9 µmol g^{-1}, for subtidal and upper zones, respectively. On the other hand, proline concentrations in *T. hemprichii* ranged from 11.05 to 16.56 µmol g^{-1} at subtidal zone and from 1.32 to 10.5 µmol g^{-1} at the upper zone in the wet season. In dry season, the concentrations ranged from 4.47 to 92.6 µmol g^{-1} and from 10.5 to 115.7 µmol g^{-1} for subtidal and upper zones, respectively. Furthermore, the population of seagrasses growing at the upper zone had slightly more proline concentrations than those at the subtidal zone, in both species (Table 2); however, the differences were not statistically significant.

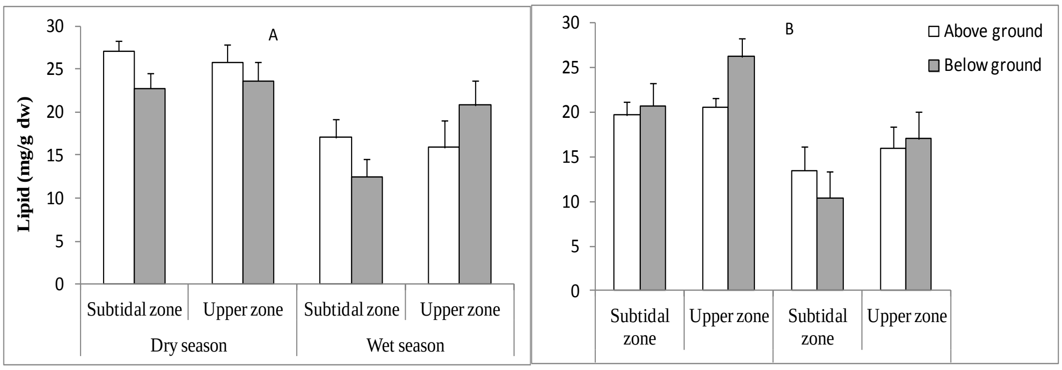

Figure 5: Concentrations of lipids in the leaves and roots of *C. rotundata* (A) and *T. hemprichii* (B) from Mjimwema seagrass meadow assessed from the subtidal and upper zones during dry and wet seasons. Values represent mean ± SE, n = 10.

Table 2: Concentrations of proline (µmol/g FW) in leaves of *Cymodocea rotundata* and *Thalassia hemprichii* collected from Mjimwema seagrass bed from two different zones during dry and wet season. Values are mean ± SE, n = 10.

Species	Zone	Dry season	Wet season
Cymodocea rotundata	Upper	49.5 ± 10.17	5.06 ± 0.71
	Subtidal	48.9 ± 9.71	2.81 ± 0.32
Thallasia hemprichii	Upper	55.4 ± 8.85	4.35 ± 0.71
	Subtidal	36.6 ± 7.76	13.6 ± 1.00

Discussion

Plants continually acquire and allocate biochemicals to different organs or tissues and to different functions involved in their growth and development (Bazzaz and Grace 1997). However, various stressors have influence in the quantity as well as the quality of biomolecules allocated in different organs with respect to the functions performed (Bazzaz and Grace 1997). The present study assessed the concentrations of different biochemicals in two tropical seagrass species in relation to seasonal changes and their vertical distribution from the shore/upper to the subtidal zones. Seasonal variations and zonation influenced the concentrations of biochemicals in the studied seagrasses.

Carbohydrate levels in plants vary depending on the prevailing environmental conditions and the impact they have on photosynthesis, thus regulating the storage and utilization regimes (Chapin et al. 1990). However, allocation of carbohydrates in the seagrass species studied did not vary significantly in response to seasons. This is an indication that seagrasses have enough storage of carbohydrates which is not fluctuating easily with environmental factors as observed by Zimmerman et al. (1995). Other studies however, have observed carbohydrate changing with increasing stress (e.g., Murphy et al. 2003; Öncel et al. 2000).

The concentrations of phosphorus varied significantly between seasons but not with zones. Although it was not investigated in this study, it has been shown that, in areas that are dominated with carbonate sediments (Short et al. 1990), phosphates are highly adsorbed and hence not freely available (Nixon 1982) for plant uptake. In such cases, availability of phosphorus will depend on other processes such as hydrolysis using enzymatic reaction, e.g., alkaline phosphatase (APA) (Pérez and Romero 1993) which will facilitate freeing of some amount into the water column and in the sediment as well as through water column mixing process (Newell 1959, Bryceson 1982). In addition, the levels of phosphates are augmented by runoff during rainy seasons. Also, it has been shown that the concentrations of phosphorus along the coasts of Western Indian Ocean peak in June just after the rainy season which tends to facilitate the discharge and runoffs from various points (McClanahan 1988). Thus, the observed higher amounts of phosphorus in this study during the wet season agree with what has been reported earlier. The concentrations of phosphorus reported here both in the wet and dry seasons were slightly lower than the previously reported concentrations in the same area (e.g. Mvungi and Mamboya 2012) and elsewhere (e.g., Fourqurean et al. 1992, Touchette and Burkholder 2000, Campbell et al. 2012) which could probably be due to limited availability of phosphorus in the studied environment (Perez et al. 1991).

Of the five biochemical compounds assessed in this study, proline accumulated the most in the leaves of both seagrass species during the dry season. Proline is a protein constituent with a “compatible solute” properties thus plays a vital role in protecting plants against various biotic and abiotic stresses (Heldt 2005) and reducing the impacts imposed by the same. The observed high accumulation of proline in the present study is an indication that the environmental conditions were stressful to the seagrasses under investigation. This is congruent with the findings of previous studies which have shown high concentrations of prolines in response to various factors in other seagrass species (e.g., Murphy et al. 2003, Sandoval-Gil et al. 2012, Sandoval-Gil et al. 2014). Likewise, in many other plant species have been reported to accumulate very high levels of proline in response to stressors such as dehydration damage, herbivory and other stressful conditions (Heldt 2005).

The ecological dynamics of coastal environments occur often due to the day to day tidal fluctuations and seasonal changes which alter the zonation and properties of intertidal and subtidal biota (Chappuis et al. 2014). The amount of light available to sustain seagrass photosynthesis varies significantly as a result of tidal events, depth and quality of water, hence regulating growth and efficiency of submerged vegetation (Hemminga and Duarte 2000). During low tide, the shallow coastal areas are exposed to high levels of irradiance and temperatures, and as a consequence, water temperature is raised substantially (Rasheed and Unsworth 2011). Therefore, plant populations at the upper (shore) zone are usually susceptible to dehydration (desiccation) stress, whereby they decrease their metabolism to circumvent direct exposure to the sun (Apichanangkool and Prathep 2014), so as to sustain tissue water balance (Shafer et al. 2007).

In this study, it was observed that seagrasses growing at the shore had more concentrations of the studied biochemicals than those at the subtidal zone even though the differences were not significant in some. Plant cells have the ability to accumulate proline speedily and degrade it quickly when needed (Trovato et al. 2008, Verslues 2010, Liang 2009) as its increased levels could lead to internal nitrogen deficiencies (Cavalieri and Huang 1981). This is in agreement with the results presented in this study which revealed significantly higher concentrations of proline in the shore zone compared to the subtidal zone, indicating that seagrasses responded rapidly to the stress of total exposure to the strong light intensity and relatively increase in temperature by up-regulating biochemicals that help in minimizing the effects. On the contrary, decline in concentrations of proline has also been reported due to other stressors such as heavy metal contamination (e.g., Prange and Dennison 2000), an indication that its concentrations depend on the nature of induced stress as well as characteristics of the species in question.

Metabolic activities of the plant cells may be stimulated by variations in the stress factors.

Studies on various species have revealed that temperature variations influence lipid productivity (Converti et al. 2009, Xin et al. 2011). This was observed in the present study where in both species, the seagrass populations found at the shore are frequently exposed on strong sunlight during low tides, thus accumulated higher lipid percentage compared to those at the subtidal zone which are mostly covered by water and thus not fully exposed to the strong sunshine. Other studies suggest that lipid productivity increases with increase in nitrate levels (Chen and Chen 2006), nitrogen starvation (Li et al. 2008, Rodolfi et al. 2009) and salinity (Takagi et al. 2006).

The present study measured biochemicals in selected seagrass species in response to seasonal variations as well as their intertidal vertical distribution from the shore to the subtidal zones. There was relatively up-regulation of biochemical constituents of the species studied here in relation to the dry season as well as the upper zone. With predicted increasing global temperature, it is anticipated that sea level will rise up to 0.18 - 0.59 m above the present level by the end of the 21st century (Church 2001, Solomon et al. 2007). Rising in sea water level might provide benefits to the seagrasses by reducing desiccation stress, especially at the upper shores, and if that is true, the seagrass beds are likely predictable redistribute shoreward (only if other factors remain constant) thus could promote higher production within the entire seagrass ecosystem. Nevertheless, unpredictable seasonal change, more runoff and the ongoing anthropogenic activities along the coast in this scenario could probably cause severe unpleasant effects by reducing light reaching seagrass beds through increased sediment loads (Short and Neckles 1999, Kaewsrikhaw et al. 2016) as well as increased eutrophication. Furthermore, with the predicted increase in ocean temperature concomitantly with the high irradiance at the intertidal zones, seagrasses and other shallow coastal vegetation will be subjected to too much stress (Collier et al. 2012). This means only tolerant species will be able to survive in such extreme conditions as well as other conflicting factors (Fitter and Hay 2002) acting at different magnitudes.

Conclusion

The findings of this study showed that, *Cymodocea rotundata* and *Thalassia hemprichii* accumulated significantly higher amounts of lipids and proline during the dry season than in wet season. Also, the populations growing at the shore have shown to be more tolerant with the help of accumulation of higher levels of lipids and proline compared to those growing at the sub-tidal zone. Of the studied biochemicals, proline was the highest chemical composition in both species when compared to others, hence could be used as one of the early warning indicators of the environmental perturbations. Generally, the trend observed in this study indicated that accumulation of different biochemicals would differ depending on the species, plant organ, season, and the conditions of the environment where they grow. Thus, biochemical characteristics would fluctuate accordingly and hence could provide valuable information reflecting the status of the seagrasses and their responses to the environmental changes. It is of paramount importance in future to undertake long term monitoring of seasonal and zonation variations on biochemical accumulation but also include more descriptors in order to fully understand their resilience patterns to the reported ever changing climatic conditions and environmental perturbations.

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