The Effect of Temperature on Leaf and Rhizome Growth Rates in the Seagrass *Halophila Ovalis* (R. Brown) Hooker f.

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Abstract: *Seagrass are marine flowering plants that complete their life cycle submerged in water. They are ecologically and economically important as habitat and food source for the majority of marine biological species. Growth and reproduction in seagrass is affected by environmental parameters such as temperature, salinity, tidal current and nutrients. Following the current global warming trend, ocean temperatures in Tanzania are predicted to increase by 2-4^{o}C from current levels of 27-28^{o}C. Changes in climate are thus likely to affect the reproduction and survival of seagrass in this region. Investigating factors affecting seagrass growth can assist to predict impacts of climate variability and change such as increase in temperature in the marine environment. In the present study Halophila ovalis (R. Brown) Hooker was studied as an indicator of the influence of temperature on growth. Samples were collected from Kunduchi beach, Dar es Salaam and grown in the laboratory under variable temperatures of 22-23, 25-26, 27-28, 30-31 and 33-34^{o}C, for 30 days. The length of new and mature leaves was measured at two days intervals; leaf number, leaf weight, rhizome length and rhizome weight were determined at the end of the experiments. Results suggest 25-26^{o}C to be the optimum temperature for growth of Halophila ovalis. Lowest growth rates were observed at temperatures of 22-23 and 30-31^{o}C, while samples grown at 33-34^{o}C survived only for four days. From these results the anticipated temperature increase of 2-4.5 ^{o}C will lead to change in seagrass distribution, abundance and possibly disappearance of higher temperature intolerant species.*

Key words: Temperature, seagrasses, *Halophila ovalis*, growth rate, biomass

INTRODUCTION

Seagrass are marine flowering plants that live and complete their life cycles submerged in seawater. They are descendant of terrestrial plants that re-entered the ocean between 100 and 65 million years ago (Bjork *et al*., 2008). They are classified under five families: Hydrocharitaceae, Cymodocea, Posidoniaceae, Zosteraceae and Rupiaceae (Kenzie *et al*., 2007). Worldwide there are about twelve genera and approximately sixty species of seagrasses (Oliveira *et al*., 2005); ten of these species are reported to occur in Tanzania, these include *Cymodocea serrulata*, *Cymodocea rotundata*, *Enhalus acoroides*, *Thalassia hemprichii*, *Thalassodendron ciliatum*, *Halophila ovalis*, *Halophila stipulacea*, *Halodule wrightii*, *Halodule uninervis* and *Syringodium isoetifolium* (Lugendo *et al*., 1999).

Seagrass communities are one of the most productive and dynamic ecosystems globally. They are referred to as "ecological engineers" as they significantly influence the physical, chemical and biological environment in which they grow (Kenzie *et al*., 2007). They provide a habitat for microbes, invertebrates and vertebrates including commercially and recreationally important species such as dugongs and sea turtles some of which are endangered. They also produce organic carbon, a large fraction of which is transported to

and sustains adjacent ecosystems (Duarte, 2002). Seagrass are also used as food sources for finfish, shellfish and mega herbivores including green turtles and dugongs (Connolly, 2009). Decomposing seagrass provide food for benthic organisms. The decaying leaves are degraded down by fungi and bacteria, which in turn provide food for other microorganisms such as flagellates and planktons (McKenzie, 2008). Seagrass rhizomes and roots bind sediments on the substrates and help to prevent erosion, and slow down water flow allowing suspended materials to settle on the bottom increasing water clarity (McKenzie, 2008).

Growth and reproduction in seagrass is affected by environmental parameters such as temperature, salinity and tidal current (McKenzie, 2008). Climate change affects oceanographic environmental parameters such as temperature, salinity and tidal currents which in turn affect growth and reproduction in seagrass (Bjork *et al*., 2008). Global mean temperatures are expected to rise by approximately range of 2-4.5°C by 2100 (IPCC, 2007). In Tanzania temperatures are predicted to increase by 2.1^{o}C in the northern parts to 4^{o}C in the central, eastern and southern parts of the country (Seitz and Nyangena, 2009). These changes are anticipated to cause seaward shifts of intertidal species such as *H. ovalis* to the sub-tidal zone. This potential shift may result in a reduction in the species diversity and abundance dependent on some mechanisms for survival of the species via both sexual and clonal reproduction (Short and Neckles, 1999). Changes in climate are thus likely to affect the reproduction and survival of seagrass (Short *et al.*, 2001). Previous studies on the effect of temperature on reproduction and growth rate of *H. ovalis* suggest that temperature affects vegetative reproduction by affecting rhizome extension (Durako and Moffler, 1987).

Temperature is also reported to be a critical factor controlling seagrass growth rate and survival (Poloczanska *et al*., 2007). However, detailed account on effect of temperature on growth parts and the optimal temperature for growth of *H. ovalis* has yet to be documented. This information can be utilized in developing appropriate *ex-situ* conservation strategies and *in-situ* seagrass management for resilience to climate change. Hence this study was carried out in light of establishing the optimal temperature for growth of *H. ovalis* and understanding the effect of temperature on its leaf and rhizome growth rate.

MATERIAL AND METHODS

The study area

Samples of *H. ovalis* were collected from Kunduchi beach, Dar es Salaam. Kunduchi beach is located at 6º 39′ – 6º 41′ S and 39º 12′ – 39º 13′ E, approximately 18 km north of Dar es Salaam harbour in Kinondoni District, Dar es Salaam Region, Tanzania (Figure 1). Kunduchi intertidal mudflat where the samples were collected is located next to Silver Sands Hotel (about 1km north of the University of Dar es Salaam Marine Biology Research Station).

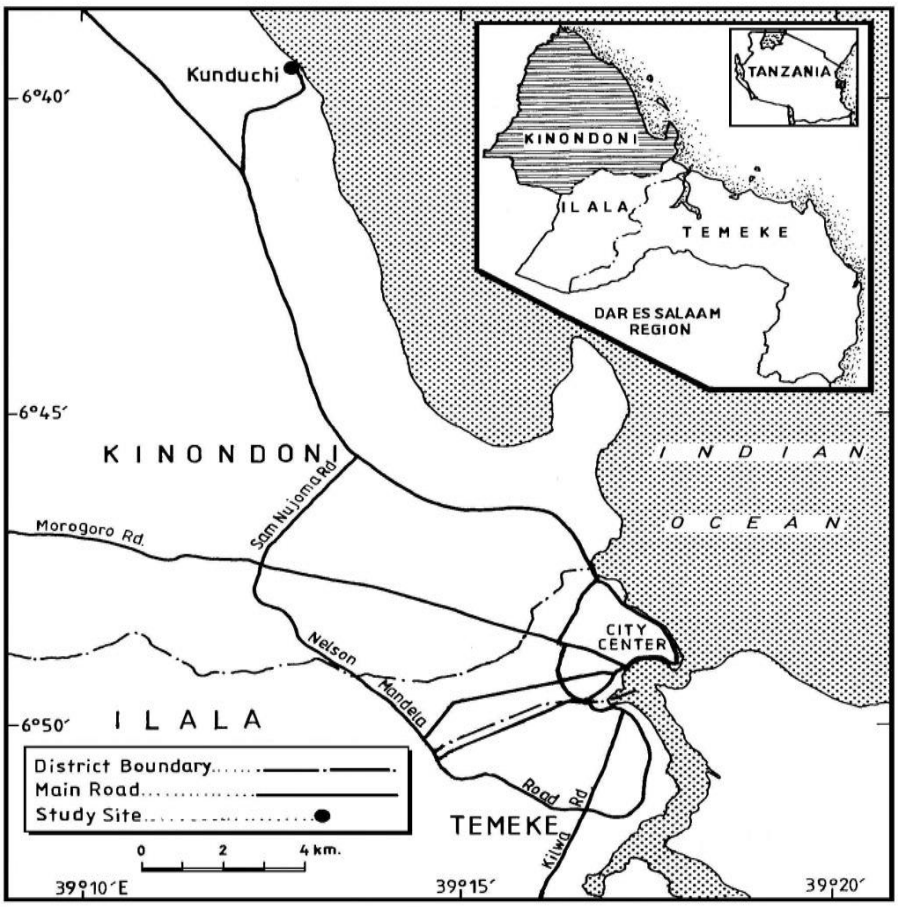

Figure 1: A map showing the study site, Kunduchi intertidal mudflat
(Source: Cartographic unit University of Dar es Salaam)

Sampling and Experimental set up

Sampling was done during the spring low tide from May to early October 2012. During every field visit temperature, salinity and light intensity were recorded. Intact cores of *H. ovalis* and seawater were collected and transported to the University of Dar es Salaam. Upon arrival cores were immediately placed into a large aquarium (70 x 70 x 25cm) with sediment and ambient sea water (salinity ca 34.6 ppt). Light intensity of 200 $\mu molm^{-2}s^{-1}$ was provided using cool Lipper type of tube light and provided with a 12:12hr light–dark rotational exposure for 4 weeks to acclimatize. Temperature in this aquarium was maintained at 27-28°C using thermostatic aquarium heaters. The air compressor was connected to the aquaria to ensure air circulation. Salinity was measured daily using a hand refractometer and maintained by addition of distilled water to compensate for losses due to evaporation. Every two days, seawater was carefully taken out and fresh seawater was added to ensure nutrient availability in the water column.

After acclimatization, the *H. ovalis* samples were transplanted to smaller aquaria (35 x 35 x 25) and subjected to varied temperature treatments. Temperature treatments were set at 22-23°C and 25-26°C to represent below optimal range, 27-28°C as the ambient temperature or control, 30-31°C and 33-34°C as above optimal range. Temperature ranges between 22°C to 31°C were achieved using thermostatic aquarium heaters placed in each aquarium whereas

33-34°C was achieved by placing the aquarium with samples into water bath in which the temperature of the water bath was regulated until the required temperature was obtained in the aquarium. Temperature and salinity in aquaria were measured daily and adjusted as needed. After every two days water in the aquarium was carefully replaced with fresh seawater using a sucking tube without disturbing the plants to ensure nutrient availability in the water column. Treatments were replicated for determination of both leaf and rhizome growth rate.

For the determination of leaf growth rate and new leaf production, six shoots of *H. ovalis* were transplanted from the acclimatization aquarium and planted into smaller aquaria (35x35x25 cm). Three shoots having new leaves of about 6 mm were selected and marked followed by the mature leaf (the second set of leaves from the new leaves). After every two days interval the lengths of new and mature leaves were measured with a string and ruler. Before taking these measurements water was carefully taken out of the aquaria using a sucking tube. After taking measurements of leaves fresh seawater was added in every aquarium to a mark to ensure nutrient availability, the experiment was conducted for 30 days. At the end of experiment marked new and mature leaves were harvested and dried at 60°C to constant weight and their dry weight recorded.

From the data collected above leaf growth rate and new leaf production were determination as follows:

The average leaf growth rate (LGR) (g dry wt/shoot/day) was calculated as:

$$LGR = \frac{\text{Total leaf weight}}{\text{Number of shoots X Number of days}}$$

New leaf production (NLP) was calculated as:

$$NLP = \frac{\text{Total number of leaves formed}}{\text{Total number of shoots}}$$

For the determination of rhizome growth rate and mean rhizome biomass, six shoots with an initial rhizome length of 15cm were carefully uprooted transplanted from acclimatization aquarium and planted in smaller aquaria having sediments and seawater for temperature treatments. The aquaria were also subjected to varied temperature (22-23°C, 25-26°C, 27-28°C, 30-31°C and 33-34°C) treatments for the period of 30 days. At the end of experimental period plants were carefully uprooted and the final rhizome lengths were measured; thereafter rhizomes were dried in an oven at 60°C to constant weight and dry weights were recorded.

Rhizome growth rate and mean rhizome biomass were determination as follows:

The rhizome elongation rate (cm/day) was calculated as:

$$\frac{\text{Final Length of the rhizome - Initial length of the rhizome}}{\text{Number of days}}$$

The mean rhizome biomass (g dry wt/ shoot) was calculated as:

$$\frac{\text{Total rhizome weight}}{\text{Total number of shoots}}$$

Data analysis

The variation in leaf length, leaf number, leaf growth rate, rhizome growth rate and rhizome biomass among different temperature treatment were analyzed using one-way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons.

RESULTS

Effect of Temperature on growth characteristics

Leaf elongation

Results of the effect of varied temperature on leaf elongation are presented in Figure 2. Variation in temperature resulted in variation in leaf elongation in new leaf and the highest leaf length was 44.3mm which was measured at 25-26ºC. However for mature leaves the difference in leaf elongation was very low hence lines showing leaf elongation at different temperatures were not well resolved as shown in figure 2B.

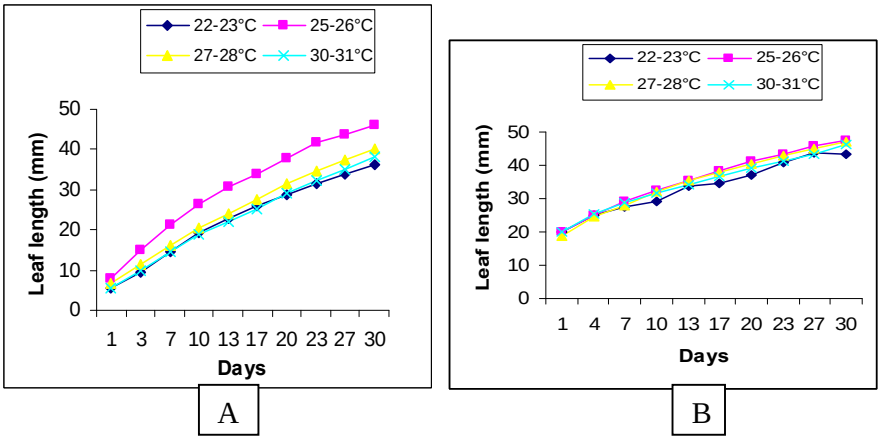

Figure 2: Leaf elongation of *H. ovalis* at varying temperature. A = Leaf elongation in newly formed leaves and B = leaf elongation in mature leaves

A distinctive difference in leaf elongation at varied temperature treatments was observed in newly formed leaves (Fig. 2 A) compared to mature leaves which showed slight difference (Fig. 2 B). This was further supported by statistical analysis whereas a significant difference in leaf elongation between temperature treatments was higher in new leaves ($p < 0.0001$, $F = 96.2$, $DF = 39$) than mature leaves ($p<0.001$, $f = 15$, $DF = 39$).

Leaf growth rate

The variation in temperature resulted in variation in leaf growth rates in both new and mature leaves as shown in figure 3.

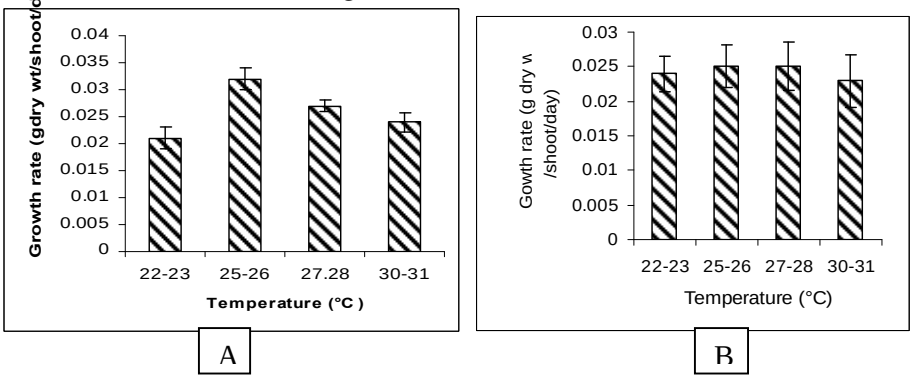

Figure 3: Mean leaf growth rate of *H. ovalis* grown at variable temperature. A = Mean growth rate in new leaves; and B = Mean growth rate in mature leaves.

Growth rates ranged between 0.022- 0.034g dry wt shoot^{-1}day^{-1} at 22-23°C and 25-26ºC, for the new leaves. For mature leaves the highest mean leaf growth rate was 0.025 g dry wt shoot^{-1}day^{-1}which was measured at a temperature of 25-26ºC. The variation in leaf growth rate was more significant in new leaves ($p < 0.0001$ f =14, DF = 35) than mature leaves ($p< 0.0052$, f =5.12, DF= 35)

New leaf production

The formation of new leaf per shoot throughout experimental time was different between temperature treatments (Figure 4).

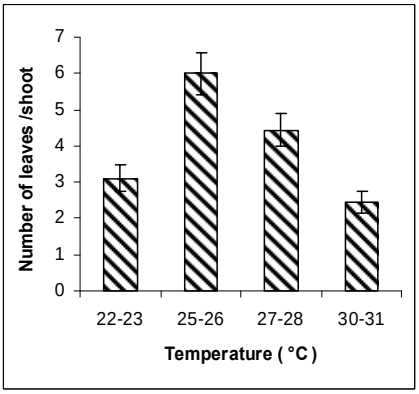

Figure 4: Mean leaf number produced per each shoot grown at variable temperature

At the end of experiment the highest mean of 6 leaves per shoot were formed at 25-26°C while the lowest mean number of leaves (2.3) was formed at a temperature of 30-31ºC. Variation in mean number of leaves produced per shoot across variable temperature was highly significant ($p<0.0001$, $f = 13$, $DF = 35$). Nevertheless Multiple Comparison test showed that the significant difference in number of leaves produced per shoot between treatments occurred at a temperature of 22-23°C vs. 25-26°C and 25-26°C vs. 30-3°C.

Rhizome growth rate

Variation in temperature resulted in variation in rhizome growth rate as indicated in figure 5.

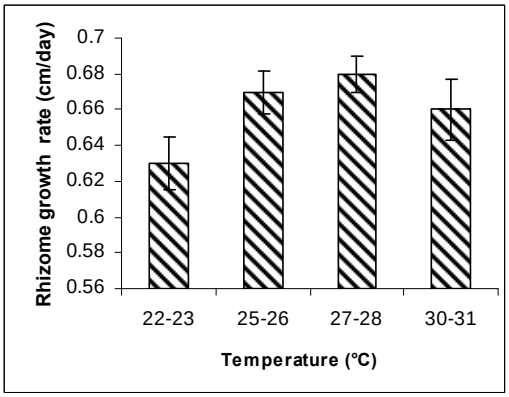

Figure 5: Rhizome growth rate at variable temperature

The highest rhizome growth rate of 0.69 cm day^{-1} was obtained at the temperature of 27-28°C while the lowest rhizome growth rate of 0.63 cm day^{-1} was obtained at 22-23°C. Statistically a significant difference in mean rhizome growth rate was observed only between temperature treatments of 22-23°C and 27-28°C ($p< 0.0391$, $f = 4.519$, $DF = 11$).

Rhizome biomass

The results of rhizome biomass of *H. ovalis* grown under variable temperature treatments are shown in the Figure 6.

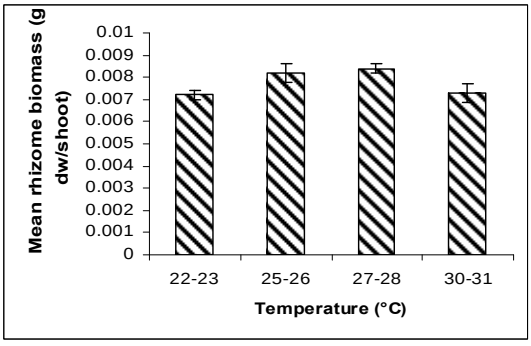

Figure 6: Mean rhizome biomass of plants grown under variable temperature

The highest rhizome biomass of 0.0084g dry wt/shoot was observed at 27-28°C and the lowest 0.0072g dry wt/shoot was observed at 22-23ºC. However, statistically there was no significant difference in rhizome biomass between temperature treatments.

DISCUSSION

This study reveals that growth in *H. ovalis* varies significantly with increase in temperature from 22-23°C. It was also noted that *H.ovalis* is sensitive to changes in temperature because a slight change from 25-26 to 27-28°C resulted in a difference in growth characteristics.

The effect of temperature on leaf growth appears to be more significant in new leaves than mature leaves a phenomenon that may be attributed to physiological activities such as photosynthesis and respiration (Wollaston, 1996). New leaves generally have higher rates of physiological activities thus increased temperatures may limit mechanisms such as respiration and photosynthesis that are enzymatically controlled leading to reduced growth rates.

In this study, the highest leaf length and highest growth rate for both new and mature leave was observed at temperatures between 25- 26°C suggesting this to be the optimum temperature for physiological processes such as photosynthesis and respiration in *H. ovalis*. The lowest growth rate at 22-23°C and 30-31°C may be explained by the effect of temperature on enzymatic control of processes such as photosynthesis and respiration. High temperature causes protein denaturation thus damaging enzymes and protein membrane reducing the efficiency of key enzymes such as Ribulose bisphosphate carboxylase (Rubisco) (Feller *et al*., 1998). Low temperatures (such as 22-23ºC in this case) cause inactivation of photosynthetic enzymes resulting in the low growth as observed in *H. ovalis* under this treatment.

In the present study it was further observed that more leaves were formed at 25-26°C. This is most likely the optimum temperature for growth of *H. ovalis* that allows rhizome extension and hence more leaf formation since rhizome elongation is of central importance for leaf formation and growth (Tomlinson, 1974). However, at lower and higher temperatures of 22-23°C and of 30-3°C, respectively fewer leaves were formed, probably because decrease or increase in temperature adversely affect important plant processes such as photosynthesis and respiration which in turn resulted to little energy production and hence limited rhizome extension and few new leaves production. These results correlate with results of Roberto and Erick (2004) who reported that the time required for leaf formation increase as temperature increases.

During this study it was observed that leaves of *H. ovalis* grown under extreme higher temperatures of 33-34°C turned blackish in colour and dropped after two days and plants eventually died within four days. The observed intolerance of *H. ovalis* to higher temperature may be attributed to temperature induced enzyme dysfunction and denaturation of important enzymes for physiological processes. Dekov *et al*. (2000) reported that extreme temperature above tolerance limits in seagrasses leads to inactivation of many chloroplasts enzymes, mainly induced by oxidative stress which causes lipid peroxidation and consequently membrane injury, protein degradation and enzyme denaturation which led to plant death. These results also conform to the observations of Campbell *et al*. (2006) who reported that temperatures above 33°C caused thermal breakdown of photosynthetic product resulting in plant death.

Unlike the leaves, which showed optimal growth at 25-26 ºC, growth rates observed in the rhizomes were optimal at temperatures of 27-28 ºC. This is probably because rhizomes lie in the sediment, which may serve as a buffer to heat absorption. Thus whilst the water temperature in the aquarium registered 27-28°C it is possible that the temperature in the sediments was lower perhaps by a degree or two (25-26ºC) which has been seen as optimal for *H. ovalis* growth. Validation of these observations is necessary in future studies.

Temperature variation did not significantly vary rhizome biomass. This is probably due to short exposure time (30 days) as growth processes that lead to biomass variation in vegetation tissue such as rhizomes is not rapid. Similar results are reported by Koch *et al*. (2006) where the variation in rhizome biomass of *Thalassodendrontestudium* cultured at variable temperature ranging between 27 to 35ºC did not show significance difference unless the plant biomass was measured, suggesting the minimal contribution of rhizome growth to overall plant growth.

CONCLUSION

From the results obtained in the present study it can be concluded that *H. ovalis* grows under optimum water temperature of between, 25-26ºC. This observation strongly suggests that in places having higher sea water temperatures such as tropical West Indian Ocean including Tanzania where the average water temperatures is 27-28 ºC the anticipated temperature increase of 2-4.5ºC (IPCC, 2007) will lead to change in distribution and abundance of *H. ovalis* and possibly disappearance of some seagrass species, which are intolerant to higher temperature. Therefore effective management and conservation strategy aimed towards improving seagrass resilience to climate change should be developed so that it allows seagrass meadows to continue supporting the marine ecosystems.

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