

SEAGRASSES AND THEIR EPIPHYTES

**Characterization of abundance and productivity
in tropical seagrass beds**

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Front cover: Photo of a shoot of the seagrass *Thalassodendron ciliatum* showing the associated epiphytic community (Photo by Patrick Mathendu).

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TO MY SISTERS

Rose, Jean, Liza and Rebecca

In the field of observation, chance favors only the prepared mind
Louis Pasteur (1822 - 1895)

ABSTRACT

Seagrass beds cover large intertidal and subtidal areas in coastal zones around the world and they are subjected to a wide variety of anthropogenic influences, such as nutrient enrichment due to sewage seepage. This study was undertaken to address specific questions focusing on whether near shore tropical seagrasses that receive a constant influx of groundwater nutrient inputs, would exhibit a higher productivity and to what extent epiphytic algae reflect the impacts of nutrient inputs. An additional aspect of study was to determine the prevalence of “acid zones” in tropical seagrasses. The productivity of the seagrasses *Cymodocea rotundata*, *Thalassia hemprichii* and *Thalassodendron ciliatum* was compared in two sites along the Kenyan coast; Nyali (a high nutrient site) and Vipingo (a low nutrient site). Of the three seagrasses *T. hemprichii* showed the most distinct differences with higher growth and biomass in the nutrient rich site whereas the growth of *C. rotundata* was similar in the two sites. A high epiphytic cover was found on the shoots of *T. ciliatum* found in the high nutrient site Nyali.

Morphological and genetic characterization of bacterial and cyanobacterial epiphytes showed specific associations of nitrogen fixing cyanobacteria on the seagrass *C. rotundata* in the low nutrient site (Vipingo). At this site, shoots of *C. rotundata* had a higher C:N ratio compared to shoots in the high nutrient site (Nyali) indicating that the association with nitrogen fixing cyanobacteria is a strategy, for this species, to meet its nutrient needs. Bacterial epiphytes belonging to the group Cytophaga-Flavobacteria-Bacteroides (CFB) were found on *T. ciliatum* and *T. hemprichii* from the two sites. CFB bacteria are characteristic of waste water, particularly from livestock farming areas, thereby confirming seepage of groundwater from surrounding catchment areas. These prokaryotic associations were specific for the different seagrasses and it appears that the establishment of epiphytic associations may not be a random encounter but a specific association that meets specific needs.

The seagrass *T. ciliatum* in the high nutrient site had an abundance of macroalgal epiphytes and the impact of the epiphytic coverage was assessed using Pulse Amplitude Modulated (PAM) fluorometry. The photosynthetic activity of seagrass parts that were covered by epiphytes was suppressed but the productivity of the whole shoot was not significantly reduced. In the nutrient rich site, epiphytes were found to contribute up to 45% of the total estimated gross productivity, during the SE monsoon season, while epiphytic contribution in the nutrient poor site, was 8%. Epiphytic abundance and contribution to productivity decreased during the NE monsoon. The photosynthetic activity of *T. ciliatum* shoots was similar in the two study sites with shoots in the nutrient rich site growing faster. *T. ciliatum*, in the low nutrient site, invested in the development of below ground root tissue which may indicate the development of a strategy to gain access to pore water nutrient pools.

Carbon uptake strategies of eight tropical seagrasses were re-evaluated to determine how common the “acid zone” mechanism is among tropical seagrasses. Six of the eight species studied showed photosynthetic inorganic carbon (Ci) acquisition based on carbonic anhydrase catalysed HCO_3^- to CO_2 conversions within an acidified diffusion boundary layer (“acid zone”). *Cymodocea serrulata* appeared to maintain its carbon uptake by extracellular carbonic anhydrase catalysed CO_2 formation from HCO_3^- without the need for acidic zones, whereas, *Halophila ovalis* appeared to have a system in which H^+ extrusion may be followed by HCO_3^- - H^+ co-transport into the cells. These findings indicate that competition for carbon, between the host seagrass species and epiphytes, could determine seagrass-epiphyte associations.



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LIST OF PAPERS

This thesis is based on one published paper and four manuscripts. The different papers will be referred to by their Roman numerals in the summary. Some of the information shown in this thesis is not included in the manuscripts.

- I** Uku, J. & Björk, M. (2005) Productivity aspects of three Kenyan seagrass species in areas of different nutrient levels in Kenya. *Estuarine, Coastal and Shelf Science* 63: 407 – 420 (Reprinted with permission from Elsevier).
- II** Uku, J., Björk, M., Bergman, B. & Díez, B. Characterization and comparison of prokaryotic epiphytes associated with seagrasses (Manuscript)
- III** Uku, J. & Björk, M. Does the abundance of epiphytes affect the productivity of the tropical seagrass *Thalassodendron ciliatum*? (Manuscript)
- IV** Uku, J. & Björk, M. Growth and photosynthetic productivity of the tropical seagrass *Thalassodendron ciliatum* and its associated macroalgal epiphytes (Manuscript)
- V** Uku, J., Beer, S. & Björk, M. Buffer sensitivity of photosynthetic carbon utilization in eight tropical seagrasses (Manuscript; accepted in *Marine Biology*)

I was involved in the planning and execution of the different projects as well as in the writing of the papers presented in this thesis. Dr. Díez developed the methodology, analysed DNA sequences and constructed the phylogenetic tree in Paper II. Prof. Sven Beer made significant contributions to the writing of Paper V.

ABBREVIATIONS

AF	Absorption factor
AZ	Acetazolamide – an external inhibitor of carbonic anhydrase
BOD	Biological Oxygen Demand
CA	Carbonic anhydrase
Ci	Inorganic carbon
ETR	Electron transport rate
F _m	Maximal chlorophyll fluorescence in a dark adapted sample
F _m '	Maximal chlorophyll fluorescence in actinic light
F _o	Minimal chlorophyll fluorescence measured in a dark adapted sample
F _o '	Minimal chlorophyll fluorescence measured after exposure to actinic light
F _v	Variable fluorescence (F _o -F _m)
F _v /F _m	Maximal quantum yield
ΔF/F _m '	Effective quantum yield
NE	North East
PAM	Pulse amplitude modulated
PAR	Photosynthetically active radiation
PSI	Photosystem I
PSII	Photosystem II
SE	South East
TRIS	Tris (hydroxymethyl) aminomethane

TABLE OF CONTENTS**PAGE**

ABSTRACT.....	4
LIST OF PAPERS.....	5
ABBREVIATIONS	6
TABLE OF CONTENTS.....	7
PREFACE.....	8
INTRODUCTION.....	9
Seagrasses as a component of the tropical marine environment.....	9
Nutrients in the seagrass environment.....	10
Carbon in the seagrass environment.....	10
THE SCOPE OF THE STUDY.....	11
Characteristics of the study areas.....	11
Selected seagrasses and methodology used in the different studies.....	12
RESULTS AND DISCUSSION.....	13
Effect of nutrient influx on intertidal seagrasses.....	13
Macroalgal epiphytes on the selected seagrasses.....	14
Prokaryotic epiphytes on seagrass leaves.....	16
Application of PAM fluorometry to determine photosynthetic activity in seagrasses and epiphytes.....	17
Impact of epiphytic shading on the productivity of the seagrass <i>T. ciliatum</i>	20
Coupling photosynthetic productivity to seagrass growth.....	21
Bicarbonate utilization by the formation of “acid zones” in seagrasses.....	22
CONCLUDING REMARKS AND FUTURE PERSPECTIVES.....	24
ACKNOWLEDGEMENTS.....	26
REFERENCES.....	27

PREFACE

Seagrass beds are a common feature in coastal ecosystems around the world. They form an important habitat for fish and other fauna and contribute to the economy of coastal populations. Due to the close proximity of seagrass beds to human habitation they are impacted by sewage seepage through the influx of groundwater into the coastal zone. This leads to nutrient enrichment of near shore coastal areas. Along the East African coastline, near shore seagrasses receive a daily input of these nutrients and the impact of this on East African intertidal seagrasses was the focus of this research.

A comparative study of the growth patterns of three seagrass species, *Thalassodendron ciliatum* (Forskål) den Hartog, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Cymodocea rotundata* Ehrenberg & Hempr. ex Ascherson, was undertaken in two sites along the Kenyan coast which were characterized by different nutrient regimes. Of the two sites, Nyali had a high concentration of hotels in close proximity to the beach while Vipingo was a site with private residences and low hotel development. Groundwater seepage characterized these sites and high nutrient levels were recorded in the water column in Nyali. The effect of nutrient inputs on the growth and productivity of the seagrasses was assessed. Enhanced nutrients, within seagrass beds, have been shown to cause a prolific growth of epiphytes and the epiphytic assemblage on the seagrasses was evaluated. Special focus was given to the characterization of prokaryotic epiphytic assemblages on the three seagrass species with particular emphasis on nitrogen fixing cyanobacteria in the two study sites.

The seagrass *T. ciliatum* dominates the coastal lagoons of the East African coast and it was found to have a high abundance of macroalgae particularly in the nutrient rich site. High epiphytic cover has been shown to affect the growth of seagrasses in several parts of the world and in this study the level of suppression of the high epiphytic cover was evaluated using Pulse Amplitude Modulated (PAM) fluorometry. During this investigation, PAM fluorometry was compared to the hole punch method (which estimates leaf growth) and to oxygen evolution (within enclosed bottles). The usefulness of combining these techniques as well as the limitations of PAM fluorometry are discussed.

Carbon uptake mechanisms of several tropical seagrasses were also evaluated. Seagrasses have been characterized as being carbon limited and studies that have reached this conclusion have used buffers in the experimental design to control pH levels. In this thesis, the buffer effect on the carbon utilization strategy of eight tropical seagrasses was reviewed in an attempt to elucidate carbon uptake mechanisms and the prevalence of “acid zones” in tropical seagrasses.

INTRODUCTION

Seagrasses as a component of the tropical marine environment

Seagrasses are marine angiosperms comprised of a group of higher plants that have adapted to live submerged in the sea. Although they form less than 0.02% of the angiosperm flora (Les et al. 1997) they are found in all coastal areas of the world except the Antarctic (Hemminga & Duarte, 2000). In tropical coastal zones, seagrass beds extend from the intertidal zone down to 20 m water depths or more and they are often located in between mangrove and coral reef ecosystems. Seagrasses are able to grow completely submerged in the marine environment. They are salt tolerant and possess an anchoring system, comprised of roots and rhizomes that enables them to withstand wave action. Seagrasses are also capable of pollination underwater (Phillips & Meñez, 1988; Hemminga & Duarte, 2000). The general morphology of seagrasses includes a well-developed rhizome, which bears roots and erect shoots that project into the water column.

There are differences in the spatial distribution of different seagrass genera with the following 7 genera being found in tropical and subtropical regions: *Cymodocea*, *Enhalus*, *Halodule*, *Halophila*, *Syringodium*, *Thalassia* and *Thalassodendron*. The remaining genera *Amphibolis*, *Heterozostera*, *Phyllospadix*, *Posidonia*, *Zostera* and *Ruppia* are found in temperate waters (Hemminga & Duarte, 2000). These seagrasses have also been grouped into floral groups based on their localities in different parts of the world (Hemminga & Duarte, 2000). The floral groups are the Temperate North Atlantic flora, the Temperate East Pacific flora, the Temperate West Pacific flora, the Temperate South Atlantic flora, the Mediterranean flora, the Caribbean flora, the Indo-Pacific flora, the South Australian flora and the New Zealand flora. Seagrass genera belong to the monocotyledon subclass Alismatidae and it is thought that they evolved from freshwater plants or from a combination of freshwater and salt-tolerant plants (Les et al., 1997).

Most temperate seagrass meadows are monospecific while those in tropical and subtropical areas are multispecific (Hemminga & Duarte, 2000). Although the species richness of seagrasses in any one tropical meadow may be high there is usually one species that dominates in terms of biomass (Hemminga & Duarte, 2000). Most of the biological diversity in seagrass beds is from the associated algae and fauna. The meadows with the richest species diversity are found in the Indo-Pacific and the Red Sea where mixed meadows are abundant (Hemminga & Duarte, 2000). The seagrasses described in this thesis belong to the Indo-Pacific floral region.

Seagrasses provide a substrate for organisms such as bacteria, microalgae, macroalgae and invertebrates (Harlin, 1975; Borowitzka & Lethbridge, 1989) and these organisms comprise a diverse epiphytic community on seagrass species. Epiphytic algae serve several functions within seagrass beds. The encrusting coralline red algae contribute to the sediment calcium carbonate levels once the leaves that they have colonized break off from the shoot and disintegrate (Orth & Montfrans, 1984). Epiphytic algae also enhance the settlement of bivalves in seagrass areas (Bologna & Heck, 1999). Epiphytes can become part of the diet of different consumer organisms through selective consumption or accidental consumption as well as through detritus food webs (Borowitzka and Lethbridge, 1989). Moncreiff & Sullivan (2001) showed the trophic importance of epiphytic algae associated with the seagrass *Halodule wrightii* (Ascherson) while Yamamuro (1999) showed that herbivores in Fijian seagrass beds depended on epiphytic cyanobacteria for their food.

Nutrients in the seagrass environment

The marine environment contains varying levels of ammonia, nitrate and phosphates both in the water column and in the pore water of the sediments (Hemminga & Duarte, 2000) therefore nutrients are available both to the leaves and to the roots that are anchored in the sediment. The uptake of nutrients by leaves or via the roots depends on the environmental conditions and sediment characteristics (Erftemeijer, 1993, Touchette & Burkholder, 2000; Hemminga & Duarte, 2000). For instance, shifts from the utilization of pore water nutrients to nutrients in the water column have been observed in areas where nutrient enrichment of the water column has occurred (Touchette & Burkholder, 2000). Carbonate sediments bind phosphates thereby seagrasses growing on such sediments are phosphorous limited (Hemminga & Duarte, 2000). Seagrasses have also been shown to retain some of their phosphorous and nitrogen through resorption from dying leaf tissue (Stapel & Hemminga, 1997).

Generally marine waters, and in particular tropical waters, are characterized by low nutrient levels (Erftemeijer, 1993; Hemminga and Duarte, 2000) and anthropogenic increases in water column nutrient levels has been shown to have negative impacts on seagrass species. The cover of seagrasses in coastal areas around the world has declined and in several areas this has been attributed to nutrient enrichment (eutrophication) caused by sewage seepage from coastal populations, industrial effluents and fertilizer inputs, (Silberstein et al, 1986; Shepherd et al., 1989; Herbert, 1999; Touchette & Burkholder, 2000). Increased interest in epiphytic algae developed due to the finding that epiphytic biomass and productivity was influenced by the nutrient content of the water column (Borowitzka & Lethbridge, 1989) and one of the early signs of increased nutrients is the enhanced coverage of seagrasses by epiphytic algae that grow on the surface of these plants (Borowitzka & Lethbridge, 1989). The heavy loads of epiphytes on seagrasses in nutrient rich sites have been shown to affect the photosynthesis of seagrasses through shading (Sand-Jensen, 1977; Bulthuis and Woelkerling, 1983; Silberstein et al., 1986; Shepherd, et al., 1989) and this is thought to be one of the causes of reduction in the coverage of seagrass beds in several parts of the world.

Carbon in the seagrass environment

Inorganic carbon (Ci) in the marine environment is present in four forms carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}). The concentrations of CO_2 in seawater are only slightly lower than in air but more importantly the diffusion rate for CO_2 in seawater is about 10,000 times slower than in air (Smith, 1988). Once CO_2 has dissolved in the seawater it reacts with water to form carbonic acid which directly dissociates further into bicarbonate and carbonate. Seawater with a pH of 8.2, salinity of 35 ‰, and a temperature of 15°C contains 90% bicarbonate and only 0.6% free carbon dioxide (Hemminga & Duarte, 2000). Like terrestrial plants, marine plants require CO_2 as this is the only inorganic carbon species that can enter the Calvin cycle. The low CO_2 content of seawater was thought to limit the photosynthetic capacity of seagrasses and early experimental work showed that seagrasses had low efficiencies in the utilization of other available sources of carbon such as HCO_3^- which are easily utilized by macroalgae (Beer & Koch, 1996). Later studies showed that seagrasses could utilize HCO_3^- via extracellularly-acting membrane bound carbonic anhydrase (CA) and that some seagrasses are capable of proton (H^+) mediated HCO_3^- uptake in “acid zones” (Beer et al., 2002) thereby making them more efficient in the utilization of HCO_3^- than was originally thought. Recent work by

Hellblom et al. (2002) showed that the use of buffers for the maintenance of stable pH levels during carbon uptake experiments interferes with the mechanisms of carbon uptake leading to the widely held perception that seagrasses are carbon limited in the marine environment.

THE SCOPE OF THE STUDY

The rising populations along the East African coastal zone have led to increased nutrient inputs into coastal lagoons and this study was designed to evaluate the implications of nutrient enrichment on the photosynthetic activity of selected seagrass species. Following the new knowledge on carbon uptake by seagrasses, a component of this study was to re-evaluate the carbon uptake capacities of some tropical seagrasses in view of their sensitivity to buffer additions in experimental studies.

The following specific questions are addressed in this thesis:

1. Is the productivity of intertidal seagrass species affected by the influx of nutrients that comes into a seagrass bed through groundwater inputs? (Paper I)
2. Are there differences in the composition of prokaryotic epiphytes associated with seagrasses in areas of different nutrient levels? (Paper II)
3. Can PAM fluorometry be used to determine photosynthetic productivity of seagrasses and their associated epiphytes? (Paper III & Paper IV)
4. What are the impacts of macroalgal epiphytes on the photosynthetic productivity of the seagrass *Thalassodendron ciliatum*? (Paper III)
5. Is photosynthetic productivity measured by PAM fluorometry, which is based on chlorophyll *a* fluorescence and electron transport rates, coupled to growth increments in the *Thalassodendron ciliatum*? (Paper IV)
6. How do tropical seagrasses deal with low carbon dioxide levels in their environment and how common is the “acid zone” mechanism for bicarbonate utilization among tropical seagrasses? (Paper V)

The results from the five papers are presented in this thesis. These results constitute findings of research work conducted, using a diverse range of methodology, in an attempt to gain an understanding of nutrient and carbon utilization in selected seagrasses growing in tropical seagrass ecosystems.

Characteristics of the study areas

The investigations on seagrass productivity and the assessment of epiphytic assemblages (Papers I to IV) were conducted in two sites found along the Kenyan coastline. The coast of Kenya is over 500 km long and lies between latitude 1 and 5°S and the selected sites for this work were Nyali and Vipingo. Nyali Beach (4° 03'S, 39° 43' E) is an important tourist centre located 2 km from Mombasa island and is characterized by numerous hotels along the beach as well as town settlements in this area. Groundwater seepage points have been found in several areas along the beach zone in the Nyali area. Vipingo (3° 45'S, 39° 50' E) is located 33 km from Mombasa Island. The site in Vipingo has a few residential houses along the beach and one beach hotel. Kenya's coastal population was found to be 1.83 million people in 1989 with an annual growth rate of 3.7% (Obura, 2001). The increasing economic activity due to shipping and tourism has drawn more people to the coast and this has created

conditions for environmental degradation and extensive seepage of pollutants into the coastal ecosystems which has been reported in several coastal areas (Mwashote, 1999; Mwangi et al. 2001). The study sites also reflected the seasonality influence of the South East (SE) monsoon and North East (NE) monsoon with variability seen in nutrient influxes, the floral composition as well as other physical characteristics of the study sites (Paper I). The SE monsoon occurs from March to October while NE monsoon begins in October and extends to March.

The evaluation of carbon utilization in seagrasses (Paper V) was conducted in Unguja (Zanzibar) Island which is located 40 miles off the coast of Tanzania between (5° 40' to 6° 30' S and 39° 10' and 39° 35' E). Seagrasses used were collected from Mbweni Beach, which is located about 5 km south of Zanzibar town. The different seagrasses studied were found in the intertidal zone close to the beach. Some of the seagrass species were found exposed during the low tide period while others were submerged closer to the subtidal zone.

Selected seagrasses and methodology used in the different studies

Several authors recognize the occurrence of twelve seagrass species in the East African coast (Isaac & Isaac, 1968; Bandeira, 2002; Ochieng & Erftemeijer, 2003). There is a thirteenth species *Ruppia maritima* L. which has been found in South African estuaries, in southern Mozambique and in Madagascar (Gullström, et al., 2002). Of these seagrass species *Thalassodendron ciliatum* (Forskål.) den Hartog, *Thalassia hemprichii* (Ehrenberg) Ascherson and *Cymodocea rotundata* Ehrenberg and Hemprich ex Ascherson were studied in Paper I and Paper II. These three seagrasses were chosen by virtue of their location in the intertidal zone, which meant that they were directly influenced, by nutrient seepage from land based sources and they were also common to the two study sites. In Paper I the environmental characteristics of the study sites in Nyali and Vipingo were determined and the productivity of the seagrasses was evaluated using the hole punch method described by Dennison (1990). In Paper II morphological and genetic methods were used to characterize the prokaryotic epiphytes found on the three seagrass species in the two sites.

The focus of Paper III and IV was the seagrass *T. ciliatum* which is dominant along the East African coastline (Ochieng & Erftemeijer, 2003; Obura, 2001). All the seagrasses found in East Africa, apart from *T. ciliatum*, have a growth morphology where leaves extend from the sediment surface. In contrast to this general growth form, *T. ciliatum* has a stem projecting from the sediment surface with its leaves attached to the tip of the stem. The stem is a host to a wide variety of epiphytes (Semesi, 1988; Bandeira 2000; Leliaert et al., 2001). PAM fluorometry was used to compare productivity patterns of this seagrass in the nutrient rich site, Nyali, where epiphytic cover was abundant, with productivity in the nutrient poor site Vipingo. The suppressive effect of the epiphytic cover on the productivity of the seagrass was also evaluated. The use of PAM fluorometry in making such measurements on seagrasses and epiphytic algae is discussed in this thesis.

In Paper V, carbon uptake mechanisms were evaluated for eight seagrasses namely *Halophila ovalis* (R. Brown) Hooker, *Halodule wrightii* Ascherson, *Cymodocea rotundata* Ehrenberg & Hempr. ex Ascherson, *Cymodocea serrulata* (R. Br.) Ascherson & Magnus, *Thalassia hemprichii* (Ehrenberg) Ascherson, *Thalassodendron cilatum* (Forskål) den Hartog, *Enhalus acoroides* (L.) Royle and *Syringodium isoetifolium* (Ascherson) Dandy with each of these species growing in different areas of the intertidal zone. Net photosynthetic productivity was

evaluated in the presence of Tris (hydroxymethyl) aminomethane (TRIS) buffer and acetazolamide (AZ - an inhibitor of carbonic anhydrase (CA) activity) which provided evidence of the presence of “acid zones” in these seagrasses.

RESULTS AND DISCUSSION

Effect of nutrient influx on intertidal seagrasses

Nutrient inputs into the two selected sites in Kenya; Nyali and Vipingo; as well as variation with the tides are shown in Paper I. Nitrate levels in Nyali were documented to reach approximately 17 μM compared to Vipingo where the nitrate level was found to be approximately 4 μM during the low tide period of the wet SE monsoon. During the drier NE monsoon period, low tide nitrate levels in Nyali reached levels of 5 μM while the levels in Vipingo were approximately 2 μM . Ammonia levels were between 3.7 μM in Nyali and 1.5 μM in Vipingo during the SE monsoon while phosphates reached approximately 0.6 μM in the two sites (Paper I). Levels of ammonium and phosphate were not significant when the two sites were compared.

Worldwide estimates of water column nutrients show that the average concentration of ammonium in seagrass beds is 3.1 μM ; the nitrate concentration is 2.7 μM while the average phosphate concentration is 0.35 μM (Hemminga & Duarte, 2000). The nitrate levels recorded in Nyali exceeded these averages while the other nutrients were within the documented levels. The tidal variation in water column nutrient levels indicated that the residence time of the nutrients was low and this has previously been reported in Kenyan lagoons and bays (Kitheka et al., 1996; Kirugara et al., 1998; Mwangi et al. 2001). In Nyali, the low BOD levels reflected a moderate level of nutrient enrichment caused by the short residence time of nutrient rich water within the seagrass beds (Paper I). Porewater nutrient levels were high indicating the availability of sedimentary nutrients in the two study sites (Paper I).

Although it seemed like the ground water coming into the nearshore areas in Nyali, the nutrient rich site, had a low residence time in the intertidal zone due to tidal flushing, the seagrasses *T. hemprichii* and *T. ciliatum* showed differences when growth of these species in the nutrient rich site, Nyali was compared to growth in Vipingo, the low nutrient site. The growth parameters of these seagrasses are documented in Paper I and this study showed notable differences in leaf production and biomass of *T. hemprichii* when the two sites were compared. Leaves of *T. hemprichii* showed leaf production rates of 0.008 g dw shoot⁻¹ day⁻¹ in Nyali and 0.004g dw shoot⁻¹ day⁻¹ in Vipingo, suggesting the influence of nutrient inputs in Nyali (Paper I). *T. ciliatum* stems were found to be around 200 mm long in Nyali whereas those in Vipingo were approximately 80 mm in length. Although the stem length was found to be within the range of stem lengths recorded in Kenyan lagoons by Kammermans et al. (2002) the abundance of epiphytes on the stems of *T. ciliatum* was higher in Nyali during the SE and NE monsoons. The composition of epiphytic algae, determined in an earlier study by Uku & Björk (2001), showed that nutrient enrichment in Nyali had an impact on the assemblage of dominant epiphytes found on the stems of this seagrass. The growth of *C.rotundata* was similar when the two sites were compared.

Macroalgal epiphytes on the selected seagrasses

The most common epiphytic algae found on the seagrass leaves and stems are the encrusting coralline red algae which appear to be the first macroalgae to colonize these substrates. The encrusting coralline red alga that is found on the leaves has been identified as *Hydrolithon farinosum* (J. V. Lamouroux) D. Penrose & Y. M. Chamberlain and it occurs on a variety of seagrasses in different localities (Penrose & Chamberlain, 1993). Stems appeared to have a thicker crust than the leaves, indicating colonization by a different encrusting coralline red alga. However, for the purpose of this thesis, all species that had an encrusting coralline growth form, whether on the stem or leaves of the seagrasses, will be referred to collectively as encrusting coralline red algae.

Before colonization by the encrusting red algae, a variety of microalgal epiphytes such as diatoms were evident on the leaves. The encrusting coralline red algae form crusts on the leaf and stem surface, which may be later colonized by larger algae such as the green algae *Cladophora* sp., red algal species like *Ceramium* sp., filamentous red algae and even sponges (Paper III). There is a lack of succession in the colonization of epiphytes on seagrasses and which was confirmed by early studies conducted by Heijs (1985, 1987) on tropical seagrass species. New epiphytic species settle on top of the pioneer species and do not replace them. Where encrusting coralline algae are found they provide an anchoring point for the spores of larger algae and they appear to provide a more favorable substrate than the leaf surface (Heijs, op cit.).

Microscopic sections of *T. ciliatum* leaves did not reveal any attachment mechanisms between the encrusting coralline red algae and the leaf and the crusts peel off easily from the leaf surface as can be seen in Plate 1.

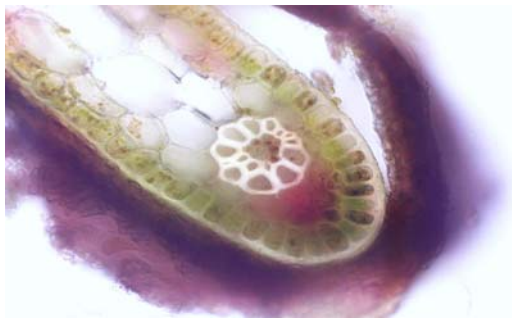


Plate 1. An old *T. ciliatum* leaf showing the encrusting coralline red algae peeling off from the leaf surface (x40)

Furthermore, the arrangement of epiphytes on the stems was not random. In Nyali, the epiphytic algae were arranged in a sequence with the encrusting coralline red algae colonizing the top part of the stem, below the leaf sheaths, followed by the filamentous red algae and then a cover of encrusting coralline red algae at the bottom of the stem close to the sediment. This colonization structure was attributed to the light gradient within the seagrass canopy where the top of the canopy (leaves) receives most light, the middle of the canopy receives up to 30% of the surface light and the

bottom of the stems receive up to 16% of the light reaching the surface seagrass bed depending on the shoot density and the season (Paper III). Similar patterns of epiphytic associations have been documented for species with stems (Borowitzka et al., 1990; Leliaert, 2001). Secondary epiphytes such as *Gracilaria corticata* (J. Agardh) J. Agardh and *Chaetomorpha crassa* (C. Agardh) Kützing were found attached on to other epiphytes on the stem.

The epiphytes on the three seagrasses exhibited seasonal variations as well as site-specific associations as shown from earlier work conducted in the Kenyan study sites (Table 1). The results from the work by Uku & Björk (2001) formed the basis for continued investigation of the seagrass-epiphyte association that is described in this thesis. The most common

macroalgal epiphytes for all seagrasses studied were *Ceramium flaccidum* (Kützing) Ardissonne and the encrusting coralline red algae in the two sites; Nyali and Vipingo (Uku & Björk, 2001). *Ulva pertusa* Kjellman and *Enteromorpha* sp. were notably abundant on seagrasses found on in the nutrient rich site Nyali during the two study seasons (Uku & Björk, 2001). Also evident were the associations of different Cyanophyta in the nutrient poor site, Vipingo, during the different study periods. The seasonality patterns of epiphytic associations of macroalgae seen during this earlier study (Uku & Björk, 2001) were evident throughout the different studies undertaken and they had implications on the estimates of carbon fixation determined later (Paper III & Paper IV). The high abundance of macroalgal epiphytes on the seagrass *T. ciliatum* is attributed to the variety of substrates (stems and leaves) that it offers for epiphytic attachment whereas the other two species only had a leaf surface.

Table 1. The species composition of epiphytic macroalgae found on leaves of *C. rotundata*, *T. hemprichii* and *T. ciliatum* from Nyali and Vipingo summarized from Uku & Björk, 2001 (SE represents presence during the South East monsoon month of August; NE represents presence during the North East monsoon month of February; 1-3 indicates epiphytic abundance on leaves based on percentage cover estimates; epiphytes on *T. ciliatum* are from the leaves and stems; * indicates abundance on stems).

Macroalgal species	<i>C. rotundata</i>		<i>T. hemprichii</i>		<i>T. ciliatum</i>	
	Nyali	Vipingo	Nyali	Vipingo	Nyali	Vipingo
RHODOPHYTA						
<i>Ceramium flaccidum</i> (Kützing) Ardissonne	SE ² NE ²	SE ¹ NE ²	SE ² NE	SE ² NE ²	SE ² NE	SE ² NE
Encrusting coralline red algae	SE ¹ NE ¹	SE ² NE ³	SE ¹ NE ¹	SE ¹ NE ¹	SE ¹ * NE ¹ *	SE ¹ * NE ¹ *
<i>Centroceras clavulatum</i> (C. Agardh)M.					SE	
<i>Amphiroa</i> sp.						NE*
Red algae (unidentified)	NE	SE	SE			SE
<i>Gracilaria</i> sp.		SE		NE		
<i>Gracilaria corticata</i> J. Ag					NE	
<i>Hypnea cornuta</i> (Lamour.) J. Ag.		NE			SE	SE* NE
<i>Wurdemanina</i> sp.					NE*	
PHAEOPHYTA						
<i>Giffordia</i> sp.	NE					SE* NE
<i>Sphacelaria rigidula</i> Kützing	SE		SE			
<i>Hincksia rallsiae</i> (Vickers) Silva	SE ³					SE
CHLOROPHYTA						
<i>Enteromorpha</i> sp.	NE	SE ³ NE	SE ³ NE ³	SE NE ³	SE ³ NE ²	SE ³ NE ³ *
<i>Cladophora mauritania</i> (Kützing)		SE	SE		NE	SE NE
<i>Ulva pertusa</i> Kjellman	NE ³	SE NE	SE NE ²		SE* NE ³ *	SE NE
<i>Chaetomorpha crassa</i> (Ag.) Kützing					NE	
CYANOPHYTA						
Blue green algae	SE					SE
<i>Rivularia</i> sp.		SE NE ¹		SE NE		SE NE ²
<i>Calothrix</i> spp.		SE		SE		
<i>Oscillatoria</i> sp.		SE		SE ³		

Notes: Blue green algae is a mixture of *Calothrix* spp. and *Oscillatoria* spp.; n=30 for each species.

Prokaryotic epiphytes on seagrass leaves

The characterization of prokaryotic epiphytes found on the leaves of the same three Kenyan seagrass species was undertaken using molecular techniques involving polymerase chain reaction (PCR) amplification, denaturing gradient gel electrophoresis (DGGE) and cloning, as well as light microscopic analysis. Such genetic studies are based on the amplification of 16S rDNA genes that are universally conserved but show enough differences to reveal the phylogenetic associations of prokaryotes. This has become a common tool in the assessment of diversity and distribution of prokaryotes in marine environments and also on seagrasses (Weidner et al., 1996; Øvreås et al., 1997; Weidner et al., 2000; Burja et al., 2001).

In this component of study (Paper II), 16S rDNA oligonucleotide primers specific for bacteria and cyanobacteria were used to amplify prokaryotes associated with seagrass leaves. The bacterial analyses revealed the presence of *Cytophaga-Flavobacteria-Bacteroides* (CFB) associated with wastes from animal farms as well as other land sources on *T. ciliatum* and *T. hemprichii* in the two study sites thereby confirming the seepage of groundwater from the surrounding catchment areas. The cyanobacterial DGGE and clone libraries of the samples exhibited an abundance of potential nitrogen fixers on the leaves of *C. rotundata* in the low nutrient site (Table 2).

Table 2. Prokaryotic species closely affiliated to clone samples from the seagrass species (*indicates known nitrogen fixing capabilities according to Bergman et al., 1997; Zehr & Paerl, 1998; Zehr et al., 2000, *? indicates that some species in this group are nitrogen fixers; the percentages indicate the abundance of clones that shared this sequence).

Prokaryotic groups	<i>Thalassia hemprichii</i>		<i>Cymodocea rotundata</i>	
	NYALI	NYALI	NYALI	VIPINGO
Bacteria	Verrucomicrobia (27%)			Verrucomicrobia (2%)
Unicellular Cyanobacteria	<i>Synechococcus</i> spp. (9%)	<i>Chroococcoidiopsis</i> sp.* (6%)		<i>Chroococcoidiopsis</i> sp.* (25%)
	Cyanobacterial symbiont (50%)	<i>Dermocapella incrassata</i> (19%)		<i>Xenococcus</i> sp.* (14%)
		<i>Myxosarcina</i> sp.* (38%)		<i>Myxosarcina</i> sp.* (12%)
Filamentous Cyanobacteria	<i>Lyngbya</i> sp.*? (5%)	<i>Anabaena cylindrica</i> * (6%)		<i>Anabaena cylindrica</i> * (2%)
	<i>Leptolyngbya thermalis</i> (5%)	<i>Leptolyngbya</i> sp. (6%)		<i>Leptolyngbya</i> sp. (2%)
	<i>Trichodesmium</i> sp.* (5%)	<i>Oscillatoria</i> sp.*? (19%)		<i>Oscillatoria</i> sp.*? (6%)
		Uncultured cyanobacteria (6%)		<i>Calothrix</i> sp.* (26%)
				<i>Trichodesmium</i> sp.* (12%)

C. rotundata was the only seagrass that showed significant differences in tissue nutrients suggesting that nitrogen fixation by the associated cyanobacteria may contribute to the sustained growth of this species in the low nutrient site. The nitrogen fixing potential of associated epiphytes has been documented by various authors (Goering & Parker, 1972; Capone, 1979; Capone, 1982; Moriarty & O'Donohue, 1993; McClathery et al., 1998; Pereg et al., 2002) and they have shown that epiphytic algae on seagrass leaves were responsible for the nitrogen fixation and were important contributors to the nitrogen budget of seagrass communities.

Differences were seen in the species composition of unicellular cyanobacteria associated with the different seagrass species. This can be seen from prokaryotic associations found on *C. rotundata* and *T. hemprichii* (Table 2) where *C. rotundata* from the two sites shared some common species thereby indicating that prokaryotic associations may be species specific. Although no cyanobacteria were amplified from *T. ciliatum* from the two sites by molecular analysis, some cyanobacterial associations were observed on the leaves of this species microscopically. Coverage of the leaves of these seagrasses by other epiphytes such as the encrusting coralline red algae did not appear to influence the abundance of prokaryotic epiphytes (Paper II).

The difference in epiphytic assemblages was attributed to the intertidal location the different species and their growth morphology (Paper II). *C. rotundata* is found higher up in the intertidal zone and has fewer leaves compared to the other two species which means that it is exposed to the highest light levels during low tide periods. Nitrogen fixing cyanobacteria (both heterocystous and non-heterocystous forms) require high irradiances due to the high energy demands of the nitrogen fixation process (Gallon & Stal, 1992) which means that attachment to *C. rotundata* is favoured.

Wahbeh & Mahasneh (1984) attributed differences in bacterial associations on the seagrasses *Halophila ovalis* (R. Brown) Hook. F., *Halophila stipulacea* (Forsk.) Aschers. and *Halodule uninervis* (Forsk.) Aschers to the quantity of inhibiting substances such as tannins and phenols as well as the soluble organic exudates released by these seagrasses. As prokaryotes are among the first colonizers of the seagrass leaves their proximity to the seagrass leaf surface means that they would be influenced by seagrass exudates. The specific associations found in Paper II could also be attributed to differences in exudates from the different seagrass species. Furthermore, the results from Paper II suggest that the establishment of prokaryotic epiphytes may not be solely a random encounter but a specific association that meets specific needs.

Application of PAM fluorometry to determine photosynthetic activity in seagrasses and epiphytes

Several methods have been used to evaluate the photosynthetic productivity of seagrasses. Some of these methods include the leaf growth measurements, the use of ^{14}C isotopes as well as gas exchange measurements (Dennison, 1990; Beer et al, 2001; Mateo et al, 2001). These methods require incubation enclosures, which subjects plants to unnatural environments and in most instances the seagrass material is uprooted (Beer, et al., 1998; Mateo et al., 2001; Dennison, 1990). In this study, Pulse Amplitude Modulated (PAM) Fluorometry was used to measure chlorophyll *a* fluorescence from Photosystem II. Chlorophyll *a* fluorescence was then used to determine electron transport rates (ETR) which was used as a quantitative measure of the photosynthetic activity of plant tissues (Ralph et al, 1998, Beer et al. 2001).

The chlorophyll fluorescence estimates made by the Diving-PAM give a parameter known as the effective quantum yield expressed as $(F_m'/F)/F_m' = \Delta F/F_m'$ (Genty, et al., 1989) where *F* is the fluorescence at a given photon irradiance (when part of the reaction centres are closed) and *F_m'* is the maximal fluorescence measured during a short (0.8 s) period of photosynthetic saturating light (when all the reaction centres are closed) (Beer & Björk, 2000; Beer et al. 2001). The effective quantum yield value can be used to determine the electron transport rate (ETR) if the absorption factor (proportion of incident quanta absorbed by the pigments of PSII) of the plant is known (Beer et al. 2001). The electron transport rate (ETR) is then

estimated as $ETR = Y * PAR * 0.5 * AF$; where Y is the effective quantum yield ($\Delta F/F_m'$), PAR is the irradiance at the leaf surface when irradiated by the PAM's internal halogen lamp (as in the rapid light curves), 0.5 is a factor that assumes that there is an equal distribution of photons in Photosystem II and Photosystem I, AF is the proportion of incident light that is absorbed by photosynthetic pigments (Beer et al, 2001). The ETR describes the ability of the Photosystems to use incident light and it can be used to compare the photosynthetic efficiencies of different leaves and different species (Maxwell & Johnson, 2000; Beer et al, 2001)

Various authors have shown the suitability of the Diving PAM (Walz) in seagrass studies (Ralph & Burchett, 1995; Ralph et al., 1998; Beer et al., 1998; Beer & Björk, 2000) and its application has become popular due to the rapid nature of the measurements it also has the added advantage of being used *in situ* so that photosynthetic patterns are obtained directly as they occur in the un-damaged plant under ambient light conditions.

The validation of ETR measurements is important as it indicates that reliability with which ETR measurements can be used as a true measure of photosynthetic rates (Silva & Santos, 2004). In this study, the validation of ETR measurements was made by determining the correlation of ETR to oxygen evolution (Paper III). This was done by measuring chlorophyll fluorescence and oxygen evolution of a seagrass section or epiphytic algae simultaneously using the Diving PAM (Walz, Germany) and a Hansatech O₂ electrode chamber (Paper III). The “perfect” relationship between ETR and oxygen evolution gives a theoretical ratio of 0.25 which is equivalent to 4 moles of electrons transported per moles oxygen evolved or carbon dioxide fixed (Beer & Björk, 2000).

Oxygen evolution by the bare parts of *T. ciliatum* leaves, leaves with encrusted epiphytes, the leaf part under the coralline epiphytes, *G. corticata* and the control plant *Ulva fasciata* Delile all came close to the theoretical “ideal” ratio of 0.25 (Paper III) and the correlation coefficients were strong. However, the encrusting coralline red algae showed the weakest correlation between the two methods (Paper III) and the ratio for this species as well as the filamentous red algae found on the stems of *T. ciliatum* did not reach the ideal ratio of 0.25 (Paper III). Oxygen evolution was low while ETR increased with increasing irradiance giving strong correlation coefficients but low ETR:O₂ ratios for these epiphytes. The inability to obtain the ideal ratio for these epiphytes has also been reported for a variety of macroalgae (Beer & Axelsson 2003; Carr & Björk, 2003) and it could be attributed to inaccurate estimates of absorption factors for the thick calcareous algae and the filamentous species. Estimation of the exact area for the filamentous algae was difficult and may be a source of error. Other sources of error that have been documented indicate that the assumption that 50% of the light absorbed by a leaf is taken up by Photosystem II (where there is a assumption of equal involvement of Photosystem I and Photosystem II in linear electron flow) may not be true for several plants (Figueroa et al., 2003; Baker & Oxborough, 2004). There could also have been errors in the measurement of light that the plants were exposed to in the oxygen chambers (Baker & Oxborough, 2004). Processes which consume oxygen such as the Mehler reaction, photorespiration and mitochondrial respiration (Beer et al., 2001) may have affected the rate of oxygen evolution.

Although the ideal ETR:O₂ ratio was not achieved for some of the epiphytic species, the ETR was still used to make comparative estimates of *T. ciliatum* productivity in the two study sites, Nyalí and Vipingo, in Paper III. The ETR information was useful in extrapolating the

contributions of the seagrass shoots and their epiphytes to primary productivity within the seagrass bed (Paper III and Paper IV).

In Paper III, the Diving PAM (Walz) was used to produce rapid light curves on seagrass components. Rapid light curve measurements are made by programming the PAM to give increasing light pulses (generated by the internal halogen lamp) to the plant part and the effective quantum yield (Y) is recorded (Beer et al, 2001). Rapid light curves provide information on the capacity of the seagrass tissue to photosynthesize under a range of different light conditions and produce a generalized relationship between irradiance and photosynthesis, measured as ETR, at different irradiance levels (Beer et al, 2001). Carr and Björk (2003) established that the durations of the irradiances given during the rapid light curve measurements (10 – 30s per irradiance) may be too short to allow effective quantum yield ($\Delta F/F_m'$) to be determined from a steady state level thereby giving an under estimate of ETR. Moreover, as rapid light curves are used on the same plant part the previous irradiance from the PAM will affect the outcome of the next irradiance therefore each effective quantum yield estimate is affected by the previous light exposure. Additionally, the incubation procedure for making a RLC which involves enclosure of the leaf in a small volume leaf clip may affect the effective yield. In view of all these factors that affect the rapid light curve measurements, the estimates of carbon fixation made in Paper III may be considered to be underestimates of the true situation found in the field. Additionally, the extrapolation of new yield estimates from the rapid light curves which were used to estimate carbon fixation also adds a bias to the data (Paper III). However, the technique was useful for the comparative assessment of productivity in the two study sites.

In view of the limitations encountered when using rapid light curves the method was improved by using point measurements to make similar estimates of carbon fixation in the two sites in Paper IV. In this work, point measurements were used to determine the effective quantum yield (Y) under ambient light conditions. Only one measurement was taken on each plant component, using the PAM, and measurements were made on new parts during each hour over the day (Paper IV). If it is true that the incubation procedure and the previous light exposure affects the yield estimate of the next irradiance in rapid light curves, then point estimates are preferable as the yield is from a new plant part and the ambient light, which is measured at the same instant as the yield, is used in the estimation of ETR. Unlike the rapid light curve estimates, yield (Y) was determined directly and not extrapolated as in Paper III. Thus the productivity estimates made using the point measurements are more realistic and could reflect the true rates of the shoots studied.

Three different methods were used to assess productivity in Paper IV; PAM fluorescence, the hole punch method and oxygen evolution in BOD bottles. All three methods showed differences in productivity between the two sites. However PAM fluorometry estimates were much higher in comparison to the other methods. Whereas aerial productivity estimates, derived from the hole punch method, consider only the growth increment of actively growing young leaves, fluorometry estimates consider the full photosynthetic activity of the leaves, which was shown to be functional in old leaves even though they did not show any growth increment. This means that PAM fluorometry estimates will be higher than those made using the aerial production which are based on growth increments of leaf tissue (Paper IV). Incubation methods produced low estimates for Vipingo indicating a possible underestimate caused by the fact that microalgae, which may contribute to the oxygen evolution, were not estimated. However, the use of all three methods enhanced the understanding of photosynthetic processes occurring within the seagrass shoots and indicates that coupling

different methods provides a better understanding of the photosynthetic functionality of plants (Paper IV).

Impact of epiphytic shading on the productivity of the seagrass *T. ciliatum*

PAM fluorometry estimates performed on seagrass leaves showed reduced electron transport rates in areas under epiphytic cover compared to areas that did not have an epiphytic cover (Paper III). However, in spite of this reduction in productivity, theoretical estimates of productivity based on the assumption that the whole seagrass shoot did not have attached epiphytes (unepiphytized seagrass) were similar to the productivity of the epiphytized seagrass shoot. This indicates that epiphytes do not reduce the overall photosynthetic output of *T. ciliatum* in both the nutrient rich site, Nyali, and the low nutrient site, Vipingo (Paper III). Figures 1A and 1B show the distribution of epiphytes on the leaves of seagrasses in the two sites during the study periods covered in Paper III and Paper IV which represent the SE monsoon and the NE monsoon.

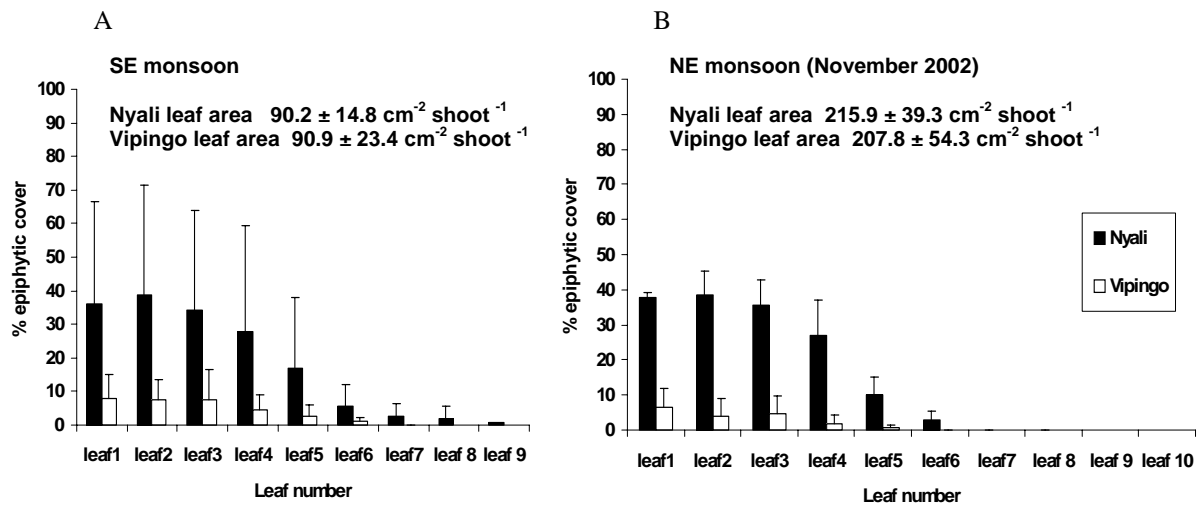


Figure 1. The percentage cover of encrusting red coralline algae on the leaves of *T. ciliatum* in the two study sites during (A) the SE monsoon and (B) the NE monsoon sampling periods (leaf 1 represents the oldest leaf and leaf 10 the youngest).

It was evident that the entire leaf surface was not covered by epiphytes and older leaves (leaf 1 to leaf 3) which had the highest epiphytic cover (comprised of encrusting coralline red algae) were found to be photosynthetically active in spite of their enhanced epiphytic cover (Paper III). The younger leaves, which had a lower epiphytic cover, also exhibited a high photosynthetic capacity, thereby indicating that there were sufficient areas on the shoot that could compensate for the loss of productivity in areas covered by epiphytes. Furthermore, the larger epiphytes which would shade the seagrass shoots grew on the stems of this species therefore they only suppressed the productivity of the stem which was in any case already light limited by shading from the overlying canopy of seagrass leaves. These factors may explain why epiphytic shading had a minimum impact on the entire shoot in Nyali where epiphytes were abundant. The abundance of epiphytes on the leaves was similar in the two seasons even when the leaf area increased in November 2002.

In Paper III, the epiphytes were found to contribute considerably to primary production in seagrass beds during the SE monsoon. The higher abundance of encrusting red algae on *T. ciliatum* leaves in Nyali is reflected an enhanced contribution of leaf epiphytes to productivity in this site. Additionally, the distribution of epiphytes on the stems was high in Nyali and these epiphytes contributed approximately 45% of the total productivity which is comparable to the epiphytic contribution of 48 – 56% in beds of *Halodule wrightii* documented by Morgan and Kitting (1984). Studies on *Posidonia australis* Hooker, indicate that over 60% of the total photosynthesis is attributed to epiphytic photosynthesis (Borowitzka & Lethbridge, 1989). The low nutrient site, Vipingo, had a lower abundance of epiphytes on the leaves and stems which was reflected in the lower shoot productivity. The epiphytic contribution to shoot productivity decreased in the NE monsoon to 12% in Nyali and is attributed to the decrease in epiphytic species and abundance particularly on the stems of *T. ciliatum*.

Coupling photosynthetic productivity to seagrass growth

Paper IV presents an attempt to link ETR estimates from PAM fluorometry to growth increments of seagrass shoots. The mean leaf ETR (mean of all leaves on a shoot) during the sampling period in November 2002, estimated from the point measurements, was 27.2 ± 3.7 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ for *T. ciliatum* shoots in Nyali while it was higher in Vipingo, the low nutrient site, at 42.6 ± 8.6 $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$. In contrast total leaf growth was higher in Nyali (23.2 ± 4.3 $\text{mm shoot}^{-1} \text{day}^{-1}$) whereas in Vipingo growth was lower (12.5 ± 2.9 $\text{mm shoot}^{-1} \text{day}^{-1}$). By comparing ETR and growth it is evident that although seagrass shoots in Vipingo had a lower growth rate compared to shoots in Nyali, they were more photosynthetically active. Maximum ETR (ETR_{max}) derived from the rapid light curves, shown in Figure 2 A and 2B, shows the activity of different components of the shoots in the two areas. ETR_{max} represents the ETR level at which the photosynthetic apparatus becomes saturated and this estimate was used to evaluate the activation level of the different parts of *T. ciliatum* at different times of the day.

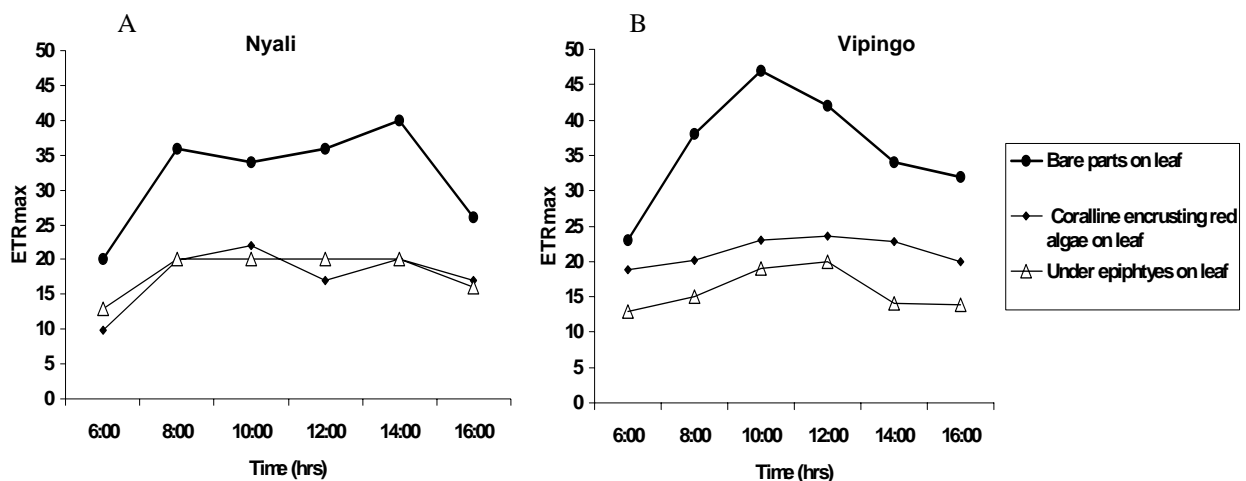


Figure 2. ETR_{max} for *T. ciliatum* leaves in (A) Nyali and (B) Vipingo showing activation at different times of the day. (The level of activation is based on the incident light which varies over the day).

The results of this study show that the bare parts on the leaves, in both study sites, had the highest photosynthetic capacity (Figure 2A and 2B). In Nyali, the bare parts on the leaves

had the highest photosynthetic capacity with most of the ETR_{max} at around $35 \mu\text{mol electron m}^{-2} \text{ s}^{-1}$ (Fig. 2A) while in Vipingo the ETR_{max} of bare parts of the leaf was $47 \mu\text{mol electron m}^{-2} \text{ s}^{-1}$ (Fig. 2B). This corresponds to the higher light levels in Vipingo where the maximum PAR over the day was $700 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ while in Nyali maximum PAR reached $400 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Higher light levels reached the canopy in Vipingo due to the fact that shoot densities in Vipingo during this period were half of that recorded in Nyali (Paper IV).

This photosynthetic activity was reflected in the oxygen evolution measured in the field (Paper IV). Plant components found to evolve the most oxygen were the same parts that had high ETR values, which confirms the validity of the PAM fluorescence estimates. It is obvious from the ETR estimates and from the growth increments of the leaves that although the plants in Vipingo seemed to be just as photosynthetically efficient as those in Nyali, the carbon fixed was not translated into growth increment of the leaves. Assessment of the below ground structure provided evidence of a denser root mass in Vipingo (Paper IV) which suggests that in this low nutrient site *T. ciliatum* is able to tap into a large area of the sedimentary nutrient pool by developing numerous roots. C:N ratios of *T. ciliatum* did not show differences between the two sites (C:N ratio: 20.7 ± 3.0 for Nyali and 20.3 ± 3.5 for Vipingo; Paper II; Uku & Björk, 2001) thereby indicating that the shoots in the low nutrient site receive adequate amounts of nutrients. As the plants in Vipingo are subjected to higher light levels due to the open canopy, which is a consequence of the lower shoot density, the seagrasses in this site may also be investing in other energy dissipation strategies and the repair of the photosynthetic apparatus from photodamage.

Bicarbonate utilization by the formation of “acid zones” in seagrasses

The inorganic carbon (Ci) utilization mechanisms of eight tropical seagrass species was studied and the carbonic anhydrase inhibitor acetazolamide (AZ) was used in order to detect if extracellular activity of the enzyme was required as part of a Ci utilisation system in which HCO_3^- was dehydrated to CO_2 prior to uptake by the seagrasses (Björk et al., 1997). However, both the controls and AZ treated samples were measured in the presence of a buffer so as to keep pH and the $\text{HCO}_3^-/\text{CO}_2$ ratio constant during the experiments. Later studies by Hellblom et al. (2001) using a temperate seagrass, *Zostera marina* L. showed that Ci acquisition of temperate seagrasses depends on H^+ extrusion therefore it became apparent that the use of buffers may have masked a similar process in the tropical seagrass species. The study presented in Paper V was undertaken to evaluate whether the same species studied in 1997 were capable of Ci uptake via the formation of “acid zones”.

The results of Paper V are summarized in Figure 3 which also shows the different carbon uptake strategies proposed by Beer et al., (2002). *C. serrulata* was not sensitive to the addition of TRIS buffer and thus appeared to support its photosynthetic carbon demand by extracellular carbonic anhydrase catalysed CO_2 formation from HCO_3^- without the need for the formation of acidic zones (carbon uptake mechanism *a* defined by Beer et al. 2002). *H. wrightii* and *C. rotundata*, which grow high up in the intertidal zone, had a significant decline in net photosynthetic activity in the presence of the buffer indicating buffer sensitivity and the presence of an acid zone. Although, *T. hemprichii*, *T. ciliatum*, *S. isoetifolium* and *E. acoroides*, which grow deeper in the intertidal area, were less sensitive to buffer additions, they were still affected by it indicating that the acid zone plays a role in carbon uptake. In addition to buffer sensitivity, these species were also sensitive to acetazolamide with 45-80% activity remaining thereby indicating that carbonic anhydrase is active in catalysing the conversion of Ci from

bicarbonate and that this conversion occurs in an acidified diffusion boundary layer. All these remaining species, except *H. ovalis*, fit into the carbon uptake mechanism *c* defined by Beer et al. 2002. *H. ovalis* was more sensitive to TRIS buffer indicating that this species may have a system in which H^+ extrusion may be followed by $HCO_3^-H^+$ co-transport into the cells in accordance to carbon uptake mechanism *b* as defined by Beer et al. (2002). *H. ovalis* showed a strong reaction to AZ after the addition of TRIS buffer (data not shown) indicating the possibility that it may also possess carbon uptake system *a* (Paper V).

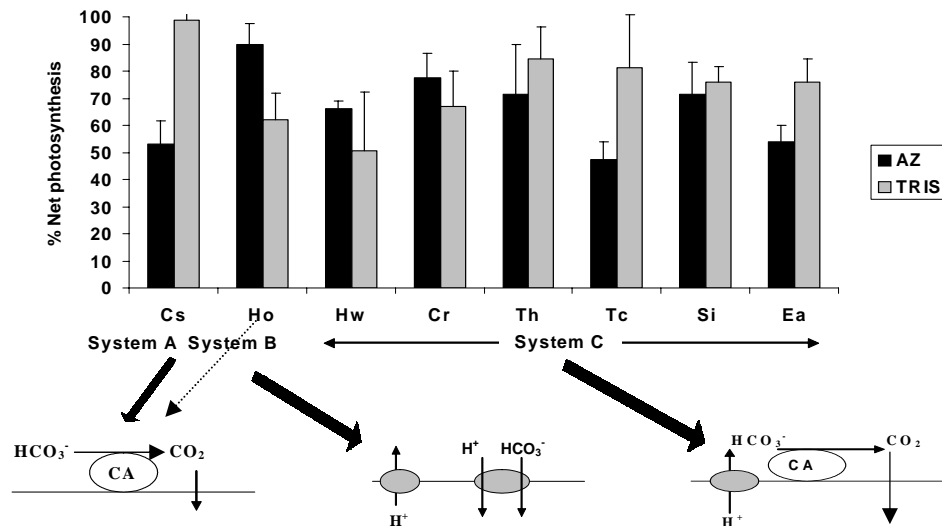


Figure 3. Trends in % net photosynthesis from the different seagrass species showing the carbon uptake strategies that have been proposed to function in them. The % net photosynthesis is the remaining photosynthetic activity compared to 100 % net photosynthetic productivity in normal seawater; Cs-*Cymodocea serrulata*, Ho-*Halophila ovalis*, Hw-*Halodule wrightii*, Cr-*Cymodocea rotundata*, Th-*Thalassia hemprichii*, Tc-*Thalassodendron ciliatum*, Si-*Syringodium isoetifolium*, Ea-*Enhalus acoroides*.

Based on the results of this study it was concluded that photosynthetic Ci acquisition in six of the eight investigated species is based on carbonic anhydrase catalysed HCO_3^- to CO_2 conversions within an acidified diffusion boundary layer. These results indicate that part of the 1997 results for the same species were due to a buffer effect on net photosynthesis. The results also confirm that seagrasses are capable of carbon uptake by the utilization of bicarbonate which is available in the water column using a variety of carbon uptake strategies. Furthermore, it is possible to speculate that there could be competition for carbon between the host seagrass species and its associated epiphytes especially when pH conditions change during the course of the day within enclosed tidal pools.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Several questions were posed at the beginning of this research work and from the different aspects investigated I conclude that:

- Nutrient enrichment, even when moderate as was the case in Nyali, appears to influence leaf growth of the seagrasses *T. ciliatum* and *T. hemprichii*.
- *T. ciliatum* and *T. hemprichii* showed productivity differences when the two sites were compared while *C. rotundata* did not. This indicates species specific responses to surrounding environmental conditions and emphasizes the importance of studying different species in multispecific seagrass beds.
- There were differences in prokaryotic associations found on seagrasses in the two sites. There was also evidence of a species specific association between prokaryotic epiphytes and the different seagrasses indicating that such associations may be determined by exudates from the seagrasses. These prokaryotes may also function to meet specific needs of particular seagrass species.
- Evidence of CFB bacterial associations which are affiliated to bacteria from farm wastes on seagrasses in the low nutrient site, Vipingo, indicates that although this site was more pristine it is still likely to be influenced by farming areas a few kilometres from the site. This shows that marine habitats may be impacted even if human activities are not directly on the beachfront.
- Some tropical seagrasses can develop different strategies to deal with nutrient limitation in their environments as can be seen by the association of nitrogen fixing cyanobacteria with *C. rotundata* in the low nutrient site and the enhanced rooting system of *T. ciliatum* in the same site. The association between *C. rotundata* and *Calothrix* spp. was persistent over the different seasons and highlights a functional relationship between the seagrass and this cyanobacterium.
- Epiphytic cover had a suppressive effect on the photosynthetic activity of the leaves of *T. ciliatum*. However, the effect on the whole shoot is minimized by the continuous production of new leaf material. This ensures that there is always new actively photosynthesising tissue to compensate for the reduced productivity in the covered parts. Furthermore, the younger leaves, which have a lower epiphytic cover, exhibited a high photosynthetic capacity therefore enhancing productivity and compensating for any suppressive effects of the epiphytes. Another consideration is that areas under the epiphytes, on the older leaves, are still photosynthetically active which means that they continue to contribute to the productivity of the whole shoot.
- Another reason that the macroalgal epiphytes did not have a detrimental effect on the overall productivity of the shoots of *T. ciliatum* in the nutrient rich site, Nyali, was that the abundance of the epiphytic cover was seasonal in nature and did not cover entire leaf surfaces. The localization of macroalgae on the stems of *T. ciliatum* also meant that the leaves were not shaded. Instead, epiphytes were found to be contributory to primary productivity within the seagrass beds and about half of the seagrass productivity estimated in the nutrient rich site was derived from macroalgal epiphytes.

- Despite the seasonal shift in epiphytic abundance, the relative amount of productivity lost due to the presence of an epiphytic cover remained relatively constant in the two sites over the NE and SE monsoon seasons (18% loss in Nyali and 4-9% loss in Vipingo) suggesting that *T. ciliatum* has achieved a balance in productivity between epiphytic suppression and productivity.
- PAM fluorometry is a useful tool in seagrass studies and its use compared well with the hole-punch method and oxygen evolution determined by incubating seagrass parts in BOD bottles. The combination of these different methods provided insights into the growth dynamics and photosynthetic activity of *T. ciliatum* in Vipingo, the low nutrient site. In this site, PAM fluorometry measurements showed that the photosynthetic productivity of this seagrass was comparable to the same species growing in the nutrient rich site, Nyali. In contrast, growth increments determined by the hole punch method revealed that leaves of *T. ciliatum* grew slowly in this site as there was an investment in the development of below ground tissue rather than above ground tissue for this species in the low nutrient site, Vipingo.
- Many tropical seagrasses are capable of bicarbonate acquisition via the formation of “acid zones” and this has implications in the acquisition of inorganic carbon under varying pH conditions that may exist periodically within seagrass beds. Moreover, the existence of carbon uptake system *c* in several of the seagrass species may indicate that this is the most advantageous system to possess.

It is evident from this work that there are new questions to be answered, for instance: Is there a true balance between epiphytic cover and seagrasses? Is this balance due to grazing by herbivores or other environmental factors? At what level would the epiphytic cover be truly suppressive on the productivity of the seagrass shoot? Are there specific attractants that drive the settlement of prokaryotes on seagrass species? What types of nitrogen fixers exist on the different seagrasses? Do the nitrogen fixing activities of both bacteria and cyanobacteria contribute to the N-budget of seagrass ecosystems? Do seagrass hosts compete with macroalgal epiphytes for inorganic carbon? If indeed there is competition for inorganic carbon does this drive epiphytic species associations within seagrass beds? Why do majority of the tropical seagrasses studied have a carbon uptake system that consists of an acidified boundary layer (carbon uptake system *c*)? Is there a competitive advantage in possessing this uptake system compared to the other systems? All these areas of research would enhance understanding seagrass species as well as the fascinating relationship between seagrasses and epiphytes.

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