Canopy-level photophysiology of the seagrass *Thalassia testudinum* in Florida Bay, Florida

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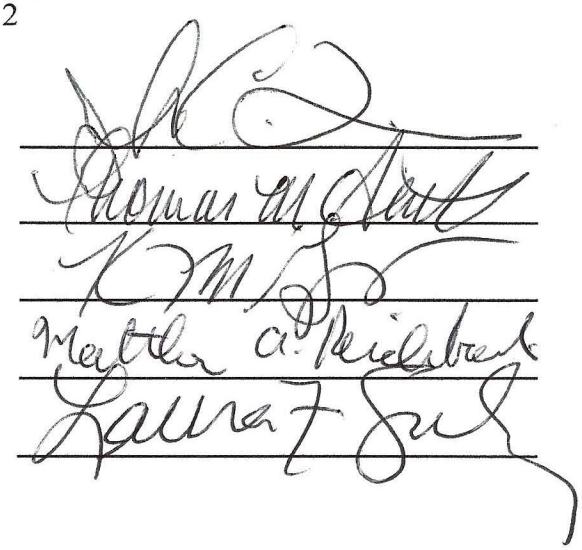

Abstract

Examination of a dense *Thalassia testudinum* meadow in Florida Bay showed that light variability can differ nearly an order of magnitude from the top to the bottom of the seagrass canopy and light penetration through the canopy is highly sensitive to the incident angle of the sun. Additionally, photosynthetically available radiation (PAR) may not be an appropriate indicator of light availability to seagrasses because this measure assumes that the leaves absorb all wavelengths equally. Becasue seagrass leaves absorb light more effectively in the red and blue regions of the spectrum and absorb green light only weakly, photosynthetically usable radiation (PUR) is a more accurate measure of light availability because it represents the differential absorption of light across the PAR spectrum. The red:far-red within a seagrass canopy increases, in sharp contrast to a terrestrial canopy. The lack of a red:far-red signal may be a key disadvantage limiting seagrasses ability to regulate canopy density.

Examination of the high frequency light fluctuations within the canopy showed that irradiance varied by an order of magnitude within a fraction of a second due to sunflecks allowing saturating light to penetrate even to the bottom of the canopy. The top of a canopy also experienced rapid fluctuations in light due to the focusing of light beams by surface waves that often exceeded double the surface irradiance.

Chlorophyll content and leaf thickness were significantly higher toward the base of adult leaves indicating a gradient in light absorbance ability through the canopy. Because of the basal growth structure of *T. testudinum* leaves, leaf biomass is strongly weighted toward the bottom of the canopy indicating the majority of leaf tissue

experiences a shaded environment. Photosynthetic performance, as measured by chlorophyll fluorescence, also varied significantly along leaves indicating interleaf acclimation to light availability.

The results of this dissertation suggest that the intercanopy variability in photosynthetic attributes plays a significant role in how a seagrass canopy adjusts to its light environment. By not representing this important factor, seagrass productivity models may significantly overestimate the gross photosynthesis in a seagrass meadow.

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PART I. INTRODUCTION

Chapter 1. Main Introduction

1.1 Problem Statement

Seagrasses, the only land plants known to have completely recolonized back to the sea, are a common feature of many coastal ecosystems throughout the world (Green and Short 2003). Extensive seagrass meadows range from the tropics to the Arctic and are some of the most productive ecosystems in the world, rivaling even intensive agricultural crops in some cases (Zieman and Wetzel 1980). Seagrasses act as a vital linkage between terrestrial and marine ecosystems performing many important ecological functions including providing habitat for a myriad of organisms, facilitating nutrient cycling, stabilizing sediments, and providing a substantial energy source for higher trophic levels (Zieman 1982). The importance of these ecosystems to local economies is emphasized where increased development and urbanization of the coastal zone is resulting in deterioration of water quality and rapid loss of seagrasses (Waycott et al. 2009). The loss of the myriad of ecosystem services provided by seagrasses has instigated an urgent need for increasing our understanding of how these ecosystems function (Duarte 1999). This research is essential in order to develop effective conservation and management strategies.

Successful management of seagrass ecosystems necessitates a detailed understanding of their distribution, abundance, and growth rates under varying conditions (Fourqurean and Zieman 1991). Light availability is often considered the most important factor influencing seagrass growth and abundance (Dennison et al. 1993; Dawes and

Tomasko 1988; Enríquez et al. 2002). Not surprising, light has been the focus of a large percentage of scientific publications looking at seagrass productivity (Koch 2001). Research typically focuses on determining the minimal light requirements for a specific seagrass species and the depth to which it can colonize (Gallegos and Kenworthy 1996; Dawes and Tomasko 1988; Dennison and Alberte 1985). When constructing seagrass productivity models, representation of light availability and the photosynthesis/irradiance relationship of the modeled species are usually the most important components (Gallegos and Kenworthy 1996; Madden and Kemp 1996; Kemp et al. 1995; Williams and McRoy, 1982; Fong and Harwell 1994).

Notwithstanding the strong consensus about the importance of light to seagrasses, there are surprising gaps in the science. Light availability to seagrasses is usually measured as the integrated irradiance across all wavelengths of photosynthetically active radiation (PAR). However, the assortment of chlorophylls and accessory pigments within leaves results in a differential absorption of light across PAR (Enríquez et al. 2002. Light within the red and blue wavelength regions are more easily absorbed while a plant may utilize little or no green light at all (Cumming and Zimmerman 2003). Using PAR may result in an overestimation of light availability especially where the light field may be depleted in the most usable wavelengths, such as is the case in aquatic environments characterized by water column chlorophyll or within a dense plant canopy (Gallegos et al. 2009).

The importance of how seagrass canopy structure affects productivity has not been adequately studied. Research in terrestrial systems stresses the importance of the

growth form and function of plant canopies (Aber and Melillo 1991). Plant canopy structure is an important factor when considering how a plant interacts with its environment (Kull 2002). In terrestrial systems, the morphological structure of a canopy and the vertical variation of leaf photosynthetic attributes through a canopy are important factors that influence how light is intercepted and utilized by plants (Hunt and Cooper 1967; Evans 1993; Jurik and Kliebenstein 1999). Surprisingly, many seagrass productivity models typically regard the canopy as a homogenous unit (Fong and Harwell 1994 for example). This simplistic treatment of the canopy may introduce a significant source of error when modeling seagrass growth. Given the importance of light availability to seagrasses, it is essential to understand the role of seagrass canopy structure in harvesting and utilizing light.

As previously stated, the most important factor influencing seagrass productivity is light. However, this point may be too simplistic. It is more accurate to say that the most important factor influencing seagrass productivity is light availability coupled with the ability of the seagrass canopy to harvest and utilize the available light. What is needed is an integrated picture of the relevant components of light, how light resources are perceived by the plant, and how plants respond mechanistically to varying light regimes.

1.2 **Goals and Objectives**

The primary goal of this dissertation was to explore the nature of canopy dynamics in a seagrass community in Florida Bay dominated by *Thalassia testudinum*. We know from growth analysis in terrestrial systems that a plant will strategically deploy

resources through a canopy. The morphological and physiological characteristics of leaves vary through a canopy in order to optimize light harvesting and photosynthetic rates. I explored this phenomenon in seagrass canopies to determine if canopy structure plays a significant role in seagrass growth. I also investigated the importance of light availability to seagrasses by examining the change in the quantity and quality of the light field through a canopy.

During this research, I developed innovative techniques for measuring the spectral irradiance within a seagrass canopy to assess both the quantity and quality of light availability. I modified an Ocean Optics USB2000 spectrometer to sample the intercanopy light field without disturbing the arrangement of leaves in the canopy. This is the first time this instrument has been deployed in this manner. During field assessment of the USB2000, I discovered that the instrument could be programmed to take near instantaneous measurements of the light field. This enabled me to observe the magnitude and spectral characteristics of the rapidly changing irradiance within the canopy. These light flecks are an important component of the intercanopy light field but have not been adequately studied in seagrass canopies (Chazdon 1988).

I also employed a portable underwater PAM fluorometer, the Diving-PAM (Heinz-Walz, Germany) to examine the vertical variation of photosynthetic characteristics within a seagrass canopy. The emergence of the Diving-PAM has expanded the field of chlorophyll fluorescence analysis to marine photosynthetic organisms including macroalgae, seagrasses, and corals (Beer and Björk 2000; Ralph et al. 1998). The Diving-PAM has been used extensively to assess responses of seagrasses

to high irradiance and other stressors (Major and Dunton 2002; Ralph and Burchett 1995). Because the photosynthetic electron transport rates calculated by the Diving-PAM are correlated to rates of photosynthetic O_2 evolution, the instrument could also be used to measure actual or relative photosynthetic rates (Silva and Santos 2004; Beer and Björk 2000).

1.3 **Scope and Organization**

This dissertation is divided into five parts. Part I is an introduction consisting of two chapters. Chapter 1 introduces the research topic to be addressed in the body of the dissertation, provides background information, summarizes the dissertation goals, and describes how the dissertation will be organized. Chapter 2 is a general introduction to the Florida Bay ecosystem where the fieldwork was conducted. I also described important ecological characteristics of seagrasses and the primary species studied during this research, *T. testudinum.*

Part II contains two chapters that investigate the nature of the light availability to seagrass. Chapter 3 examines the characteristics of the light field in the water column. I explored the nature of spectral attenuation of light through water columns and seagrass canopies with differing characteristics. Plants can learn important traits about their environment, such as stand density and vicinity of neighboring plants, by detecting changes in the spectral quality of light. Therefore, an important aspect of this research is the examination of the relative distribution of the wavelengths within the intercanopy light field. This chapter also covers the development and refinement of techniques for field deployment of the Mini-spec. Chapter 4 examines the rapid fluctuation of light

within the seagrass canopy related to both intercanopy light flecks as well as wave focusing of light. Both the magnitude and spectral distribution of light were investigated.

Part III deals with the variation of morphological traits and photosynthetic characteristics along seagrass leaves. Chapter 5 is an assessment of the geographic variation of canopy structure in mono-specific stands of *T. testudinum* across a gradient of environmental conditions in Florida Bay. The goal of this chapter was to investigate the relationship between *T. testudinum* canopy structure and plant productivity. Seagrasses are known to exhibit vastly different plant morphologies in response to varying environmental factors such as depth, light availability, nutrient availability, soil type, and the presence of competing species. Like terrestrial plants, seagrasses may be able to actively partition photosynthetic resources such as leaf chlorophyll and nutrient content or alter leaf morphology (i.e. leaf width and thickness) in response to variations in light availability. Because *T. testudinum* leaves grow from a basal meristem, there is another a layer of complexity to canopy structure. The leaf tissue at the top of a *T. testudinum* canopy is older, experiences higher light intensity, and is more affected by hydrological forces than the leaf tissue at the bottom of the canopy. Any vertical variation in leaf attributes might also be a function of the vertical gradient in the age of leaf tissue along individual *T. testudinum* leaves. Chapter 6 takes a closer look at the interleaf variation of nutrients along *T. testudinum* leaves. The goal of this chapter is to investigate how leaf physiological processes coupled with external environmental factors influence the partitioning of nutrients along seagrass leaves.

Part IV includes one chapter that examines photosynthesis of *T. testudinum* leaves using chlorophyll fluorescence. In Chapter 7, I will explain how chlorophyll fluorescence is used to elicit valuable information about the photosynthetic activity of a plant. I developed new and innovative techniques to investigate seagrass photosynthetic activity using the Heinz-Walz Diving-PAM. I completed several investigative studies that explored the variability of photosynthetic activity at sites along gradients of nutrient availability, depth, as well as interleaf variability. I also investigated the change in photosynthetic activity along leaves as they age by marking and following individual leaves over their entire lifespan. Another objective was to determine the relationship between the photosynthetic parameters generated by the Diving-PAM and the actual leaf morphological and physiological characteristics. The results may elicit important aspects of how chlorophyll florescence relates to photosynthesis in seagrasses and may determine if the instrument can be used to assess important leaf parameters.

Part V includes two chapters that detail field experiments designed to help answer some of the questions raised during the previous studies. Chapter 8 explores the effect of decreasing light availability on the interleaf variability of photosynthetic attributes. I altered the light reaching a *T. testudinum* meadow using shade canopies and then followed changes in photosynthetic activity using the Diving-PAM. Leaf samples were also taken at intervals over the next two months to examine changes in leaf morphology and physiological attributes. The objective of this study is to determine if a seagrass can alter canopy level photosynthetic attributes in response to changing light conditions over a period of weeks or months. This study will also help determine if active partitioning of

resources drives the interleaf variation of photosynthetic attributes or if it is merely a result of the age gradient along leaves. Chapter 9 investigates the diurnal variation of light availability and photosynthetic activity through a seagrass canopy. This study will determine the ability of *T. testudinum* to acclimate to changing light conditions within hours.

The final chapter is a synopsis that describes how this dissertation contributed to the understanding of the importance of light to seagrass. It summarize the key objectives and unique accomplishments that I achieved. It makes connections between the various chapters by developing a conceptual model of the life history of leaves including how photosynthetic characteristics change over time. I also discuss the implications that this research may have on the development of accurate seagrass productivity models. The goal of any scientific research is to answer questions, but results sometimes simply reveal new questions. I conclude by making suggests about what additional research could contribute to this dissertation's results.

Chapter 2. The seagrass ecosystem of Florida Bay

2.1 Location description

The majority of the research for this dissertation was conducted at sites throughout Florida Bay and the near-shore waters of the Florida Keys. Some additional work was included from research trips with the Smithsonian Ecosystem Research Consortium to Carrie Bow Key, Belize. Laboratory work was completed at the Florida Bay Interagency Science Center in Key Largo and the University of Virginia in Charlottesville, Virginia. The research sites were accessed by small boats deployed from the UVA Key Largo bunkhouse. The seagrass beds were reached using SCUBA or snorkel depending on water depth.

Florida Bay is an approximately 1,800-km^2, triangular shaped estuary located at the southern end of the Florida peninsula between 24° and 25° N, just north of the Tropic of Cancer (Figure 2.1). Florida Bay is intricately linked to and influenced by its neighboring terrestrial and marine ecosystems. The northern border is defined by the transitional boundary with the vast Everglades ecosystem that comprises the entire watershed for the estuary (Nuttle et al. 2000). The transitional boundary between these two ecosystems is characterized by mangrove forests interlaced with many small creeks that are the primary freshwater source to the bay. To the southeast, the bay is bounded by the islands of the Florida Keys. Narrow channels between the islands, some of which are manmade, link the bay to the near shore marine waters, coral reef ecosystems, and eventually to the warm tropical waters of the Florida Current. To the west, the bay transitions into the more temperate and sub-tropical waters of the Gulf of Mexico.

Figure 2.1. Location of Florida Bay.

Florida Bay formed over the last 4,000 years as sea levels rose and flooded a limestone shelf (Wanless and Tagett 1989). The islands of the Florida Keys themselves are actually ancient coral reefs dating to the Pleistocene when sea level was much higher. The bay is crisscrossed by irregularly shaped, shallow mud banks that delineate deeper basins. These mud banks are composed of living plant tissue, dead organic matter, and

carbonate mud sediments layered on top of the gradually sloping shelf (Wanless and Tagett 1989). The average depth across the bay is only 1.5 m and rarely exceeds 2 m (Zieman 1982). The deepest points are the deep channels that cut across the mud banks linking the basins. Small mangrove islands are scattered throughout the bay and mangrove habitat is a common feature of most of the coastal areas.

The Florida Bay ecosystem is characterized by a tropical to subtropical climate and a dry/wet seasonality (Porter and Porter 2002). Air temperatures range from 29.4°C in August to 20.8°C in January with an annual average of 24.5°C because of the shallow depth of the bay (Zieman 1982). Water temperature closely matches air temperature most of the year (Holmquist et al. 1989). Approximately 2/3 of the annual precipitation occurs during the wet season from May to October (Nuttle et al. 2002). Florida Bay experiences considerable seasonal variation in salinity (Zieman 1975). During the wet season, fresh water mixing lowers salinity to below that of seawater throughout most of the bay (Fourqurean and Robblee 1999). During the dry season, from November to April, evaporation exceeds precipitation, meaning Florida Bay essentially becomes a negative estuary for part of the year. During exceptionally dry years, water salinity can exceed 50‰ in isolated basins (Fourqurean et al. 1992). South Florida experiences the highest frequency of tropical storms and hurricanes within the United States with a return frequency of 7 to 8 years (Porter and Porter 2002). These periodic storm events have played an important role in shaping the ecology of Florida Bay over decades and centuries (Fourqurean and Robblee 1999).

Florida Bay is considered a phosphorous-limited system (Fourqurean et al. 1992). This is a common characteristic of tropical carbonate systems, while temperate estuaries are typically N-limited (Day et al. 1989). Highly charged phosphate anions (PO_4^{3-}) readily bind to the surfaces of the positively charged carbonate ions within sediments. This effectively removes large amounts of phosphorous from the available nutrient pool (Day et al. 1989). In Florida Bay, phosphorous availability typically decreases along a gradient from the west to the northeast (Fourqurean and Zieman 1992). However, phosphorous availability can also be driven locally by the presence of bird rookeries or anthropological sources (Fourqurean et al. 1995).

Florida Bay is physically linked to its neighboring ecosystems through mixing of water bodies by tides, currents, and winds (Porter and Porter 2002). Tides are most pronounced along the western edge of the bay and in vicinity to the inlets through the Keys (Corbett et al. 1999). Tides are negligent in the northeast region of the bay where the persistent seasonal wind direction is the primary influence on water depth. The ecosystems are linked chemically through the exchange of dissolved nutrients and the transport of organic materials (Brand 2002). The systems are also ecologically linked with many species living, eating, or breeding across the ecosystem boundaries as they progress through their lifecycles (Ault et al. 2009; Heck et al. 2003).

2.2 **Seagrass community**

Florida Bay is characterized by extensive meadows of seagrass that reach areal densities comparable to the densest meadows in the world (Zieman et al. 1989). Typically, seagrass abundance follows the gradient in nutrient availability across the bay.

The densest meadows are in the western part of the bay where they benefit from nutrients transported in from the Gulf of Mexico. The seagrass *Thalassia testudinum* (Turtle grass) is the dominant marine plant species in Florida Bay, accounting for nearly 90% of the total biomass in many areas (Zieman 1982). Two other seagrasses species, *Syringodium filiforme* (Manatee grass) and *Halodule wrightii* (Shoal grass), are commonly found intermixed with *T. testudinum*, in areas that are not favorable to *T. testudinum*, or during successional phases following disturbance (Zieman 1982). The land-water interfaces are almost entirely dominated by red mangrove (*Rhizophora mangle*). Seagrass species diversity also varies along the nutrient gradient with *T. testudinum* being more dominant in areas with low P and *H. wrightii* occupying the areas with high P (Fourqurean et al. 1995).

2.3 **Florida Bay research sites**

This research was conducted at sites across Florida Bay and the offshore waters of the Florida Keys (Figure 2.2). These sites represent the diverse plant morphologies that *T. testudinum* can exhibit and the various environmental conditions it can inhabit. Table 2.1 shows the abbreviations that will be used to identify the sites. Rabbit Key Basin (RKB) is located in a semi-enclosed basin located in the west-central section of Florida Bay, averages ~1.5 m in depth, and is surrounded by a wide shallow bank. RKB is dominated by a large continuous monospecific meadow of *T. testudinum*. RKB experienced a large-scale seagrass die-off during the 1980s and has subsequently been the site of extensive research. The BANK site is located on a shallow bank approximately 1 km north of RKB and contains approximately the same seagrass coverage as RKB.

BANK experiences high-energy disturbance from tidal fluctuations varying the water depth from 0.3 m to 1 m, partially exposing leaf tips at low tide. Both of these sites are characterized by very deep sediments and extensive belowground biomass. Water clarity is typically quite clear with Secchi depths that exceed the water depth.

The sampling site at Barnes Key (BAR) has a highly transparent shallow water column, <1 m in depth, and experiences only slight tidal variation, ~5 cm. The site is populated by very dense monospecific *T. testudinum*. Johnson Key Basin (JKB) contains sparse *T. testudinum* in ~1.2 m of water with large short shoots similar to those found at the previous sites. Eagle Key Basin (EKB) is located in 1.5 m of water near the southern edge of the Everglades. This site experiences high inputs of fresh water and salinity is typically below 10 ‰. The water column is quite turbid with Secchi depth of only 20-30 cm. Small short shoots of *T. testudinum* are found intermittently in very low densities. The Duck Key (DUCK) site, located in the northeastern portion of the bay where P availability is lowest, is characterized by sparse *T. testudinum* with relatively small short shoots and short leaves. Rankin Key (RAN) is located in the northwestern part of the bay is characterized by intermediate density and leaf lengths.

Tavernier Key (TAV), Carysfort Reef (CFT), and Alligator Reef (ALG) are located on the offshore of the Florida Keys with water depths of 2.5 m and 6 m, respectively. These sites contain sparse *T. testudinum* with relatively small short shoots and short leaves growing in coarse carbonate sand. Salinity and water temperature are not strongly influenced by watershed runoff and seasonal fluctuations are not as pronounced as the bayside sites.

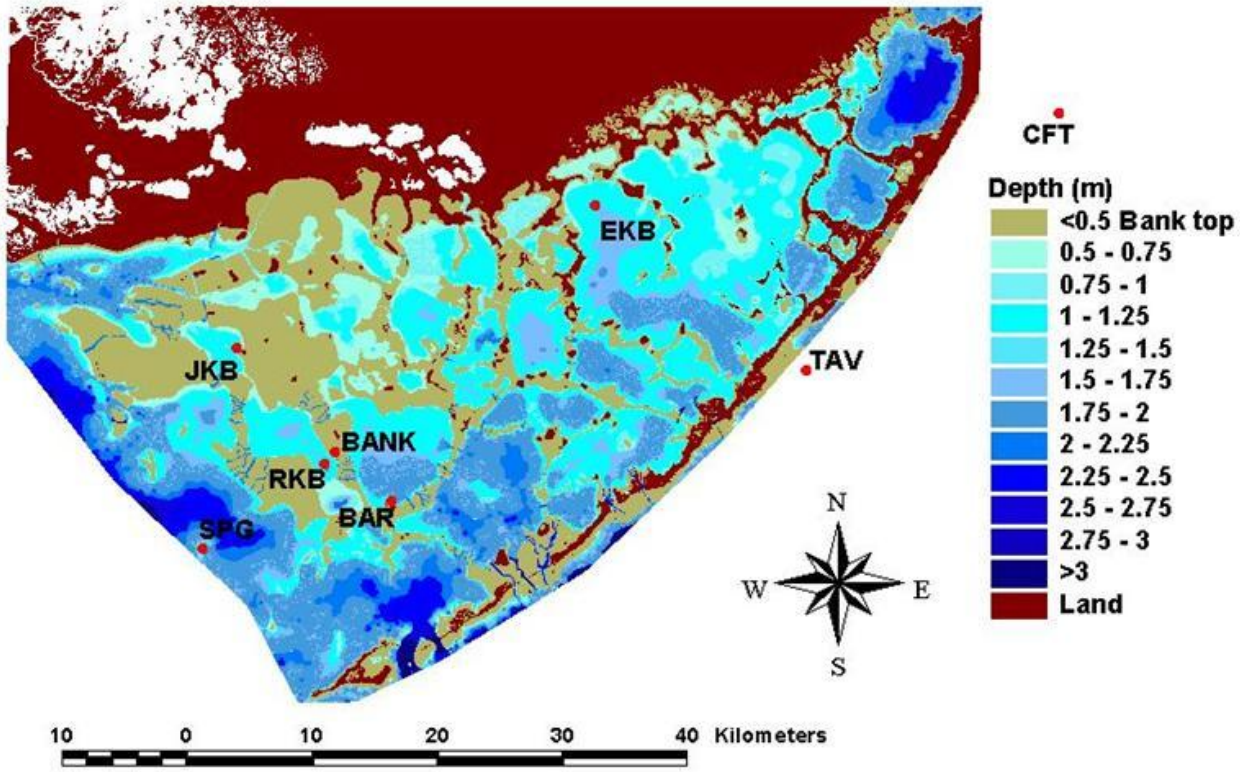

Figure 2.2. Location of Florida Bay and offshore Florida Keys research sites

Table 2.1. Florida Bay and offshore Florida Keys research sites.

Abbreviation	Site name
ALG	Alligator Reef
BANK	Rabbit Bank
BAR	Barnes Key
CFT	Carysfort Reef
DUCK	Duck Key
EKB	Eagle Key Basin
JKB	Johnson Key Basin
RAN	Rankin Lake
RKB	Rabbit Key Basin
SPG	Sprigger Bank
TAV	Tavernier Key

2.4 **Seagrass ecology**

Seagrasses perform an important role in the cycling of nutrients in coastal zones and estuaries (Hemminga et al. 1999; Short et al. 1990). Seagrass leaves serve as the major sink for nutrients and are a primary source of dead organic matter to the detrital nutrient pool (Thresher et al. 1992). Nutrients occur in five basic pools in a seagrass ecosystem: 1) gaseous form within the water or sediment, e.g. CO_2; 2) living organic matter, i.e. biomass; 3) dead organic matter, i.e. detritus; 4) available nutrients; and 5) unavailable nutrients (Likens and Borman 1972). Seagrasses also facilitate decomposition in sediments by pumping photosynthetically produced oxygen to the rhizosphere (Wigand et al. 1997; Hume et al. 2011; Short et al. 1990). This helps avoid anoxic conditions in the sediments that can lead to anaerobic decomposition and accumulation of toxic levels of sulfide (Carlson et al. 1994; Day et al 1989).

The physical structure of a seagrass canopy provides ideal habitat for incredible diversity of creatures including fish, copepods, decapods, crustaceans, mollusks,

gastropods, and the larval phases of many reef-dwelling and pelagic species (Cocheret de la Moriniere et al. 2002; Nagelkerken et al. 2001; Hemminga and Duarte 2000; Ogden and Zieman 1977). Seagrass meadows act as a nursery for reef dwelling and pelagic species many of which have immense commercial value (Heck and Thoman 1984; Unsworth et al. 2010). The dense foliage provides cover from predators and shelter from currents and waves, increasing the survival of young (Heck et al. 2003; Fonseca et al. 1990). Juveniles often dominate the fish community in a seagrass bed (Murphey and Fonseca 1995; Hemming and Duarte 2000). Seagrasses also pump photosynthetically produced oxygen to the sediments providing suitable conditions for a diverse infauna community (Reynolds et al. 2007).

Seagrass canopies create a positive feedback mechanism that improves conditions for their continued presence by increasing nutrient retention and light availability in their local environment (Hemminga and Duarte 2000). Friction from the leaves baffles currents and waves slowing the water velocity within the canopy to below the settling velocity for particles that would otherwise remain suspended in the water column (Short and Short 1984). Additionally, the extensive belowground structure of a seagrass bed, consisting of an interconnected matrix of roots and rhizomes, stabilizes sediments inhibiting erosion and increasing nutrient retention (Koch 1999).

Seagrass habitats are vital to the ecological functioning of their ecosystems. The high rates of primary production and substantial leaf standing crop of seagrass beds offer a tremendous source of energy and nutrients to higher trophic levels through direct and indirect pathways (Hemminga and Duarte 2000; Duarte 1990; Day et al. 1989) and represents a significant component of the global carbon cycle (Smith 1981). Seagrass

leaves and rhizomes offer a direct food source to a diverse faunal community including sea turtles, manatees, and many species of fish (Ogden 1980). Additionally, the large leaf surface area provides a substrate for a substantial epiphytic community that can attain biomass levels equal to the leaf standing crop and may offer grazers a more assimilable energy source than the seagrass leaves themselves (Frankovich and Fourqurean 1997). Most seagrass material is not grazed directly, instead becoming available to higher trophic levels following senescence via the detrital food web (Knauer and Ayers 1977).

Seagrasses have three key adaptations that allow them to successfully survive in the marine environment: 1) the leaves are adapted for saline environments (Jagels 1973); 2) they are capable of submarine pollination (Cox and Tomlinson 1988; Ducker and Knox 1976); and 3) the leaves contain internal cavities called lacunae that promote an erect canopy structure and allow for internal gas exchange between leaves and belowground structures (Hemminga and Duarte 2000). Seagrasses exhibit considerable plasticity allowing the plants to alter morphology and physiology to cope with stress and heterogeneity in their environment (Hemminga and Duarte 2000).

Seagrasses are clonal plants that grow mostly vegetatively by extension of a belowground horizontal rhizome. Seagrass morphology varies greatly across species although they all follow a general modular structure (Hemminga and Duarte 2000). Individual ramets, consisting of a vertical rhizome segment topped by a bundle of leaves, grow at regular intervals along a subsurface horizontal rhizome and project vertically through the sediment into the water column (Zieman 1982). Ramets of an individual clone share resources via transport through the rhizome. All seagrass leaves grow from a basal meristem (i.e. from the bottom) usually shrouded by protective leaf sheathes (Kuo

and den Hartog 2006). Roots extend from the rhizome and occasionally from the vertical ramet itself. The roots and rhizomes of adjacent clones enmesh below ground creating a secure hold on the sediment (Koch 1999). Seagrass ramets are commonly referred to as short shoots, colloquially and in literature.

Seagrasses have a major advantage over terrestrial grassland ecosystems in that water, often a limiting factor in terrestrial environments, is present in abundance (Hemminga and Duarte 2000). The tradeoff is that submersion in water presents some major obstacles chiefly the fact that light decreases sharply in water (Kirk 1994). Another potential drawback is that carbon dioxide concentration is much lower in water than air, making CO_2 limitation more likely (Beer and Waisel 1979). However, seagrasses are able to utilize dissolved bicarbonate (HCO_3^-) as an alternative carbon source to CO_2 reducing the possibility of carbon limitation (Beer et al. 2002). The aquatic environment can also experience very high energy from waves and currents. Essential nutrients can be lost when leaves are broken or when nutrients are leached into the water column (Hemminga et al. 1999). Another common limitation is that aquatic sediments are often oxygen depleted (Day et al. 1989). Seagrasses can alleviate this stress by pumping photosynthetically produced oxygen to the root zone. The unique morphology of seagrasses (i.e. highly plastic leaf canopy and extensive belowground structure) allow them to compete effectively in the aquatic environment.

2.5 **Decline and loss of seagrass meadows**

Coastal and estuarine seagrass ecosystems throughout the world are in decline due to increased turbidity from anthropogenic euthrophication related to the substantial population growth along coastlines (Robblee et al. 1991; Giessen et al. 1990; Orth and

Moore 1988; Lapointe et al. 1994). Researchers often use seagrass distribution and abundance as bioindicators of habitat health because of its sensitivity to changes in submarine illumination (Dawes 1998; Dennison et al. 1991). Seagrasses have been called "coastal canaries", referring to the canaries once taken into coalmines to serve as early indicators of bad air, because declines in seagrass abundance can be an early indicator of deterioration of water quality (Orth et al. 2006). The continued presence of seagrass habitat in coastal zones is essential for the ecological functioning of important fisheries and consequently instigates considerable attention from the scientific community, conservation groups, and government agencies (Waycott et al. 2009; Orth et al. 2006; Duarte 2002).

2.6 **Economic and ecological value of seagrasses to Florida Bay**

The diversity and abundance of the fish community make the seagrass beds of Florida Bay and the near-shore waters of the Florida Keys one of the most popular recreational fishing destinations in the world (Ault et al. 2009). In Florida, the overall economic impact of recreational fisheries greatly surpasses revenues from commercial fisheries and is even greater than the famed Florida citrus industry (FFWCC 2005). In 2008, the Florida Keys recreational bonefish fishery alone contributed approximately $1 billion to Florida's economy (Ault et al. 2009). Because bonefish are typically a catch and release species and can be caught twenty or more times during their life, the value of a single bonefish has been estimated to be over $70,000 (Ault et al. 2009). This dollar figure includes all the economic activity associated with the recreational anglers that travel from around the world to target this fish including professional guides, fishing supplies, boat fuel, and food and lodging. Consider also that these anglers often bring

families, who spend money on food, lodging, and entertainment. Bonefish and many of the other fish species targeted by recreational anglers are dependent on a healthy seagrass ecosystem for survival (Crabtree et al. 1998).

The value of the recreational fishing industry alone makes the health of the Florida Bay seagrass community a significant concern to local populations. The Florida Bay seagrass ecosystem is also vital to the offshore recreational and commercial fishing community because of its role as a nursery for many pelagic species (Heck et al. 2003). When considering the essential linkages with the Everglades and coral reef ecosystems, the value of Florida Bay's seagrasses are immeasurable.

The Everglades, Florida Bay, and the greater Florida Keys ecosystem are of national and global significance. The United States has designated a number of federal protected areas to protect the unique wildlife and the ecosystem services that they support including four National Wildlife Refuges, two National Parks, and the Florida Keys National Marine Sanctuary. Reflecting its global significance, the Everglades National Park has been designated as a UNESCO World Heritage Site.

2.6.1 Recent history

The region surrounding Florida Bay has experienced significant changes over the last century (Fourqurean and Robblee 1999). Over the last 75 years, the population of the Miami metropolitan area has increased ten-fold and the area has seen tremendous coastal development (Porter and Porter 2002). Subsequently, as developable land became scarce, the "swampy" Everglades were viewed as wasted space. Beginning in the 1920´s the Army Corps of Engineers began draining the wetlands to build roads, allow development, and control flooding (Light and Dineen 1994). These projects consisted of a complex

network of canals that diverted the historical water flow of the Everglades. As a result, nearly 90% of the water flow that previously emptied into Florida Bay is now diverted into the Atlantic Ocean and Gulf of Mexico (Porter and Porter 2002). Additionally, many miles of roads were built on elevated embankments that acted essentially as dikes, further restricting the natural water flow (Kushlan 1987). In the Florida Keys, the construction of the Florida Overseas Railway, the Overseas Highway, population increases, and expanded public use of the ecosystem has resulted in further disturbance (Fourqurean and Robblee 1999).

The mixing of the freshwater system with the salt water system is critical to the ecological health of Florida Bay's plant and the wildlife (Nuttle et al. 2000). The significant ecological decline observed in Florida Bay is widely considered the result of the long-term alteration of the natural water flow (Nuttle et al. 2000). Because of these significant alterations, the Florida Bay ecosystems that we see today may be quite different then in pre-Columbian time (Fourqurean and Robblee 1999; Zieman 1982).

At the time, these projects may have seemed like the right thing to do. Most of the people living in the region would not live there if not for this project. However, in recent decades it has become evident that the entire interlinked everglades/bay/reef ecosystem may be at risk (Robblee et al. 1991). Frequent hypersalinity events, elevated water temperature, overfishing, boating pressures, sewage discharge, and coastal runoff have ravaged the Everglades, with cascading effects on the neighboring Florida Bay and corals reefs.

During the 1980s, extensive die-off of *T. testudinum* was reported in Florida Bay eventually denuding 4000 ha and disturbing 23,000 ha to a lesser degree (Zieman et al.

1999). The die-offs were followed by a significant decrease in water clarity, massive algae blooms, and subsequent loss of seagrass cover. The cause of the die-off, though still in some dispute, is widely believed to be a result of high salinity and temperatures, hypoxic sediments, and loss of biodiversity of seagrass communities (Fourqurean et al. 2003; Robblee et al. 1991). This overdevelopment is believed to have resulted from a reduced disturbance regime because the die-off immediately followed a period of historically low hurricane frequency and lowered freshwater input from the Everglades watershed (Durako et al. 1994). Viewed in tandem with global climate change, the array of stressors on the entire regional ecosystem may be reaching a zenith. This is apparent from the enormous Everglades fires, seagrass die-offs, and coral bleaching on the reefs (Fourqurean and Robblee 1999).

In response, scientists, resource managers, conservation groups, politicians, and user groups came together to seek solutions (Davis and Ogden 1994). Between 1996 and 1999, these groups were involved in a comprehensive process to formulate a plan to restore and preserve south Florida's natural ecosystem. In the late 1990's legislation was passed granting nearly $10 billion toward the restoration of the Everglades (U.S. Congress 2000). The Comprehensive Everglades Restoration Plan (CERP) was developed with the purpose of restoring and preserving south Florida's natural ecosystems while enhancing water supplies and maintaining flood control (USACE and SFWMD 1999). CERP provides a framework through which the U.S. Army Corps of Engineers and the South Florida Water Management District will work with other partners to restore, protect, and preserve the water resources of south Florida. This project is of such significance and magnitude that implementation is expected to take

more than 30 years. The goal of this project is to "replumb" the Everglades by restoring the natural water flow by removing canals and raising roadways. The restoration project will attempt to effectively reestablish the hydrological regime of Florida Bay to near historical levels (Perry 2004). However, this water flow could now be laden with nutrient runoff from the extensive agricultural region that has developed north of the Everglades (Herbert et al. 2011). Concerns about the effects of this restored water flow on the health of the seagrass populations in Florida Bay have instigated much research (Ogden et al. 2005).

Because of the known sensitivity of *T. testudinum* to shifts in salinity and nutrient availability, there is concern that this radical change in hydrologic and nutrient regime may have a dramatic effect on the ecological communities in the estuary (Herbert and Fourqurean 2009). As recently as the 1960's, the Florida Bay seagrass communities in the northern and eastern parts of the bay were dominated by *H. wrightii* (Zieman 1982). The community has since shifted to sparse *T. testudinum* possibly in response to less variable fluctuations in salinity resulting from the diversion of the natural freshwater flow (Fourqurean et al. 2003). The historical change in Florida Bay's salinity regime has been demonstrated through paleo-ecological reconstructions using stable isotopes in coral cores and changes in the diversity and abundance of benthic infauna in sediment cores (Brewster-Wingard and Ishman 1999). Many of the negative ecological changes in Florida Bay are a direct result of the past water management activities. The goal of the CERP is to unravel this water management to benefit the ecosystems. However, the specific effects on the Florida Bay ecosystem can only be predicted (Herbert et al. 2011).

PART II. THE NATURE OF LIGHT IN A TROPICAL SEAGRASS ECOSYSTEM

Chapter 3. Examination of attenuation and alteration of down-welling light through an estuarine water column.

3.1 Abstract

This study examined the alteration of the quantity and quality of the down-welling light field through a dense *T. testudinum* canopy and overlying water column using an Ocean Optics USB 2000 "Mini-spec". Light quality was determined by weighting the photosynthetically available radiation (PAR) spectrum against a typical absorbance spectrum of a *T. testudinum* leaf to calculate the photosynthetically usable radiation (PUR), a representation of the light that the leaf actually absorbs and utilizes in photosynthesis. The results suggest that PUR is a more accurate indicator of light availability to seagrasses than PAR. The study also revealed that a seagrass canopy lacks the lower red:far-red characteristic in terrestrial canopies questioning the role of the phytochrome system in seagrass meadow development.

3.2 Introduction

Plant growth is dependent on their ability to absorb solar energy and convert it into chemical energy via photosynthesis. Understandably, the most important factor in understanding seagrass growth is knowledge of the light environment. However, estimating the light availability to seagrass can be incredibly complex owing to the overlying water column among other factors.

3.2.1 Nature of Light

We usually define "light" as being the portion of solar energy that is visible to the human eye. We have known since ancient times that light originating from the sun

influences plant growth (Hart 1988). Solar energy is produced during nuclear fusion when hydrogen nuclei within the sun combine to form a helium atom (Häder and Tevini 1987). The small amount of mass that is lost in this process is converted to energy then radiated into space at the speed of light as electromagnetic waves. This field of energy, called the electromagnetic spectrum, includes energy ranging from short wavelength cosmic rays (10^{-12} m) to extremely long wavelength radio waves (10^{4} m) (Häder and Tevini 1987).

The electromagnetic energy that travels through space is far different then the solar energy that reaches the surface of the earth (Kirk 1994). A large portion of the shortest wavelengths are reflected back to space while the radiation that transmits through the Earth's atmosphere is attenuated due to the scattering from air molecules and dust particles and absorption by water vapor, oxygen, ozone, and carbon dioxide in the atmosphere (Smith and Morgan 1981). The absorption and scattering processes reduces both the magnitude and the spectral distribution of the solar radiation (Kirk 1994). Ozone at the top of the atmosphere eliminates a band of short wavelengths that would make life on earth impossible. The solar energy that reaches the earth's surface is also affected by the distance it travels through the atmosphere and the angle of incidence upon the surface. This pathlength fluctuates diurnally as a function of solar elevation angles while the angle of incidence is a function of latitude and time of year (Kirk 1994).

Quantum theory, first put forth in 1675 by Isaac Newton, states that radiation consists of discrete particles called photons (Kirk 1994). Huygens, Fresnel and finally Maxwell maintained that electromagnetic energy travels in waves (Häder and Tevini 1987). In 1900, Max Planck concluded that light displays characteristics of both a

particle and a wave, unifying the two theories. Light exhibits both a frequency and a wavelength, like a wave, but exists in discrete units. Planck Law's states that a single photon of light, also called a quanta, has a defined energy, which is a function of the frequency and the speed of the light:

$$E = h\nu = h\, c/\lambda$$

where h, Planck's constant is 6.63×10^{-34} J s. This means that a photon of blue light (400 nm) has nearly twice the energy of a red photon (700 nm) (Kirk 1994). Later in his law of photochemical equivalence, Einstein stated that a single molecule will react only after absorbing the energy of a single quanta (Hall and Rao 1999). This concept led to measuring light in units of quanta, termed an Einstein or one *mol* (6.02×10^{23}) of photons. Conversion to SI units dropped the use of the term Einstein in favor of *mols*. Light available to plants is typically measured as irradiance, the flux of photons incident upon a horizontal surface over time, generally presented as µmol of photons per m^2 per second (µmol m^{-2} s^{-1}).

The term "visible" light refers to the portion of the electromagnetic spectrum ranging from 400 nm to 700 nm, also termed photosynthetically active radiation (PAR) (Hart 1988). PAR is defined as the integration of the light energy from 400 nm to 700nm (Kirk 1994). The measuring of PAR has long been a fundamental practice in plant ecology (Baird 1923). The ultraviolet and far-red regions bordering PAR spectrum are also of interest to plant researchers (Hart 1988). Although plants are unable to utilize this light for photosynthesis, it can reveal valuable information about a plant's environment (Balleré 1999). It is useful to refer to wavelength regions of PAR light and the adjacent

wavelength regions of the spectrum by their corresponding color within the visible spectrum (Figure 3.1).

Table 3.1. Color ranges of the visible portion of the electromagnetic spectrum.

Color	Wavelength Range (nm)
Near UV	300 - 400
Violet	400 - 438
Blue	438 - 475
Blue-green	475 - 513
Green	513 - 550
Yellow-green	550 - 588
Yellow	588 - 625
Orange	625 - 663
Red	663 -700
Far-red	700 - 800

3.2.2 Light in Aquatic Systems

The behavior of light in aquatic ecosystems is much different from in air. To begin with, a portion of the down-welling light field is reflected directly by the water surface ranging from 2% when the angle of incidence is perpendicular to the surface to nearly 100% at the sun nears the horizon. Pure water absorbs light strongly in the red and far-red portions of the spectrum and less at wavelengths below 600 nm (Kirk 1994). The optical properties of a particular water column are a function of the concentration of dissolved organic matter, phytoplankton, and suspended particulate matter in the water (Gallegos et al. 1990). Because these water properties absorb or scatter photons of

different wavelengths at different rates, the spectral distribution of light is also significantly altered (Spence 1981).

Light decreases exponentially with depth in water and follows Beer's Law:

$$I_z = I_0 * \exp^{-Kd*z}$$

where z is water depth, I_z is the irradiance at depth z, I_0 is the initial irradiance at depth 0, and K_d is the diffuse attenuation coefficient (Kirk 1994). The diffuse attenuation coefficient (K_d) is the most common parameter used to characterize the optical properties of a water column.

3.2.3 PAR versus PUR

In some cases, using PAR as an indicator of light availability for plants may be inadequate because not all quanta within the PAR spectrum are equally absorbed by leaf photosynthetic pigments (Zimmerman 2003). Much of the measured PAR spectrum may be completely unusable in photosynthesis (Gallegos et al. 2009). Seagrasses, due to their apparent lack of specialized accessory pigments for absorbing green light (Cummings and Zimmerman 2003), are particularly dependent on a narrower band of radiation than PAR represents (Gallegos 1994). Light availability should also consider the ability of leaves to absorb the incident light, or the quality of the light. A good quality light field is abundant is red and blue wavelengths, those most easily absorbed by leaf pigments.

A more useful indicator of light availability to plants is photosynthetically usable radiation (PUR) (Morel 1978). PUR weights the light spectrum using the absorbance spectrum of the seagrass or other target species. The term photosynthetic light harvesting efficiency (φL) is used to define the spectrally weighted portion of down-welling light that is absorbed by a leaf (Cummings and Zimmerman 2003). Good light quality implies

that a high percentage of PAR is usable by plants and poor light quality means that the light is mostly unusable. While PAR is an indication of light availability, PUR considers a plant's ability to absorb the light. Distinguishing PAR from PUR may be especially important in the case of aquatic plants because of the selective attenuation of the most usable wavelengths by water column chlorophyll, suspended organic matter, and other attributes (Gallegos 1994).

3.2.4 Seagrass canopies

Seagrasses often form dense beds where self-shading by leaves significantly modifies both the quantity and quality of the inter-canopy light field (Enríquez and Pantoja-Reyes 2005). The extent to which the quantity of light is attenuated is a function of the canopy architecture and leaf orientation, while selective attenuation of the primary photosynthetic by leaf pigments alters the spectral quality (Zimmerman 2003; Holmes 1981). The growth form of seagrasses further complicates the canopy structure as leaf pigments, photosynthetic capacity, and epiphytic growth vary along leaf blades (Enríquez et al. 2002). However, the Lambert-Beer Law is still an appropriate method for describing the attenuation of light through a canopy (Zimmerman 2003). Furthermore, seagrasses provide an ideal growing surface for epiphytic algae, which further alter the quantity and quality of the light available to the leaves (Frankovich and Zieman 2005).

Terrestrial plants that grow beneath a leaf canopy experience a reduction in irradiance as well as a lower light quality because of selective absorption of blue and red wavelengths by leaf pigments (Schmitt and Wulff 1993). The inter-canopy light field is characterized by a very low level of diffuse light interspersed with intense bursts of direct light that last only a second or less (Pearcy 1988). The importance of sunflecks to

terrestrial plants has long been well appreciated (Lundegarth 1927). These sunflecks can contribute a substantial portion of the total light available to light-limited understory plants (Chazdon and Pearcy 1991).

The inter-canopy light field in an aquatic environment is more complicated because of the additional modification by the water column (Spence 1981). The down-welling light field within a canopy is highly variable consisting of both direct and diffuse light. Diffuse light can better penetrate through gaps in a canopy because it is multi-directional while direct light is unidirectional and more likely to intercept a leaf (Enríquez and Pantoja-Reyes 2005). Cloud cover, although greatly decreasing the total irradiance, increases the percentage of sunlight that is diffuse and causes substantial changes in the spectral quality of light in the canopy (Holmes 1981). Canopy light attenuation is also strongly affected by solar angle (Zimmerman 2006). At high solar angles, direct light is more able to penetrate a canopy of erect leaves. At low solar angles, light has to pass through a longer pathlength of water to reach the plants as well as more leaves (Holmes 1981).

3.2.5 Red:far-red

The development and structure of a plant community is strongly influenced by competition for light (Schmitt and Wulff 1993). Plants not only obtain energy from light, they also obtain important information about their environment. For highly productive plant communities, the most important characteristic of their environment is often the vicinity of other plants (Balleré 1999). Plants respond to shading by altering leaf area and increasing leaf pigment concentration (Givnish 1988). However, a plant can also respond to shading by positioning leaves out of the shade (Vandenbussche et al. 2005).

A plant's ability to sense the quantity and quality of light in its environment may be as crucial as vision is to animals (Smith 2000).

An important indicator of light quality in a plant canopy is the red:far-red light ratio (R:FR). Because leaf pigments tend to absorb red light and reflect far-red light, the amount of red light relative to far-red light decreases within a plant canopy (Holmes 1981). The R:FR of unaltered solar irradiance is approximately 1.2 while the ratio within a dense terrestrial plant canopy can be only 0.05 (Schmitt and Wulff 1993). Changes in R:FR is a more reliable indicator of the proximity of neighboring plants than the reduction of light intensity, as it allows plants to differentiate between shade caused by leaves as opposed to shade caused by solar angle, clouds, or other objects (Tomasko 1992).

Plants possess a number of photosensory systems whose function is to acquire information about the plant's light environment (Balleré 1999). A plant's primary means for detecting and responding to changes in the R:FR of their light environment are the photoreversible pigments of the phytochrome system, a family of photoreceptors found in all higher plants (Smith 2000). The phenomena of a reversible photoaction within plants was discovered by Borthwick et al (1952) while studying seed germination of lettuce seeds under differing light regimes. Seed germination was found to be promoted by red light and inhibited by far-red light suggesting a photoreversible process.

The phytochrome system regulates molecular and physiological processes during many stages of plant growth and development but is not involved in light harvesting for photosynthesis (Smith and Whitelam 2006). Phytochrome allows a plant to continuously assess and adapt to changing light environment (Quail 2002). The phytochrome system

provides plants with temporal signals that activate phases of their biological development. Many of the most vulnerable points in a plant's life cycle, such as flowering, germination, and dormancy are highly time sensitive (Orozco-Segovia et al. 1993; Smith and Whitelam 2006). Because the phytochrome system can interpret the difference between seasonal or diurnal fluctuations in R:FR and fluctuations due to cloud cover, it can function as a reliable timing agent (Chambers and Spence 1984). The precise timing of many important plant processes may be so important that plants may have evolved numerous redundant photosystems to guarantee success under all possible conditions (Smith 2000).

Phytochrome consists of a low molecular weight protein attached to a photoreversible pigment (Hall and Rao 1999). Phytochrome shifts between two forms when exposed to red (P_r) or far-red (P_{fr}) light (Figure 3.1) (Schmitt and Wulff 1993). The absorbance peak of the P_r form is 660 nm while the peak for P_{fr} is 720 nm (Morgan and Smith 1978a). Plant morphogenesis occurs as a function of the equilibrium state between these two forms. Higher amounts of P_{fr} relative to P_r provokes gene expressions that modify the plant canopy structure to effectively reach out of the shade (Vandenbussche et al. 2005). Plants react to reduced R:FR via a range of plastic responses including enhanced elongation, increased apical dominancy, elevated leaf angle, and altered resource allocation (Smith 2000).

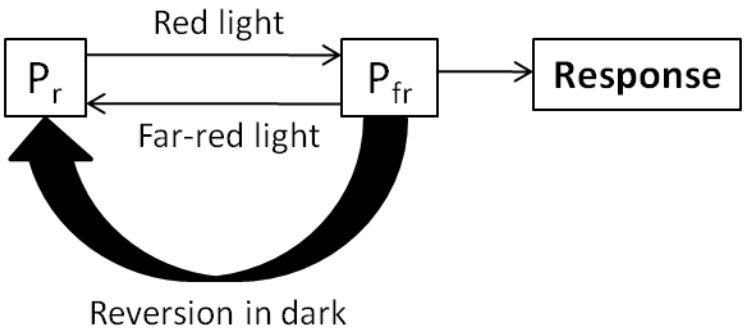

Figure 3.1. Diagram illustrating the photoconversion of the two forms of phytochrome.

3.2.6 Objectives

Although the spectral quality of light may have a profound effect on the development and growth of seagrass, surprisingly little is known about the spectral quality of light within a seagrass canopy. The purpose of this study is to measure how the intensity and spectral distribution of light changes through a water column and within a seagrass canopy. Light quality will be ascertained using important indicators of light quality including spectral attenuation of light, PUR as compared to PAR, and R:FR. This study will present evidence concerning whether or not light quality is an important component of light availability in a seagrass meadow.

3.3 **Methods**

The "Mini-Spec"

The primary instrument used in this study to measure spectral irradiance was the Ocean Optics USB2000 spectrometer, "the Mini-spec" (Ocean Optics 2005). The Mini-spec is a miniature spectrometer, about the size of a deck of cards that measures light intensity across wavelengths from 189 nm to 867 nm at 0.3 nm intervals (Figure 3.2).

The Mini-spec is connected to a computer via the USB port and is operated through special software from Ocean Optics. It draws power from the host PC, eliminating the need for an external power supply. The light to be measured is accumulated through a CC-3 Cosine Corrector that collects light from a 180° field of view and then passes it through a thin fiber optic cable into the spectrometer. Within the instrument, the light passes through a slit and filtering device that narrows the light to a specific wavelength region. The light is reflected onto a grating that differentially diffracts as a function of wavelength. The diffracted light is focused across a detector array that transmits a digital signal to the software. The software compares the measured spectra against the reference spectrum set at the factory.

3.3.1 Calibrating the Mini-spec

To attain actual measurements of quanta, the Mini-spec must be calibrated with a standardized light source (LS-1-Cal, Ocean Optics). The LS-1-Cal is a tungsten halogen light source that provides a known absolute irradiance from 300 nm to 800 nm. Once activated the light source must be allowed to warm up for at least 15 minutes to ensure a stable output. Then the cosine collector is inserted into the SMA connector of the light source. After selecting a suitable integration period, the software is switched to "Irradiance" mode. The integration time is comparable to a shutter speed in a camera. The detector array receives incoming photons for the period of integration, displays the reading, clears the array and then starts the next scan. The integration time should be adjusted so that the maximum signal in the displayed spectrum does not exceed 3500 counts. If the integration time is set too high, detector arrays will become saturated and yield an erroneous reading. To complete a calibration scan, the light path to the

spectrometer is completely blocked for a dark reading. Separate calibration files must be created for each integration time to be used. When conducting a scan, the Mini-spec software can be set to average a series of scans to remove the effects of boat rocking, wave focusing, or other variability. The scans to average typically range from 200 to 1000 scans.

Figure 3.2. Image of the Ocean Optics USB2000, the Mini-spec.

3.3.2 Light sampling assembly

To facilitate deployment of the Mini-spec setup in the field, I constructed a light sampling assembly. The vertical section of the assembly consists of a ½" PVC pipe approximately two feet long with a 90° angle connector attached at the bottom. A short section of ½" PVC is inserted into the flange and a thin ¼" aluminum rod was securely attached forming a right angle to the vertical section. The end of the aluminum rod was bent to point upward. The 10 m fiber optic was attached to the assembly with cable ties and the end with the cosine corrector positioned toward the vertical. The terminal end of

the fiber optic was attached to the Mini-spec and computer. To measure down-welling irradiance in water deeper than 3 m, the assembly was attached to a thin rope and lowered over the side of the boat. To measure upwelling irradiance the horizontal part of the assembly can simply be reversed so that the cosine corrector points down.

In shallower water, the assembly was anchored to the sediment to reduce the effect of boat rocking on the measurement (Figure 3.3). The assembly is slid over a 3/8" aluminum rod that is securely driven into the sediment forming a 90° angle. The assembly is positioned at the desired depth within the water column or seagrass canopy and secured with a wing nut. The small size of the assembly allows the cosine corrector to be deployed within a canopy without disturbing the normal posture of the leaves. When the assembly is set at the lowest position, the top of the cosine corrector is positioned approximately five cm above the sediment.

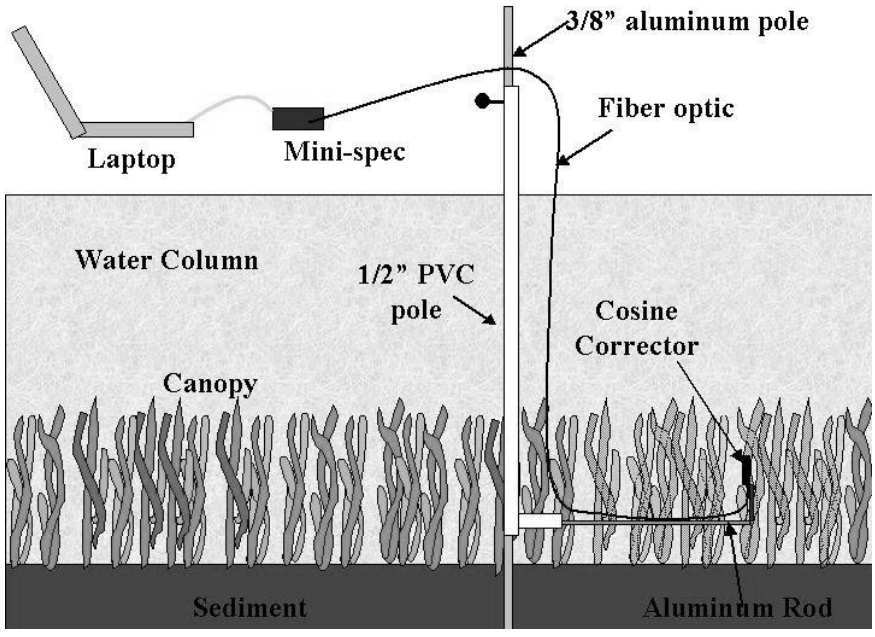

Figure 3.3. Light sampling assembly.

3.3.3 Light profiles

Water column or canopy light attenuation was calculated by conducting light profiles. A profile begins with a scan of the irradiance directly above the surface and followed by a succession of irradiance scans at increasing depths. Typically, a profile will include 4-8 scans. If clouds obstructed the sun during the profile, the profile was repeated. The Mini-spec calculates irradiance as units of light energy ($\mu W/cm^2/s$). These values were converted to quantum units for each wavelength interval using the following equation (Kirk 1994):

$$Q = 5.03 * I * \lambda * (1/N_A) * 10^{19}$$

Q: irradiance in $\mu mol\ m^{-2}\ s^{-1}$
I: irradiance in $\mu W / cm^{-2}\ s^{-1}$
λ: wavelength in nm
N_A: Avogadro's number = 6.02×10^{23} photons / mol

Spectral diffuse attenuation coefficients ($K_d(\lambda)$) were calculated as the slope of the plot of the natural log of irradiance versus depth for each wavelength. The water surface spectra were not used in the regressions in order to negate the effect of surface reflection. Separate calculations were made for the water column and canopy. Profiles were conducted between noon and 2 pm for all sampling sites. Profiles resulting in regression coefficients (R^2) lower than 0.70 were considered erroneous and discarded.

Total PAR for each spectrum was calculated by integrating the irradiance from 400 to 700 nm. PUR was calculated by weighting the PAR spectrum with the average leaf absorption spectrum for *T. testudinum* normalized to its peak at approximately 675 nm and to unit sum. For comparison of attenuation rates, PAR and PUR were both

normalized to their values at immediately below the water surface. R:FR was calculated by integrating across 660 to 670 nm for red light and 725 to 735 nm for far-red light (Smith 2000).

3.3.4 Sampling Sites

Light profiles were conducted at Alligator Reef and Rabbit Key Basin. Alligator Reef is located offshore of the Florida Keys with a depth of approximately 8 m and is characterized by clear oligotrophic marine water. The light assembly was deployed on the side of the boat facing the sun and water column scans were conducted at 1 m intervals. Measurements through the seagrass canopy were not completed at this site. Considering the depth and the sparse short shoots and short leaves, there is likely little canopy self-shading occurring. The other sites were located approximately 1 km apart in central Florida Bay. The plant community at Rabbit Key Basin was a dense (1330 short shoot m^{-2}) monospecific stand of *T. testudinum* with a canopy height of approximately 40 cm. The 1.8 m water column was characterized by water column chlorophyll and some suspended carbonate sediment. The water column was mostly transparent with some suspended carbonate sediments. I conducted Mini-spec scans of the water column and seagrass canopies at 10 cm intervals. To measure the upwelling irradiance at the sites, I reversed the orientation of the light sampling assembly.

I also conducted Mini-spec scans in a terrestrial grass canopy and mangrove forest to compare between relative spectral distribution of the intercanopy light fields of submerged and terrestrial plant communities. The field next to Key Largo science center was used as an example of a terrestrial grass meadow with similar density. I sampled the intercanopy light field with the sampling assembly positioned under approximately 30 cm

of canopy. I also sampled the light field under a mangrove canopy located nearby the science center with the light sampling assembly positioned with approximately 2 m of canopy above it. I compared the relative irradiance spectra by normalizing them to 1.0 at 500 nm. The R:FR ratios for each canopy light field were calculated and compared.

3.4 **Results**

3.4.1 Alligator Reef

The irradiance profile conducted at Alligator Reef showed the nature of light attenuation through a water column mostly affected by the attenuation properties of pure water (Figure 3.4). The irradiance spectrum decreased with depth across all wavelengths but most noticeably at wavelengths greater than 600 nm. Most wavelengths above in the far-red region were attenuated within the first 3 m of the water column. At a depth of 6 m, all orange, red, and far-red light were nearly eliminated. The peak irradiance at all depths was at approximately 450 nm consistent with the visual appearance of the deep blue water of the site. The spectral attenuation coefficient calculated for Alligator Reef profile clearly shows the abrupt increase in absorbance above 600 nm (Figure **3.5**). Absorbance is relatively unchanged from 450 nm to 575 nm and lowest below 450 nm. Values for attenuation of the UV region are unreliable due to considerable noise in the scan.

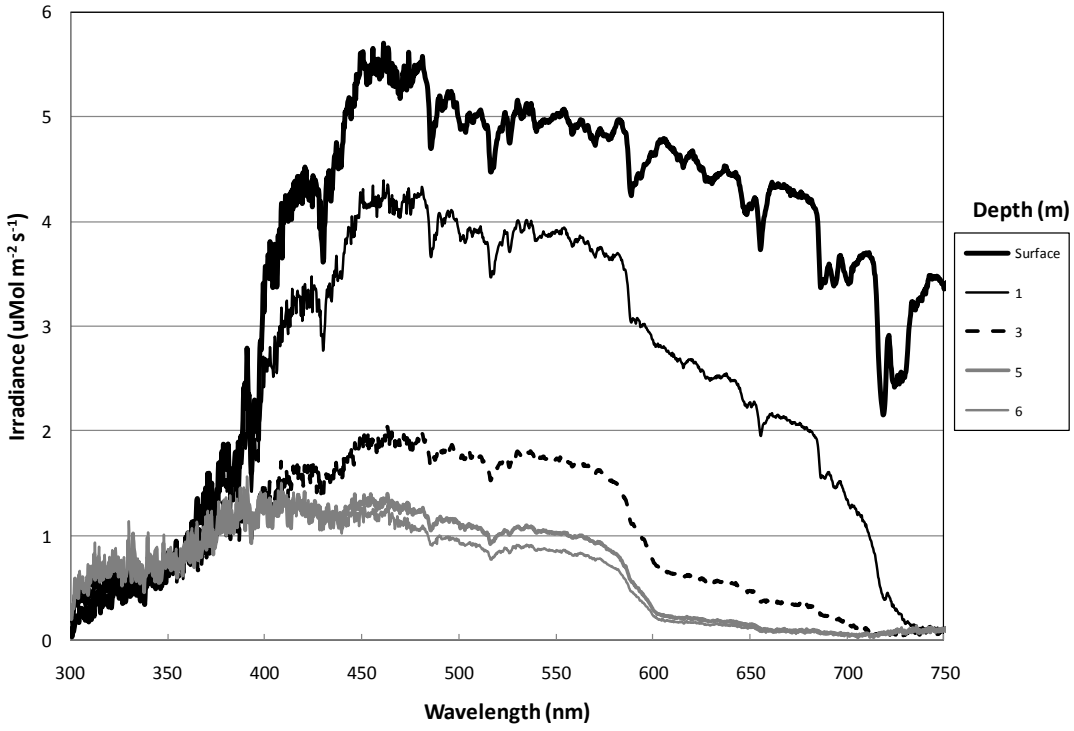

Figure 3.4. Irradiance profile above a sparse (600 Short shoot m^{-2}) *Thalassia testudinum* canopy at Alligator Reef at midday. The surface scan was taken with the light sensor immediately below the water surface followed by scans at 1-2 m intervals.

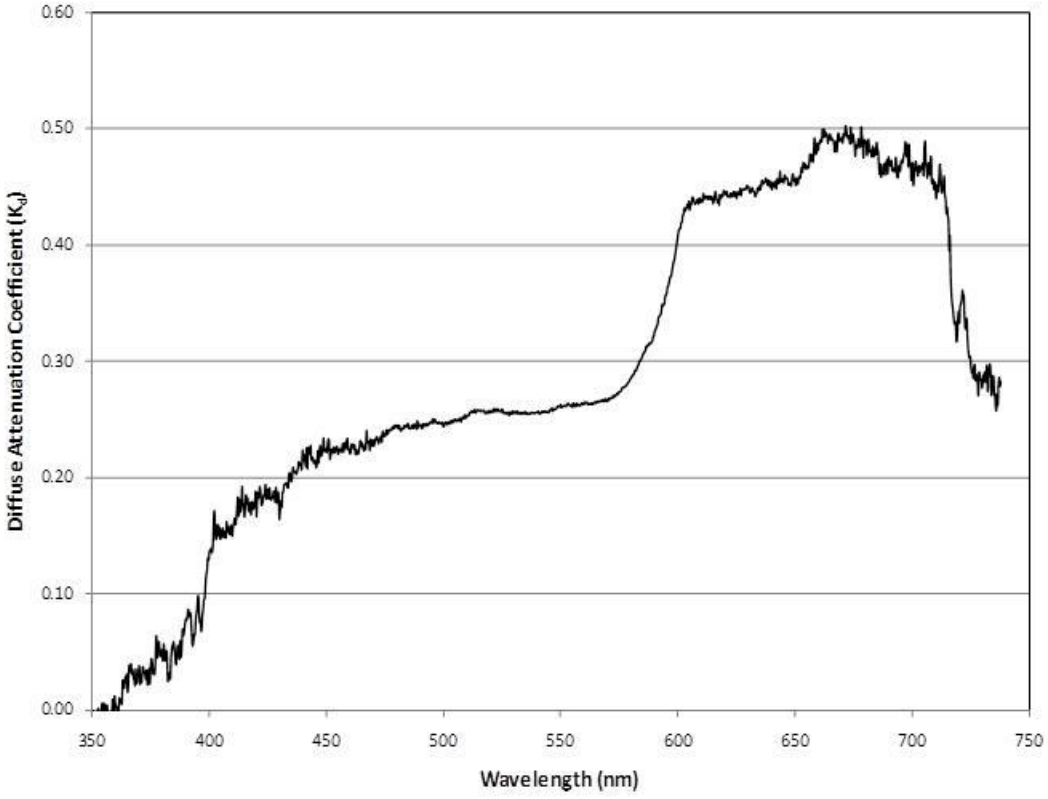

Figure 3.5. Diffuse spectral attenuation coefficients of water column at Alligator Reef, Florida Keys at midday calculated from the irradiance profile in Figure 3.4.

3.4.2 Rabbit Key Basin

The irradiance at Rabbit Key Basin became weighted more toward green wavelengths with increasing depth (Figure 3.6). This is characteristic of attenuation from water column chlorophyll and alteration of the light by leaf absorption. The attenuation spectra for the water column and seagrass canopy are combined in Figure 3.7. Water column attenuation was highest in the violet and blue regions starting at approximately 2.0 at 400 nm and declining steadily toward longer wavelengths to a minimum of 0.8 at 550 nm. There was a distinct ridge at 600 nm but only a slight ridge at 660 nm. Attenuation increased again in the far-red region. There was a distinct indent in the

attenuation spectrum at 760 nm. This indent was not found at Alligator Reef (Figure 3.5) mainly because down-welling irradiance at wavelengths above 750 nm was too noisy to allow for accurate calculation of the attenuation coefficient. The canopy attenuation Rabbit Key Basin was 2- to 3-fold higher than for the water column. This is partly due to the continued attenuation by the water column but also to the increased probability of interception of light by leaves.

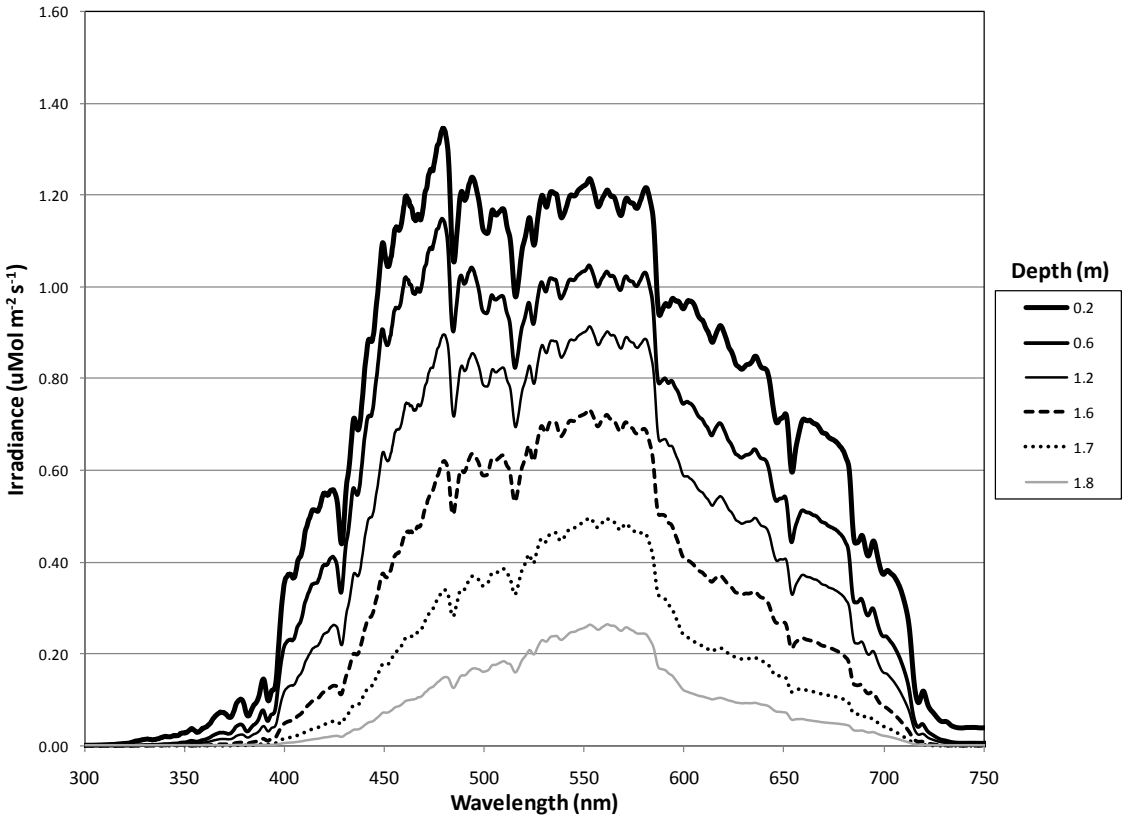

Figure 3.6. Spectral irradiance profile at Rabbit Key Basin, Florida Bay at noon. The spectra at 1.6 m, 1.7 m, and 1.8 m are within the *T. testudinum* canopy.

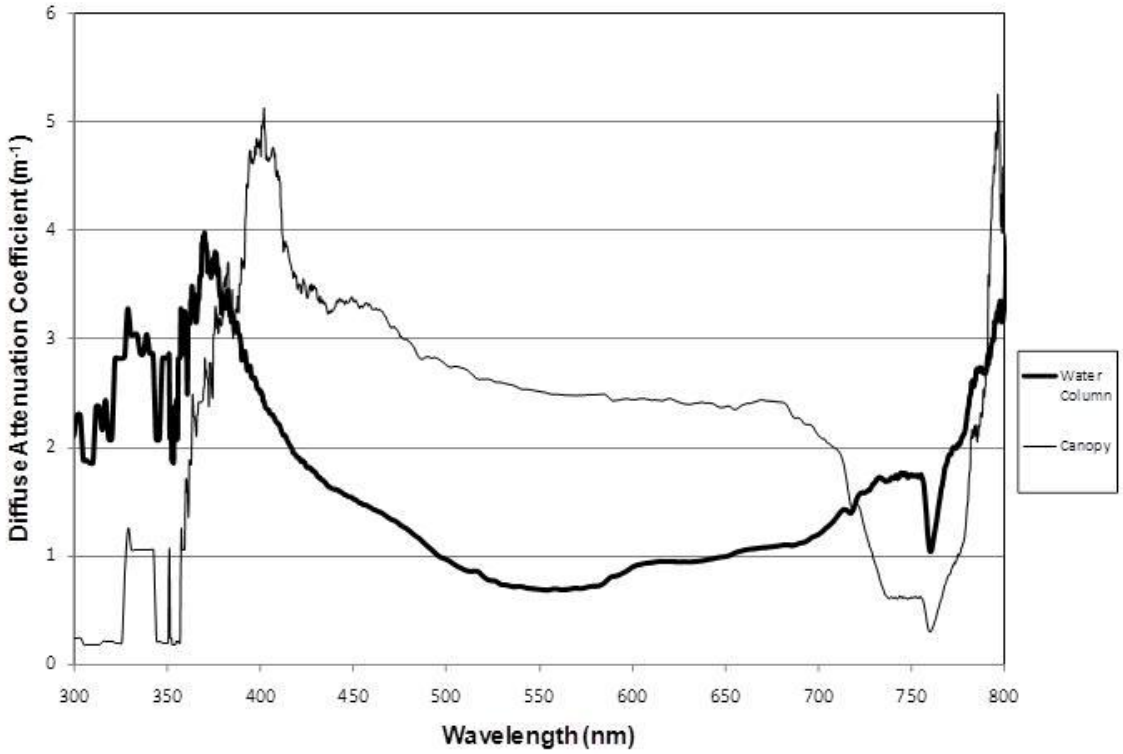

Figure 3.7. Diffuse attenuation coefficient of water column and seagrass canopy at Rabbit Key Basin, Florida Bay.

3.4.3 Red:far-red versus depth

Figure 3.8 compares the R:FR versus depth through the water columns and seagrass canopy at Alligator Reef and Rabbit Key Basin. The R:FR at just below the surface was approximately 1.4 at both sites. At Alligator Reef, the R:FR showed an abrupt increase from just below the surface to 1 m depth. R:FR decreased after 3 m but these values are not reliable because far-red light was nearly eliminated at these depths. At Rabbit Key Basin, the R:FR increased to nearly 4.0 at only 60 cm in depth. At the top of the seagrass canopy, at an overall depth of approximately 1.6 m, the R:FR was nearly 6.0. With increasing depth in the canopy the R:FR decreased to 3.5, however at this point

most of the far-red was attenuated. These results show that the R:FR in a seagrass canopy is inconsistent with the R:FR within a terrestrial canopy (Schmitt and Wulff 1993).

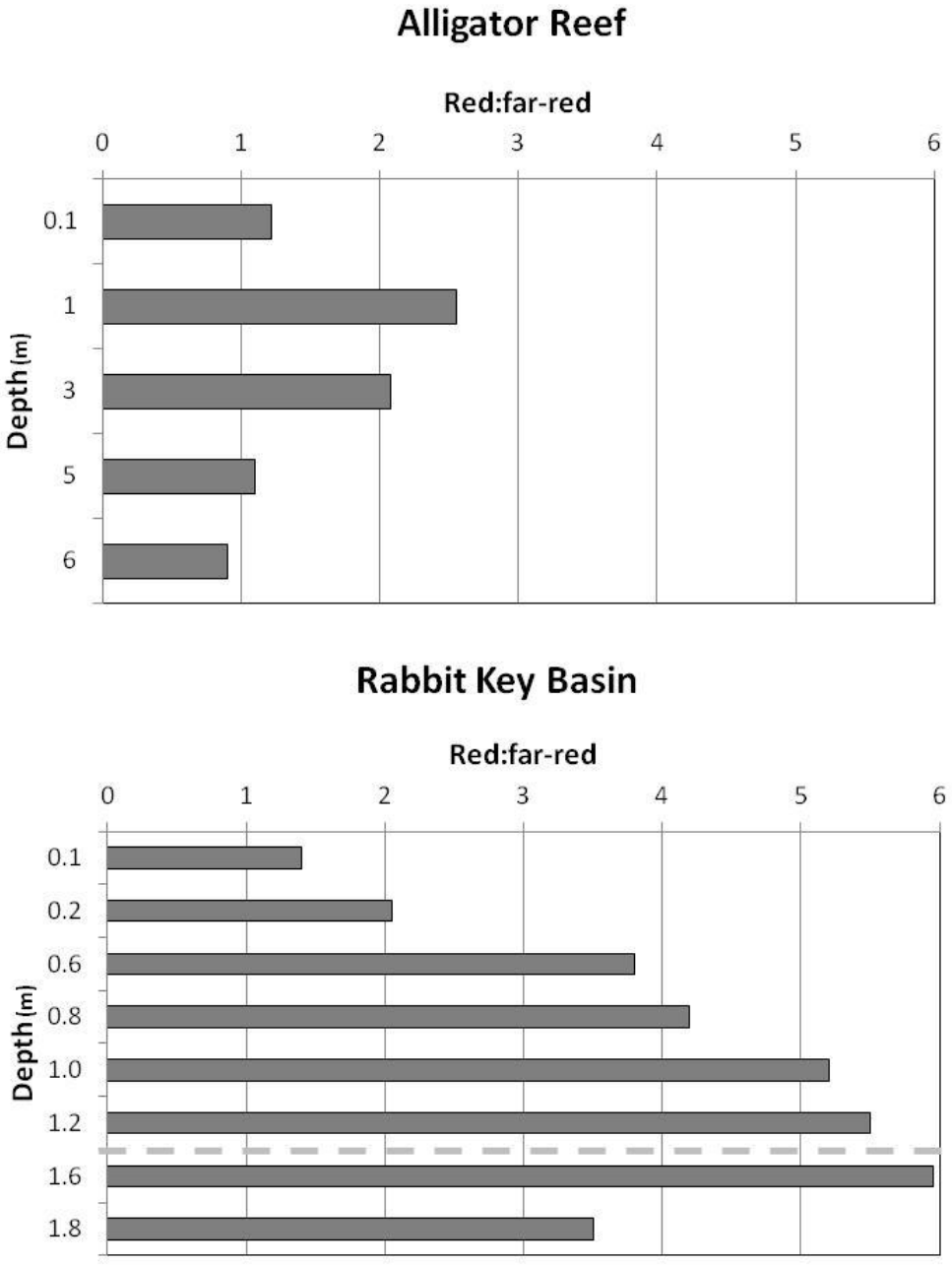

Figure 3.8. Red:far-red at Alligator Reef and Rabbit Key Basin. The values below the grey dashed line represent red:far-red within the seagrass canopy.

3.4.4 PAR versus PUR

The normalized absorption spectrum for *T. testudinum* leaves exhibited a peak in the red wavelengths at approximately 680 nm, a broad maximum at blue wavelengths (400 – 490 nm), and a trough at green wavelengths (525 – 625 nm) (Figure 3.9). This spectrum was used to calculate PUR from the scans of down-welling spectral irradiance. However, even at the minimum absorbance at 555 nm, measured absorption was still nearly 40% of the maximum. Considering that *T. testudinum* has no accessory pigments that absorb green wavelengths (Zimmerman 2003), I constructed a hypothetical scenario using the absorption spectrum of pure chlorophyll and assumed no absorbance in the green wavelengths (525 - 600 nm). This hypothetical spectrum is expected to produce the maximum difference between PAR and PUR.

The light attenuation coefficients were calculated for PAR (K_{PAR}), PUR adjusted with the *T. testudinum* leaf absorption spectrum (K_{PURm}), and PUR adjusted using the hypothetical absorption spectrum (K_{PURh}) for each of the sites (Table 3.2). The percentage of the surface irradiance reaching the top of the canopy and the bottom canopy are also shown. At Alligator Reef, 9.1% of the surface PAR reached the seagrass canopy, while 7.1% of the PURm, and 6.1% of the PURh reached the same depth. At Rabbit Key Basin, 18.1% of the surface PAR irradiance reached the top of the seagrass canopy while only 2.6% reached the bottom of the canopy. When considering the PURm, 13.9% of usable light reached the top of the seagrass canopy and only 1.9% reached the bottom of the canopy. When considering PURh, 8.4% of usable light reached the top of the seagrass canopy and only 1.0% reached the bottom of the canopy.

These results suggest that far less light reaches the seagrass canopy if only the photosynthetically usable portion of the PAR spectrum is considered. Using PAR rather than PURh would overestimate light availability to the seagrass at Alligator Reef by approximately 50%, 9.1% of surface PAR versus vs. 6.1% of surface PAR. The light availability at the top of the seagrass canopy at Rabbit Key Basin would be overestimated by approximately 54% (18.1% versus 8.4%). The light availability at the bottom of the seagrass canopy at Rabbit Key Basin, where the majority of the leaf tissue is found, would be overestimated by 160% (2.6% versus 1.0%).

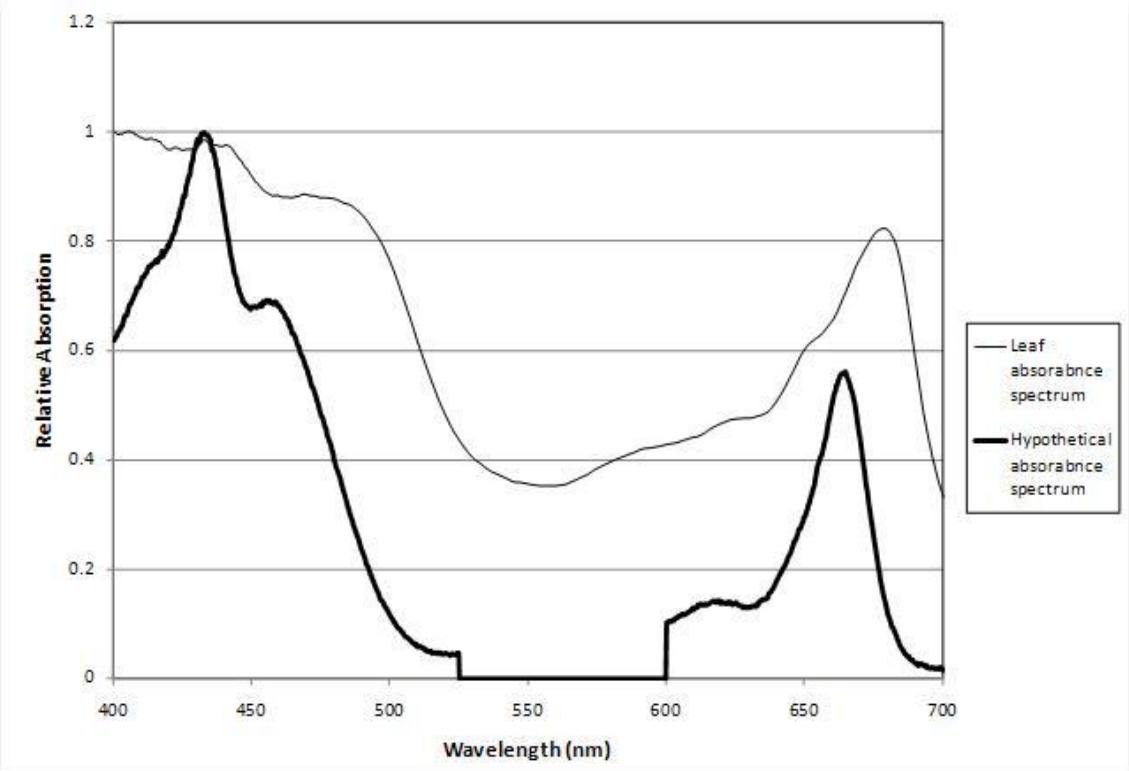

Figure 3.9. Average relative absorption spectrum of *Thalassia testudinum* leaves from Rabbit Key Basin and the hypothetical absorption spectrum based on chlorophyll alone. Values were normalized to 1.0 at the peak wavelength.

Table 3.2. Diffuse attenuation coefficients for photosynthetically active radiation (PAR) and photosynthetically usable radiation (PUR) at Alligator Reef, Florida Keys and Rabbit Key Basin, Florida Bay. The diffuse attenuation coefficients for PUR were created by weighting the relative absorption spectrum of *Thalassia testudinum* (K_{PURm}) and a hypothetical spectrum that assumes no absorption of usable light in the green wavelengths (K_{PURh}). The percent of the surface irradiance that reaches the bottom of water column and seagrass canopy is shown in parentheses.

Site		Depth[a] (m)	K_{PAR} (m^{-1})	K_{PURm} (m^{-1})	K_{PURh} (m^{-1})
Alligator Reef	Water Column	8	0.3 (9.1%)	0.33 (7.1%)	0.35 (6.1%)
Rabbit Key Basin	Water Column	1.6	1.22 (18.1%)	1.41 (13.9%)	1.77 (8.4%)
Rabbit Key Basin	Seagrass Canopy	0.4	4.82 (2.6%)	4.96 (1.9%)	5.22 (1.0%)

3.4.5 Upwelling irradiance

The upwelling irradiance at Rabbit Key Basin and Rabbit Bank were nearly identical, spectrally. The magnitude of the upwelling light was approximately 1% of the down-welling light at the same depth. The plot of the Rabbit Key Basin and Alligator Reef were normalized to 1.0 at 500 nm in order to compare the relative irradiances across the spectrum between the sites (Figure 3.10). The spectra were smoothed considerably in order to eliminate the extreme noise in the scans. The upwelling spectrum at Rabbit Key Basin included all wavelengths within PAR and showed a distinct peak at approximately 550 nm reflecting the relative green to yellowish-green of the water column. There was no UV and only a very small amount of far-red light, from 700-725 nm, in the upwelling light field. The upwelling irradiance at Alligator Reef was restricted to wavelength between 400-600 nm and showed a distinct peak approximately 450 nm in the blue region of the visible spectrum.

Although the cosine corrector measures irradiance from a 180° field of view, the upwelling irradiance is too low to be considered a significant source of error. The light that was present was strongly weighted to the green and yellow regions of the spectrum. No red or far-red light was detected in the upwelling spectrum. It is not likely that the upwelling irradiance is a significant component of the light field at either site. The estimates of R:FR would not be affected by not including the upwelling irradiance in the calculations.

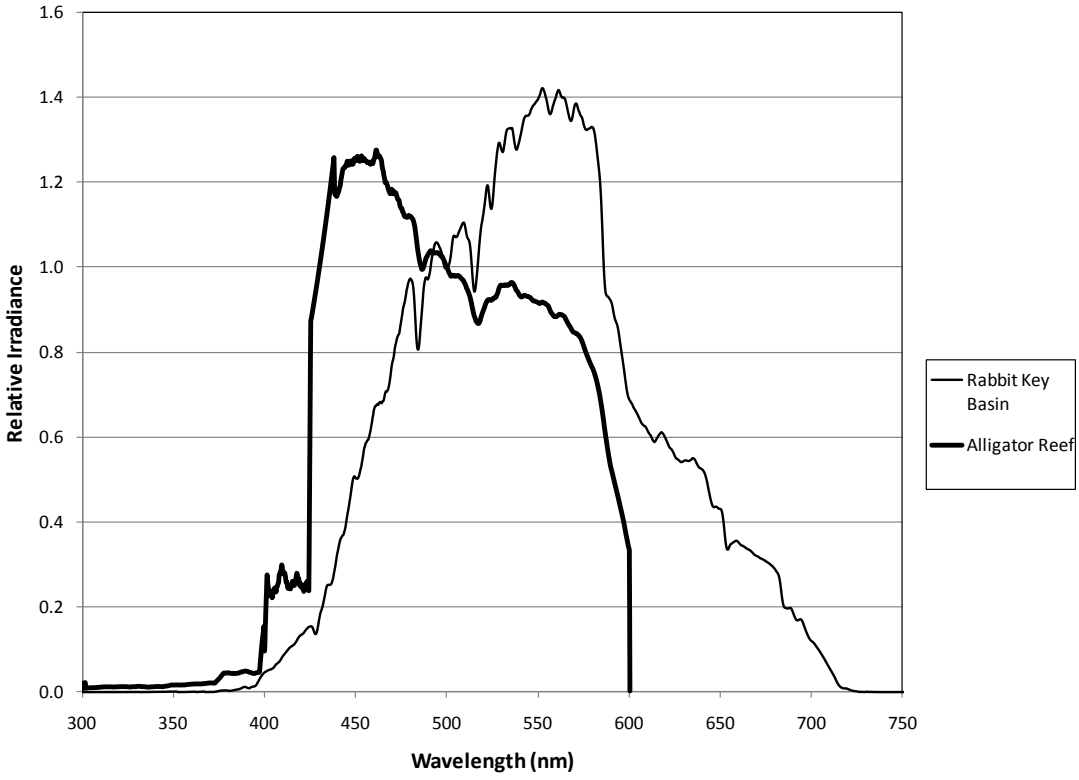

Figure 3.10. Relative upwelling irradiance at Rabbit Key Basin and Alligator Reef at noon. Spectra were normalized to 1.0 at 500 nm.

3.4.6 Comparison of seagrass canopy and terrestrial canopy light fields

In order to compare light quality with a seagrass canopy to terrestrial plant canopies, the irradiance scans for a seagrass canopy, mangrove canopy, terrestrial canopy, and uninterrupted sunlight, were normalized to 1.0 at 500 nm (Figure 3.11). The relative irradiance of unobstructed sunlight was relatively flat and peaked at approximately 450 nm. Light in the mangrove canopy was enriched in shorter wavelengths from 300 nm to 475 nm and highly enriched in far-red light. For the terrestrial grass canopy, peak irradiance within the visible spectrum was at approximately 550 nm. Far-red light was approximately 10 times higher than other light regions. The seagrass canopy was slightly enriched in green light but showed no enrichment of far-red.

Figure 3.11 clearly shows that the spectral distribution of light within a seagrass canopy is radically divergent from a terrestrial canopy. This is also evident when comparing the R:FR for the different canopy light fields (Figure 3.12). The R:FR for the mangrove canopy and terrestrial grass canopy were considerably lower than for unobstructed sunlight, at 0.41 and 0.24, respectively. These values are consistent with the expected values indicated in the literature (Hart 1988). The R:FR for the seagrass canopy was greater than 4.0. This suggests that seagrass canopies do not experience the characteristic light field of terrestrial canopies where lowered R:FR indicates the presence of neighboring plants.

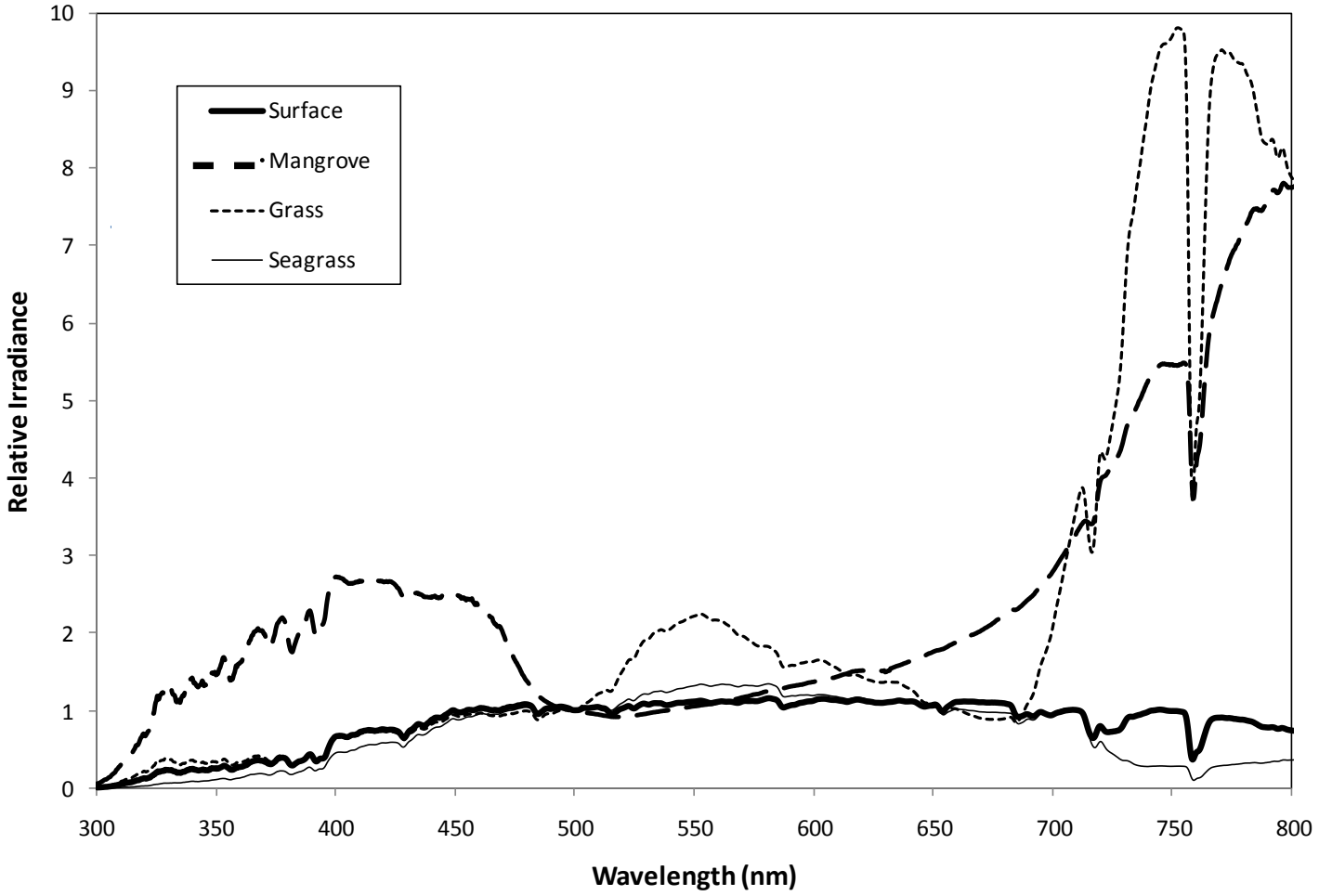

Figure 3.11. Relative irradiance spectra of surface irradiance, 30 cm within a dense *Thalassia testudinum*, 30 cm within a terrestrial grass canopy, and underneath a dense mangrove canopy. Spectra were measured at solar noon and are normalized to 1.0 at 500 nm.

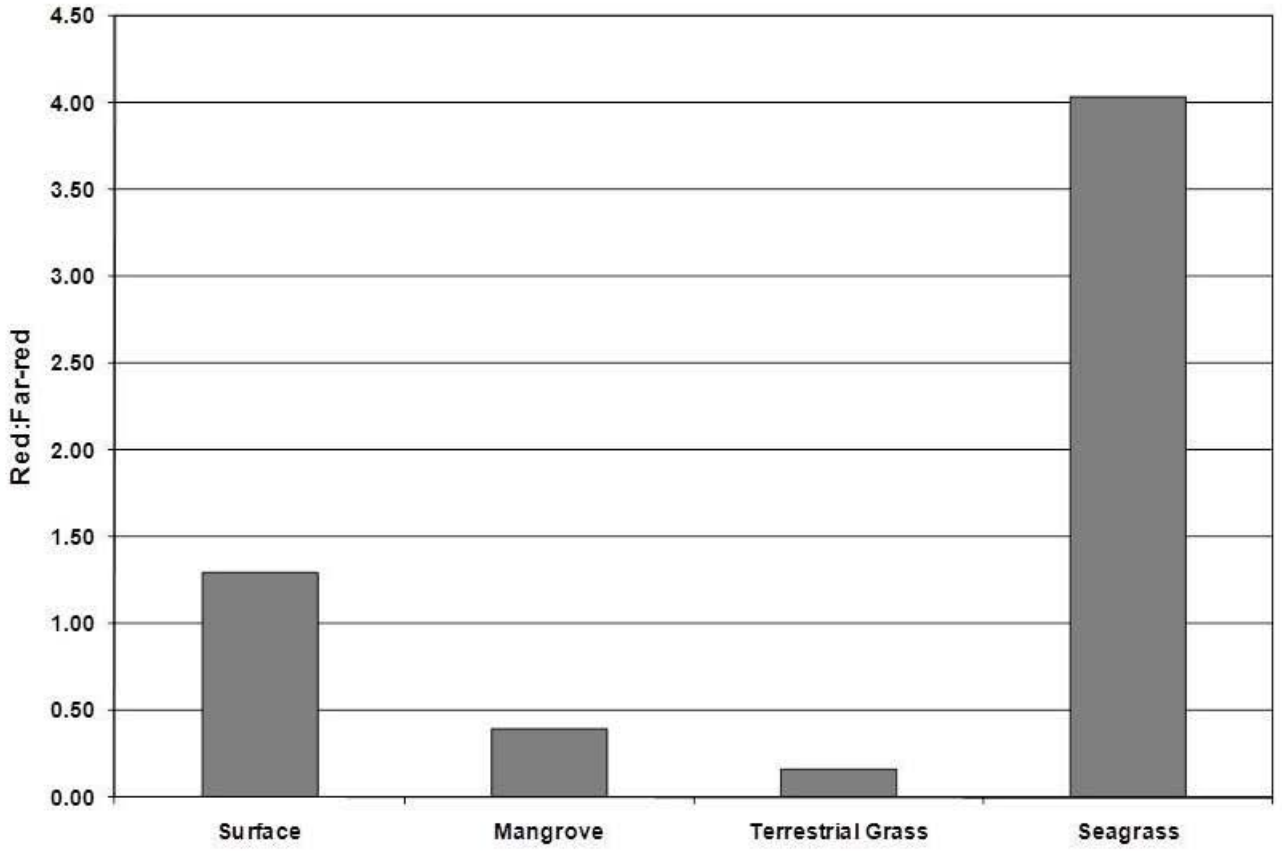

Figure 3.12. Red:far-red of the down-welling solar irradiance at solar noon for unobstructed surface light, within a mangrove canopy, within a terrestrial grass canopy, and within a submerged *Thalassia testudinum* canopy.

3.5 **Discussion**

3.5.1 PAR versus PUR

This study showed that both the quantity and quality of light declines significantly with depth through a water column and through a seagrass canopy. Although this study was relatively conventional, there were a number of important findings. These results illustrate the importance of considering the spectral distribution of the light available to seagrass leaves rather than only the integrated PAR. PAR has long been the primary measure of light availability to plants and it may be suitable for general characterizations of total visible light. PAR considers that all photons from 400-700 nm are the same, despite the fact that plants do not absorb or use all photons equally. This may be satisfactory for comparing light availability between plants that experience similar light fields. Because photons are differentially absorbed by the inherent and apparent optical properties of a water column (Kirk 1994), the relative spectral distribution of the down-welling light field changes with increasing depth.

Water column constituents, including suspended particulates, dissolved organics, and algae, preferentially absorb photons that correspond to the peaks of the chlorophyll absorbance spectrum. The PAR spectrum within a water column can be dominated by green photons and depleted in the red and blue photons. Therefore, PAR may not be a suitable measure for estimating light availability to seagrasses and other submerged photosynthetic organisms. This is particularly the case for seagrasses, whose leaves do not appear to contain accessory pigments that allow for absorption of light outside of the primary chlorophyll absorption spectrum (Cummings and Zimmerman 2003).

As Morel (1978) and Gallegos et al. (2009) have suggested, photosynthetically usable radiation (PUR) is a more accurate estimation of light availability that takes into account a plant's ability to absorb available light. Although beyond the scope of this study, PUR could be further refined by determining the action spectrum of quantum yield for an organism. Morel (1978) referred to this as Photosynthetically Stored Radiation (PSR). The results of this study suggested that using PAR might overestimate light availability to the seagrass leaves by as much as 160%. This suggests that an important component of seagrass productivity models should be the absorbance spectrum of the seagrass leaves.

3.5.2 Seagrass canopy light field

This study showed that light incident along an individual seagrass leaves can vary significantly. This may have implication with how a seagrass canopy acclimates to light availability. In order to acclimate to variation in light availability through a canopy, a plant will selectively allocate resources to leaves to optimize light harvesting and utilization. For example, a terrestrial tree canopy will supply the shaded lower canopy leaves with higher chlorophyll concentrations, while the upper canopy leaves will receive supplementary photosynthetic enzymes to support higher maximum photosynthetic rates. In order to achieve the same optimization to the gradient in light availability through its canopy, a seagrass must make these adjustments along an individual leaf. This phenomenon will be investigated in Part III of this dissertation.

This study showed that the peak irradiance experienced by seagrass leaf tips can be well in excess of the light saturation point and may induce chronic photoinhibition and a decline in quantum efficiency (Enríquez et al. 2002; Major and Dunton 2002). At the

same time, the lower section of a leaf experiences shaded conditions within the canopy. Considering the growth form of *T. testudinum*, the top section of an individual leaf will have experienced a drastically different light regime during its development. When the leaf first emerges, it is exists in a highly shaded, light-limited environment. As the leaf grows, it experiences increasingly higher light as it reaches near the top of the canopy. Once full grown the top of the leaf may experience irradiance an order of magnitude higher than it did when it emerged. This light history may have a significant effect on the how a seagrass leaf allocates photosynthetic resources. This is further complicated by the effects of shading from epiphytes, which accumulate on the leaves over time.

3.5.3 Red:far-red in a seagrass canopy

This study showed that the spectral quality of the light field within a seagrass canopy is drastically different from the light field within a terrestrial canopy (Figure 3.11). The light field within a seagrass canopy exhibits a far different R:FR than within a terrestrial canopy. As light passes through a terrestrial canopy, R:FR declines from 1.2-1.4 to 0.5 or lower as red light is absorbed by leaves and far-red light remains relatively unchanged. However, R:FR within a seagrass canopy is several times greater than for full sunlight because the overlying water column preferentially absorbs far-red light. The R:FR signal typical of terrestrial canopies is not found within a seagrass canopy.

Seagrasses have been shown to respond morphologically to experimentally reduced R:FR (Rose and Durako 1994; Tomasko 1992). Rose and Durako found that the marine macrophyte *Ruppia maritima* transplanted in aquaria showed significantly greater internode length and branching frequency when exposed to a R:FR of 0.55. Tomasko observed that *H. wrightii* exhibited different morphology when growing in a

monospecific stand compared to plants growing within a canopy of *T. testudinum*. Tomasko found similar results in aquaria where reduced R:FR was accomplished by floating *T. testudinum* leaves above transplanted *H. wrightii*.

Despite the conclusions of Rose and Durako (1994) and Tomasko (1992) that seagrasses can respond to lowered R:FR, there is no evidence for a reduced R:FR signal within an *in situ* seagrass canopy. Furthermore, this study showed that the down-welling light field in a clear water column is almost entirely absent of any far-red after a depth of only 3 m. Within a more turbid water column and within a seagrass canopy, far-red light can attenuate completely within 1 m. This does not dispute the results of Rose and Durako (1994) or Tamasko (1992). Seagrasses may indeed have the ability to detect and react to decreased R:FR. However, these studies do not address the absence of the lower R:FR *in situ*.

The phytochrome system is capable of reacting to very low levels of red and far-red light (Vandenbussche et al. 2005). However, the threshold for inducing a morphological response from reduced R:FR is thought to be around 0.5 (Balleré 1999). It is also thought that shade-tolerant understory plants are less responsive to R:FR than species from high light environments (Schmitt and Wulff 1993). Besides the R:FR phytochrome system, plants also possess photoreceptors for detecting blue (B) and UV radiation (Balleré 1999). Although the mechanism of the UV/B photoreceptors are not as understood as phytochrome, it is unlikely that the UV/B photosystem plays the same role in seagrass systems as in a terrestrial system given that UV is also strongly attenuated in water.

Phytochrome mediated photomorphogenic responses to lowered R:FR give plants the ability to balance canopy development and reach out of shaded conditions without overdeveloping canopy structures that become self-defeating by blocking out light (Vandenbussche et al. 2005). This study suggests that seagrass canopies lack a recognizable R:FR signal that indicates the extent of canopy self-shading. This may be a critical disadvantage for dense seagrass meadows and may play a role in the overdevelopment of seagrass meadows that have led to primary die-off events. Without other controlling factors such as competition or disturbance, the *T. testudinum* meadows in Florida Bay may be unable to regulate stand density. Although living in an aquatic environment has some advantages for higher plants, the loss of the R:FR signal may be a key disadvantage and may explain why seagrasses are the only land plant to have migrated into the marine environment.

3.5.4 Effect on community production

The results of this study showed that the tips and upper portions of individual *T. testudinum* leaves experience super-saturating light while the lower parts of leaves receive light well below saturation levels. It is possible that the tips of leaves are photoinhibited while the lower part of the leaves compensate for this photoinhibition so that the integrated production of the community is unaffected (Binzer et al. 2006). Light use efficiency in a seagrass canopy depends first on the canopy structure and the distribution of light through the canopy (Aber and Melillo 1991). Secondly, it depends on the photoacclimation of the leaves to the ambient light conditions (Kull 2002.).

Terrestrial canopies photoacclimate by controlling the relative distribution of resources to leaves depending on the leaves light environment (Aber and Melillo 1991).

Leaves higher in the canopy receive resources to maximize light saturated photosynthetic rate, while the shaded lower canopy leaves receive resources to maximize light harvesting. However, the growth dynamic of *T. testudinum* leaves prevents the plant from optimizing individual leaves. To effectively photoacclimate leaves, *T. testudinum* must adjust attributes along the lengths of individual leaves.

3.5.5 Conclusions

Despite the prevalence of statements in the primary literature asserting that light availability is the most important factor influencing seagrass distribution and growth, the relationship is not straightforward and requires additional explanation. A major reason for the lack of correlation between productivity and light availability in seagrasses is that the irradiance at the top of the canopy is a poor indicator of actual light utilized by the plant (Enríquez and Pantos-Reyes 2005). The total photosynthesis of a *T. testudinum* meadow is a function of the irradiance distributed through the canopy and the ability of the leaves to absorb and utilize this available light. To understand the response of *T. testudinum* to light availability, it is also essential to understand the vertical distribution of photosynthetic capacity along seagrass leaves.

Chapter 4. Light fluctuations in a seagrass canopy (Manuscript).

4.1 **Abstract**

The Ocean Optics USB2000 “Mini-spec” was utilized to examine the high-resolution light fluctuations experienced within a shallow seagrass canopy. Two coinciding phenomena are responsible for the observed light fluctuations. Within the seagrass canopy, the light environment is characterized by intermittent sunflecks caused by the oscillation of leaves by the waves and currents. The magnitude of light can fluctuate more than five standard deviations from the mean in a fraction of a second due to sunflecks. Shallow seagrass canopies also experience a unique fluctuation caused by the focusing of direct beams of light by surface waves. These narrow focused bands of light can exceed double the surface irradiance and may lead to photoinhibition or photodamage in the seagrass leaves.

4.2 **Introduction**

The magnitude of light availability to a plant varies on a number of scales due to seasonal fluctuation in the declination of the sun, diurnal periodicity of solar angle, or shorter-term variations due to changes in cloud cover. Within a plant canopy, irradiance can be even more dynamic on the scale of minutes to seconds. As light passes through a canopy, it is absorbed by, transmitted through, or reflected from leaves, stems, and other plant structures altering both the quantity and spectral quality of the light field (Pearcy 1988). However, some light beams penetrate through small gaps in the canopy and pass all the way to the forest floor completely unaltered (Chazdon 1988). The light field within a canopy is characterized by irregular diffuse light interrupted by brief instances of bright light (Holmes 1981).

Variations in canopy architecture results in an extremely heterogeneous light environment within a plant understory (Pearcy et al. 1994). Hiking through a dense forest canopy illustrates this. Looking up will reveal small glimpses of the sky through openings in the canopy. While looking down you will observe bright irregular patterns of light that have penetrated these openings and reached the forest floor unaltered (Figure 4.1). These bursts of light that reach the lower canopy unaltered are commonly called light flecks, sunflecks, or sun patches (Miller and Norman 1971; Chazdon 1988; Kubiske and Pregitzer 1997).

4.2.1 Sunflecks

Sunflecks vary in size, shape, duration, frequency, and intensity depending on canopy structure, changing solar angle, wind speed, as well as other factors (Watling and Press 2000; Chazdon and Pearcy 1991). Sunflecks attributable to large gaps in a canopy might last minutes or hours and recur consistently at the same time and location each day as the sun lines up with a gap in the canopy (Pearcy et al. 1994). Other sunflecks might last only a brief second as wind moves some branches briefly rearranging some leaves. Sunflecks in forest understories are often clustered occurring in rapid secession followed by periods of no sunfleck activity (Pearcy et al. 1994). Because the shaded light in some forest understory can be extremely low, sunflecks can contribute the majority of daily irradiance that reaches the canopy floor (Watling and Press 2000). In tropical rainforest canopies, as much as 90% of the total daily irradiance reaching the forest floor can be attributed to sunflecks (Leakey et al. 2005; Chazdon and Pearcy 1991).

Figure 4.1. Sunflecks in the understory of a tropical rainforest, El Yunque National Forest, Puerto Rico.

The question of the ecological consequences of sunflecks in forest canopies was mostly ignored until the 1930's. Sunflecks were commonly considered background noise in the irradiance measurement that could easily be accounted for by averaging over time (Atkins and Poole 1926; Carter 1934; Walton 1936). Evans (1939) was the first researcher to measure and characterize sunflecks and to question their impact on plant productivity. It has since been established that the heterogeneous light environment created by intercanopy sunflecks significantly influences the photosynthetic responses of the understory leaves and plants (Leakey et al. 2005; Pearcy et al. 1994). Traditional

analysis of plant productivity considered that plant growth rate was proportional to the total amount of light absorbed by a plant. The timing of the light received by a plant, not just the amount of light, is now considered important (Morgan and Smith 1978b; Chazdon 1988; Leaky et al. 2005). However, because of the wide variability of sunflecks, a universal standard for describing their activity has not been developed.

Differences in a species ability to use sunflecks significantly influences competition for light and other resources (Woods and Turner 1971; Elias 1983; Knapp 1992), chiefly CO_2 and water, and often controls the structure of understory communities (Miller and Norman 1971). Sunflecks provide understory plants with the opportunity to increase their daily carbon gain beyond what they would be able to achieve in steady low light (Chazdon and Pearcy 1991). Sunflecks are also of higher light quality than the diffuse light field because they have not been modified by transmission through or reflection from leaves (Holmes 1981). Many understory species have evolved specific morphological and physiological adaptations for exploiting this highly heterogeneous resource (Chazdon and Pearcy 1991).

4.2.2 Sunflecks in aquatic environments

The sunfleck phenomenon has also been researched in aquatic environments most notably in kelp forests (Wing and Patterson 1993). Light distribution in aquatic canopies is strongly influenced by properties of the sea surface and water column (Gerard 1984). Light penetration through an aquatic canopy has the added component of attenuation by water and the components of the water column such as suspended particulate matter and water column chlorophyll (Holmes 1981). This would suggest that the magnitude of sunflecks within an aquatic canopy would be lower than in a terrestrial canopy.

However, aquatic environments with a roughened water surface have an additional factor to consider.

Shallow aquatic ecosystems experience a unique irradiance flux due to the convergence of light rays refracted by surface waves (Stramska and Dickey 1998). As direct beams of light pass through the air-water interface, they are refracted. Light passing through a shifting air-water interface is refracted in many different directions. The consistent wave pattern of a wind-roughened surface causes some light beams refracted on either sides of a wave to be directed toward a focus point similar to how a lens focus light. In shallow water, bands of focused light can be observed moving across the sediment in sync with the wave oscillation (Figure 4.2). These temporal and spatial fluctuations in the light field are usually treated as noise and filtered out (Snyder and Dera 1970). The magnitude of wave-focusing peaks at a depth of approximately 1 m then decreases gradually but the phenomenon has been detected as deep as 150 m (Dera and Gordon 1968). Light fluctuations due to wave-focusing are several orders of magnitude greater under clear sky conditions then when the sun is covered by clouds (Snyder and Dera 1970). The spectral distribution of light is also altered by wave-focusing because of differential refraction of light as a function of wavelength (Gordon et al. 1971; Stramska and Dickey 1998).

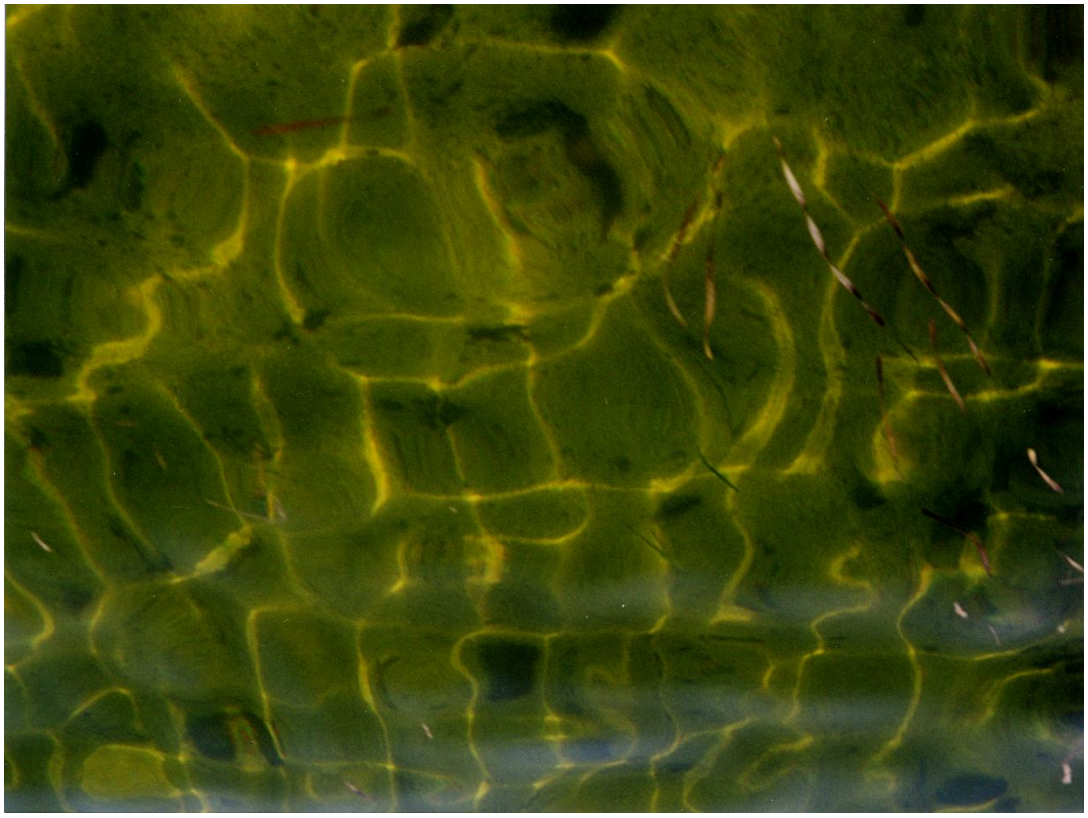

Figure 4.2. Patterns of wave-focused light in shallow water. Water depth = ~1.5 m.

4.2.3 Light variations to seagrasses

Accurate knowledge of the distribution of light through a seagrass canopy is essential to predict landscape-level productivity (Gallegos et al. 2009; Zimmerman 2003). However, little attention has been given to the effect of the timing of light on seagrass productivity. Many seagrasses grow in very dense meadows where self-shading significantly reduces the amount of light that reaches to the bottom of the canopy (Enríquez and Pantoja-Reyes 2005; Carruthers and Walker 1997). Some comparisons can be made between seagrasses and terrestrial grass species, as both grow in dense large monospecific stands with dense canopy structures (Aber and Melillo 1991). The obvious difference is the water column overlying the seagrass and its effect on radiative transfer.

4.2.4 Photosynthesis in fluctuating light

Researchers have attempted to understand how the heterogeneous light environment created by sunflecks may affect the growth of understory plants (Morgan and Smith 1978b; Pearcy 1988; Chardon and Pearcy 1986; Pearcy et al. 1994; Zipperlen and Press 1997; Küppers et al. 1999; Brantley and Young 2009). To utilize sunflecks, plants must be able to intercept them, absorb the light, and effectively utilize that light for photosynthesis (Watling and Press 2000). Many understory plants have leaves with specialized morphology and photosynthetic physiology that serve to efficiently intercept, absorb, and utilize sunflecks (Zipperlen and Press 1996; Watling and Press 2000). These plants must be able to acclimate quickly to drastically divergent light intensities within seconds, or less.

Most findings agree that photosynthetic response to changes in light availability is not linear (Zipperlen and Press 1997; Vierling and Wessman 2000). Many scientific studies suggest that variability of light on the scale of 0.1 Hz to 10 Hz influences primary production and the photosynthetic physiology of plants (Chardon and Pearcy 1986; Green and Gerard 1990). However, results have ranged broadly from no significant effect to increases of greater than 100% in photosynthetic efficiency when intermittent light was substituted for continuous light (Brantley and Young 2009; Sager and Giger 1980). Some evidence suggests that wave-induced light flashes may contribute to significant gains in primary productivity and light utilization efficiency of macroalgae and phytoplankton (Gerard 1984, Greene and Gerard 1990; Gallegos et al. 1980).

4.2.5 Chapter objectives

Many studies have addressed the effects of sunflecks on photosynthesis in terrestrial plants (Pfitsch and Pearcy 1992; Pearcy 1988; Gross 1982). Additionally, the effects of the wave induced light burst phenomena has been well studied in phytoplankton (Walsh and Legendre 1983; Gallegos et al. 1980), macroalgae communities (Green and Gerard 1990; Wing and Patterson 1993), and kelp forests (Gerard 1984; Wing et al. 1993). However, there is little research to characterize the rapidly fluctuating light within a shallow seagrass canopy (Enríquez and Pantoja-Reyes 2005).

One reason for this is that there are few suitable instruments and methodologies to measure accurately the seagrass intercanopy light field *in situ*. Common light sensors are too large to be deployed within a dense seagrass canopy without altering the canopy structure. Additionally, their slow response times are insufficient for measuring irradiance fluctuations on the time scale of seconds or less. The typical method for measuring aquatic light is to suspend the light sensor from above the water surface. This predictably results in a sampling error as the sensor oscillates with surface waves. Although this study will not directly evaluate the effects of the heterogeneous light field on seagrass photosynthesis, some inferences may be made based on similar research and intuitive examination of the fine-scale temporal dynamics and other characteristics of the intercanopy light field.

The primary objectives of this chapter are as follows: 1) modify the USB 2000 "Mini-spec" to measure high-resolution irradiance fluctuations and to develop methods to eliminate sources of error that will interfere with accurately measuring the rapid light

fluctuations within the seagrass canopy; 2) accurately measure and characterize the temporal heterogeneity of the light environment within a dense seagrass canopy; 3) examine the effects of wave-focusing and intercanopy sunflecks on the relative spectral distribution of the intercanopy light field; and 4) examine whether the short-term variations in irradiance intensity in a seagrass canopy may lead to errors in estimating light availability to seagrasses.

4.3 **Methods**

4.3.1 Instrument setup

The fine-scale temporal changes in down-welling irradiance through a seagrass canopy were measured utilizing an Ocean Optics USB 2000 "Mini-spec". The Mini-spec was calibrated using the LS-1-Cal tungsten halogen light source and equipped with a 200 nm fiber optic cable and a CS-3 cosine corrected sensor. By setting the Mini-spec OOIIRad software to the lowest integration period, 4 ms, and selecting the option to save scans continuously, a sampling resolution of approximately 6 Hz was accomplished. A light sampling assembly was constructed that allowed the cosine corrector to be positioned at desired locations through the water column and canopy (Figure 4.3). Because the sampling assembly is inserted into the sediment and independent of the boat, the effects of boat rocking or swaying of the sensor are eliminated. This ensures that measurements accurately reflect the variable nature of the down-welling irradiance. The Mini-spec and light sampling assembly were always deployed on the same side of the boat as the sun to eliminate shading from the boat.

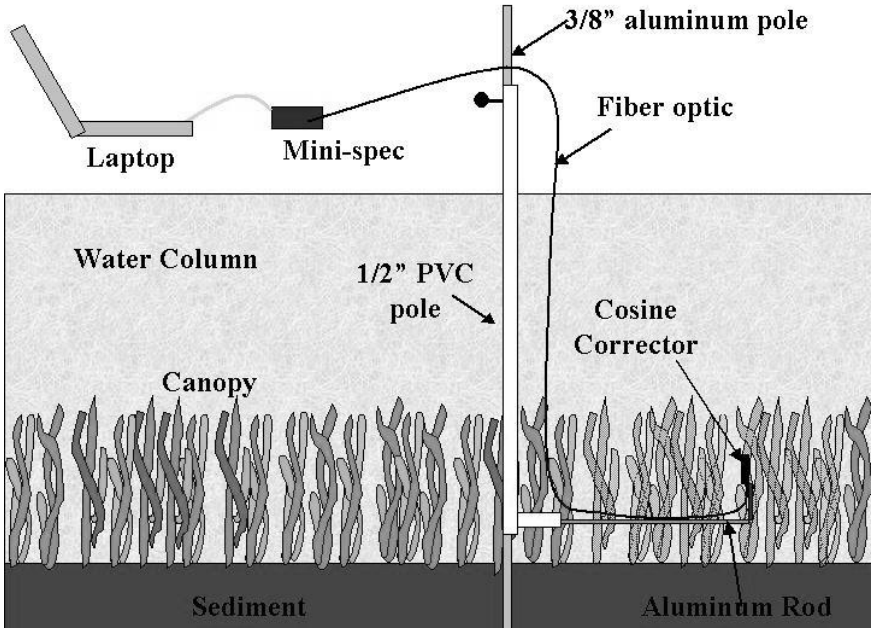

Figure 4.3. Seagrass canopy light sampling assembly for the Ocean Optics USB2000.

4.3.2 Time series scans

A time series scan of down-welling irradiance was completed by positioning the cosine-corrected sensor toward the vertical and at the desired location then initiating a scan via the software. The Mini-spec records continuous irradiance scans until the software is interrupted. Each individual irradiance scan is saved into a separate text file that includes irradiance at individual wavelengths from 300 nm to 800 nm. A typical time series is 20 seconds, which results in approximately 120 individual output files. These files were combined into a single data array using a routine developed in MatLab. A time series could then be plotted and analyzed for total PAR irradiance, individual wavelengths, or ranges of wavelength.

4.3.3 Field sampling

The study was conducted in a dense monspecific stand of *T. testudinum* at Rabbit Key Basin in central Florida Bay. Time series irradiance scans were conducted at the top, middle, and bottom of the seagrass canopy at ten locations across a transect. The top of canopy measurements were recorded with the cosine corrector at 40 cm from the sediment and approximately level with the top of the canopy where the leaves would not obstruct the light to the sensor. The middle canopy measurements were taken with the cosine corrector positioned 20 cm from the sediment with approximately 20 cm of seagrass canopy above it. The bottom of canopy measurements were completed with the cosine corrector positioned at 5 cm above the sediment with 35 cm of canopy above it. Each canopy profile was preceded and followed by a scan of the irradiance above the water surface. The study was completed between 1100 h and 1300h on the side of the boat facing the sun and while the sun was unobstructed by clouds. If the sun became obstructed by clouds during the profile, the time series was repeated.

For comparison, time series scans were conducted at the top of the canopy during a period when the sun was completely obstructed by clouds, at a nearby site where the water column was highly turbid due to suspended carbonate sediments, and within the canopy but at a low solar angle (approximately 15^{o}).

4.3.4 Data analysis

The ten time series profiles were compiled into an Excel spreadsheet and descriptive statistics (mean, standard deviations, maximum, and minimum) of integrated PAR were calculated for each. Total PAR was plotted versus time to examine the absolute changes in irradiance at each layer of the seagrass canopy. Standard deviations

from the mean irradiance of the time series were also plotted to indicate the relative magnitudes of the peaks and troughs of the time series. The number of distinct peaks and troughs were visually identified for each time series and the frequency estimated as the number of peaks per seconds. To examine for changes in the spectral distribution of the irradiance over the time series, the wavelength measurements were summed for specific color regions (see Table 3.1). R:FR was also calculated and plotted versus time to investigate changes in light quality.

The density of the seagrass canopy was estimated by counting the number of short shoots within a 10 cm quadrat placed at the location of each time series profile. Leaf lengths were calculated from three randomly selected short shoots from the middle of each quadrat. Water depth was calculated by using a PVC pole labeled with graduated marks. Surface wave height and frequency were estimated by observing the water rise and fall on the PVC pole.

4.4 **Results**

The mean *T. testudinum* canopy density at the sampling site was 1303.3 short shoots m^{-2} with a standard deviation of 192.9. The mean length of adult leaves was 35.1 cm with a standard deviation of 3.7. The total effective canopy height was approximately 0.4 m owing to the long vertical rhizome sections from which the leaves extend. The water depth was 1.8 m while the estimated average wave height was 0.2 m with a frequency of approximately 2-3 Hz.

4.4.1 Time series scans

A representative time series was selected from the ten profiles to plot. The 3-dimensional plot of water column spectral irradiance versus time shows that the Mini-

spec can amply capture the rapid light fluctuations from wave focusing (Figure 4.4). The plot includes only six seconds of data because of data limitations of the SigmaPlot application used. However, even this brief time series captures an accurate assessment of the fluctuations in the magnitude of the light field. The intervals between the peaks of the light fluctuations above the canopy appear mostly uniform, consistent with the observations of the patterns of wave-focusing on bare sediment (Figure 4.2). The light bands vary in brightness and propagate in concurrence with the surface waves. The 3-dimensional plot of the spectral irradiance within the seagrass canopy illustrates the lower light within the canopy and captures the periodic flashes from sunflecks (Figure 4.5). The intervals between the peaks within the canopy appear less regular than the time series at the top of the canopy owing to the random swaying of leaves.

The descriptive statistics for the light time series are shown in Table 4.1. Surface irradiance averaged 2502.6 µmol m^{-2} s^{-1} during the course of the sampling. Surface measurements were not recorded when the sun was obstructed by the intermittent clouds. The irradiance fluctuated ranged from 2322.5 µMol m^{-2} s^{-1} at the beginning of the sampling to 2654.1 µMol m^{-2} s^{-1} at the end. Mean irradiance decreased 75% from the top to the bottom of the canopy from 1511.7 µMol m^{-2} s^{-1} to 369.5 µMol m^{-2} s^{-1}. The maximum instantaneous irradiance at the top of the canopy was 5369.3 µmol m^{-2} s^{-1}, more than twelve times greater than the light saturation point of 438 µmol m^{-2} s^{-1} for *T. testudinum* (Fourqurean and Zieman 1991) and approximately twice the intensity of the surface irradiance. The minimum irradiance at the canopy top, 583.4 µmol m^{-2} s^{-1}, also exceeded the saturation point. The maximum irradiance for the middle of the canopy was seven times greater than the saturation point at 3101.2 µMol m^{-2} s^{-1} while the minimum

irradiance was only 85.0 µMol m^{-2} s^{-1}. The maximum instantaneous irradiance at the bottom of the canopy was 74% lower than for the top of the canopy while the minimum irradiance decreased 87%. The maximum instantaneous irradiance at the bottom of the canopy exceeded the saturation point by more than three-fold while the minimum irradiances was 73.0 µMol m^{-2} s^{-1}, approximately 80% lower than the light saturation point. The Coefficients of Variation suggest only small differences between the ten time series reflecting the uniformity of the seagrass meadow. The frequencies of the light fluctuations ranged from 2.15 Hz for the top of the canopy, 1.65 Hz for the middle of the canopy, and 1.95 Hz for the bottom of the canopy.

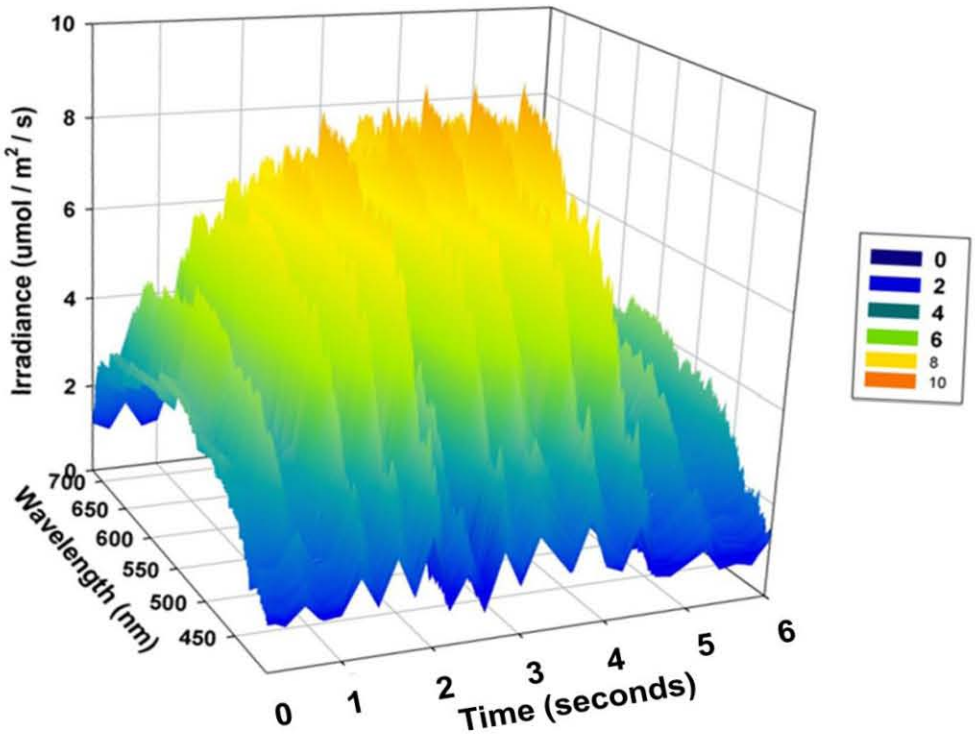

Figure 4.4. Spectral distribution of the high frequency fluctuations in down-welling irradiance at a depth of 1.1 m at Rabbit Key Basin. Resolution of time series is approximately 6 Hz.

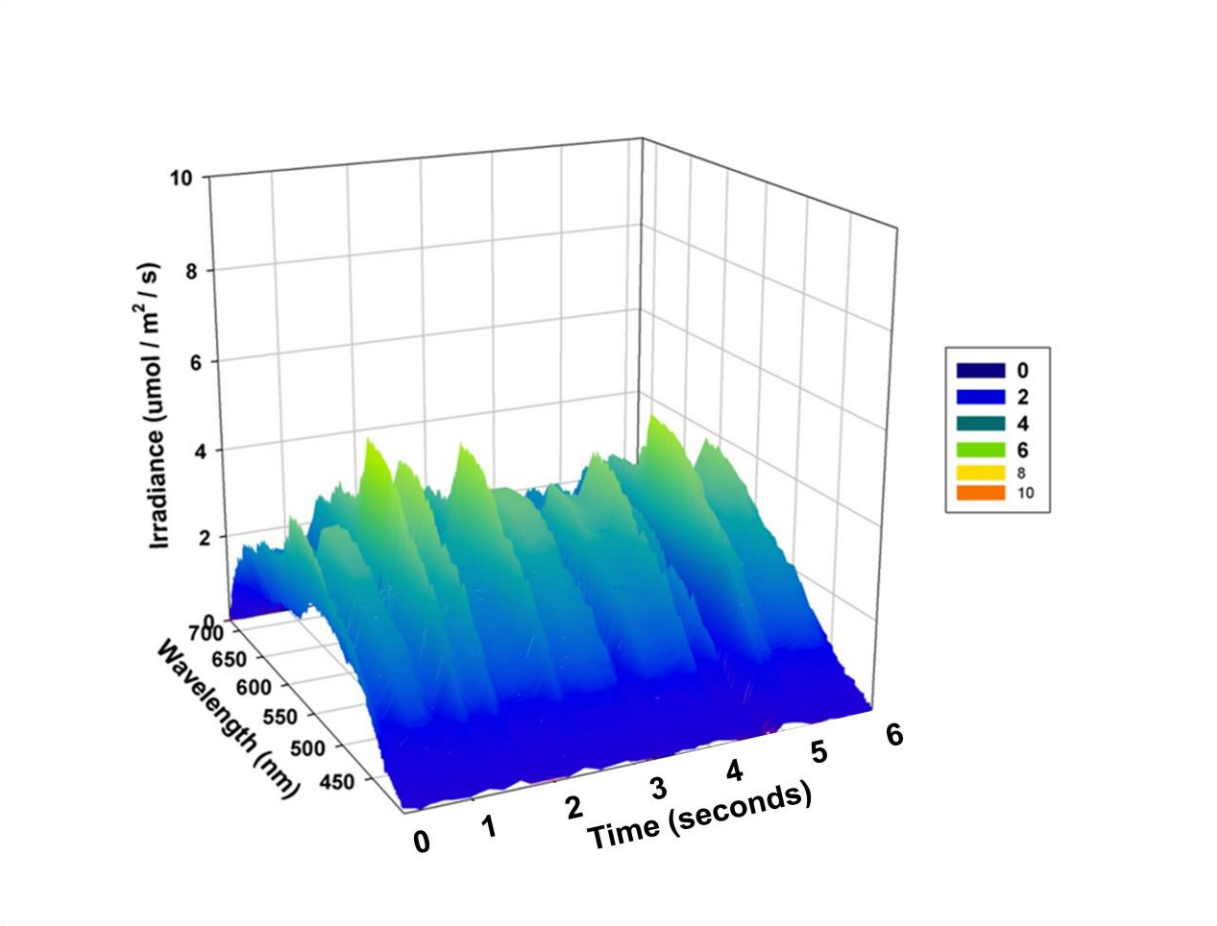

Figure 4.5. Spectral distribution of high frequency fluctuations in down-welling irradiance at a depth of 1.4 m within a canopy of *Thalassia testudinum* at Rabbit Key Basin. Resolution of time series is approximately 6 Hz.

Table 4.1. Mean irradiance values (PAR) of time series from the top, middle, and bottom of the canopy at random locations (n = 10) across a *Thalassia testudinum* meadow at Rabbit Key Basin, Florida Bay in July 2005. Units for irradiance are µMol m^{-2} s^{-1}. Total water depth was 1.8 m. Canopy locations are as follows: Top, 40 cm above sediment approximately level with the top of the canopy; Middle, 20 cm from the sediment with approximately 20 cm of seagrass canopy above it; and Bottom, 5 cm above the sediment and 35 cm of overlying canopy. Standard deviations are in parentheses.

		Canopy Location		
	Above Surface	Top	Middle	Bottom
Mean Irradiance	2502.6 (195.2)	1511.7 (136.2)	940.9 (275.8)	369.5 (303.2)
SD	136.7 (20.1)	901.6 (89.7)	571.5 (268.1)	217.2 (161.3)
CV	0.055 (0.008)	0.596 (0.110)	0.607 (0.312)	0.588 (0.291)
Max	2654.1 (85.1)	5369.3 (404.8)	3101.2 (625.9)	1409.2 (817.9)
Min	2322.5 (19.5)	583.4 (279.9)	85.0 (90.1)	73.1 (89.4)
Median	2497 (31.0)	1206.2 (148.3)	800.3 (221.6)	316.4 (116.6)
Estimated frequency	NA	2.15 (0.71)	1.65 (1.19)	1.49 (1.15)
Number of Peaks	NA	43.0 (2.7)	33.2 (6.7)	29.7 (9.9)

4.4.2 Analysis of peaks and troughs

While the mean irradiance exceeded the light saturation point at the top and middle of the canopy for all time series, the mean irradiance at the bottom of the canopy did not. A simple photosynthetic-irradiance curve would calculate a photosynthetic rate using this mean irradiance as if all irradiance was available for absorption and utilization. However, this time series exceeded the light saturation point during peaks. By summing across the time series for all irradiance that exceeded the saturation point, I found that approximately 15.6% of the total measured irradiance over this time series exceeded the saturation point. Using a simple photosynthetic-irradiance relationship, this would result in a 15.6% overestimation of gross photosynthesis.

The time series plots for total PAR and standard deviations (SD) from the mean illustrate the relative magnitude of the peaks and troughs of the light fields (Figure 4.6). The top of canopy time series showed thirty-nine distinct peaks. Eighteen of these exceeded one SD, eight of these exceeded two SD, three reached three SD, and one reached over four SD. The peaks in the time series within the canopy showed lower and less frequent peaks. For the thirty distinct peaks in the middle of the canopy time series, twelve exceeded one SD, four exceeded two SD, and two exceeded three SD. For the twenty-nine peaks in the bottom of the canopy time series, fourteen exceeded one SD, three exceed two SD, and one exceeded five SD. Analysis of the time series troughs also reveals noticeable differences. The time series troughs at the top of the canopy tended to reach but not fall below -1 SD. The troughs at the middle and bottom of the canopy

varied more and typically dropped below -1SD but no troughs exceeded approximately -1.5 SD.

To examine the sunflecks for wavelength specific focusing, I calculated the relative amount of each spectral color band for the distinct peaks and troughs identified in Figure 4.6. Table 4.2 compares the relative distribution of light color bands of peaks, troughs, and the average for the time series at the top, middle, and bottom of the canopy. The values for peaks and troughs were compared against the time series means using t-tests. Above the canopy, the time series peaks showed enrichment (i.e. significant differences from the mean) in the longer wavelengths, yellow, orange, and red. The troughs showed enrichment in the violet and blue regions. The color regions in the peaks or troughs only showed significantly higher amounts of light, no color region was found to be significantly lower. The within canopy time series showed similar results.

4.4.3 Fluctuations in Red:far-red

The times series showed only slight fluctuation in R:FR (Figure 4.7). At the top of the canopy, R:FR fluctuated from 4.1 to 4.4. R:FR at the middle of the canopy was higher owing to the selective attenuation of far-red relative to red light through water. However, the R:FR fluctuated approximately the same amount from 5.0 to 5.3. R:FR was lowest at the bottom of the canopy because reflection of red light from leaves was now offsetting the loss of far-red light. The R:FR showed a wider range at the bottom of the canopy fluctuating from 3.1 to 3.9 probably due to the introduction of intense sunflecks. In fact, the highest R:FR for this time series corresponds with the intense five SD sunfleck seen at the 6-sec point of the time series in Figure 4.6. While the lowest

R:FR at approximately 4-sec, 12 sec, and 17-sec correspond with the lowest points in the irradiance time series. The R:FR for all of the time series is well above the range known to instigate acclamatory responses to in plants (Balleré 1999).

4.4.4 Time series under clouds and in turbid water

The irradiance time series at the top of the canopy under cloudy conditions (Figure 4.8A) was noticeably different then under full sunlight. This time series was accomplished at the same location, under the same wave conditions, and only minutes later than the earlier time series. The purpose of this measurement was to investigate the nature of the wave-focusing of light, when the light source is diffuse. In this case, the irradiance was diffused by the cloud cover before entering the water column. Although irradiance showed rapid fluctuations, the magnitude of most peaks did not exceed approximately 1.8 SD showing no substantial spikes. The troughs were typically one standard deviation below the mean consistent with the clear sky time series. The time series conducted at highly turbid water column showed very rapid fluctuations but no large spikes (Figure 4.8B). While all the other time series showed higher peaks than troughs, the maximum fluctuations of the time series for the turbid water column were often below -1 SD. These results suggest that the intensity of the wave-focusing is decreased when the light source is diffused.

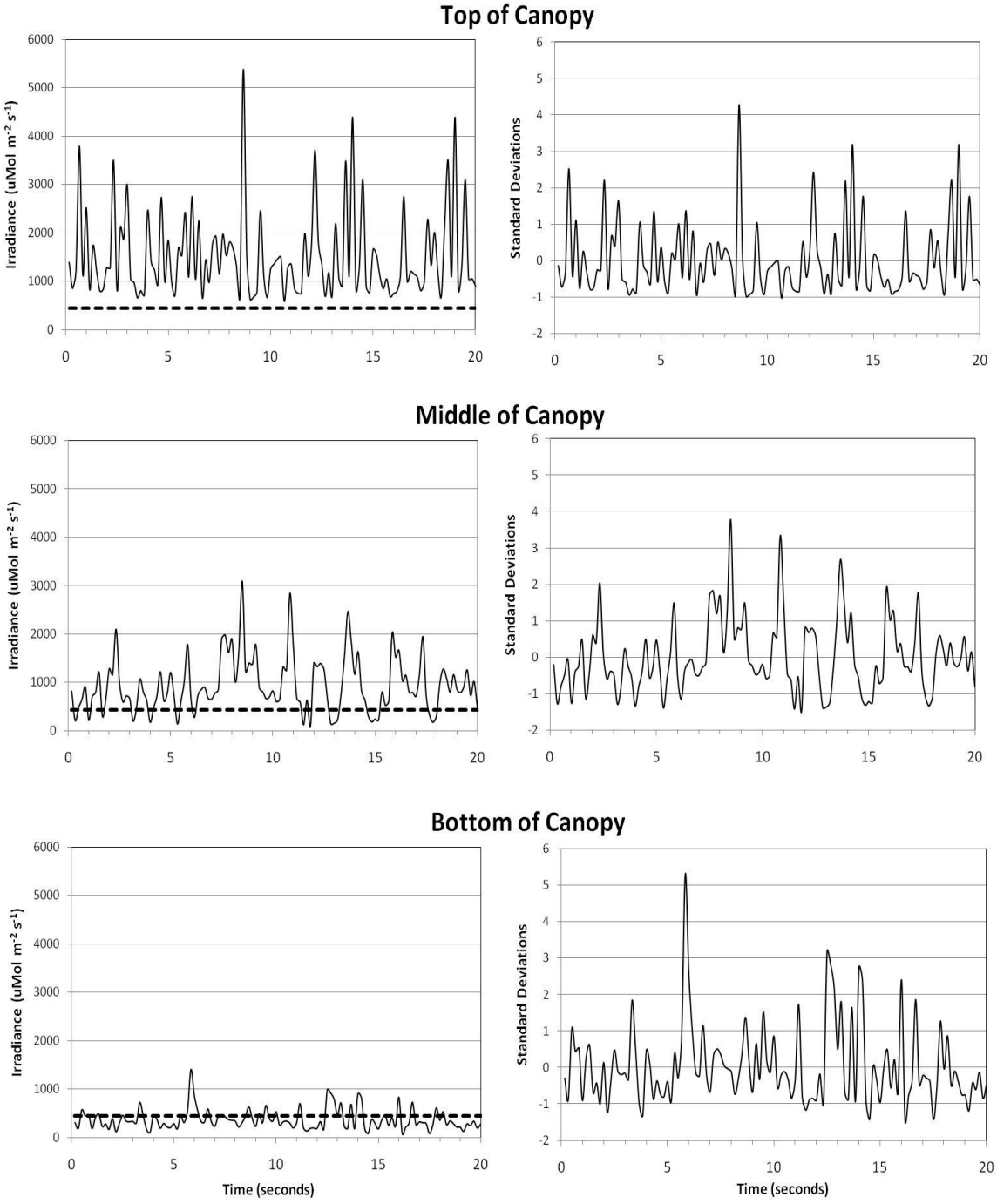

Figure 4.6. Irradiance time series measurements at the top, middle, and bottom of a *Thalassia testudinum* canopy. Figures in the left column indicate total instantaneous photosynthetically active radiation (PAR). The horizontal dashed line indicates the approximate light saturation point for *Thalassia testudinum* (Fourqurean and Zieman 1991). The figures in the right column indicate the standard deviations from the mean of the time series. The y-axes were kept consistent to assist in comparing between the canopy layers.

Table 4.2. Comparison of the peaks and troughs of inter-canopy irradiance time series within a *T. testudinum* canopy at Rabbit Key Basin, Florida Bay. Values are the percent of total photosynthetically active radiation that falls within the color region (standard deviation). Values in bold are significantly higher ($P < 0.05$).

	Top of Canopy			**Middle of Canopy**			**Bottom of Canopy**		
Color Region	Troughs	Peaks	Mean	Troughs	Peaks	Mean	Troughs	Peaks	Mean
Violet	**8.7 (0.23)**	7.3 (0.34)	8.0 (0.67)	**8.7 (0.10)**	7.4 (0.21)	7.8 (0.60)	**7.8 (0.38)**	6.4 (0.51)	7.3 (0.65)
Blue	**12.2 (0.23)**	11.2 (0.42)	11.6 (0.52)	**12.1 (0.09)**	11.2 (0.22)	11.5 (0.51)	**11.5 (0.27)**	10.6 (0.50)	11.2 (0.54)
Blue-green	13.1 (0.14)	12.7 (0.25)	12.9 (0.16)	13.0 (0.07)	12.8 (0.13)	12.9 (0.26)	12.9 (0.12)	12.6 (0.30)	12.8 (0.28)
Green	13.7 (0.04)	13.3 (0.80)	13.6 (0.05)	13.6 (0.04)	13.7 (0.05)	13.6 (0.30)	14.1 (0.20)	13.8 (0.13)	14.0 (0.20)
Yellow-green	14.5 (0.06)	14.6 (0.51)	14.6 (0.17)	14.4 (0.03)	**14.8 (0.06)**	14.6 (0.21)	15.2 (0.33)	15.3 (0.14)	15.2 (0.30)
Yellow	13.4 (0.12)	**14.2 (0.33)**	13.7 (0.34)	13.4 (0.06)	**14.0 (0.13)**	13.8 (0.36)	13.8 (0.18)	**14.5 (0.31)**	14.1 (0.34)
Orange	12.7 (0.20)	**13.8 (0.42)**	13.2 (0.41)	12.8 (0.08)	**13.5 (0.19)**	13.3 (0.49)	12.9 (0.22)	**14.0 (0.45)**	13.3 (0.47)
Red	11.9 (0.25)	**12.9 (0.50)**	12.3 (0.39)	12.0 (0.11)	**12.7 (0.22)**	12.4 (0.53)	11.8 (0.30)	**12.9 (0.53)**	12.2 (0.54)

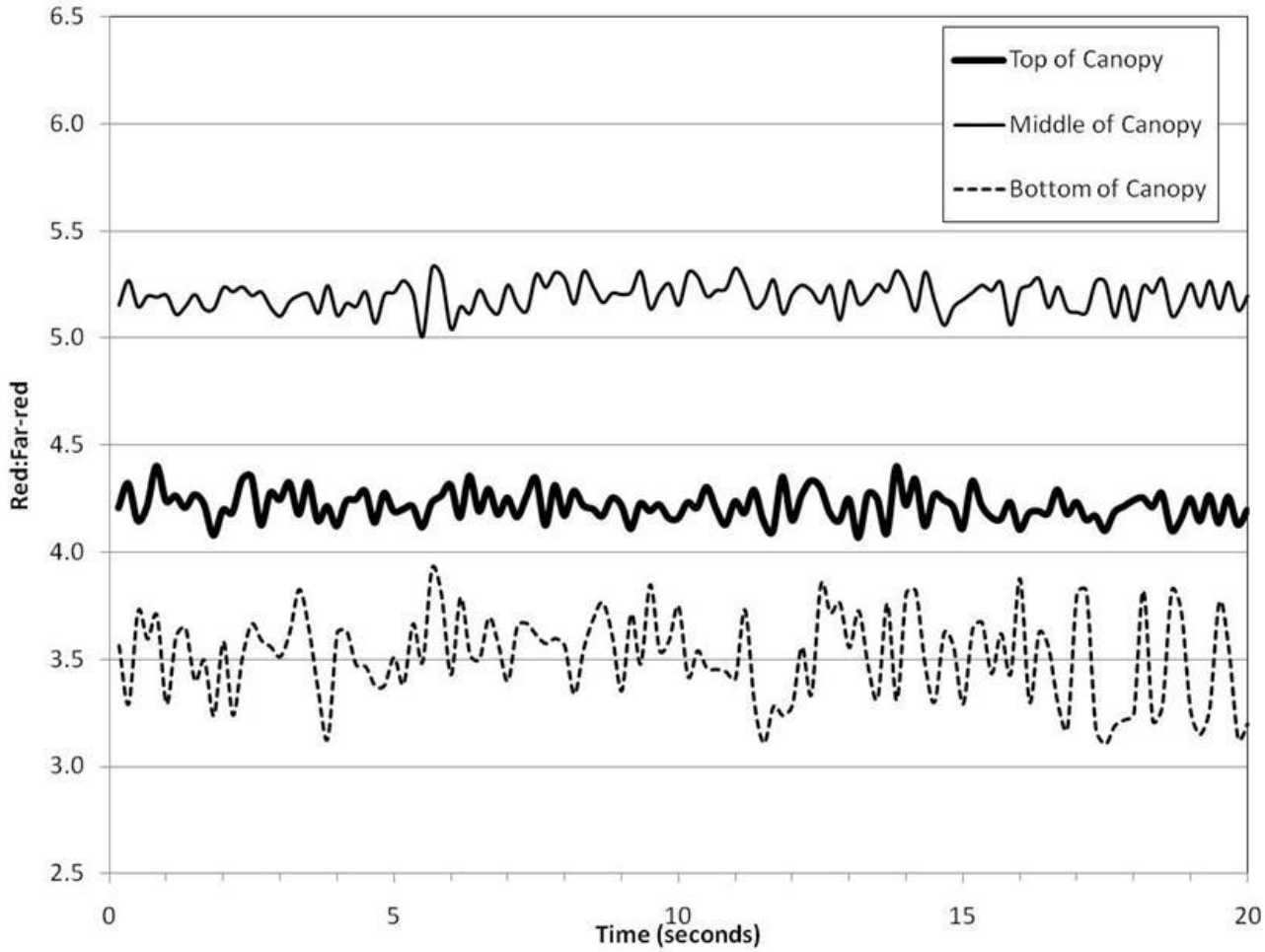

Figure 4.7. Time series of Red:Far-red within a *Thalassia testudinum* canopy at Rabbit Key Basin, Florida Bay, Florida. The vertical distance from the sediment of the light measurements are as follows: Bottom of canopy = 5 cm; Middle of Canopy = 20 cm; Top of Canopy = 40 cm, approximately level with the longest leaves.

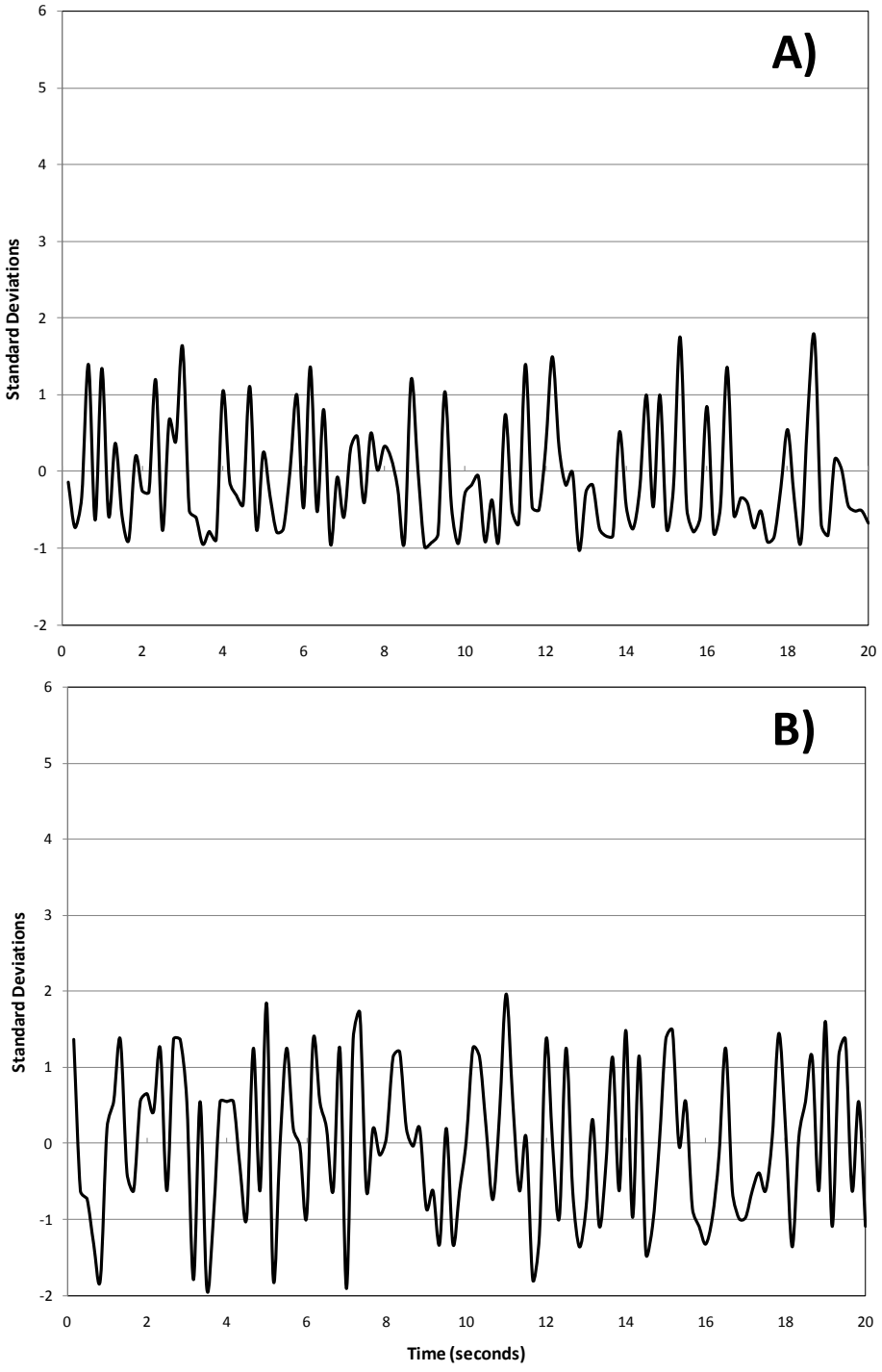

Figure 4.8. High-frequency time series of irradiance at the top of a *Thalassia testudinum* canopy with the sun obstructed by clouds (A) and within a highly turbid water column (B). Light probe was positioned at a depth of 1.2 m.

4.5 **Discussion**

4.5.1 Utilization of the Mini-spec

This study demonstrated that with only slight modifications the Mini-spec is capable of accurately assessing the high frequency light fluctuations within the aquatic environment. The results showed that the light field within a dense *T. testudinum* canopy is highly variable with irradiance varying nearly three orders of magnitude within a fraction of a second. With the wide wavelength range offered by the Mini-spec, this is the first time that the spectral characteristics of sunflecks have been examined within a seagrass canopy.

The study also showed that the light field at the top of the seagrass canopy is as variable as the intercanopy light field owing to the focusing of light by surface waves. The high intensity narrow bands of wave-focused light can exceed twice the magnitude of the surface irradiance raising questions about whether this light, though brief, could be damaging to the photosynthetic apparatus of the seagrass leaves. The irradiance peaks at the bottom of the canopy that reached more than twice the mean of the time series and two or more standard deviations greater than the mean are likely instances where intercanopy sunflecks correspond with wave-focused light. The super-saturating light is able to penetrate even to the bottom of the canopy despite the overlying water column and self-shading from the canopy.

4.5.2 Comparison to terrestrial canopy light field

Despite some similarities, the light field within a seagrass canopy is quite different from the light field in a forest understory. While leaves at the top of a terrestrial canopy experience mostly steady state irradiance, the top of a seagrass canopy

experiences a rapidly fluctuating light field due to wave-focusing. Sunflecks under a forest canopy can persist for minutes with intensities equivalent to unobstructed sunlight (Chazdon and Pearcy 1991). Maximum instantaneous irradiance in a terrestrial canopy could never exceed the intensity of unobstructed sunlight, while the brief sunflecks within a seagrass can exceed surface sunlight even within the canopy.

4.5.3 Alteration of the spectral distribution of the light field

The time series troughs within the canopy time were expected to have relatively less red and blue light than at the top of the canopy. At the top of the canopy, the troughs in the time series are caused entirely by the defocusing of light. However, within the canopy, troughs are should also be created when leaves shaded the sensor. Light transmitting through the leaves and reaching the sensor should be depleted in red and blue wavelengths that are preferentially absorbed by leaf pigments. However, there was no significant change in the spectral distribution of the light within troughs at increasing depth in the canopy. This may indicate that the light fluctuations at the bottom of the canopy are highly influenced by wave-focused light.

The significantly higher amount of long wavelengths relative to shorter wavelengths could have minor effects on photosynthesis since wavelengths are shifted away from the most efficiently absorbed wavelengths (i.e. blue). The small variations of the R:FR within the canopy is not considered significant enough to induce morphological changes via the phytochrome system (Schmitt and Wulff 1993).

4.5.4 Potential implications to photosynthesis

Consider the light environment that an undercanopy leaf experiences. The leaf is busy absorbing low intensity diffuse light when suddenly a sunfleck occurs bathing it full

sunlight. Leaf temperature suddenly climbs, water rapidly evaporates from the leaf surfaces and photosynthesis rate increases. Then just as quickly, the sunfleck disappears returning the leaf back to low diffuse light. Only to return a few seconds later to full sunlight. This makes it very difficult for a plant to acclimate and achieve maximum photosynthetic efficiency. Within a seagrass meadow, we must also consider that the top of the canopy is rapidly pulsed with supersaturating light, at least under a clear sky and water column. This supersaturating light also penetrates to the bottom of the seagrass canopy where the majority of leaf tissue is located.

The light environment experienced by a dense *T. testudinum* canopy is quite different than the irradiance represented in common seagrass productivity models that are driven by a simple photosynthetic-irradiance relationship (Madden and Kemp 1996; Fong and Harwell 1994). These models assume steady-state irradiance while in reality the light availability to seagrasses fluctuates significantly on the order of seconds and even milliseconds. This study suggested that as much as 15.6% of the irradiance at the bottom of the *T. testudinum* canopy might be unusable in photosynthesis because the light exceeds the light saturation point. However, a simple irradiance measurement that averages over time to eliminate sampling error due to sunflecks and wave-focusing would yield an estimation of irradiance that was well below the saturation point. The results of this study suggest that by not accounting for the short-term fluctuations of light photosynthetic rate could be overestimated by as much as 15.6%. However, this error may be even higher since the light saturation point used for these estimates was calculated from light-adapted leaves. The light saturation point for shade-adapted leaves is typically lower than for sun-adapted leaves (Givnish 1988). Because leaf tissue is

strongly weighted toward the bottom of a seagrass canopy, the majority of the leaf tissue experiences a sub-canopy light environment.

4.5.5 Photosynthetic utilization of sunflecks

Most research concerning plant adaptations to shaded environments focuses on responses to steady light conditions not the dynamic light environment typical of many understories (Pearcy 1988). Responses to rapidly changing light is an important aspect of how some sub-canopy plants adapt to shaded conditions because a high percentage of the available light in a dense understory exists as sunflecks (Chazdon and Pearcy 1991). In order to take advantage of sunflecks, the response time of the photosynthetic apparatus within a leaf must be adapted to the duration of the sunflecks.

Even in saturating light, maximum photosynthetic rates are not achieved immediately. The photosynthetic apparatus of a plant requires an induction period to ramp up to maximum rates (Krause and Weiss 1991). The induction period results from the need for light activation of Rubisco and other photosynthetic enzymes, the opening of stomates, and CO_2 uptake (Pearcy 1988). Sunflecks are not usually of sufficient duration to allow for full induction but continuous light is not always needed for induction to occur (Pearcy 1988). The rapid light fluctuation within a seagrass canopy may act to keep the induction state of the leaves fully charged and at optimal response rate.

Limitation of sunfleck utilization in terrestrial canopies has been widely connected to the role of stomata (Woods and Turner 1971). In terrestrial tree canopies, sunflecks have been shown to create spatial variation in stomatal opening in response to rapid (seconds) changes in light (Küppers et al. 1999). Seagrasses do not have stomata

achieving gas exchange directly through the leaf epidermis (Cummings and Zimmerman 2003). This may give seagrasses an advantage in utilizing sunflecks.

Sunflecks are generally considered a net positive for canopy-integrated photosynthesis (Pearcy et al. 1994). Total plant productivity is optimized when most irradiance is intercepted by the canopy and utilized in photosynthesis. The dense canopy of the *T. testudinum* meadow in this study experiences significant self-shading. Because the majority of the leaf tissue is located at the base of the canopy, *T. testudinum* essentially acts as a shade-adapted plant. Optimization of canopy density is essential for survival since an overly dense canopy would absorb the majority of light in the upper canopy. The lower canopy leaf tissue would absorb light well below its capacity while remaining a significant respiratory burden on the plant. Sunflecks offer the lower canopy leaf tissue and opportunity to significantly increase photosynthesis. The wave-focusing affect would also act to increase the amount of light that penetrates to the base canopy layer. The implications of this is that shallow dense *T. testudinum* meadows may be reliant on sunflecks and wave-focusing to maintain carbon balance.

Studies of canopy irradiance in terrestrial forests and kelp forests address the effects of the canopy on light availability to sub-canopy species. The dense seagrass canopy at Rabbit Key Basin did not contain sub-canopy plant or macroalgae species. The sub-canopy community consists primarily of benthic and suspended microalgae and epiphytic algae growing on the seagrass leaves. Although the sub-canopy community was not considered as part of this study, the results accurately describe the light availability to these sub-canopy species. The unique intercanopy light field of a shallow

seagrass canopy in clear water also may have physiological effects on the epiphytic community on seagrass leaves, which are often highly sensitive to light availability.

4.5.6 Photoinhibition and photodamage

Seagrasses have many distinctive attributes that allow for efficient harvesting of light (Enríquez and Pantoja-Reyes 2005; Cumming and Zimmerman 2003) suggesting that seagrasses function as shade-adapted plants. However, this study showed that a shallow *T. testudinum* in a clear water column experiences supersaturating light greatly exceeding the surface irradiance suggesting that they may also possess well-developed photoprotective mechanisms to avoid significant photodamage from the high intensity light, a common attribute sun-adapted plants (Givnish 1988).

The high magnitude fluctuations generated by sunflecks and wave-focusing may be damaging to the photosynthetic apparatus of seagrass leaves (Öquist et al. 1992; Wing and Patterson 1993). When more photons are absorbed than can be utilized in photosynthesis, light no longer is the limiting factor for photosynthesis. Above this light saturation point, photosynthesis becomes limited by the rate of carbon metabolism (Hall and Rao 1999). Several protective mechanisms are induced that prevent permanent damage to the photosynthetic apparatus but causes temporary photoinhibition (Powles 1984). This reversible decline in photosynthesis occurs nearly instantaneously but it may take several minutes to return fully to the maximum photosynthetic rate (Krause 1988).

Photoinhibition imposes an energy cost on a plant, due to a decrease in the light-use efficiency and the cost of maintaining the protein synthesis apparatus, which allows for recovery after the high light (Raven 1989). The sensitivity to high light stress and photoinhibition is typically higher in shade-adapted plants and leaves (Krause 1988).

The reversibility of photoinhibition is limited. Long-term exposure to supersaturating light can result in destruction of photosynthetic pigments and damage to the photosynthetic apparatus leading to leaf and/or plant mortality (Powles 1984).

These results present an interesting question. How do seagrasses, which are often considered to function as shade-adapted plants, survive in such a high light environment (Larkum et al. 2006). Shade-adapted characteristics would certainly seem to be advantageous to seagrasses that grow in deep water or in dense canopies with significant self-shading like the *T. testudinum* at Rabbit Key Basin. Shade-adaptation is thought to be a major evolutionary factor that allows the seagrass' ancestors to migrate into to aquatic environment. However, shade-adapted plants are also known to be more susceptible to photoinhibition and photodamage in high light environments (Öquist et al. 1992). In shallow clear water columns such as Rabbit Key Basin where irradiance at the top of the canopy can reach many times the light saturation point, shade-adapted characteristics could be a serious detriment to a plant. This study showed that because of the wave-focusing of light, a seagrass canopy can experience short bursts of sunlight that exceed twice the intensity of even the maximum sunlight at noon of a summer day and more than twelve times the light saturation point for the study species. Super saturating light was even experienced at the bottom of the dense canopy. Plants must compromise between acclimation and photoinhibition (Anderson and Osmond 1987).

4.5.7 Limitations of this study

Investigating the complex nature of the light field within a seagrass canopy is not easily accomplished. Many factors can affect the characteristics of the light field including canopy density, leaf orientation, solar angle, cloud cover, and water turbidity.

The frequency and magnitude of surface waves increases the complexity of the intercanopy fluctuations. Although this study successfully characterized the highly dynamic light environment within a seagrass canopy, it did not characterize the spatial characteristics of this variability. These time series measurements represent only a snap shot of the light field and other environmental factors for this site. Variations in wave conditions and water column clarity would no doubt influence the results. However, this Rabbit Key Basin sampling site is visited regularly and the conditions reported on the sampling day are quite common. Other factors such as deeper or shallower seagrasses, less dense canopies, or shorter leaves, would also influence the results.

The study was also not designed to make inferences about whether seagrasses are able to utilize sunflecks like many sub-canopy plants. To understand the utilization of sunflecks by seagrasses, it is necessary to study the photosynthetic induction response of the leaves on near instantaneous time scales (Belshe et al. 2007). Are seagrasses able to acclimate along leaves based on the micro light environment? These questions are discussed in later chapters where I look for evidence of vertical variation of leaf photosynthetic attributes that may indicate sun or shade adaptations.

4.5.8 Further research

Future research should be directed toward determining the benefits to seagrass productivity by sunflecks and wave-focused light since this study suggests that declines in water clarity will considerably reduce the occurrence of these phenomena in a seagrass canopy. Most studies consider sunflecks only as a potential source of light energy to the understory. However, the nature of sunflecks may also contain valuable information about a plant's environment similar to how a plant reacts to variations in light quality.

Reliable assessment of photosynthetic activity in rapidly fluctuating light may be accomplished by analyzing chlorophyll fluorescence characteristics of leaves (Schreiber et al. 1997). Measurements of chlorophyll fluorescence parameters can be completed near instantaneously and reflect the immediate short-term light history of the leaf (Ralph and Gademann 2005).

PART III. EXAMINATION OF PHOTOSYNTHETIC CHARACTERISTICS ALONG *THALASSIA TESTUDINUM* LEAVES

Chapter 5. Examination of the vertical variation of leaf characteristics in *Thalassia testudinum* in Florida Bay.

5.1 Abstract

This study examined the interleaf variation in chlorophyll content along leaves of *T. testudinum* at eight sites across Florida Bay. Chlorophyll content typically increased from base to tip in the youngest leaves and declined significantly from base to tip along older leaves. Although this suggests evidence for interleaf photoacclimation in response to the decline in irradiance through the canopy, this variation may due to the growth form and vertical age structure of the seagrass leaves. Also, a portion of the decline may be due to exposure to high light and other environmental stresses at the leaf tips. The *T. testudinum* in sparse meadows where canopy self-shading is low or nonexistent also showed decline in chlorophyll at the tips. Additionally, leaf tissue is weighted toward the base of the canopy. Coupled with the vertical variation in leaf attributes, this creates a vertical gradient in leaf absorption ability through the canopy.

5.2 Introduction

5.2.1 Canopy heterogeneity

Total plant photosynthesis depends on the efficiency with which the plant can absorb and utilize available solar radiation, provided water and nutrients are not limiting (Hunt and Cooper 1967). Total irradiance absorbed by a plant depends on how the photons in the light field are distributed through the plant canopy (Russell et al. 1989). A canopy is defined as the 3-dimensional organization of aboveground structures of a plant responsible for light harvesting and gas exchange (Aber and Melillo 1991). Light

interception by a canopy is considered proportional to the leaf area index (LAI), a unitless ratio calculated as the total leaf surface area over a unit of horizontal area (Russell et al. 1989). This method assumes that leaf attributes related to photosynthetic activity, such as leaf morphology and pigment content, are homogeneous through the canopy. In fact, many physiological attributes vary significantly through a canopy (Kull and Niinemet 1998). The accurate assessment of whole-plant productivity requires knowledge of the heterogeneity of canopy structure and leaf photosynthetic responses to environmental variables (Kull and Kruijt 1999).

The most obvious environmental variable that varies through a canopy is light. Both the magnitude and spectral distribution of the down-welling light field are modified by passage through a plant canopy (Holmes 1981). This produces a corresponding gradient in leaf photosynthetic attributes driven by variations in photoacclimatory responses through the canopy (Kull and Niinemets 1998). Plants have the ability to adjust leaf photosynthetic attributes during growth in a process called photomorphogenesis (Hart 1988). The role of photomorphogenesis is to optimize plant morphology and resource allocation to maximize whole plant photosynthesis (Hart 1988). Although canopy structure is largely determined during initial growth, the influence of light availability on plant development continues through all stages of growth (Meir et al. 2002). Plants also have mechanisms to photoacclimate to short-term changes in irradiance ranging from minutes to hours (Kull and Kruijt 1999).

5.2.2 Sun versus shade plants

Plant species are commonly considered either sun- or shade-adapted referring to the presence of traits that afford a competitive advantage in either high light or shaded

environments (Öquist et al. 1992). These adaptations influence the spatial distribution of species within a community and are often important factors, which may determine a species' role in secession (Givnish 1988). Sun/shade adaptations include leaf, canopy, and plant level traits (Table 5.1) (Givnish 1988). Sun-adapted leaves are able to achieve high light saturated photosynthetic rates but they also have higher respiratory demand resulting in a relatively high light compensation point. Shade-adapted leaves are able to maintain positive net photosynthesis even at low light levels because respiratory demand is minimized by having thinner and lighter leaves with lower stomatal density (Givnish 1988). The trade-off is that shade plants are more susceptible to photoinhibitory stress when exposed to full sunlight (Öquist et al. 1992).

At supersaturating irradiance, photosynthesis is limited by the rate of non-photochemical dark reactions (Hall and Rao 1999). Optimization to high light is achieved through increases in the concentration of Rubisco and other photosynthetic enzymes in leaves (Pearcy and Sims 1994). Light-limited leaves optimize by allocating more resources toward photochemical activities involving light harvesting (Kull and Kruijt 1999). Lower and upper canopy leaves of an individual plant share many of the same adaptations that distinguish sun- and shade-adapted plants. Lower canopy leaves behave as a shade-adapted plants while leaves at the top of a canopy exhibit attributes of sun-adapted plants.

5.2.3 Seagrass canopy development

Seagrass canopy development differs from that of a tree canopy in a number of ways. Because seagrass leaves grow from a basal meristem (i.e. from the bottom), total biomass and leaf area are weighted toward the bottom of a seagrass canopy (Tomlinson

1980). An emerging seagrass leaf initially grows under shaded conditions deep within the canopy then subsequently experiences greater irradiance as it matures and extends into the upper canopy (Enríquez et al. 2002). This means that an emergent seagrass leaf must initially adapt to shaded conditions then adjust to high light conditions once reaching full canopy height. This growth structure also means that the oldest portions of leaves are found at the tips. While leaves from different levels of a tree canopy are independent from each other, a single adult seagrass leaf spans the entire vertical span of the canopy. This means that photoacclimation to the canopy light gradient would have to occur along individual seagrass leaves.

Table 5.1. Characteristics of sun- and shade-adapted plants. Derived from Givnish 1988.

Trait	Sun	Shade
Leaf-level photosynthetic light response		
Light-saturated rate	High	Low
Compensation irradiance	High	Low
Saturation irradiance	High	Low
Biochemistry		
N, Rubisco, and soluble protein / mass	High	Slightly lower
Chlorophyll *a* / *b* ratio	High	Low
Chlorophyll / N ratio	Low	High
Anatomy and ultrastructure		
Chloroplast size	Small	Large
Thylakoid / grana ratio	Low	High
Morphology		
Leaf mass / area	High	Low
Leaf thickness	High	Low
Stomatal size	Small	Large
Stomatal density	High	Low
Palisade / spongy mesophyll ratio	High	Low
Mesophyll cell surface / leaf area ratio	High	Low
Leaf orientation	Erect	Horizontal
Canopy-level		
Leaf area index	High to low	Low
Phyllotaxis	Spiral	Distichous
Twig orientation	Erect	Horizontal
Asymmetric leaf bases	Very rare	Infrequent
Plant-level		
Fractional allocation to leaves	Low	High
Fractional allocation to roots	High	Low
Reproductive effort	High	Low

Seagrass leaves display considerable plasticity in photosynthetic attributes in response to differing and changing light regimes (Backman and Barilotti 1976; Czerny and Dunton 1995; Dennison and Alberte 1982; Gordon et al. 1994; Kraemer and Hanisak 2000). Seagrass leaves harvested from shallow and deep-water sites show characteristics of sun or shade adaptations with resource allocation shifting to light harvesting apparatus as light availability declines (Dawes 1998; Dawes and Tomasko 1988). Seagrasses also show considerable seasonal variation in leaf constituents, due to seasonal variations in environmental variables such as temperature and light availability (Macauley et al. 1988; Dawes and Lawrence 1980).

Several ecological and physiological traits may drive interleaf variability in seagrass leaves. First, the vertical age structure of a seagrass leaf means that the leaf tip is older than the base. Therefore, age related declines in photosynthetic ability would vary along a leaf. Second, leaf attributes may have been altered as a photoacclimatory response to the canopy light gradient. Lastly, leaf tips may experience photodegradation due to the supersaturating irradiance often experienced at the canopy top (Enríquez et al. 1992).

5.2.4 Chlorophyll

The rate of photosynthesis of a plant is proportional to amount of light intercepted by a canopy of leaves (Russell et al. 1989). Leaves contain light harvesting complexes within the chloroplasts that absorb light and then subsequently transfer the energy to photosynthetic reaction centers (Horton et al. 1996). Leaves of photosynthetic organisms contain an assortment of pigments that work to harvest solar energy, including chlorophylls, and accessory pigments such as phycobilins, and carotenoids (Green et al.

2003). Of these, only chlorophyll *a* is essential and present in all oxygen evolving photosynthetic organisms because of its involvement in the electron transfer processes of photochemistry (Scheer 2003).

Chlorophyll *b* is an accessory pigment found in all higher plants but only in peripheral light-harvesting complexes and has no role in electron transport (Eggink et al. 2001). Chlorophyll *b* is present in the leaves usually in a ratio of 1:3 to chlorophyll *a*. Accessory pigments absorb light but can only transfer the energy to chlorophyll molecules as opposed directly to photosynthesis reaction centers (Green et al. 2003). The role of accessory pigments is to widen the absorption spectrum of the light harvesting apparatus to absorb more effectively in the green region of the spectrum. Carotenoids have an additional function in protecting the photosynthetic apparatus from photodamage by dissipating excess light energy as heat (Hall and Rao 1999).

The Chlorophyll *a* molecule is composed of a porphyrin ring, which includes a magnesium (Mg) atom surrounded by four nitrogen atoms, and a phytol tail (Green et al. 2003) (Figure 5.1). The molecular formula for chlorophyll *a* is $C_{55}H_{72}N_4O_5Mg$, which gives it a molecular weight of 892 g mol^{-1}. The molecular formula of chlorophyll *b* is $C_{55}H_{70}N_4O_6Mg$, which gives it slightly heavier molecular weight of 906 g mol^{-1}. The molecular structure of chlorophyll is analogous to that of hemoglobin where an atom of iron is found in place of the Mg atom (Hall and Rao 1999).

Chlorophyll was first discovered in 1817 by French chemists Pelletier and Caventou as the pigment giving plants their green color. In the 1880's, the German botanist Engelmann proved that chlorophylls were the primary photoreceptive pigments responsible for photosynthesis (Hall and Rao 1999). In Engelmann's famous experiment,

a filament of the green alga *Spirogyra* was isolated in the absence of air on a microscope slide with a suspension of motile oxygen dependent bacteria. When the slide was illuminated, the bacteria surrounded the filaments of alga. In a later experiment, the alga was illuminated by light separated into the components of the spectrum by a prism. The largest number of bacteria moved to the areas the area illuminated by red and blue light. Very few bacteria were found in the area illuminated by green light. In this experiment, Engelmann showed that absorption of light was not equal across the PAR spectrum.

Chlorophyll tends to absorb strongly in the red and blue portions but very weakly in the green. The absorption spectrum of chlorophyll *a* shows distinct peaks at 430 nm and 660 nm while chlorophyll *b* has peaks at 460 nm and 650 nm (Green et al. 2003). The light harvesting complexes of leaves have a combination of chlorophylls and accessory pigments (Hall and Rao 1999). Accessory pigments like phycobilins and carotenoids absorb strongly in the orange and green regions of the spectrum. The variation in the absorption spectrums leads to greater integrated absorption of PAR when the pigments are combined in light harvesting complexes (Green et al. 2003). Plants are able to alter the relative amounts of chlorophyll *a* and *b* (Chl *a*:*b*) within leaves to more efficiently harvest light (Green et al. 2003). The Chl *a*:*b* is an important trait that may indicate active acclimation to changing light conditions of a leaf.

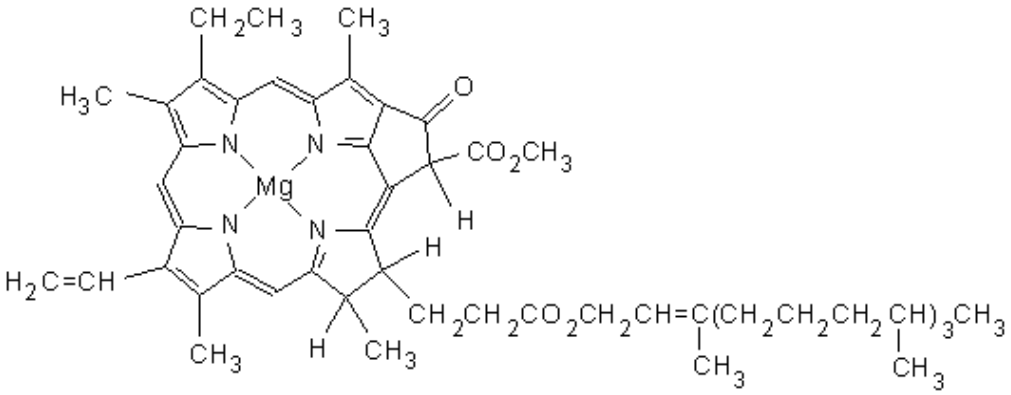

Figure 5.1. Chlorophyll *a* molecular structure.

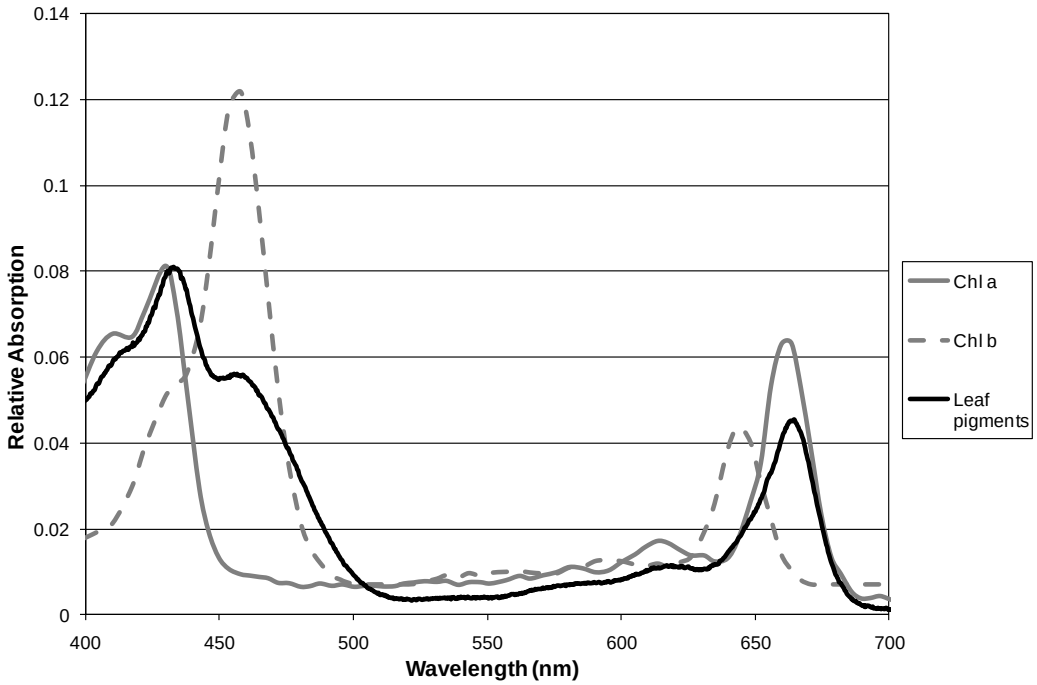

Figure 5.2. Absorption spectrum of Chl *a* and Chl *b* extracted from a spinach standard (Sigma-Aldrich) and leaf pigments extracted from *Thalassia testudinum* leaf tissue. Extractions made in 90% acetone.

5.2.5 Chapter Objectives

The main objective of this chapter is to determine the interleaf variation in chlorophyll content along leaves of *T. testudinum*. Sampling sites were selected across Florida Bay and the Florida Keys to represent the wide distribution of environmental conditions that *T. testudinum* can inhabit. I also determined interleaf variation in specific leaf weight (SLW), total chlorophyll concentration (Chl *a* and Chl *b*), Chl *a*:*b*, and leaf elemental composition (C:N:P). The relationship between these attributes and plant morphology and productivity was examined. The results are expected to vary between sites depending on the leaf length, canopy density, depth, light and nutrient availability.

5.3 **Methods**

5.3.1 Sampling method

I collected ten *T. testudinum* short shoots from eight sampling sites (BANK, BAR, CFT, EKB, JKB, RKB, SPG) in Florida Bay and the offshore of the Florida Keys. Healthy short shoots that were representative of the majority found at the sites were selected. The water depth, salinity, and temperature at the sites were measured and recorded. The short shoots were kept in seawater inside a dark cooler, transported to the Key Largo lab, and processed immediately. Maximum time from sample collection to processing was less than four hours. Leaves were carefully separated from the short shoot samples and cleaned of epiphytes by scraping both sides with a razor blade.

The leaves were ranked by age as follows: Leaf e, emergent leaves less than approximately 5 cm in length; Leaf 1, the youngest adult leaf; Leaf 2, the next older leaf, and so forth. Not all short shoots had a leaf that qualified as emergent. The leaves were

then cut into 5 cm segments starting from the leaf base. The segmentation technique is illustrated in Figure 5.3. The leaf base can be clearly identified as the interface between the green chlorophyll-containing leaf and the white leaf sheath. The length and width of each segment were measured and recorded. The segments were freeze-dried, weighed, and ground to a fine powder using a mortar and pestle. The ground samples were stored in 3 ml microcentrifuge tubes in a freezer until analysis.

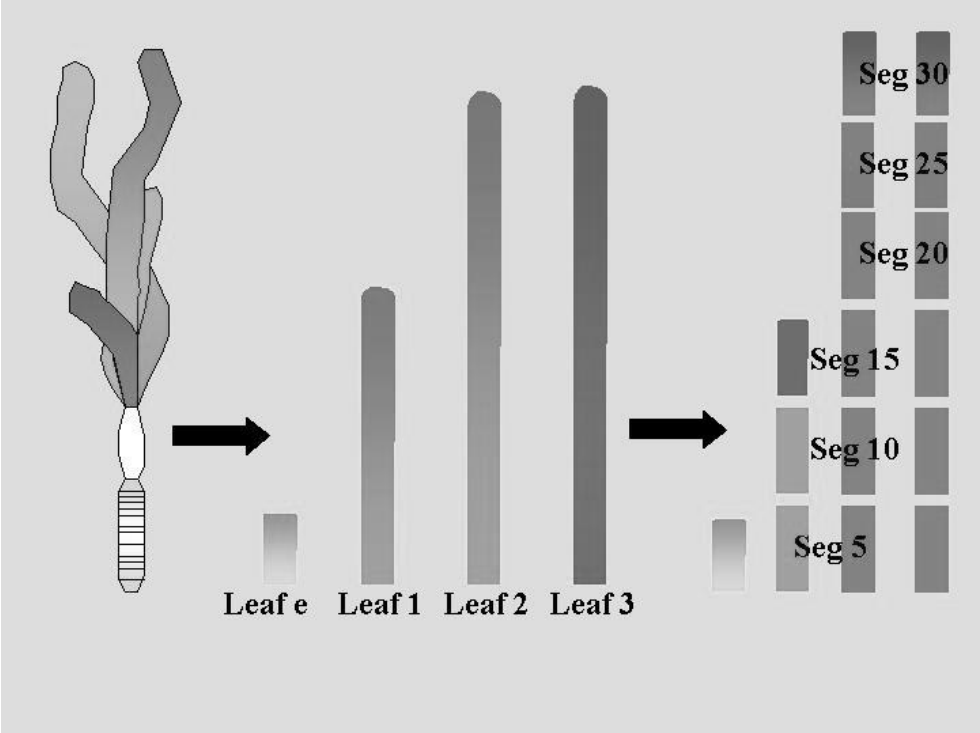

Figure 5.3. Seagrass segmentation technique.

5.3.2 Productivity analysis

Seagrass leaf productivity was measured at each site using the leaf hole-punch method (Zieman and Wetzel 1980). For each site, six rectangular wire quadrats (10 cm x 20 m) were inserted into the sediment along an approximately 5 m transect within the

same area where the short shoots were sampled. Special attention was needed so that only short shoots originating within the area of the quadrat were included. A small hole was punched with a hypodermic needle at the base of each leaf bundle where the non-pigmented sheath and the pigmented portion effectively marking each leaf in the bundle. All of the short shoots within each qaudrat were harvested after approximately 2 weeks of growth. For each quadrat, short shoots were counted and the leaf bundles were cut at the same location as the original needle hole. For leaves that had already reached maximum height, the hole remained stationary at the leaf/sheath interface. For leaves that continued to elongate the hole migrated upward. The biomass located between the leaf base and the needle hole was defined as new leaf growth. Leaf biomass from the hole to the leaf tip was identified as old growth. Occasionally, new leaves emerged during the 2-week growth period had no hole. These three biomass groups, new leaves, new growth, and old growth, were placed in pre-weighed aluminum envelopes, dried at 60ºC, and weighed. Total growth for a quadrat was calculated as the sum of the new growth and the new leaves.

Standing crop, or the aboveground dry biomass, was calculated as the sum of all three biomass subgroups. Specific leaf productivity was calculated as the dry weight of the total growth divided by the dry weight of the standing crop and the number of days of growth (g g^{-1} d^{-1}). Short shoot productivity was calculated as the total growth divided by the number of short shoots in the quadrat and the number of days of growth (g SS^{-1} d^{-1}). Areal productivity was the dry weight of the total growth expressed on a per area basis (g m^{-2} d^{-1}). Productivity data for CFT and SPG were provided by the Seagrass Ecological

Research Center (SERC) at Florida International University (FIU). Productivity between sites was compared using a one-way ANOVA and Tukey's post hoc analysis.

5.3.3 Chlorophyll determination

The chlorophyll concentration of the leaf segments was determined using a variation of an acetone extraction technique (Dennison 1990). Because chlorophyll degrades quickly when exposed to light, it was imperative that the sample be kept away from direct light. The laboratory was kept as dark as possible during all phases of the chlorophyll analysis. Approximately 5 mg of a ground sample was placed in a scintillation vial and 10 ml of 90% acetone was added using a calibrated pipette. The samples were covered in foil and placed in a freezer overnight to allow for full chlorophyll extraction, shaking occasionally.

Sample chlorophyll concentration was calculated using a Shimadzu RF-Mini 150 filter fluorometer (RF-Mini). The RF-Mini operates by the application of an excitation light, which is followed by the emission of a fluorescent signal from the chlorophyll sample. This signal is measured by a detector and displayed in units of relative fluorescence (RF). Photosynthetic pigments, such as Chl *a* or Chl *b*, become excited by specific wavelengths of light and subsequently remit this light, via fluorescence, at longer wavelengths. The RF-Mini uses two glass filters to limit the wavelength ranges of both the excitation light reaching the sample and the emitted light reaching the detector. The filters used for Chl *a* determination are 440 nm for excitation and 670 nm for emission. For Chl *b* determination, the required filters are 460 nm and 650 nm. Standard curves for Chl *a* and Chl *b* concentrations were generated using pure Chl *a* and Chl *b* extracted from spinach (Sigma-Aldrich).

Chlorophyll concentration was determined by dispensing 3 ml of the sample into quartz cuvette, which was then placed inside the sample chamber of the RF-Mini. If the sample concentration is beyond the linear range of the standard curve, the sample was diluted. This dilution factor ranged from 1.0 to 30. Leaf chlorophyll concentration per leaf dry weight and per leaf area were calculated using the sample concentration, the volume of acetone added to the vial, the weight of the sample added to the vial, and the segment dry weight to area ratio using the following equations:

µg Chl / mg leaf dry wt. = µmol Chl in sample * Vol. (L) / dry weight of leaf segment (mg) * dilution factor

µg Chl / cm^2 = µmol Chl in sample * Vol. (L) / (dry wt. / area (mg/cm^2)) * dilution factor

5.3.4 Testing of the dry grind method for chlorophyll extraction

The dry grind technique in this study was used as opposed to extracting the chlorophyll from wet leaf tissue for a number of reasons. The high number of samples generated during this study would have made it difficult to complete the processing in a reasonable amount of time following the sample collection. Additionally, the chlorophyll concentration of the samples is significantly affected by the total time a sample was allowed to extract in the acetone. By pregrinding 100 dried samples then adding the acetone to all using a calibrated bottle-top dispenser, the extraction time could be standardized. Using the dry grind technique exposes the samples to light for less time minimizing photodegradation of the chlorophyll. Furthermore, the volume of acetone to each sample can be more accurately measured with a calibrated dispenser.

In the wet grind method, the volume of acetone in a sample is controlled by filling to a level on a graduated test tube. There are inherent inaccuracies involved in using this

technique and the graduated test tubes themselves can be inaccurate. Finally, acetone is highly volatile and must be used under a ventilated hood to avoid exposure to the fumes. However, the Key Largo laboratory where the work was completed was not equipped with a ventilated hood.

The dry grind method was tested to establish its accuracy in comparison to the wet grind method. Fifty healthy adult *T. testudinum* leaves were collected from Barnes Key Basin being careful to select leaves with similar thickness and color. Five cm segments were cut from the middle of each of the leaves. Then twenty of the segments were immediately analyzed for chlorophyll using a wet grind technique. The fresh tissue was ground with a mortar and pestle after adding a few ml of 90% acetone. The extractant was poured into a graduated test tube, fresh acetone was poured into the mortar, and grinding repeated until leaf material appeared to be absent any remaining chlorophyll. The mortar and pestle were then rinsed with acetone and the rinse acetone with any remaining leaf tissue added to the test tube. The test tube was then topped off to 10 ml with acetone. The test tubes were stored in a dark freezer overnight to allow for full extraction. Chlorophyll concentration of each sample was determined using the RF-mini.

The remaining leaf segment were freeze-dried, ground into a fine powder with a mortar and pestle, and then stored in twenty 10 ml scintillation vials. A small sample from each vial was analyzed for chlorophyll content using the dry grind technique. The vials were covered with aluminum foil and stored in a freezer. To assess the stability of the chlorophyll in the freeze-dried sample, each was reanalyzed after one week, one month, and two months.

5.3.5 Elemental determination

Leaf phosphorous content was determined using a variation of a hot acid extraction technique (McGlathery et al. 1994). Approximately 5 mg of each ground leaf sample was weighed into borosilicate tubes, sealed tightly with foil, then ashed at 550º C for 6 hours in a furnace oven. After allowing the samples to cool to room temperature 2 ml of HCl and 10 ml of DIW were added to each. The tubes were again capped tightly with foil and placed in an oven at 100º C for 2 hours. After cooling to room temperature, the test tubes were topped off to 10 ml with DIW to replace any volume lost due to evaporation. A set of dilutions of a standard stock solution of potassium dihydrogen phosphate (KH_2PO_4) was prepared to generate a standard curve. One ml of a mixed coloring reagent was added to each sample and standard dilution. Color development was allowed to proceed for at least ½ hour and sample absorbance at 885 nm was read on a Hewlett Packard Model 8453 UV/VIS Spectrophotometer. If a sample concentration exceeded the linear range of the standard curve, it was diluted and absorbance reread. Samples were processed in batches of approximately one hundred so that the spectrometer readings could be completed in a reasonable amount of time ensuring that color development remained approximately standardized.

The molar concentration of PO_4 in a sample was calculated using the linear equation from the standard curve. The total mass of P in the sample was determined by multiplying the sample PO_4 concentration by the sample volume (13 ml) and the molecular weight of P, 30.97. The percent P content in a leaf segment was calculated by dividing total P by the mass of dry leaf sample used. Samples were run in duplicate where the quantity of sample allowed. Citrus leaf standards were run, concurrently, to

determine analytical error. The recovery of phosphorous in the reference standard was 96±7% using this method.

The percent content of N and C in the leaf segment samples was determined utilizing a Carlo-Erba Elemental Analyzer. Replicates were run when adequate sample was available. C, N, and P contents were converted to molar ratios (C:N, C:P, and N:P) using the respective molecular weights as follows:

C:N = (%C / 12.01115) / (%N / 14.0067)

C:P = (%C / 12.01115) / (%P / 31)

N:P = (%N / 14.0067) / (%P / 31)

5.3.6 Statistical analysis

The leaf attribute data were compiled in an Excel spreadsheet and sorted by site, leaf rank, and segment number. Variations along individual leaves and variability between sites as a function of leaf and segment number were assessed using one-way ANOVA after checking for normality and homogeneity of variance. If the tests failed, the data were log transformed. Tukey's multiple comparison tests were used to identify specific differences along leaves at each site and between sites at specific leaf and segment locations. The total chlorophyll content and total leaf area per m^2 at the sites was compared to the site productivity measurements using Pearson correlation analyses. A correlation analysis was also conducted comparing chlorophyll concentration, leaf area, elemental content, and the site physical characteristics. All statistical analyses were performed using SAS statistical software version 9.1 (SAS Institute, Cary, NC).

Vertical profiles of leaf area at each site were produced by adding the average leaf area across all leaves at each segment location, multiplying by the frequency probability

of the segment, and then multiplying by the short shoot density of the site. Vertical canopy profiles of total chlorophyll were produced by multiplying the average leaf area of a segment by the chlorophyll concentration ($\mu g/cm^2$) at the segment and then summing across all leaves for each canopy level.

5.4 **Results**

5.4.1 Site characteristics

The basic structure of a *T. testudinum* short shoot was similar at all sampling sites consisting of a bundle of two to five leaves of incremental ages. The older leaves were, heavily epiphytized, contained lesions toward the tips, and were often fragmented. The physical characteristics at the sites are summarized in Table 5.2 Water depth ranged from 0.5 m on the banks, SPG and BANK, to 6.0 m at the reef site CFT. Water temperature showed little between-site variability ranging from 28 ºC at CFT to 30.5 ºC on the banks and averaging 29.4 ºC. Salinity varied little from seawater salinity except for EKB where salinity was 9.0 ‰ due to the freshwater input from the nearby Everglades.

Table 5.2. Water column characteristics of Florida Bay sampling, September 2002.

Site	Depth (m)	Temperature (ºC)	Salinity (‰)
BANK	0.5	30.5	38.0
BAR	1.2	29.5	37.5
CFT	6.0	28.0	36.0
EKB	1.5	29.5	9.0
JKB	1.2	29.0	33.5
RKB	1.8	29.5	37.5
SPG	0.5	29.0	36.0
TAV	3.0	30.5	36.0

5.4.2 Leaf and canopy morphology

T. testudinum leaf morphology varied significantly between sites but not within sites (Table 5.3). The longest leaves were Leaf 3 at JKB at 44.6 cm while the shortest adult leaves were at EKB where Leaf 3 averaged only 13.6 cm. The short leaf length recorded for Leaf 3 at SPG was due to frequent leaf fragmentation. No short shoot sampled from CFT had more than two leaves. Some short shoots at JKB, RKB, and BANK had fourth or fifth leaves. However, these were not present in sufficient numbers to allow for statistical comparisons. Leaf widths also varied significantly between sites ranging from 0.55 cm at EKB to 1.03 at SPG ($P < 0.001$) (Table 5.3). However, leaf widths were consistent between the leaves of individual short shoots and within sites (p-value ranged from 0.89 to 0.99).

Figure 5.4 shows the vertical profile of leaf area through the canopies at the sampling sites. All sites showed leaf area heavily weighted toward the bottom of the canopy. Leaf biomass is highly weighted toward the base of the canopy with approximately 60% of the photosynthetic tissue contained in the bottom 15 cm. This is due to an inherent growth property of seagrasses where leaves grow from a basal meristem (Hemminga and Duarte 2000). This vertical gradient in age structure of the canopy is a significant driver of many of the within leaf and between leaf variations discussed later. Interestingly, JKB had slightly lower leaf area at the base of the canopy compared to the segment directly above it. This is due to a unique feature of leaf morphology found only at JKB, where the leaves were less wide at the bottom.

The significant variation in leaf productivity, shoot density and leaf standing crop between the sites is reflective of the adaptable morphology of *T. testudinum* to a variety

of growing conditions (Table 5.4). At SPG, the *T. testudinum* was growing within a canopy of *S. filiforme*. The short shoot, standing crop, and productivity measurements are for *T. testudinum* only. Table 5.5 shows the results of the ANOVA and Tukey comparisons testing for significant differences between sites. To make comparisons easier the sites are ranked from highest to lowest. BAR had the highest short shoot density at 1583 SS m^{-2} while the lowest density was at SPG with only 200 SS m^{-2}. Interestingly, the standing crop at the sites did not follow the same ranking as for short shoot density. While BAR remained highest on the list and SPG the lowest, JKB ranked higher than for short shoot density owing to the longer leaves and higher number of leaves per short shoot. While RKB had ~20% higher short shoot density than BANK, the standing crop at BANK was ~65% higher than RKB. The highest areal productivity was at BAR with 2.91 g m^{-2} d^{-1} followed closely by TAV with 2.68 g m^{-2} d^{-1}. These values were nearly twice as large the next closest site. SPG and EKB both had exceptionally low areal productivity, 0.57 and 0.29 g m^{-2} d^{-1}, respectively. However, leaf productivity was highest at SPG, 39.6 mg g^{-1} d^{-1}. This was approximately double the leaf productivity found at any other site. The highest short shoot productivity was found at JKB at 3.37 mg SS^{-1} d^{-1} while the lowest was at EKB with 0.65 mg SS^{-1} d^{-1}.

Table 5.3. Morphological characteristics of *Thalassia testudinum* leaves at Florida Bay sampling sites. Leaf rank denotes leaves of increasing age while 'e' represents immature emergent leaves ~5cm in length. Leaf widths are combined because no short shoots showed significant difference in width of different aged leaves. Values are means ±SD. Values with the same letter are not significantly different as determined by Tukey's multiple comparison tests ($P < 0.05$).

	Leaf Width	Leaf Lengths (cm) by Leaf Rank			
Site	(cm)	e	1	2	3
BANK	0.74±0.11d	no data	13.7±3.1bc	19.0±4.5cd	17.1±4.1c
BAR	0.97±0.11b	4.4±0.55a	15.0±1.5b	26.4±1.5b	29.9±1.6b
CFT	0.82±0.08c	5.3±0.78a	13.8±2.1cd	17.0±1.4cd	no data
EKB	0.55±0.09e	4.3±0.67a	8.6±0.51d	10.9±0.65e	13.6±1.7cd
JKB	0.99±0.11ab	4.8±2.5a	25.9±3.8a	40.8±3.0a	44.6±2.7a
RKB	0.81±0.11c	5.8±1.2a	17.3±6.7a	27.8±3.5b	27.1±3.8b
SPG	1.03±0.10a	5.2±0.55a	18.6±2.5ab	20.1±1.7cd	6.8±0.8d
TAV	0.83±0.07c	4.5±0.58a	9.9±0.39cd	14.6±0.46de	14.7±0.6cd

Table 5.4. Mean productivity and standing crop of *Thalassia testudinum* at Florida Bay sampling sites, Sept. 2002. Values are means ±SD (n = 6).

Site	Short Shoot Density (#SS m^{-2})	Standing Crop (g m^{-2})	Areal Productivity (g m^{-2} day^{-1})	Short Shoot Productivity (mg SS^{-1} day^{-1})	Leaf Productivity (mg g^{-1} day^{-1})
BANK	1133±192	141.1±30.1	1.34±0.43	1.25±0.29	13.3±1.8
BAR	1583±268	169.2±51.1	2.91±0.80	1.86±0.20	17.4±1.4
CFT	675±220	41.8±8.3	0.79±0.24	1.25±0.48	18.7±3.1
EKB	592±222	27.4±15.6	0.29±0.10	0.65±0.22	11.5±4.7
JKB	458±191	90.6±31.2	1.46±0.49	3.37±0.66	16.2±1.3
RKB	1367±327	85.5±14.6	1.38±0.20	1.04±0.16	16.3±1.3
SPG	200±84	14.3±10.8	0.57±0.45	2.55±1.2	39.6±7.5
TAV	708±211	52.7±38.2	2.68±1.20	0.81±0.15	23.1±4.7

Table 5.5. Site rankings for above ground productivity of *Thalassia testudinum* at Florida Bay sampling sites. Values with the same letter are not significantly different as determined by Tukey's multiple comparison test ($P < 0.05$).

Rank	Short Shoot Density (SS m^{-2})	Standing Crop (g m^{-2})	Areal Productivity (g m^{-2} day^{-1})	Short Shoot Productivity (mg SS^{-1} day^{-1})	Leaf Productivity (mg g^{-1} day^{-1})
Highest	BAR a	BAR a	BAR a	JKB a	SPG a
	RKB ab	BANK ab	TAV ab	SPG b	TAV b
	BANK b	JKB bc	JKB abc	BAR c	CFT c
	TAV c	RKB b	RKB abc	BANK c	BAR cd
	CFT c	TAV cd	BANK abc	CFT d	RKB de
	EKB c	CFT cd	CFT bc	RKB d	JKB cd
	JKB cd	EKB d	SPG c	TAV de	BANK f
Lowest	SPG d	SPG d	EKB c	EKB e	EKB ef

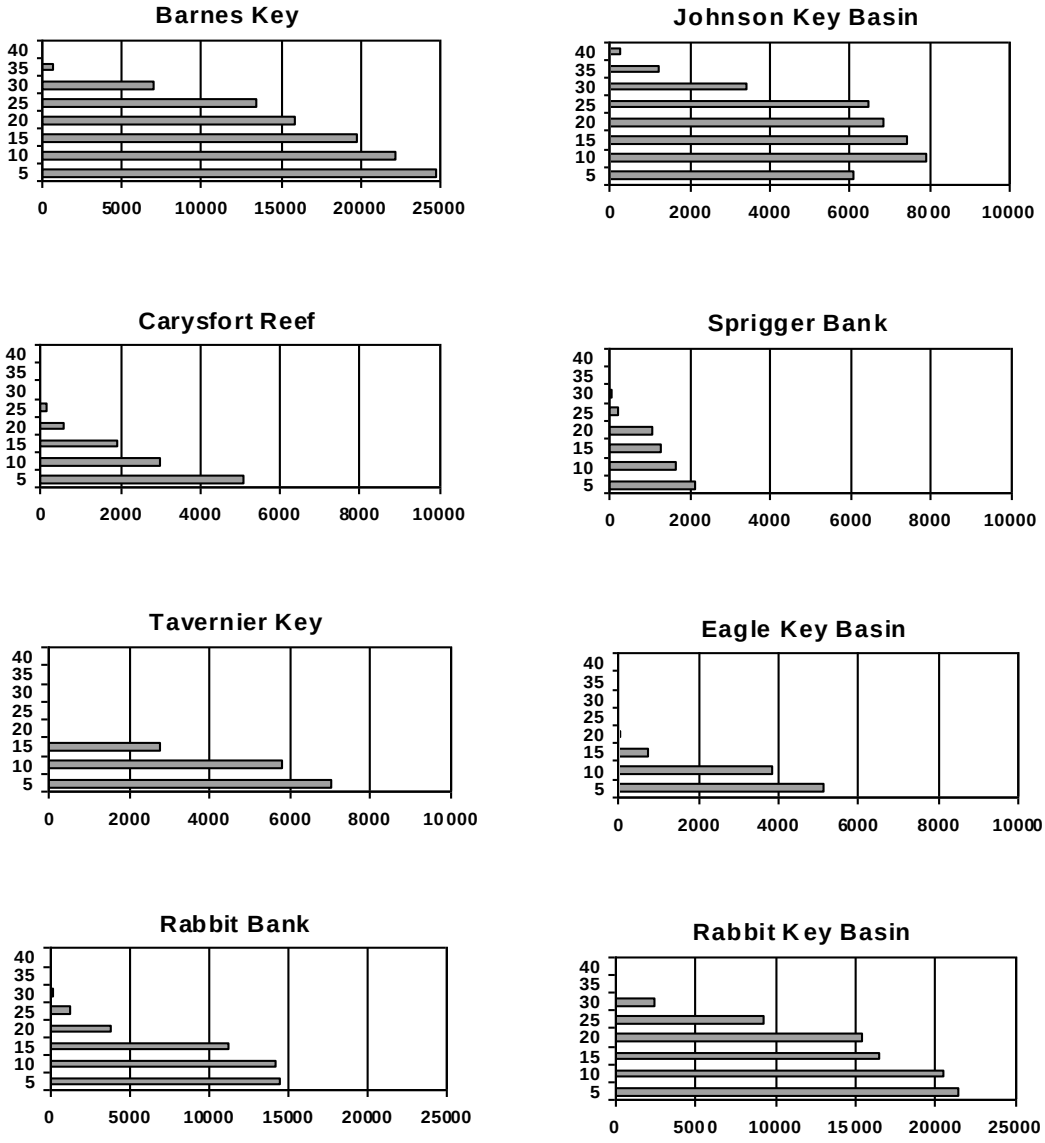

Figure 5.4. Vertical profiles of leaf area through *Thalassia testudinum* canopies at Florida Bay sampling sites, X-axis: leaf area (cm^2); Y-axis: height in canopy (cm).

5.4.3 Leaf chlorophyll content

There was no significant difference in chlorophyll concentration found between samples analyzed using the wet grind method and the dry grind method (P = 0.87). There was also no significant difference found between the freeze-dried samples after one week (P = 0.37), one month (P = 0.54), and two months (P = 0.49). Therefore, the chlorophyll within the dried and ground samples was considered stable for periods of at least two months and the dry grind technique for extracting chlorophyll is suitable for this study.

Leaf chlorophyll concentration, µg of total chlorophyll (Chl *a* + *b*) per leaf dry weight, varied along leaf blades and between different aged leaves at all sampling sites (Figure 5.5). The typical profile of a short shoot showed very low chlorophyll concentration in emergent leaves, concentrations increasing base to tip along Leaf 1, and concentrations decreasing from base to tip along older leaves. As leaves age, a significant decline in chlorophyll concentration is observed at the tips of leaves, however, total chlorophyll at the base of leaves continues to increase. Although the total sum of chlorophyll in full-grown adult leaves declines with age, chlorophyll concentration at the leaf bases increased or only slightly decreased as leaves aged. The highest chlorophyll concentration was typically found at the middle or tip of Leaf 1. The lowest concentrations were found at the tips of older leaves and in emergent leaves. The highest within-leaf variability was at BAR where chlorophyll concentration varied two-fold along younger leaves. Standard deviations were relatively small indicating consistency in canopy traits within sites. Chlorophyll per unit area basis closely matched the values for chlorophyll per unit leaf weight (data not shown).

5.4.4 Site comparisons

The range of chlorophyll values among the sample sites demonstrates the ability of *T. testudinum* to control pigment production based on environmental conditions. Usually, leaf chlorophyll concentration is inversely correlated with light availability. Comparing leaf chlorophyll at CFT and TAV validates this hypothesis. While CFT and TAV have similar short shoot densities and standing crop, CFT experiences lower light availability due to a deeper water column (6.0 m vs. 3.0 m). Reflecting the expected effect of photoacclimation, leaf chlorophyll concentration at CFT was significantly higher than that found at TAV. The relationship between light availability and leaf chlorophyll concentration is not maintained when comparing RKB to BANK.

Short shoot productivity (g SS^{-1} d^{-1}) at BANK was 21% compared to RKB. Conversely, RKB has a 23% higher rate of leaf productivity (mg g^{-1} d^{-1}) than BANK. This explains why the two sites achieve equivalent areal productivity despite their differences in leaf morphology. The leaves at BANK experience approximately double the daily irradiance as RKB but the leaf chlorophyll concentration at BANK is nearly double that for RKB. There may be an explanation for this departure from the expected trend. The leaf C:P values at BANK suggest that BANK has higher nutrient availability than RKB. This may be due to the vicinity of the BANK site to outside nutrient sources compared to the isolation of the RKB site. In addition, the shallow water column at BANK means that the seagrass canopy can slow the tidal and current flow, often trapping senesced seagrass leaves and drifting macroalgae along with the nutrients that they contain. The higher nutrient availability may allow leaves at BANK to achieve higher

maximum rates of carboxylation. This allows the leaf to allocate additional resources toward light harvesting apparatus increasing the light saturated maximum photosynthetic rates (Field 1983).

Given higher leaf chlorophyll, P availability, and irradiance, productivity at BANK would be expected to be higher than at RKB. However, the areal productivity (g m^{-2} day^{-1}) of BANK and RKB is nearly identical. Only by examining the canopy profile of total chlorophyll for the two sites can the question be answered. BANK and RKB actually deploy similar quantities of total canopy chlorophyll per area. RKB has both longer and wider leaves with lower chlorophyll content while BANK has higher leaf chlorophyll concentration but smaller leaves. Because of the higher leaf chlorophyll concentration, BANK is able to deploy a comparable amount of total chlorophyll within its canopy even though there is lower leaf area present.

Interleaf variation of Chl *a*:*b* was not significant at most sites. These data are shown in the Appendix. Because of the small size of the leaf segments from some sites, there was not always enough leaf tissue to perform nutrient analysis. The results for interleaf variation in SLW are also included in the appendix. Evaluation of these the interleaf variation in C, N, and P and SLW is accomplished more precisely in Chapter 6.

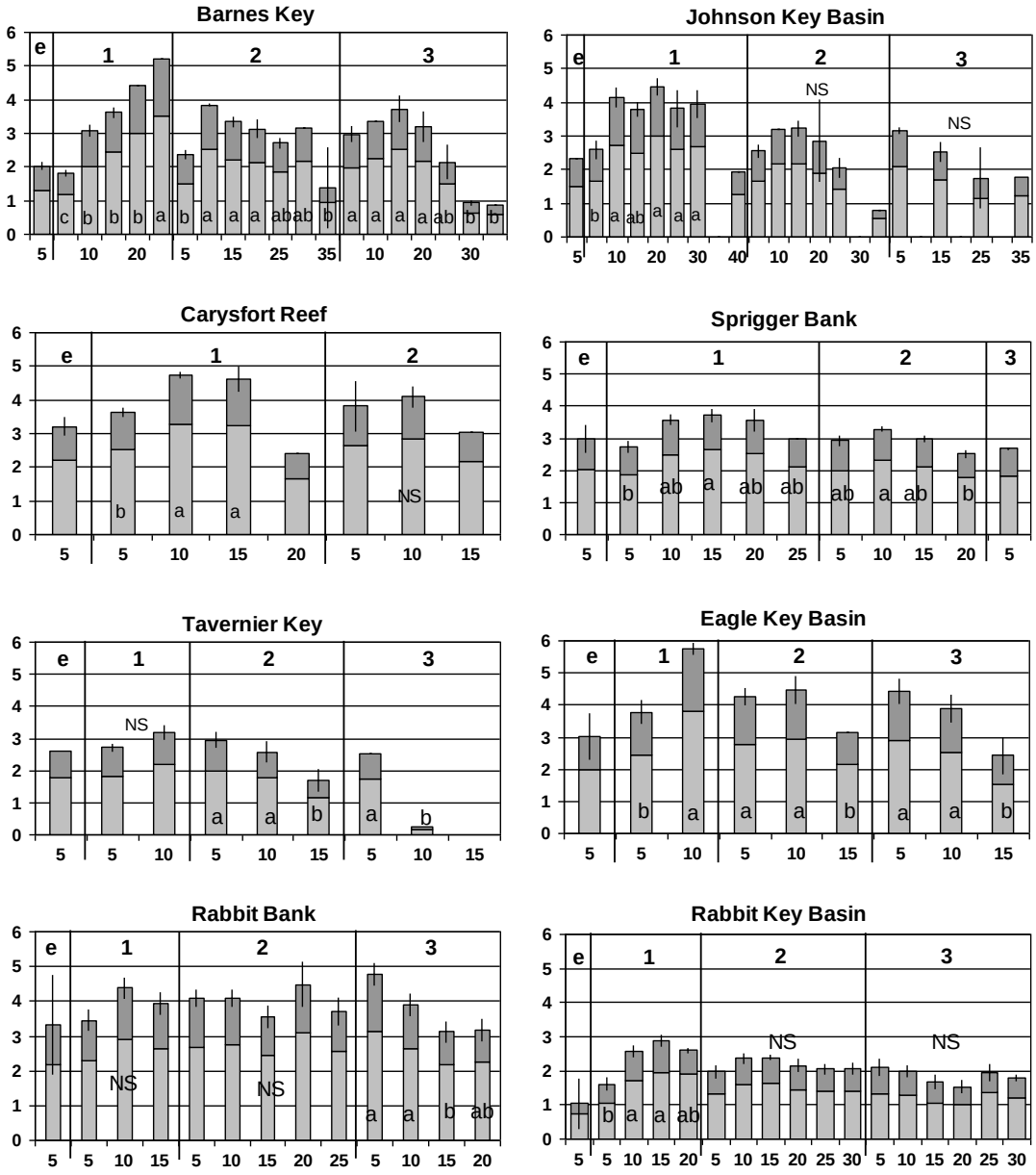

Figure 5.5. Interleaf variation of total chlorophyll concentration in leaves of *Thalassia testudinum* short shoots at Florida Bay sampling sites, September 2002 . X-axis indicates segment distance (cm) from leaf base, Y-axis is total chlorophyll concentration (µg Chl (*a* + *b*) / mg leaf dwt.). Light gray bar represents Chl *a* and dark gray bars represent Chl *b*. Error bars are standard deviations. Leaf rank denotes leaves of increasing age while e represents immature emergent leaves ~5cm in length. Letters indicate significant variation along individual leaves as determined by one-way ANOVA and Tukey's multiple comparison test ($P<0.05$).

5.4.5 Leaf Chl:N and Chl:P

The ratio of total chlorophyll (Chl *a* + Chl *b*) to N and P, expressed as mmol of total chlorophyll per mol of N or P, is shown for the four sampling sites where there was sufficient material to complete enough C:N:P analysis (Figure 5.6). Chl:N and Chl:P were closely correlated but patterns varied between sites. There were few statistically significant differences found along leaves due to the small sample sizes. However, noticeable trends were observed. For the sites with long leaves and dense canopies (i.e. BANK and RKB), the lowest leaf Chl:N and Chl:P was consistently found in emergent leaves. Chl:N and Chl:P increased from base to tip along Leaf 1. Along older leaves, Chl:N and Chl:P typically decreased from base to tip. The statistically significant interleaf Chl:N at BANK is evidence of interleaf photosynthetic optimization. Although other sites show similar trends sample size were not sufficient to make conclusions.

5.4.6 Summer/winter comparison of leaf chlorophyll content

The comparison of leaf attributes from summer versus winter at RKB is shown in Figure 5.7. The water temperature at the time of the winter sampling was 20º C in January 2005 versus 29.5º C during the summer sampling. Salinity was 34‰ during the winter sampling versus 37.5‰ for the summer. Water clarity was slightly higher during the winter sampling with a K_d(PAR) of 0.51 m^{-1} versus 0.65 m^{-1} for the summer. Significant differences were found for total chlorophyll concentration in emergent leaves and the base and middle of adult. Significant differences were also found for SLW along the entire lengths of adult leaves (Leaf 1 and Leaf 2). No significant differences were found for Chl *a*:*b*.

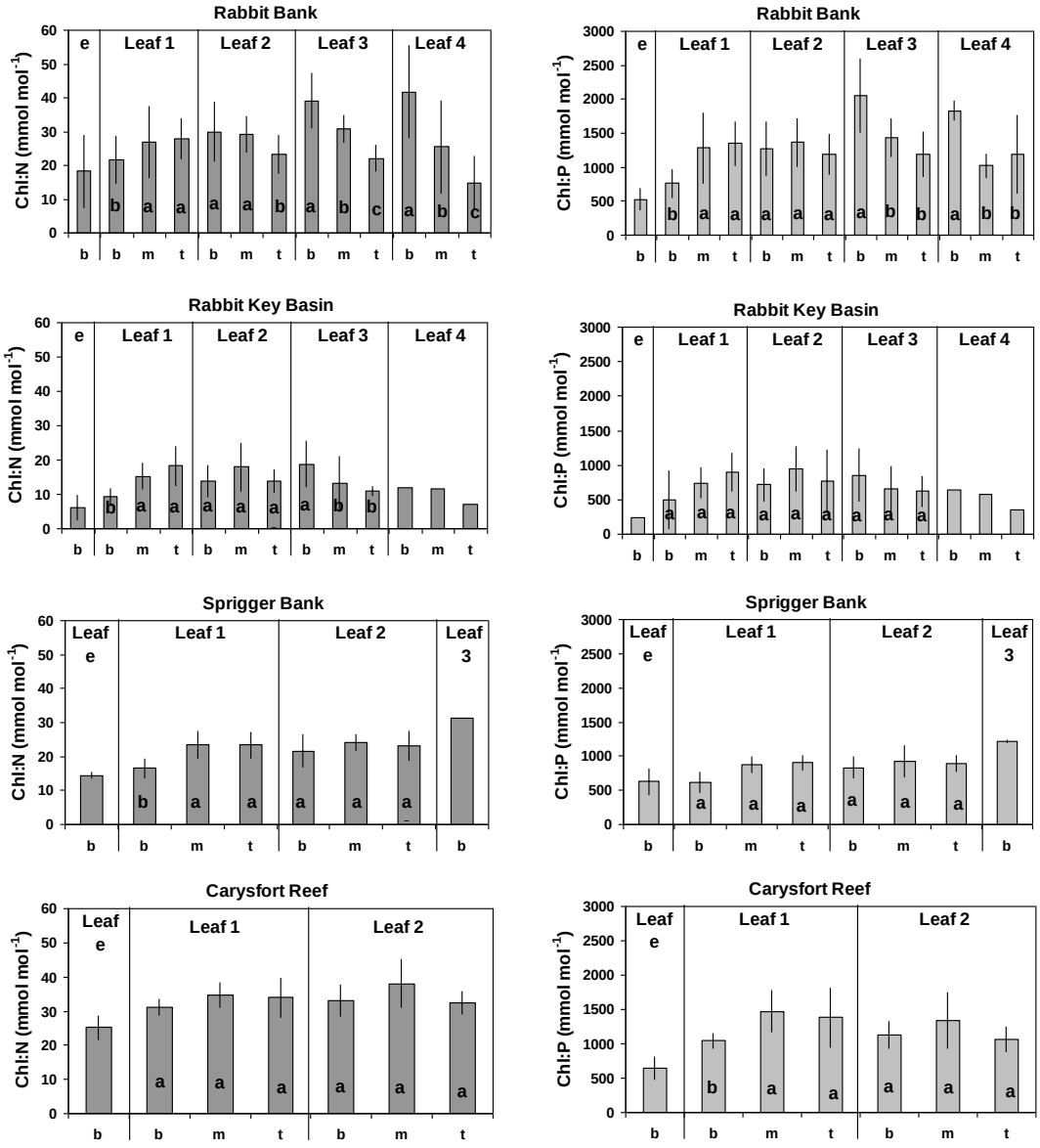

Figure 5.6. Interleaf variation of Chl:N and Chl:P along Thalassia testudinum leaves from Florida Bay sampling sites. Values on x-axis represent relative position along leaves, b: bottom; m: middle; t: top. Leaf rank denotes leaves of increasing age while e represents immature emergent leaves ~5cm in length. Letters indicate significant variation along individual leaves as determined by one-way ANOVA and Tukey's multiple comparison test ($P<0.05$).

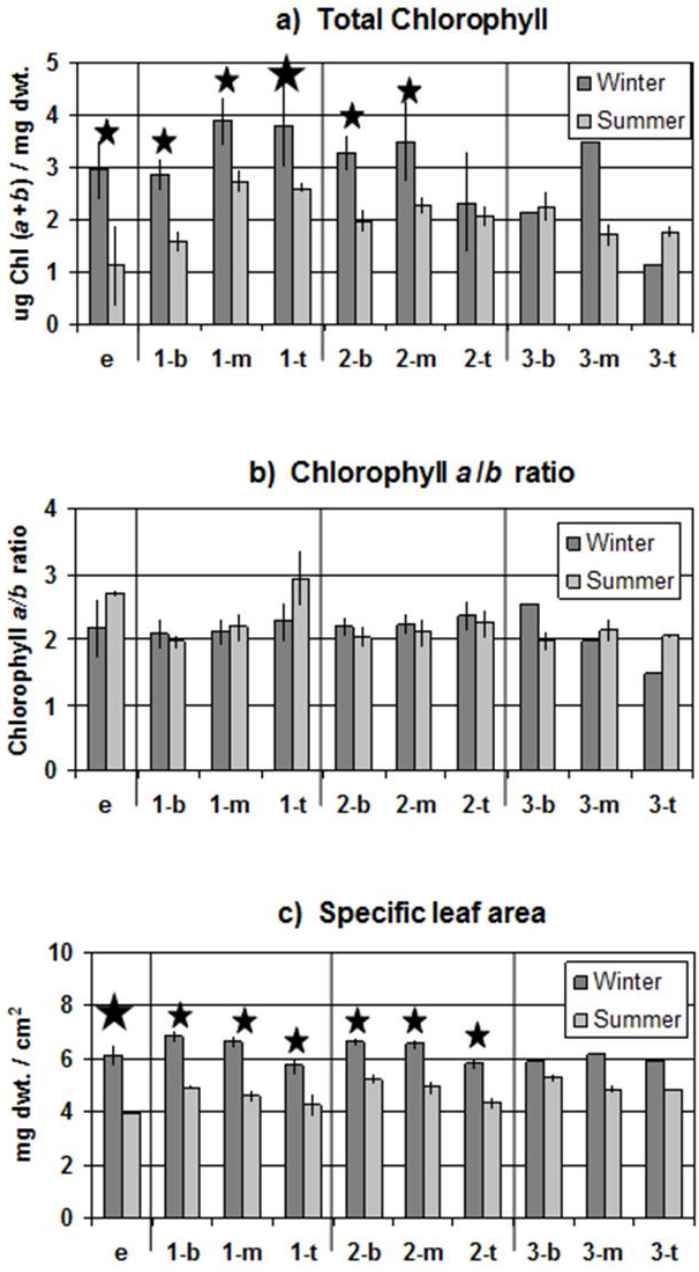

Figure 5.7. Summer/winter (August/January) comparison of the interleaf variation in *Thalassia testudinum* leaf attributes at Rabbit Key Basin, Florida Bay. X-axis indicates the Leaf Rank and segment location. Stars indicate significant differences for the leaf and segment location ($P < 0.05$).

5.4.7 Correlation of leaf attributes to site characteristics

The results of the correlation analysis comparing site productivity with leaf attributes are shown in Table 5.6. Short shoot productivity was significantly correlated to the mean length of adult leaves (R = 0.80). SLW was significantly correlated to standing crop (R = 0.62) and areal productivity (R = 0.63). Leaf chlorophyll was also significantly correlated also to areal productivity but negatively (R = -0.64) indicating that sites with lower leaf chlorophyll content typically had higher areal productivity. This would seem to be counter intuitive because lower chlorophyll content implies less efficient light harvesting.

Standing crop, a measure of canopy density and to some extent the degree of self-shading, was negatively correlated to Chl *a*:*b* (R = -0.66). This is consistent with the literature that suggests that a common response to canopy self-shading is an increase in Chl *b* relative to Chl *a* (Kull 2000). Leaf productivity was positively correlated to Chl *a*:*b* (R = 0.79) indicating that leaves with higher Chl *a* relative to Chl *b* grow faster. Short shoot productivity was negatively correlated to C:P indicating lower productivity at sites that were more P-limited.

Table 5.6. Correlation matrix comparing site attributes and productivity data versus leaf attributes. Correlation coefficients in bold indicate significant relationships (P<0.05).

Productivity Measurements	Depth To canopy	Adult leaf length	SLW	Chl conc. (*a* + *b*)	Chl *a*:*b*	C:N	C:P
Short shoot density	-0.09	0.13	0.35	-0.44	-0.49	-0.31	0.04
Standing crop	-0.34	0.45	**0.62**	-0.39	**-0.66**	-0.11	-0.16
Areal productivity	-0.05	0.27	**0.63**	**-0.64**	-0.23	0.47	0.11
Short shoot productivity	-0.38	**0.80**	0.51	-0.18	0.03	0.10	**-0.74**
Leaf productivity	-0.10	-0.08	0.00	-0.33	**0.79**	-0.01	-0.35

5.5 **Discussion**

This study showed that chlorophyll content and other leaf characteristics vary significantly along *T. testudinum* leaves sampled from a variety of environmental conditions and canopy structures. A portion of this interleaf variation is likely due to the vertical age structure of seagrass leaves that grow from a basal meristem. Chlorophyll degradation is a principal symptom of senescence of leaf tissue (Nooden 1988). Additionally, the oldest section of the leaf (i.e. the tips) have been subjected longer to environmental stressors like wave energy, grazers, and disease, which may damage leaf tissue. The high irradiance at the top of the canopy that is often well in excess of the saturation intensity may result in chronic photoinhibition, damage to the photosynthetic apparatus, and break down of chlorophyll molecules (Enríquez et al. 2002; Krause 1988; Barko and Filbin 1983). Lastly, as shown in Chapter 3 light availability varies significantly along individual *T. testudinum* leaves. This suggests that canopy photoacclimation may play a role in the interleaf variation in chlorophyll content and other leaf characteristics.

Comparing between leaves of increasing age reveals a picture of the accumulation and degradation of chlorophyll over the life of a *T. testudinum* leaf. Chlorophyll accumulates rapidly in young emergent leaves. As the leaf elongates and reaches Leaf 1 status, chlorophyll continues to increase at the base but begins to decline at the tip. This is true except for sites with shorter leaves, such as EKB and TAV. When a leaf reaches Leaf 2 status, chlorophyll has declined significantly at the tip and has started to decline at all leaf segments except at the base, which still is increasing chlorophyll in most cases.

When the leaf reaches Leaf 3 status, chlorophyll is still increasing at the base, except at SPG and TAV. The change over time of the relative distribution of chlorophyll content along leaves suggests an active photoacclimation process.

This previous exercise assumes that the leaves all experienced the same environmental conditions during growth. However, adjacent leaves on a short shoot may have grown during different seasons despite the relatively short lifespan of *T. testudinum* leaves of approximately 52 days (Fourqurean and Zieman 1991). While Florida Bay is sub-tropical, there is still considerable seasonal variation in many environmental variables including irradiance, nutrient availability, salinity and water temperature. The seasonal comparison at RKB demonstrates the extent to which leaf chlorophyll content can vary from summer (August) to winter (January). The higher chlorophyll content during the winter sampling is probably mostly due to lower irradiance during winter months. However, nutrient availability is higher during winter due to lower demand. This may allow the seagrass to deploy more resources toward light harvesting apparatus.

Chlorophyll can decline in leaves due to both age related senescence and photooxidation (Rontani et al. 1996). Additionally, many studies have shown that seagrasses can photoacclimate to changing light regimes by altering leaf chlorophyll content (Dennison and Alberte 1982; Gordon et al. 1994; Major and Dunton 2002). This study provides evidence that all of these processes may actually occur simultaneously. Further evidence of this is the decline in Chl *a* at some leaf tips. Because Chl *a* degrades faster than Chl *b*, a decline in chlorophyll due entirely to senescence or photooxidation would result in a lower Chl *a*:*b* (Maunders and Brown 1983). This suggests that the

lower Chl *a*:*b* at the leaf bases is a photoacclimatory response. Higher specific leaf weight and leaf thickness are common adaptations seen in sun-adapted leaves (Givnish 1988). However, both of these attributes declined from base to tip in *T. testudinum* leaves. This attribute may make seagrasses more buoyant allowing them to maintain an erect canopy.

5.5.1 Seagrass canopies versus terrestrial canopies

Terrestrial canopies are able to optimize photosynthesis by deploying canopies that maximize light harvesting and leaf photosynthetic capacity (Givnish 1988). Plants can modify leaf structure and vary arrangement of leaves through a canopy (Aber and Melillo 1991). Leaf photosynthetic attributes through the canopy are dependent on the light environment within which they are found (Kull 2002). Leaves at the top of the canopy exhibit traits of a sun-adapted plant while lower canopy leaves display shade-adapted characteristics (Evans 1993). The result of this canopy-level photoacclimation is that a canopy performs as a layered system as opposed to the 'big leaf' oversimplification often considered (Kull 2002).

A seagrass canopy exhibits some of these same attributes but possess some limitations due to morphological properties unique to seagrasses. Because of the characteristics of basal growth, leaf area in a *T. testudinum* meadow is heavily concentrated toward the bottom of the canopy where the light availability is lowest. The distribution of the dry biomass through a canopy is further augmented because of the within-leaf variation of SLW, which finds denser leaf tissue at the base of leaves. Other

important inter-canopy variations may also include temperature and leaf conductance. These features were not evaluated in this study.

Another major difference between a seagrass canopy, or a terrestrial grass canopy for that matter, and a tree canopy is that a seagrass leaf spans the entire canopy. While individual leaves distributed in a tree canopy photoacclimate based on their specific light environment, seagrass must acclimate along the length of an individual leaf. Furthermore, a seagrass leaf section must first acclimate to the shaded conditions within the canopy then acclimate to the high light conditions after reaching full length.

5.5.2 Canopy-level photoacclimation

Canopy-level photoacclimation also includes the differential distribution of nutrients. Leaf N content is typically higher toward the top of a canopy reflecting higher resource allocation toward enzymes associated with the 'dark' reactions of photosynthesis (Givnish 1988; Kull et al. 1995). Although there is no evidence in the primary literature, I expected that leaf P content would follow the same trend as N content in P-limited seagrasses. The plots of leaf C:N and C:P in this study do not suggest that *T. testudinum* selectively allocates nutrients along leaves in response to the canopy light gradient. Although the data are not overwhelming, higher ratios were consistently found at the tips of leaves. Furthermore, there was a general trend of higher ratios in older leaves.

The strongest evidence for within-leaf photoacclimation in *T. testudinum* is seen in the plots of Chl:N. Photoacclimation to lower irradiance is associated with reduction of the quantity of soluble protein relative to the total chlorophyll content, which is

reflected in lower Chl:N (Evans 1993). Nitrogen partitioning indicates the relative distribution of N toward light harvesting and electron transport activity as opposed to ATP synthesis and CO_2 carboxylation. Although Chl:N increased from base to tip along Rank 1 leaves at BANK and RKB, the ratios decreased along older leaves. This suggests that the leaves photoacclimated along their lengths as they aged and became more optimized to the canopy light gradient over time. The sites with short leaves and sparse short shoot densities (i.e. CFT and SPG) do not exhibit significant differences along leaves in the relative content of chlorophyll and nutrient content. Canopy-level photoacclimation is less important at these sites because the inter-canopy light gradient is insignificant due to the short leaves and low short shoot density.

The large disparity in Chl:N between BANK and RKB requires some explanation. Because of the negligible water depth, the light availability at BANK is much higher than at RKB. The common assumption would expect a lower Chl:N at the site with the higher light availability (Evans 1993; Givnish 1988). In fact, the Chl:N at BANK is considerably higher than RKB at all sections along leaves and is comparable to the Chl:N at CFT. This is despite the fact that CFT has a depth of 6 m compared to the 0.5 m depth at BANK. This indicates that despite the relatively high irradiance productivity at BANK is light-limited. This could only mean that nutrient availability at BANK exceeds the potential demand of the seagrass. Therefore, photoacclimation responses of plants are not driven solely by differences in light availability.

5.5.3 Future research

Measurement of the photosynthetic-irradiance relationship for seagrasses is typically accomplished by removing individual short shoots from the environment and performing analysis in the laboratory. An interesting research topic would be to examine the effect of the removal of light completion on individual *T. testudinum* short shoot. However, because *T. tetstudinum* is a clonal plant, the community production is a summation of the interconnected individual elements. Therefore, the effect of different light regimes on the photosynthetic status of a *T. tetstudinum* is best determined in the field. A potential field experiment may involve reducing the effects of canopy self-shading by physically bending surrounding short shoots away from an individual short shoot. This would greatly increase the light reaching the lower section of leaves. If the vertical variation in leaf chlorophyll content were simply due to the age structure of the leaves, there would be little change compared to control short shoots. However, if the chlorophyll content at the bottoms of the leaves were to decline significantly compared to control, this would be an indication of active photoacclimation of the leaf tissue to the higher light availability.

5.5.4 Conclusions

The data from this study show strong evidence for photoacclimation along *T. testudinum* leaves in Florida Bay. Other competing factors may influence the variation in photosynthetic characteristics along leaves that are principally age related. These age-related factors include interleaf variation in the state of senescence, break down from exposure to harsh environmental conditions, and prolonged exposure to super-saturating

light. The ability to photoacclimate along an individual leaf is an essential attribute that would help alleviate the effects of leaf self-shading in dense canopies.

Chapter 6. Examination of the interleaf variation in nutrient content in *Thalassia testudinum* leaves.

6.1 Abstract

This study examined the vertical variation of nutrient content along *T. testudinum* leaves at three sites in Florida Bay representing a gradient in nutrient availability. Decreases in the N and P leaves and between leaves of increasing age was used to estimate the amount of these nutrient resorbed by the plant before the leaves detach. Leaf resorption of N ranged from 19.5 to 24.1, while resorption of P ranged from 29.3 to 48.6. Resorption was higher at the sites that had the lowest nutrient availability, as indicated by C:N:P of the leaf tissue. Considering the extreme P-limited conditions found in Florida Bay, the recycling of P from older leaves could be an important process that helps this seagrass species meet its nutrient requirements for primary production and reduces its dependence on external sources

6.2 Introduction

The main macronutrients utilized in photosynthesis are carbon (C), nitrogen (N), and phosphorus (P), primarily available in the environment in the forms carbon dioxide (CO_2), ammonium (NH^{4+}), and phosphate (PO_4^{3-}) (Hall and Rao 1999). Sediment porewater is the primary source of N and P for seagrasses and seagrass leaves are usually the largest sink of nutrients in their systems (Hemminga et al. 1999; Stapel et al. 1996). Some studies have offered evidence that seagrasses may take up nutrients through their leaves directly from the water column, however, the importance of this is questionable in oligotrophic systems (Cornelison and Thomas 2004; Stapel et al.1996). An ecosystem is considered nutrient limited if the addition of a nutrient results in an increase in rate of net

primary production (Howarth 1988). Temperate seagrass meadows are often N limited while tropical systems are typically limited by P availability (Powell et al. 1989; Short 1987; Short et al. 1990). In severely nutrient limited seagrass meadows, nutrients are taken up immediately as they become available. Consequently, measurements of sediment nutrient concentrations do not accurately reflect nutrient availability because they do not account for the exceptionally fast turnover of the nutrient pools (Fourqurean et al. 1992).

The elemental content of plant tissue provides insight into the nutrient regime experienced by the plant during growth (Gerloff and Krombholz 1966). Plants that are N limited should have tissues depleted in nitrogen, likewise for P limitation. Scaling leaf N and P content to C content is a more useful technique commonly used to evaluate the relative limitations of these nutrients (Duarte 1990). The C:N and C:P of leaf tissue shifts from high to low as the supply of N and P meet plant demands (Howarth 1988). These ratios trend toward an optimum when resources other than nutrients are limiting. For example, Redfield (1958) found that the optimum C:N:P ratio of 106:16:1 for phytoplankton. Atkinson and Smith (1983) established an equivalent "Redfield" ratio for seagrasses of 550:30:1 suggesting that the amount of nutrients required to support a particular rate of primary production is substantially lower for marine plants than for phytoplankton (Atkinson and Smith 1983). Duarte (1990) found that the median elemental content of 27 species of seagrass from 30 different locations (1.8% for N and 0.20% for P) accurately separated nutrient limited seagrasses from those that would not

respond to nutrient enrichment. This corresponds to a C:N:P ratio of 474:24:1 or about 20% lower than the Atkinson and Smith optimum.

Nutrient resorption from senescing leaves is one of the most important functions performed by plants to conserve nutrients (Chapin and Kedrowski 1983). Nutrient resorption efficiency is defined as the percentage of nutrients reclaimed from leaf tissue and transported to new plant tissue or internal nutrient pools (Aerts 1996). The simplest method for estimating nutrient resorption from leaves is to measure the difference between the nutrient content of the leaf with the highest nutrient content with the nutrient content of the oldest leaf, which is usually the leaf with the lowest nutrient content (Killingbeck 1996). This method assumes that the decline in absolute nutrient content of aging leaves indicates nutrient resorption from the leaves. In the case of seagrasses, leaching of nutrients to the water column is considered insignificant (Stapel and Hemminga 1997). In addition, it is assumed that the nutrients remaining in the oldest leaf are lost when the leaf detaches. Active recycling of nutrients from seagrass leaves has been reported in a number of studies including some using isotope tracers (Lepoint et al. 2002; Stapel and Hemminga 1997; Hemminga et al.1999; Alcoverro et al. 2000).

Despite thriving in nutrient limited systems, seagrasses have not evolved efficient nutrient resorption or other strategies for conserving nutrients such as extended leaf life (Hemminga et al. 1999). Additionally, seagrasses can lose nutrients via hydrologic stress, leaf fragmentation, and leaching (Hemminga et al. 1999). The export of seagrass leaves has been found to be a significant input of nutrients to nearby coral reef systems highlighting the nutrient loss due to leaf abscission (Zieman et al. 1979). Hemminga et

al. (1999) found that nutrient resorption varied widely across different seagrass species and ecosystems averaging 20.4% for N and 21.9% for P. P resorption has been found as high as 51% in the tropical seagrass *Cymodocea rotundata* and as low as 0% for *Zostera marina* in temperate systems (Hemminga et al. 1999). There was often wide variability found within seagrass species sampled from different locations. For example, resorption of N in *Zostera marina* leaves ranged from 3.8% to 36% depending on location sampled (Hemminga et al. 1999). This may show the variability of nutrient resorption from seagrass leaves but may also reveal inconsistencies in the methods used to estimate nutrient resorption.

T. testudinum is the dominant primary producer in the shallow estuarine waters of Florida Bay (Zieman et al. 1989). Florida Bay, bounded by the Florida Keys to the south and east, the Everglades to the north, and the Gulf of Mexico to the west, contains one of the largest seagrass populations found anywhere in the world. It is believed that the marine waters of the Gulf of Mexico are the primary source of P to the bay (Fourqurean and Zieman 1992). The mean C:P ratio for *T. testudinum* leaf tissue in Florida Bay is 1070 and ranges from 448 to in the southwest to the 1271 in the northeast region suggesting a gradient in P-limitation that also follows a gradient in seagrass distribution and abundance (Zieman et al. 1989; Fourqurean et al. 1992). Despite this severe P-limitation, *T. testudinum* in Florida Bay achieves shoot densities and productivity rates as high as any found in the world (Zieman et al. 1989). This is especially remarkable since *T. testudinum* has a short leaf lifespan compared to other tropical seagrasses (Duarte 1991).

Considering the high primary production and dense meadows of *T. testudinum* in Florida Bay, it is hypothesized that nutrient resorption plays an important role in nutrient cycling in this system. However, no current data exist for nutrient resorption rates for *T. testudinum* leaves in Florida Bay. This study will use the methods from Stapel and Hemminga (1997) to estimate nutrient resorption in leaves of *T. testudinum* from sites along the nutrient availability gradient in Florida Bay. Within-leaf nutrient dynamics were further assessed by determining the vertical variation of elemental content (C, N, and P) along leaves.

6.3 **Methods**

T. testudinum short shoots were harvested during July 2005 from Rankin Lake (RAN), Duck Key (DUCK), and Barnes Key (BAR) (see Figure 2.2), which represent the southwest to northeast gradient in phosphorous availability in Florida Bay (Fourqurean et al. 1992). The sites were visited within the same week. Twelve healthy short shoots representative of the majority within the meadow were haphazardly selected from each site. The short shoots were stored submerged in seawater in a dark cooler, transported to the lab, and processed immediately.

Leaves were separated from the short shoots and ranked incrementally by age with the youngest leaf called Leaf 1, the next older leaf called Leaf 2, and so forth. Young leaves, usually less than 5 cm in length, with low pigmentation and undeveloped vascular structure were ranked as emergent leaves (Leaf e). Not all short shoots had a leaf that qualified as emergent. Leaves that were brown, noticeably fragmented, or partially detached were disregarded. Leaves were cleaned of epiphytes by scraping both

sides with a razor blade, submerging them in a 5% HCl solution for three minutes, then thoroughly rinsing with fresh water. The leaf was cut at the interface of the white non-photosynthetic sheath and the green leaf, which was then cut into multiple segments ranging from 5-7 cm long depending on the length of the leaf. The length and width of each segment was measured using calipers then frozen and freeze-dried. After weighing the dry tissue, the leaf segments were ground into a fine powder with a mortar and pestle and stored for later elemental analysis.

Total P content (% dry wt.) of the leaf segments was determined colormetrically using a variation of a hot acid extraction technique (McGlathery et al. 1994). Approximately 5 mg of dry tissue was used and duplicates were run when the quantity of sample allowed. Citrus leaf (%P = 0.137) standards were run, concurrently, to determine analytical error. The recovery of P in the reference standard was 96.1% using this method. The percent content of N and C in the leaf segments was determined utilizing a Carlo-Erba CHN analyzer. The total elemental content of each leaf segment was calculated by multiplying the percent elemental content for the segment with the corresponding segment dry weight. Total elemental content of each leaf was calculated by summing all of the segments for each leaf. Percent content of C, N, and P were converted to molar ratios (C:N, C:P, and N:P) using the respective molecular weights. The relationship between C:N and C:P ratios of the leaf segments and their N and P content were examined using regression analyses. The statistical significance of within-leaf and between-site differences were analyzed using one-way ANOVA and Tukey's multiple comparison tests.

Resorption of nitrogen and phosphorous from leaves was estimated using a variation of the methods of Stapel and Hemminga (1997) where the decrease in nutrient content between leaves of increasing age on a single short shoot was assumed to be due to translocation from leaves toward new growth or the plant nutrient pool. The Stapel and Hemminga method calculated the percent resorption of nitrogen and phosphorous using the following equation:

$$\%R = \frac{K_{\max} - K_j}{K_{\max}} * 100\%$$

%R is the percentage of the maximum leaf nutrient content that is resorbed, K_{max} is the nutrient content (mg N or P) of the leaf, and K_j is the content of the tip of the oldest leaf. Resorption was calculated with and without including the sheath as part of the total leaf nutrient pool. Between-site comparisons were conducted using one-way ANOVA. All statistical analyses in this study were conducted using SAS software, version 9.1 (SAS Institute Inc., Cary, NC, USA).

6.4 **Results**

6.4.1 Site characteristics

The general morphology of the *T. testudinum* short shoots at the three sampling sites differed significantly in leaf length but not number of leaves per short (Table 6.1). No short shoots from DUCK had a leaf categorized as Leaf 4, however, many had leaves ranked as emergent. While BAR and RAN both had some short shoots that possessed Rank 4 leaves, these particular short shorts did not have leaves that fell into the emergent category. Short shoots at BAR contained the longest leaves for all leaf ranks except for

the emergent leaves. Usually leaves did not reach full length until reaching Leaf 2 status, however, the length of the oldest leaves (Leaf 3) at BAR were shorter than the younger mature leaves (Leaf 2). This difference was not due to fragmentation of leaf tips. The dense leaf canopy at BAR (>1500 SS m^{-2}) and RAN (~1000 SS m^{-2}) was dense enough to conceal the sediment surface. The sediment at these sites was comprised mostly of dead organic matter and carbonate mud with an extensive layer of leaf litter. DUCK had less than 500 SS m^{-2} with sediment comprised mostly of coarse carbonate sand and less leaf litter. No seagrass species other than *T. testudinum* were present at any of the sites.

6.4.2 Leaf attributes

There were statistically significant differences ($P<0.0001$ for all) between sites in specific leaf weight (SLW), elemental contents, and elemental ratios when pooling data for all leaves and segments (Table 6.2). SLW (mg leaf dwt cm^{-2}) was highest at DUCK at 4.91, which was 35% higher than at RAN (3.65) and 45% higher than at BAR (3.38). All elemental contents are expressed as the mean percentage of dry weight ± 1 SE. C content was 40.0±0.29 at BAR and 36.7±0.20 for RAN both above the mean for all seagrass of 33.6±0.31 (Duarte 1990). Leaf C content at DUCK was 33.6±0.24 corresponding closely with the mean for seagrasses. The low mean C content at DUCK is noteworthy because DUCK also had lower N and P content than the other sites and the relative contribution of C is expected to be higher in tissue depleted in N or P. Leaf N content varied significantly ($P < 0.001$) between sites the highest, 2.22%, found at RAN followed by 2.10% at BAR and 1.90% at DUCK. All of these values were in line with the mean N content for seagrasses of 1.92% (Duarte 1990). The P contents for the

Florida Bay sites were well below the seagrass wide mean of 0.23±0.11 (Duarte 1990) reflecting P-limitation of Florida Bay. Leaf P content at BAR and RAN, 0.087±0.002 and 0.10±0.003 respectively, compared closely to the average for *T. testudinum* in Florida Bay of 0.11 while the P content at DUCK, 0.059±0.003, was well below the seagrass average and on the lower end of the frequency distribution of P content in Florida Bay (Fourqurean and Zieman 2002). Leaf C and P contents showed strong statistically significant relationships with the corresponding C:N and C:P ratios for all the sites (Figure 6.1). The best regression fit for the N content versus C:N plot was a logarithmic function. The regressions for DUCK and RAN overlapped and had high R^2 values of 0.88 and 0.89, respectively. The regression plot for BAR was noticeably separated from the other two and had a lower R^2 of 0.66. The plots of P content versus C:P ratio showed an extremely close relationship via a power function. Again, the data points for DUCK and RAN trended closely together and had higher R^2 values (R^2 = 0.95 and 0.97), than for BAR (R^2 = 0.91). In the N plot, the data points were clustered between 1.5% and 2.5% N content and C:N of approximately 15 to 25. This contrasts with for phosphorous where the data points were well distributed.

Table 6.1. Short shoot and leaf morphological characteristics for *Thalassia testudinum* short shoots from three sites in Florida Bay, BAR: Barnes Key Basin, DUCK: Duck Key, and RAN: Rankin Key. Values are means ±1 SE. Values within each column with different letters are significantly different (P<0.05) using one-way ANOVA and Tukey's post-hoc comparison.

Site	# Leaves / Short Shoot	Leaf lengths (cm) by Leaf Rank				
		e	1	2	3	4
BAR	3.4±0.2 a	4.6±0.5 b	23.6±2.0 a	40.9±1.7 a	37.4±2.5 a	31.5±6.3 a
DUCK	3.4±0.2 a	5.4±0.7 a	14.4±0.9 b	16.1±0.7 c	14.5±1.1 c	NA
RAN	3.9±0.1 a	5.7±0.2 a	16.3±1.9 b	24.0±1.4 b	25.0±1.7 b	26.0±3.3 a

Table 6.2. Mean (± 1 SE) leaf elemental content and elemental ratios in leaves of *Thalassia testudinum* from sites in Florida Bay. Values within each row with different letters are significantly different (P<0.05) using one-way ANOVA and Tukey's post-hoc comparison.

Leaf Attributes	Site		
	BAR	DUCK	RAN
SLW (mg cm^{-2})	3.38±0.04 c	4.91±0.08 a	3.65±0.06 b
% C	40.0±0.29 a	33.6±0.24 c	36.7±0.20 b
% N	2.10±0.02 b	1.90±0.04 c	2.22±0.03 a
% P	0.087±0.002 b	0.059±0.003 c	0.101±0.003 a
C:N	22.6±0.21 a	21.6±0.44 b	19.9±0.27 c
C:P	615.3±18.6 c	1907.6±178.5 a	1099.9±42.4 b
N:P	60.1±1.6 b	87.2±6.8 a	54.5±1.6 b

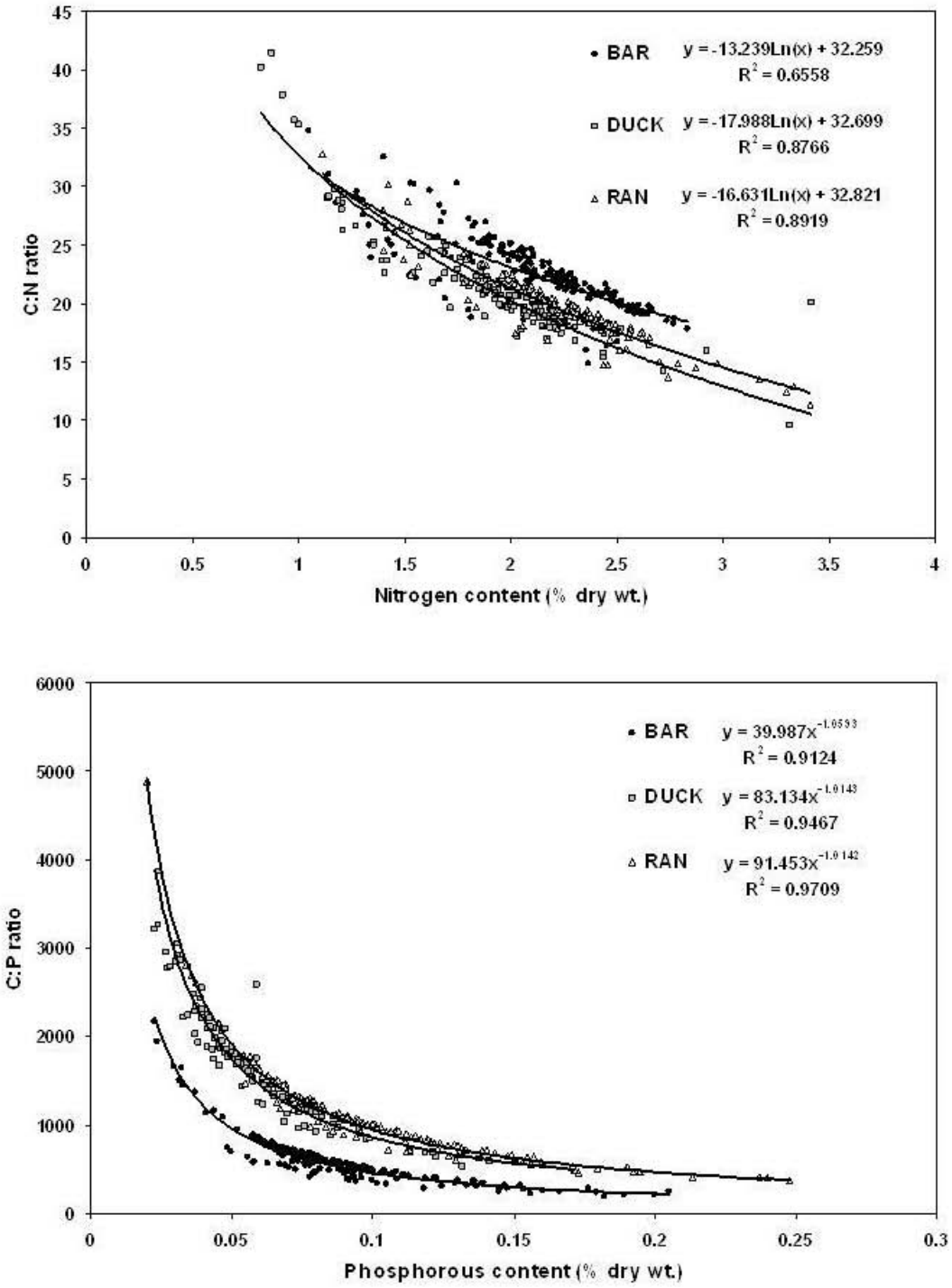

Figure 6.1. Relationship between C:N and C:P ratios and the nitrogen and phosphorous contents of *T. testudinum* leaves. Best regression line fit for nitrogen relationship was a logarithmic function while the best fit for phosphorous relationship was a power function.

Figure 6.2 shows the within-leaf variation in SLW along leaves of successive ranks. Emergent leaves were excluded but in all cases had lower SLW than the next older leaf rank reflecting their underdeveloped leaf vascular structure. DUCK had the highest SLW ranging from 4 to 6 mg cm^{-2} along leaves while BAR and RAN were comparable ranging from 3 to 4 mg cm^{-2} along leaves. Typically, SLW increased from the sheath to the leaf base then decreased toward the tip in Leaf 1. This was also the case for older leaves except for those at BAR where Leaf 2 was heaviest at the sheath and then decreased toward the tip. However, an increase in SLW toward leaf tips was seen in Leaf 2 and Leaf 3 at BAR. Typically, SLW increased with leaf age for all sites.

Figure 6.3 shows the within-leaf variation of C, N, and P content (as % of total dry weight of segment) in successive leaf ranks for each sampling site. C content increased sharply from sheath to leaf base for all leaf ranks generally followed by an upward trend toward leaf tips. N content typically increased from base to tip along younger leaves and decreased at the tips of older leaves. The higher N content in the sheaths of young adult leaves (Leaf 1) at DUCK and RAN may be partly attributed to the small concentration of chlorophyll in some sheaths of juvenile leaves. The N content of sheaths decreased with leaf age at all sites. There was little difference seen in N content between sites in the green portion of Leaf 1 and 2, however, DUCK had noticeably lower N along Rank 3 leaves. In Leaf 1, the highest P content was found in sheaths followed by a decline toward the tips. In mature leaves, P content was generally slightly lower in sheaths and declined sharply towards tips. P content decreased across all leaves with increase in leaf age.

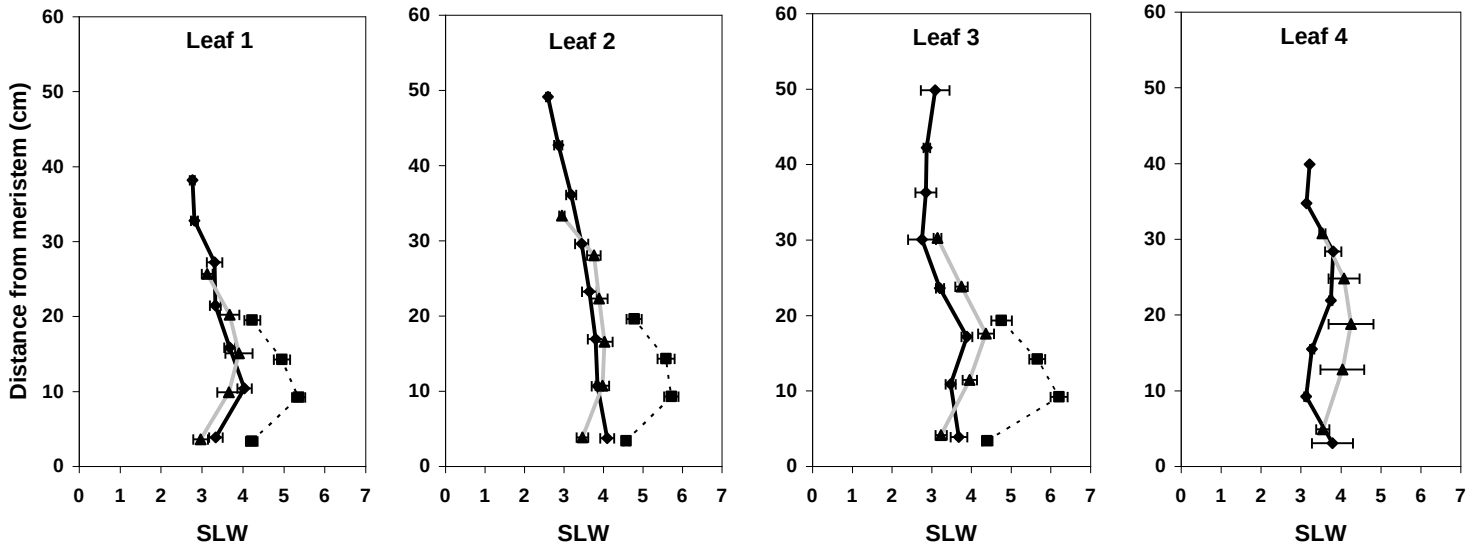

Figure 6.2. Within leaf variation of specific leaf weight (mg/cm^2) in *Thalassia testudinum* leaves of increasing age from three sites in Florida Bay. The bottom value for each leaf represents the leaf sheath. Sites represented as follows: BAR, bold black line with circles; DUCK, broken black line with squares; RAN, grey line with triangles. Bars are standard errors.

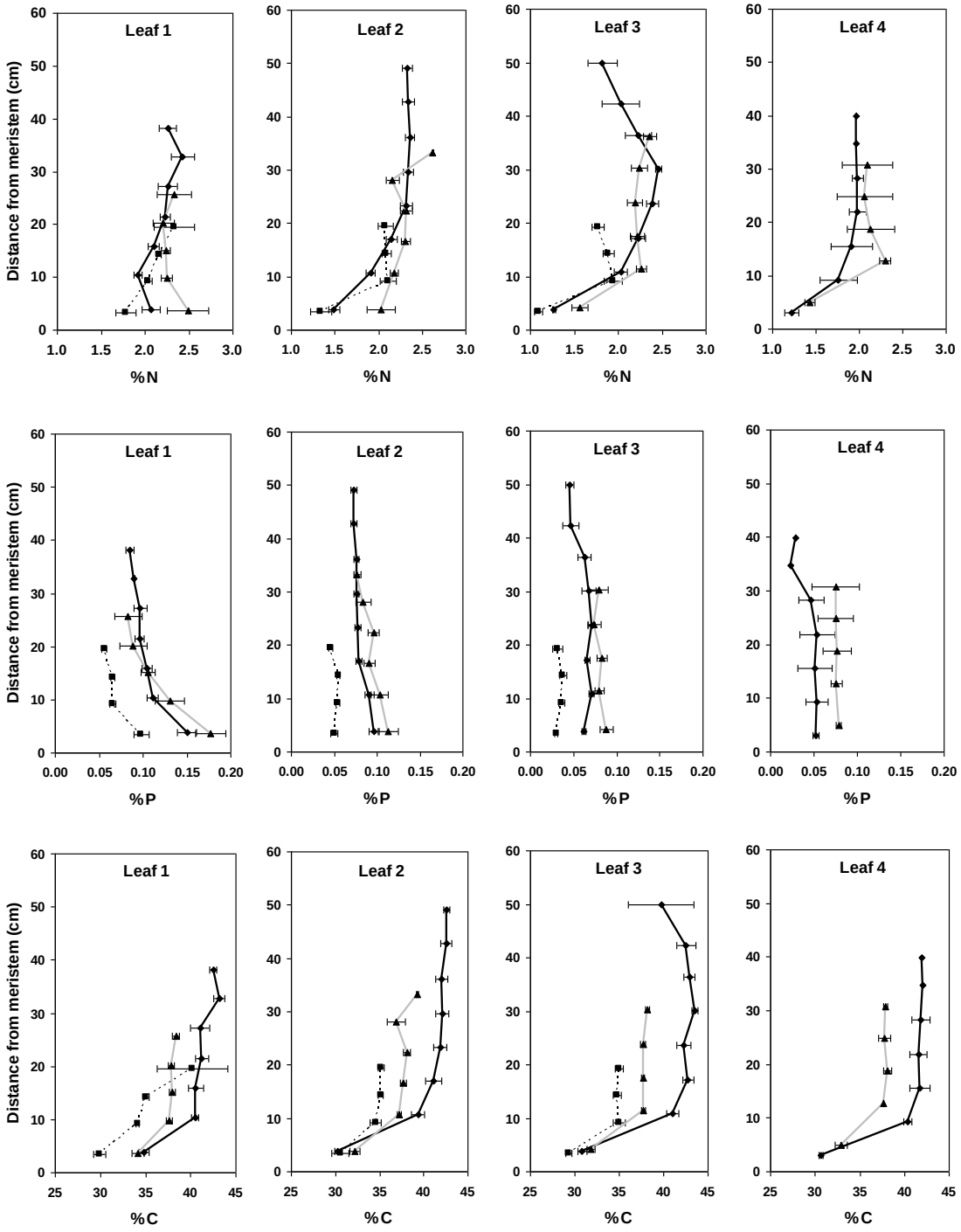

Figure 6.3. Within leaf variation of C, N, and Ps content (% dwt.) in *Thalassia testudinum* leaves of increasing age. Bottom values for each leaf represents the leaf sheath. Sites represented as follows: BAR, bold black line with circles; DUCK, broken black line with squares; RAN, grey line with triangles. Bars are standard errors.

Total N content (μg) within relative locations for leaves of increasing ages, expressed as the mass of N in a 1 cm^2 section of leaf, is shown in Table 6.3. Leaves were compared at relative vertical locations starting with the leaf sheath, the base or lowest segment of the green leaf, the middle, defined as the segment just below the segment at the tip, and the top segment or leaf tip. Because *T. testudinum* leaves exhibit basal growth, new tissue forms at the meristem located at the top of the short shoot and bottom of the sheath. This makes between leaf comparisons problematic because the younger leaf may still be growing and receiving substantial nutrient inputs from the plant. These data should be interpreted with this in mind.

In most cases total N increased with increasing leaf age. The exception was for the leaf bases at DUCK and RAN where total N increased from Leaf 1 to Leaf 2. Resorption from leaves was calculated for each vertical segment location as the absolute change in N between the segment with the highest total and the corresponding segment in the oldest leaf. Emergent leaves were not used in resorption calculation even though they occasionally had higher total N. Resorption was considerably higher for leaf sheaths than for the green leaf segments with $\%R_N$ ranging from 28.3 at RAN to 36.9 at DUCK while leaf tips had higher $\%R_N$ than leaf bases ranging from 0 to 17.1. Table 6.4 shows the total P (μg) found in one cm^2 of leaf tissue at relative locations along leaves of increasing age. The highest total P, ignoring emergent leaves, was always found in Leaf 1 and the lowest in the oldest leaves making resorption calculations straightforward. For all sites, the highest $\%R_P$ was found in the sheaths, ranging from 45.9 at RAN to 68.4 at DUCK, and declined toward leaf tips.

Table 6.3. Total nitrogen (µg) in one cm^2 of leaf tissue at locations along successive leaf ranks in *Thalassia testudinum* from three sites in Florida Bay. Values calculated by multiplying N content (% dry wt. x 100) by specific leaf weight (µg cm^{-2}). Resoprtion was calculated as the percent change between the nutrinet content of the leaf rank with highest nutrient content and nutrient content of the oldest leaf. Asterices indicate the values used for the calculation of $\%R_N$.

	Segment	Leaf Rank					
Site	Location	e	1	2	3	4	$\%R_N$
BAR	Sheath	50.5±7.0	68.4±4.1*	61.7±4.3	47.0±3.4*		31.3
	Base	58.4±4.4	77.5±3.1*	73.2±2.8	69.6±3.0*		10.2
	Middle		73.4±2.8*	71.7±2.2	65.3±5.6*		11
	Tip		68.1±3.3*	64.4±2.3	57.4±5.0*		15.7
DUCK	Sheath	79.7±7.1	75.3±5.6*	61.2±5.5	47.5±2.5*		36.9
	Base	83.3±4.9	109.0±2.5	120.7±6.3*	120.1±7.4*		0.5
	Middle		110.8±3.7	116.1±2.6*	108.3±9.1*		6.7
	Tip		99.8±8.4*	96.1±3.3	90.3±4.0*		9.5
RAN	Sheath	75.8±6.0	74.6±8.1*	70.6±6.9	51.0±4.2*	50.6±2.4	28.3
	Base	99.0±19.1	82.3±6.8	86.5±3.9	89.5±4.3	93.0±12.7	na
	Middle		91.6±6.6*	88.0±4.3	86.5±3.9*	82.9±7.3	5.8
	Tip		73.9±2.7	81.9±2.9*	67.8±2.6*	67.9±6.6	17.1

Table 6.4. Total phosphorous (µg) in one cm^2 of leaf tissue along leaves of *Thalassia testudinum* in Florida Bay. Values calculated by multiplying P content (% dry wt. x 100) by specific leaf weight (µg cm^{-2}) for each segment location. Resorption was calculated as the percent change between the nutrinet content of the leaf rank with highest nutrient content and nutrient content of the oldest leaf. Asterices indicate the values used for the calculation of $\%R_p$.

Site	Segment Location	Leaf Rank e	1	2	3	4	$\%R_p$
BAR	Sheath	4.48±0.55	4.96±0.37*	3.9±0.26	2.30±0.15*		53.6
	Base	4.07±0.34	4.50±0.30*	3.50±0.22	2.49±0.17*		44.7
	Middle		3.22±0.17*	2.31±0.12	1.77±0.30*		45.0
	Tip		2.52±0.14*	2.09±0.11	1.66±0.29*		34.1
DUCK	Sheath	4.69±0.36	4.12±0.35*	2.28±0.19	1.30±0.11*		68.4
	Base	3.28±0.16	3.49±0.20*	3.09±0.13	2.24±0.19*		35.8
	Middle		3.49±0.12*	2.94±0.14	2.20±0.43*		37.0
	Tip		2.40±0.10*	2.22±0.13	1.67±0.19*		30.4
RAN	Sheath	4.17±0.52	5.19±0.53*	3.92±0.48	2.90±0.35	2.81±0.18*	45.9
	Base	6.66±1.04	4.71±0.72*	4.11±0.43	3.17±0.29*	2.98±0.16*	36.7
	Middle		3.92±0.28*	3.60±0.22	3.24±0.25*	3.12±0.75*	20.4
	Tip		2.87±0.33*	2.77±0.21	2.24±0.26	2.35±0.82*	18.1

6.4.3 Nutrient resorption

Table 6.5 shows the N and P resorption calculated using entire leaves as in Stapel and Hemminga (1997). These estimates were calculated differently than for the within leaf variations found in Table 6.3 and Table 6.4 where segment total N and P were pooled before calculating resorption. Whole leaf resorption was estimated by calculating the change in total N and P across leaves for each short shoot separately. This allowed assessment of within site variability and for statistical comparison between sites. Resorption was also calculated considering the green leaf and sheath together and for the green leaf only. There was no significant difference found between the two methods. Only the values for leaf and sheath calculations will be discussed.

The highest $\%R_N$ (mean ± SE) was found at BAR at 24.1±5.7 followed by 19.5±5.7 at DUCK and 20.3±5.3 at RAN. The highest $\%R_P$ was found at DUCK at 48.6±4.7 where the highest P limitation, suggested by a C:P of 1908, was also found. The $\%R_P$ for BAR and RAN were 33.4±6.7 and 29.3±6.4, respectively. The average $\%R_N$ found in this study, 20.3±3.0, compared closely with the average value found for all seagrasses of 20.4 (Hemminga et al. 1999). However, the average $\%R_P$ found for the three Florida Bay sites in this study, 36.9±3.7, was 68% higher than the seagrass wide average of 21.9 reported by Hemminga et al. (1999).

Table 6.6 compares the C, N, and P of live leaf sheaths with those of old sheaths that have remained attached to the short shoot after their green leaf sections have broken off. Old sheaths had significantly higher C content than live sheaths at BAR ($P<0.001$), 30.8 versus 36.2, but not significantly higher at DUCK ($P = 0.33$) and RAN ($P = 0.25$).

N content was significantly lower in old sheaths at DUCK (P = 0.01) and RAN (P = 0.03) showing declines of 20.2 and 17.5, respectively while BAR showed only a modest decline of 7.2% (P = 0.09). P content was significantly lower in old sheaths at all three sites. The decline was highest at DUCK (P < 0.001) where P content declined 67% from 0.030 to 0.010. RAN declined by 58.1% (P = 0.01) while BAR declined by 30% (0.04). Interestingly, the P content of the old sheaths at BAR and RAN was higher than the P content found in mature sheaths at DUCK.

Although these data are well below the resorption efficiencies found for terrestrial grasses, $\%R_N$ of 58.5 and $\%R_P$ of 71.5 (Aerts 1996), the $\%R_P$ of 48.6% found at DUCK was more than twice the average for seagrasses reported by Hemminga et al. (1999). The only other seagrass close to this level of $\%R_P$ was *Cymodocea rotundata* from Indonesia with 51%. Strong resorption of P from older leaves would be a great advantage for plants in a highly P-limited system such as those at DUCK. is consistent with the of 0.059% was considerably lower than that found for the *C. rotundata* leaves, which ranged from 0.26% in young leaves to 0.09% in the oldest leaves.

Table 6.5. Nutrient resorption (%) in leaves of *Thalassia testudinum* from three sites in Florida Bay. Resorption was calculated by summing the N and P within leaves both with and without including the sheaths. Resorption was calculated separately for each short shoot as opposed to the pooling of leaves as in the method of Stapel and Hemminga (1997). No significant differences were found between sites using one-way ANOVA ($P<0.05$).

	Leaf and sheath		Leaf only	
Site	$\%R_N$	$\%R_P$	$\%R_N$	$\%R_P$
BAR	24.1±5.7	33.4±6.7	25.4±6.7	36.0±7.0
DUCK	19.5±5.7	48.6±4.7	20.4±6.5	43.3±7.0
RAN	17.7±5.3	29.3±6.4	15.0±6.0	29.7±7.0
Combined	20.3±3.0	36.9±3.7	20.0±3.6	36.3±4.1

Table 6.6. Carbon, nitrogen, and phosphorous concentration in the living leaf sheaths and sheaths with detached leaves that remain attached to the short shoot. Live sheaths are from the oldest living leaves of a short shoot. Old sheaths are from the more recent detached leaves. Sites with different letters indicates significant differences ($P<0.05$) using one-way ANOVA and Tukey's post-hoc comparison. Values in bold indicate significant differences between live and old sheaths using t-tests ($P<0.05$).

% element content	Site	Mature sheaths	Old sheaths	% change
%C	BAR	30.8±0.42ab	36.2±0.82a	**17.5**
	DUCK	29.3±0.32b	31.8±1.55b	8.5
	RAN	32.1±0.41a	33.7±1.34ab	5
%N	BAR	1.25±0.02b	1.16±0.04a	-7.2
	DUCK	1.09±0.05c	0.87±0.04b	**-20.2**
	RAN	1.53±0.07a	1.26±0.08a	**-17.6**
%P	BAR	0.060±0.002b	0.042±0.01a	**-30**
	DUCK	0.030±0.002c	0.010±0.00c	**-66.7**
	RAN	0.086±0.006a	0.036±0.01b	**-58.1**

6.5 **Discussion**

This study presents evidence that *T. testudinum* leaves can actively resorb N and P from leaves before abscission. Given the extreme P-limited conditions found in Florida Bay, the recycling of P from older leaves could be an important process that helps this seagrass species meet its nutrient requirements for primary production and reduces its dependence on external sources (Hemminga et al. 1991). This could be a key adaptation that provides *T. testudinum* with a competitive advantage that controls its distribution in this ecosystem.

*T. testudinu*m follows a similar life cycle as other plants. Generally, plant leaves experience three life stages: 1) adolescence, a period of growth, 2) maturity, a period of constancy of photosynthetic traits, and 3) senescence (Hill 1980). In newly emergent leaves, leaf growth is supported by nutrients and glucose transport from older leaves or reserves in rhizomes. As the leaf elongates it reaches a point where it can maintain itself and transport of external nutrients and glucose ceases. Upon reaching maturity, a leaf shifts from a sink to a source of photosynthate to the rest of the plant and the direction of flow in the phloem reverses direction. Eventually, the leaf begins a gradual deterioration as leaf nutrient and chlorophyll content continues to decline ending with the death and abscission of the leaf. Our results suggest that the resorption process in T. testudinum leaves begins even before a leaf reaches full maturity. The total N and P declined from Leaf 1, which is still elongating, to Leaf 2. This indicates that nutrient decline in T. testudinum leaves is independent of senescence and is a process that occurs throughout the life of a leaf.

In terrestrial deciduous species, leaf senescence occurs as an endogenously controlled degenerative process starting with the retranslocation of nutrients and ending with the death of the leaf (Noodén 1988). Senescence in deciduous plants usually begins abruptly and proceeds quickly. Most evergreen species do not experience this internally programmed senescence but instead experience gradual degeneration processes driven by exogenous factors associated with aging (Noodén 1988). The triggering mechanism for nutrient resorption in seagrasses may be influenced by changes in the nutrient demand of the short shoot meristem as it diverts resources to the growth of new tissue.

It may seem inefficient for a plant to transport nutrients into a leaf, which are then promptly remobilized and transported out of the leaf as may be the case with *T. testudinum* in this study (Hill 1980). Nutrient remobilization and resorption is an energy intensive process so the benefit must outweigh the cost. There may be an alternative explanation for the decline in N and P in *T. testudinum* leaves as opposed to active translocation of nutrients associated with senescence. The resorption of nutrients from *T. testudinum* leaves may be more of a passive process as opposed to the active translocation assumed in some studies. Nutrient decline in leaf tissue may be attributed to transport of N and P compounds within the phloem solute. While mainly composed of sucrose, phloem solute also contains a relatively high concentration of amino acids and phosphate (Hall and Baker 1972; Chapin et al. 1990). This would explain why the decline starts early in the maturity of the leaves. The rapid remineralization of organic nutrients from decomposing seagrass leaves may be a more efficient method of recycling seagrass leaf nutrients than the energy intensive process of resorption from attached

leaves. Resorption efficiency may be more important in systems with slow leaf turnover not necessarily low N or P availability.

Studies of *T. testudinum* leaves often ignore the role of the non-photosynthetic leaf sheath. This study showed that sheaths contain a substantial amount of N and P. Additionally, nutrient resorption from sheaths is higher than for the green parts of leaves. The decline in N and P in sheaths cannot be explained as upward transport to the leaf because the N and P of this tissue are declining at this point. The higher nutrient decline from sheaths is more likely due to the shorter distance and thus lower energy required for transport and the close proximity of the sheath to the basal meristem.

A *T. testudinum* leaf detaches from the short shoot at the interface with the sheath because sheaths contain high lignin content so they can remain attached to the short shoot for a considerable amount of time. There is significant difference between the total N and P within live sheaths and dead sheaths. However, it is not known if or for how long nutrient resorption continues from sheaths after leaf abscission. Although it is evident that the N and P content of older sheaths could be a substantial source for the nutrients, some of this decline is likely due to leakage to the water column and the start of decomposition as opposed to internal transport.

The methods used in this study to estimate leaf nutrient resorption have several potential sources for error. This method assumes that leaves experience no leaching to the water column, however, up to 3% of the nutrient loss in leaves can be attributed to leakage from leaves (Penhale and Thayer 1980). Additionally, the leakiness of leaves probably increases with age due to hydrodynamic stresses and damage from grazers.

Other studies have found evidence of significant uptake of N and P by seagrass leaves (Borum et al. 1989; Brix and Lyngly 1985; Gras et al. 2003). It is unlikely that any measurable leaf uptake of nutrients occurs in the extremely nutrient limited water of Florida Bay.

The C:N:P ratios of seagrass leaves may reflect not only the environmental conditions during growth but also the biological processes after the leaf reaches maturity (Yamamuro et al. 2004). Even though *T. testudinum* leaves have a relatively short life span the individual leaves on a short shoot may still have grown under significantly different environmental conditions (Duarte 1991; Durako 1994). The distribution, productivity, and standing crop of *T. testudinum* in Florida Bay show significant seasonal variability (Fourqurean et al. 2001) and the C:N:P of the leaf tissue also reflects the variation in nutrient demand (Powell et al. 1989) . Given the period of the sampling of this study, i.e. late summer, it is less likely that seasonal differences in growth rate attributed to any variation in leaf nutrient content. However, nutrient availability in the system reaches a low at late summer as areal productivity peaks.

PART IV. EXAMINATION OF THE VERTICAL VARIATION OF PHOTOSYNTHETIC ACTIVITY IN SEAGRASS LEAVES USING CHLOROPHYLL FLUORESCENCE.

Chapter 7. Examination of photosynthetic activity in *Thalassia testudinum* leaves using chlorophyll fluorescence.

7.1 Abstract

This study examined the interleaf variation of photosynthetic performance along *T. testudinum* leaves via chlorophyll fluorescence analysis. The Heinz-Walz Diving-PAM was utilized to measure dark-adapted maximum quantum yield (F_v/F_m) and to perform rapid light curves yielding measures of photosynthetic performance (ETR_{max} and alpha). F_v/F_m and alpha declined while ETR_{max} increased base to tip of the leaves indicating interleaf photoacclimation through the seagrass canopy. Comparisons were also made between deep and shallow canopies and between nutrient-enriched and control plots, which showed that *T. testudinum* photoacclimates along a light gradient and in response to nutrient availability. The study also showed a strong correlation between dark-adapted fluorescent measurements and leaf photosynthetic attributes including chlorophyll content, specific leaf weight, leaf thickness, and light absorbance.

7.2 Introduction

In order to fully understand the vertical variability of photosynthesis through a seagrass canopy, we must consider the amount of light available to the leaves, the ability of a leaf absorb the available light, and the ability of the leaves to utilize the absorbed light in photosynthesis. For a plant to utilize light energy absorbed by the light harvesting complexes, the chlorophyll excitation energy must move through the electron transport chain to the photosynthetic reaction centers and result in photochemical energy

conversion (Krause and Weiss 1991). However, not all light absorbed by the light harvesting complexes follows this path. A portion is lost or dissipated by other mechanisms related to inherent inefficiencies in the photosynthetic process (Krause and Weiss 1991).

The ratio of absorbed photons that are utilized in photosynthesis to the total photons absorbed is called quantum efficiency, or quantum yield, and is expressed as a unitless ratio from zero to 1.0 (Hall and Rao 1999). Optimal quantum yield is typically around 0.80 to 0.84 for higher plants and as high as 0.90 for some algae (Beer and Björk 2000; Demmig and Björkman 1987). Stress factors such as heat, cold, drought, pollutants, nutrient deficiency, or natural senescence can cause decreases in effective quantum yield (Genty et al. 1989).

Quantum yield is also highly sensitive to the near-term light history of the plant leaf (Krause and Weiss 1991). The quantum yield under ambient light conditions is called the effective quantum yield while maximum potential quantum yield is achieved after a leaf has been subjected to a period of darkness (Durako and Kunzelman 2002). Investigating variations in quantum yield has become an important method for evaluating the physiological state of the photosynthetic apparatus of seagrass leaves (Belshe et al. 2007; Silva and Santos 2004; Beer and Björk 2000; Ralph et al. 1998; Ralph and Burchett 1995).

7.2.1 Chlorophyll fluorescence

The most common way to assess the quantum yield in green plants is by analyzing chlorophyll fluorescence (Krause and Weis 1991). Chlorophyll fluorescence

arises from the deactivation of an excited chlorophyll molecule (Maxwell and Johnson 2000). Each photon of light absorbed by a chlorophyll molecule raises an electron in the chlorophyll molecule from a ground state to one of two excited states (Hall and Rao 1999). An excited state is when an electron traveling around the porphyrin ring of a chlorophyll molecule rises to a higher orbital. There are specific differences in the energy between the ground state and the excited states of chlorophyll molecules (Hart 1988). For the increase in orbital to be achieved, the energy of the photon must match the difference in energy between the ground state and either of the two excited states. A red photon moves a chlorophyll molecule to excited state 1, while a blue photon with higher energy excites a chlorophyll molecule to excited state 2 (Hall and Rao 1999). This along with a range of suborbitals results in the indicative chlorophyll absorption spectrum (Hart 1988). An excited state is unstable and quickly returns to the ground state releasing the absorbed energy in the process (Krauss and Weiss 1991).

There are three competing pathways for the energy released from an excited chlorophyll molecule. The energy can be transferred to other chlorophyll molecules, dissipated as heat, or reemitted as a photon with longer wavelength and lower energy (Figure 7.1). This reemission of light energy is called chlorophyll fluorescence (Hall and Rao 1999). Most of the light energy absorbed by a leaf is utilized in photosynthesis or dissipated as heat, but approximately 1-3% of the absorbed light is reemitted, or fluoresced, as red and far-red light from 660-760 nm (Krause and Weis 1991). Generally, fluorescence is highest when photochemistry is lowest (Genty et al. 1989). Most chlorophyll fluorescence is emitted by Photosystem II (PSII), which extracts

electrons from water and feeds them to the electron transfer chain connecting the two photosystems (Maxwell and Johnson 2000).

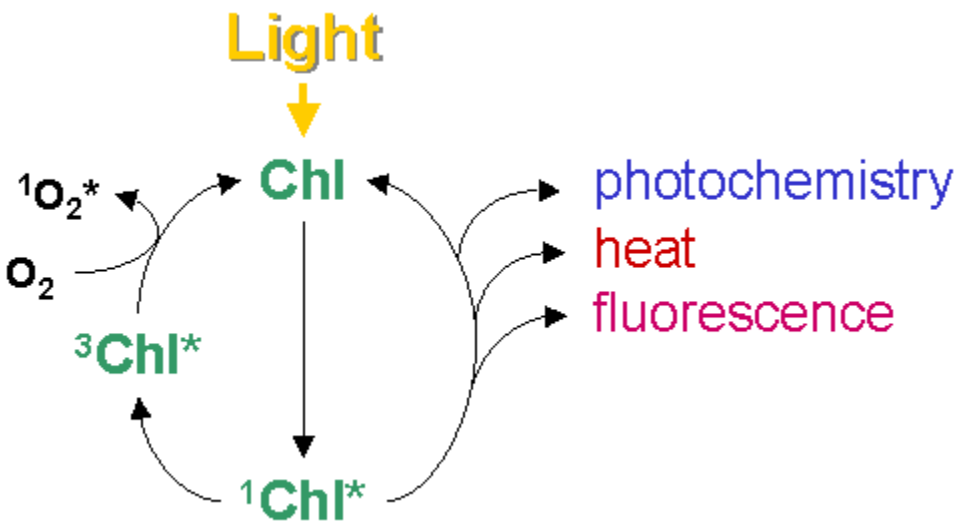

Figure 7.1 Schematic illustration of primary energy conversion in photosynthesis, which governs *in vivo* chlorophyll fluorescence.

Starting from darkness, the fluorescent signal from leaf in vivo increases rapidly after exposure to a light source then slowly decreases to a steady state. This initial fluorescence curve (Figure 7.2), termed the *Kautsky effect* after its discoverer (Kautsky and Hirsch 1931; Govindjee 1995), corresponds to the gradual ramp up of CO_2 uptake (Govindjee 2004). Once the enzymes involved in the dark reactions of carbon metabolism become fully light activated, the light energy is consumed in photochemistry and fluorescence decreases (Krause and Weiss 1984). This progressive ramp up of

photosynthesis, which can take minutes to hours depending on the species, is called the induction period (Chazdon and Pearcy 1986).

The term quenching refers to any process that lowers the fluorescence signal (Maxwell and Johnson 2000). A decrease in fluorescence due to an increase in the light energy transferred to photochemistry is call photochemical quenching, while non-photochemical quenching refers to all other sources including heat and photoinhibitory responses (Krause and Weis 1991). Non-photochemical quenching is the primary mechanism that allows plants to dissipate excess light energy and prevent damage to the photosynthetic apparatus (Krause 1988).

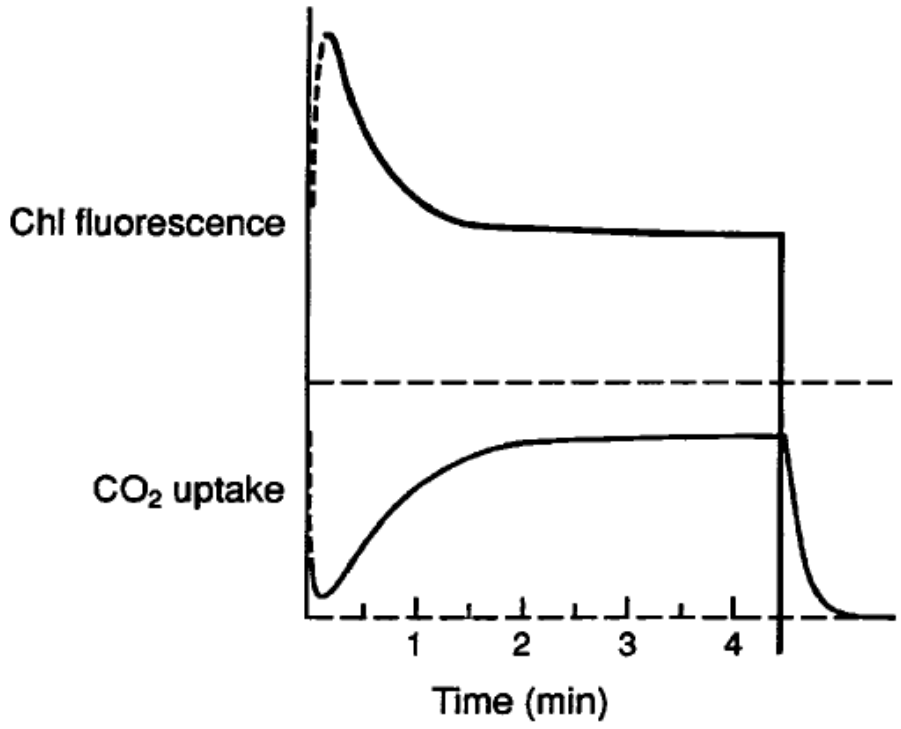

Figure 7.2. Chlorophyll fluorescence induction curve, the Kautsky Effect (Govindjee 2004).

7.2.2 PAM Fluorometry

Following the discovery of the relationship between chlorophyll fluorescence and photosynthesis, considerable interest grew in the scientific community for developing methods to measure it. For many years, the use of fluorescence as an analytical tool was confined to basic biophysical research because there was only limited instrumentation to measure the fluorescent signal and no methodology to interpret the data (Schreiber 2004). Chlorophyll fluorescence emissions extend from 660 nm to 760 nm, and if shorter wavelength excitation light is used, separation of fluorescence from the measuring light is readily achieved with the help of optical filters (Schreiber et al. 1986). The main problem was the inability to segregate the fluorescent signal from the ambient light.

Today, the most common system for measuring chlorophyll fluorescence is the pulse amplitude modulated (PAM) technology (Schreiber et al. 1986). To understand the principle of PAM, it is important to distinguish between fluorescence intensity and fluorescence yield. Fluorescence intensity varies several orders of magnitude depending on the intensity of the ambient light but offers only arbitrary value in photosynthetic research. Fluorescent yield is the difference between the fluorescent intensity under ambient light and the fluorescent signal after a pulse of saturating light fully reduces all photosynthetic reaction centers. Fluorescent yield indicates important information about the state of the photosynthetic apparatus (Krause and Weiss 1991).

To measure fluorescent yield the detection system must distinguish between fluorescence resulting from excitation from the measuring light and the much stronger

signals from ambient light. In a PAM fluorometer, an actinic light source is switched on and off rapidly, or modulated, a sensor is calibrated to only detect fluorescent signals matching this frequency (Heinz-Walz 1988). This allows the measurement of fluorescence in the presence of background light and especially under natural light conditions (Maxwell and Johnson 2000). A PAM fluorometer effectively measures the fluorescence emanating from Photosystem II offering an intrinsic probe of the state of photosynthetic apparatus at a sub-molecular level (Krause and Weis 1984).

7.2.3 The fluorescence parameters

A PAM fluorometer measures the yield of photochemical energy conversion (Y) of a leaf by comparing the chlorophyll fluorescent signal before (F') and immediately after (F'_m) the application of a pulse of saturating light (Heinz-Walz 1998). Effective Y, the rate of electron transfer at a given irradiance when a portion of photosynthetic reaction centers are closed, is calculated as $(F'_m - F') / F'_m$. The discovery of this simple but accurate measurement of quantum yield via analysis of chlorophyll fluorescence was a major breakthrough (Krause and Weis 1984).

Effective Y is extremely sensitive to the recent light history of the leaf presenting a significant source of diurnal variation in the measurements (Ralph et al. 1998). To remove the effects of ambient light, leaves are subjected to a period of darkness, or dark-adapted, before taking the measurement. Dark-adapting a leaf allows all the photosynthetic reaction centers to return to their initial redox state or become "open" minimizing fluorescence yield (Krause and Weis 1991). Dark-adapting samples effectively provides a consistent way of assessing the photosynthetic state of a leaf

regardless of the recent light history. To distinguish between light-adapted samples, the fluorescence parameters for dark-adapted samples become F for initial fluorescence and F_m and maximum fluorescence (Heinz-Walz 1998). F and F_m by themselves are of empirical use only. Their magnitudes are variable and depend on fluorometer settings and the intensity of the excitation light source. The maximum potential quantum yield, or the yield when all reaction centers are open, is calculated as $F_v/F_{m,}$ where F_v is the variable fluorescence (F_m-F) (Durako and Kunzelman 2002). $F_v/F_{m,}$ if properly assessed, is a reliable measure of the maximum photosynthetic efficiency of a leaf and can indicate the degree of photodamage or inactivation of reaction centers (Krause and Weis 1991).

A PAM fluorometer can be used to measure actual photosynthetic rates by calculating the electron transport rate (ETR) of a sample (Heinz-Walz 1998). Calculating the ETR depends on the accurate estimation of the photosynthetically active radiation (PAR) at the leaf surface and the fraction of the incident PAR (μmol m^{-2} s^{-1}) absorbed by the leaf (AF). ETR is calculated by multiplying the effective Y of a leaf under ambient light conditions by the PAR, AF, and 0.5 (ETR = Y * PAR * AF * 0.5). Because a PAM fluorometer only measures the yield of PS II, multiplying by 0.5 accounts for the fact that only half of the absorbed light will be utilized by PS II, the other half being apportioned to PS I (Heinz-Walz 1998). Beer and Björk (2000) found that ETR was highly correlated to the rate of photosynthetic O_2 evolution in three species of seagrasses.

Characteristics of the photosynthesis-irradiance (P/I) relationship can be ascertained via a PAM fluorometer using rapid light curves (RLC) (Belshe et al. 2007). A RLC is the plot of ETR versus increasing PAR intensities and is analogous to a P/I

curve. Three important values can be acquired from a RLC: 1) maximum photosynthetic capacity (ETR_{max}); 2) photosynthetic efficiency (alpha), the initial slope of the RLC; and 3) the light saturation point (I_k), calculated as ETR_{max} / alpha.

7.2.4 Current research

The PAM fluorometer was considered a major breakthrough in photosynthetic research and led to progressive expansion of practical research using chlorophyll fluorescence in plant science (van Kooten and Snel 1990). In recent years, PAM fluorometry has become a standard technique used in plant ecophysiology offering a non-intrusive method for measuring photosynthetic performance *in situ* (Maxwell and Johnson 2000; Beer et al. 1998). Chlorophyll fluorescence can be used as a proxy of plant stress because environmental stresses including temperature extremes, high light, and water availability can reduce the ability of a plant to metabolize normally creating an imbalance between the absorption of light energy by chlorophyll and the use of energy in photosynthesis (Genty et al. 1989).

The photosynthetic characteristics of seagrasses have traditionally been assessed in the laboratory setting (Enríquez et al. 2002; Rose and Durako 1994; Fourqurean and Zieman 1991). Seagrass leaves are commonly transferred to enclosed chambers where the photosynthetic/irradiance relationship is determined by measuring the rate of O_2 evolution within the chamber at varying irradiance intensities (Beer and Björk 2000; Fourqurean and Zieman 1991). This method is limited in that the leaves must be removed from their natural environment. With the introduction of the Heinz-Walz

Diving-PAM (Figure 7.3), PAM fluorometry research has been expanded to include submerged ecosystems including coral reefs, macroalgae, and seagrasses.

Recent work by Beer and Björk (2000) has shown that measurements of chlorophyll fluorescence are correlated with actual rates of photosynthesis calculated as O_2 production. In most cases, researchers have calculated only relative measurements of photosynthesis as opposed to actual values of photosynthesis calculated as net C flux. This does not limit the usefulness of chlorophyll fluorescence in photosynthetic research. These measurements still allow for relative comparisons between populations or between species (Ralph et al. 1998). Based on the large experience with chlorophyll fluorescence analyses of terrestrial plants, investigations using the DIVING-PAM have shaped a clearer understanding of underwater photosynthesis under natural conditions.

Figure 7.3. The Heinz-Walz Diving-PAM.

7.2.5 Objectives

In this study, I assessed the effectiveness of the Diving-PAM for investigating the photosynthetic ability along *T. testudinum* leaves. I expected that F_v/F_m is optimal in younger leaf tissue and decreases as a leaf ages. Since *T. testudinum* leaves grow from a basal meristem, the gradient in the age of leaf tissue may produce a gradient in F_v/F_m along the leaf with higher values at the bottom of leaves. This gradient would also be evident when comparing leaves of increasing age on an individual short shoot. It is also evident from previous studies, that *T. testudinum* leaves are sensitive to variations in light availability and nutrient availability (Dawes 1998; Fourqurean et al. 1995). Acclimation to variations in environmental conditions may be reflected by differences in chlorophyll fluorescence parameters. Since chlorophyll fluorescence is a function of absorption of light by leaves, the fluorescence parameters may also be correlated to the light absorbance attributes of leaf tissue.

Calculation of actual ETR by the Diving-PAM is dependent on the accurate estimation of the AF of the leaf location being sampled. The Diving-PAM's manual suggests calculating the AF using the attached PAR sensor by comparing PAR before and after placing a leaf over the sensor (Heinz-Walz 1998). This is highly problematic as the ambient light source is highly variable. The Diving-PAM sets a default value of 0.84 for AF and unless the actual value of AF is known the calculated ETR values are relative and of limited use. I hypothesized that the dark-adapted fluorescence parameters are correlated with leaf chlorophyll content and leaf light absorption characteristics. Determining this relationship could provide a method for estimating the AF without

making any additional measurements. This would greatly expand the options for utilizing the Diving-PAM.

This chapter includes five independent studies that assessed the photosynthetic characteristics of *T. testudinum* leaves under various conditions as indicated by chlorophyll fluorescence parameters. The first study determined the interleaf variation in F_v/F_m and RLC parameters along *T. testudinum* at Rabbit Key Basin (RKB) and Sprigger Bank (SPG). For the second study, I followed the F_v/F_m along individual *T. testudinum* leaves over their entire life span to determine the time response of the decline in F_v/F_m. The third study measured chlorophyll fluorescence parameters of *T. testudinum* leaves along a depth gradient. The fourth study assessed the affects of experimental nutrient enrichment on chlorophyll fluorescence parameters. The last study determined the relationship between the dark-adapted chlorophyll fluorescence parameters and leaf characteristics that may affect light absorption (i.e. specific leaf weight, leaf thickness, chlorophyll content, and light absorbance).

7.3 **Methods**

7.3.1 Operation of the Diving-PAM

The Diving-PAM uses a special leaf clip to allow the instrument's optical fiber to be positioned at the proper angle to and distance from the leaf surface (Figure 7.4). The leaf clip has a shutter that when closed blocks the light to the measurement location on the leaf. I completed a preliminary study to assess the optimal dark-adaptation period for *T. testudinum* leaves. If the dark period is too short, then the reaction centers within the leaf will not be fully opened and the calculation of F_v/F_m will be low. If the dark period

is too long, the microenvironment at the leaf sampling location may become anoxic because of the leaf clip may restrict gas exchange affecting the measurement (correspondence with Peter Ralph). The shortest adequate dark adaptation period is also desired because it will allow for the most measurements during a sampling session. To determine the optimal dark adaption period for in situ *T. testudinum* leaves, I randomly selected ten short shoots at Sunset Cove and conducted saturation pulse measurements at the middle of the youngest adult leaf after dark-adapting leaves for 2, 5, 7, 10, and 15 minutes. I compared the results using a one-way ANOVA.

One of the challenges using the Diving-PAM, is that the time needed to dark-adapt limits the number of measurements that can be completed during a single field trip or a single SCUBA dive. I developed a routine where I used ten leaf clips simultaneously to maximize the number of measurements during a single dive or sampling session while also ensuring a consistent dark-adaption period. Importantly, to get an accurate reading the leaf epiphytes must be carefully removed from the location where the measurement will be taken before attaching the leaf clip.

Upon completion of the dark-adaptation period, the fiber optic is connected to the leaf clip and the shutter is opened. By pressing the START button on the Diving-PAM, the initial fluorescence under actinic light is recorder followed by a pulse of saturating light. The instrument records the maximum fluorescence and calculates variable fluorescence and the fluorescent yield. For each measurement, the Diving-PAM saves these variables along with water depth (m), water temperature (ºC), and down-welling PAR (µmol m^{-2} s^{-1}).

For RLC measurements, the appropriate command is selected from the menu and a series of nine fluorescence measurements are automatically taken at increasing irradiance values at ten second intervals. ETR_{max} and alpha are estimated via a MATLab routine that uses a least squares curve fit to the plot of ETR versus irradiance. I_k is calculated as ETR_{max} / alpha. Data is stored in the Diving-PAM's internal data recorder that can be downloaded via an interface box and WinControl software.

7.3.2 Study 1 – Interleaf variation of F_v/F_m and RLC parameters

Chlorophyll fluorescence parameters were measured along *T. testudinum* leaves at Rabbit Key Basin and Sprigger Bank during peak summer at the same sampling locations as used in Chapter 5. The leaf age cohorts were categorized as in Chapter 5. For each site, thirty short shoots were haphazardly selected and dark-adapted F_v/F_m measurements were taken at the top, middle, and bottom of the first four adult leaves. One-way ANOVA were used to compare between the vertical locations along each leaf. RLCs were also performed at the bottom, middle, and top of the first adult leaf on thirty short shoots from each site. T-tests were used to compare between the sites at each vertical leaf location.

Figure 7.4. Positioning of the Diving-PAM leaf clip.

7.3.3 Study 2 - Leaf life history

The change in chlorophyll fluorescence parameters along the individual leaves over time was examined in a *T. testudinum* meadow at Sunset Cove from August to October. The seagrass bed was located in ~2.5 m of water and ~50 m from the mangrove-edged shoreline. Thirty *T. testudinum* short shoots were haphazardly selected along two transects parallel to the shoreline. Short shoots were selected with the criteria that they were representative of the population and contained an emergent leaf less than ~5 cm long. Numbered bird bands were placed around the base of the short shoots and orange flags were positioned nearby to mark the locations. The youngest leaf of each

selected short shoot was marked by punching a small hole with a needle near the top of the leaf.

The study site was visited every two or three days. The lengths of the marked leaves were measured and dark-adapted Diving-PAM measurements were conducted at the top, middle, and base of the marked leaves. These leaves were measured until they completely senesced. The mean F_v/F_m was plotted over time for each vertical leaf location.

7.3.4 Study 3 - Variation in chlorophyll fluorescence along a depth gradient

The variability in chlorophyll fluorescence parameters in *T. testudinum* leaves was examined along a submerged slope at the Blue Ground Station near the Smithsonian Tropical Research Institute (Carrie Bow Cay, Belize). Site and meadow characteristics were determined via a parallel study by Gallegos et al. 2009. Diving-PAM measurements were completed at depths of 1.5 m (~800 short shoots m^{-2}), 5.6 m (~350 short shoots m^{-2}), and 11m (~50 short shoots m^{-2}). The deepest sampling site corresponded to the light-limited edge of seagrass growth. Approximate light availability at 1.5 m was approximately 70% of surface light, approximately 20% at 5.6 m, and <5% at 11 m. At each sampling location, thirty dark-adapted F_v/F_m measurements and ten RLCs were completed at the middle of the youngest fully-grown adult leaf. ETR_{max} and alpha were calculated The sites were compared using a one-way ANOVA and Tukey's multiple comparison tests.

7.3.5 Study 4 – Effects of nutrient enrichment on chlorophyll fluorescence

To evaluate the effects of nutrient enrichment on the chlorophyll fluorescence parameters of *T. testudinum* leaves, I conducted Diving-PAM measurements within a previously established experimental design framework at Duck Key, Florida Bay

(Armitage et al. 2005). The experiment site included four treatment (N and P enriched) plots and four control (untreated) plots. Ten F_v/F_m measurements and five RLC's were completed within each plot at the middle of the youngest fully-grown adult leaves (Leaf 1) and the next older leaves (Leaf 2). Comparisons between the nutrient enriched leaves and untreated leaves were accomplished via one-way ANOVA.

7.3.6 Study 5 - Correlation of fluorescence parameters and leaf attributes

This study was carried out at in *T. testudinum* meadows at three sampling sites across Florida Bay including Sunset Cove, Rabbit Key Basin, and Duck Key. The intention of taking samples at three different sites was not to make inter-site comparisons but rather to include samples with a wide variety of leaf morphologies and pigment content. At each site, dark-adapted F_v/F_m measurements were completed on fifty haphazardly selected leaves making sure to include leaves of various ages and pigment content. The locations of the measurements were marked by punching a hole at the base of where the leaf clip was positioned on the leaf. The leaf was then removed from the short shoot and placed in a labeled bag. The leaves were kept in seawater and in the dark and processed with two hours. Two Diving-PAM settings were kept constant throughout the measurements with the measuring light intensity and the gain, set at three and nine respectively. The distance from the fiber optic sensor to the leaf was also kept constant.

Segments, 1 cm to 1.5 cm long, were cut from the leaves at the locations of the Diving-PAM measurements. The absorbance spectrum of each segment was determined using the Ocean Optics Mini-spec and a halogen light source (Frankovich and Zieman 2005). The light source was positioned over a 30-gallon tub filled seawater. A sampling

apparatus was used to secure the Mini-spec fiber optic sensor perpendicular to the light source. The apparatus was then placed in the tub. A sample of the source light spectrum was saved. Absorbance spectra of each segment were determined by placing it over the fiber optic sensor of each instrument and comparing to the source light spectrum. Twenty leaf sheaths were also scanned. The sheaths are located at the bottom of leaves and are non-pigmented but are of similar tissue and thickness as the pigmented portion of the leaf. The average absorbance from leaf sheaths was subtracted from the leaf absorbance of leaves to account for light absorbed by ancillary leaf tissue rather than chlorophyll or other photosynthetic pigments. The leaf segment area and thickness were measured and recorded. Dry weight was determined after freeze-drying the samples.

The segment were quickly freeze-dried and stored in the dark. The chlorophyll content of the segments was determined using the acetone extraction method from Chapter 5. The segment chlorophyll concentration, segment area, and dry weight, were matched with the segment's corresponding chlorophyll fluorescence parameters taken in the field. A correlation matrix was completed comparing the variables. This was followed by stepwise multiple regression analyses to determine the relationship between leaf chlorophyll content and light absorbance versus F, F_m, and F_v/F_m values. For the Diving-PAM to be adequate in estimating leaf absorbance and chlorophyll content, I would expect regression coefficient of at least 0.70.

7.4 **Results**

The analysis determined that the optimal dark-adaptation period for *T. Testudinum* leaves was ten minutes (Figure 7.5). Although a 15-minute dark adaptation

achieved a slighter higher F_v/F_m, ten minutes was considered an adequate dark period and was the dark adaptation period used for all F_v/F_m measurements during this study.

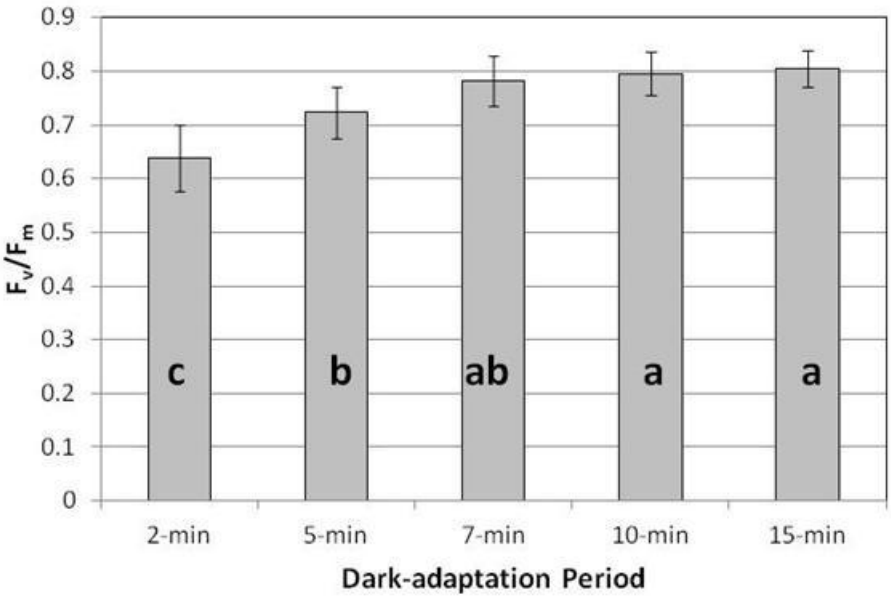

Figure 7.5. Comparison of the effects of increasing dark-adaptation period on the F_v/F_m of *Thalassia testudinum* leaves.

7.4.1 Study 1 - Interleaf variation of F_v/F_m and RLC parameters

All short shoots sampled at RKB had at least four adult leaves. There was significant interleaf variation in dark-adapted chlorophyll fluorescence parameters (F, F_m, and F_v/F_m) at RKB (Figure 7.6). Mean F varied from 175 at bottoms of younger leaves to 100 at the tips of the oldest intact leaves. Mean F_m ranged from over 700 at the middle of the youngest leaves and bottoms of the older leaves to 225 at the tip of the oldest leaves. F and F_m declined significantly from the bottom to tips in all leaves except the youngest adult leaves. The highest F_v/F_m was along the youngest leaves and the base of Leaf 2 and

Leaf 3. Older leaves showed declines along the leaf blades most notably at the leaf tips. F_v/F_m declined approximately 36% from a maximum of 0.76 at the middle of Leaf 1 to 0.49 at the tip of Leaf 4.

All selected short shoots at SPG included at least three adult leaves. The fluorescence parameters at SPG showed similar trends as at RKB (Figure 7.7). F did not vary significantly along any leaf cohort. However, F_m showed a significant decline at the middle and tips of Leaf 2 and Leaf 3. F_v/F_m declined significantly along Leaf 2 and Leaf 3 ranging from 0.68 at the top of Leaf 2 to 0.39 at the top of Leaf 3. At RKB, the decline at the tops of older leaves resulted from declines in both F and F_m. In sun-adapted plants, F often remains unchanged with declines F_v/F_m mainly the result of a reduced F_m (Ralph and Burchett 1995). The decline in F_v/F_m along the older leaves at SPG was mostly the result of declines in F_m,, however, F was also generally higher in older leaves. This pattern of F_v/F_m is more indicative of photoinhibition (Demmig and Borkman 1987).

There were significant differences between the RLC parameters (ETR_{max}, alpha, and I_k) for RKB and SPG (Figure 7.8). RKB exhibited significantly higher alpha than SPG at all vertical leaf locations. This is consistent with the higher light conditions at SPG. Alpha also declined slightly along the sampled leaves at both sites. ETR_{max} was significantly higher at the bottom and middle of leaves at SPG. This would be consistent with acclimation to the higher light availability at SPG. However, ETR_{max} at the top of leaves was significantly higher at RKB. The general trend at both sites saw increasing ETR_{max} toward leaf tips, the exception being that the top of leaves at SPG showed a

pronounced decline. This could be the result of photodamage or physically damaged leaf tips at the high-energy SPG site.

I_k generally trended upward from base to leaf tips except at the tips of leaves at SPG. At RKB, I_k increased from 110.9 µmol m^{-2} s^{-1} at the leaf base to 194.7 µmol m^{-2} s^{-1} at leaf tips, a 78% increase. At SPG, I_k increased from 226.6 µmol m^{-2} s^{-1} at the leaf base to 321.1 µmol m^{-2} s^{-1} at the middle of the leaf but declined to 248.5 µmol m^{-2} s^{-1} at the top of the leaf. I_k was significantly higher at SPG than RKB at all leaf locations. The lowest I_k at SPG, at the bottom of the leaf, was still higher than the leaf tips at RKB indicating a higher degree of sun-adaptation at SPG. The values found for F_v/F_m were consistent with values found in other studies for *T. testudinum* (Durako and Kunzelman 2002; Enríquez et al. 2002). The values for ETR_{max} and alpha are consistent with the values found for *T. testudinum* (Belshe et al. 2007) and other seagrasses species (Silva and Santos 2004; Ralph and Gademann 2005).

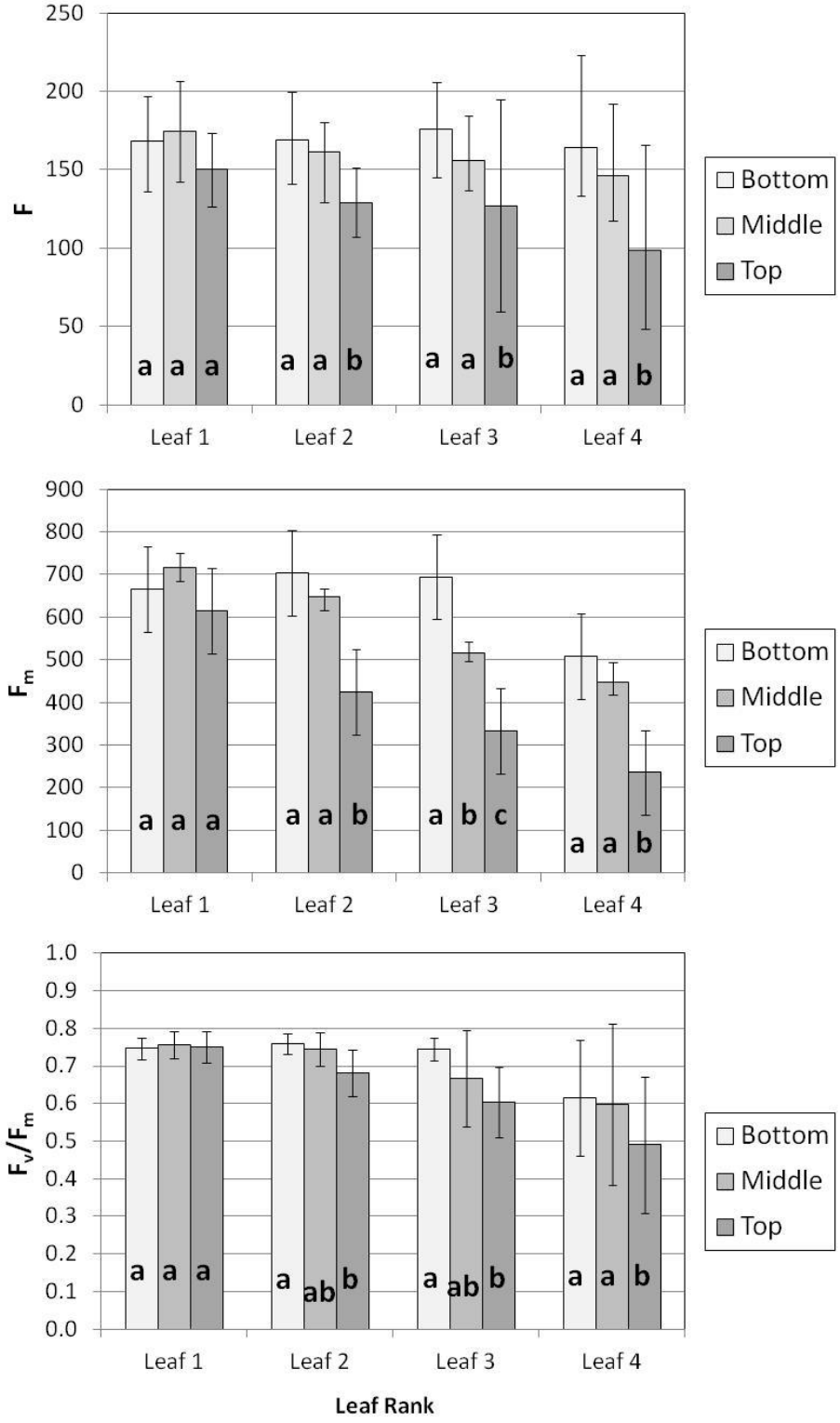

Figure 7.6. Interleaf variation of dark-adapted chlorophyll fluorescence parameters along *Thalassia testudinum* leaves at Rabbit Key Basin. Values are means ±SD of twenty haphazardly selected short shoots. Values with the same letter are not significantly different as determined by Tukey's multiple comparison test ($P < 0.05$).

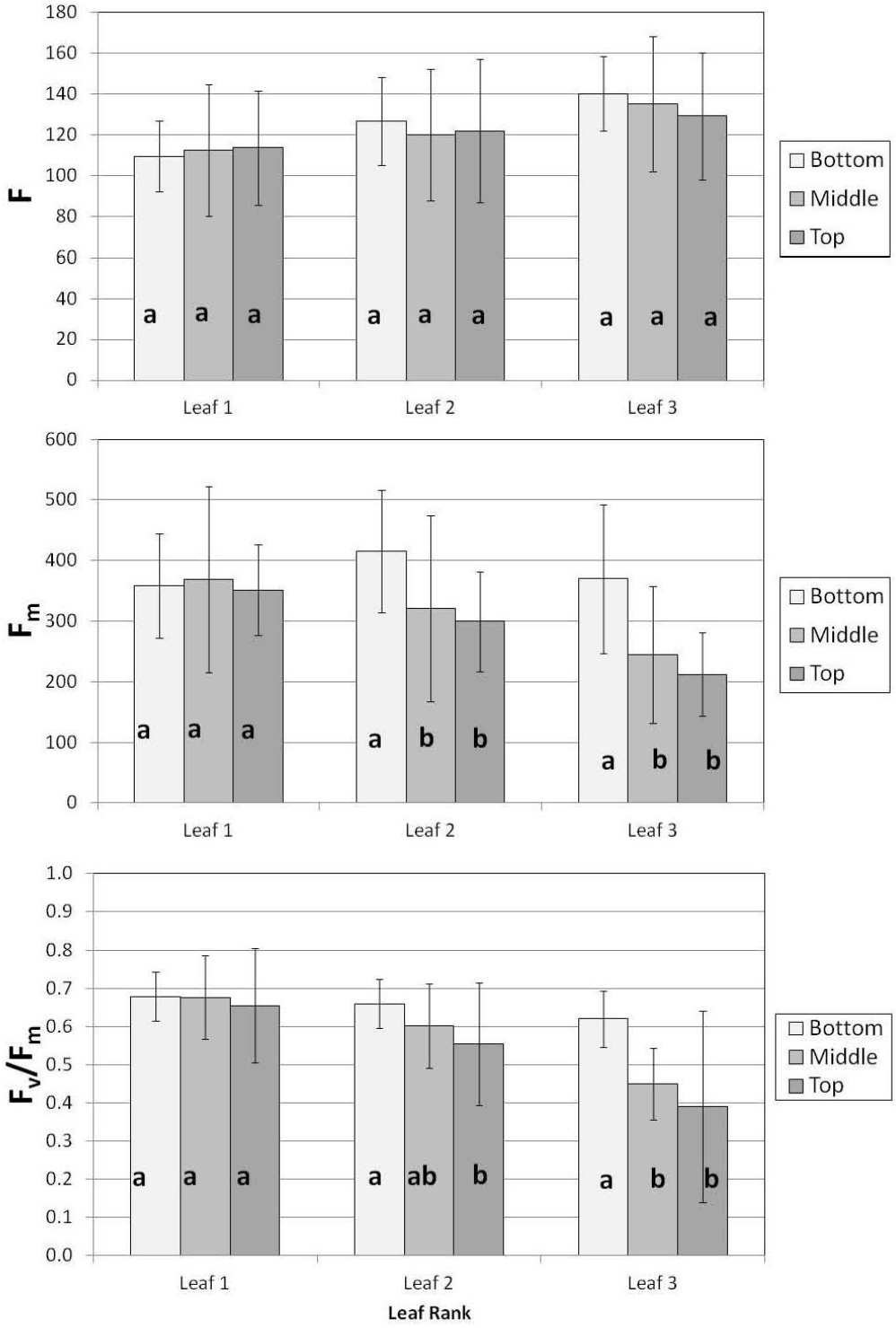

Figure 7.7. Interleaf variation of dark-adapted chlorophyll fluorescence parameters along *Thalassia testudinum* leaves at Sprigger Bank. Values are means ±SD of twenty haphazardly selected short shoots. Values with the same letter are not significantly different as determined by Tukey's multiple comparison test ($P < 0.05$).

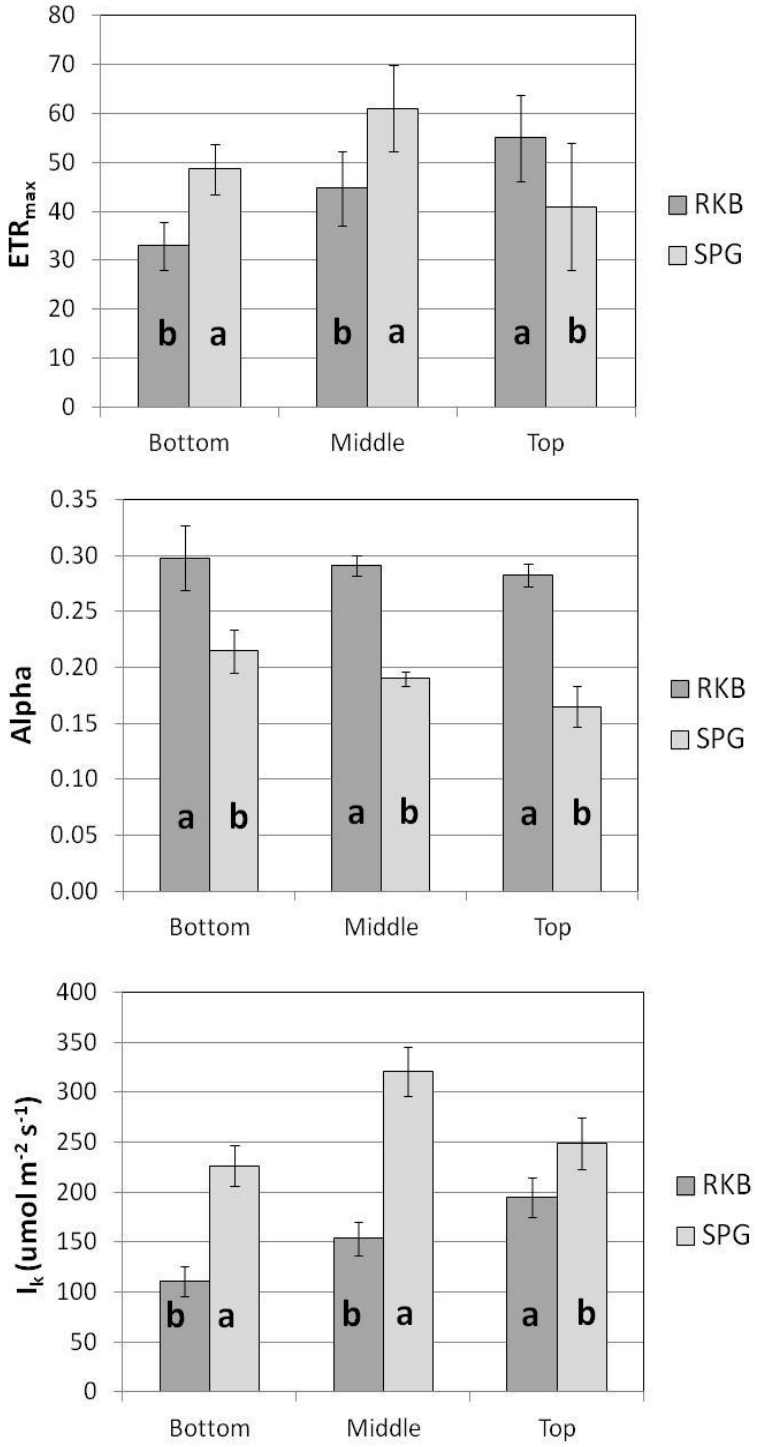

Figure 7.8. Interleaf variation of RLC parameters along *Thalassia testudinum* leaves at Rabbit Key Basin (RKB)and Sprigger Bank (SPG). Values are means ±SD of twenty haphazardly selected short shoots. Values with the same letter are not significantly different.

7.4.2 Study 2 - Life history of chlorophyll fluorescence in leaves

The life history analysis showed the time response of the decline in F_v/F_m along leaves (Figure 7.9). At the leaf base, F_v/F_m increased rapidly reaching a maximum of approximately 0.81 at Day 5 and remained steady until Day 32. Measurements at the middle and tops of leaves began after Day 5. At the middle of leaves, F_v/F_m remained steady at approximately 0.80 until starting a decline after Day 24. F_v/F_m at the top of leaves began to decline at Day 15 corresponding to the point where leaf length reached 20 cm. Sampled leaves did not reach full length until approximately Day 35 after which point F_v/F_m at the top of leaves began to rapidly decline. By Day 39, the tops of leaves had lost all pigment and begun to slough off. The middle of leaves remained photosynthetically active until Day 55. F_v/F_m at the bottom of leaves remained above 0.5 until Day 55. By Day 60, all sampled leaves had completely senesced. The rapid decline in F_v/F_m at the top of leaves was closely associated with their emergence at the top of the canopy. The abrupt decline may be due to the sudden increase in light availability after being shaded within the canopy.

7.4.3 Study 3 - Variation in chlorophyll fluorescence along a depth gradient

The variation in dark-adapted chlorophyll fluorescence parameters (F, F_m, and F_v/F_m) and RLC variables (ETR_{max}, alpha, and I_k) along a depth gradient is shown in Figure 7.10. No variation in F was found, however, F_m declined significantly with decreasing depth. F_v/F_m declined from 0.805 at 11 m to 0.71 at 1.5 m as expected with the increasing light availability. ETR_{max} nearly doubled along the depth gradient from 20.8 at 11m to 43.9 at 1.5 m while alpha declined by more than half from 0.312 to 0.140.

I_k increased from 66.2 µmol m^{-2} s^{-1} at a depth of 11 m to to 310.1 µmol m^{-2} s^{-1} at 1.5 m. The trend in the RLC variables is consistent with acclimation to increasing light availability with the deep site showing the strongest shade-adapted characteristics, higher F_m and alpha but lower ETR_{max} and I_k.

7.4.4 Study 4 - Effects of nutrient enrichment on chlorophyll fluorescence

T. testudinum leaves from the nutrient enriched plots showed significantly higher F_v/F_m when compared to leaves from the control plots (Figure 7.11). In Leaf 1, nutrient enrichment showed no effect on F while F_m showed a 30% increase resulting in 7% increase in F_v/F_m. In Leaf 2, F was 13% higher in nutrient enriched leaves while F_m was 58% higher resulting in a 14.5% increase in F_v/F_m. Being in the northeast part of Florida Bay, Duck Key is extremely nutrient limited. Figure 7.12 shows that nutrient enrichment also has a positive effect on photosynthetic capacity as shown by higher ETR_{max} and alpha in nutrient enriched leaves. For Leaf 1, ETR_{max} and alpha were both approximately 10% higher in the nutrient enriched leaves. In Leaf 2, ETR_{max} showed an increase of 11%and alpha increased 15.5%. This study showed that nutrient enrichment has a strong positive effect the *T. testudinum* leaves and increases the ability of the leaves to utilize the available light. The results of Chapter 6 showed that the N and P in leaf tissue declines toward leaf tips. Therefore, this study is evidence that the similar declines in F_v/F_m observed along leaves may be partially due to declines in N and P in leaf tissue.

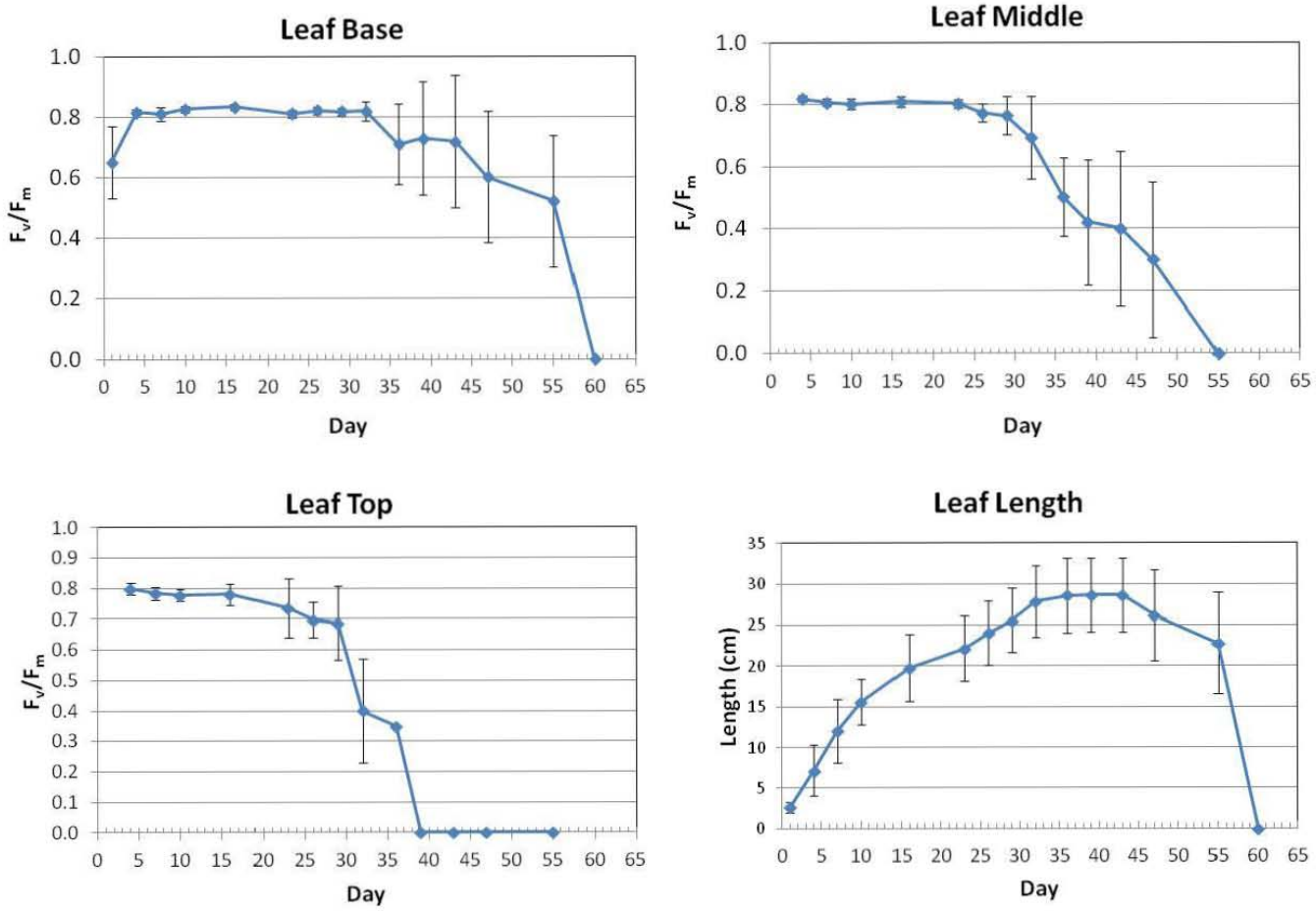

Figure 7.9 Life history of F_v/F_m along individual leaves of *Thalassia testudinum* at Sunset Cove, Florida Bay. Error bars are ±SD.

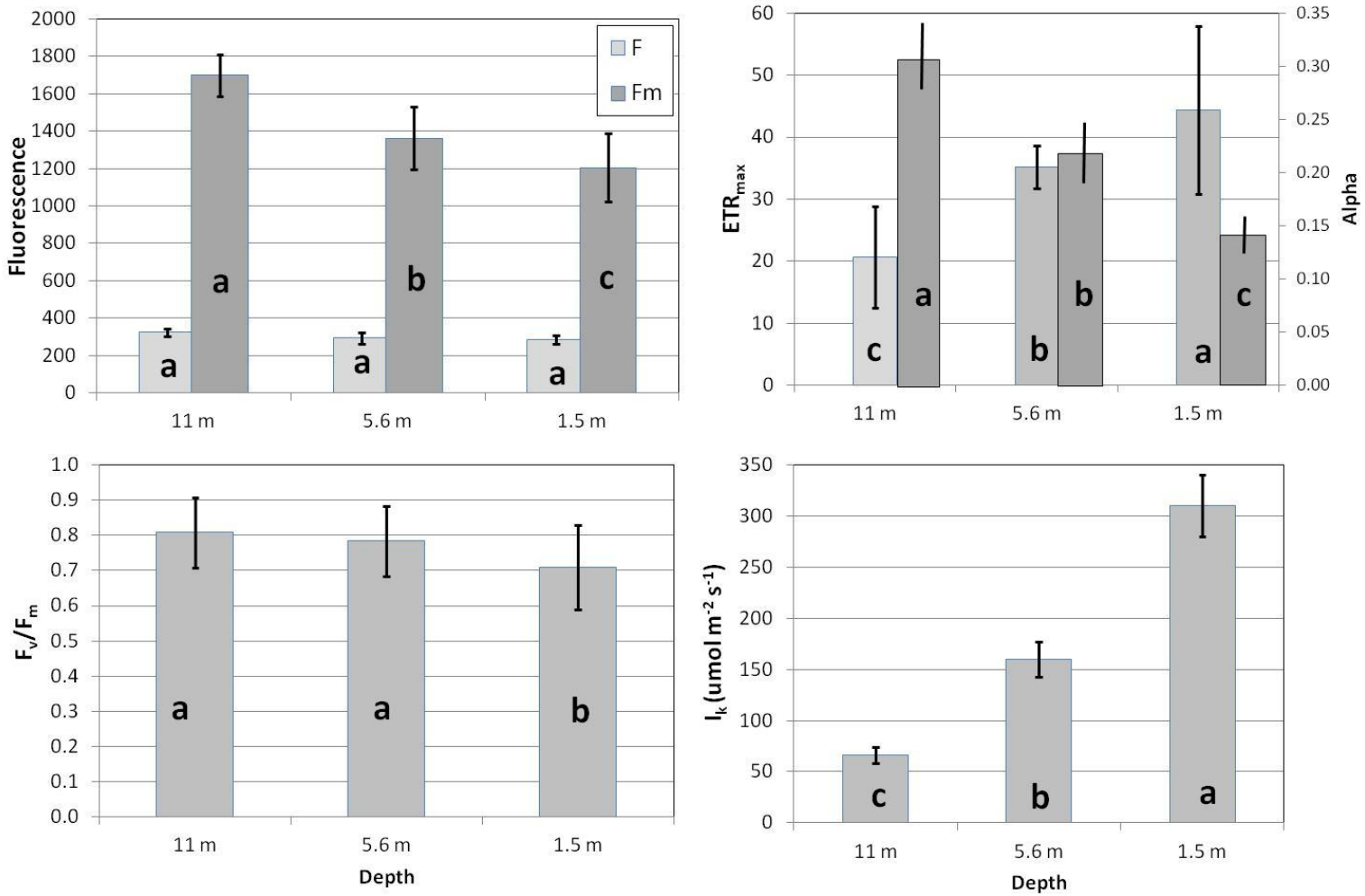

Figure 7.10. Variation in chlorophyll fluorescence parameters in Thalassia testudinum leaves along a depth gradient at Carrie Bow Key, Belize. Values are means ±SD of twenty haphazardly selected short shoots. Values with the same letter are not significantly different.

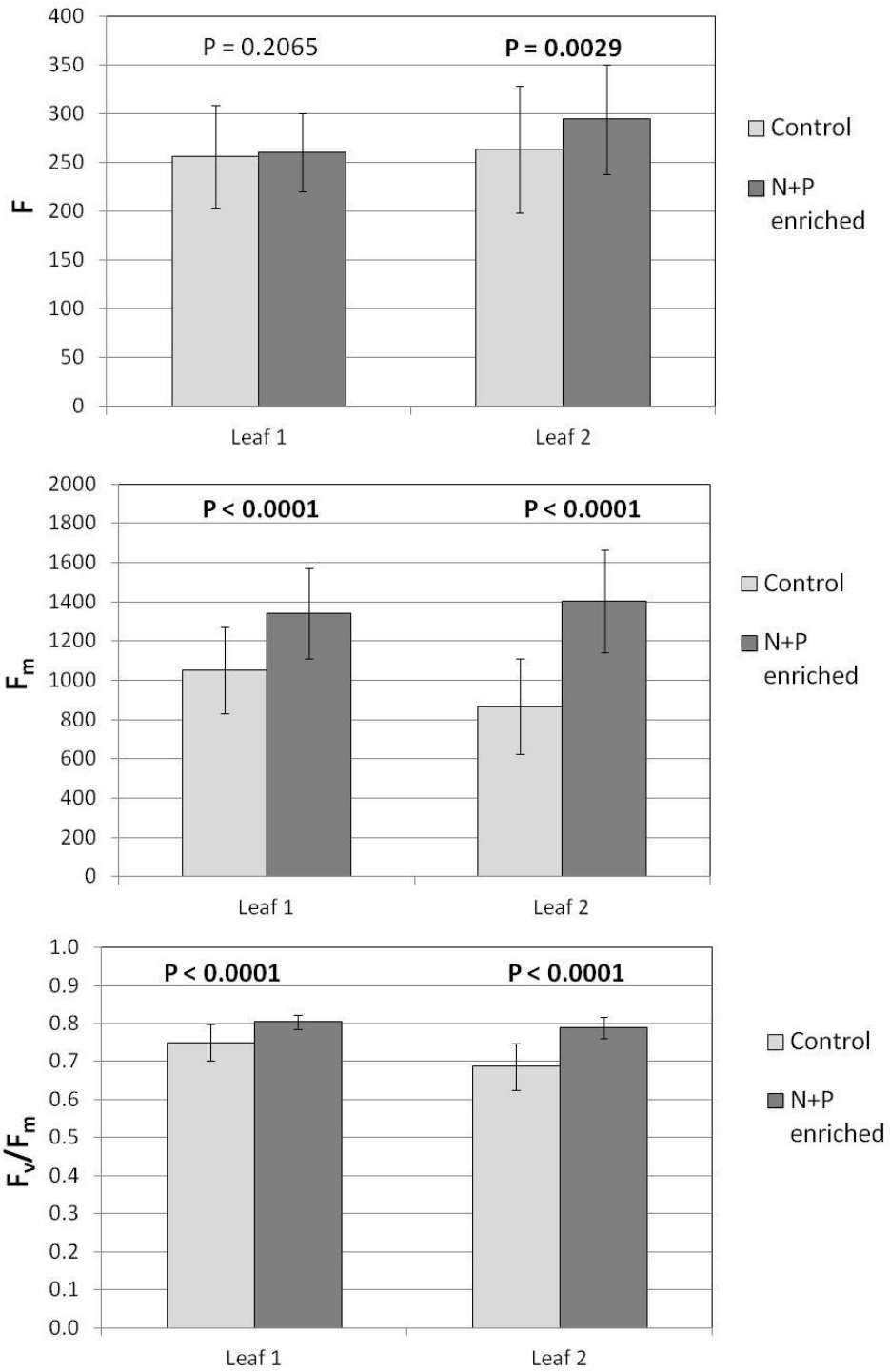

Figure 7.11. Comparison of chlorophyll fluorescence parameters (F, F_m, and F_v/F_m) between *Thalassia testudinum* leaves from nutrient (N and P) enriched plots and control plots at Duck Key, Florida Bay. Values are means ±SD. Comparison were made using t-tests.

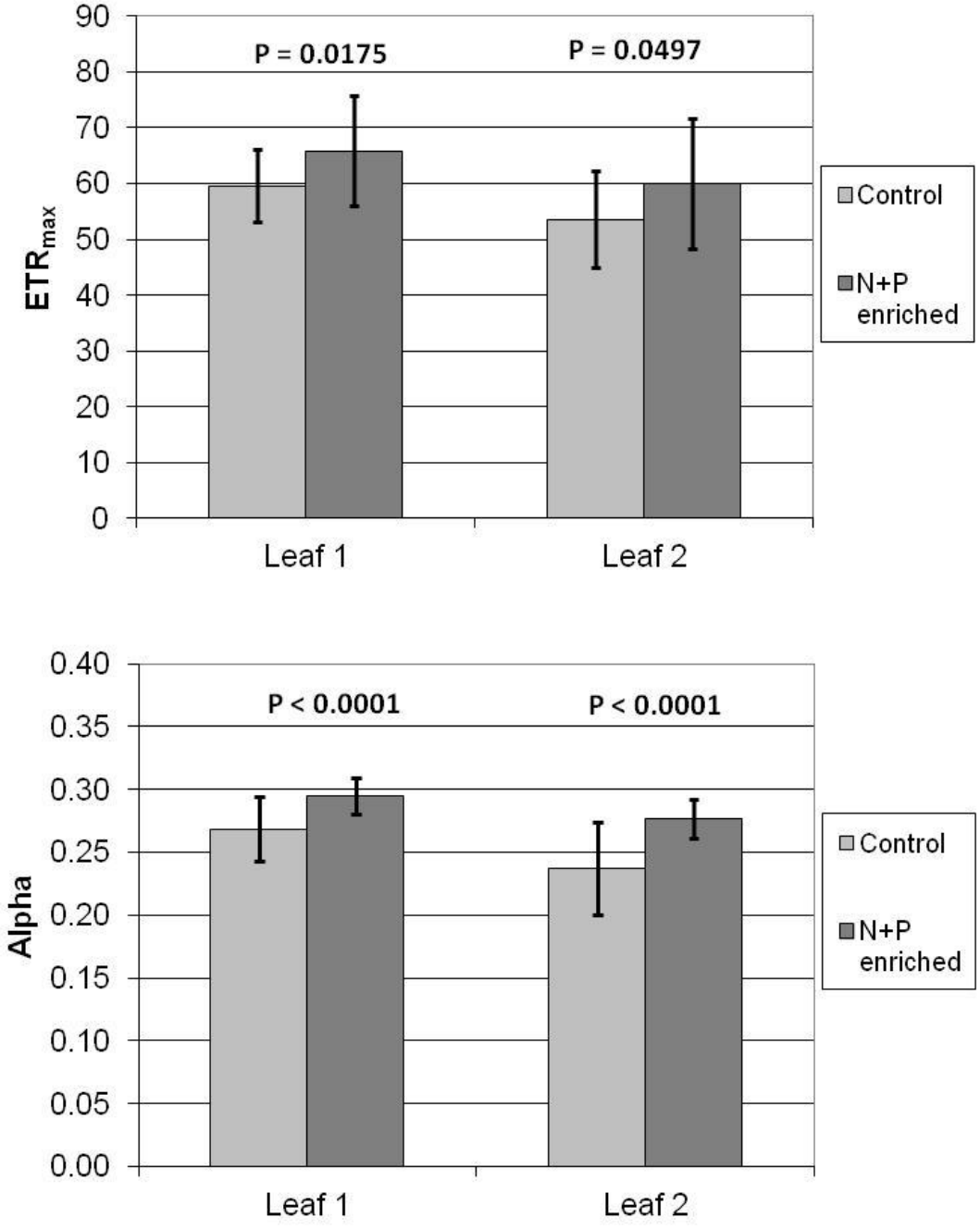

Figure 7.12. Comparison of RLC variables (ETR_{max} and alpha) between *Thalassia testudinum* leaves from nutrient (N and P) enriched plots and control plots at Duck Key, Florida Bay. Values are means ±SD. Comparison were made using t-tests.

7.4.1 Study 5 - Correlation of fluorescence parameters and leaf attributes

The average absorbance spectrum of the *T. testudinum* segments is shown in Figure 7.13. Absorbance of leaf sheaths was fairly even across the PAR spectrum. Subtracting absorbance of the leaf sheaths reduced the leaf absorbance spectrum by approximately 20%. The absorbance spectrum showed a distinct peak at 674 nm and a broad ridge from 400-500 nm. The lowest absorbance was at approximately 550 nm.

The results of the correlation analysis comparing dark-adapted chlorophyll fluorescence parameters of leaf segments with the physical leaf attributes are shown in Table 7.1. Leaf physical characteristics included leaf thickness (mm), specific leaf weight (SLW) (mg cm^{-2}), total chlorophyll concentration by weight (µg mg^{-1}), total chlorophyll by leaf area (µg cm^{-2}), Chl *a*:*b*, total PAR absorbance, absorbance at 674 nm, and absorbance at 435 nm.

Leaf thickness was positively correlated to SLW and Total Chl by area but not well correlated to light absorbance. However, SLW was well correlated to PAR absorbance at 0.47. Total Chl by weight showed strong positive correlation to light absorbance with the highest coefficient, 0.57, at 674 nm. However, Total Chl by weight was not correlated to leaf thickness or SLW. Total Chl by area was strongly correlated to light absorbance with the highest coefficient, of 0.67, also at 674 nm. However, Total Chl by area was well correlated to leaf thickness. Chl *a*:*b* was negatively correlated to chlorophyll content by weight and area. This indicates that higher leaf chlorophyll concentration typically coincided with greater increases in Chl *b* relative to Chl *a*.

All the chlorophyll fluorescence parameters were well correlated to leaf chlorophyll content with correlation coefficients ranging from 0.51 to 0.65. F and F_m were also significantly correlated to leaf thickness. Absorbance of PAR was significantly correlated to F and F_m but only at 0.28 and 0.23, respectively. Absorbance at 435 was also correlated to F and F_m at 0.27 and 0.24, respectively. PAR absorbance and absorbance at 435 nm showed no correlation to F_v/F_m. Absorbance at 674 nm was significantly correlated to F, F_m, and F_v/F_m at 0.40, 0.41, and 0.28, respectively.

The results of the regression analyses comparing the chlorophyll fluorescence parameters with leaf chlorophyll content and leaf light absorbance are shown in Table 7.2. These results suggest that dark-adapted Diving-PAM measurements can accurately predict leaf light absorbance and leaf chlorophyll content. The best fit was for absorbance at 674 nm where F, F_m, and F_v/F_m accounted for 71.8% of the variation. The regression fits for absorbance of PAR and absorbance at 435 nm were also statistically significant with R^2 of 0.313 and 0.521, respectively. The regression for F, F_m, and F_v/F_m versus Total Chl by area versus resulted in an R^2 of 0.725 while the R^2 for the regression for Total Chl by weight was lower at 0.407.

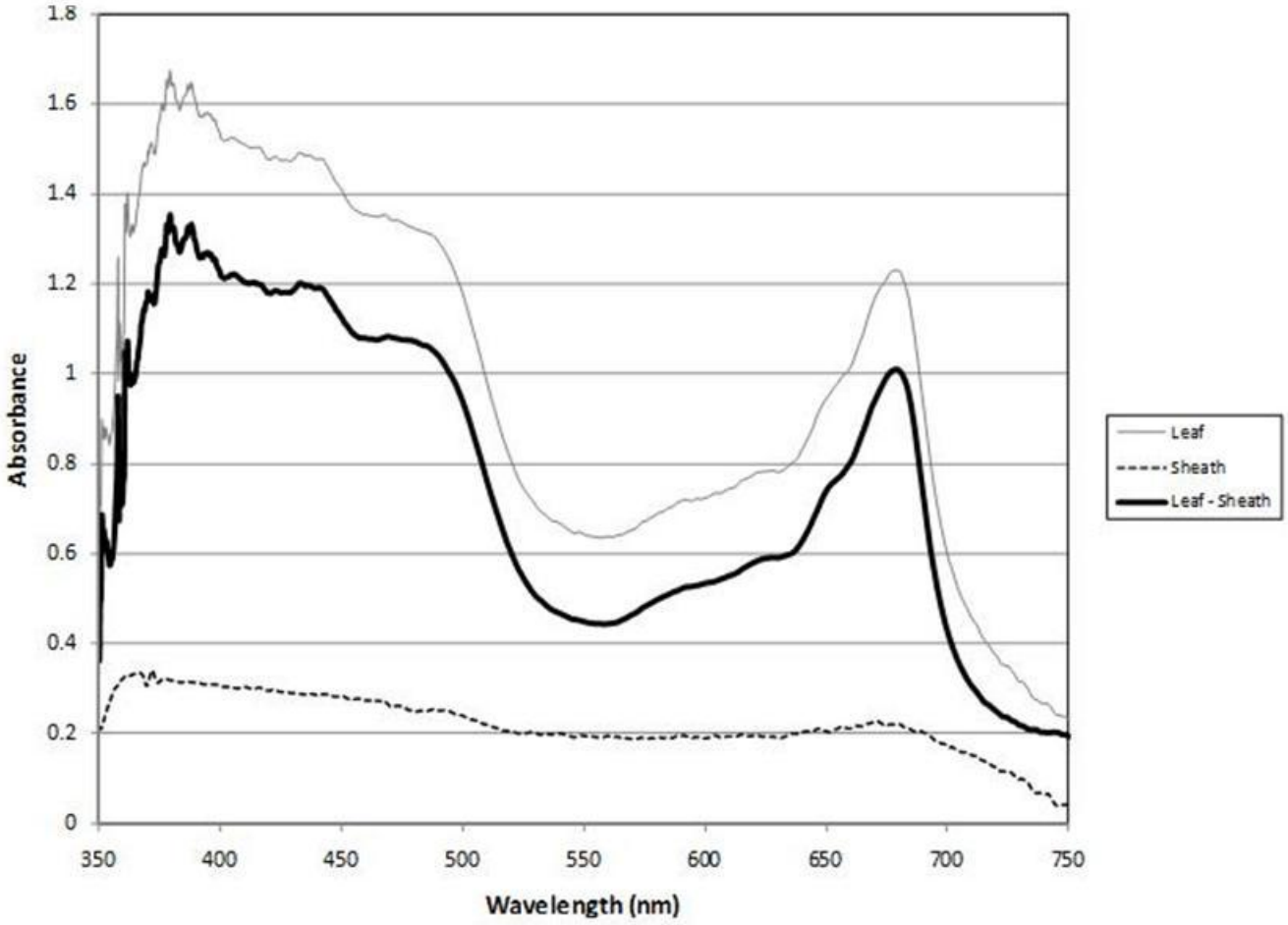

Figure 7.13. Absorbance spectrum of *Thalassia testudinum* leaves from Florida Bay. The plot shows the correction for absorbance from ancillary leaf tissue by subtracting the absorbance spectrum of the non-pigmented leaf sheaths. Plotted values are the average of 75 adult leaf segments.

Table 7.1. Correlation analysis comparing leaf attributes and synchronized fluorescence parameters. Correlation coefficients in bold are statistically significant at $P < 0.01$, values in italic are significant at $P < 0.05$.

	Leaf Thickness	SLW	Total Chl by wt.	Total Chl by area	Chl *a*:*b*	Absorbance (PAR)	Absorbance (674 nm)	Absorbance (435 nm)
Leaf Thickness		**0.36**	*0.26*	**0.43**	*-0.22*	*0.21*	*0.29*	*0.20*
SLW	**0.36**		-0.06	*0.21*	*-0.19*	**0.47**	**0.39**	**0.43**
Total Chl by wt.	*0.26*	-0.06		**0.95**	*-0.31*	**0.33**	**0.57**	**0.38**
Total Chl by area	**0.43**	*0.21*	**0.95**		**-0.33**	**0.46**	**0.67**	**0.49**
Chl *a*:*b*	*-0.22*	*-0.19*	**-0.31**	**-0.33**		-0.17	*-0.22*	*-0.22*
F	**0.43**	*0.23*	**0.51**	**0.56**	-0.15	**0.28**	**0.40**	**0.27**
F_m	**0.39**	*0.19*	**0.61**	**0.65**	-0.17	*0.23*	**0.41**	*0.24*
F_v/F_m	*0.23*	0.04	**0.51**	**0.51**	*-0.19*	0.09	**0.28**	0.10

Table 7.2. Regression analysis predicting light absorbance and chlorophyll content of leaf segments using in situ chlorophyll fluorescence parameters measured via the Diving-PAM.

Independent Variable	Dependent Variables					
	F	F_m	F_v/F_m	Intercept	R^2	p-value
Absorbance (PAR)	1.01E-03	3.80E-05	NA	4.35E-01	**0.313**	<0.0001
Absorbance (435 nm)	1.17E-03	3.75E-04	-5.43E-01	9.06E-01	**0.521**	<0.0001
Absorbance (674 nm)	1.90E-03	1.51E-04	8.70E-01	5.73E-01	**0.718**	<0.0001
Total Chl by area (µg cm^{-2})	-3.41E-01	2.70E-01	-7.79E+02	5.26E+02	**0.725**	<0.0001
Total Chl by wt. (µg mg^{-1})	-6.12E-05	1.76E-03	5.26E+00	-2.77E+00	**0.407**	<0.0001

7.5 **Discussion**

This study showed that chlorophyll fluorescence analysis via the Diving-PAM can reveal important information about the photosynthetic state of *T. testudinum* leaves. Because they are easy to accomplish, chlorophyll fluorescence parameters are often over interpreted. In order for chlorophyll fluorescence parameters to be utilized as accurate indicators of the physiological condition of seagrass leaves, the variation in the parameters must be associated with known changes in the photophysiology of leaves. This study found evidence that variations in F_v/F_m and RLC variables (ETR_{max}, alpha, and I_k) are related to changes in light and nutrient availability and can indicate the state of senescence. The study also found that the dark-adapted fluorescence parameters are significantly correlated to the chlorophyll content of leaves and may be able to be used to predict leaf light absorbance.

7.5.1 Chlorophyll fluorescence analysis as a measure of leaf aging

Like most sub-tropical and tropical plant species, *T. testudinum* in Florida Bay is not deciduous and the plants do not shed a substantial number of leaves at any particular time of year, although there is seasonal variation in productivity, standing crop, plastochrone interval, and leaf chlorophyll content (Zieman 1975; Barber and Behrens 1985). But at approximately 52 days, *T. testudinum* leaves have a relatively short lifespan even for seagrasses (Hemminga et al. 1999).

This study showed that an optimal F_v/F_m of around 0.80 was attained as a leaf reaches maturity and then generally decreased as leaf tissue aged. This was demonstrated by comparing F_v/F_m along leaves of increasing age (i.e. leaves on the same short shoot)

and by following individual leaves over their lifespan. This study showed that *T. testudinum* leaves actually lose a substantial amount of their photosynthetic capacity well before abscission. This means that the effective life span may be even shorter. The leaves at Sunset Cove had lost at least half of the their maximum potential quantum yield after only thirty days while the middle leaves had lost half by 40 days. However, the base of the leaves remained photosynthetically vibrant until approximately five days before leaf abscission. Furthermore, *T. testudinum* leaves appear to begin losing photosynthetic ability before they have even reached full length.

A short leaf lifespan would seem to be inefficient because creating new leaves is very energy intensive and requires additional nutrients. However, a short leaf life span has some advantages. Leaf lifespan is inversely related to net photosynthesis (Reich et al. 1991). So the trade off is that short-lived leaves may cost energy and nutrients to replace frequently, but the plants are able to maintain optimum photosynthetic performance by continuously producing new leaves.

7.5.2 Canopy-level photoacclimation

Variations in F_v/F_m can lend insight to how *T. testudinum* leaves acclimate to different light environments. Variations in RLC parameters along leaves at RKB and SPG also show evidence of interleaf photoacclimation to the intercanopy gradient in light availability. The comparison between RLC parameters at SPG and RKB imply that the leaves at RKB, with higher alpha but lower ETR_{max} and I_k, are more shade-adapted than the SPG leaves. These results suggest that using a standard P-I relationship derived from mesocosm experiments or single sites will not accurately predict photosynthetic rate for

T. testudinum across its entire range (Fourqurean and Zieman 1991; Dennison 1987; Fong and Harwell 1994). The interleaf variation in photosynthetic performance may be substantial enough to introduce a significant error in seagrass photosynthetic models that use a standard P-I relationship to represent the entire canopy.

Changes in fluorescence parameters can provide insight into photoinhibitory processes within a leaf (Krause 1988). These processes include photoprotective responses and permanent photodamage, which can occur simultaneously (Öquist 1992). Decline in F_v/F_m can result from photoprotective responses to the supersaturating light experienced in shallow seagrass meadows (Ralph and Burchett 1995). The declines in F_v/F_m can be further assessed by considering the corresponding changes to F and F_m. A decline in F_v/F_m can be the result of an increase in F and/or a decrease in F_m (Krause and Weis 1991). The two sites investigated in Study 1 both showed declines in F_v/F_m at the tips of older leaves but the declines resulted from different causes. The decline along leaves at SPG was due to increases in F and decreases in F_m indicating photoprotective processes were primarily occurring. The F_v/F_m decline at the tips of older leaves at RKB was due to significant decreases in both F and F_m indicating photodamage.

Photodamage occurs when absorbed light energy exceeds the photoprotective capacity of the photosynthetic apparatus. Photoprotective processes dissipate excess excitation energy through non-photochemical quenching. Photoprotection protects the photosynthetic apparatus from permanent damage until more effective acclimation mechanisms can adjust to higher irradiance conditions (Franklin et al. 1992). Declines in

F_v/F_m can reveal a leaf under stress. However, it is difficult to segregate these changes in F_v/F_m from the natural variation without additional information.

Chapter 5 demonstrated evidence that *T. testudinum* may photoacclimate along leaves in response to the intercanopy gradient in light availability. These results lend further evidence of interleaf photoacclimation in *T. testudinum*. However, just as in the variation of chlorophyll content along leaves, the declines in F_v/F_m are certainly attributable to multiple factors. The interleaf variation in RLC parameters (ETR_{max}, alpha, and I_k) lend additional support of the hypothesis of interleaf photoacclimation in *T. testudinum* leaves. However, the RLC data does not indicate if the changes in parameters are due to alterations at the level of the photosystems or the result of long-term changes in leaf photosynthetic characteristics (e.g. chlorophyll content, leaf thickness) during photomorphogenesis.

7.5.3 Fluorescence parameters versus leaf attributes

Dark-adapted fluorescent measurements made with the Diving-PAM showed an exceptional ability to predict chlorophyll content of *T. testudinum* leaves. F_m is a better predictor of chlorophyll concentration than F. This may be purely statistical given the higher magnitudes and range of values for F_m or it may be related to the physiological source of the rise from F to F_m in leaves. F is a measurement of fluorescence during the application of a very weak light source that is unlikely to instigate photosynthesis (Heinz-Walz 1995). Most chlorophyll molecules are not actively absorbing photons and most excitation energy is transferred to photochemical activity as opposed to fluorescence pathways. F_m is the fluorescent signal measured after the application of a saturating light

pulse that completely suppresses photochemical yield and all reaction centers are fully reduced. The fluorescent signal is directly related to the number of reaction centers rather as opposed to only. The results also showed that the fluorescence parameters are a reasonable predictor for leaf light absorbance. However, this was only at wavelengths corresponding to the absorbance peaks of chlorophyll making it difficult to use the fluorescence parameters to substitute AF values in the calculations of ETR.

The Diving-PAM is an incredibly useful instrument for investigating photosynthetic attributes in *T. testudinum* leaves. However, researchers must be careful in how they design experiments and interpret results because the chlorophyll fluorescence parameters can be rationalized to fit just about any hypothesis.

PART V. EXPERIMENTAL ANALYSIS OF CANOPY DYNAMICS IN SEAGRASS

Chapter 8. Effect of experimental shading on the interleaf variation of photosynthetic attributes of *Thalassia testudinum* leaves.

8.1 Abstract

This study examined the effect of experimentally reduced light availability on the vertical variation of photosynthetic attributes of *T. testudinum* leaves. The four shade canopies that were deployed in a dense *T. testudinum* meadow reduced light availability by 32%. After eight weeks of shading, chlorophyll content declined significantly along the entire lengths of younger leaves that would have emerged and grown under shaded conditions while older leaves saw declines only toward the leaf base. The significantly higher F_v/F_m for shaded leaf tips suggests a lower degree of light stress compared to the unshaded leaves. The lack of change at lower leaf locations may be due to either the lower light levels deeper within the canopy or to the fact that the lower parts of the leaf are younger and healthier than the tips.

8.2 Introduction

8.2.1 Effects of light reduction on seagrass

The significant declines in seagrass populations in many parts of the world are often attributed to reduced light availability resulting from decreased water quality or shading from the excessive growth of macroalgae and epiphytes (Waycott et al. 2009; Dennison et al. 1993). To adequately plan for ecosystem changes, natural resource managers require a thorough knowledge of how seagrass meadows will react to declines in light availability (Gallegos 1994).

T. testudinum exhibits a high degree of phenotypic plasticity in how it responds to the various light environments that it inhabits. From the edge of the light-limited depth to the supersaturating light experienced in shallow water, *T. testudinum* can survive and thrive by optimizing leaf and canopy morphology (e.g. leaf length, short shoot density, and chlorophyll content) through the process of photomorphogenesis (Gallegos et al. 2009). Comparing light-limited *T. testudinum* plants to those inhabiting shallow high-light environments, it can be surprising that the two plants are even the same species.

Chapter 3 demonstrated that *T. testudinum* experiences a gradient in light availability along an individual leaf. Considering the ability of *T. testudinum* to acclimate to a variety of light environments, the initial thought is to attribute the vertical variation of photosynthetic attributes along the *T. testudinum* leaves to interleaf photoacclimation. However, the basal growth of *T. testudinum* leaves also produces a vertical variability in the age of leaf tissue. Chapter 7 showed that *T. testudinum* leaf tissue begins to lose photosynthetic ability before it reaches full length. Also, older leaf tissue has been exposed longer to the high-energy marine environment, disease, and herbivory, all of which can adversely affect photosynthetic attributes. Additionally, the oldest part of leaves (i.e. the tips) often experience supersaturating light intensities, which may result in chronic photoinhibition, photodegradation of chlorophyll, and permanent damage to the photosynthetic apparatus (Krause 1988).

The effects of light decline on seagrasses have been extensively studied (Ruiz and Romero 2001; Lee and Dunton 1997; Tomasko and Dawes 1989; Neverauskas 1988). Experimentally-reduced light availability using shade canopies resultsd in lower

productivity, decreased standing crop, shorter plastochrone interval, and increased leaf chlorophyll content (Lee and Dunton 1997; Czerny and Dunton 1995; Dennison and Alberte 1982). These studies focus on the ability of the leaves to adjust photosynthetic traits to declines in light availability similar to seasonal declines or to changes in waer column clarity. However, none of these studies examined the effect of shading on the vertical variation of leaf photosynthetic characteristics.

8.2.2 Objectives

The purpose of this study is to investigate the effects of imposed shading on the vertical variation of leaf attributes in including: 1) chlorophyll content; 2) Chl *a*:*b*; 3) C:P and C:N; 4) specific leaf weight (SLW); and 5) chlorophyll fluorescence characteristics. The productivity and epiphyte density of shaded and unshaded short shoots will also be compared. The results will be studied to assess if *T. testudinum* alters vertical canopy structure in response to shading. Experimental shading typically results in significant decreases in leaf productivity and standing crop. Evidence of shade adaptation in the new leaves within the shaded plots would include higher chlorophyll content, higher alpha, and lower ETR_{max}. The shaded leaves may also exhibit higher F_v/F_m especially at the leaf tips indicating less chronic photoinhibition.

8.3 Methods

8.3.1 Shade canopies

To manipulate the irradiance reaching seagrass, four shading devices (Figure 8.1) were constructed and placed in a *T. testudinum* bed located in RKB. The 1.5 m square canopies reduced down-welling irradiance without altering other environmental factors

and consisted of ½" PVC frames with ¾" plastic mesh for the shading material. Four control plots equal in size to the canopies were established by placing PVC poles vertically in the sediment at the corners of a 1.5 m square. In order to eliminate any effects that clonal integration may have on the experiment, rhizomes were severed around the perimeter of the canopies and control plots using a saw. The canopies were cleaned every two weeks of accumulated epiphytic growth. The Mini-Spec was used to compare the down-welling irradiance under and outside the canopies to determine the extent of irradiance reduction.

8.3.2 Sample collection

The site was visited after four and eight weeks of shading. Five short shoot leaves were removed from the center of each plot and cut into 5 cm segments and the chlorophyll concentration and elemental content were determined using the methods from Chapter 5. Dark-adapted chlorophyll fluorescence measurements (F, F_m, and F_v/F_m) and five RLCs were conducted with the Diving-PAM at the base, middle, and top of the three youngest adult leaves on five short shoots from the center of each plot.

At week 8, the productivity was determined using a leaf punching method (Zieman and Wetzel 1980). All of the leaves inside a 10 cm by 20 cm quadrat were placed at the center of the each plot were punched. The short shoots were harvested after two weeks and productivity was calculated as areal productivity (g new growth^{-2}), specific productivity (mg new growth / g leaf dry weight), and short shoot productivity (mg new growth / short shoot). Epiphyte density at week eight was determined by gently scraping the epiphytes from the leaves of five short shoots per plot and then accurately

measuring the total leaf area of the short shoots. The epiphytes were put into preweighed scintillation vials and freeze-dried. After freeze-drying, the samples were weighed to determine epiphyte dry weight, then 10 ml of 90% acetone was added to each vial. Concentrations were read on the RF-mini after waiting 24 h for chlorophyll extraction. Chl *a* concentrations of the epiphyte samples were measured using the RF-Mini. Epiphyte load was determined as both epiphyte dry weight and Chl *a* per short shoot and leaf area. The difference within the plots was compared using a one-way ANOVA. If the plots within each treatment were not found to be significantly different, the data was pooled and treatments were compared using t-tests.

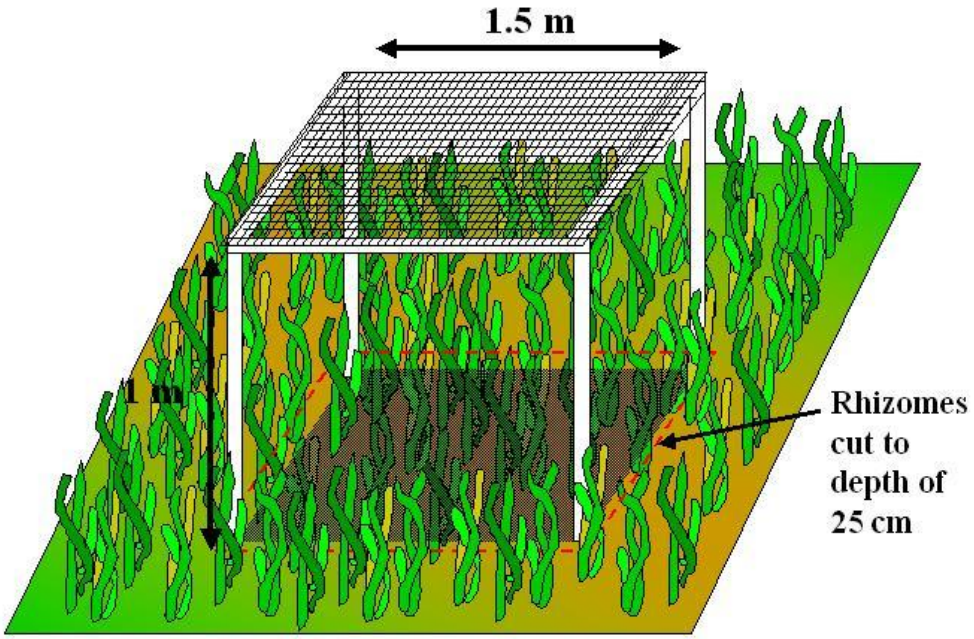

Figure 8.1. Diagram of shade canopies

8.4 **Results**

The shade canopies reduced the down-welling irradiance incident upon the top of the *T. testudinum* canopy by 32% (Figure **8.2**). The canopies did not alter the spectral distribution of the light or the red:far-red. After four weeks of shading, there were no statistically significant differences found in fluorescence parameters or leaf chlorophyll content. Only the data after eight weeks are shown in the results. Epiphyte density did not change significantly after eight weeks ($P = 0.213$).

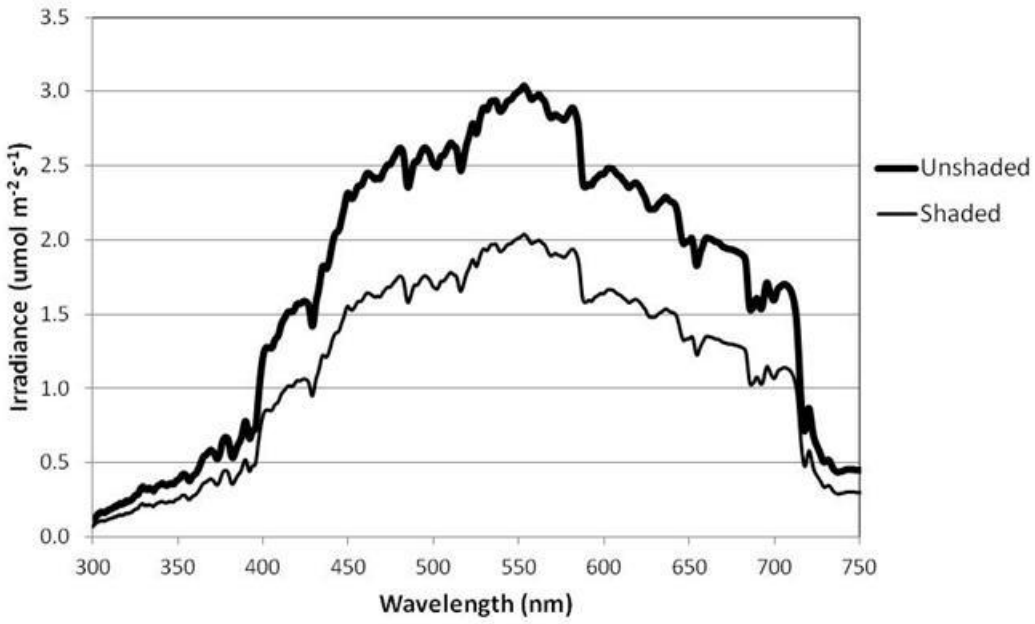

Figure 8.2. Comparison of spectral irradiance incident at the top of the *Thalassia testudinum* canopy at Rabbit Key Basin, Florida Bay in unshaded plots and plots shaded by canopies devices.

8.4.1 Comparison of productivity

The results of the productivity analysis of the plots after 8 weeks of shading are shown in . Although short shoot density was unchanged, there was a 20.2% decrease in leaf standing crop after 8 weeks of shading. While specific productivity did not show significant change, short shoot productivity declined by 26.6% in the shaded plots. While the unshaded short shoots produced new leaves approximately every 32.6 days, the shaded short shoots produced new leaves only on average every 46.8 days. Additionally, the growth of new leaves was 56.2% smaller than in the unshaded plots.

Table 8.1. Comparison of mean productivity measurements (±SD) of shaded and unshaded plots of *Thalassia testudinum* at Rabbit Key Basin, Florida Bay. Values in bold are significantly different ($P < 0.05$).

	SS density ($SS\ m^{-2}$)	Standing Crop ($g\ m^{-2}$)	Specific Productivity ($mg\ g^{-1}\ day^{-1}$)	SS Productivity ($mg\ SS^{-1}\ day^{-1}$)	New Leaf Growth ($mg\ SS^{-1}\ day^{-1}$)	Plastochrone Interval (day^{-1})
Unshaded	1183±284	***70.5±10.2***	28.9±4.0	**1.67±0.25**	**0.26±0.05**	32.6±6.4
Shaded	1183±189	***56.3±1.1***	25.8±4.5	**1.23±0.06**	**0.11±0.03**	46.8±12.9
% Diff	0.0	-20.2	-10.6	-26.6	-56.2	43.4
P-value	1.0000	0.4168	0.4312	0.0406	0.012	0.2455

8.4.2 Comparison of leaf attributes

Leaf chlorophyll content increased along the length of Leaf 1 and Leaf 2 after eight weeks of shading (Figure 8.4). Leaf chlorophyll content increased from base to tip along Leaf 1 ranging from 2.7 to 5.9 $\mu g\ mg^{-1}$. This suggests an initial green up period as a leaf matures. Chlorophyll content at the tips of leaves was approximately double the content at the leaf base for both shaded and unshaded leaves. Shaded leaves had significantly higher chlorophyll content across the entire length of the leaves. The difference was greatest at the leaf tips where shaded leaves had approximately 18% higher chlorophyll content. The average difference across the entire Leaf 1 was 12.5%.

Leaf chlorophyll content generally decreased along Leaf 2 except at the leaf base, which appeared to have not reached full maturity. Shaded leaves had significantly higher chlorophyll content at every vertical leaf segment except at the bottom of the leaves. Chlorophyll content ranged from 2.7 to 5.8 $\mu g\ mg^{-1}$. At the 10 cm segment of Leaf 2, shaded leaves had 61% higher chlorophyll content than unshaded leaves. Along the rest

of the leaf chlorophyll content averaged approximately 20% higher in shaded leaves. Leaf chlorophyll content generally decreased from base to tip and was noticeably lower than Leaf 1 and Leaf 2.

The results for the other leaf attributes are shown in Table 8.2. Chl *a*:*b* was significantly lower in shaded plots for Leaf 1 and the middle and bottom of Leaf 2. Specific leaf weight and C:N were not significantly different along any leaf. C:P was significantly lower in shaded plots for the entire Leaf 1 and bottom of Leaf 2 ranging from 1:562 in younger leaves to over 1:1500 in older leaves. P-limitation in seagrasses is indicated by C:P greater than 1:474 (Duarte 1990).

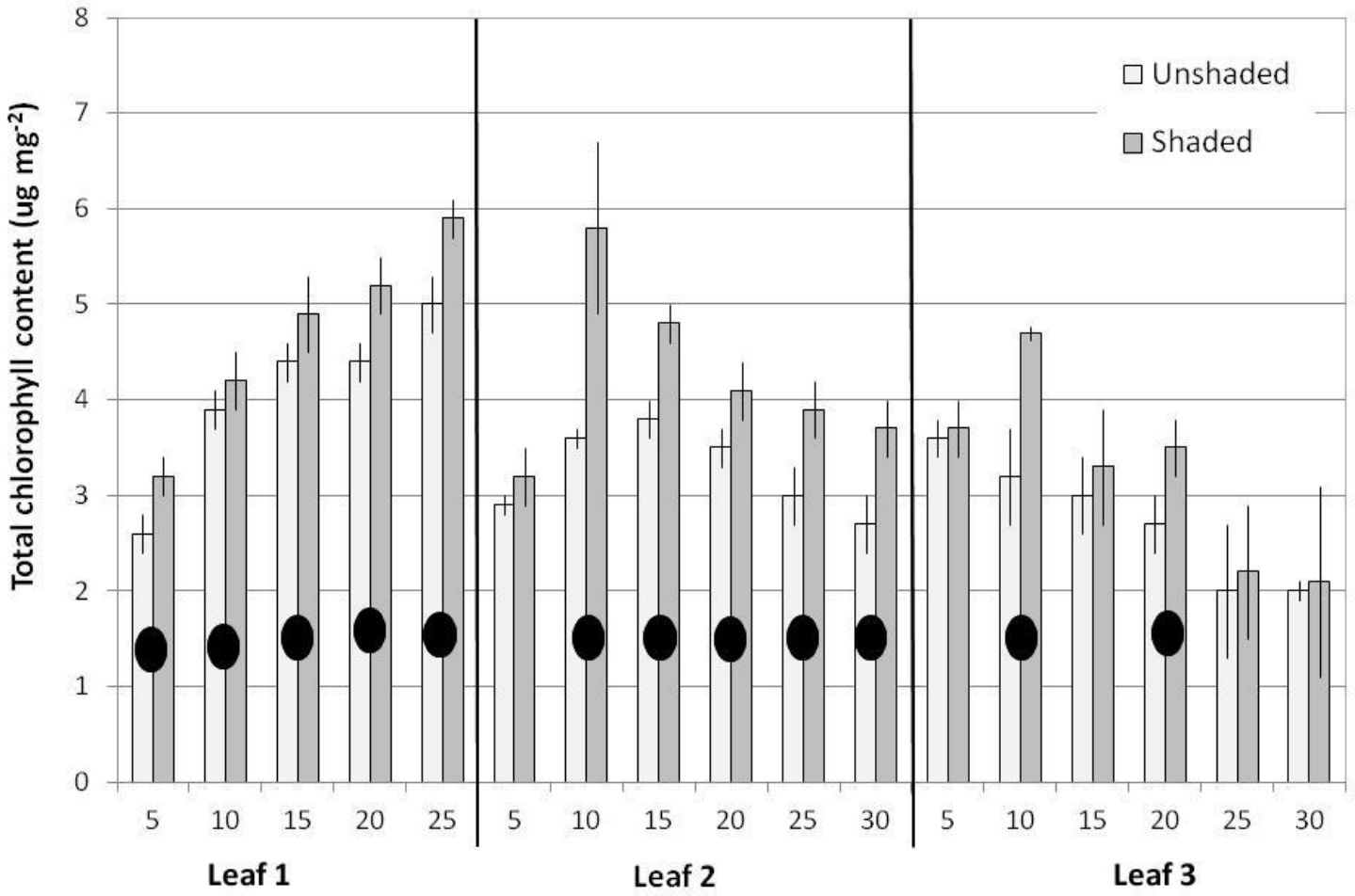

Figure 8.3. Comparison of leaf chlorophyll content along shaded and unshaded *Thalassia testudinum* leaves at Rabbit Key Basin, Florida Bay. Black dots indicate statistically significant differences ($P < 0.05$).

Table 8.2. Comparison of leaf attributes along shaded (S) and unshaded (U) *Thalassia testudinum* leaves. Shaded leaves received approximately 32% less light for eight weeks. Values are means ±SD. Bold are statistically significant ($P < 0.05$).

Leaf	Segment (cm)	Plot	Chl *a* (μg mg^{-1})	Chl *b* (μg mg^{-1})	Chl *a*:*b*	SLW (mg cm^{-2})	C:N	C:P
1	5	U	**1.8±0.1**	**0.9±0.1**	**2.05±0.03**	5.3±0.2	14.2±0.5	585±20
		S	**2.1±0.1**	**1.1±0.1**	**1.95±0.02**	5.0±0.2	13.6±0.4	562±21
1	10	U	2.6±0.1	1.3±0.1	**2.09±0.03**	4.7±0.2	15.8±0.5	**730±40**
		S	2.8±0.2	1.4±0.1	**1.91±0.04**	4.6±0.2	14.4±0.4	**568±34**
1	15	U	**3.0±0.1**	**1.4±0.1**	**2.08±0.03**	4.7±0.3	15.5±0.6	**797±47**
		S	**3.3±0.2**	**1.7±0.1**	**1.96±0.05**	4.7±0.3	15.3±0.5	**681±17**
1	20	U	**3.0±0.2**	**1.4±0.1**	**2.17±0.03**	5.1±0.2	16.7±0.5	**936±45**
		S	**3.5±0.1**	**1.7±0.1**	**2.07±0.05**	5.2±0.3	15.3±0.2	**719±14**
1	25	U	**3.4±0.2**	**1.6±0.1**	**2.12±0.05**	4.1±0.3		
		S	**3.9±0.1**	**2.0±0.1**	**1.99±0.06**	3.9±0.3		
2	5	U	1.9±0.1	1.0±0.1	**1.94±0.02**	5.6±0.1	16.5±0.4	**764±41**
		S	2.1±0.2	1.1±0.1	**1.81±0.02**	5.3±0.2	15.8±0.4	**662±34**
2	10	U	2.4±0.1	**1.2±0.1**	**2.08±0.01**	5.4±0.0	17.3±0.7	758±19
		S	3.8±0.6	**2.1±0.3**	**1.82±0.02**	5.6±0.7	16.4±1.0	758±34
2	15	U	**2.6±0.1**	**1.2±0.1**	**2.11±0.02**	5.8±0.2	17.5±0.5	919±13
		S	**3.2±0.2**	**1.6±0.1**	**1.99±0.02**	5.4±0.2	22.4±5.3	857±35
2	20	U	2.4±0.1	**1.1±0.1**	**2.15±0.03**	5.6±0.2	18.4±1.0	982±90
		S	2.8±0.3	**1.5±0.2**	**2.03±0.03**	4.8±0.5	18.6±0.9	933±86
2	25	U	**2.1±0.2**	**1.0±0.1**	2.13±0.04	**5.2±0.2**	19.7±0.9	1059±102
		S	**2.7±0.2**	**1.3±0.1**	2.12±0.02	**4.5±0.1**	20.2±1.8	1238±160
2	30	U	**1.8±0.2**	**0.8±0.1**	2.18±0.04	4.5±0.2		
		S	**2.5±0.2**	**1.2±0.1**	2.13±0.03	4.4±0.2		
3	5	U	2.4±0.2	1.2±0.1	1.99±0.03	6.0±0.2	19.3±1.1	1122±93
		S	2.5±0.2	1.3±0.1	1.99±0.05	5.7±0.2	19.5±1.0	863±33
3	10	U	2.2±0.5	1.1±0.2	2.08±0.06	5.9±0.3	20.3±1.8	1325±234
		S	3.1±0.1	1.6±0.1	1.93±0.01	6.1±0.1	21.7	996
3	15	U	2.1±0.2	1.0±0.1	2.10±0.03	**6.1±0.2**	20.7±1.1	1307±80
		S	2.2±0.4	1.1±0.2	1.93±0.05	**5.2±0.2**	23.2±2.3	1511±320
3	20	U	**1.8±0.2**	**0.9±0.1**	2.08±0.06	5.3±0.2	21.3±0.2	1298±64
		S	**2.0±0.2**	**1.5±0.1**	2.01±0.09	4.9±0.4	19.1	1081
3	25	U	1.4±0.5	0.6±0.2	2.18±0.07	5.1±0.6	25.3	1411
		S	1.5±0.5	0.8±0.2	1.84±0.17	3.9±0.2	21.3±3.5	2366±937
3	30	U	1.4±0.1	0.6±0.0	2.16±0.04	4.2±0.1		
		S	1.4±0.7	0.7±0.3	2.06±0.08	4.2±0.4		

8.4.3 Comparison of chlorophyll fluorescence parameters

The comparison of dark-adapted chlorophyll fluorescence parameters is shown in Figure 8.5. There were no significant differences found between shaded and unshaded leaves in F. The only significant difference in F_m was found at the tip of Leaf 3, which was approximately 32% higher in the shaded plots. F_v/F_m was significantly higher in the tips of Leaf 1, 2, and 3. The magnitude of the difference was noticeably higher in the older leaves. At the tip of Leaf 3, F_v/F_m under the shade canopy was 0.61 versus 0.51 in the unshaded plots, an approximately 20% difference. The results for Leaf 1 and Leaf 2 could be due to the increase in chlorophyll content in the shaded leaves. However, this would not explain the difference at the tip of Leaf 3, which did not show significant change in chlorophyll content. Another explanation is that the tips of the shaded leaves experience less exposure to supersaturating light and the increase in F_v/F_m is due to a lesser degree of photoinhibition.

The comparison of RLC variables is shown in Figure 8.6. The shaded leaves showed significantly lower ETR_{max} along all leaves except at the bottom of Leaf 3. The highest ETR_{max} in the unshaded plot, 55.5 at the tip of Leaf 1, was 20.1% higher than in the shaded. On average shaded leaf sections were approximately 25% lower than unshaded. Alpha was significantly higher along the older leaves with the greatest difference at the leaf tips. The alpha at the tip of Leaf 3 was 29.4% higher in shaded plots. I_k was significantly higher in the unshaded leaves along all leaves while the highest I_k was at the tips of leaves. The difference between shaded and unshaded leaf tips

increased with leaf age with the shaded tips 21% lower for Leaf 1, 32.3% lower for Leaf 2, and 38.2% for Leaf 3.

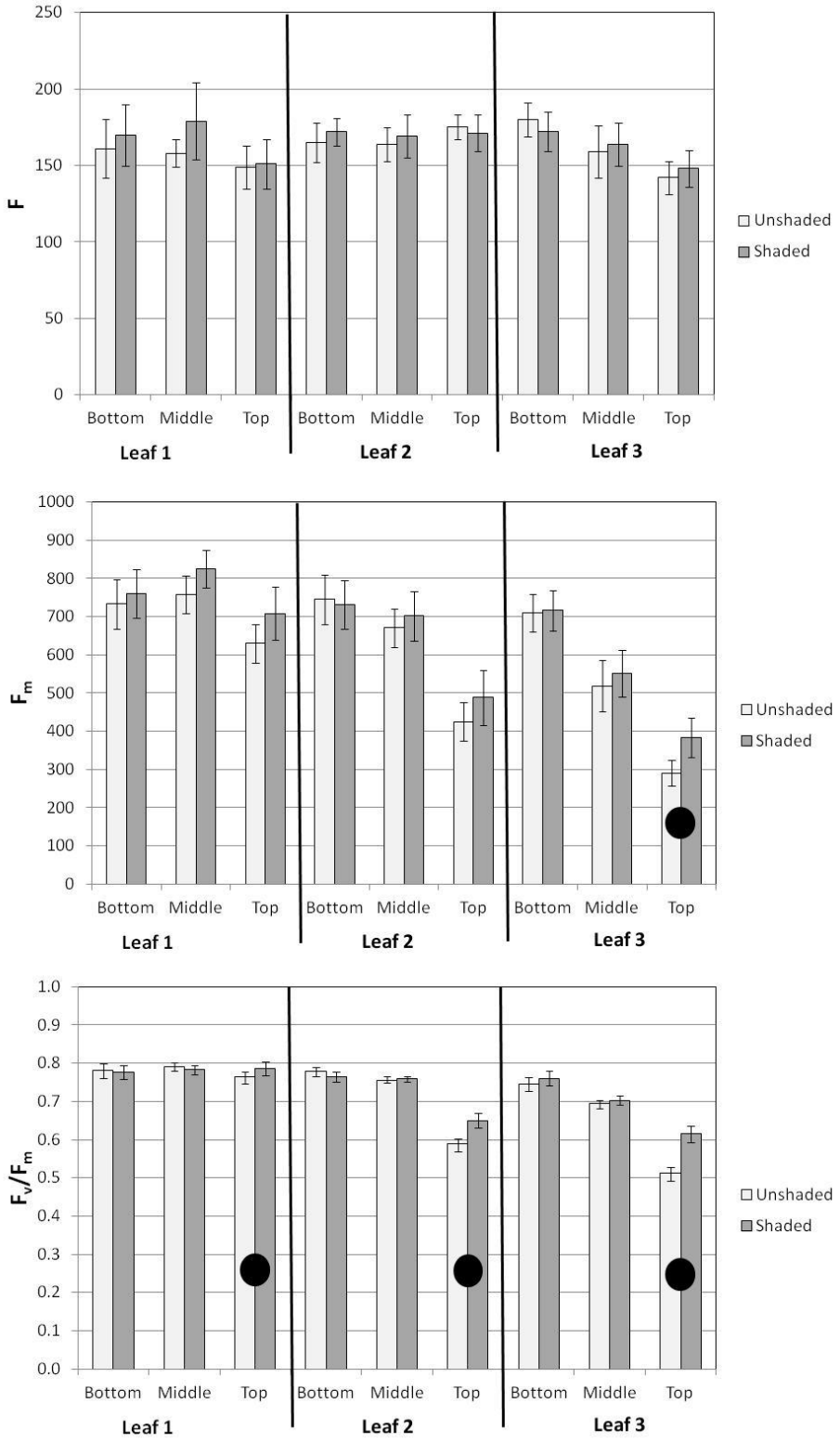

Figure 8.5. Comparison of dark-adapted chlorophyll fluorescence parameters along shaded and unshaded leaves of *Thalassia testudinum* Rabbit Key Basin, Florida Bay. Black dots indicate statistically significant differences ($P < 0.05$).

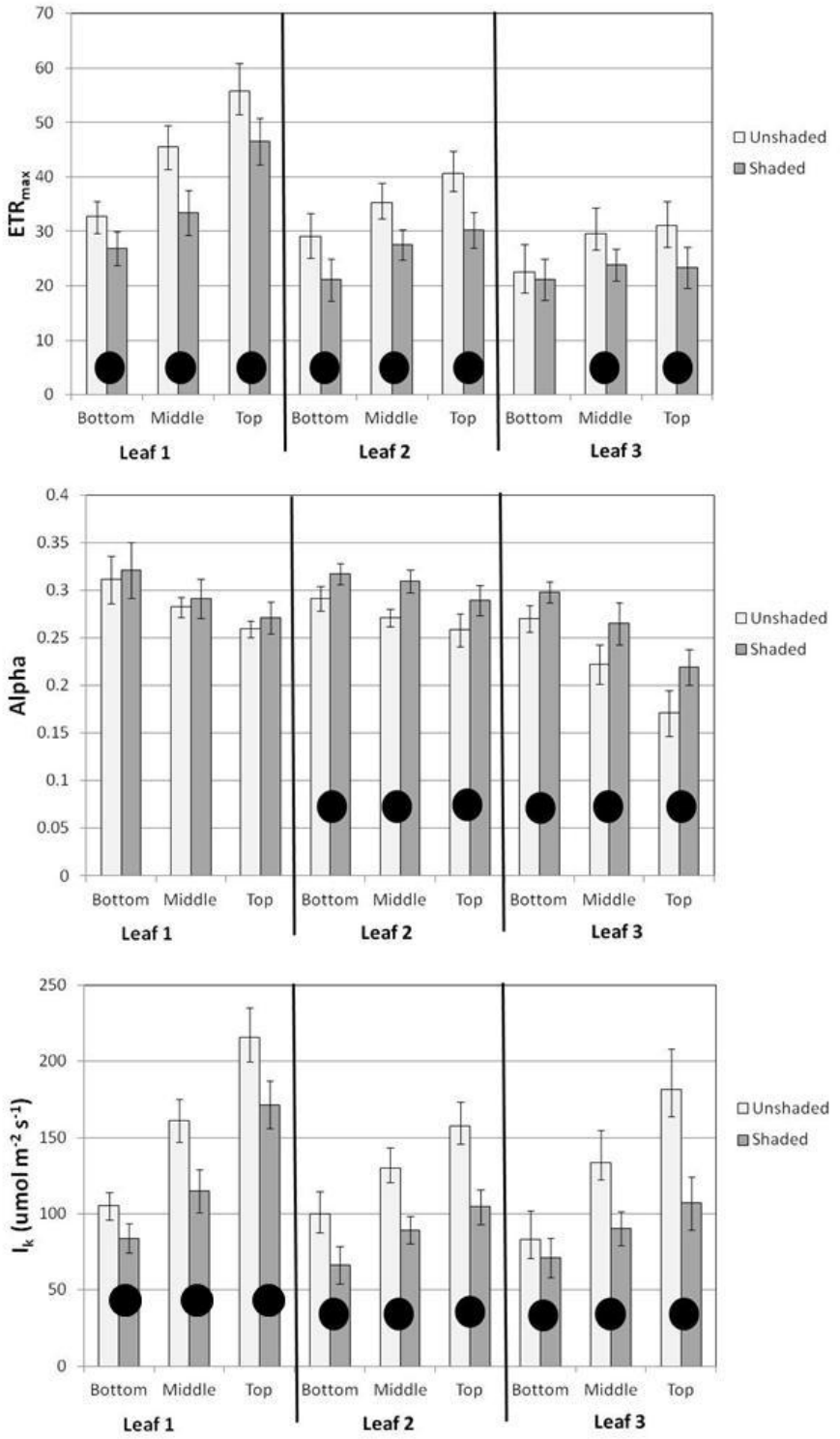

Figure 8.6. Comparison of ETR_{max} and Alpha along shaded and unshaded leaves of *Thalassia testudinum* Rabbit Key Basin, Florida Bay. Black dots indicate statistically significant differences ($P < 0.05$).

8.5 **Discussion**

The experimental reduction of light by 30% for eight weeks to *T. testudinum* resulted in a 20.2% decrease in leaf standing crop, a 26.6% decrease in short shoot productivity, and 57.7% decrease in the growth of newly emerged leaves. *T. testudinum* leaves showed various morphological and physiological responses to the reduction in light availability. The shaded leaves reacted to the imposed shading by increasing leaf chlorophyll content to increase light harvesting ability. This is consistent with other studies (Lee and Dunton 1997; Dennison and Alberte 1982) and with the general model for shade-adapted plants (Givnish 1988). Generally, the increase in chlorophyll content was found in younger leaves. The younger leaves (Leaf 1 and Leaf 2) likely emerged after the introduction of the shade canopies while Leaf 3 likely emerged and elongated prior to the shading.

There would appear to be some difference in how a leaf adjusts to shade conditions depending on the light environment under which it developed to maturity. Considering the expected lifespan of a *T. testudinum* leaf of approximately 52 days and the eight weeks of imposed shading, the youngest leaves would have been exposed to the treatment effects for nearly their entire existence while older leaves would have been exposed to the shade treatment after they had already reached full length and pigmentation. *T. testudinum* appears to acclimate to a reduction in light availability primarily through changes in leaf attributes during photomorphogenesis. Acclimation to changing light through production of chlorophyll or other process is energy intensive. It makes sense that the plant would expend most of its energy on acclimating younger

leaves. Older leaves have already lost a large amount of their photosynthetic capacity, the plant would gain little by expending energy to acclimate these leaves. It may also be more efficient to acclimate a leaf as it develops as opposed to after it has reached maturity.

The lower C:P in the younger shaded leaves suggests that they are less nutrient limited and more light-limited than the unshaded leaves. As photosynthetic ability declines due to the lower light, less P is required and this is reflected by a lower C:P in the leaf tissue. Considering that C:N did not change in the shaded leaves, Rabbit Key Basin would not be considered N-limited.

One of the primary process that a plant uses to induce photomorphogenic response is through detection of the red:far-red of the light field through the phytochrome system. However, as Chapter 3 showed, a seagrass canopy does not experience a change in the red:far-red that could drive the photoreversible phytochrome system. Plants have redundant processes for detecting variations in light availability.

8.5.1 Evidence of photoinhibition

One of the objectives of this study was to use the results to consider whether the *T. testudinum* leaves exhibit evidence of photoinhibition under natural light conditions. Some evidence does suggest photoinhibitive processes primarily at the tips of older leaves. This evidence includes significant decline in chlorophyll content coinciding with declines in F_m and F_v/F_m. The tips of Leaf 3 were considered the most likely to exhibit evidence of photoinhibition since they experienced the highest light for the longest time. If the tips of unshaded leaves were experiencing photodamage, chlorophyll content would

likely have been higher in the shaded tips. However, chlorophyll content was higher at the leaf tips only in the younger leaves, which is likely due to variations in photoacclimation rather than photodamage.

8.5.2 Sun versus shade plants

There has been some debate concerning the identification of seagrass as either a sun plant or a shade plant (Cumming and Zimmerman 2003; Dennison and Alberte 1982; Cayabyab and Enríquez 2007). Shade plants are characterized by a sensitivity to high light that induces photoinhibition and an ability to acclimate leaf morphology and photosynthetic physiology (Givnish 1988). The *T. testudinum* in this study showed evidence of being both a sun and shade plant. The plants adjusted pigment concentration due to shading but fluorescence measurements did not suggest photoinhibition. The significantly higher F_v/F_m for shaded leaf tips suggests a lower degree of light stress compared to the unshaded leaves. Photoinhibition in the unshaded short shoots would be evident in lower F_v/F_m due to higher F values. F values did not change significantly while F_m and F_v/F_m showed significant change. The lack of change at lower leaf locations may be due to either the lower light levels deeper within the canopy or to the fact that the lower parts of the leaf are younger and healthier than the tips.

Chapter 9. Diurnal variation of light availability and photosynthetic activity in a *Thalassia testudinum* canopy at Barnes Key, Florida Bay

9.1 Abstract

This study examined the diurnal variation in leaf photosynthetic activity and canopy light availability in a dense *T. testudinum* meadow at Barnes Key, Florida Bay. The results showed that surface light reflection and light penetration through the canopy are strongly related to solar elevation angle. ETR_{max} and alpha also varied significantly through the canopy in response to diurnal variation in light variability. This study revealed the problem of relying on single calculations of photosynthetic attributes to parameterize seagrass productivity models.

9.2 Introduction

Light availability is often considered the most important factor influencing seagrass distribution, growth, and abundance (Dennison and Alberte 1985; Dennison 1987; Dawes 1998). However, tropical seagrasses found in clear oligotrophic waters can experience irradiance intensities well in excess of those needed to maximize photosynthetic rates and are often considered nutrient limited (Duarte 1990; Powell et al. 1989; Fourqurean et al. 1992). These seagrasses show limited correlation between light availability and leaf productivity challenging the theory of light's central role in these systems (Enríquez and Pantos-Reyes 2005; Tomasko 1992; Herzka and Dunton 1997).

Recent studies have suggested that estimation of light availability to dense seagrass meadows has been inaccurately described. Enríquez et al. (2002) showed that canopy self-shading can account for a 3 order of magnitude variation in the light intensity

from the top of the canopy to the base leading to a potential overestimation of integrated canopy photosynthesis of approximately 50%.

Another potential source of error in quantifying light availability is that the standard measure of light availability to plants, photosynthetically active radiation (PAR) does not account for the disproportionate absorption of blue and red light by photosynthetic pigments; i.e. all quanta are considered equal. A more accurate measure is photosynthetically usable radiation (PUR), which is the PAR spectrum weighted against the leaf absorbance spectrum of the plant of interest (Morel 1978). This method takes into account not just the light available to the plant but also the ability of the plant to absorb the available light. The correspondence of PAR and PUR is an indicator of the quality of light (Gallegos 1994).

Seagrasses are highly adaptive to varied light environments allowing them to colonize along light gradients ranging from full exposure in intertidal areas to the light-limited depth limit (Dawes and Tomasko 1988; Duarte 1991). Seagrasses adapt to seasonal or other long term changes light availability by altering leaf morphology, short shoot density, pigment concentration, and rate of leaf formation (Gordon et al. 1994; Dennison and Alberte 1982). They are also able to acclimate to short term changes in light due shading from floating algal mats or diurnal variation by altering the photosynthesis-irradiance (P-I) response of the leaves (Belshe et al. 2007; Silva and Santos 2003). Seagrasses also use photoprotective responses to photoacclimate to supersaturating irradiance (Major and Dunton 2002).

Thalassia testudinum, the dominant seagrass in the shallow waters of coastal and estuarine waters of southern Florida and the Caribbean Sea, forms dense meadows where the extensive belowground matrix and other non-photosynthetic tissues can account for more than 85% of the total biomass (Zieman 1982). This living tissue requires substantial quantities of oxygen during maintenance respiration and thus requires an ample supply of photosynthetically produced oxygen (Fourqurean and Zieman 1991). The minimal light requirement for *T. testudinum* is higher than other seagrasses because of the significant oxygen demand of the extensive below-ground tissues (Duarte et al. 1991). During the 1980's, extensive die-off of *T. testudinum* meadows occurred in Florida Bay eventually denuding 4000 ha and disturbing 23,000 ha to a lesser degree (Robblee et al. 1991). These events generally occurred in extremely dense meadows and may have been precipitated by periods of unusually high water temperatures, and consequently higher plant respiration rates, coinciding with decreasing sunlight and lower photosynthesis during late summer and early fall (Durako 1994; Zieman et al. 1999). Studies have suggested that these die-offs may be caused when imbalances in oxygen demand, possibly from overgrowth, result in anoxic condition in the sediment resulting in the toxic accumulation of sulfide within the leaf meristem (Borum et al. 2005). In order to evaluate the susceptibility of a dense seagrass meadow to a die-off event, it is necessary to accurately estimate the total gross photosynthesis of the canopy.

The distribution of light in a seagrass canopy is strongly affected by the angle of the direct light beam relative to the canopy top and the orientation of the leaves (Zimmerman 2003). At high solar angles, gaps in a vertically erect canopy are more

easily penetrated by down-welling light beams while at low solar angles light must pass through a longer pathlength of water and leaves. Seagrass leaves grow from a basal meristem, meaning the oldest sections of leaves are located at the top of the canopy, which significantly influences the vertical canopy structure and the distribution of photosynthesis through the canopy (Dalla Via et al. 1998; Enríquez et al. 2002). Because of the vertical age structure and variation in light exposure, photosynthetic pigment concentration and photosynthetic performance vary significantly along seagrass leaves (Enríquez et al. 2002; Dalla Via et al. 1998; Durako and Kunzelman 2003).

An important component of light quality in a plant canopy is the red:far-red light ratio (R:FR). Because green leaves tend to absorb red light (600 to 700 nm) and reflect far-red light (700 to 800 nm), the amount of red light relative to far-red light decreases with depth through a canopy (Holmes 1981). R:FR is approximately 1.2 for daylight and can be as low as 0.05 within a dense plant canopy (Smith and Morgan 2001). Plants detect and respond to changes in R:FR via the phytochrome system, a family of reversible photoreceptors found in all higher plants (Smith 2000). Phytochrome allows a plant to detect shade conditions by provoking gene expressions that modify the plant canopy structure to effectively reach out of the shade instilling a fitness advantage by maximizing light capture (Vandenbussche et al. 2005; Skalova et al. 1999). Physiological and morphological responses to reduced R:FR has been extensively researched in terrestrial plants and includes biomass reallocation, stem and leaf elongation, and rate of clonal branching (Smith 2000; Schmitt and Wulff 1993;

Vandenbussche et al. 2005). Rose and Durako (1994) and Tamasko (1992) found similar morphological responses by exposing seagrasses, *in vitro*, to lowered R:FR.

9.2.1 Objectives

Despite the wide attention given to the importance of light availability for seagrass survival and given the extreme shoot densities in which they are often found, until recently little attention has been directed toward changes in light availability through a seagrass canopy or variation in photosynthetic ability through the canopy. While Enriquez et al. (2002) measured P-I response along leaves, they performed these measurements in the laboratory after removing the leaves from their natural light environment. Studies have shown that P-I parameters are extremely sensitive to the recent light history suggesting that to achieve accurate results measurements must be made *in situ* without altering the natural light regime (Durako and Kunzelman 2002). Belshe et al. (2007) showed significant diurnal variation of P-I attributes in *T. testudinum* leaves but did not compare along leaves.

This study will attempt to integrate the vertical and diurnal variations of light quality and quantity and photosynthetic ability through a seagrass canopy. This study will examine the vertical structure of the canopy as well as the interleaf variation of leaf attributes including chlorophyll concentration, leaf thickness, specific leaf weight, and leaf light absorbance. It will also assess the diurnal vertical variation of photosynthetic performance along leaves from early morning to sunset. The variation of light availability through the canopy will be calculated by conducting vertical irradiance profiles throughout the day.

9.3 **Methods**

9.3.1 Study Site

This study was carried out in a dense monospecific meadow of *T. testudinum* at Barnes Key, Florida Bay, FL, which is the site of past and current die-off events. Water depth at this site is <1 m with ~30-40 cm of water column above the dense canopy. The insignificant tidal variation means that the leaves remain submerged throughout the day. The sediment is comprised mostly of live belowground structures, dead organic matter, and calcium carbonate mud. This site experiences insignificant tidal variation with the leaves remaining entirely submerged throughout the day. The minimal wave energy allows the canopy to maintain an erect status experiencing little undulation. The thickness of the standing crop is such that the sediment surface is not observable from above the canopy. The heavy layer of leaf litter also helps to conceal the highly reflective carbonate mud and greatly lowers the reflectivity of the sediment surface. The low tidal and wave energy typical of this site allows the canopy to remain perpendicular to the sediment unlike high-energy meadows where the leaves are oriented to near horizontal or sway back and forth. The exceptionally long short shoots and leaf sheaths produce an effective canopy that starts approximately 5 to 8 cm above the sediment surface.

9.3.2 Canopy and leaf attributes

Leaf standing crop and productivity were determined via the leaf punching method using six 10 cm by 20 cm quadrats and a 15 day growth period (Short and Duarte 2001). For three quadrats, all leaves were carefully removed from short shoots, scrapped

of epiphytes with a razor blade, and separated by age. The length and width of each leaf was measured with a ruler and calipers. Leaf lengths were measured from the tip to the interface where the green leaf meets the white sheath, which was also measured. These measurements were used to determine the vertical structure of the canopy.

Ten additional short shoots were haphazardly selected and transported in seawater, within three hours of harvest, to the lab at Key Largo for processing. These short shoots were representative of the majority of short shoots found at the site. Leaves were ranked by age with the youngest adult leaf being Leaf 1 and the next older leaf being Leaf 2. Young leaves between 4 cm and 8 cm in length were identified as emergent leaves or Leaf e. Leaves less than 4 cm in length were disregard. After measuring leaf length and width, a 5 cm segment was cut from base, middle and tips of the leaves. The thickness of the segment was measured with a dial thickness gage (Peacock) accurate to 0.01 mm. Leaf spectral absorbance was calculated on each segment using the methods from Enríquez et al. (2002) utilizing the Ocean Optics USB 2000 (Mini-spec) and a halogen light source. The PAR absorbance factor (AF) for each segment was calculated by integrating the spectral absorbance from 400 to 700 nm. Chlorophyll concentration was determined for each segment via acetone extraction using as in Chapter 5.

9.3.3 Light profiles

Down-welling irradiance was measured using the Mini-spec and the light sampling assembly. By inserting the assembly into the sediment on the sunny side of the boat, the effects of wave rocking and reflection from the boat were negated. Irradiance

profiles were conducted throughout the day from early morning to sunset by taking 30 sec average readings of the down-welling light spectrum at 10 cm intervals through the water column and canopy. If clouds obstructed the sun during the profile, the profile was repeated. Cloud cover, water depth, and wave conditions at the time of the profile were recorded. For comparison, light measurements were also conducted in a terrestrial grass meadow at solar noon with the light sensor positioned at the bottom of the canopy.

Total PAR for each spectrum was calculated by integrating the irradiance from 400 to 700 nm. PUR was calculated by weighting the PAR spectrum with the average leaf absorption spectrum for *T. testudinum*. The peak wavelength of the absorption spectrum was given a value of 1.0 with the remaining wavelengths valued as a proportion of this peak wavelength. The irradiance spectra were then multiplied by this relative absorption spectrum to determine PUR. Spectral diffuse attenuation coefficients ($K_d(\lambda)$) were calculated as the slope of the plot of the natural log of irradiance versus depth. Surface spectra were not used in the regressions in order to negate the effect of surface reflection. Separate calculations were made for the water column and canopy. Profiles resulting in regression coefficients (R^2) lower than 0.70 were discarded. R:FR was calculated by integrating across 660 to 670 nm for red light and 725 to 735 nm for far-red light (Smith 2000). The solar elevation at the time of each profile was calculated as a function of latitude, day of year, and time of day using the equations from Kirk (1994). Differences in the wavelength distribution between spectra were compared by normalizing the spectra to 1.0 at 500 nm. Linear regressions were performed to find the

effect of solar elevation on R:FR, K_d(PAR), K_d(PUR), and K_d for 10 nm bands centered at a number of primary wavelengths (350, 440, 550, 675, and 735 nm).

9.3.4 Photosynthetic performance

Diurnal variation in photosynthetic characteristics of *T. testudinum* leaves was measured *in situ* using the Diving-PAM (Heinz-Walz, Germany). Rapid light curves (RLC), comparable to P-I curves, were performed along the leaves of three haphazardly selected short shoots at times throughout the day (Ralph and Gademann 2005). Measurements were made at the base, middle, and tip of the adult leaves and the middle of emergent leaves. The relatively low leaf epiphytic growth at the site was easily removed before attaching the leaf clip by gently passing the leaf between the thumb and index finger. The RLC was conducted immediately after attaching the clip in order to avoid dark-adapting the leaf segment. The electron transport rates (ETR) calculated by the Diving-PAM were corrected using corresponding segment specific AF calculated from the harvested leaf samples. The photosynthetic efficiency (alpha), the initial slope of the curve, and photosynthetic capacity (ETR_{max}), peak ETR and corresponds with P_{max}, were estimated by fitting the ETR and PAR data for each RLC to a exponential decay function (Ralph and Gademann 2005). The half saturation coefficient was estimated using the equation $I_k = ETR_{max} / \text{alpha}$.

9.3.5 Data and statistical analysis

Customized MATLAB scripts were used to analyze and display spectra data including the conversion of Mini-spec irradiance output into quanta units, integration across wavelength bands, and calculation of diffuse attenuation coefficients. The

irradiance values generated by the Mini-spec were converted to quanta units using Planck's constant and the equations from Kirk (1994). Within leaf and between leaf comparisons were analyzed using ANOVA and Tukey's multiple comparison tests. Linear regression and correlation analyses were performed to assess the influence of solar elevation and time of day on light attenuation, R:FR, alpha, and ETR_{max}. All statistical analyses were performed using SAS 9.1 (SAS Institute Inc.).

9.4 **Results**

9.4.1 Leaf attributes

The shoot density (1583 SS m^{-2}), standing crop (169.2 g dwt. m^{-2}), and areal productivity (2.91 g m^{-2} day^{-1}) of the *T. testudinum* meadow at Barnes Key are among the highest reported for Florida Bay (Zieman et al. 1989). The thickness of the standing crop is such that the sediment surface is not observable from above the canopy. The heavy layer of leaf litter also helps to conceal the highly reflective carbonate mud and greatly lowers the reflectivity of the sediment surface. The exceptionally long short shoots and leaf sheaths produce an effective canopy that starts approximately 5 to 8 cm above the sediment surface.

Leaf biomass is highly weighted toward the base of the canopy with approximately 60% of the photosynthetic tissue contained in the bottom 15 cm (Figure 9.1). This is due to an inherent growth property of seagrasses where leaves grow from a basal meristem (Hemminga and Duarte 2000). This vertical gradient in age structure of the canopy is a significant driver of many of the within leaf and between leaf variations

discussed later. The mean leaf photosynthetic characteristics along the first four leaves are shown in

Table 9.1. There was significant within leaf variation in leaf light harvesting characteristics including leaf thickness, specific leaf weight, chlorophyll concentration, and Chl *a*:*b*. This creates a vertical gradient in light absorbance ability through the canopy. Chlorophyll concentration increases from base to tip in younger leaves reflecting accumulation of pigments as the leaf matures. As leaves age, a significant decline in chlorophyll concentration is observed at the tips of leaves, however, total chlorophyll at the base of leaves continues to increase. Chlorophyll can decline in leaves due to both age related senescence and photodegradation (Rontani et al. 1996). Additionally, many studies have shown that seagrasses can photoacclimate to changing light regimes by altering leaf chlorophyll content (Dennison and Alberte 1982; Gordon et al. 1994; Major and Dunton 2002). This study provides evidence that all of these processes may actually occur simultaneously.

Chl *a*:*b* showed no significant variability in younger leaves, however, older leaves saw a significant increase from the base to tip. Chl *a* and Chl *b* have slightly different absorbance peaks in the red and blue light regions so a lower Chl *a*:*b* results in a broader absorbance spectrum and increased light harvesting efficiency (Agustí et al. 1994). Chl *b* is an accessory pigment found only in peripheral light-harvesting complexes and has no role in electron transport (Eggink et al. 2001). Because Chl *a* degrades faster than Chl *b*, a decline in chlorophyll due entirely to senescence or photooxidation would result in a lower Chl *a*:*b* (Maunders and Brown 1983). This suggests that the lower Chl *a*:*b* at the

leaf bases is a photoacclimatory response. Higher specific leaf weight and leaf thickness are common adaptations seen in sun-adapted leaves (Givnish 1988). However, both of these attributes declined from base to tip in *T. testudinum* leaves. This attribute may make seagrasses more buoyant allowing them to maintain an erect canopy. The peak irradiance experienced by leaf tips is well in excess of the light saturation point leading to chronic photoinhibition and declines in quantum efficiency (Enríquez et al. 2002; Major and Dunton 2002). This may be responsible for a portion of the chlorophyll decline.

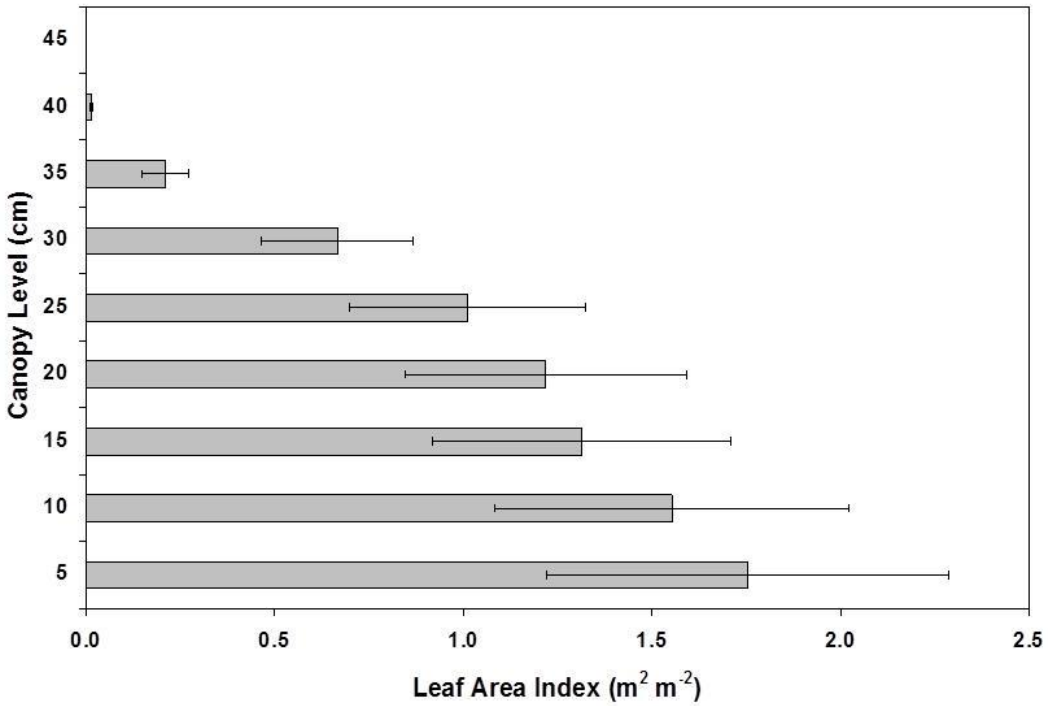

Figure 9.1. Vertical distribution of leaf area through the Thalassia testudinum canopy at Barnes Key, Florida Bay. Bars are means of three 10 x 20 cm quadrats. Error bars are standard error.

Table 9.1. Interleaf variation in *Thalassia testudinum* leaf attributes at Barnes Key Basin. Values are means ±SD. Leaf rank denotes leaf age: e, emergent; Rank 1, youngest adult leaf; Rank 2, next oldest leaf. Segment location indicates relative vertical location along the leaf. Letters denote significant differences along individual leaves using Tukey's multiple comparison test ($p<0.05$). Comparisons for leaf length are between different leaf ranks.

Leaf rank	Segment location	Leaf Length (cm)	Leaf Thickness (mm)	SLW (mg/cm^2)	Total Chl (mg/g)	Chl *a*:*b*	AF
e	Middle	4.4±0.6 c	0.25±0.04	5.28±0.37	2.03±0.14	1.84±0.06	0.68±0.09
1	Base	15.0±1.5 b	0.38±0.09 a	6.59±0.83 a	1.81±0.36 b	1.87±0.17 a	0.73±0.08 a
1	Middle	NA	0.27±0.06 b	6.21±0.79 ab	3.25±0.64 a	1.90±0.27 a	0.68±0.11 b
1	Tip	NA	0.17±0.04 c	5.29±1.10 b	3.59±0.85 a	2.07±0.13 a	0.63±0.03 c
2	Base	26.4±4.8 a	0.41±0.07 a	6.96±0.53 a	2.36±0.50 b	1.78±0.18 b	0.75±0.09 a
2	Middle	NA	0.28±0.07 b	7.00±0.45 a	3.46±0.52 a	1.96±0.11 ab	0.66±0.11 b
2	Tip	NA	0.17±0.05 c	4.59±0.29 b	2.56±0.93 b	2.12±0.20 a	0.64±0.13 b
3	Base	29.9±4.3 a	0.41±0.07 a	6.87±0.62 a	2.98±0.65 a	1.92±0.15 b	0.74±0.14 a
3	Middle	NA	0.27±0.08 b	5.80±0.41 b	3.60±1.27 a	2.16±0.17 a	0.67±0.12 b
3	Tip	NA	0.18±0.04 c	4.20±0.53 c	1.13±0.73 b	2.13±0.12 a	0.60±0.09 c

9.4.2 Light field

The solar elevation angle as a function of time of day was calculated for the time of year and latitude at Barnes Key (Figure 9.2). Time of day was adjusted to standard time from daylight savings time. The rate of attenuation through the water column at increasing solar angle is shown in Figure 9.3. Water column attenuation is only slightly affected by changes in solar elevation most likely due to the increase in the pathlength of the direct beam radiation through the water column (Spence 1981). The magnitude of the canopy attenuation varies 2- to 3-fold due to changes in the angle of entry of the direct beam radiation and the canopy driven by diurnal changes in solar elevation (Figure 9.4). This is due partly to the continued increase in the pathlength for direct light beams through the water column and canopy but also to the increased probability of interception of direct beam radiation by leaves. At low solar angles, direct light is less likely to penetrate to the subcanopy through gaps (Canham 1988). Although diffuse light is able to more effectively penetrate a closed canopy, intercanopy diffuse radiation is much lower intensity than sunfleck light. Additionally, the effective surface area of leaves increases as the angle of the direct radiation approaches perpendicular to the leaf surface (Zimmerman 2003).

Red:far-red generally increased with depth through the water column and canopy and varied as a function of time of day (Table 9.2). The daily mean for red:far-red increased from 1.44 above the water surface to 3.52 at 60 cm depth, which corresponded to 30 cm within the canopy. At the bottom of the canopy, the red:far-red increase slightly to 3.18. However, at this depth there is little detectable far-red light. Above the surface,

red:far-red showed a slight decrease at low solar elevation. However, within the water column and especially within the seagrass canopy, red:far-red increased at low solar elevation.

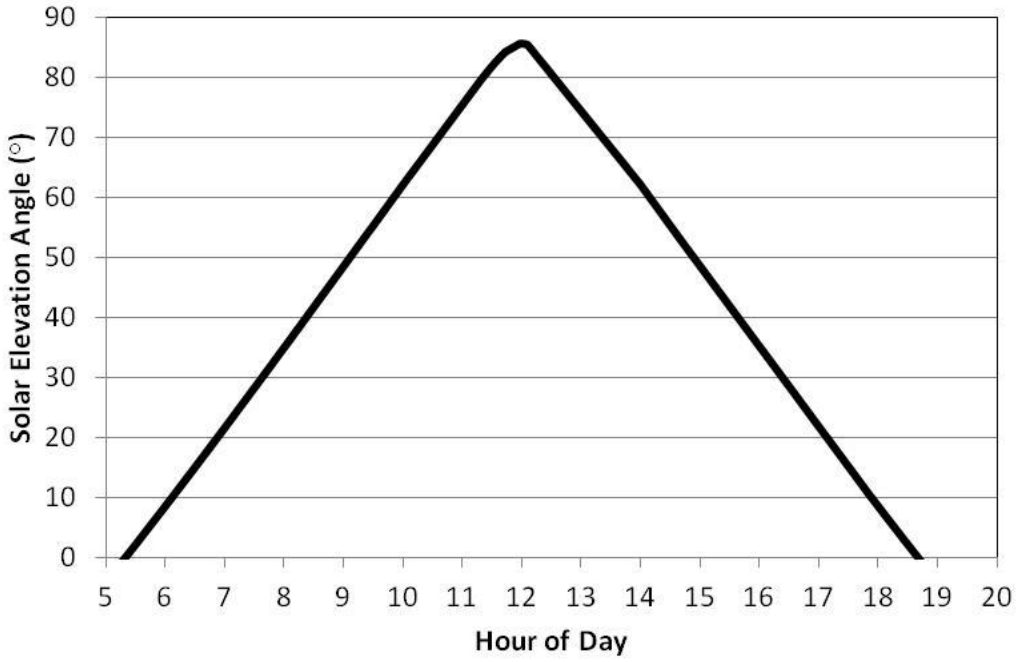

Figure 9.2. Solar angle versus hour of day on July 24 at Barnes Key, Florida Bay (25° N Latitude). Calculated from Kirk 1994.

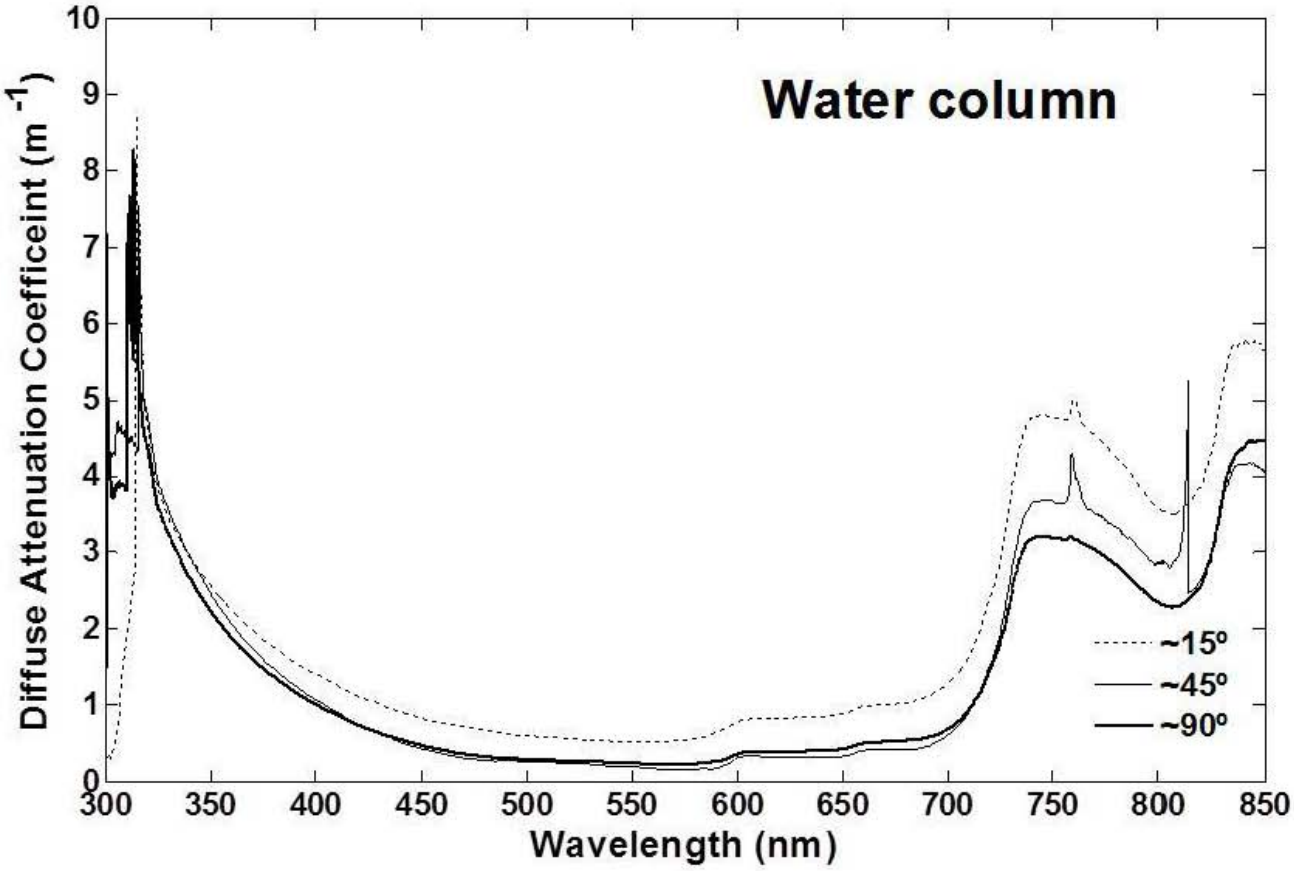

Figure 9.3. Spectral attenuation coefficients through the water column at Barnes Key, Florida Bay at high (~90º), mid (45º), and low (15º) solar elevations.

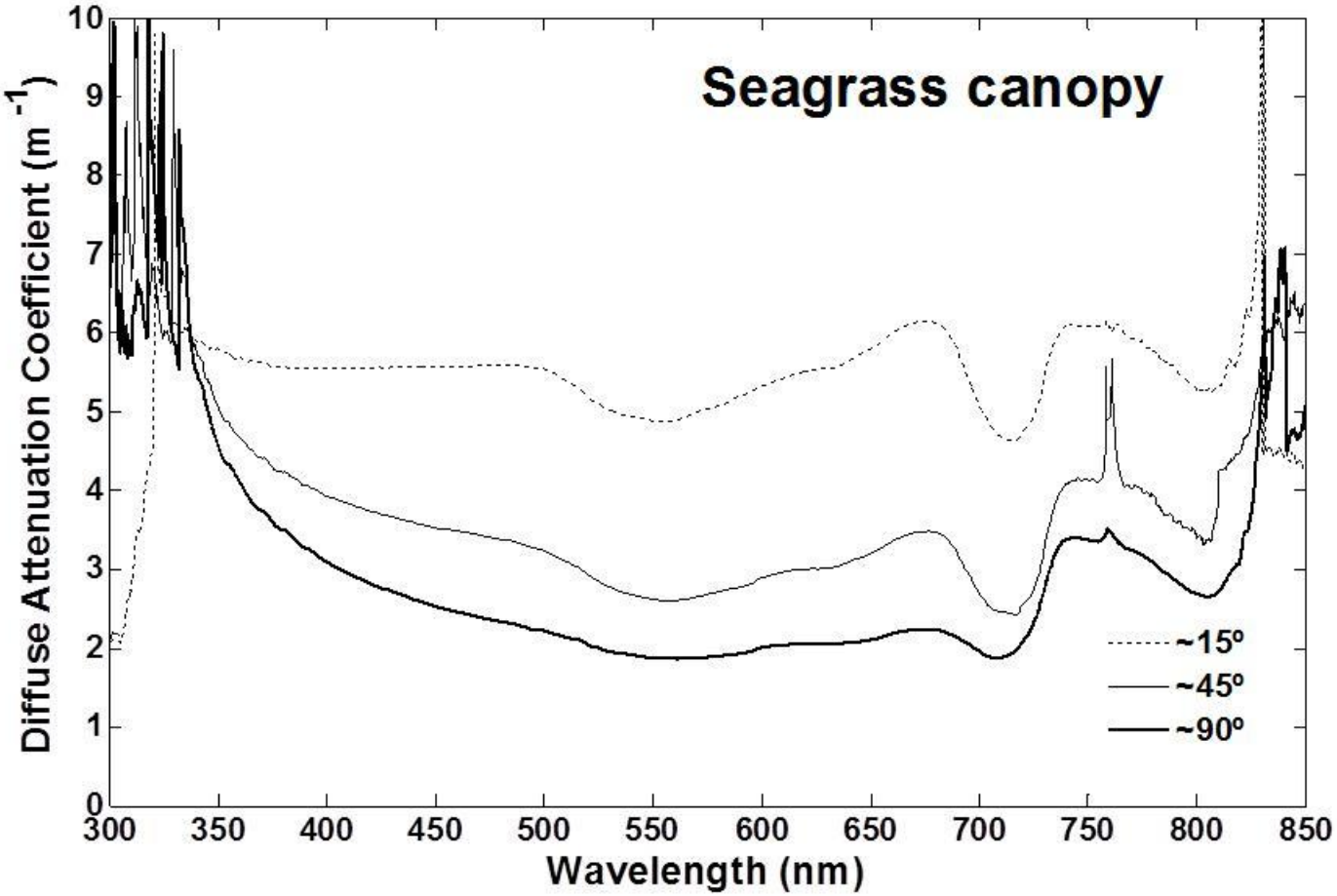

Figure 9.4. Spectral diffuse attenuation coefficient through a *Thalassia testudinum* canopy at Barnes Key, Florida Bay at high (~90º), mid (45º), and low (15º) solar elevations.

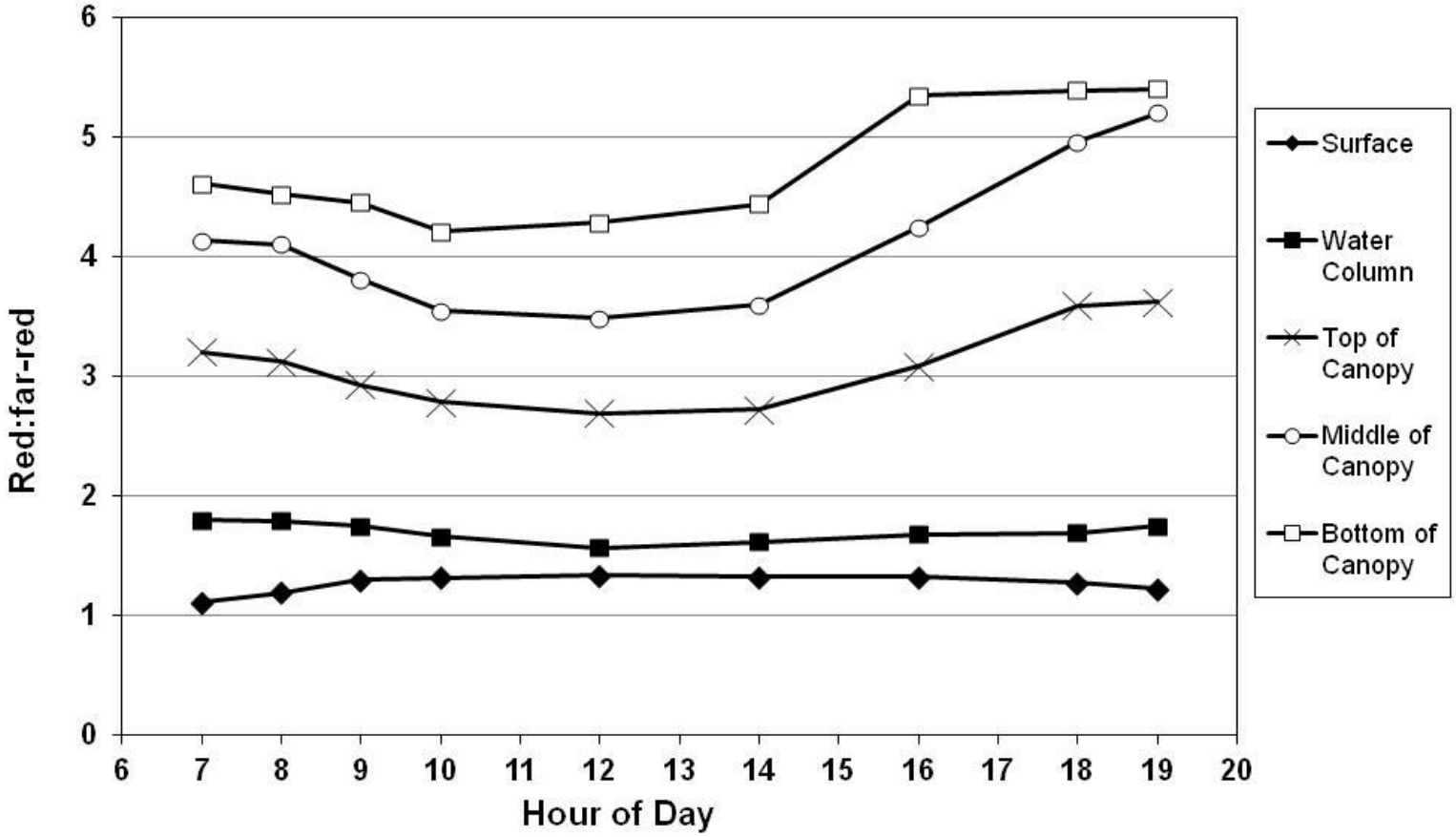

Figure 9.5. Diurnal variation of red:far-red from above the water surface to the bottom of the water column at Barnes Key, Florida Bay.

Table 9.2. Red:far-red (R:FR) versus depth and solar elevation through the water column and *Thalassia testudinum* canopy at Barnes Key, Florida Bay. The shaded rows are for the measurements within the seagrass canopy.

	R:FR	R:FR vs. solar elevation	
Depth	Mean±SD	R	R^2
Surface	1.44±0.09	**-0.845**	**0.714**
10 cm	1.70±0.15	**-0.852**	**0.726**
20 cm	2.14±0.26	**-0.892**	**0.796**
30 cm	2.64±0.42	**-0.853**	**0.728**
40 cm	3.26±0.60	**-0.884**	**0.781**
50 cm	3.63±0.53	**-0.876**	**0.767**
60 cm	3.52±0.50	-0.322	0.104
70 cm	3.18±0.61	0.200	0.040

9.4.3 Diurnal variation in surface reflectance

The spectral reflectance from the water surface at increasing solar elevation angle is shown in Figure 9.6. At high solar angles, reflectance from the surface was only 3 to 4% of the irradiance and the reflectance was fairly even across the wavelengths from 400 to 800 nm. However, when the sun was closer to the horizon the reflectance increased and the amount of reflectance increased at higher wavelengths. These results show that nearly a third of the reduction in red light at the beginning and end of the day is due to reflectance from the water surface.

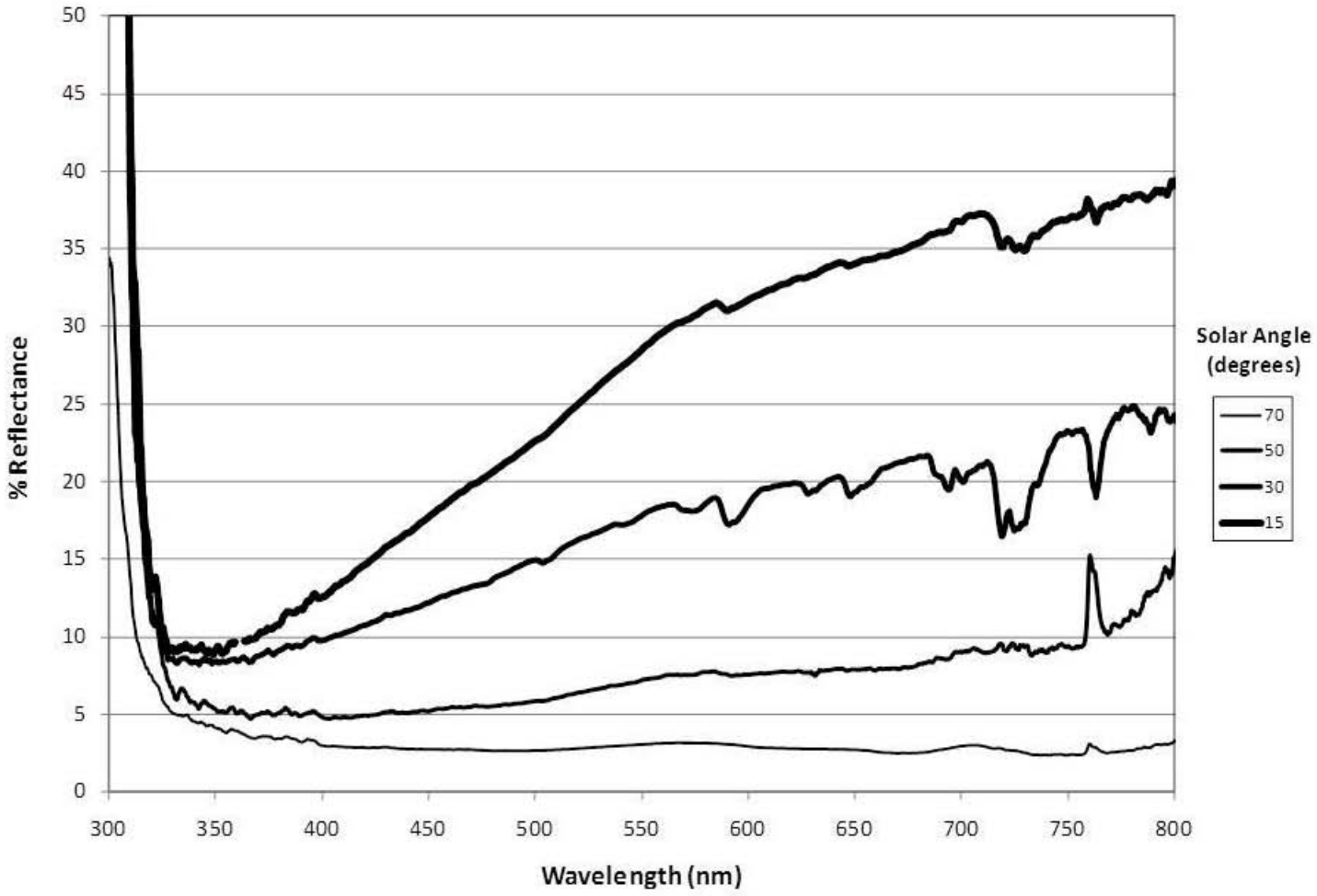

Figure 9.6. Spectral reflectance of light from the water surface at Barnes Key, Florida Bay at increasing solar angles.

9.4.4 Chlorophyll fluorescence measurements

The initial measurement of photosynthetic parameters, alpha and ETR_{max}, suggested that the highest photosynthetic activity occurred at the tips of leaves. However, after correcting the ETR values of the RLC using the vertical variation in leaf light absorbance, peak photosynthetic capacity shifted toward the middle of leaves (Figure 9.7). This is important to note because there is significantly greater leaf area located at the middle of the canopy then at the top. As observed with the leaf attributes, the vertical variability of photosynthetic activity is driven by a number of factors including variation in tissue age along leaves and differences in light availability (Durako and Kunzelman 2002; Enríquez et al. 2002). While the tips of *T. testudinum* leaves display characteristics of a sun-adapted plant, the majority of the seagrass canopy actually functions as a shade-adapted plant (Major and Dunton 2002; Givnish 1988). Figure 9.8 shows an example of the plot of ETR_{max} and alpha versus the hour of day. The regression parameters were compiled in Table 9.3 and Table 9.4

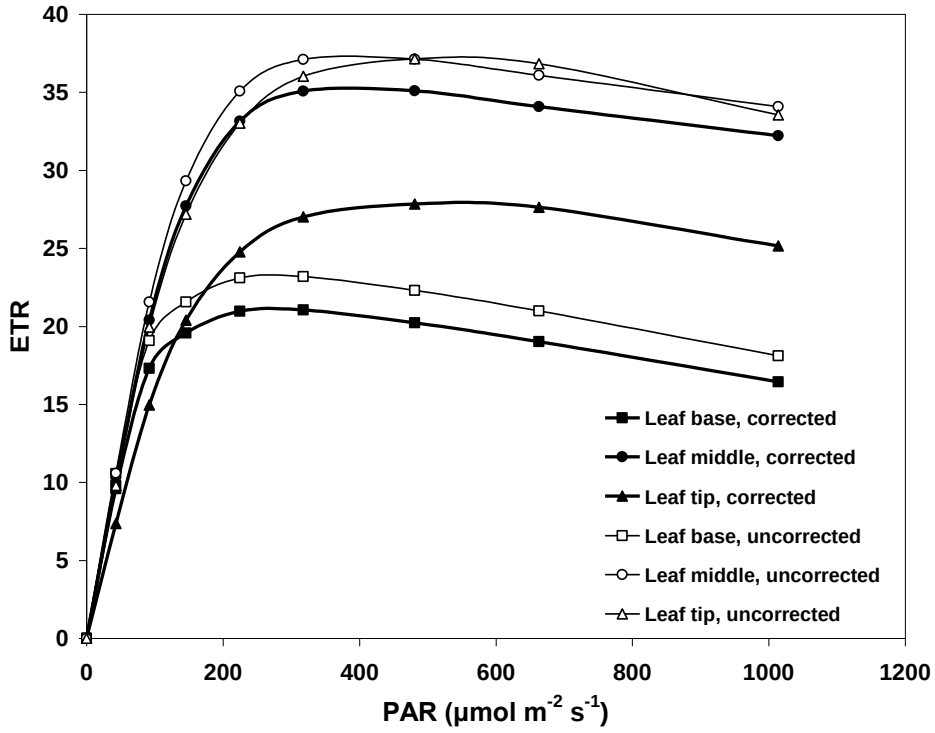

Figure 9.7. Rapid light curves along *Thalassia testudinum* leaves (Leaf Rank 1) at Barnes Key, Florida Bay before and after correcting for interleaf variation in leaf light absorbance.

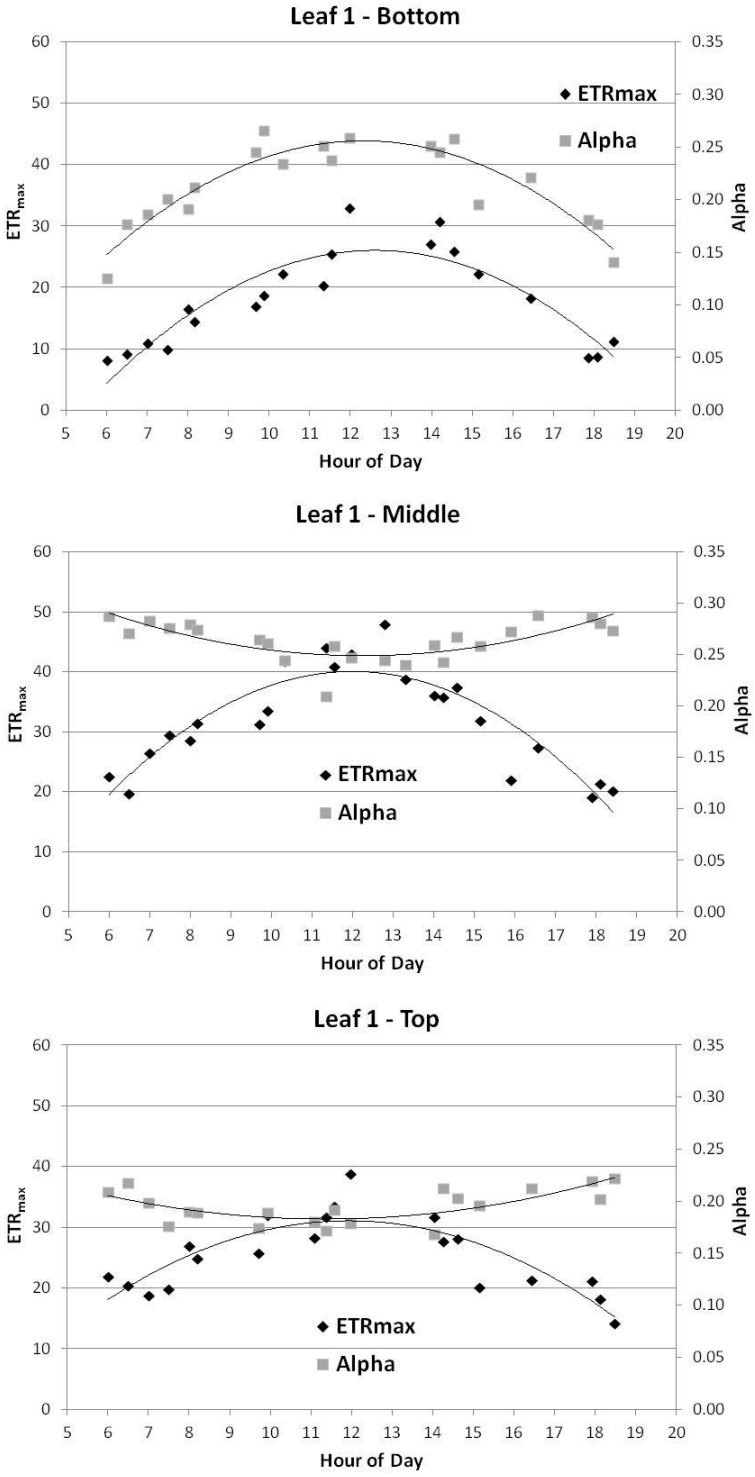

Figure 9.8 Diurnal variation in ETR_{max} and alpha along the youngest adult leaf at Barnes Key, Florida Bay. Equations are 2nd order polynomials. Regression parameters are included in Table 9.2 and 9.3.

Table 9.3. Regression parameters for ETR_{max} versus time of day fitting the 2nd order polynomial equation $ETR_{max} = X^2 + X + Intercept$.

Leaf Segment	x^2	x	Intercept	R^2
Leaf e	-0.6211	15.403	-67.593	0.7682
Leaf 1 – Bottom	-0.4981	12.558	-53.123	0.8163
Leaf 1 – Middle	-0.5700	13.684	-42.064	0.8141
Leaf 1 – Top	-0.3685	8.7928	-21.397	0.7131
Leaf 2 – Bottom	-0.3364	8.4227	-34.236	0.7396
Leaf 2 - Middle	-0.4481	11.507	-45.155	0.6451
Leaf 2 - Top	-0.4314	9.8549	-25.393	0.7945

Table 9.4. Regression parameters for Alpha versus time of day fitting the 2nd order polynomial equation $Alpha = X^2 + X + Intercept$.

Leaf Segment	x^2	x	Intercept	R^2
Leaf e	-0.0023	0.0567	-0.1337	0.7468
Leaf 1 – Bottom	-0.0027	0.0668	-0.1558	0.8612
Leaf 1 – Middle	0.0011	-0.026	0.4088	0.5661
Leaf 1 – Top	0.0008	-0.0178	0.2838	0.5447
Leaf 2 – Bottom	-0.0018	0.0476	-0.0594	0.6099
Leaf 2 - Middle	0.0005	-0.0157	0.344	0.2403
Leaf 2 - Top	0.0019	-0.0538	0.5278	0.8466

9.5 **Discussion**

This study revealed the problem of relying on single calculations of photosynthetic attributes to parameterize seagrass productivity models. Light penetration through a water column is highly sensitive to the incident angle of the sun. For example, a calculation of the light attenuation coefficient through a water column while the sun is at a solar angle of approximately 90º would overestimate the light penetrating one meter of water at 15º solar angle by approximately a third. Light penetration through the seagrass canopy is even more sensitive to solar angle. A canopy attenuation coefficient calculated at noon would overestimate the light reaching the bottom of the canopy by an order of magnitude. This is significant considering that the majority of the leaf tissue is contained in the bottom third of the canopy. Also, consider that an increasing amount of down-welling light is reflected from the water surface as the sun approaches the horizon. Using single calculations for water column attenuation coefficients is not adequate for estimating diurnal light penetration through the water column.

This study also showed the importance of understanding the role of the interleaf variability in the ability to absorb available light. The initial measurement of photosynthetic parameters, alpha and ETR_{max}, suggested that the highest photosynthetic activity occurred at the tips of leaves. However, after correcting the ETR values of the RLC using the vertical variation in leaf light absorbance, peak photosynthetic capacity shifted toward the middle of leaves (

Figure 9.7). This is important to note because there is significantly greater leaf area located at the middle of the canopy then at the top. As observed before with the leaf

attributes, the vertical variability of photosynthetic activity is driven by a number of factors including variation in tissue age along leaves and differences in light climate (Durako and Kunzleman 2002; Enríquez et al. 2002).

Diurnal variation of canopy light penetration also affects the red:far-red within the canopy. Literature suggests that red:far-red is lower when the sun is closer to the horizon (Chambers and Spence 1984). This consistent diurnal light signal is considered a possible mechanism by which aquatic plants might use the phytochrome system to detect the length of the photoperiod. This could be an important means for controlling the timing of reproduction. This study did show a slight decline in red:far-red at the surface. However, within the seagrass canopy the red:far-red increased, questioning whether the red/far-red reversible phytochrome plays a role in regulating seagrass morphology.

T. testudinum leaves are highly adaptable to changes in light availability. The leaves can photoacclimate along individual leaves in response to intercanopy variations in light availability. The *T. testudinum* leaves can also photoacclimate to diurnal changes in light availability. The values of ETR_{max} and alpha can be used to construct a Photosynthesis-Irradiance curve to estimate the actual ETR at a given irradiance. Typical seagrass photosynthesis models use values for maximum photosynthetic rate and alpha that have been derived by measuring oxygen evolution of leaves in a mesocosm at increasing irradiances. This is problematic in that *T. testudinum* leaves must be removed from their environment. This method also assumes that the P-I relationship is static throughout the day. This study showed that ETR_{max} and alpha vary significantly throughout the day in response to changes in irradiance. This variation is not consistent

through the canopy. The photosynthetic performance at the base and middle of leaves is much more sensitive to diurnal changes in irradiance than the tops of leaves. To represent accurately the photosynthetic performance of *T. testudinum* leaves, the ETR_{max} and alpha of productivity models should be dynamic to account for the intercanopy and diurnal variability of these important parameters.

Chapter 10. Dissertation synopsis and synthesis

The primary objective of this dissertation research was to investigate how photosynthesis varies through a seagrass canopy. The results showed that photosynthesis varies significantly through a seagrass canopy due to the decline in the quantity and quality of light through the canopy, the interleaf variation in light harvesting ability, and vertical photoacclimation of photosynthetic performance through the canopy. Within a dense *T. testudinum* meadow, the intercanopy light environment is highly dynamic and the canopy structure extremely complex. The typical seagrass productivity models do not include a module to represent the canopy structure. This dissertation showed that variations of photosynthetic attributes through a seagrass canopy are significant and should be an essential component of any seagrass model.

10.1 **PAR versus PUR**

Light availability is commonly considered the primary factor influencing the productivity, distribution, and abundance of seagrass meadows. This dissertation showed that this is overly simplistic. Seagrass photosynthesis depends on the amount of light available and the ability of the seagrass leaves to absorb and utilize this light. The typical measure of light availability to plants, PAR, is inadequate for representing the light available to a seagrass canopy. Seagrass leaves absorb light primarily in the red and blue wavelength regions and very weakly or not at all in the green region. Because red and blue is preferentially attenuated through a water column, the light field reaching a seagrass canopy is depleted in the primary wavelengths used in photosynthesis.

Photosynthetically usable irradiance, or PUR, takes into account the usability of the light field.

10.2 **Sun versus shade-adapted**

Because of their ability to acclimate to low irradiance, seagrasses are often considered a shade-adapted plant. The attributes of a shade-adapted plant would seem to be beneficial to a plant that grows under a water column. Generally, maximum photosynthetic rates for seagrass species are considerably lower than the range for terrestrial C_3 shade plants (Hemminga and Duarte 2000), which supports the idea that seagrasses are shade-adapted plants. However, seagrass species such as *T. testudinum* have relatively high light requirements to meet the demand of extensive belowground structures. Sun-adapted attributes including high light-saturated photosynthetic rate would be of benefit. Shade-adapted have a low tolerance to high light stress and can quickly become photoinhibited. Shallow *T. testudinum* leaves can withstand very high irradiance well in excess of the light saturation point, as well as the super-intensified pulses of light from wave-focusing, without experiencing permanent photodamage, an important attribute of sun-adapted plants. Rather than being solely a sun- or shade-adapted plant, *T. testudinum* exhibits attributes of both allowing it to colonize deeper habitats where light is limiting and shallow habitats where light is beyond saturating.

A seagrass leaf experiences a highly variable light environment over its lifespan. As a leaf emerges, it experiences the highly shaded environment of the bottom of the canopy. As it elongates it reaches out of the shade and into the open light at the top of the canopy. However, an individual adult leaf also experiences a drastically different

light environment along its length with the base being highly shaded and the tip experiencing unobstructed light. This dissertation showed that the base of a *T. testudinum* exhibits shade-adapted attributes at the same time the tip of the leaf is exhibiting sun-adapted attributes. The conventional approach for characterizing a plant as either sun- or shade-adapted does not adequately describe seagrass leaves.

10.3 **Red:far-red in seagrass canopies**

This dissertation showed that the light field within a seagrass canopy does not exhibit a lowered ratio of red light to far-red light. The red:far-red is an important indicator of the vicinity of neighboring plants and of canopy density and all higher plants possess the phytochrome system that detects changes in the red:far-red (Balleré 1999). Although research has shown that seagrasses can react to experimentally decreased red:far-red in a laboratory setting (Rose and Durako 1994; Tomasko 1992), a lower red:far-red is not observed in an *in situ* seagrass canopy. This suggests that a seagrass canopy may have other mechanisms for detecting canopy density, or it may mean that seagrasses lack an important mechanism that terrestrial plants use to regulate canopy density. The recolonization of plants to the marine environment brought with it many advantages and disadvantages. The inability to use the decrease of red:far-red as an indicator of canopy density may be one of the disadvantages.

10.4 **Sunflecks in a seagrass canopy**

Shallow *T. testudinum* canopies experience a highly dynamic light environment. The intercanopy light environment is characterized by intermittent sunflecks caused by the oscillation of leaves by the waves and currents. Seagrass canopies also experience a

unique fluctuation caused by the focusing of direct beams of light by surface waves. This wave-focusing causes the noticeable patterns of bright light seen on the sediment surface in clear shallow waters. Seagrass canopies are intermittingly pulsed with bands of light that can exceed three times the magnitude of the mean irradiance. This super-saturating light may lead to an increase in photoinhibition or photooxidation of leaf chlorophyll.

The light fluctuations have an effect on the measured irradiance where the mean irradiance does not exceed the light saturation point. A simple photosynthetic-irradiance curve calculates photosynthetic rate using the mean irradiance as if all irradiance was available for absorption and utilization. However, the peaks of the dynamic irradiance field regularly exceeded the light saturation point. By summing across the time series for all irradiance that exceeded the saturation point, I found that approximately 15.6% of the total measured irradiance over this time series exceeded the saturation point. Using a simple photosynthetic-irradiance relationship, this would result in a 15.6% overestimation of gross photosynthesis.

The magnitude of sunflecks and wave-focusing are muted when the incident light is diffused by clouds or by a turbid water column. While direct solar irradiance comes primarily from one direction, diffuse light has been scattered and travels in many different directions. Although diffuse light is often lower intensity, it statistically has a better chance of penetrating a canopy (Gutschick 1984). The contribution of sunflecks and diffuse light to the total light availability to a leaf also increases significantly at lower solar angles (Canham et al. 1990).

10.5 **Photoacclimation along leaves**

Chlorophyll content declines significantly from base to tip along *T. testudinum* leaves. This suggests that *T. testudinum* acclimates along leaves in response to the decline in irradiance with depth through the canopy. However, the growth form of *T. testudinum* leaves means that the oldest leaf tissue is at the top of the leaves. The vertical variation in chlorophyll content may simply be due to a decline in chlorophyll over time as the leaf ages and is exposed to high light and other environmental stresses. The *T. testudinum* in sparse meadows where canopy self-shading is low or nonexistent also showed decline in chlorophyll at the tips.

Photosynthetic performance as measured by chlorophyll fluorescence also varied significantly along leaves with the tips of leaves exhibiting sun-adapted traits and the bottoms of leaves acting as shade-adapted. This is a clear indication of photoacclimation along leaves. Even as leaf tips lose chlorophyll and show some sign of photoinhibition (i.e. lower F_v/F_m), the photosynthetic performance (i.e. ETR_{max}) remains high. The diurnal variation in chlorophyll fluorescence parameters and RLC variables showed that *T. testudinum* will acclimate to diurnal variations in ambient irradiance.

10.6 **Seagrass leaf life history**

The photosynthetic attributes of *T. testudinum* leaves change considerably over their lifespan. *T. testudinum* leaves are considered short lived even for seagrasses. Leaf lifespan is considered of ecological significance (Reich et al. 1992). The relatively short lifespan of *T. testudinum* may be a mechanism for maintaining optimal leaf tissue. Net carbon gain from a leaf over its lifetime depends on the total construction and

maintenance cost, net photosynthetic rate, and leaf lifespan (Mooney and Gulmon 1982). In seagrasses, the energy and resources of the seagrass plant may be more efficiently used to create new leaves as opposed to maintaining old leaves. This blade abandonment strategy has been suggested as a strategy for controlling the density of leaf epiphytes in seagrass leaves (Littler and Littler 1999).

Leaves with shorter life spans tend to have higher photosynthetic rates (Reich et al. 1991). However, short leaf lifespan is not a beneficial attribute for nutrient limited plants (Hemminga et al. 1991). The decline in P content as leaves aged suggests that *T. testudinum* actively resorbs P from older leaves (Stapel and Hemminga 1997), an important attribute that may moderate the effects of nutrient loss when older leaves slough off.

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