Effects of hydrodynamic regime on photosynthesis in the green alga Caulerpa

Mark D. Driscoll

University of South Florida

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Effects of hydrodynamic regime on photosynthesis in the green alga *Caulerpa*.

by

Mark D. Driscoll

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
Department of Biology
College of Arts and Science
University of South Florida

Major Professor: Florence I.M. Thomas, Ph.D.
Kevin S. Beach, Ph.D.
Clinton J. Dawes, Ph.D.

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Dedication

This thesis is dedicated to my wife, Jodie Corsetti Driscoll, for her patience, encouragement and support.
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ABSTRACT

The delivery of nutrients to the surface of marine algae can be controlled by the local hydrodynamic regime: in higher flow velocities, the Diffusive Boundary Layer (DBL) at the uptake surface is thinner, which can increase the flux of dissolved chemicals to the algal surface. If the primary productivity of an alga is controlled by the availability of a dissolved chemical, increased water flow should result in greater primary productivity due to increased chemical flux. To test the hypothesis that increased water flow will increase Photosystem II kinematics (PSII) in the green alga *Caulerpa* we used a Diving Pam Fluorometer to measure the maximum relative electron transport rate (Pmax), Saturation Irradiance (Ik), Non-photochemical quenching (NPQ), the light limited slope of photosynthesis vs. irradiance curve (α) and photo-chemical quenching (qP) and compared these measured values among treatments of varying flow speeds in a portable laboratory flume. We also measured the influence of water flow on values of Pmax, Ik, α, qP and NPQ in the field. Results showed that in *C. racemosa* collected from Tampa bay, and tested in a laboratory flume, values of Pmax and Ik were positively correlated to increase water flow, possibly indicating mass-transfer limitation. *C. mexicana*, collected from the Florida Keys, showed a decrease in values of Pmax, and Ik
with increasing water velocity in flume experiments, indicating that the increased flow was resulting in physiological stress. This result was supported with field measurements for *C. sertularioides*, which showed a negative correlation between Pmax and flow velocity and *I*ₚ and flow velocity.
Introduction

Marine macro-algal communities represent a highly productive and often overlooked component of marine systems (Hader 1999, Hurd 2000). As primary producers, providers of food and shelter to a wide variety of marine life and as sediment stabilizers (Williams 1990), macroalgae play an integral role in the health and development of many coastal marine ecosystems (Wheeler and Björnsäter 1992, Gacia et al. 1996, Campbell 2001). Rates of algal productivity have been shown to be some of the highest on the planet (Littler 1980, Hurd 2000) and in near shore marine communities, macroalgae play an important role in overall production (Wing and Patterson 1993, Hader et al. 2001). While the vast majority of macroalgae are a healthy component of marine ecosystems, some are dangerous invaders, destroying native eco-systems and remaining unresponsive to eradication efforts (Hill et al. 1998, Argyrou et al. 1999, Aussem and Hill 1999, Ceccherelli et al. 2000, Uchimura et al. 2000, Stimson et al. 2001, Harris and Tyrell 2001).

Members of the genus Caulerpa (Bryiopsidacea) are important components of tropical marine ecosystems worldwide (Collado-Vides and Robledo 1999, Collado-Vides 2002). With approximately 70 species within the genus, Caulerpa is often one of the dominant macro-algal species within tropical shallow water habitats (Gacia et al. 1996). Recently coastal marine systems in California and the Mediterranean Sea have been
invaded by a rogue strain of *C. taxifolia* (Vahl) C. Agardh, which has overgrown native seagrass beds and detrimentally affected the native marine ecosystems (Ceccherelli and Cinelli 1999, Ceccherelli et al. 2000, Meinesz et al. 2001, Ceccherelli and Sechi 2002, Ceccherelli and Campo 2002, Boudouresque et al. 2002). It is clear that macroalgae are important components of healthy marine systems, as well as devastating components of some disturbed systems. Due to the substantial impact that macro-algae can have on coastal marine systems it is important that we understand the factors that control algal production.

**Limits of Algal Productivity:**

Marine macrophyte physiology shows an incredible amount of diversity (Littler 1980, Littler and Arnold 1982, Saffo 1987, Koehl and Alberte 1988, Jensen et al 1985, Hader et al 1996, Beach et al 1997, Stevens and Hurd 1997, Larned 1998, Williams and Carpenter 1998, Hurd 2000, McCook et al 2000, Stimson and Larned 2000, Hillis 2001,). This diversity allows algal communities to flourish in a vast range of environmental conditions. Researchers have shown that macroalgae are able to change their overall morphology (Koehl and Alberte 1988), pigment quality and quantity (Beach and Smith 1996a) and photosystem structure (Fork and Satoh 1986, Beach and Smith 1996b) in response to varying environmental conditions. However, even with the highly plastic nature of these organisms, light and nutrients can severely limit photo-production

The availability of saturating irradiance is a controlling factor for photosynthesis in marine macroalgae (Engleman 1883, Beach and Smith 1997a, Beach and Smith 1997b, Beach et al. 1997, Gomez et al. 1998, Cloern 1999, Yentsch et al. 2002). The availability of light also contributes to the vertical zonation of marine algae (Engleman 1883). While it is well known that not enough light can limit the primary production of algae, it must also be considered that an overabundance of light can be detrimental given certain circumstances (Jensen et al 1985, Beach et al 1995, Beach and Smith 1996, Hader et al 1996, Beach and Smith 1997a, Beach and Smith 1997b, Beach et al 1997, Hader et al. 1999, Hader et al. 2000, Gorbunov et al. 2000). Species that are low light acclimated can see a drastic drop in photosynthetic activity when brought into drastically higher light regimes (Hader et al. 1996). It is believed that this decrease is caused by damage to the D1 protein complex within photosystem II of the electron transport chain (Greene et al. 1994, Hader 1996, Hader 1999). If damage to the D1 protein occurs, the protein must be replaced before normal functions resume. Light inhibition can often be overcome if the algae are acclimated in small steps to the desired level of irradiance (Hader et al 1996). Researchers have shown that given enough time to acclimate to a new light regime, macroalgae are able to adjust pigment levels and pigment ratios, allowing the organism to more efficiently utilize the available light regime (Riechert and Dawes 1986).

When light levels are saturating, nutrient availability becomes the major limiting factor to macroalgal production (Chapman 1974, Duke et al. 1989, Björnsäter and

Many studies have experimentally examined the effect of nutrient limitation in algae; however, due to the metabolic requirements of individual species, nutrient limitation varies by species and study site. For example increased productivity was seen when samples of the coral reef algae Chlorodesmis fastigiata were artificially fertilized with nitrate and phosphate (Kinsey and Dommm 1974). Similarly, chlorophyll content and overall biomass was shown to increase after N and N+P additions to the water column (Menendez et al. 2002). Greene et al. (1994) proposed that in areas where C, N and P are not limiting, Fe is the main limiting nutrient to phytoplankton growth. By seeding the open ocean with Fe Green et al. (1994) showed that plankton growth and productivity was shown to drastically increase.
Nutrient enrichment is not always beneficial to marine vegetative assemblages. Taylor et al. (1995) showed that while nutrients may positively affect the growth of one component of an ecosystem, the same nutrient pulse could be detrimental to other components. Their research showed that the biomass of *Nannochloropsis spp.* increased as a result of N fertilization as well as N+P fertilization, the nutrient pulse resulted in a loss of below ground biomass for the associated seagrass community.

Certain species of algae are known to display mechanisms that allow them to obtain nutrients from sources other than the water column. The thallus of *Dictyospheria cavernosa* grows in such a manner as to isolate a parcel of water between it and the benthos (Larned and Stimpson 1996). This “chamber” provides two sources of nutrients other than what is available from the water column. The first source is metabolic waste products from invertebrates that colonize the inside of the chamber, and the second source is the efflux of nutrients from the sediment that is trapped under the algal thallus. The nutrient concentrations inside these chambers were found to be ten times higher than the surrounding water column (Larned and Stimpson 1996). This mechanism was hypothesized to be the factor that allows *D. cavernosa* to thrive in waters where the ambient water column nutrients are not high enough for algal growth (Larned and Stimpson 1996). Transport of nutrients from the sediment to the vegetative tissue of algae has also been seen. Uptake of labeled $^{15}$N by the rhizoids of the alga *Caulerpa cupressoides* was shown by Williams (1984), demonstrating that in some species of *Caulerpa* sediment nutrients can play a large role in the productivity of the algae.
In terms of nutrient availability, organisms can be considered to be either physically limited, biologically limited or in between the two. In a physically limited system, the organism is not experiencing a high enough nutrient flux to saturate its physiological processes (e.g. uptake potential, enzyme loads). Biologically limited systems receive more than enough nutrient flux to saturate their processes; however, these organisms do not have the capability to process all of the available nutrients (e.g. not enough uptake sites, insufficient enzyme stores).

**How hydrodynamics affects algal productivity:**

Many researchers have proposed that the hydrodynamic regime experienced by an organism can have a large effect on its metabolic processes (Odum 1956, Wheeler 1980, Riber and Wetzel 1987, Fonseca and Kenworthy 1987, Koehl and Alberte 1988, Patterson et al. 1991, Koch 1993, Williams and Carpenter 1998, Kuffner 2001). Studies have shown that relative irradiance levels (Dromgoole 1987, Koehl and Alberte 1988, Patterson 1992, Wing and Patterson 1993, Wing et al. 1993) and nutrient delivery (Koehl and Alberte 1988, Bilger and Atkinson 1992) can be drastically changed depending on the ambient hydrodynamic regime of a given area. Changes in the local hydrodynamic regime can affect an organism in two major ways. The three dimensional orientation of the organism can be changed, affecting the relative light levels and the structural reaction to flow (Koehl and Alberte 1988). Secondly, the nutrient delivery to the organism’s
surface can be affected by disruptions to the boundary layers surrounding the organism (Gerard 1982, Thomas and Cornelisen 2003)

The flow of water past a submerged organism results in drag forces acting on that particular organism, which can have a major impact on its physiology. Organisms located in high-energy areas generally experience more drag than those found in sheltered areas. Temperate kelps are able to protect themselves from this increased drag by changing the morphology of their blades so as to reduce form drag (Koehl and Alberte 1988). In high velocity areas the blades of *Nereocystis leutkiana* take on a smooth and narrow form that allows the blades to clump together into a streamlined cone, reducing drag. In low velocity areas the same blades take on a more ruffled, wider form (Koehl and Alberte 1988). This clumping in high velocity reduces the cumulative amount of light that reaches the entire plant due to self-shading. In low velocity areas the blades do not clump together, resulting in a higher surface area exposed to irradiance.

The clumping of blades into a more streamlined shape is not the only condition related to hydrodynamics regimes that can be detrimental to plants. Dromgoole (1987), Wing and Patterson (1993) and Wing et al. (1993) showed that hydrodynamic regimes can drastically alter the light climate reaching a benthic community. Light flecks are periods of intense light caused by canopy movement or surface waves. Surface waves in particular can cause a short term, intense focusing of light, up to five times the ambient levels onto the benthos (Sagert and Schubert 2000, Schubert et al. 2001). It is possible that under certain conditions, these periods of intense light are strong enough to cause
permanent damage to the photosystems of aquatic primary producers, resulting in photo-inhibition.

The local hydrodynamic regime can affect the nutrient delivery to an algal thallus by increasing advection of nutrients over the surface and by decreasing the thickness of the diffusive boundary layer (DBL (a gradient in concentration where chemical transport is controlled only by molecular diffusion, not momentum.)). Hydrodynamics controls the thickness of the DBL and therefore greatly affects nutrient delivery (Kohel and Alberte 1988, Bilger and Atkinson 1992). If nutrient delivery is influenced by hydrodynamics, then metabolic processes dependent on that delivery are then linked to hydrodynamics as well.

The momentum boundary layer (MBL) is usually a completely turbulent region, where the rates of chemical transport are dominated by inertial forces. The turbulence in this layer is a function of water velocity and the properties of the benthic roughness elements. Due to the presence of turbulence in the water column, the rates of chemical transport in this layer are much higher than would be expected from molecular diffusion alone (Kays and Crawford 1993). Eddies vertically mix dissolved chemicals through this layer to the surface of the viscous sub layer, transporting them through the water column faster than molecular convection or molecular diffusion.

The viscous sub layer (VSL) is a thin region immediately next to the surface of a solid. In this layer, the vertical transport of momentum due to turbulence is no longer effective, often resulting in a region of laminar flow. Chemical transport in this region is governed by viscous transport, or molecular conduction between the layers (Kays and
The concentration of solutes within the viscous sub layer is controlled by the delivery of chemicals from the momentum boundary layer. In areas of low turbulence, the transport of these solutes may be slower, resulting in a lower concentration within the viscous sub layer.

Below the viscous sub layer is the diffusive boundary layer (DBL). In this region, the transport of solutes to and from a solid is completely reliant on molecular diffusion. If an organism uses a chemical faster than it can be delivered through this layer, it will become physically limited. Similarly, if an organism excretes a metabolic bi-product into this layer faster than it can diffuse out, then an elevated concentration of that bi-product will occur in the water next to the tissue surface. For plants, boundary layers can cause a major problem. All photosynthesis results in the production of oxygen, which can compete with carbon for binding sites on the enzyme Ribulose 1-5 Bisphosphate Carboxylase Oxygenase (RUBISCO). When this happens, primary productivity may be reduced due to the oxygen build up in the DBL.

Hydrodynamic regimes may greatly affect the nutrient limited metabolic processes such as photosynthesis under some circumstances. As the boundary layers surrounding a submerged solid are broken up by turbulent energy, or made thinner by increased velocity, molecular diffusion to that solid is increased. In this manner, even in low nutrient waters, the relative nutrient flux experienced by an organism can be quite high if the area is swept by high velocity water flow. Conversely, if there is little turbulent energy or velocity in the water column, an organism in a nutrient rich environment may experience nutrient limitation due to the thickness of the diffusive
boundary layer. Two branches of research have developed models of how water flow affects aquatic organisms. The first branch focuses on the effects of water flow on photosynthesis and gross morphology, and the second branch has demonstrated the dependence of nutrient uptake on water velocity.

Wheeler (1980) demonstrated that increased water velocity influenced the photosynthetic rates of *Macrocystis pyrifera* during laboratory experiments using 3.0 cm tissue discs. Oxygen evolution was shown to increase from a level of 0.3 µmol O₂ cm⁻² in stagnant water to a level of 1.3 µmol O₂ cm⁻² in water moving at the equivalent of 7.0 cm s⁻¹. Similar results were reported by Gerard (1982), who determined that small tissue samples of *M. pyrifera* is mass transfer limited in flows less than 3.0 to 4.0 cm s⁻¹. These studies were followed by Koehl and Alberte (1988) who demonstrated increased photosynthetic activity in tissue discs of *Nereocystis leutkiana* as a result of increased flow regimes in laboratory experiments. Koehl and Alberte (1988) also demonstrated that the morphology of *N. leutkiana* is highly variable depending upon environmental conditions. In high flows, blades were found to be smooth and without marginal spines, allowing the blades to clump together and reduce form drag, where as in low flow, blades were found to have large marginal spines and ruggose morphology, increasing turbulence around the blade. This increased turbulence acts to disrupt the viscous sub-layer and increase the nutrient flux to the edge of the diffusive boundary layer. In several studies that utilized whole plants, instead of dissected tissue disks and blades, rates of primary production ([µgO₂ {µg Chl a}⁻¹ h⁻¹]) and acetylene reduction, an indicator of nitrogen fixation, in tropical algal turfs have been positively correlated to water velocity (0-22 cm
s \textsuperscript{-1}) (Carpenter et al. 1991, Williams and Carpenter 1998). The effect of water velocity on photo-production in marine algae is not uniform, Koch (1993) demonstrated that increasing friction velocities (u* (a measure of the intensity of the turbulence in a system)) from 0.0 cm s \textsuperscript{-1} to 0.3 cm s \textsuperscript{-1} significantly increased the photosynthetic rate of \textit{Ulva lactuca}. However, increasing µ* beyond 0.3 cm s \textsuperscript{-1} had no effect on the algae, and it was postulated that under less than saturating irradiance increased water flow could be detrimental to photosynthesis. Koch postulated that this decreased performance in higher flows may have been due to the algae being accustomed to the flow regime in the area where it was collected. By changing this regime, the plants may have become stressed, reducing their efficiency (Koch 1993).

In addition to measuring primary productivity in response to varying hydrodynamic regimes, several studies have investigated the affects of water flow on nutrient uptake rates. Increased N and P uptake rates as a function of velocity have been widely reported in the literature (Atkinson and Bilger 1992, Bilger and Atkinson 1992, Bilger and Atkinson 1995, Thomas and Atkinson 1997, Thomas et al. 2000, Thomas and Cornelisen 2003). Atkinson and Bilger have mainly worked on organisms in flume systems. These studies noted dependence of the first order rate constant for the uptake of important nutrients by coral reef assemblages on hydrodynamic regime. Along the same lines, Thomas and Cornelisen have focused on field based experiments using a large field flume capable of encompassing 3.7 m\textsuperscript{2} of the benthos. These experiments have demonstrated that nutrient uptake of an entire community \textit{in situ} is directly correlated to water velocity. Additionally, these studies showed that the uptake rates of individuals
within the community are correlated to water flow and to whole community uptake (Cornelisen and Thomas 2002).

**Objectives:**

To clarify the role of hydrodynamics in photo-production new technology that allows for measurements of hydrodynamic regimes and direct measurements of photosystem II kinematics will be used *in situ*. By measuring PSII kinematics in laboratory experiments, I intend to show that a Diving PAM Fluorometer is capable of measuring changes in PSII kinematics due to varying hydrodynamic regimes. I also plan on utilizing field measurements to directly measure PSII kinematics without removing the plants from their natural environmental conditions, which has been suggested as a stressor to aquatic vegetation (Koch 1993). These studies will focus on the genus *Caulerpa* due to its extensive presence in near-shore tropical and sub-tropical marine systems. The detailed goals of this thesis are:

1. To determine if hydrodynamic regime affects photosystem II kinematics *in situ* and in a controlled laboratory flume.

2. To determine if the effects of hydrodynamic regime on photosystem II kinematics can be measured by integrating data collected with an Acoustic Doppler Velocimeter and a Diving Pam Fluorometer.
3. To determine the magnitude of the dependence of photosystem II kinematics on hydrodynamic regime within the genus *Caulerpa*.

**Background information on *Caulerpa* spp.**

Research has shown that members of the genus *Caulerpa* are important components of tropical marine ecosystems around the world (Collado-Vides and Robledo 1999, Collado-Vides 2002). The morphology of the genus *Caulerpa* is highly diverse, and within species the morphology is amazingly plastic (Gacia et al. 1996, Benzie et al. 2000, Collado-Vides 2002), changing drastically in accordance to environmental conditions. As early as 1906 water motion and ambient light levels were being considered as important factors affecting the morphology of *Caulerpa* (Svedelius 1906). Barilotti (1970) showed that the morphology of *C. prolifera* (Forskål) Lamouroux was determined by available light levels; Peterson (1972) then showed a similar dependence on ambient light levels in *C. racemosa*.

(Ceccherelli and Sechi 2002). *Caulerpa racemosa*, which is an immigrant species from the red sea, has shown a similar pattern as *C. taxifolia* in the Southern Mediterranean Sea, out-competing *C. nodosa*, *Zostera noltii* (Ceccherelli et al. 2002) and *Posidonia oceanica* (Ceccherelli et al. 2000). The negative effect of these algae has not been limited to the overgrowth of submerged aquatic vegetation. Arigoni et al (2002) showed that native labrid fishes underwent a drastic color change in *C. taxifolia* bed as compared to normal color morphs in *P. oceanica* beds. Decreases in invertebrate abundance (Bellan-Santini et al. 1996), species richness, and fish biomass (Francour et al. 1995, Relini et al. 2000) were also seen within *C. taxifolia* beds as compared to natural seagrass assemblages. The high levels of secondary metabolites present in *Caulerpa spp* may explain this decline in species diversity. The acetylenic sesquiterpene metabolite caulerpenyne (Figure 1), which is the main anti-herbivory defense in *Caulerpa spp*. has been shown to compromise a substantial (0.2-13.0%) component of the frond weight depending on the season (Dumay et al. 2002). The high levels of secondary metabolites in *Caulerpa* is not surprising, given that the genus is mainly found in tropical waters which are known for high rates of herbivory (Pennings and Paul 1992, Cronin and Hay 1996).

Data collected on *Caulerpa spp*. has demonstrated in many systems its ability to colonize a wide range of substrate types, ranging from muddy unconsolidated sediments to rocky intertidal areas (Meinesz et al. 1995, Chisholm and Jaubert 1997, Ceccherelli and Cinelli 1998, Arigoni et al. 2002). *Caulerpa* has been reported at depths of 50 meters, well below the photic zone of most green algae (Arigoni et al. 2002). This flexibility allows *Caulerpa* a vast range of options when settlement of fragments or
zygotes occurs, giving *Caulerpa* an advantage over species that are more selective in their substratum choices. Ecologically, *Caulerpa* has shown that it is an important component in the succession of tropical seagrass beds (Williams 1990). By consolidating loose sediments, *Caulerpa* makes it possible for climax species, such as seagrasses to colonize an area.

The growth strategy of *Caulerpa* is based on an extremely fast rate of vegetative expansion (Chisholm and Jaubert 1997, Ceccherelli and Piauzzi 2001). This rapid growth allows the alga to quickly extend new tissue into a large area immediately surrounding the initial settlement plot. *Caulerpa* is known to grow at night, as is seen by the white growing tips visible in the early morning (Dawes and Barilotti 1969). As light levels increase, cytoplasm replaces the “meristemplasm” that occupies the white areas of the cell and Chloroplasts re-position themselves in this newly available area (Dawes and Barilotti 1969). Like many members of the Bryopsidales, *Caulerpa* has the ability to reproduce both sexually via holocarpy (Zuljevic and Antolic 2000) and asexually through vegetative fragmentation (Smith and Walters 1999, Ceccherelli and Piauzzi 2001, Walters et al. 2002). Vegetative fragmentation is an extremely effective mechanism of reproduction in *Caulerpa*, fragments as small as 2.0 mm have been shown to successfully settle, attach and grown into full size plants (Goddard and Dawes 1983, Smith and Walters 1999, Ceccherelli and Piauzzi 2001). The effectiveness of this growth strategy is seen in *C. taxifolia*, which, in the Mediterranean has spread solely through vegetative fragmentation, as only male gametes have been observed (Zuljevic and Antolic 2000). The same is true for *C. racemosa* in the southern Mediterranean; no gamete production
has been seen in the alga, meaning that while sexual reproduction of *C. racemosa* is important in tropical waters, vegetative growth and fragmentation are solely responsible for the alga’s success in the Mediterranean Sea (Ceccherelli and Piazzi 2001).

**Acoustic Doppler Velocimeter**

Early studies utilized methods such as hot film anemometers, plaster of paris dissolution, and “life saver” dissolution to measure bulk flow velocities (Wheeler 1980, Koehl and Alberte 1988). Plaster of paris and life saver dissolution give a general idea of water velocity by measuring the amount of mass lost over a given time. Identical samples can be calibrated at a known water velocity and compared to samples tested (Wheeler 1980, Koehl and Alberte 1988). Hot film anemometers measure the advection of heat from the probe tip and correlate it with velocity (Koch 1993). These techniques, while informative, do not measure the turbulent energy in a system, which affects the DBL. Acoustic Doppler Velocimeters measure water flow in three vectors simultaneously; “x” which is parallel to the bulk flow, “y” which is perpendicular to the bulk flow, and “z” which is the vertical perpendicular to the bulk flow. The amount of variation between these measures allows researchers to collect data on the amount of energy, or turbulence in the system.
The PAM Fluorescence Measuring Technique

Traditional methods of measuring primary production have relied on gas exchange or increased biomass as the primary indicating factor. While looking at an increase of biomass or gas exchange over time can give a gross estimate of primary productivity, these estimates may not give accurate insights into the efficiency of algal photosystems. The advances seen in chlorophyll fluorescence technology in the past decade have drastically changed the ability of eco-physiologists to discern photosystem II (PSII) kinematics in the laboratory and in situ.

Pulse amplitude modulated fluorescence technology is based on exposing photosynthetic tissue to a 655nm light source that is rapidly switched on and off at a rate of 20 kHz. A photodiode then detects only the fluorescence at wavelengths greater than 700 nm to determine the fluorescent output of the sample (Walz manual 1998, Maxwell and Johnson 2000). The ability to collect a non-invasive, real time fluorescence reading from samples in situ has expanded the scope of the questions that can be addressed concerning plant physiology.

The basics of chlorophyll fluorescence are relatively uncomplicated. Energy that enters the photosynthetic apparatus of a given plant can follow three different pathways (Figure 2). These pathways control the photochemistry of the system or the actual conversion of light energy to glucose, and the dissipation of excess energy before it can cause irreparable damage to the photosystem. Energy entering the first such pathway is used to drive photosynthesis (production of ATP and NADPH) and is known as
photochemical quenching (qP). Energy that enters the second such pathway is dissipated as heat; this pathway is linked to photo-protection, the dissipation of excess energy before it can cause permanent damage to PSII (NPQ). The third option is for the energy to be re-emitted from the PSII antennae complex at a higher wavelength (fluorescence).

Fluorescence is thought to be responsible for the dissipation of approximately 5% of excess the energy present in a given PSII antennae complex. The three energy pathways of PSII are competing circuits, if the amount of energy entering one increases, it will in turn decrease the amount entering the other two (Maxwell and Johnson 2000, Krouse and Weis 1991). By looking at the relative amounts of energy in each pathway, it is possible to look at the overall efficiency of PSII.

The performance of PSII can be determined from data collected from the changes in fluorescent levels between times of no, low and high light (Figure 3). PAM fluorescence can be used with the plants either in a dark acclimated or light acclimated state. In dark acclimated conditions, the vegetative tissue is kept in complete darkness for a varying length of time; this darkness ensures that all of the photo-receptors are in a non-oxidized or open state. A small measuring light (0.15µ mol photon per m²) is applied to the sample to determine the background fluorescence level (Fo). A pulse of light with intensity high enough to oxidize (close) all available photo-receptors is then applied to obtain the maximum fluorescent yield (Fm) for the sample. The difference between the values of Fm, and Fo is known as the variable fluorescence or Fv. After the saturating light pulse, a photosynthesis driving light is applied to the sample for a prescribed length of time. A second saturating pulse is then switched on and the samples
maximum fluorescence in the light (Fm’) is measured. Immediately before the second saturation pulse is applied a measure of the samples residual fluorescence (Ft) is taken. The comparison of these values allows one to investigate the efficiency of the photosystem.

The effective quantum yield of energy conversion in a given sample is determined from the following equation Yield = (Fm’-Ft)/Fm’ (Maxwell and Johnson 2000, Waltz Manual 1998). This value can be used to interpret how much energy is being consumed in photochemistry. The potential quantum yield of PSII (ΦPSII) can be determined by dividing $F_v/F_m = (F_m-F_o)/F_m = PSII/\eta_P$. This measure can be used to determine the possible yield for a sample if all of its reaction centers are “open” (Maxwell and Johnson 2000). Photochemical quenching ($\eta_P$) is an indication of how many reaction centers are open, or able to accept solar energy. Calculated as $(Fm’-Ft)/ (Fm’-F_o’)$, $\eta_P$ allows one to look at the actual processes affecting yield (Maxwell and Johnson 2000). Non-photochemical quenching or NPQ, measures the amount in heat dissipation in dark acclimated samples.

$F_m$ cannot be measured without the presence of non-photochemical quenching (Maxwell and Johnson 2000). Due to this fact if an accurate measure of $F_m$ is desired, samples must be dark acclimated prior to the initial reading, and a relative measure taken from this dark adapted state. Dark acclimation ensures that all available photo-centers are in the “open” state, meaning that they are capable of accepting photo-chemical energy. $\eta_N$ can also affect the level of $F_o$ in some situations, which in turn leads to a reduction in the yield of $F_o’$. When $\eta_N$ reaches a point above 0.4, $F_o$ can be quenched,
reducing the yield of F_o`. This quenching of F_o` can be addressed only by the use of a far red light pulse to enhance Qa-reoxidation. This light source however, is not available in the diving PAM. The diving PAM does however give a second measure of non-photochemical quenching (NPQ) that is not as sensitive to F_o` quenching. Where qN is affected by this quenching at 0.4, NPQ is affected by quenching at 4.0 (Maxwell and Johnson 2000). Due to this increased tolerance, the value of NPQ has become the measure of choice for heat dissipation analysis.

Field studies using PAM fluorometry have demonstrated the usefulness of this technology in measuring the parameters surrounding marine primary productivity. Much of the present work has focused on the effects of increased irradiance on PSII (Hader et al. 1996, Hader et al. 1999). This work has indicated that the diving PAM fluorometry system is able to measure in situ changes in quantum yield and qN in PSII as well recovery time due to photo-inhibition. A correlation between increased photokinetics and irradiance levels was also shown in seagrasses (Ralph et al. 1998), sponges (Beer and Ilan 1998a) and corals (Beer et al. 1998b). This work has set the base for continued work using PAM technology to look at PSII kinematics as a function of various other environmental fluctuations (e.g. water flow and salinity).

The development of chlorophyll fluorescence technology along with advances in acoustic doppler velocimeters has made research questions that were not measurable a short time ago valid research avenues. The ability to take detailed in situ measurements of hydrodynamic regime and tie them in with direct measures of photosystem kinematics may allow eco-physiologists, physical biologists, and oceanographers to produce more
accurate models of aquatic productivity. This thesis investigates the hypothesis that increased water flow will increase PSII kinematics in Caulerpa spp. This hypothesis is based on the fact that in higher water flow; the boundary layer is reduced, allowing for a higher rate of nutrient transport to the algal thallus, or a higher rate of O₂ removal from the surface. This study is the first to use a direct measure photosystem activity to look at the effects of flowing water on aquatic vegetation.
Materials and Methods

To determine the effects of water flow on the photoproduction of the genus Caulerpa. I preformed three sets of experiments. These experiments were conducted with specific goals in mind. The first set of experiments was conducted to determine whether photosystem efficiency was dependent on changes in water velocity in a laboratory flume. The second set of experiments was conducted to determine the effects of water flow on photosynthesis in a natural setting, and the third set of experiments was conducted in order to determine the effects of water flow on photosynthesis over varying lengths of time.

Measurements on several species of macroalgae within the genus Caulerpa from the Florida Keys and Tampa Bay were used to characterize the effects of hydrodynamic regime on photosystem II (PSII) kinematics. Laboratory measurements were collected in order to determine if photosystem kinematics are a function of hydrodynamic regime and field measurements were collected in order to elucidate the role of hydrodynamics in algal productivity in situ. All photosynthetic parameters analyzed in this study were measured using rapid light curves produced by a Diving PAM Fluorometer. PSII kinematics measurements were taken approximately half way up the frond from the rhizome, and in no case was new tissue (complete lack of pigment) used for measurement of photosynthetic activity. Unlike past studies on productivity in flowing waters, the development of the Diving PAM Fluorometer allows for non-
invasive, direct measurements of PSII kinematics. In all experiments, values of maximum electron transport rate (Pmax), the initial slope of the photosynthetic curve (\(\alpha\)) and saturation irradiance (I\(_s\)) were analyzed. In several experiments photochemical quenching (qP) and non-photochemical quenching (NPQ) were also analyzed.

In all of the experiments conducted, rapid light curves were collected and analyzed with two different non-linear models using the sigma plot software suite. The Platt inhibition model, \(f=p^x(1-\exp((-a^x)/p))\times(\exp(-b^x/p))\) (Henely 1993) was used when light curves showed a marked inhibition of photosystem structure (Figure 4) and the hyperbolic tangent model \(f=P_{\text{max}}\tanh((\alpha^x)/P_{\text{max}})+R_d\) (Henely 1993) was used when there was no inhibition in the light curve (Figure 5). Only curves where the model fit with a \(r^2\) value of 0.90 or better were used in the data analysis.

The recent advances in technology used to measure hydrodynamic regimes have also drastically changed the scope of the questions that can be raised concerning the interactions of biological processes and water flow. In all flume experiments, the water velocity was measured by taking a velocity profile (measures of velocity were collected at 1.0 cm intervals with an ADV) through the water column and calculating a depth average velocity. Field measurements of velocity were bulk flow measures taken at the approximate midpoint of the water column. Care was taken when near a surface, to ensure that the ADV signal was not being intercepted by the substrate (Finelli 1999).
Caulerpa

_Caulerpa racemosa_, _C. sertularioides_ and _C. mexicana_ were chosen for this study due to their abundance in Florida’s coastal eco-systems. The unifying characteristics of _Caulerpa spp._ is a coenocytic thallus, a rhizomatous mass used to anchor the algae to the substrate and trabeculae supporting the exterior cell walls. _Caulerpa spp._ often grow in an intertwined matt of rhizomes, acting to stabilize unconsolidated sediments and allowing the settlement of climax species such as seagrasses which require solid sediments.

_Caulerpa sertularioides_ (Figure 6) is characterized by an erect featherlike frond up to 20.0 cm in length and 1.0-2.0 cm in width. The branchlets or pinnules are oppositely branched and approximately 3.0-11.0 cm long and pointed at the tip. The rhizome is up to 2.5 mm in diameter and can grow up to 2.0 m in length. _C. sertularioides_ is most often found in shallow waters on sandy substrates. _C. sertularioides_ is found from the shallow subtidal down to a depth of 10.0 m. The range of _C. sertularioides_ is from Florida in the North throughout the Southern Caribbean (Littler and Littler 2000).

_Caulerpa racemosa_ (Figure 7) is characterized by a creeping or erect frond that is 1.0-5.0 cm tall. The pinnules of _C. racemosa_ are highly diversified and may include rounded, mushroom capped, or oval shaped apices. The stolon of _C. racemosa_ is thick (2.0-3.0 mm) and creeping with a large number of branched rhizoids. _C. racemosa_ is often found attached to rocks in areas of considerable surf as well as sandy sediments that
experience little to no water flow, such as in thick seagrass beds. *C. racemosa* is found from the intertidal zone to a depth of 50.0 m. The range of *C. racemosa* is from Florida in the North throughout the Southern Caribbean (Littler and Littler 2000).

*Caulerpa mexicana* (Figure 8) is characterized by an erect frond that can range from 2.0 cm in height in high-energy areas to 25.0 cm in height in low-energy environments. Pinnules are opposite of each other and resemble the fronds of a fern. The stolon of *C. mexicana* can be relatively thin ranging from 0.6 mm to 1.5 mm in diameter. The rhizoids are thin and numerous, allowing the algae to anchor in very soft sediment. The depth range of *C. mexicana* is reported as the shallow subtidal through a depth of 15.0 m. The species range of *C. mexicana* is the same as the other two species mentioned, with a northern limit of Florida throughout the Southern Caribbean (Littler and Littler 2000).

**Measurement of PSII kinematics**

Before the start of each experiment, samples were dark acclimated in order to normalize the light history of individual plants. This dark acclimation involved securing the sample inside the magnetic leaf clip of the Diving PAM Fluorometer for a period of ten minutes. The acclimation period ensured that all of the reaction centers within PSII were oxidized, or open for the capture of photochemical energy. The sample clips required by the Diving PAM Fluorometer to dark acclimate samples present an immediate problem when studying the effects of hydrodynamic regime on photosystem
function. Samples must be held in the clips for at least 11.5 minutes (10 minutes for dark acclimation and at least 1.5 minutes for a rapid light curve) during which time the boundary layer around the thallus is increased due to the added size of the leaf clip. This increased boundary layer may act to slow the acquisition of nutrients by the algae. To ensure that this increased boundary layer around the plants due to the presence of the magnetic leaf clip was not a source of nutrient (e.g. C, N, P) limitation a small flume experiment was conducted to determine if there was a difference in Pmax when samples were acclimated using the clips vs. when they were acclimated in a dark room. Samples of *Caulerpa racemosa* were collected from the South East jetty of the Sunshine Skyway Bridge at the mouth of Tampa Bay (Figure 9). All samples were transported in aerated seawater back to the lab within one hour, cleaned of all macro-epiphytes and kept for 24 hours in aerated seawater. The samples were then placed into a 110L racetrack flume filled with artificial seawater with a salinity of 35ppt (Figure 10). Samples were held in place using zip ties attached to a false floor in the flume and allowed to acclimate to the hydrodynamic regime for 30 minutes before measurements were taken. A light bank of 10,000 K Coralife© full spectrum grow lights suspended above the flume provided illumination. Samples were dark acclimated using either the magnetic leaf clip (Waltz, Germany) under full artificial illumination or with the room in complete darkness. Water flow in the flume was controlled using a small trolling motor and kept at approximately 15 cm s⁻¹. After dark acclimation, rapid light curves using light levels of 0, 8, 12, 22, 30, 48, 66, 104, 155 µmol quanta m⁻² s⁻¹ were conducted. To determine if the magnetic leaf
clip was negatively affecting Pmax between the two treatments, an ANOVA (SYSTAT) was run after assessing the normality of the data (SYSTAT).

An initial, small scale experiment was conducted in order to determine whether or not a diving pam fluorometer could be used to measure short-term changes in photosystem kinematics due to changes in water velocity. A series of small flume experiments were conducted at the Keys Marine Lab (KML), on Long Key Florida (Figure 11). Samples of Caulerpa mexicana were collected from the Thalassia testudinum bed behind KML over five days of December of 2002. Samples were transferred within five minutes to a re-circulating seawater table and stored for 24 hours under natural light conditions before experiments were conducted. Samples were cleaned of all macro-epiphytes and placed into a 110L racetrack flume filled with un-filtered seawater (Figure 10), where the water velocity was controlled using a small trolling motor. High velocity trials were run at 25.0-30.0 cm s\(^{-1}\), and low velocity trials were run at 5.0-10.0 cm s\(^{-1}\). Water velocity profiles were measured using a Sontek Acoustic Doppler Velocimeter (ADV) at a sample rate of (25 hz) and a depth average velocity was calculated. The samples were held in place using zip ties attached to a false floor in the flume. The plants were allowed to acclimate to the flow conditions for one hour before measurements were taken. A bank of overhead fluorescent lights placed approximately 30.0 cm above the flume provided irradiance. Samples of C. mexicana were placed in a magnetic leaf clip (Waltz, Germany) so that no pinnules were overlapping and dark acclimated for 10 minutes. Rapid light curves using a Diving PAM Fluorometer were taken using light levels of 0, 48, 66, 99, 138, 212, 311, 463, 734 \(\mu\)mol
quanta m$^{-2}$ s$^{-1}$. Light curves were analyzed for maximum electron transport rate (Pmax), saturation irradiance (I_k) and the initial ascending slope of the curve (a) by fitting the data to either the hyperbolic tangent model or the Platt inhibition model (Henely 1993). Data were tested for normality using SYSTAT 10 statistical packages and values of I_k were normalized to the square root of the data (SIGMA STAT). An ANOVA (SYSTAT) was used to assess the affect of hydrodynamic regime on Pmax, I_k and a.

**Field Experiments**

Unlike laboratory experiments that use constant uni-directional flow, organisms living in natural conditions experience constant changes in the magnitude and direction of water flow due to tidal changes and wind driven waves (Hearn et al. 2001, Wing and Patterson 1993). To assess the role of hydrodynamics in algal photo-physiology in situ a series of field measurements were taken on samples of *Caulerpa sertularioides* growing on a carbonate shoal approximately 2.0 km North-West of the Long Key Channel Bridge. Rapid light curves were measured on samples of *Caulerpa sertularioides* growing on Old Sweat Bank, on the Florida Bay side of Long Key, Florida (Figure 11) between June 6, 2002 and June 9, 2002. Much of the water from Florida Bay, including water originating from the Everglades empties into the Atlantic Ocean through the Long Key Channel (Wang 1998). Water velocities on the shoal range from near 0.0 cm s$^{-1}$ at slack tides to greater than 1.0 m s$^{-1}$ during flood tides. Old Sweat Bank is characterized as a carbonate shoal composed mainly of *Halimeda spp.* segments and crushed coral. Vegetative
growth on the shoal is comprised of *Thallassia testudinum* and *Syringonia filiforme*, extensive *Halimeda spp.* and *Penicillus spp.* beds, and intermittent patches of *Caulerpa sertularioides*. *Caulerpa* patches were approximately 1.0 m in diameter and were located along the midline of the shoal. Large patches of *Porites porites* are scattered over the shoal along with various species of sponges. The bank is surrounded on all sides by dense *T. testudinum* beds, and cut into three sections by two tidal channels (Figure 11). Water depth over the shoal ranges from approximately 1.0 m at flood tide to <10 cm at neap tide. All light curves were run between 7:30 am and 8:00 pm to ensure that a variety of water flow velocities (>1.0 ms\(^{-1}\) < 0.05 cms\(^{-1}\)) were encountered at all times of photosynthetic activity for the algae.

Samples of *Caulerpa sertularioides* were placed into the magnetic leaf clip in such a manner that no pinnules overlapped and dark acclimated in situ for ten minutes (Waltz, Germany). Rapid light curves were then run on all samples using a diving pam fluorometer at light levels of 0, 48, 66, 99, 138, 212, 311, 463, 734 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\). Light curves were analyzed for maximum electron transport rate (Pmax), saturation irradiance (I\(_s\)) and the initial ascending slope of the curve (\(\alpha\)) by fitting the data to either the hyperbolic tangent model or the Platt inhibition model (Henely 1993). Ambient light levels were measured using the external light sensor on the Diving PAM Fluorometer. Light levels at the algal thallus ranged from 40-1167 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\). The 2\(\pi\) quantum sensor was mounted onto the top of fluorometer, which was the approximate height of the algae off the substrate. Simultaneous acoustic doppler velocimeter (ADV, Nortek) readings were taken adjacent to the algae. To measure bulk flow over the shoal
at mid water depth, the ADV sensor head was mounted perpendicular to the substrate when water height above the bottom was >15cm (Figure 12). When water height was <15cm the sensor was mounted parallel to the substrate (Figure 12). ADV samples were taken at 25hertz with burst intervals of 5 minutes. Using the internal clocks features of the fluorometer and the ADV, individual light curves were matched by time to the corresponding bulk flow measurement over the shoal. Water velocities over the shoal were separated into three discreet categories, <20 cm s\(^{-1}\), 20-30 cm s\(^{-1}\), and >30 cm s\(^{-1}\). To determine if photosystem kinematics were dependent on hydrodynamic regime a two way ANOVA (SYSTAT) was used to test for differences in Pmax, I_k, and \(\alpha\), with time of day as a covariate. Data was tested for normality (SYSTAT) and subsequently normalized (SIGMA STAT) to the square root of the data. All outliers were removed according to Grubbs outliers test (Rolph and Sokal 1981) before analysis was preformed.

**Time in flow**

In an attempt to isolate the effects of hydrodynamics on the photosystem kinematics, and to determine the effects of “time in flow” on the PSII kinematics of *C. racemosa*. a series of small flume experiments was conducted in a controlled laboratory setting. In this manner the amount of light, nutrient levels and salinity can be controlled with hydrodynamic regime as the only fluctuating component.

Samples of *Caulerpa racemosa* were collected from the South East jetty of the Sunshine Skyway Bridge at the mouth of Tampa Bay (Figure 9) between July 2, 2002
and August 5, 2002. Samples were collected just after sunrise and were returned to the lab within one hour. Samples were cleaned of macro-epiphytes and kept in aerated seawater for 24 hrs before experiments were conducted. Only samples that had not been damaged in the collection process were used for experiments. Three individual plants were then placed into each of two identical 110L racetrack flume (Figure 10) filled with artificial seawater (35 ppt). The sea water was fertilized with 0.0161g l⁻¹ PO₄, 0.031 g l⁻¹ NH₄, and 0.005 g l⁻¹ Nitrate. These nutrient levels correspond to a ten year average during the summer months at the Sunshine Skyway Bridge (http://www.floridamarine.org, 2003). Water velocity within the flumes was controlled by a small trolling motor. Velocity profiles within the flume were taken using an ADV (SONTEK) and a depth average velocity was calculated for use as a bulk flow measurement. Illumination was provided by an overhead bank of 10,000 K Coralife© grow lights suspended over the flumes. Samples were allowed to acclimate to the hydrodynamic regime for a period of one hour before Rapid Light Curves were collected using light levels of 0, 8, 12, 22, 30, 48, 66, 104, 155 µmol quanta m⁻² s⁻¹. Rapid light curves were taken every hour for 7 hours. Light curves were analyzed for maximum electron transport rate (Pmax), saturation irradiance (Ik) and the initial ascending slope of the curve (α) by fitting the data to either the hyperbolic tangent model or the Platt inhibition model (Henely 1993). Photochemical quenching (qP) and non-photochemical quenching (NPQ) values were obtained for each sample by selecting the set of data within each curve nearest the saturation irradiance for the sample. To assess the role of hydrodynamic regime on the photosystem kinematics of C. racemosa a two way repeated
measures ANOVA (SIGMA STAT) was preformed with water flow as one factor and the amount of time in the flow as the second factor. The measurements from each of the three plants in each flume were averaged to form one set of data per trial. The data was then assessed for normality (SYSTAT) and transformed (SIGMA STAT) to the square root of the data for Pmax, I_k, and NPQ. Data was transformed to the ln of the data for \( \alpha \) and qP. All outliers were removed using Grubbs outliers test (Rohlf and Sokal 1981).

The ratio of Pmax in high flow to Pmax in low flow was plotted for each of the seven time periods (FIGURE 13). A distinct drop in the ratios was seen after two hours of immersion. The data for measurements three through seven was then assessed for normality (SYSTAT) and transformed (SIGMA STAT) to the square root of the data for Pmax, I_k, and NPQ. Data was transformed to the ln of the data for \( \alpha \), and qP. All outliers were removed using Grubbs outliers test (Rohlf and Sokal 1981). A two way repeated measures ANOVA was then used to assess the role of hydrodynamics after two hours of immersion.
Results

Does the magnetic sample holder affect values of Pmax?

The differences in values of Pmax between trials using a magnetic leaf clip to dark acclimate a sample and trials using a dark room to dark acclimate samples were not significantly different (P=0.790, F=0.073, Figure 14). Samples acclimate with clips had a mean Pmax of 8.6 (SE = 1.4, n=9) and samples acclimated in a darkened room had a mean Pmax of 8.9 (SE=3.1, n=9).

Laboratory measurements of PSII kinematics on C. mexicana after a short exposure to hydrodynamic regime.

The effect of water velocity on values of Pmax from C. mexicana was significant (P<0.05, F=6.811, Figure 15). Samples run at high (25-30cm s⁻¹) and low (5-10 cm s⁻¹) velocities had mean values of Pmax of 26.5 (SE=7.0, n=4) and 52.3 (SE=7.0, n=4) respectively. Values of α from C. mexicana in the two different hydrodynamic regimes were not significantly different. The effect of water velocity on saturation irradiance was very close to being significant, (P=0.055, F=5.66). The mean of Iᵦ in high flow was 16.2 (SE=1.7 n=4) and the mean of Iᵦ in low flow was 22.0 (SE=1.7, n=4).
In situ field measurements of PSII kinematics on *C. sertularioides*

Water velocity had a significant affect on the values of $P_{\text{max}}$ and $I_k$ in *Caulerpa sertularioides* measured in situ ($P<0.001$, $F=9.387$, Figure 16, Table 1 and $P<0.05$, $F=3.754$, Figure 17, Table 1 respectively). Water flow did not affect values of $\alpha$ ($P=0.570$, $F=5.67$) Table 1). There was no significant interaction of time on the values of $P_{\text{max}}$, $\alpha$ or $I_k$ (Table 1).

Laboratory measurements of PSII kinematics in *C. racemosa* after long term exposure to hydrodynamic regime.

Values of $P_{\text{max}}$ ($P=0.067$, $F=3.865$), $\alpha$ ($P=0.100$, $F=3.044$), $I_k$ ($P=0.893$, $F=0.0188$), $qP$ ($P=0.198$, $F=1.800$) and $NPQ$ ($P=0.490$, $F=0.497$) for *C. racemosa* run in laboratory flumes at different water flow velocities over a period of seven hours were not significantly different (Table 2). There was no significant difference in Values of $P_{\text{max}}$ ($P=0.343$, $F=1.143$), $\alpha$ ($P=0.104$, $F=1.818$), $I_k$ ($P=0.674$, $F=0.669$) and $qP$ ($P=0.135$, $F=1.677$) due to the time spent in the flow (Table 2). There was a significant effect of time in flow for values of $NPQ$ ($P<0.005$, $F=4.825$, Figure 18, Table 2, Table 3). The interaction of time spent in water flow and water velocity for $P_{\text{max}}$ ($P=0.266$, $F=1.297$), $\alpha$ ($P=0.409$, $F=1.032$), $I_k$ ($P=0.078$, $F=1.972$), $qP$ ($P=0.164$, $F=1.570$) and $NPQ$ ($P=0.286$, $F=1.259$) was not significant (Table 2).

The ratio of $P_{\text{max}}$ in fast flow to $P_{\text{max}}$ in slow flow was calculated to determine if this value changed over time (Figure 13). The ratio was found to be relatively constant for the first two hours, then the ratio dropped, indicating that there may be a delay in
response to changes in water flow. If the data is analyzed for the time after two hours there is a significant difference between Pmax in fast flow and Pmax in slow flow. A second repeated measures ANOVA was run for measurements three through seven. Water velocity significantly affected values of Pmax values at times three through seven (P<0.05, F=4.816, Figure 19, Table 4). The mean value of Pmax in low flow velocity was 3.5 (SE=0.201, n=45), the mean value of Pmax in high flow was 4.7 (SE=0.278, n=45). Water velocity did not affect the values of α, I_k, qP and NPQ (P=0.098, F=3.096, P=0.971, F=0.00138, P=0.098, F=3.096 and P=0.321, F=1.039 respectively Table 4). The amount of time spent in the hydrodynamic regime did not affect the values of Pmax, α, I_k, and qP(P=0.264, F=1.342, P=0.167, F=1.65, P=0.665, F=0.618 and P=0.167, F=1.675 respectively, Table 3). The amount of time in the hydrodynamic regime did affect values of NPQ significantly (P=.001, F=4.197, Figure 20, Table 4, Table 5). The interaction of water velocity and time spent in flow did not affect the values of Pmax, α, I_k, qP and NPQ (P=0.273, F=1.318, P=0.458, F=0.920, P=0.139, F=1.810, P=0.403, F=1.023 and P=0.374, F=0.826 respectively, Table 3).
Discussion

The objectives of this thesis were to determine if hydrodynamic regime affects photosystem II kinematics in three species of the green algae Caulerpa, and to determine the extent of the dependence of PSII kinematics on hydrodynamic regime. Experiments also showed that the effect of hydrodynamics on members of the genus Caulerpa may be species, or location specific.

The results of these experiments indicate that photosystem II kinematics of Caulerpa sertularioides, C. mexicana, and C. racemosa are influenced by hydrodynamic regime in lab experiments and in situ. Lab experiments on C. racemosa (Figure 19) collected from Tampa Bay, support the findings of Wheeler (1980), Gerard (1982), Koehl and Alberte (1988), Carpenter et al. (1991) and Williams and Carpenter (1998) that increased water flow positively correlates with Pmax (figure b) and I_k (figure c) in marine algae. These results support our hypothesis that increased water flow would positively influence PSII kinematics. A possible explanation is that in Tampa Bay, C. racemosa is physically limited. As water velocity was artificially increased more nutrients were delivered to the plant surface, allowing for an increase in the electron transport rate.

Field measurements of C. sertularioides from the Florida Keys and lab measurements of C. mexicana did not support our hypothesis. Results showed a drastic decrease in Pmax as a function of water velocity (Figure 13, Figure 15). These findings are supported by several studies on the effects of water flow on marine and freshwater
algae. Gerard and Mann (1979) noted that the growth of *Laminaria longicrursis* in exposed sites was significantly slower than the growth of *L. longicrursis* in protected sites. More recently, Borchardt (1994) demonstrated that in freshwater plants which are N and P limited, flows greater than 30 cm s\(^{-1}\) were physiologically costly. Several mechanisms may be responsible for these results, including nutrient limitation due to the high water velocity, light flecking, and self shading.

Nutrient limitation is often thought to occur only during periods of low flow, when nutrient delivery is impeded by the thickness of the diffusive boundary layer. However, nutrient limitation can occur during periods of relatively high water flow if there are competing mechanisms within an organism that are triggered by the increase in water velocity. Gerard and Mann (1979) concluded that in high flowing waters N was more limiting than in slow flows in *L. longicrursis* because of specialized structures the algae needed to produce and maintain in order to survive in high velocity environments. If the plants need N to build or maintain these structures, then less is available for the cells photosynthetic pathways. Borchardt (1994) proposed that the actual nutrient requirements of nutrient limited *Spirogyra fluviatilis* changed with changes in hydrodynamic regime; the plants needed more of a limiting nutrient when flow was increased. This conclusion supports the conclusions of Gerard and Mann (1979), that while nutrient delivery is increased in higher flows, the actual cellular or organismal quota is also increased. Carbon limitation may actually increase with flowing waters depending on the form of C that a plant is capable of using (Borchardt 1994). If a plant utilizes an extra cellular bi-carbonate converting mechanism to convert bi-carbonate to
CO₂, increased water flow may actually increase C limitation. These conversion mechanisms often rely on the algae actively pumping a high gradient of protons (H⁺) into the diffusive boundary layer next to the thallus surface. These H⁺ are needed to convert bi-carbonate to CO₂. If water velocity increased and these H⁺ were transported away from the thallus, then higher levels of N and P may be needed to increase H⁺ transport across the cell membranes.

The light field experienced by any photosynthetic organism can drastically change its rate of photosynthesis. Sun flecks are intense burst of irradiance that result from the movement of canopy structures and surface waves that can account for significant differences in photosynthetic capacity of marine plants (Dromgoole 1987, Wing and Patterson 1993, Wing et al. 1993). Due to their focusing effect, surface waves result in high-frequency light fluctuations (millisecond range ), while the movement of canopy structures results in slower frequency and longer duration fluctuations (seconds to minutes ) (Green and Gerard 1990, Wing and Patterson 1993, Sagert and Schubet 2000). These fluctuations in light levels and intensity are known to be both beneficial and costly to algae. Under high frequencies of fluctuating light, the photosynthetic rates of many species have been shown to increase (Dromgoole 1987, Dromgoole 1988, Green and Gerard 1990). Conversely, other studies have demonstrated a negative correlation between light flecks and photosynthetic rates (Wellnitz and Rinne 1999). For a short period of time (milliseconds) focusing of irradiance by surface waves can increase the amount of light reaching the benthos by as much as five times the surface irradiance (Sagert and Schubert 2000, Schubert et al. 2001). During periods of high water flow on
Old Sweat Bank, small surface waves ran over the shoal with a very high frequency (Driscoll personal observations). The light regime resulting from this intense focusing of light may cause photo-inhibition in *C. sertularioides* due to temporarily damage the antennae complexes of PSII.

*Caulerpa*, like several other genera of the siphonous green algae have the ability to translocate their chloroplasts in times of stress and growth (Dawes and Barilotti 1969, Drew and Abel 1990, Chisholm and Jaubert 1997). This chloroplast movement was witnessed in *C. sertularioides* and *C. racemosa* during periods of high water flow. This chloroplast movement may have induced self-shading (of plastids) effects which could have resulted in an underestimation of PSII kinematics in high flow conditions.

In high velocity flows marine algae have been shown to “clump” together or bend to reduce the effects of form drag (Koehl and Alberte 1988). This clumping, while reducing drag can also decrease the photosynthetic activity of a plant by limiting the amount of light reaching the individual photosynthetic surfaces of the plant. The frond axis of *C. sertularioides* in the Florida Keys remained upright at all water velocities, however, observations showed the pinnules bending with the bulk flow so that they were almost parallel to the main velocity vector. This bending of the pinnules, while not quantified, may have caused self-shading during times of high water flow, decreasing values of Pmax.

Comparison between sites in the Florida Keys and Tampa Bay are made difficult by the differences in light regime and ambient nutrient concentration. The collection site in Tampa Bay is often extremely turbid due to terrestrial run-off and storm activity,
limiting the amount of light penetrating the water column. Nutrient levels are also high
due to extensive anthropogenic inputs. Conversely, Old Sweat Bank has very little
turbidity in the water column, allowing high levels of irradiance to penetrate the water.
Like most tropical systems the nutrient levels of carbonate shoals in the keys are deplete
(Lapointe and Clark 1992). These factors make comparisons between locations
equivocal, continued experimentation at both sites will be needed to draw conclusions on
a wider scale than species level.

These experiments are the first to use a direct measure of PSII kinematics to look
at the role of hydrodynamics in photo-production. Previous studies have relied on
oxygen evolution as the measure of photosynthetic activity (Raven et. al. 1979, Carpenter
et. al. 1991, Garcia et al. 1996). The measure of oxygen evolution as a function of water
velocity creates a situation that may drastically affect the results of experiments. Just as
Nitrogen, Phosphate, and Carbon are mass transfer limited to photosynthetic surface,
oxygen may be mass transfer limited from the same surface. This limitation would mean
that in low flows a gradient of oxygen would form within the boundary layer with higher
levels near the tissue surface. By increasing water velocity this oxygen would be carried
into the water column as the boundary layer thickness is decreased, erroneously
increasing the measured rates of oxygen evolution. By measuring chlorophyll
fluorescence this artifact is removed since fluorescence originates in the PSII antennae
complex and the measure itself is not affected by associated boundary layers.

This research is the first set of data that looks at direct measures of photo-
production as a function of hydrodynamic regime. Our findings support the hypothesis
that hydrodynamic regimes can influence physiological processes in marine algae. The method of this influence and the degree of the dependence of PSII on water flow conditions are still unclear, however, the results of these experiments and observations suggest that the use of PAM Fluorescence could be applied to elucidate the answers to these questions. Future directions for this project may involve altering the concentrations of nutrients in a variety of hydrodynamic regimes in an attempt to isolate the limiting factor, if any, in the photosynthetic pathways of Caulerpa spp. In situ measurements on several more species of Caulerpa would also be beneficial in determining the role of hydrodynamics in photosystem function.
**Figure 1.** Diagram of the anti-herbivory metabolite caulerpenyne. This compound is one of the main factors allowing for the success of *Caulerpa spp.* In certain cases caulerpenyne may compose up to 13% of the wet frond weight of *C. taxifolia*, making the algae in-edible to any known herbivore.
Figure 2. The three main energy pathways for photosystem II. (1) Energy is shunted off to the electron transport chain, (2) energy is dissipated as heat before it damages the photosystem and (3) energy is re-emitted from photosystem II at a higher wavelength (Fluorescence).
Figure 3. Schematic diagram of the pulse amplitude modulated fluorescence measuring principle in both light and dark adapted samples. (Walz 2001).
Figure 4. Representative curve that would be analyzed using the Platt inhibition model. A distinct decrease in levels of Pmax (photo-inhibition) is seen. μmol photons m$^2$s$^{-1}$
Figure 5. Representative curve that would be analyzed using the hyperbolic tangent model. No photo-inhibition is seen in the curve.
Figure 6. Drawing of *Caulerpa sertularioides* from Caribbean Reef Plants (Littler and Littler 2000). The pinnules are clearly much finer than those found in *C. mexicana* and *C. racemosa*. 
Figure 7. Drawing of C. racemosa from Caribbean Reef Plants (Littler and Littler 2000). This diagram depicts the variety in pinnule shapes and formations encountered in Tampa Bay, Florida.
Figure 8. Drawing of *Caulerpa mexicana* from Caribbean reef plants (Littler and Litter 2000). The pinnules of *C. mexicana* grown in a very flat broad shape, which is very similar to that of *C. taxifolia.*
Figure 9. Collection site for *C. racemosa* at the Sunshine Skyway Bridge south causeway. Specimens were collected from a depth of approximately 0.5m and returned to the lab within one hour of collection.
Figure 10. 110L racetrack flume. Water velocity within the flume is created by a small trolling motor and augmented using a diode control. Turbulence within the flume is dampened using three sets of flow straighteners.
**Figure 11.** Maps of Study sites in the Florida Keys. Upper image shows the Florida Keys and Florida Bay. Lower cutout diagrams Old Sweat Bank. *C. sertularioides* collection sites are indicated as dark shapes in each section of the shoal.
Figure 12. Orientation of the acoustic doppler velocimeters during field measurements. The ADV was attached to a large ring stand device with cable ties and hose clamps, and height was adjusted using several ring stand clamps. The sensor head was placed perpendicular to the substrate when water depth was greater than 15 cm and it was oriented parallel to the substrate when the water depth was less than 15 cm.
Figure 13. Ratio of Pmax in fast flow to Pmax in slow flow at each measuring interval. A clear drop in the ratio of Pmax fast/ Pmax slow is seen between times two and three indicating a possible lag time for the effects of hydrodynamics to be seen in PSII.
Figure 14. Results of dark acclimation trial using magnetic leaf clips under full illumination and a dark room. There was no significant difference (P value = 0.790) between trials utilizing the magnetic leaf clips and a dark room to dark acclimate tissue samples. These results indicate that the samples used were not nutrient limited by the presence of the magnetic leaf clip.
Figure 15. Values of Pmax for *C. mexicana* run at high and low velocities in a laboratory flume. Water velocity was shown to significantly affect values of Pmax (P value = 0.04). High flow was 20 – 25 cm s$^{-1}$ and low flow was 5-10 cm s$^{-1}$. Values of Pmax were significantly higher in slow hydrodynamic regimes.
Figure 16. In situ measures of $P_{\text{max}}$ in *C. sertularioides*. PSII kinematics parameters were measured without removing the plant from its natural environment. Water velocity was shown to significantly affect values of $P_{\text{max}}$ measured in the field using a Diving PAM Fluorometer (P value <0.001). Low flow was less than 20 cm s$^{-1}$, medium flow was 20 – 30 cm s$^{-1}$ and high flow was above 30 cm s$^{-1}$. The highest values of $I_k$ were seen when velocity was less than 20 cm s$^{-1}$. The lowest values of $P_{\text{max}}$ were measured when water velocity was greater than 30 cm s$^{-1}$.
Figure 17. *In situ* measurements of Saturation irradiance in *C. sertularioides*. Saturation irradiances measured on *C. sertularioides* measured in situ were significantly different as a function of water velocity (P value = 0.018). Low flow was less than 20 cm s⁻¹, medium flow was 20 – 30 cm s⁻¹ and high flow was above 30 cm s⁻¹. The highest values of $I_k$ were seen when velocity was less than 20 cm s⁻¹.
Figure 18. Laboratory measurements of NPQ in *C. racemosa*. Samples were collected from Tampa Bay, and PSII kinematics parameters were measured in a laboratory flume every hour for seven consecutive hours. Values of NPQ, measured from hours three through seven were shown to be significantly different as a function the amount of time spent in the water flow (P value = 0.002).
Figure 19. Laboratory measurements of Pmax in *C. racemosa*. *C. racemosa* was collected from the Sunshine Skyway jettie, and exposed to different water flow regimes in a small laboratory flume. After three hours in a hydrodynamic regime, values of Pmax from *C. racemosa* were shown to be significantly different as a function of water velocity (P value = 0.022). High flow was considered velocities above 15 cm s⁻¹ and low flow was considered velocities lower than 15 cm s⁻¹. Higher values of Pmax were measured in velocities greater than 15 cm s⁻¹.
Figure 20. Laboratory measurements of NPQ in *C. racemosa*. Samples were collected from Tampa Bay, and PSII kinematics parameters were measured in a laboratory flume. Values of NPQ, measured from hours three through seven were shown to be significantly different as a function the amount of time spent in the water flow (P value = 0.002). Hours labeled “A” are significantly different than hours labeled "B".
Table 1. Values of Pmax, α, and I_k measured on C. sertularioides in situ.
Measurements were taken on plants that had not been removed from their natural habitats, and that were exposed to natural water flow and irradiance conditions. Values of Pmax, measured in situ were found to be significantly different as a function of water velocity (P value <0.001). Values of α and I_k were not found to be significantly different in different water flows when measured in situ.
Table 2. Values of Pmax, α, Ik, qP, and NPQ measured on *C. racemosa* in a laboratory flume over seven hours. Samples were collected from Tampa Bay and measurements were taken in a laboratory flume. Measured values of Pmax, α, Ik, qP and NPQ were not found to be significantly different as a function of water velocity. The values of Pmax, α, Ik, and qP were not found to be significantly different as a function of the amount of time spent in a given hydrodynamic regime. Values of non-photochemical quenching were found to be significantly different as a function of the amount of time spent in a given flow regime (P value <0.005). There was no significant interaction between velocity and time.

<table>
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<tr>
<th>Flow Speed</th>
<th>Flow Speed</th>
<th>P value</th>
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<tr>
<td></td>
<td>High</td>
<td>Low</td>
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<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
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<tr>
<td>Pmax (µmol electron m²s⁻¹)</td>
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<td>4.0</td>
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<tr>
<td>α (µmol electron m²s⁻¹)</td>
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<tr>
<td>Ik (µmol quanta m²s⁻¹)</td>
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<tr>
<td>QP</td>
<td>0.238 (0.00973)</td>
<td>0.226</td>
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<tr>
<td>NPQ</td>
<td>0.083 (0.00547)</td>
<td>0.098</td>
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Table 3. Values of NPQ at each measurement hour. Measurements were taken on samples in a laboratory flume. NPQ values are significantly different as a function of time immersed in flow. Values of NPQ at measurement intervals of 3 and seven hours were significantly different than at the other five measurement times.
Table 4. Values of Pmax, α, I_k, qP, and NPQ measured on *C. racemosa* over five hours in a laboratory flume. Samples were collected from Tampa Bay, and measurements were taken while the samples were immersed in a given water flow regime in a laboratory flume. Measurements were taken once every hour for seven hours. Measured values of Pmax for times three through seven were found to be significantly different as a function of water velocity (P value <0.05). There was a higher electron transport rate in samples experiencing a faster hydrodynamic regime. Values of α, I_k, qP and NPQ at times three through seven in fast and slow hydrodynamic regimes were shown to not be significantly different. Measured values of NPQ were shown to be significantly different as a function of the amount of time spent in a given hydrodynamic regime (P value = 0.002). Values of Pmax, α, I_k and qP were not significantly different in at different measuring periods. There was no interaction of time and velocity.
<table>
<thead>
<tr>
<th>Time</th>
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**Table 5.** Values of NPQ after 2 hours of immersion. PSII kinematic parameters were measured on samples collected from Tampa Bay were measured once an hour for seven hours. Values of NPQ are significantly different as a function of time immersed in flow (P value = 0.002).
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