*CASSIOPEA XAMACHANA* AS A BIOINDICATOR OF DISSOLVED INORGANIC PHOSPHATES IN SEAWATER

by

BRIAN DANIEL TODD

(under the direction of Dr. William K. Fitt)

## ABSTRACT

*Cassiopea xamachana* is a scyphozoan jellyfish that harbors endosymbiotic algae known as zooxanthellae. The uptake of dissolved inorganic phosphates (DIP) by *Cassiopea* requires a symbiotic relationship with zooxanthellae of the genus *Symbiodinium*. Animals were collected from Florida Bay, Key Largo, Florida for analysis. Pre-exposure to high levels (2μM and greater) of DIP *in hospite* caused a decreased rate of phosphate uptake. The animals continued to show a decreased rate of uptake for up to 5 hours after being removed from elevated DIP levels. This suggested a suitability for use of *Cassiopea xamachana* as a bioindicator of DIP in seawater. Subsequent field studies involved placing animals on a fore reef, a patch reef, and nearshore in Florida Bay. Animals that were farther from shore exhibited significantly greater ($p<0.05$) rates of DIP uptake after 4 days than those in nearshore Florida Bay, suggesting that DIP levels decrease significantly with distance from shore.

INDEX WORDS: *Cassiopea xamachana*, Bioindicators, Phosphate, Florida Bay, Coral reefs, Zooxanthellae, Symbiosis, *Symbiodinium*

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# CHAPTER 1

# INTRODUCTION

Nutrients are implicated both directly and indirectly in the decline of coral reefs. With increased oceanic nutrients, plankton biomass grows and the resulting increase in turbidity and decrease in light can limit coral productivity and survival (Hallock and Schlager, 1986). A change in reef community structure can also occur in areas of elevated nutrients (Kinsey and Davies, 1979). Macroalgae proliferate faster than slow-growing corals and they shade the corals while also reducing suitable substrate for larval settlement (Lewis, 1986; Hughes, 1994). A more direct result of increased nutrients is seen when phosphate levels are elevated. Phosphate acts as a poison in the crystal lattices of calcification (Simkiss, 1964; Belda and Yellowlees, 1995). Studies illustrate that elevated phosphate can depress deposition of calcium carbonate while increasing community production due to algal proliferation (Kinsey and Davies, 1979; Kinsey and Domm, 1974; Tomascik and Sander, 1987).

Some authors have hypothesized that there are threshold levels of nutrients (1 μM dissolved inorganic nitrogen and 0.1 μM soluble reactive phosphorus) that, if exceeded, result in coral loss through direct and indirect actions (Bell, 1992; Lapointe, 1997). While the specific threshold levels and the overriding generality of these proposed levels are disputed (Hughes *et al*, 1999), even these authors concede that elevated nutrients are detrimental to corals among reef communities. Nutrient levels in

the Florida Keys have been on the rise in the past few decades and there is much debate over exact sources (Lapointe *et al*, 2002; Boyer and Jones, 2002). Some researchers contend that nutrients reaching the reefs are from anthropogenic inputs of sewage and phosphate mining on Florida and the Florida Keys (Lapointe *et al*, 1990; Lapointe *et al*, 1992; Brand, 2002), while others feel that natural causes like resuspension of nutrient-rich sediments or upwelling are the cause (Szmant and Forrester, 1996). It is clear that nutrient levels and their effects on Florida's coral reef communities warrant future monitoring at appropriate spatial and temporal scales.

Bioindicators are commonly used in oceanographic work to aid researchers in investigating ecosystem health. Chlorophyll *a* is used as a predictor of nutrient enrichment (Harding and Perry, 1997), alkaline phosphatase is used to determine phosphorus limitation, and seaweeds and seagrasses are assayed to determine nutrient enrichment and limitation in surrounding seawater (Lapointe, 1989; Lapointe *et al*, 1994; Lapointe *et al*, 1987). The use of a cnidarian-zooxanthellae symbiosis as a bioindicator of elevated nutrient levels has many added advantages over traditional water column nutrient measurements.

Firstly, traditional approaches such as point sampling provide only a spatial and temporal snapshot of nutrient concentrations. They cannot account for transitory or episodic events unless samples are drawn in the midst of the event. Secondly, elevated phosphates in seawater may be testable using current methods, but do not immediately identify the impact on the nutrient dynamics of key ecological indicators, such as corals. Bioindicators integrate local temporal environmental influences and offer a more detailed picture of resulting impacts. They are also able to retain the nutrient signal for a finite

period of time. Finally, bioindicators can reveal biological effects at exposure levels that may be below analytically detectable levels.

This thesis is designed as a series of separate experiments that illustrate the efficacy of using *Cassiopea xamachana* as a bioindicator of dissolved inorganic phosphate levels in seawater. *Cassiopea xamachana* is a scyphozoan jellyfish common to shallow mangrove habitats of the Caribbean Sea and Florida Keys. Members of this genus harbor the symbiotic dinoflagellates *Symbiodinium microadriaticum*, also known generically as zooxanthellae (Freudenthal, 1962). Like many cnidarian-dinoflagellate symbioses, *Cassiopea* actively take up dissolved nutrients, including phosphate, from surrounding seawater (Yonge and Nicholls, 1931; Pomeroy and Kuenzler, 1969; D'Elia, 1977; Muller-Parker *et al*, 1990). Nutrient history influences the rate of phosphate uptake by zooxanthellae (Deane and O'Brien, 1981; Kelty and Lipschultz, 2002). Aposymbiotic and non-symbiotic cnidarians release dissolved phosphates (Muller-Parker *et al*, 1990), suggesting that phosphate uptake in symbiotic animals is algal-driven. Thus, uptake of dissolved phosphate by intact medusae of *Cassiopea xamachana* is likely influenced by environmental concentrations of dissolved inorganic phosphates. It is possible that the medusae of *Cassiopea xamachana* may be used as an indicator of phosphate concentrations in seawater by examining the relationship between dissolved phosphate concentrations in seawater and concomitant uptake rates of the intact symbiosis..

# CHAPTER 2

# ANATOMY OF THE HOST-SYMBIONT RELATIONSHIP IN *CASSIOPEA XAMACHANA*

## Introduction

*Cassiopea xamachana* is a scyphozoan jellyfish common to shallow mangrove habitats of the Caribbean Sea and Florida Keys. Like many scyphozoans, it has a lifecycle characterized by an alternation between medusa and polyp. All *Cassiopea* begin their life as free-swimming planula larvae. The planulae receive chemical cues for settlement and metamorphosis from decaying plant material (*e.g*., mangrove leaves) forming aposymbiotic polyps known as scyphistomae (Fleck *et al*, 1999). The scyphistomae are ingestive heterotrophs capable of reproducing by asexual budding. The jellyfish will remain in this stage until ingestion of dinoflagellates in the genus *Symbiodinium*, a subset of brown symbiotic algae generically known as zooxanthellae (Freudenthal, 1962; Trench, 1993). Scyphistomae of *Cassiopea* begin strobilation and create tiny medusae known as ephyras that carry with them the acquired zooxanthellae. Medusae are either male or female, and reproduce sexually with the female holding the developing eggs in specialized oral tentacles until they are ready to be released (Hofmann *et al*, 1996).

The symbiont associated with *Cassiopea xamachana* is the unicellular dinoflagellate *Symbiodinium microadriaticum* (Freudenthal, 1962). The dominant phase of the algae while in the host is a non-motile coccoid cell, which resides mostly in

amoeboid host cells in the mesoglea of *Cassiopea* (Colley and Trench, 1983). In culture, the algae cycle between this phase and a more typical gymnodiniod morphology with a flagellum that encircles the central axis and another flagellum propels the dinoflagellate forward (Trench and Blank, 1987). The symbiosis is established when the aposymbiotic *Cassiopea* schyphistoma, or polyp stage, ingests the algae and is phagocytosed by digestive cells. *Symbiodinium* sp are photosynthetic autotrophs and they can translocate carbon to their host jellyfish, which benefits in terms of increased growth rate and reproduction.

Zooxanthellae are common symbionts in tropical and some temperate cnidarians, and are also found among mollusks in the giant clams and their relatives. In most all cases the algae act to meet some or all of the host's carbon needs. The relationships between hosts and zooxanthellae have been the subject of many studies including work on hermatypic corals, sea anemones, and giant clams (Jokiel and Morrissey, 1986; Muscatine *et al*, 1979; Muller-Parker, *et al* 1990; Fitt *et al*, 1993).

The purpose of the current study is to investigate the physiological parameters associated with populations of zooxanthellae and different sizes of the host jellyfish *Cassiopea xamachana*. Zooxanthellae densities and chlorophyll *a* concentrations will be correlated with jellyfish wet weight and bell diameter.

## Materials and Methods

**Animal collection:** *Cassiopea xamachana* specimens were collected from a near-shore mangrove lagoon on the Atlantic coast of Key Largo, FL near mile marker 99.5. The mangrove lagoon is ocean fed and has a series of man made navigable canals to allow ship passage. All specimens were transported to the Key Largo Marine Research

Laboratory for analysis. An attempt was made to select a range of individuals representing a diversity of sizes.

**Host biomass measurements:** Bell diameter was measured with bell extended to the nearest millimeter using a ruler. Specimens were blotted dry once to remove excess water and then weighed on a Metler electronic scale to determine wet weight to the nearest milligram.

**Symbiont measurements**: All specimens were diced and homogenized in Instant Ocean seawater using a Virtis tissue grinder. A 1 mL aliquot was taken from the homogenate and preserved with 0.1mL formalin for later determination of zooxanthellae density. Using a Neubauer hemacytometer, average zooxanthellae densities for each specimen were calculated after making 6 replicate counts per animal. Two 15 mL samples of the homogenate were also collected for chlorophyll *a* analysis. Each sample was centrifuged to remove animal tissue and then algal pellets were frozen for preservation until analysis. The pellets were subsequently resuspended and extracted with 90% acetone overnight and then read on a Pharmacia spectrophotometer. Total chlorophyll *a* was calculated from absorbance at 663 and 630 nm by the method of Jeffrey and Humphrey (1975).

## Results

Wet weight of *Cassiopea xamachana* medusae increased with bell diameter in the characteristic log-linear fashion (Fig. 2.1).

There is an exponential increase in density of zooxanthellae in relationship with bell diameter (Fig. 2.2A). However, total number of zooxanthellae increases linearly as a function of wet weight up to 7cm in diameter jellyfish (Fig. 2.2B). Total zooxanthellae

numbers range from approximately 12 million in a small medusa (2.8cm diameter) to upwards of 150 million in a larger medusa (7.0cm diameter). Although numbers of zooxanthellae increase with both jellyfish biomass and bell diameter, the relative density as expressed per gram wet weight remains rather constant with only a slightly downward trend as bell diameter increases (Figure 2.2C).

Figure 2.3A shows the exponential increase in total chlorophyll *a* as bell diameter increases, similar to the pattern seen with density of zooxanthellae. Chlorophyll density (μg/gWW) remains relatively constant as bell diameter increases (Figure 2.3B). A look at the relationship between zooxanthellae and chlorophyll *a* (Figure 2.3C) reveals a positive linear correlation, such that chlorophyll *a*/zooxanthellae remained relatively constant over the size range of jellyfish investigated.

## Discussion

Wet weight of jellyfish increases exponentially with increase in bell (Fig. 2.1). This can be attributed primarily to the allometric relationship of volume increasing faster than surface area as observed in all animals (Schmidt-Nielsen, 1974). Work on the giant clam *Tridacna gigas* showed a similar trend with wet weight of the clams increasing exponentially with increase in clam shell length (Fitt *et al*, 1993). Total zooxanthellae found in jellyfish increased exponentially with wet weight and bell diameter (Fig. 2.2). Similarly, Fitt *et al* (1993) demonstrated that smaller tridacnid clams have significantly higher densities of zooxanthellae per gram wet weight than do larger clams. Verde and McCloskey (1998) also found that *Cassiopea xamachana* follows this same trend, with smaller animals having much higher densities of zooxanthellae compared to larger jellyfish. Figure 2.2C emulates this trend but does not illustrate it as clearly, perhaps due

to the limited size range of jellyfish used in the current study. Since newly settled planulae larvae and early scyphistomae polyps contain no zooxanthellae, and presumably release phosphate in the environment, the maximum density of symbionts and maximum potential for phosphate uptake per unit biomass appears to occur in relatively small jellyfish (Verde and McCloskey, 1998; Fig. 2.2C)

Levels of chlorophyll in host-zooxanthellae symbioses correspond to the abundance of zooxanthellae within the host tissue. It follows that chlorophyll *a* trends should parallel those found with zooxanthellae. Figure 2.3C embodies this relationship showing a strong linear correlation between zooxanthellae numbers and chlorophyll *a* concentrations ($R^2$= 0.44, $p$<0.05). Also, both the zooxanthellae numbers and chlorophyll *a* levels increase exponentially with bell diameter (Figure 2.2A and 2.3A).

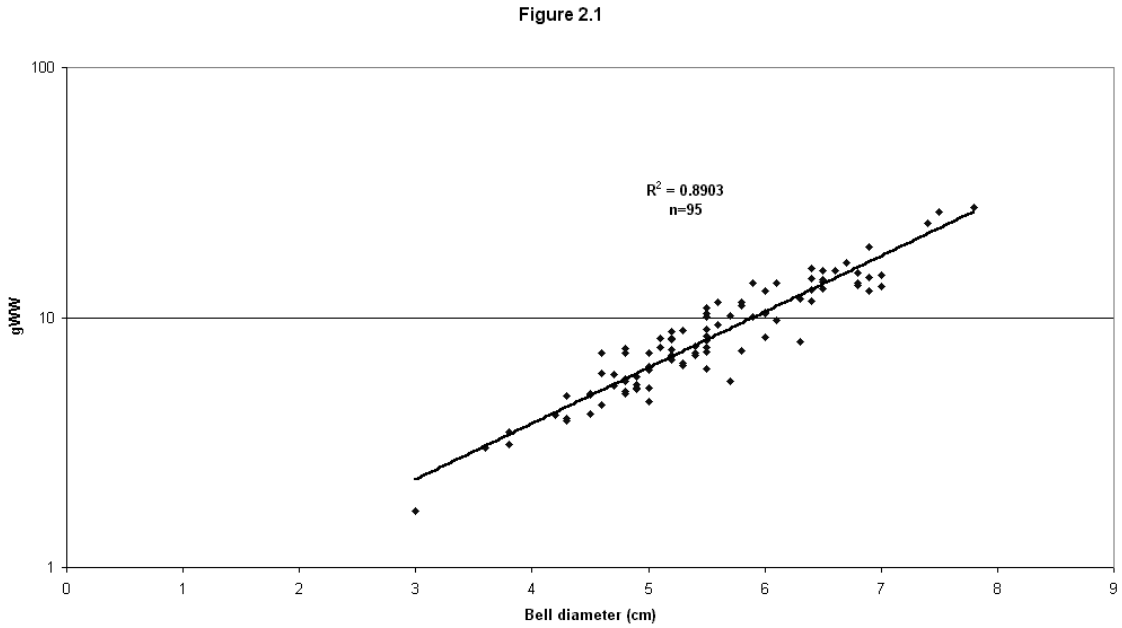



Figure 2.1. Log linear increase of wet weight in grams with bell diameter (cm).

Figure 2.2. Increase in total zooxanthellae per animal as a function of bell diameter (A) and wet weight (B).

Figure 2.2A

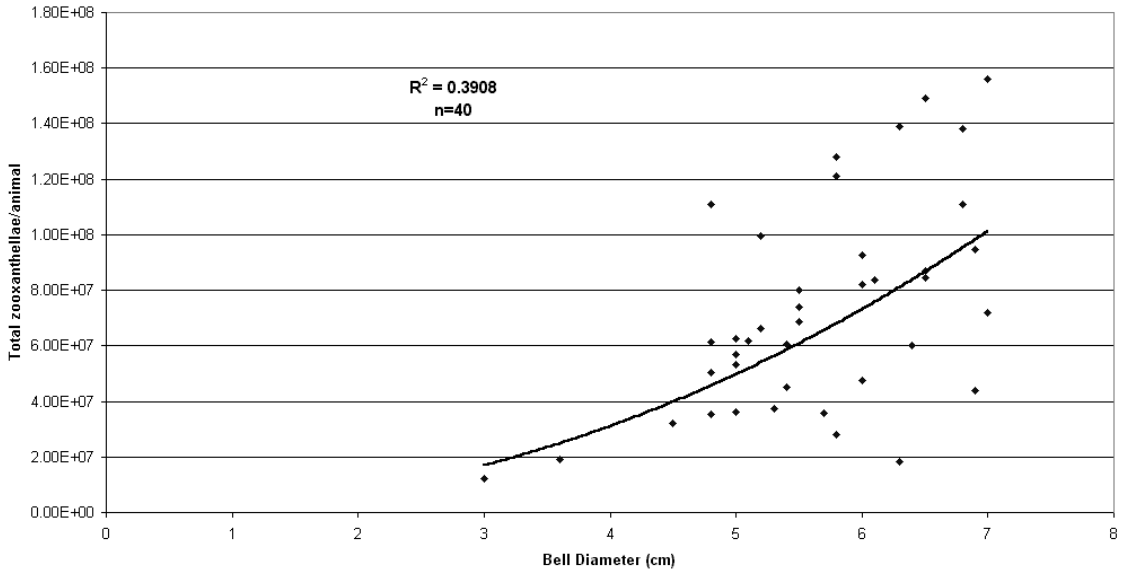
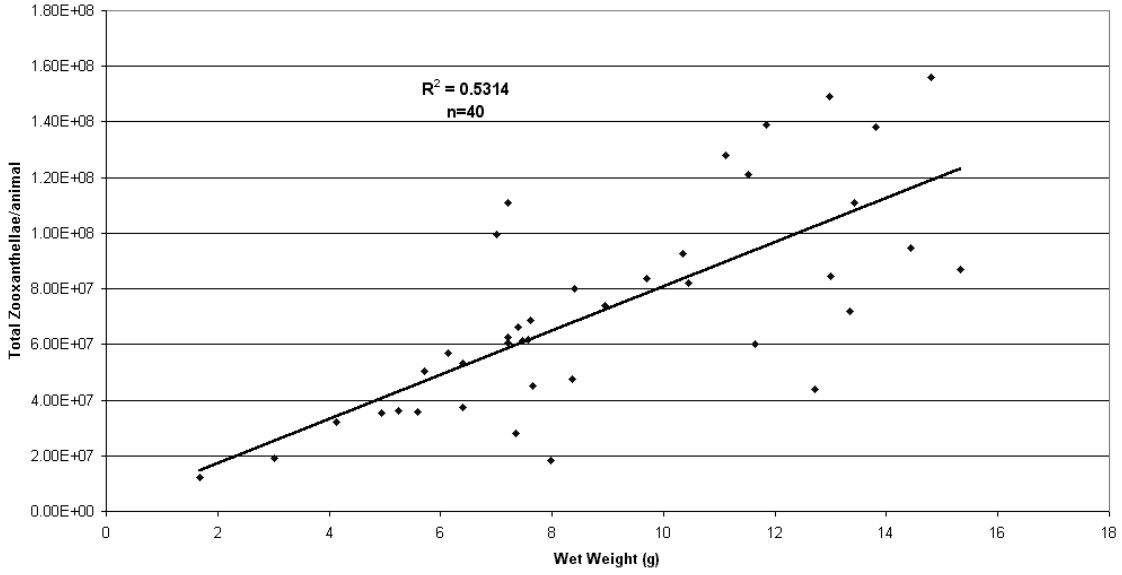



Figure 2.2B



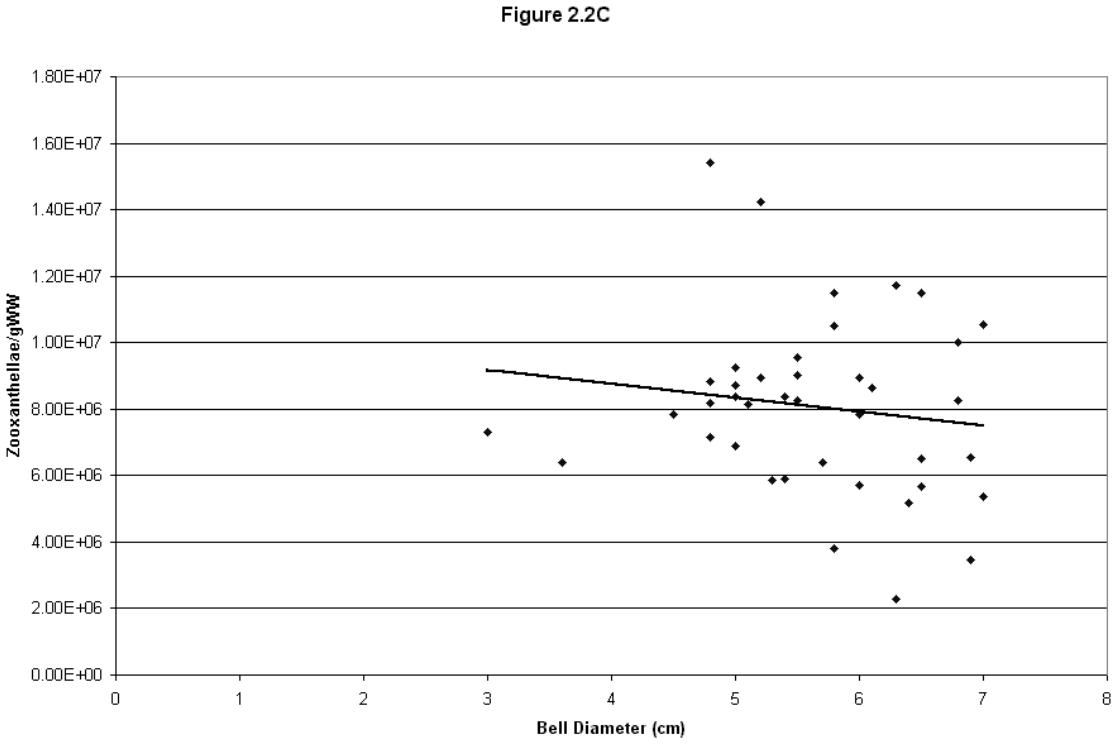



Figure 2.2C  Zooxanthellae density per gram wet weight as a function of bell diameter.

Figure 2.3. Total chlorophyll *a* per animal as a function of bell diameter (A). Chlorophyll *a* per gram wet weight as a function of bell diameter (B).

Figure 2.3A

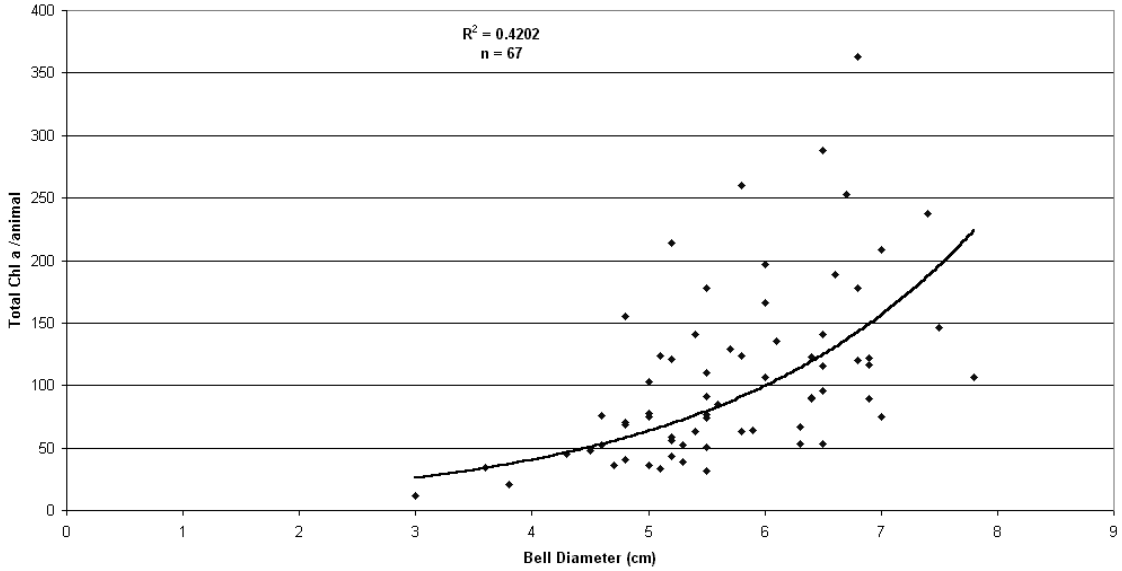



Figure 2.3B

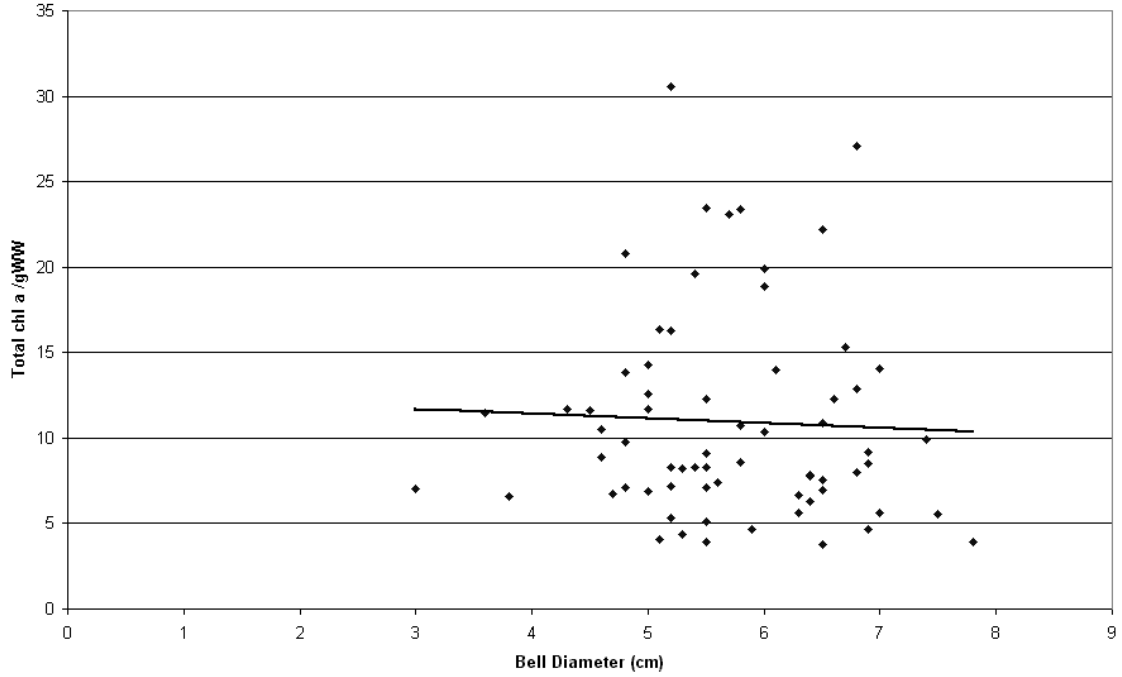


Figure 2.3C

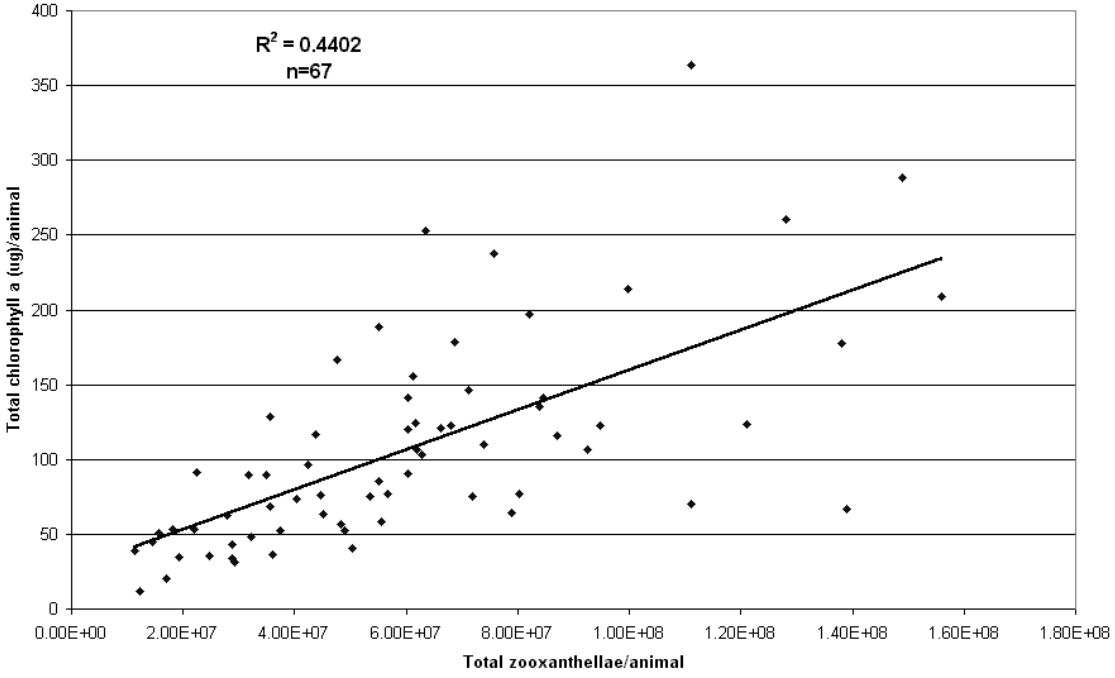



Figure 2.3C. Linear increase of total chlorophyll a per animal with total zooxanthellae per animal.

# CHAPTER 3: SIZE MATTERS

## Introduction

*Cassiopea xamachana* is a scyphozoan jellyfish common to mangrove habitats in the Caribbean Sea and Florida Keys. The medusae are often observed pulsating upside-down on the bottom substrates of muddy, shallow mangrove bays, a habit that has earned them the common name of "upside-down jellyfish". Members of this genus harbor the symbiotic dinoflagellates *Symbiodinium microadriaticum*, also known as zooxanthellae (Freudenthal, 1962). As early as 1908, Perkins observed the jellyfish using the bell as a sucker to aid the medusa in adhering to the substratum (Perkins, 1908). It has since been postulated that the jellyfish rest in such a manner to expose their algal symbionts to sunlight while simultaneously absorbing nutrients from the often decaying and nutrient-rich ocean bottom (Drew, 1972).

The role of algal symbionts and nutrient relationships in invertebrate-algal symbioses has long been a topic of interest. Yonge and Nicholls (1931) were among the first to note that hermatypic corals with symbiotic zooxanthellae can actively remove phosphate from the surrounding seawater. Some time later, Pomeroy and Kuenzler (1969) implicated the zooxanthellae in the uptake of dissolved inorganic phosphate (DIP, essentially soluble reactive phosphorus) by comparing uptake rates of both symbiotic and aposymbiotic corals. Further studies have documented the importance of the symbiotic relationship in phosphorus uptake by showing that uptake is dependent on light through

photosynthetic roles (D'Elia, 1977; Muller-Parker *et al*, 1990). Verde and McCloskey (1998) have pointed out that symbiont relationships of attached benthic invertebrates and unialgal symbionts (*e.g.* corals) have been more widely studied than those of motile symbiotic invertebrates. However, studies as early as 1936 on *Cassiopea frondosa* have shown that these jellyfish exhibit similar phosphate uptake phenomena as reef corals (Smith, 1936).

Previous research has shown that nutrient uptake rates in some marine algal-invertebrate symbioses are dependent upon animal size. Fitt *et al* (1993) found that smaller *Tridacnid* clams had higher rates of nitrogen uptake per body mass than larger clams. Similarly, Verde and McCloskey (1998) found smaller *Cassiopea xamachana* to have significantly higher rates of respiration per body mass than larger ones. In both studies there was also a marked increase in weight-specific algal density, with smaller individuals having greater densities of symbionts than larger animals.

The purpose of this experiment is to examine the relationship between DIP uptake rates and jellyfish size. Because zooxanthellae densities and numbers vary with the mass of the jellyfish (see Chapter 2), uptake rates should similarly be size dependent.

## Materials and Methods

**Animal collection:** *Cassiopea xamachana* specimens were collected from a near-shore mangrove lagoon on the Atlantic coast of Key Largo, FL near mile marker 99.5. The mangrove lagoon is ocean fed and has a series of man made navigable canals to allow ship passage. Ambient levels of $PO_4$ were typically less than 0.2 μmol/L. All specimens were transported to the Key Largo Marine Research Laboratory in seawater from the lagoon. Analyses and assays were performed within two hours of collection

unless otherwise noted. An attempt was made to select a range of individuals representing a diversity of medusa sizes, ranging from 3.0cm to 8.0cm.

**Biomass measurements:** Bell diameter was measured to the nearest millimeter using a ruler. Specimens were blotted dry once to remove excess water and then weighed on a Metler electronic scale to determine wet weight to the nearest milligram. The animals were then diced and homogenized in Instant Ocean seawater (35 ppt) using a Virtis tissue grinder. A 1 mL aliquot was taken from the homogenate and preserved with 0.1mL formalin for later determination of zooxanthellae density. Using a Neubauer hemacytometer, average zooxanthellae densities for each specimen were calculated from 6 replicate counts per animal.

Two 15 mL samples of the homogenate were also collected for chlorophyll-a analysis. Each sample was centrifuged to remove animal tissue and then algal pellets were frozen for preservation until analysis. The pellets were subsequently resuspended and extracted with 90% acetone overnight and then read on a Pharmacia spectrophotometer. Total chlorophyll a was calculated from absorbance at 663 and 630 nm by the method of Jeffrey and Humphrey (1975).

**Phosphate uptake:** The medusae were transferred to acid-washed glass Petri dishes of adequate size at the start of the experiment so that the bell of the animal would have room to extend. Each animal was placed in 200 ml of Instant Ocean seawater (35 ppt). Phosphate concentrations in the salt mix are known to be negligible (<0.03 μM as tested by AquaCraft) and were undetectable by our methods (<0.03μM). The experimental seawater was mixed to contain approximately 2 μmol/L phosphorus in the form of dissolved $K_2HPO_4$ and three 50 ml samples of the starting concentration were

taken in order to ascertain the exact initial concentration of the mixture. Once the animals were placed in their respective Petri dishes, duplicate 25 ml water samples were taken every ten minutes for thirty minutes giving 6 total samples for each medusa. All samples were placed in a –20$^{o}$C freezer to prevent bacterial growth from impacting phosphate levels before phosphate concentrations were determined.

Less than 24 hours later, samples were thawed and prepared for DIP determinations using the addition of a molybdate compound and following the heteropoly blue formation method (Strickland and Parsons, 1968). Standards were also prepared at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 μM $KH_2PO_4$. A standard curve was constructed and the resulting regression was used to calculate DIP concentrations from absorbance readings. All absorbances were measured at a wavelength of 885 nm. The resulting concentration values were used to determine the rates of uptake in micromoles per hour with the following formula:

$$\frac{(conc\ t_x) - (conc\ t_{x+10})}{1{,}000\ ml} \times \frac{V(t_{x+10})}{10\ min} \times \frac{60\ min}{1\ hr}$$

where '*conc* $t_x$' is the concentration (μmol/L) at time '*x*' minutes (10,20, or 30) and '*V*' is volume (200, 150, or 50 ml).

## Results

**Depletion of dissolved inorganic phosphate (DIP) from seawater:**

The decrease in concentration of phosphates in seawater surrounding the jellyfish through the duration of the 30-minute assays was typically more rapid during the first 10 minutes than in subsequent time intervals (Figure 3.1). Approximately 80% of the animals followed this trend with the most marked changes in concentrations, and thus the

highest rates of uptake, occurring in the first 10 minutes of an animal's contact with the starting solution. Some animals exhausted the available phosphate before the 30 minutes ended. For these reasons, comparisons between all animals were made using the rates found in the first 10 minutes of the uptake assays. This also aided in reducing variability.

**DIP uptake rates versus animal size:**

Uptake rates of DIP per hour per gram wet weight were higher in smaller animals than in larger animals (Figure 3.2A). This weight-specific trend is the same when uptake rates are plotted against bell diameter (Figure 3.2B). However, larger animals exhibited a greater overall uptake of phosphate than did smaller specimens (Figures 3.3A and 3.3B).

## Discussion

The results of the current study indicate that medusa size does play an important role in determining the uptake rates of DIP by symbiotic *Cassiopea xamachana*. The most probable explanation is that zooxanthellae within the animals are driving the uptake of DIP and that uptake rates correlate with animal size because the zooxanthellae populations follow animal wet weight closely. Smaller specimens have a lower overall uptake rate, yet exhibit a higher uptake rate per gram wet weight. Since zooxanthellae densities per gram wet weight were also greater in smaller jellyfish (see Chapter 2, Figure 2.2), the greater rate of uptake per gram wet weight can be explained by this higher zooxanthellae density. Fitt *et al* (1993) demonstrated similar trends in a symbiotic clam *Tridacna gigas*, where smaller host animals had greater zooxanthellae densities per gram wet weight and were probably responsible for greater uptake rates of nitrate and ammonium from seawater.

D'Elia (1977) implicates zooxanthellae in DIP uptake in his study of phosphate flux in reef corals, which demonstrated that five different symbiotic reef corals readily removed reactive phosphates from seawater, while an asymbiotic coral, *Tubastrea coccinea*, released reactive P. Similarly, Muller-Parker *et al* (1990) found that an anemone, *Aiptasia pallida*, with no zooxanthellae (*i.e*., aposymbiotic) would not remove phosphates from seawater, whereas symbiotic anemones readily removed phosphates from solution. Earlier, Pomeroy and Kuenzler (1969) postulated that zooxanthellae were directly responsible for uptake of phosphates by their coral hosts when they observed uptake of DIP by symbiotic corals in light but decreased rates of uptake in the dark.

While there is still some variability in DIP uptake rates at each given jellyfish size, targeting a specific size class and using animals within these limited boundaries for uptake comparisons will likely alleviate many discrepancies caused by different sized animals.

Figure 3.1

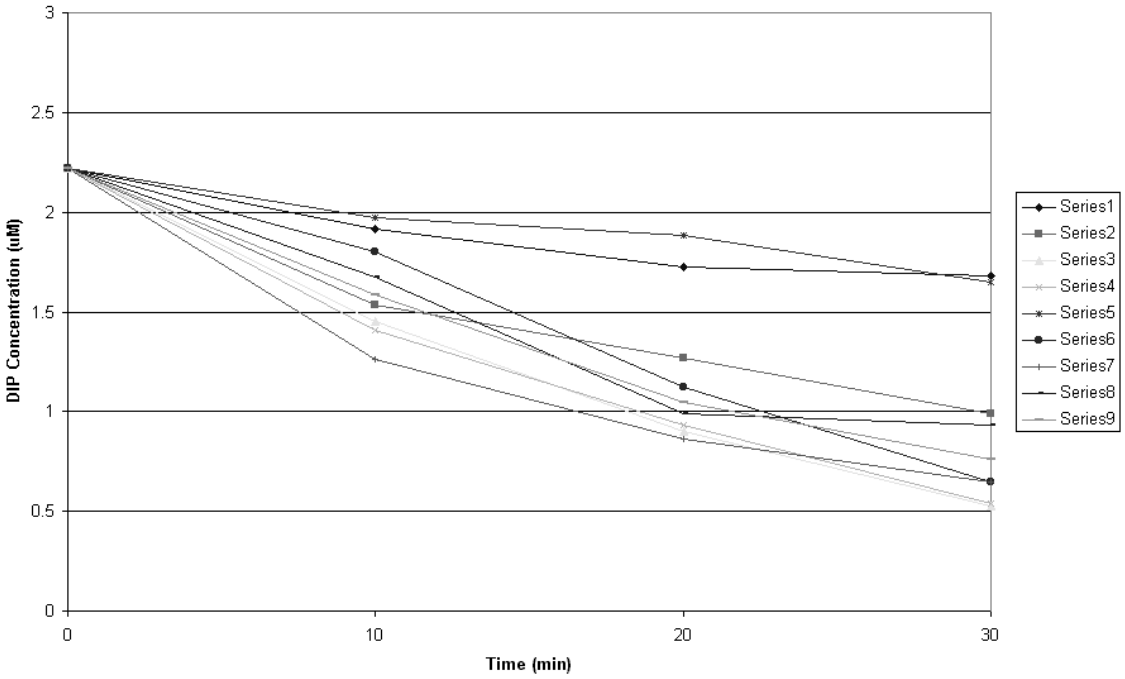



Figure 3.1. Decrease in dissolved inorganic phosphate (DIP) from seawater with time. Series 1-9 represent 9 different jellyfish. Decrease in DIP concentrations is quickest in first 10 minutes.

Figure 3.2. Uptake rates of DIP per gram wet weight as a function of wet weight (A) and bell diameter (B).

Figure 3.2A

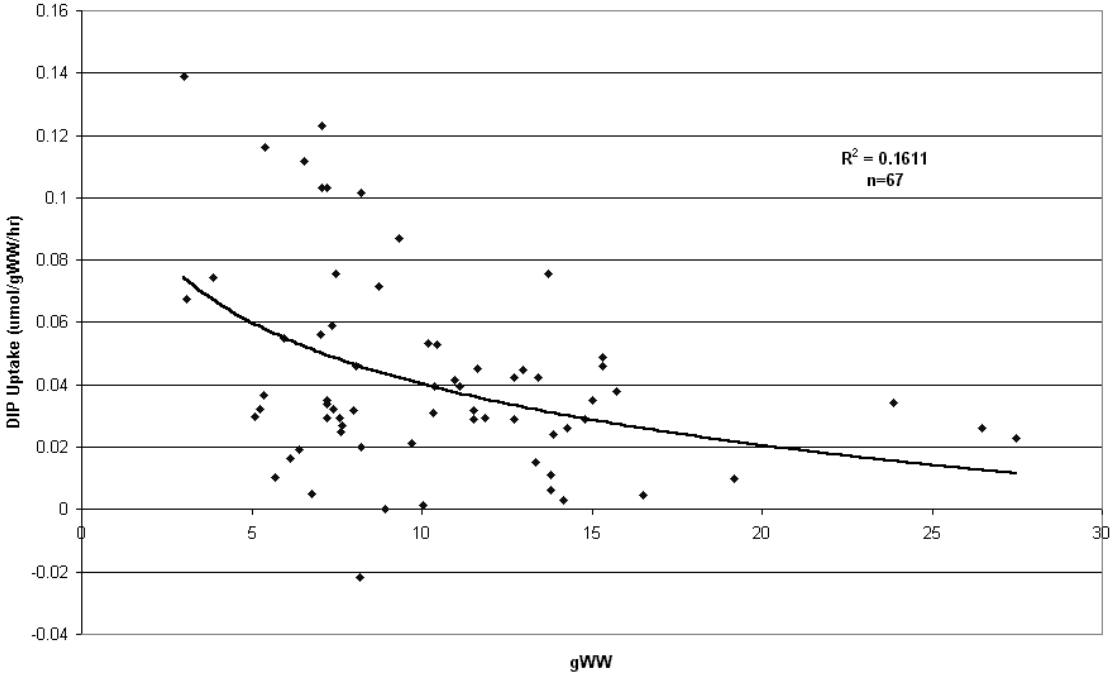



Figure 3.2B

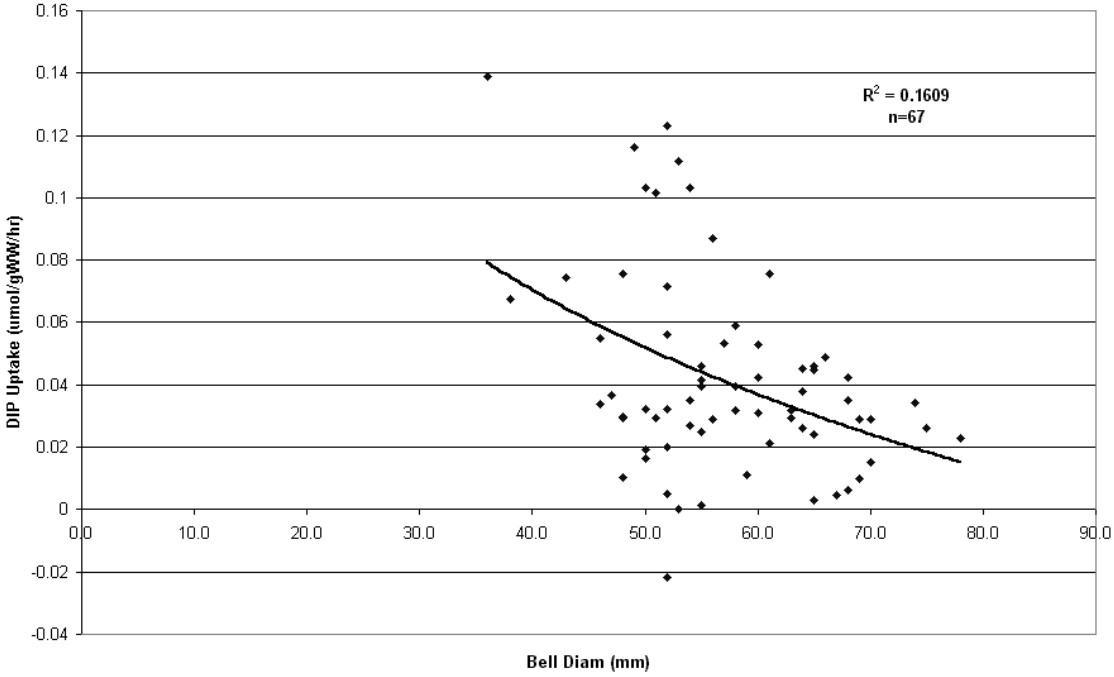


Figure 3.3. DIP uptake in micromoles/hour as a function of wet weight (A) and bell diameter (B).

Figure 3.3A

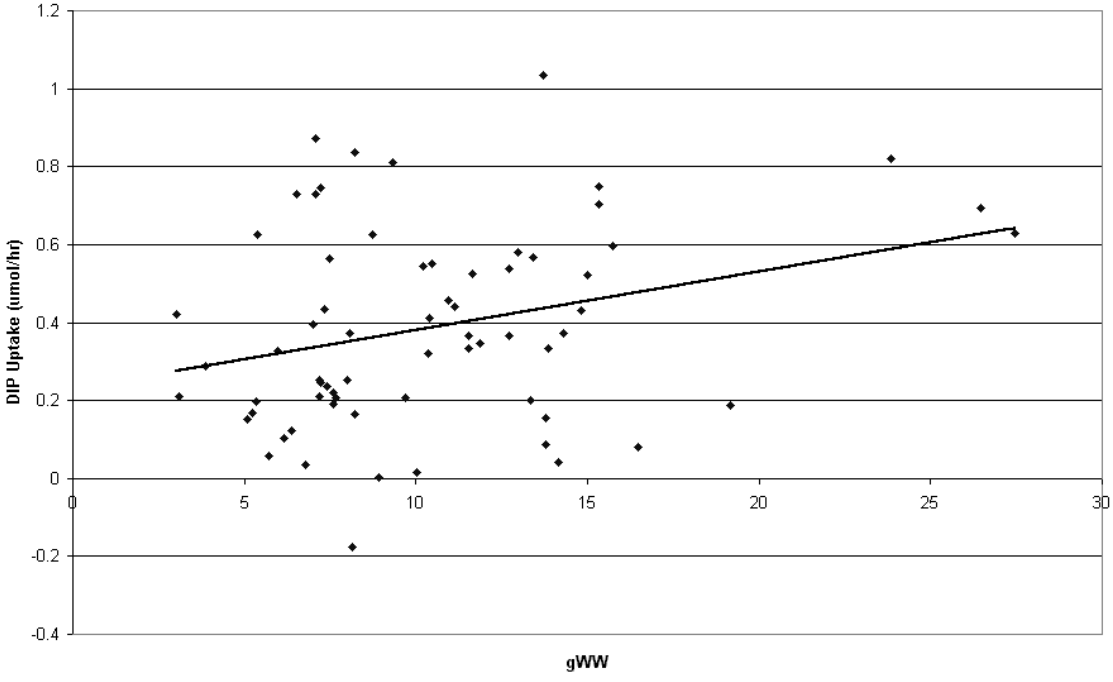
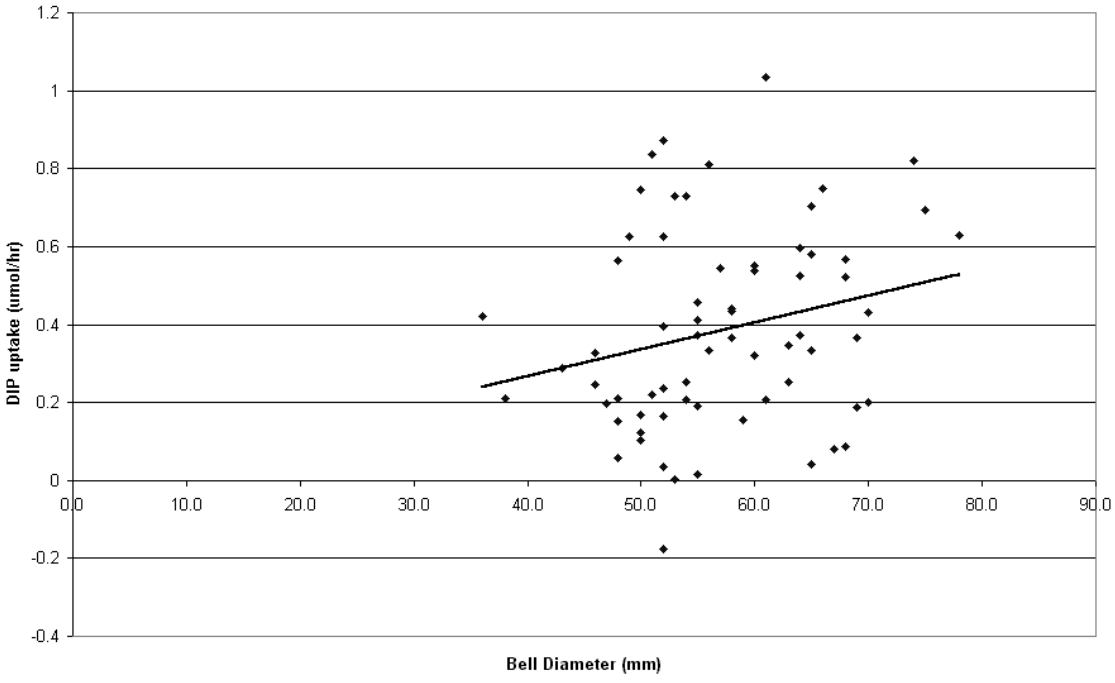



Figure 3.3B



# CHAPTER 4

# EXPOSURE OF CASSIOPEA XAMACHANA TO ELEVATED CONCENTRATIONS OF DIP: SUITABILITY AS A BIOINDICATOR

## Introduction

*Cassiopea xamachana* is a scyphozoan jellyfish common to mangrove habitats in the Caribbean Sea and Florida Keys. Members of this genus harbor the symbiotic dinoflagellates *Symbiodinium microadriaticum*, also known as zooxanthellae (Freudenthal, 1962). The zooxanthellae are contained in gastrodermal and amoeboid cells in the mesoglea of the medusa and its inverted benthic posture ensures exposure of these endosymbionts to sunlight (Smith, 1936; Drew, 1972). The zooxanthellae are important to the symbiosis because they translocate photosynthetically fixed carbon to their hosts (Muscatine and Hand, 1958; Fisher *et al*, 1985). Zooxanthellae isolated from their host exhibit uptake of dissolved nutrients in proportion to their photosynthetic activities, with uptake rates being higher in the light compared to the dark (Kelty and Lipschultz, 2002; Jackson and Yellowlees, 1990). Zooxanthellae within a host are also implicated in the uptake of nutrients, including dissolved ammonium and soluble reactive phosphorus, from seawater (Yonge and Nicholls, 1931; Muscatine, 1980; Pomeroy and Kuenzler, 1969).

Verde and McCloskey (1998) noted that symbiont relationships of attached benthic invertebrates with unialgal symbionts such as corals have been more widely

studied than those of motile invertebrates. However, preliminary studies on *Cassiopea frondosa* showed that these jellyfish exhibited similar phosphate uptake phenomena (Smith, 1936). More recently, Vodenichar (1991) documented the uptake of dissolved ammonium from seawater by *Cassiopea xamachana*, and results from Chapter 3 (pp. 16-26) indicate that this species also takes up soluble reactive phosphorus.

Kelty and Lipschultz (2002) report that nutrient history and uptake by zooxanthellae are tightly coupled. They found that zooxanthellae isolated from the host anemone *Aiptasia pallida* for 24 hours were able to take up phosphate 75 times faster per cell than freshly isolated zooxanthellae (<1hr). They also measured phosphate concentrations available to the zooxanthellae *in situ* higher than phosphate concentrations in filtered Bermudian seawater, and speculated that the more recently isolated zooxanthellae exhibited suppressed uptake rates probably related to higher phosphate concentrations within the host tissue (Kelty and Lipschultz, 2002). Deane and O'Brien (1981) also showed that very recently isolated zooxanthellae from the giant clam *Tridacna maxima* had significantly lower rates of phosphate uptake than those isolated from the host for more than 24 hours.

The purpose of this study was to examine the effects of elevated phosphate concentrations on the uptake rates of intact symbiotic jellyfish *Cassiopea xamachana*. Results may be useful in determining environmental nutrient history by examining uptake rates of *in situ Cassiopea xamachana*.

## Materials and Methods

**Animal collection:** *Cassiopea xamachana* specimens were collected from a near-shore mangrove lagoon on the Atlantic coast of Key Largo, FL near mile marker 99.5 on

June 22, 2001. The mangrove lagoon is ocean fed and has a series of man made navigable canals to allow ship passage. Ambient levels of $PO_4$ were typically less than 0.2 μmol/L. All specimens were transported to the Key Largo Marine Research Laboratory in seawater from the lagoon. Analyses and assays were performed within two hours of collection unless otherwise noted. An attempt was made to select individuals of the same size (45mm to 60mm).

**Biomass measurements:** Bell diameter was measured to the nearest millimeter using a ruler. Specimens were blotted dry once to remove excess water and then weighed on a Metler electronic scale to determine wet weight to the nearest milligram.

**Incubations in elevated phosphate concentrations**: Eighteen individuals were exposed in groups of three to different μM concentrations of dissolved $KH_2PO_4$ in Instant Ocean seawater (35ppt). Phosphate concentrations in the salt mix are known to be negligible (<0.03 μM as tested by AquaCraft) and were undetectable by our methods (<0.03 μM). The six predetermined concentrations were 0, 0.2, 0.5, 1.0, 2.0, and 20 μM dissolved inorganic phosphate (DIP). Specimens were left in their respective solution for one hour and then removed. All specimens were rinsed gently in filtered seawater to prevent phosphate carry-over from incubation solutions. Assays were then performed.

**Phosphate uptake assays:** The medusae were transferred to acid-washed glass Petri dishes of adequate size at the start of the experiment so that the bell of the animal would have room to extend. Each animal was placed in 200 ml of Instant Ocean seawater (35 ppt). Phosphate concentrations in the salt mix are known to be negligible (<0.03 μM as tested by AquaCraft) and were undetectable by our methods (<.03 μM). The experimental seawater was mixed to contain approximately 2 μmol/L phosphorus in

the form of dissolved $K_2HPO_4$ and three 50 ml samples of the starting concentration were taken in order to ascertain the exact initial concentration of the mixture. Once the animals were placed in their respective Petri dishes, duplicate 25 ml water samples were taken every ten minutes for thirty minutes giving 6 total samples for each medusa. All samples were placed in a –20°C freezer to prevent bacterial growth from impacting phosphate levels before phosphate concentrations were determined.

Less than 24 hours later, samples were thawed and prepared for DIP determinations using the addition of a molybdate compound and following the heteropoly blue formation method (Strickland and Parsons, 1968). Standards were also prepared at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 μM $KH_2PO_4$. A standard curve was constructed and the resulting regression was used to calculate DIP concentrations from absorbance readings. All absorbances were measured at a wavelength of 885 nm. The resulting concentration values were used to determine the rates of uptake in micromoles per hour with the following formula:

$$\frac{(\text{conc } t_x) - (\text{conc } t_{x+10})}{1{,}000 \text{ ml}} \times \frac{V(t_{x+10})}{10 \text{ min}} \times \frac{60 \text{ min}}{1 \text{ hr}}$$

where '*conc* $t_x$' is the concentration (μmol/L) at time '*x*' minutes (10,20, or 30) and '*V*' is volume (200, 150, or 50 ml).

## Results

Animals incubated at elevated phosphate concentrations exhibited diminished uptake rates (Figure 4.1). Specimens from the 20 μM incubation actually excreted phosphate during the course of the experiment, while those incubated at 2 μM showed no

active uptake or excretion. Animals incubated at less than 2 μM concentrations took up phosphate during the course of the assay.

Figure 4.2 illustrates the same concept with a different graphical presentation. A regression line has been fitted to the scatter diagram using the SAS package. The correlation coefficient ($R^2$) with n=18 shows $p>0.05$ significance level, indicating that the shape of the line is not significantly different from zero due to variability in the data. When an ANOVA test was chosen to further analyze the data, the uptake rates for both the 20 μM and 2 μM groups were found to be lower than all others using a least significant difference test at 95%.

## Discussion

The results of the experiment indicate that pre-exposure of *Cassiopea xamachana* medusae to elevated phosphate levels can inhibit and reduce the animal's uptake rates of dissolved phosphate. Environmental exposure to 20 μM and 2 μM phosphate concentrations for one hour produced a significant depression of the uptake capacity of the animals. Uptake of dissolved nitrogen and phosphorous has previously been attributed to the presence of zooxanthellae (Muscatine and Marian, 1982; Muscatine et al, 1979; Pomeroy and Kuenzler, 1969). Thus, the results of the current study agree with previous research, which shows that nutrient history influences zooxanthellae phosphate uptake rates (Deane and O'Brien, 1981; Kelty and Lipschultz 2001).

Implications of this study include the possibility of detecting and estimating environmental phosphate levels based on observed uptake rates of *Cassiopea xamachana* collected from the site. Ambient levels of phosphate in the Florida Keys are reported to be in the 0.02-0.25 μM range depending on exact location as recorded in Long Key

(Szmant and Forrester, 1996). Based on the current study, it would be difficult to detect phosphate concentrations in this range with *Cassiopea xamachana* if exposure was limited to one hour. However, it is our speculation that increasing the time of exposure could effectively increase the sensitivity of *Cassiopea xamachana* to dissolved inorganic phosphates.

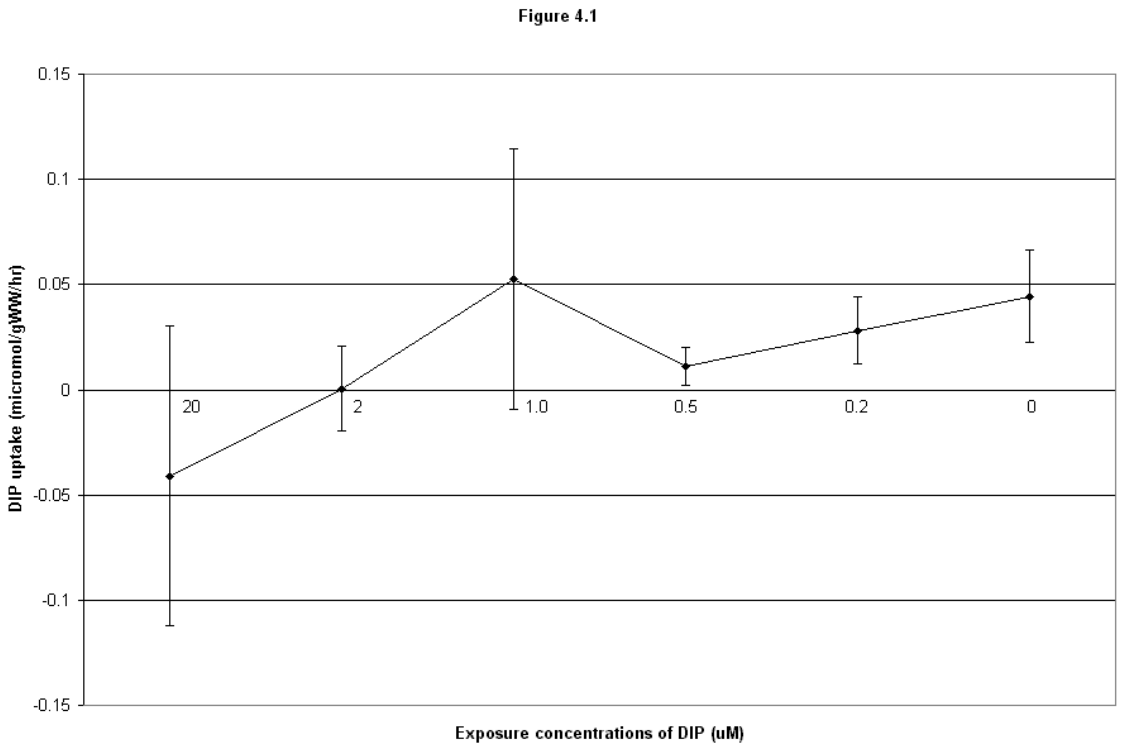



Figure 4.1. DIP uptake (μmol/gWW/hr) as a function of pre-exposure to different concentrations of DIP for one hour.

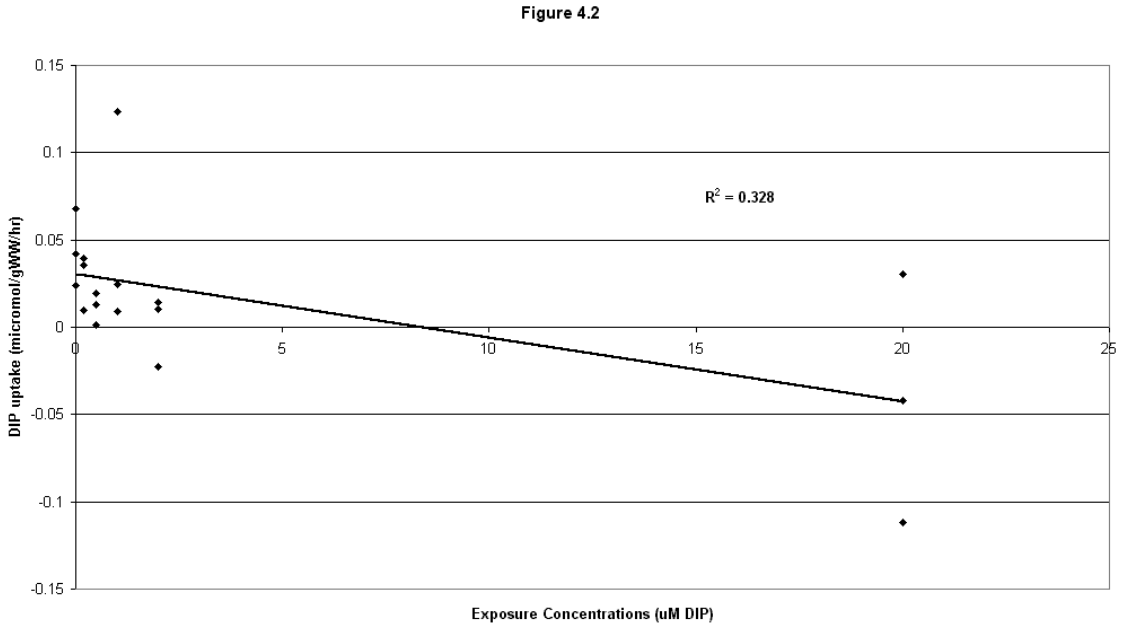



Figure 4.2. Regression of DIP uptake rates (μmol/gWW/hr) as a function of pre-exposure to different μM concentrations of DIP.

# CHAPTER 5

# DURATION OF PHOSPHATE UPTAKE SIGNAL IN *CASSIOPEA XAMACHANA*

## Introduction

Medusae of *Cassiopea xamachana* have been demonstrated to actively take up dissolved inorganic nutrients from surrounding seawater as described in the previous chapter and in other studies (Vodenichar, 1991; Smith, 1936). Preliminary work also indicates that the potential exists to use these animals as bioindicators of phosphates in seawater (see Chapter 4). The medusae develop a diminished or increased rate of phosphate uptake depending on time of exposure and the level of phosphates in the surrounding seawater. This signal allows the researcher to deduce ambient phosphate levels by measuring the uptake rates of exposed *Cassiopea xamachana*. The purpose of the current study is to determine the length of time *Cassiopea xamachana* will exhibit this uptake signal following removal from their *in situ* environment.

## Materials and Methods

**Animal collection:** *Cassiopea xamachana* specimens were collected from a near-shore mangrove lagoon on the Atlantic coast of Key Largo, FL near mile marker 99.5 on June 28, 2001. The mangrove lagoon is ocean fed and has a series of man made navigable canals to allow ship passage. Ambient levels of $PO_4$ were typically less than 0.2 μmol/L. The specimens were transported to Athens, GA in seawater from the lagoon.

Analyses and assays were performed the following day. An attempt was made to select individuals of the same size (4.0cm to 6.0cm).

**Biomass measurements:** Bell diameter was measured to the nearest millimeter using a ruler. Specimens were blotted dry once to remove excess water and then weighed on a Metler electronic scale to determine wet weight to the nearest milligram.

**Incubation in elevated phosphate**: Fifteen specimens of similar size (4.0 – 5.0cm) were selected and exposed to a 2 μM concentration of dissolved inorganic phosphate for one hour. After one hour, all animals were rinsed in artificial Instant Ocean seawater and then transferred to a holding tank of Instant Ocean artificial seawater (35ppt). Phosphate concentrations in the salt mix are known to be negligible (<0.03 μM as tested by AquaCraft) and were undetectable by our methods (<0.03 μM). Three individuals were assayed immediately upon removal from the 2 μM incubation tank. One hour after removal, three more animals were assayed. The remaining *Cassiopea xamachana* were assayed in groups of three exactly 5 hours, 24 hours, and 48 hours after removal from the 2 μM incubation tank.

**Phosphate uptake assays:** The medusae were transferred to acid-washed glass Petri dishes of adequate size at the start of the experiment so that the bell of the animal would have room to extend. Each animal was placed in 200 ml of Instant Ocean seawater (35 ppt). The experimental seawater was mixed to contain approximately 2μmol/L phosphorus in the form of dissolved $K_2HPO_4$ and three 50 ml samples of the starting concentration were taken in order to ascertain the exact initial concentration of the mixture. Once the animals were placed in their respective Petri dishes, duplicate 25 ml water samples were taken every ten minutes for thirty minutes giving 6 total samples

for each medusa. All samples were placed in a –20°C freezer to prevent bacterial growth from impacting phosphate levels before phosphate concentrations were determined.

Less than 24 hours later, samples were thawed and prepared for DIP determinations using the addition of a molybdate compound and following the heteropoly blue formation method (Strickland and Parsons, 1968). Standards were also prepared at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 μM $KH_2PO_4$. A standard curve was constructed and the resulting regression was used to calculate DIP concentrations from absorbance readings. All absorbances were measured at a wavelength of 885 nm. The resulting concentration values were used to determine the rates of uptake in micromoles per hour with the following formula:

$$\frac{(\text{conc } t_x) - (\text{conc } t_{x+10})}{1{,}000 \text{ ml}} \times \frac{V(t_{x+10})}{10 \text{ min}} \times \frac{60 \text{ min}}{1 \text{ hr}}$$

where '*conc* $t_x$' is the concentration (μmol/L) at time '*x*' minutes (10,20,30) and '*V*' is volume (200, 150, or 50 ml).

## Results

Medusae assayed immediately upon removal from the 2 μM phosphate incubation tank excreted phosphate (Figure 5.1). Those assayed one hour after removal still excreted phosphate, although at a lower rate. After 5 hours, the jellyfish had returned to actively taking up dissolved phosphates.

## Discussion

*Cassiopea xamachana* medusae exhibit diminished phosphate uptake capacity after one hour of exposure to 2 μM phosphate (See Chapter 4). High levels of ambient phosphate diminish the uptake capacity of the jellyfish. The results of the current study indicate that this diminishment is not permanent and is in fact short lived. Figure 5.1 indicates that some time between 1 and 5 hours after removal from a high phosphate exposure to phosphate-poor seawater, the jellyfish return to taking up phosphate instead of excreting it. It is also possible that this expressed uptake rate, or signal, will endure longer and not diminish if the animal is kept in the exposure water until the assays are performed.

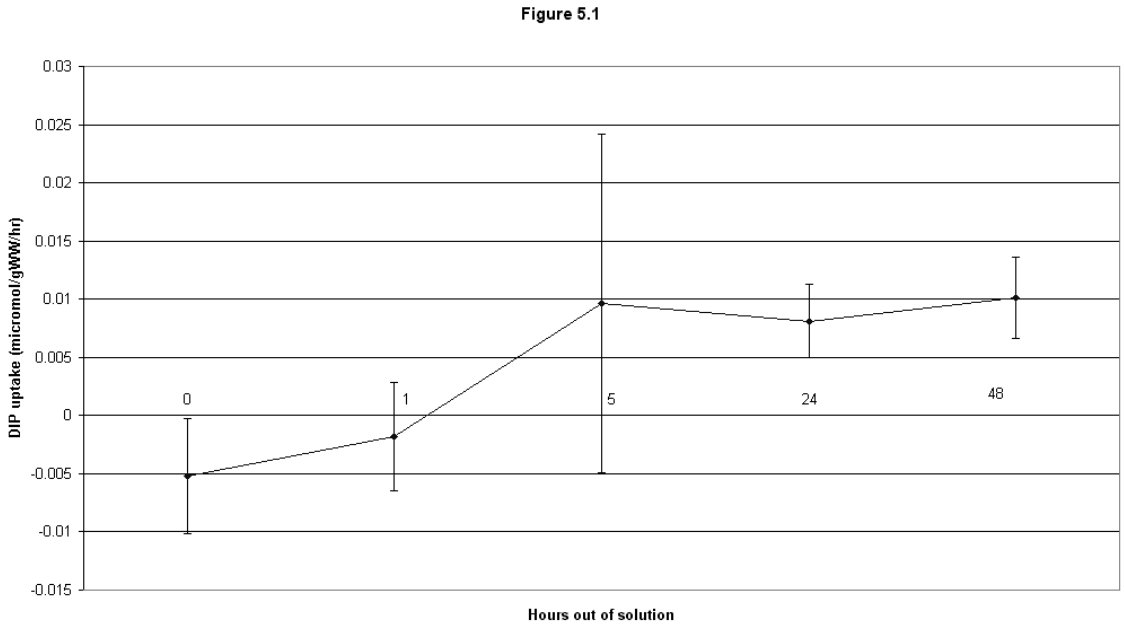



Figure 5.1. DIP uptake rates (µmol/gWW/hr) as time out of incubation tank increases.

# CHAPTER 6

# APPLICABILITY OF *CASSIOPEA XAMACHANA* AS A BIOINDICATOR OF PHOSPHATES: FIELD TRIALS

## Introduction

Many recent studies indicate that Florida's coral reefs are declining in coral cover and are in peril of further degradation and loss (Porter and Meier, 1992; Stahl, 1999). One of the most extensive ongoing reef health projects, the Coral Reef Monitoring Project (CRMP) reports that the percent of stony coral cover decreased roughly 40% in the upper and lower keys from 1996-1999, and as much as 25% in the middle keys (Porter *et al*, 2002). There is much contention over the specific causes for decline, but recent authors tend to agree that the interconnectedness of the Florida Bay and nearshore waters to the offshore reefs is a negative influence in the survival of reef-building corals (Porter *et al*, 1999; Cook *et al*, 2002). High turbidity, variable temperatures and salinity, as well as elevated nutrients combine with flow through Keys' Channels to negatively influence water quality, eventually impacting the offshore reefs (Szmant and Forrester, 1996; Porter *et al*, 1999). The degree of influence and flow to offshore reefs is debatable, and depends on season, wind, and current patterns. Additionally, Szmant and Forrester (1996) contend that elevated nutrient levels found on reefs are likely not a result of shore-based or Florida Bay influenced sources, but rather upwelling. Regardless, nutrients are still suspected of playing an important role in the decline of Florida's coral reefs and even

seagrass beds (Lapointe and Clark, 1992; Lapointe *et al*, 2002; Brand, 2002). In addition, the fact that a human pathogen has been identified as the cause of "white pox" disease in *Acropora palmata* corals suggests that pollutants from local sources impact nearshore reef corals (Patterson *et al*, 2002).

Nutrients are implicated both directly and indirectly in the decline of coral reefs. With increased oceanic nutrients, plankton biomass grows and the resulting increase in turbidity and decrease in light can limit coral productivity and survival (Hallock and Schlager, 1986). A change in reef community structure also occurs in areas of elevated nutrients. Macroalgae proliferate faster than slow-growing corals and they shade the corals while also reducing suitable substrate for larval settlement (Lewis, 1986; Hughes, 1994). A more direct result of increased nutrients is seen when phosphate levels are elevated. Phosphate acts as a poison in the crystal lattices of calcification (Simkiss, 1964). Studies illustrate that elevated phosphate can depress coral growth while increasing community production due to algal proliferation (Kinsey and Davies, 1979; Kinsey and Domm, 1974; Tomascik and Sander, 1987). These reasons are also believed to influence the biogeography and occurrence of coral reefs. Because of shore based inputs and runoffs, corals are typically found far from shore and in nutrient poor waters (Shinn *et al*, 1994). Drainage of the adjacent continental basins onto near-shore environments has often been suggested as the reason reefs such as the Great Barrier Reef flourish some 50-70 miles from the Australian shoreline.

Some authors have hypothesized that there are threshold levels of nutrients (1 μM dissolved inorganic nitrogen and 0.1 μM soluble reactive phosphorus) that, if exceeded, result in coral loss through direct and indirect actions (Bell, 1992; Lapointe, 1997).

While the specific threshold levels and the overriding generality of these proposed levels are disputed (Hughes *et al*, 1999), even these authors concede that elevated nutrients are detrimental to corals among reef communities. Nutrient levels in the Florida Keys have been on the rise in the past few decades and there is much debate over exact sources (Lapointe *et al*, 2002; Boyer and Jones, 2002). Some researchers contend that nutrients reaching the reefs are from anthropogenic inputs of sewage and phosphate mining on Florida and the Florida Keys (Lapointe *et al*, 1990; Lapointe *et al*, 1992; Brand, 2002), while others feel that natural causes like resuspension of nutrient-rich sediments or upwelling are the cause (Szmant and Forrester, 1996). It is clear that nutrient levels and their effects on Florida's coral reef communities warrant future monitoring at appropriate spatial and temporal scales.

Findings of several investigators suggest that phosphorus is often the limiting nutrient in nearshore areas in the Florida Keys. Lapointe (1989) compared C:N:P ratios in four different macroalgae in the Florida Bay and found that the ratios supported primary limitation by P rather than N. Cook *et al* (2002) report similar findings; this time on the actual symbiotic reef corals. Redfield ratios of sampled zooxanthellae from *Montastraea annularis* again support the theory that P is limiting in the surrounding waters (Cook *et al*, 2002). Miller *et al* (1999) discovered that there was no increase in macroalgae growth following experimental nitrogen and phosphorus enrichment for 41 days on Pickles Reef, Key Largo. They suspect that elevated nutrient levels on the surrounding reefs were already high enough to promote maximal macroalgae growth without enrichment. It is clear that nutrient levels and their effects on Florida's coral reef communities are an area that warrants future monitoring.

Previous experiments confirm that medusae of *Cassiopea xamachana* respond to elevated soluble reactive phosphates (SRP) in seawater (see Chapters 4 and 5). Following exposure to higher levels of SRP, the uptake rates of the animals decrease or the medusae actually excrete phosphate. This suggests *Cassiopea xamachana* may be a useful indicator of seawater quality in relation to elevated levels of phosphates. Bioindicators are commonly used in oceanographic work to aid researchers in investigating ecosystem health. For instance, chlorophyll *a* is used as a predictor of nutrient enrichment, alkaline phosphatase is used to determine P limitation, and seaweeds and seagrasses are assayed to determine nutrient enrichment and limitation in surrounding seawater (Harding and Perry, 1997; Lapointe, 1989; Lapointe *et al*, 1994). The use of a cnidarian-zooxanthellae symbiosis as a bioindicator of elevated nutrient levels has many added advantages over traditional water column nutrient measurements.

First of all, traditional approaches cannot account for transitory or episodic events. Secondly, elevated phosphates in seawater may be testable using current methods, but do not immediately identify the impact on the nutrient dynamics exhibited by cnidarian-zooxanthellae hosts such as corals. Also, bioindicators can reveal biological effects at exposure levels that may be below analytically detectable levels. Finally, using *Cassiopea xamachana* instead of a scleractinian coral has the added advantage of being easily moveable, and significantly less destructive to already jeopardized coral populations.

The purpose of this experiment is to examine the applicability of using medusae of *Cassiopea xamachana* as a bioindicator of ambient SRP levels on coral reef tracts.

Animals were placed in flow through chambers in an environment for a known time interval and phosphate uptake rates were determined.

## Materials and Methods

**Animal collection:** *Cassiopea xamachana* specimens were collected from a near-shore mangrove lagoon on the Atlantic coast of Key Largo, FL near mile marker 99.5 on June 12 and 13, 2001. The mangrove lagoon is ocean fed and has a series of man made navigable canals to allow ship passage. Ambient levels of $PO_4$ were typically less than 0.2 μmol/L. All specimens were transported to the Key Largo Marine Research Laboratory in seawater from the lagoon. Analyses and assays were performed within two hours of collection unless otherwise noted. An attempt was made to select individuals of the same size (4.5cm to 6.0cm).

**Transplanting of animals and sampling schedule**: Thirty animals were used in preparing a baseline for phosphate uptake comparison to transplanted animals. Twenty-seven specimens were selected for relocation to one of three different environmental areas. Nine animals were taken from the nearshore Atlantic coast and placed in Florida Bay approximately 10m from shore. Nine animals were taken to a patch reef approximately 3 miles from shore known as Admiral Reef. The last nine animals were placed 5 miles from shore on a fore reef called Little Grecian. The animals were kept in clear plastic chambers 1,500 $cm^3$ with numerous large holes drilled in the sides and a screened top to allow exchange of water and adequate flow-through. Animals were assayed to determine phosphate uptake rates after four days and again after a total of eight days had passed. At four days, the assays were performed in the boat within minutes of collection. This allowed us to quickly return the animals to their environment.

After eight days, the animals were collected and transported to Key Largo Marine Research Laboratory. Assays were then performed about 2 hours after collection.

**Biomass measurements:** Bell diameter was measured to the nearest millimeter using a ruler. Specimens were blotted dry once to remove excess water and then weighed on a Metler electronic scale to determine wet weight to the nearest milligram. At the end of the experiment, animals were diced and homogenized in Instant Ocean seawater (35 ppt) using a Virtis tissue grinder. A 1 mL aliquot was taken from the homogenate and preserved with 0.1mL formalin for later determination of zooxanthellae density. Using a Neubauer hemacytometer, average zooxanthellae densities for each specimen were calculated from 6 replicate counts per animal.

**Phosphate uptake assays:** The medusae were transferred to acid-washed glass Petri dishes of adequate size at the start of the experiment so that the bell of the animal would have room to extend. Each animal was placed in 200 ml of Instant Ocean seawater (35 ppt). Phosphate concentrations in the salt mix are known to be negligible (<0.03 μM as tested by AquaCraft) and were undetectable by our methods (<.03 μM). The experimental seawater was mixed to contain approximately 2 μmol/L phosphorus in the form of dissolved $K_2HPO_4$ and three 50 ml samples of the starting concentration were taken in order to ascertain the exact initial concentration of the mixture. Once the animals were placed in their respective Petri dishes, duplicate 25 ml water samples were taken every ten minutes for thirty minutes giving 6 total samples for each medusa. All samples were placed in a $-20^{o}C$ freezer to prevent bacterial growth from impacting phosphate levels before phosphate concentrations were determined.

Less than 24 hours later, samples were thawed and prepared for DIP determinations using the addition of a molybdate compound and following the heteropoly blue formation method (Strickland and Parsons, 1968). Standards were also prepared at concentrations of 0, 0.5, 1, 1.5, 2, and 2.5 μM $KH_2PO_4$. A standard curve was constructed and the resulting regression was used to calculate DIP concentrations from absorbance readings. All absorbances were measured at a wavelength of 885 nm. The resulting concentration values were used to determine the rates of uptake in micromoles per hour with the following formula:

$$\frac{(conc\ t_x) - (conc\ t_{x+10})}{1{,}000\ ml} \times \frac{V(t_{x+10})}{10\ min} \times \frac{60\ min}{1\ hr}$$

where '*conc* $t_x$' is the concentration (μmol/L) at time '*x*' minutes (10,20,30) and '*V*' is volume (200, 150, or 100 ml).

A SAS package was used to perform further statistical analysis of the data.

## Results

Uptake rates are plotted using the first 10 minutes of the assays. Figure 6.1 suggests there is a correlation between sites and uptake rates of *Cassiopea xamachana*.

**Little Grecian Reef**: Uptake rates were significantly higher ($p$=0.0014) after four days on the fore reef compared to the baseline uptake rates from animals freshly collected from the nearshore canal. However, there was no significant difference in uptake rates eight days after translocation to the fore reef compared to the initial uptake rates.

**Admiral Reef**: Uptake rates were significantly higher ($p$=0.006) after four days on the patch reef compared to the baseline uptake rates from animals freshly collected in

the nearshore canal. This trend was reversed after eight days on the patch reef, with uptake rates being significantly lower ($p$=0.0015) after eight days on Admiral Reef.

**Florida Bay**: There was no significant difference in uptake rates after either four or eight days incubation in nearshore Florida Bay when compared to freshly collected nearshore animals from the oceanside canal.

**Zooxanthellae Numbers**: Animals collected from Little Grecian after eight days had significantly lower zooxanthellae densities ($p$=0.00025) by about half than animals freshly collected from the nearshore canal. Similarly, animals collected from Admiral Reef after eight days had significantly lower zooxanthellae densities ($p$=0.00003) by about half than animals freshly collected from the nearshore canal. There was no significant difference in zooxanthellae densities between animals transplanted to Florida Bay and those freshly collected from the nearshore canal.

## Discussion

Healthy coral reefs are "uniquely adapted to low levels of nutrients in the water" (Stahl, 1999). Because they often occur far from shore, land-based influences such as freshwater runoff, sediments, and dissolved nutrients are minimized. Szmant and Forrester (1996) reciprocated this idea when they demonstrated that nutrient levels in Key Largo were highest in canals and decreased as samples were taken further from shore. Cook *et al* (2002) found that offshore corals transplanted to inshore reefs exhibited lower rates of calcification. They cite elevated nutrient levels, related to lower light penetration, inshore as a possible cause for this trend. Risk and Sammarco (1991) also report that skeletal density of hermatypic corals in the Great Barrier Reef decreased with increasing proximity to shore, possibly due to nutrient increases inshore.

The results of the current study demonstrate the efficacy of *Cassiopea xamachana* as a bioindicator of phosphates in seawater. The animals exhibit lower DIP uptake capacities in nearshore waters with higher ambient DIP. As the animals are moved offshore to coral reefs with lower DIP concentrations, the uptake rates increase. Soluble reactive phosphate levels were below detectable concentrations using our methods (< 0.03 μM) in both reef environments. Concentrations of DIP in the collection canal and in the nearshore Florida Bay were almost the same and often measured below 0.1 μM, yet Florida Bay tended to be slightly lower. Even when phosphate levels are undetectable by current analytical methods, the medusae were able to develop a characteristic uptake expression, or signal, that allows the researcher to know the phosphate levels of the ambient water.

There was no significant impact on DIP uptake rates when animals were removed from the nearshore oceanside collection canal and transplanted to the nearshore Florida Bay. This indicates that between the two environments, there is not a significant difference in ambient DIP concentrations.

When animals were relocated to the nutrient-poor reefs, there was a significant increase in uptake rates following four days of gestation. This indicates that phosphate levels were extremely reduced in the ambient seawater. Furthermore, it is interesting to note that the animals placed on Little Grecian fore reef exhibited higher uptake rates than those on Admiral patch reef (Figure 6.1). This could be attributed to the nutrient gradient that exists as distance from the shore increases (Cook et al, 2002).

Uptake rates after eight days on either of the two reefs were not higher than those from freshly collected nearshore jellyfish. A careful look at the zooxanthellae densities

reveals a possible reason. Both groups of animals had significantly reduced zooxanthellae densities after spending 8 days on their respective reefs (Little Grecian $p$=0.0025; Admiral $p$=0.00003). Environmental stress from eight days of reduced temperatures ($4^{o}$ C cooler), decreased light from depth, and heavy surge and wave activity likely caused a physiological change in the host-symbiont relationship. Loss of zooxanthellae has long been reported as a consequence of temperature change in corals (summarized by Buddemeier and Fautin, 1993). The resulting stress causes a response in the host-symbiont relationship whereby zooxanthellae numbers decrease. Because zooxanthellae are responsible for the uptake of nutrients (see Chapter 3 for discussion), such a large decrease in the densities of zooxanthellae leads to a decrease in phosphate uptake capacity. Animals relocated from the collection canal to the Florida Bay had no significant change in zooxanthellae numbers after eight days. This is presumably because environmental stimuli did not change between the two similar environments.

Four days incubation on the reefs provided useable results that correctly identified the decreased ambient DIP concentrations in the seawater. Complications arise when animals are left longer, because physiological changes resulting from environmental stress affect uptake rates. *Cassiopea xamachana* is a useful bioindicator that integrates environmental phosphate concentrations over time and provides a signature uptake rate during assay that corresponds to different elevations of DIP.

Figure 6.1

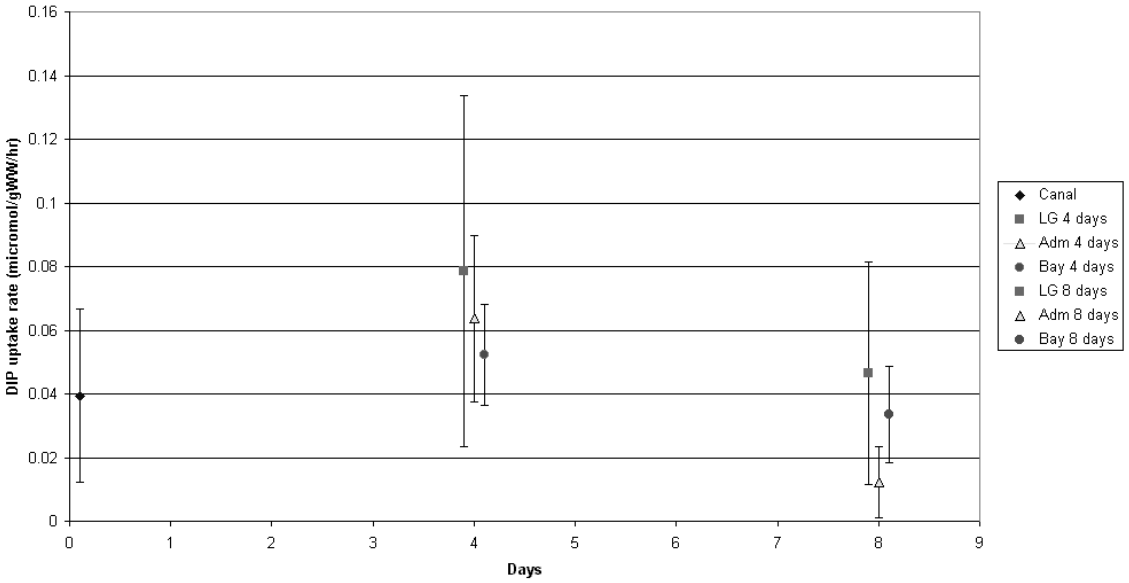



Figure 6.1. DIP uptake rates (umol/gWW/hr) as a function of environmental differences. The first plotted point represents 30 specimens collected from the nearshore canal and assayed to provide a baseline uptake rate. Subsequent points represent groups of 9 animals placed in one of three environments after 4 or 8 days of exposure in the environment. The three environments included nearshore Florida Bay (Bay), Little Grecian fore reef (LG), and Admiral patch reef (Adm).

# CHAPTER 7

## SUMMARY

The purpose of these experiments was to determine the applicability of *Cassiopea xamachana* as a bioindicator of dissolved inorganic phosphates (DIP) in seawater. Like many cnidarian-dinoflagellate symbioses, *Cassiopea* actively take up dissolved nutrients from surrounding seawater (Yonge and Nicholls, 1931; Pomeroy and Kuenzler, 1969; D'Elia, 1977; Muller-Parker *et al*, 1990). It has been suggested that nutrient history impacts the phosphate uptake rates of zooxanthellae (Deane and O'Brien, 1981; Kelty and Lipschultz, 2002). After determining the effect of animal size on phosphate uptake rates in the first chapters, the next experiment was designed to investigate the impact of nutrient history on the animals' uptake rates. The results show that highly elevated levels of DIP inhibit the uptake rates, with very high levels (20μM) causing the animals to release DIP.

The jellyfish change their uptake rates with increasing and decreasing levels of DIP, so field tests were used to determine their efficacy at indicating seawater DIP levels outside of a laboratory setting. After being exposed to different environmental gradients, the animals displayed uptake rates that varied based on distance from shore. Szmant and Forrester (1996) found that highest levels of phosphates occur nearshore in the Keys, and decline with increasing distance from shore, with lower levels existing on reefs. This pattern was apparent in the uptake rates of the experimental *Cassiopea xamachana*. It is

possible to use *Cassiopea xamachana* as an indicator of DIP levels in seawater by examining the uptake rates after exposing the animal to an environment.

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