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Nutrient Limitation of Bioluminescent Dinoflagellates in Mangrove Lagoon, Salt River Bay, St. Croix, USVI

Michelle Kimberlin
University of South Carolina - Columbia

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NUTRIENT LIMITATION OF BIOLUMINESCENT DINOFLAGELLATES IN MANGROVE
LAGOON, SALT RIVER BAY, ST. CROIX, USVI

by

Michelle Zimmerlin

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Accepted by:

James L. Pinckney, Director of Thesis

Claudia Benitez-Nelson, Reader

Ronald Benner, Reader

Lacy Ford, Vice Provost and Dean of Graduate Studies

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DEDICATION

I would like to thank my sister, Jessica, she has been extremely supportive of me throughout my life and especially in graduate school. She always provides encouragement and words of wisdom. My parents, for encouraging and supporting me throughout my life. My brother for being there. I would not have been able to get through graduate school without my friends and my fantastic roommate, Chelsea Wegner. Emily Osborne – our time together was too short, thank you for letting me hang out in your lab for far too long, your friendship means so much to me! Charley and Max, they have been the best!

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ABSTRACT

Bioluminescent bays resulting from dense concentrations of bioluminescent dinoflagellates are rare, with only 14 documented systems worldwide. One of these bays, Mangrove Lagoon, is located in Salt River Bay National Park in St. Croix, USVI. Very little is known about the environmental factors responsible for maintaining the high dinoflagellate densities in this lagoon. In order to assess the dynamics of the dinoflagellates, *in situ* nutrient addition bioassays were conducted to determine which nutrients regulate the phytoplankton community, which includes the bioluminescent dinoflagellate *Pyrodinium bahamense*. Bioassays were conducted in two different seasons, (January 2013 and May 2013) to examine seasonal responses to nutrient additions. The three hypotheses that were addressed in this project are (1) is the phytoplankton community is phosphorus-limited and (2) does mangrove leachate stimulates the growth of dinoflagellates relative to the other phytoplankton, and (3) does vitamin B₁₂ will stimulate the growth of the dinoflagellate *Pyrodinium bahamense* var. *bahamense* in comparison to the other phytoplankton present. Photopigment biomarkers were used to assess community composition and biomass in the bioassays as well as ambient waters. The phytoplankton communities, except the dinoflagellates were co-limited in nitrogen and phosphorus. The ANOVAs indicated nutrient addition effects on each individual group except dinoflagellates and non-peridinin dinoflagellates ($p < 0.01$). A posteriori multiple comparisons indicated a significant positive effect of N+P for diatoms and green algae and significantly higher responses to B₁₂ for cyanobacteria and

haptophytes. There was a significant negative effect of leaf additions for all groups except dinoflagellates, which showed a significant positive response to the 6 yellow and 3 orange leaf treatments ($p < 0.05$). Responses to mangrove leaf color and number did not show a discernible trend for the other algal groups other than the general negative response to all leaf additions. Dinoflagellates seem to be best suited for Mangrove Lagoon, as they are not nutrient limited, possibly because they are able to vertically migrate and the not significantly inhibited by mangrove organic matter.

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LIST OF ABBREVIATIONS

Biobay.....	Bioluminescent Bay
N.....	Nitrate (NO_3^+)
P.....	Phosphate (PO_4^-)
NP.....	Nitrate (NO_3^+) + Phosphate (PO_4^-)
C.....	Control
DOM.....	Dissolved Organic Matter
B_{12}	Vitamin B_{12} (Cobalamin)
BP.....	Bathypotometer
TN.....	Total nitrogen
TP.....	Total phosphorus
TOC.....	Total organic carbon

CHAPTER 1

MANGROVE LAGOON

Bioluminescent bays:

Bioluminescence is a naturally occurring chemical reaction, in which a burst of light is emitted from a living organism. There are only fourteen known bioluminescent bays, or biobays, worldwide. Biobays are a rare and beautiful example of a tropical inshore environment where phytoplankton and mangrove forests are the major primary producers (Burkholder & Burkholder 1958). Biobays require specific environmental parameters in order to thrive, such as prolonged water retention time, nutrient availability, shallow basin bathymetry, and limited tidal range (U.S Dept. of Interior 1968; Walker 1998). Biobays are surrounded by mangroves where specific biological interactions take place, such as the release of nutrients by bacteria that may be essential for bioluminescent dinoflagellates (O'Connell et al. 2007; Viruet 2007; U.S Dept. of Interior 1968). There are four to six biobays where bioluminescence has been observed year round, indicating that these bays must maintain a stable environment to support bioluminescent organisms throughout a full annual cycle.

Pyrodinium bahamense var. *bahamense* is the dinoflagellate species that is responsible for bioluminescence in Mangrove lagoon, St.Croix, USVI. In order for the dinoflagellate population to remain stable, the influx of water must exceed evaporation rates, which are typically high in the study region (Walker 1998). Mangrove lagoon is characterized by a small mouth, this is mechanism prevents loss of phytoplankton due to

tidal flushing. At Puerto Mosquito, a biobay in Puerto Rico, it was found that wind is the primary driver in supplying water into the bay which also helps to retain the population of dinoflagellates (Walker 1998). Walker (1998) also found that biobays are subject to large fluctuations in pH because they are small, enclosed, and experience heavy rainfall during wet seasons. The wide range in pH suggests that the phytoplankton community must be adaptable to a highly variable environment.

The distribution of *Pyrodinium* in a biobay is common to all bioluminescent bays in the Caribbean (Gasparich 2007). The highest concentration of *Pyrodinium* cells is usually found furthest from the mouth of the bay due to a more stable environment and lower flushing rates (Algeo 2008, Gasparich 2007). Fluctuations of pH, temperature, O₂, and nutrients are greater at the mouth of the bay due to higher flushing rates resulting in a less stable environment where dinoflagellates are typically found in lowest abundance.

In addition to the above described abiotic needs, some studies have suggested that dinoflagellates in biobays also have specific biotic requirements, such as the supply of mangrove dissolved organic matter and vitamin B₁₂(cobalamin) (Florentine 2007, Bernache-Baker 1989). Furthermore, Bernache-Baker (1989) suggested that B₁₂ is essential for *Pyrodinium* to produce bioluminescence. The production of B₁₂ results from bacteria processing nutrients from the leachate of mangrove leaves and producing the necessary nutrients for bioluminescence (Bernache-Baker 1989).

Biobays are fragile ecosystems that can and have been destroyed by anthropogenic actions or natural disasters, such as dredging and hurricanes (Walker 1998). Hurricanes have the ability to wipe out a biobay quickly, while anthropogenic activities can affect biobays on longer time-scales. Heavy rainfall, tidal fluctuations, and

changes in nutrients, temperature, salinity, wind, and sunlight can result in the destruction of a biobay (Walker 1998). In addition, increased boat traffic, increased sediment supply and decreased sunlight all have the potential to have negative impacts on bioluminescence in a biobay. For example, in a biobay in the Bahamas, the bioluminescent dinoflagellate population was not able to maintain a high enough concentration after the mouth of the bay was dredged, in order to allow for more boat traffic (Harvey 1952, U.S Dept. of Interior 1968). Evidence suggests that biobays rely on mangroves and other external sources to provide organic material to the bay (U.S Dept. of Interior 1968). If this balance is altered the system could be impaired and become unable to support the bioluminescent dinoflagellates. Changes in nutrient fluxes not only have the ability to destroy a biobay but can also adversely affect economic markets, such as recreation and tourism that depend on the nightly bioluminescence in biobays as an attraction.

An important yet unanswered ecological question is “why is *Pyrodinium bahamense* var. *bahamense* the dominant species present?”. Possible explanations include: 1) the tannins released from the mangroves inhibit the growth of other phytoplankton, 2) *Pyrodinium* produces saxitoxin that inhibits growth of other phytoplankton or reduces mortality due to grazing (Usup et al. 2012), 3) the large size and motility allows *Pyrodinium* to search for and store nutrients, resulting in them out-competing other phytoplankton (Usup et al. 2012), 4) biobays favor flagellated phytoplankton over diatoms due to the low vertical mixing and stability of the water mass, 5) Specific nutrient(s) released by red mangroves are essential for *Pyrodinium* growth and bioluminescence. The purpose of this study was to investigate the importance

of macronutrients, DOC, and vitamin B₁₂ on *Pyrodinium bahamense* var. *bahamense* growth and compare the results with the responses of the phytoplankton community in Mangrove Lagoon.

Bioluminescence:

Bioluminescence occurs in fourteen phyla, and is common in bacteria, fish, crustaceans, and cnidarians. Bioluminescence is present in the phylum Dinoflagellata, in about 81 species (Marcincko et al. 2013). The dinoflagellate bioluminescent reaction occurs in scintillons, organelles that are part of the vacuolar membrane (Lambert 2006). Protons are released into the scintillon from the vacuole and cause a drop in pH from 8 to 5.7, which facilitate the bioluminescence reaction (Lambert 2006, Smith et al. 2011,). The reaction is catalyzed by the enzyme luciferase, which oxidizes luciferin and results in light emission and the by-product of oxyluciferase (Lambert 2006; Pain 2008,). Bioluminescence is energetically expensive to dinoflagellates and estimated to consume about 255 Jmol⁻¹ per reaction (Rees et al. 1998). Although costly, dinoflagellates expend energy on bioluminescence at the expense of growth, leading researchers to believe the purpose of bioluminescence is necessary and beneficial for the dinoflagellate.

There are two main hypotheses explaining dinoflagellate bioluminescence. The ‘burglar alarm’ hypothesis suggests that when a zooplankter is close to phytoplankton, the phytoplankton will bioluminesce, alerting potential predators of the zooplankter. The predator will hopefully eat the zooplankter resulting in the dinoflagellate to escape predation. The second hypothesis suggests that light emission at night startles zooplankton, allowing the dinoflagellate to escape. Both these hypotheses are accepted

by the scientific community as possible explanations for why bioluminescence occurs, though the exact reason is unknown.

Dinoflagellates emit light in the range of 474-476 nm as a burst of light lasting on average 100 msec (Marcinko et al. 2013). The duration, wavelength, and intensity vary among species and depend on cell's history (Marcinko et al. 2013). Throughout the night, the stored luciferin is depleted and the light emitted will decrease as the night passes (Marcinko et al. 2013). In addition, the amount of irradiance from the previous day can affect the intensity and amount of bioluminescence the following night. Buskey et al. (1994) showed that cultured dinoflagellates held without food were unable to bioluminesce suggesting that the nutritional state of the cell may affect the ability to bioluminesce. In addition, dinoflagellates were shown to preferentially use energy to bioluminesce rather than to grow. Latz and Jeong (1996) found that *Pyrodinium.cf. divergens* turned to cannibalism when starved and suggests that dinoflagellates invest heavily in bioluminescence (Marcincko et al. 2013).

Bioluminescence can be quantified using a bathyphotometer (BP), which measures the amount of light emitted following a mechanical stimulus (Marcincko et al. 2013). The BP can provide estimates of the bioluminescence potential within a known volume of water. However, there are no standards for different types of BPs, and the lack of common units makes comparisons of different types of bioluminescence difficult (Marcincko et al. 2013). It is near impossible to compare new values to old values because of the lack of standardized units and different techniques for sampling. In the future, biobay measurements should be made and standardized in order to compare the

‘brightness’ of each bay throughout the year; and compare future values to current values. This would allow a way to quantify biobay variation or deterioration.

Pyrodinium bahamense var. *bahamense*:

Pyrodinium bahamense var. *bahamense* is a bioluminescent dinoflagellate ranging in size from 30 to 60 μm (Gasparich 2007) and has a generation time of 3-3.5 days (Seliger et al. 1970). *Pyrodinium bahamense* is relatively slow growing, and will be outcompeted by faster growing phytoplankton if the water residence time within a lagoon is short (Usup et al. 2013). However, the large size and mobility of *P. bahamense* may be advantageous when there is long water residence time, a stratified water column, and limited nutrient concentrations (Usup et al. 2012). This species is thought to be positively phototactic when in tropical sunlight and migrates to depths at night, allowing for nutrient uptake from the depths at night (Seliger et al. 1971). However, *Pyrodinium* that is present in the biobays is seen in high concentrations at the surfaces at night, as they bioluminesce. Soli (1966) found that *P. bahamense* migrated to the surface around 1800, however individuals could be found throughout the water column. Around midnight, the dinoflagellates started their descent to the depths by morning, and came up higher in the water column around 0900, and descend again by 1600, where they would ascend back up (Soli 1966), making two cycles in a 24 hour period. *Pyrodinium* has a ‘biological clock’ resulting in the inability to produce light during the night (Hasting, 1975). In order for *Pyrodinium* to produce light at night they require a significant amount of light exposure to produce energy during the day (Walker 1998). After a series of cloudy days the intensity of bioluminescence produced by dinoflagellates decreases (Walker 1998). Seixas (1988) reported that dinoflagellate population abundance declines when there is

high precipitation and high cloud coverage. This was also observed in the *Pyrodinium* population in Bahia Fosforescente, a bay located in Puerto Rico, that nearly disappeared after 25.4 cm of rain fell in less than a two week period (Walker 1998). Other observations in Florida have contradicted these findings, with an increase in *Pyrodinium* abundances following a rainfall event (Philips et al. 2006).

Pyrodinium is found in warm tropical water, warmer than 20°C, but blooms in waters warmer than 25°C (Philips et al. 2006). *Pyrodinium* has a wide salinity tolerance ranging from 14 to 46 ppt. *Pyrodinium* populations, in the Indian River Lagoon, Florida, were not found to have a significant correlation with phosphorus levels (ranging from 8 µg l⁻¹ to 100 µg l⁻¹) (Philips et al. 2006). However, blooming populations were found at phosphorus levels greater than 300µg l⁻¹ (Philips et al. 2006). *Pyrodinium* population concentrations, in addition, did not have a significant correlation with nitrogen levels with peak population concentrations at 600µg l⁻¹ (Philips et al. 2006).

A close relative of *Pyrodinium bahamense* var. *bahamense*, *Pyrodinium bahmanese* var. *compressum*, produces saxitoxin. This species is found in the Indo-Pacific, the Pacific coast of Central America, and recently in the Indian River Lagoon, Florida (Usup et al. 2012, Landsberg et al. 2006). *Pyrodinium bahmanese* var. *compressum* is a significant cause of seafood toxicity, paralytic shellfish poisoning (PSP), in Southeast Asia, Pacific and Atlantic Coasts along Central America (Usup et al. 2012). In 2006, *Pyrodinium bahamense* var. *bahamense* was reported to produce saxitoxin in the Indian River Lagoon, Florida. However, saxitoxin has not been reported in bioluminescent bays at a significant level. The production of PSP may be an allelopathic

mechanism by which *Pyrodinium* inhibits or slows the growth of competing phytoplankton species.

Mangroves:

Mangroves are extremely important ecosystems that protect coastlines, filter pollutants, and hinder excess sediment from entering the bay (Algeo 2008). Mangroves produce large amounts of organic matter (up to 2500 mg C m⁻² d⁻¹) which is an important source of nutrients for biobays (Gonneea et al. 2004). All biobays are surrounded by the mangrove species, *Rhizophora mangle*, also known as the red mangrove. Red mangroves appear to provide nutrients that are essential to the dinoflagellates (Trainer 2007, Bernache-Baker 1995, Philips et al. 2006); but it is unknown which nutrient or compound is mainly responsible. Mangroves are a significant source of dissolved organic matter (DOM), which can be used by not only by the phytoplankton present, but also bacteria. Bacteria absorb these materials and convert them into essential nutrients needed by *Pyrodinium*, such as vitamin B₁₂. Dinoflagellates must acquire vitamin B₁₂ and other nutrients from their environment as they cannot produce them on their own.

Florentine (2007), in an investigation of nutrient budgets for three tropical bays, observed plant material accumulation and decomposition differences between seagrass and mangroves, as a possible explanation for the differences in abundances of bioluminescent dinoflagellates. This study also analyzed the stable isotopes of the sediment from each bay to determine the respective contributions of seagrass and mangrove to organic matter. The biobay, Puerto Mosquito, Puerto Rico, was found to have the lowest carbon isotope ratios and TIC (total inorganic carbon) values suggesting

that, relative to the organic matter in the other bays, Puerto Mosquito is dominated by mangrove derived organic matter and the sediment is primarily from a terrestrial source (Florentine 2007). The other two bays are not considered biobays because the population concentration was not high enough to cause the same brightness as Puerto Mosquito. This study supports the hypothesis that organic matter from red mangrove trees may be necessary for *Pyrodinium* to thrive in a biobay.

Ramos et al. (2007) investigated mangrove leaf litter as a source of nitrogen and phosphorus, in a northern Brazil estuary (Ramos et al. 2007). Once leaves fall into the water, chemical and physical reactions occur changing the composition of the leaves. In the first 24 hours, $17 \pm 2\%$ of the initial dry mass of mangrove leaf litter was leached (Davis et al. 2003). Leaf litter may be a possible source of nitrogen, phosphorus, and dissolved organic matter for the phytoplankton community. In the first two days of the leaching experiments there was a general increase in TOC, TN, TP in the water surrounding the leaves (Davis et al. 2003). As the leaves fall off the trees into the water, there is an immediate chemical and physical reaction leading to an increase of nutrients in the water system. The more trees surrounding the bay the more nutrients are made available to be leached into the water. Mangrove leaves also leach a relatively large amount of dissolved organic matter, and are an extremely important source of carbon to aquatic ecosystems (Benner et al. 1990). Mangrove leaves are a source of labile organic matter and also a source of sugars, protein, polyphenols, and inorganic nutrients to the surrounding waters (Maie et al. 2008). Microbial action and the loss of water soluble compounds are primarily responsible for the decomposition of mangrove leaves (Benner

et al. 1990b). Mangrove leachate is quickly converted into biomass and moved into the food web (Benner et al. 1986).

Tannins are made by terrestrial plants as a defense mechanism against herbivores, and are leached from mangrove leaves; and they make water look brown in color and can affect the biogeochemistry in the marine environment by sequestering proteins. Tannins stay in the water for about a day before being transformed either biotically or abiotically (Maie et al. 2008). They are known to positively and negatively affect phytoplankton primary production but the response is species specific (Ake-Castillo & Vazquez 2008). Because tannins are water soluble and reactive, they can inhibit microbial activity and effect the N cycle (Maie et al. 2008). When tannins bind with proteins, they slowly degrade and release N; which may serve as a source of N in the ecosystem (Maie et al. 2008). They bind with metals and may precipitate out by forming aggregations. Tannins are also, highly reactive in water and transform rapidly, (Maie et al. 2008); and contribute to the DOC pool after considerable decomposition. They may be important in biobays as an inhibitor to other phytoplankton groups. Mangroves, which are known producers of tannins, may be responsible for inhibiting the growth of other phytoplankton in bioluminescent bays, allowing for dinoflagellates to maintain a sustainable biomass.

Mangroves go through a series of predictable color changes. While attached, the leaves are green, then turn yellow and fall, changing from yellow to orange. Yellow leaves collected from the trees were found to have C:H:O:N of 82:10:61:1 while orange leaves collected from the water were found to have C:H:O:N of 94:12:66:1 (Benner et al. 1990). The various colors leach different nutrients into the water and one color may be

more important to biobays. The color change in the leaves may be linked to a ‘brighter’ period in the biobay.

Nutrients in a tropical system:

Dinoflagellates and other phytoplankton in marine systems are usually limited by sunlight or, more commonly, nutrients. Nitrogen and phosphorus are the most common limiting macronutrients for phytoplankton. Phosphorus is usually thought to be the limiting nutrient in tropical, carbonate rich waters. Sediments in the tropics commonly act as a P sink because phosphorus binds to calcium carbonate forming a more stable form called apatite, making it less bioavailable to organisms (Pain 2008). Porewater that is undersaturated with calcite or aragonite would promote phosphorus dissolution, making the sediment a source of phosphorus (Pain 2008). Commonly, phytoplankton are found to have the nutrient ratio of C:N:P of 106:16:1. In environments where nitrogen is limiting the N:P ratio is <16, while phosphorus limited environments have a ratio greater than 16. However, stoichiometric bases for nutrient limitation are frequently flawed and do not predict nutrient limitation.

HYPOTHESES AND OBJECTIVES:

The aim of this study was to assess the dynamics of dinoflagellates using in situ nutrient addition bioassays to determine which nutrients regulate the phytoplankton community including the bioluminescent dinoflagellate *Pyrodinium bahamense* in a biobay in St. Croix, USVI. This research provides insights into the primary nutrients regulating phytoplankton community structure including dinoflagellate abundance and satisfies the need for baseline data prior to planned changes in the immediate watershed.

A goal of this project was to provide insights into the effect of excess nutrients on the phytoplankton community. In addition, this research will help support or disprove the suggested relationship between vitamin B₁₂ and organic matter from red mangroves to the bioluminescent dinoflagellates, *Pyrodinium*.

The primary hypothesis was that dinoflagellate growth and abundance was phosphorus-limited in the lagoon, because phosphorus is often limiting in the tropics, and in another biobays. This hypothesis was tested using nutrient addition bioassays with different treatments: N, P, NP, and a control. The second hypothesis was that nutrients leached from degrading mangrove leaves stimulate the growth of dinoflagellates relative to other phytoplankton groups. This was tested using bioassays with additions of red mangrove (*Rhizophora mangle*) leaves, from the surrounding area in different concentrations. The third hypothesis was that vitamin B₁₂, cobalamin, will stimulate growth of the bioluminescent dinoflagellates in comparison to rest of the present phytoplankton community. B₁₂ addition bioassays were used to test this hypothesis. The main objectives were to determine whether nitrogen or phosphorus regulate phytoplankton community in the lagoon, to determine if mangrove leaves stimulate growth of dinoflagellates, and what is the effect of B₁₂ on the phytoplankton community composition.

METHODS AND MATERIALS:

Study site:

St. Croix is the largest of the U.S. Virgin Islands, located 151 km southeast of Puerto Rico. It is 39 km long and 9 km wide, a total of 207 sq. km. (Fig. 1) The island is

composed primarily of cretaceous volcanic sedimentary rocks and carbonate sediments (Hubbard et al. 2008). It lies on the Caribbean Plate, close to the fault that separates it from the North American Plate (Hubbard et al. 2008). The dry season is from February to April. St. Croix receives 35-45 inches of rain annually during the wet season from August to November, occurring mostly in short but intense thunderstorms. The strongest winds occur during December through February. Tropical storms and hurricanes occur between June and November, with peak abundance in August and September. Intense thunderstorms or hurricanes cause flash flooding and result in a large freshwater discharge, which temporarily reduces salinity and increases turbidity in the near-shore environment.

Salt River Bay is a 4.10 km² National Park created in 1992 (Fig. 1) and has many different types of habitats, including mangroves, seagrass beds, reefs, and submarine canyons. Mangrove Lagoon is a small (250 m x 130 m), shallow (<4 m) human-made embayment on the east side of Salt River Bay National Park. The bottom sediments are a mixture of calcium carbonate sediment, sand and fine silt (Reidhaar, personal communication). The waters of Mangrove Lagoon are an ecotourism feature for St. Croix therefore there is local interest for a more complete understanding of the bioluminescent *Pyrodinium bahamense* var. *bahamense* in the lagoon.

Experiment 1: To determine the limiting nutrient for the phytoplankton community and the effect of eutrophication on the phytoplankton community.

Water from the upper 1-2m of the water column was collected from the lagoon using an integrated water sampler and put into 1L Nalgene clear polycarbonate bottles

Bioassay bottles were washed with 5% hydrochloric acid, and washed three times with lagoon water. Pre-mixed nutrients were added to the respective treatments of 1) NO₃; 2) PO₄-3; 3) NO₃ + PO₄-3; and 4) Control (nothing added). In the January experiment, the final concentrations were 20 μM, and 10 μM, N and P, respectively (Table 1). In the May experiment, the final concentrations were 40 μM and 20 μM, N and P, respectively. There were four replicates of each treatment. In May, an additional treatment for B₁₂ was added to the bioassay at a concentration of 0.1 μM, with 4 replicates. The bottles were put into mesh bag lined with a fiberglass screen in order to reduce ambient irradiance by ~40%. For the May incubations, a floating corral covered with 2 layers of fiberglass screen was used for the incubations. Figure 2 shows the corral. The corral was placed in the middle of the lagoon to minimize shading from shoreline vegetation. The bottles were incubated for a total of 48 hours, and subsampled at 24 hours. Water from the bottles was filtered onto glass fiber filters (Whatman gf/f, 25 mm dia.) using a gentle vacuum. The filters were put into labeled centrifuge tubes, and kept in the dark at -80° C and shipped to the USC lab in a Dewar flask cooled with liquid N₂.

Experiment 2: To determine if mangrove leaves stimulate the growth of dinoflagellates

Water was collected and dispensed into bottles as described above. Fresh leaves were collected from mangrove trees surrounding the lagoon and placed into bioassay bottles 48hrs. The treatments were as followed: 1) 3 yellow leaves; 2) 3 orange leaves; 3) 6 yellow leaves; 4) 6 orange leaves and 5) control (nothing was added). The color variation is indicative of nutrient levels, age, and what will be leached. There were 3 replicates of each treatment. For the January experiment, bottles were put into the mesh

bag that was lined with fiberglass to reduce ambient irradiance by ~40%, the bags were placed by the side of the lagoon. May incubations were conducted using the floating corral. Samples were filtered and stored as described above.

The water used in the bioassays was taken from a depth of 1-2m down in the back of Mangrove Lagoon, as it was predetermined this was the area that had the highest intensity of bioluminescence at night. Additional water was taken from the surface and bottom waters during the night and day to determine biomass distributions. In addition, water was sampled in various locations map the spatial distribution of phytoplankton and dinoflagellate biomass in Mangrove Lagoon.

Laboratory Methodology:

Data Analysis:

All samples for nutrients analysis were taken initially and for each treatment at hour 24 and 48. Water samples were shipped on dry ice to the Hollings Marine Laboratory. DOC was measured with a Shimadzu TOC-V analyzer with an attached ASI-V autosampler. The samples were acidified with HCl to a pH <2 prior to analysis. Nitrate and phosphate was measured by a Lachat Series 8000 nutrient analyzer.

Photopigments were analyzed following the format from Pinckney et al. (1996) using a Shimadzu High Performance Liquid Chromatography (HPLC). Total chlorophyll a was used as an indicator for phytoplankton biomass. Biomarker pigments were used to determine the community composition using ChemTax, a factor analysis program to determine relative phytoplankton group abundance using characteristic pigment ratios

(Mackey et al. 1997, Pinckney et al. 2001, Wright & Jeffery 2006). Identifying pigments with their corresponding phytoplankton groups are shown in table 2.

The nutrient addition bioassay results were analyzed using a randomized complete block design two-factor MANOVA with month as the blocking factor (January, May) and nutrient treatment as the second factor (control, N, P, N+P, B₁₂). Algal group responses to the mangrove leaf addition bioassays were analyzed using a randomized complete block design two-factor MANOVA with month as the blocking factor (January, May) and leaf treatment as the second factor (control, 3 orange, 6 orange, 3 yellow, and 6 yellow leaves).

RESULTS:

In January, the lagoon water consisted of: 64% diatoms and chrysophytes, 18% cyanobacteria, and 8% dinoflagellates, but in May there was a shift to 46% diatoms and chrysophytes, 48% cyanobacteria, and a decrease to 4% dinoflagellates (table 3). Phytoplankton community composition in the surface and bottom waters was similar in May.

Nutrient Addition Bioassay:

The nutrient addition bioassay results were analyzed using a randomized complete block design two-factor MANOVA with month as the blocking factor (January, May) and nutrient treatment as the second factor (control, N, P, N+P, B₁₂). The multivariate tests indicated a significant nutrient effect (Pillai's trace = 1.114, $F = 3.309$, $p < 0.001$) and block effect (Pillai's trace = 0.811, $F = 34.98$, $p < 0.001$). Subsequent univariate ANOVAs indicated nutrient addition effects on each individual group except

dinoflagellates and non-peridinin dinoflagellates ($p < 0.01$). A posteriori multiple comparisons indicated a significant positive effect of N+P for diatoms and green algae and significantly higher responses to B₁₂ for cyanobacteria and haptophytes. In the B₁₂ addition treatments, cyanobacteria increased from 0.63 ± 0.08 to $3.48 \pm 2.42 \mu\text{g/l}$, while diatoms and chrysophytes increased from 0.06 ± 0.08 to $3.34 \pm 2.11 \mu\text{g/l}$. Dinoflagellate were not as impacted, they increased from 0.046 ± 0.011 to $0.124 \pm 0.233 \mu\text{g/l}$. Cryptophytes did not show a clear response to any of the treatments. Table 4 shows the results of the nutrient addition bioassays. Figure 3 shows the chl a variation due to nutrient addition in January and May, and figure 4 shows the dinoflagellate variation due to nutrient addition in January and May.

Mangrove Leaf Bioassays:

DOC concentration increased from C, Y3, Y6, O3 to O6 (Table 5) and more ambient DOC in the lagoon in May than in January, $2212.98 \mu\text{M C}$ and $79.19 \pm 43 \mu\text{M C}$, respectively. Leaves of the same color also leached more DOC in May than January (Table 5). The DOC values were significantly different ($F= 5.029$, $p<0.001$) from January to May. Chl a decreased in concentration with increasing number of leaves added, and as the DOC increased (fig. 5). The RCB 2 factor ANOVA indicated that treatment had a significant effect on chl a values ($F_{5, 58}=17.219$, $p < 0.001$) and there was a significant block effect (Month, $F_{1, 58}=11.152$, $p<0.001$).

Algal group responses to the mangrove leaf addition bioassays were analyzed using a randomized complete block design two-factor MANOVA with month as the blocking factor (January, May) and leaf treatment as the second factor (control, 3 orange,

6 orange, 3 yellow, and 6 yellow leaves). The multivariate tests indicated a significant leaf addition effect (Pillai's trace = 1.971, $F = 5.97$, $p < 0.001$) and block effect (Pillai's trace = 0.831, $F = 28.15$, $p < 0.001$). Subsequent univariate ANOVAs indicated leaf addition effects on each individual group except non-peridinin dinoflagellates ($p < 0.01$). A posteriori multiple comparisons indicated a significant negative effect of leaf additions for all groups except dinoflagellates, which showed a significant positive response to the 6 yellow and 3 orange leaf treatments ($p < 0.05$). Responses to mangrove leaf color and number did not show a discernible trend for the other algal groups other than the general negative response to all leaf additions. The results of the leaf addition bioassay are shown in table 6. Chl a variation from leaf additions is shown in fig 6, and dinoflagellate variation is shown in figure 7.

DISCUSSION:

In order to determine which nutrient is limiting growth of the dinoflagellate population in Mangrove Lagoon, bioassays were performed in January 2013 and May 2013. It is concluded that the dinoflagellates in Mangrove Lagoon are not nutrient limited as they showed no significant change between treatments. Interestingly, most other phytoplankton groups showed an increase in abundance in the N+P treatments only, suggesting a co-limitation. This suggests that the dinoflagellates are best suited for the nutrient levels in the lagoon, while the other present phytoplankton are co-limited for nitrogen and phosphorus. Dinoflagellates did not appear to be the most abundant phytoplankton species present, but seems to thrive in the environment. This population is found near the bottom of the water column during the day (0900- 1100h), possibly to

avoid photoinhibition, as the sun is very intense in the tropics. Because dinoflagellates are able to use their flagella to migrate vertically, they have the ability to find nutrients at the bottom of the water column, while other phytoplankton are immobile. The dinoflagellates at this location maybe lying on the top of the sediment absorbing nutrients and are still able to photosynthesize as light is penetrating throughout the water column. At night (2000- 2200 h), the species is found throughout the water column and near the surface where the bioluminescence can be seen by kayakers.

Chl a, an indicator of biomass, also showed nitrogen and phosphorus co-limitation. There was more biomass in May than January, $4.957 \pm 0.200 \mu\text{g/l}$ and $1.385 \pm 0.141 \mu\text{g/l}$, respectively. Possibly because of an increase in nutrients, an increase in rain fall and more hours of sunlight, there was also a shift in population. In January, the lagoon consisted of 64% diatoms and chrysophytes, 18% cyanobacteria, and 8% dinoflagellates; while in May there was a shift of cyanobacteria to 48%, 46% diatoms and chrysophytes, and 4% dinoflagellates. The bay was also less turbid in May than January for allowing for deeper light penetration, the increase of biomass in May could be due to the increase in water clarity as it creates a more stable environment for the phytoplankton. There was little variation between the control, nitrate and phosphate treatments. However, in the NP treatment there was a significant increase in biomass, suggesting the co-limitation. There was also an increase in biomass in the B₁₂ treatment, however, there was not an increase in dinoflagellate abundance. Cyanobacteria and diatoms/chrysophytes showed the biggest increase of abundance due to B₁₂ addition.

Selinger et al. (1975) stated that *Pyrodinium bahamense* var. *bahamense* are positively phototactic in the tropical sunlight, meaning that it swims towards the depths at

night, and comes to the surface waters during daylight. However, this study found more peridinin, the biomarker for dinoflagellates, at the depths during daylight and spread throughout the water column at night. This study supports the findings of Soli (1966), who saw a ‘double’ vertical cycle. The dinoflagellates may swim towards the depths during daylight in order to avoid photoinhibition and gather nutrients.

The purpose of this study was to determine what mechanism maintains dinoflagellate populations in a biobay. Five mechanisms were suggested: 1) the tannins released from the mangroves inhibit the growth of other phytoplankton, 2) *Pyrodinium* produces paralytic shellfish poisoning (PSP) that inhibits growth of other phytoplankton or reduces mortality due to grazing (Usup et al. 2012), 3) the large size and motility of *Pyrodinium* allows it to search for and store nutrients (Usup et al. 2012), 4) biobays favor flagellated phytoplankton over diatoms due to the nature of the water mass, 5) there is a specific nutrient that is released by the red mangrove trees that is essential for *Pyrodinium*. Not all of these hypothesis were tested in this study, but important pieces of the complex puzzle can be added. Tannins were not directly tested in this study, however, there is a connection between the mangroves and the biobays, as every biobay is surrounded by red mangroves. DOC could be the important factor that allows for dinoflagellates to thrive in this environment, as all phytoplankton groups showed a decrease in biomass, suggesting that DOC may be inhibiting their growth; while dinoflagellates increased or stayed relatively constant with high amounts of DOC. Or the tannins leached from the leaves could be impacting the phytoplankton community composition, the exact mechanism is still unknown. The mangrove leaves could be leaching other nutrients that are essential to the bioluminescent dinoflagellates, however

those nutrients were not specifically studied. PSP has never been reported in the area, and was not tested in this study. The 3rd mechanism, the large size and motility allows *Pyrodinium* to search for and store nutrients could easily be occurring in the lagoon, as they did not show a response to the excess nutrients present. The dinoflagellates have the ability to vertically migrate and search for nutrients elsewhere in the water column such as on the sediment surface. The dinoflagellates are still able to photosynthesize on the bottom as the light was still able to penetrate to the bottom, allowing for nutrient uptake from the sediment. In the past B₁₂ has been thought to be essential for bioluminescent dinoflagellates, may very well be true, but further research needs to be done to understand the complex relationship.

This research is part of a larger research project ongoing in Mangrove Lagoon. The project is attempting to determine what are the biotic and abiotic factors controlling or affecting this biobay. With a proposed research facility to be built nearby the site, it is imperative to determine what are the possible effects of the new facility would have on the bay. Having a facility next to the biobay could do more harm than good; there would be an increase in boat traffic, possible destruction of mangrove trees, an increase in sediment and nutrient runoff in to the biobay. In addition, there would be an increase in light population which would decrease the relative brightness of the biobay; this could negatively impact the kayaking ecotourism groups. With the many possible negative impacts on the biobay the risks are too great to construct a new research lab in close proximity to the biobay and a new site should be chosen.

Overall, it seems that *Pyrodinium* is best suited for the environment. The species does not seem to be stressed for nutrients; while the other phytoplankton were stressed

for both nitrogen and phosphorus. It seems while DOC inhibits the other phytoplankton present, it does not seem to significantly affect dinoflagellates. *Pyrodinium* is mostly found at the bottom of the water column during the day, and spread throughout during the night. In the future, more bioassays should be performed in the middle of the bay where there would be little chance of the phytoplankton being light limited. Future studies could look at other nutrients, such as ammonia, and other vitamins that maybe necessary for bioluminescence. In the future, collecting water samples from outside the bay to compare nutrient values to inside the bay would be interesting to see if there were any significant differences. In the future, using a BP to obtain 'brightness' levels would be interesting, this value could be used to compare to other biobays found in the area, and to compare to future values found at Mangrove Lagoon. Future studies could compare bioassays done in Mangrove Lagoon to Altoona Lagoon, another biobay in St. Croix.



Figure 1.1. Map of Salt River Bay. Mangrove lagoon can be found in the upper right of the map. C is the site of water collection for the bioassays. Water was collect 2m down in the water column. The yellow star on the subset map of St. Croix shows the location of Salt River Bay in St. Croix, USVI. Google Images 2013

Table 1.1. Nutrient values ($\mu\text{M/l}$) from the nutrient addition experiments, both January and May. SD values were not calculated in May as only one water sample was analyzed.

Nutrient Addition Experiment Values			
January Nutrient Values			
Time/Treatment	N+N $\mu\text{M/L}$	PO4 $\mu\text{M/L}$	N:P
Ambient	2.31 \pm 0.25	0.50 \pm 0.04	5:01
Time 48 N	17.48 \pm 3.99	0.35 \pm 0.03	49:1
Time 48 P	1.54 \pm 0.60	6.86 \pm 1.71	1:04
Time 48 N+P	15.70 \pm 4.64	6.64 \pm 1.16	2:01
Time 48 C	1.50 \pm 0.27	0.33 \pm 0.05	4:01
May Nutrient Values			
Ambient	1.1	0.5	2:01
Time 48 N	39.7	0.4	99:1
Time 48 P	1.3	16	1:12
Time 48 N+P	24.8	17.5	1:01
Time 48 C	0.7	0.5	1:01
Time 48 B ₁₂	0.3	0.4	1:01



Figure 1.2. Floating Corral located in the middle of the lagoon.

Table 1.2. Identifying biomarker pigments with corresponding phytoplankton groups

Group	Identifying Pigment
Diatoms	Fucoxanthin
Dinoflagellates	Peridinin
Prymnesisophytes	19'-HF
Prochlorophytes	DV Chl a and DV chl b
Chlorophytes	Lutein
Cryptophytes	Alloxanthin
Chrysophytes	19'-BF
Prasinophytes	Prasinoxanthin
Cyanobacteria	Zeaxanthin

Table 1. 3. Ambient phytoplankton percentage of community composition in January and May. Also shown is the phytoplankton percentage community composition in the surface and bottom waters in May.

	DiatomsChryso	Cyanobacteria	Dinoflagellates	Cryptophytes	Haptophytes	Eugleno & Chloro & Prasino	NonPeridDinos
January	64.28 ± 6.03%	18.20 ± 2.12%	8.12 ± 3.56%	6.51 ± 0.70%	0.71 ± 0.50%	2.12 ± 0.41%	0.05 ± 0.03%
May	45.56 ± 3.06%	48.76 ± 3.55%	3.56 ± 0.68%	0.05 ± 0.02%	1.44 ± 0.10%	0.57 ± 0.28%	0.06 ± 0.05%
May- Top	48.27 ± 0.62%	42.93 ± 0.50%	1.73 ± 0.26%	4.85 ± 0.21%	1.70 ± 0.16%	0.45 ± 0.02%	0.06 ± 0.00%
May- Bottom	44.26 ± 1.98%	47.43 ± 1.11%	1.01 ± 0.18%	5.65 ± 0.02%	1.07 ± 1.06 %	0.53 ± 0.02%	0.06 ± 0.00%

Table 1.4. Nutrient addition bioassay phytoplankton biomass results.
Phytoplankton biomass ($\mu\text{g/l}$), in January and May.

January	Cyanobacteria	EuglenoChloroPrasino	Dinoflagellates	Haptophytes	Cryptophytes	DiatomsChryso	NonPeridDinos
Initial	0.16 \pm 0.07	0.02 \pm 0.01	0.07 \pm 0.04	0.01 \pm 0.01	0.06 \pm 0.02	0.58 \pm 0.16	0.00 \pm 0.00
Control	0.23 \pm 0.15	0.02 \pm 0.01	0.16 \pm 0.11	0.02 \pm 0.02	0.12 \pm 0.05	1.41 \pm 0.53	0.00 \pm 0.00
Nitrate	0.25 \pm 0.12	0.02 \pm 0.00	0.16 \pm 0.04	0.03 \pm 0.01	0.08 \pm 0.04	1.45 \pm 0.24	0.00 \pm 0.00
Phosphate	0.19 \pm 0.05	0.02 \pm 0.01	0.19 \pm 0.06	0.02 \pm 0.01	0.12 \pm 0.03	1.22 \pm 0.20	0.00 \pm 0.00
N+P	0.26 \pm 0.12	0.03 \pm 0.01	0.26 \pm 0.05	0.04 \pm 0.02	0.19 \pm 0.08	1.91 \pm 0.66	0.00 \pm 0.00
<hr/>							
May	Cyanobacteria	EuglenoChloroPrasino	Dinoflagellates	Haptophytes	Cryptophytes	DiatomsChryso	NonPeridDinos
Initial	0.63 \pm 0.08	0.01 \pm 0.00	0.05 \pm 0.01	0.02 \pm 0.00	0.00 \pm 0.00	0.06 \pm 0.08	0.00 \pm 0.00
Control	0.70 \pm 0.10	0.03 \pm 0.05	0.04 \pm 0.06	0.11 \pm 0.12	0.00 \pm 0.00	2.89 \pm 4.76	0.02 \pm 0.02
Nitrate	0.78 \pm 0.26	0.01 \pm 0.00	0.00 \pm 0.01	0.08 \pm 0.02	0.00 \pm 0.00	1.20 \pm 0.19	0.01 \pm 0.00
Phosphate	0.46 \pm 0.47	0.02 \pm 0.05	0.00 \pm 0.01	0.10 \pm 0.11	0.00 \pm 0.00	2.68 \pm 3.93	0.01 \pm 0.02
N+P	1.17 \pm 0.28	0.10 \pm 0.07	0.00 \pm 0.01	0.25 \pm 0.18	0.00 \pm 0.00	10.99 \pm 5.92	0.04 \pm 0.02
B12	3.48 \pm 2.42	0.01 \pm 0.01	0.12 \pm 0.23	0.24 \pm 0.17	0.00 \pm 0.00	3.34 \pm 2.11	0.03 \pm 0.03

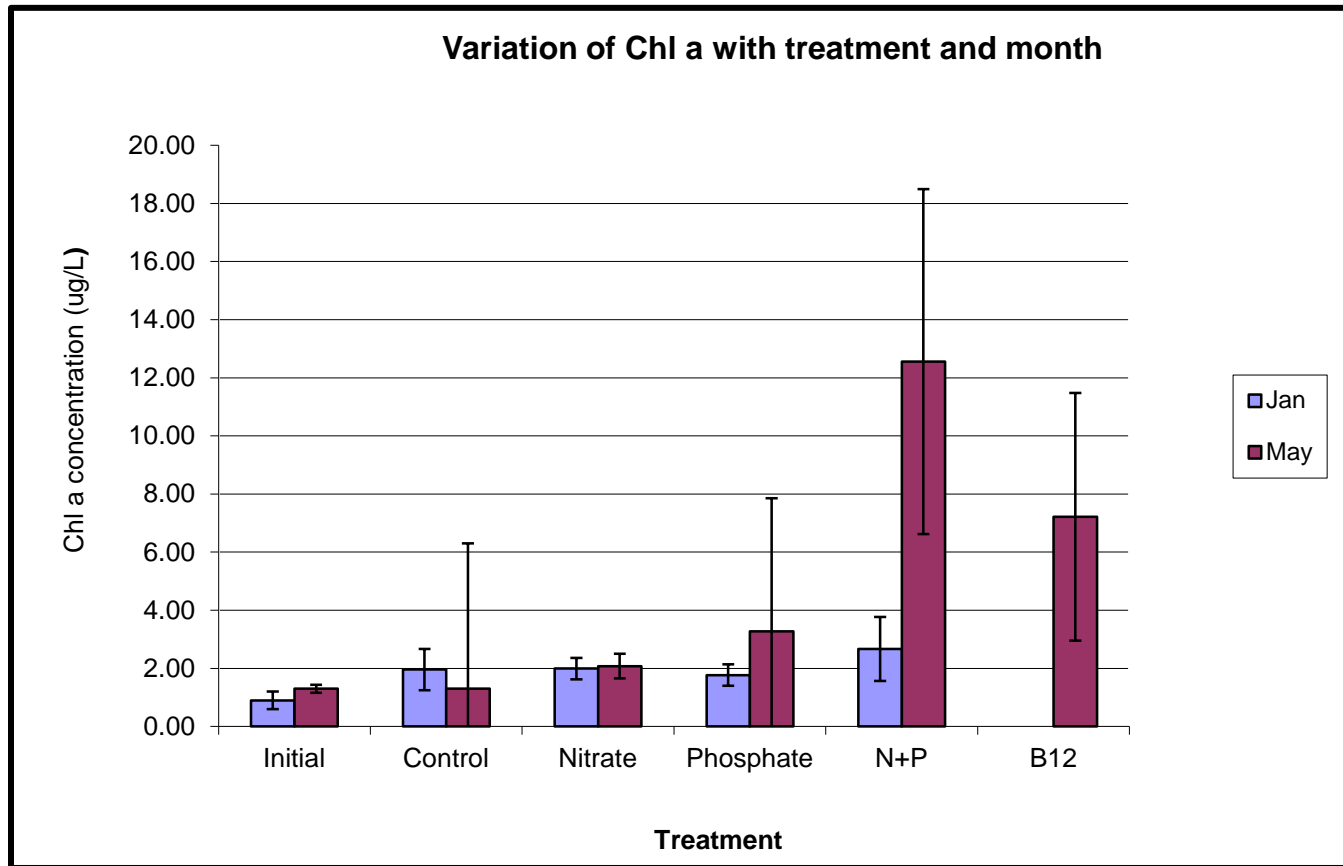


Figure 1.3. Variation of Biomass with treatment and month. Biomass was measure from Chl a, $\mu\text{g/L}$.
B₁₂ was not tested in January. N+P= Nitrogen and phosphorus

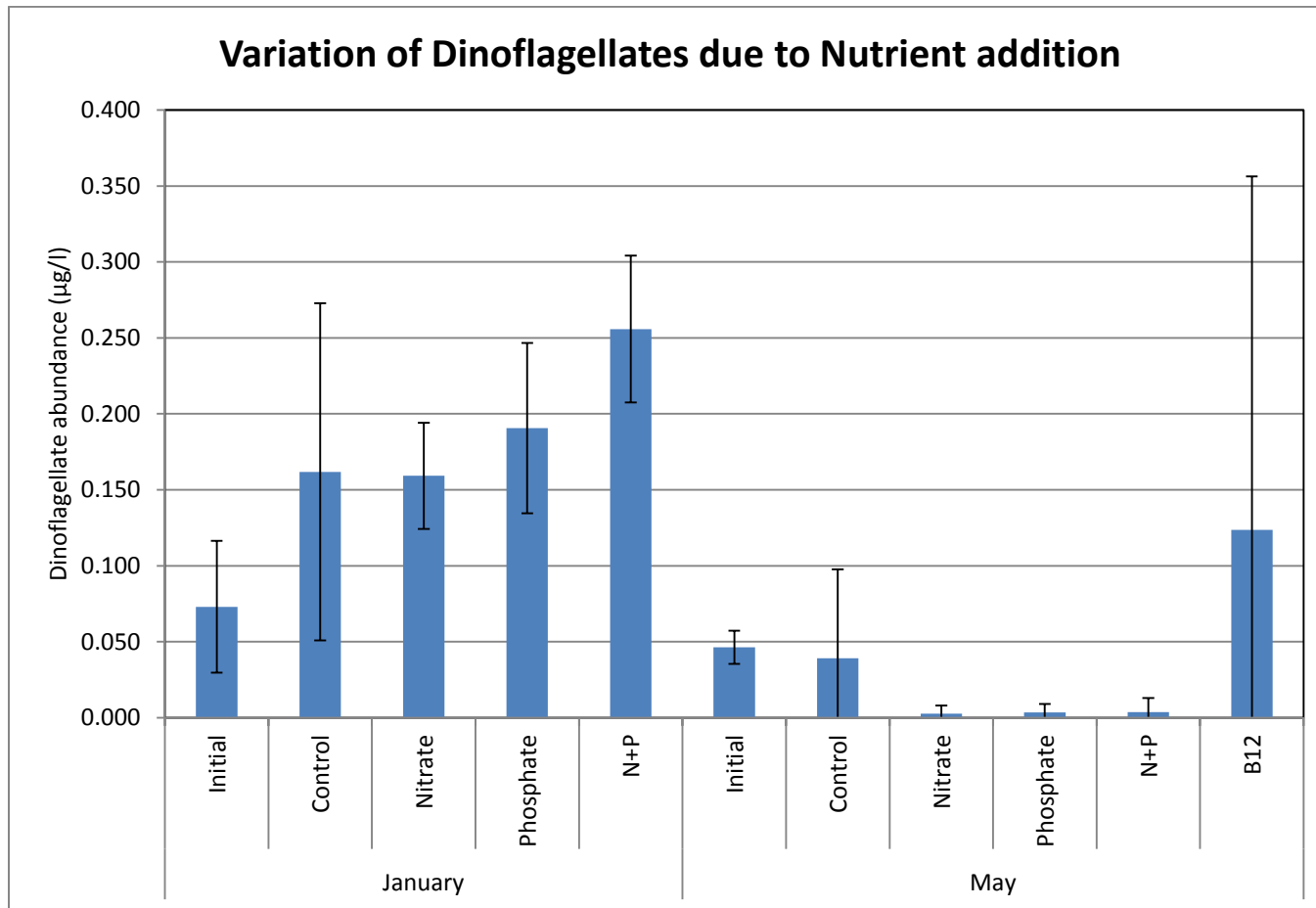


Figure 1.4. Dinoflagellate abundance variation due to nutrient addition, measured in µg/l, in January and May. B₁₂ was not measured in January.

Table 1.5. DOC values ($\mu\text{M C}$) from different treatments in January and May. These values are for the 48 hour treatment, except time 0.

May time 0 has no SD because only one measurement was taken.
 Y3= 3 yellow leaves, Y6= 6 yellow leaves, O3= 3 orange leaves, O6= 6 orange leaves.

DOC Values $\mu\text{M C}$		
Treatment	January	May
Ambient	79.19 \pm 43.75	2212.98
Control	194.35 \pm 30.29	1870.23 \pm 175.11
Y3	539.26 \pm 160.87	3193.74 \pm 1247.37
Y6	1414.45 \pm 875.12	4599.40 \pm 1316.10
O3	1306.03 \pm 219.97	5381.73 \pm 2743.91
O6	2199.37 \pm 842.68	6465.74 \pm 2215.21

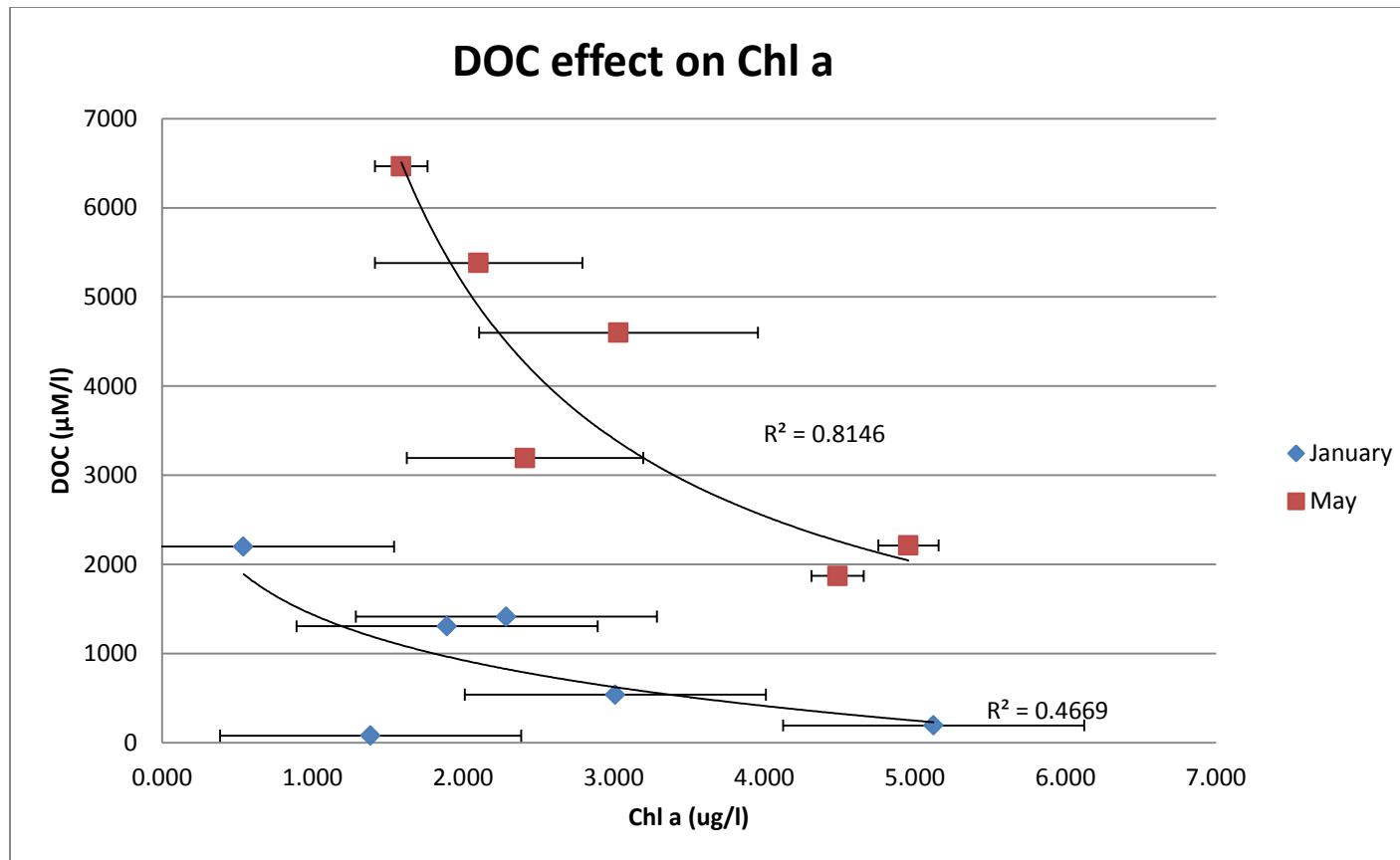


Figure 1.5. The effect of DOC on chl a. Chl a is an indicator of biomass.

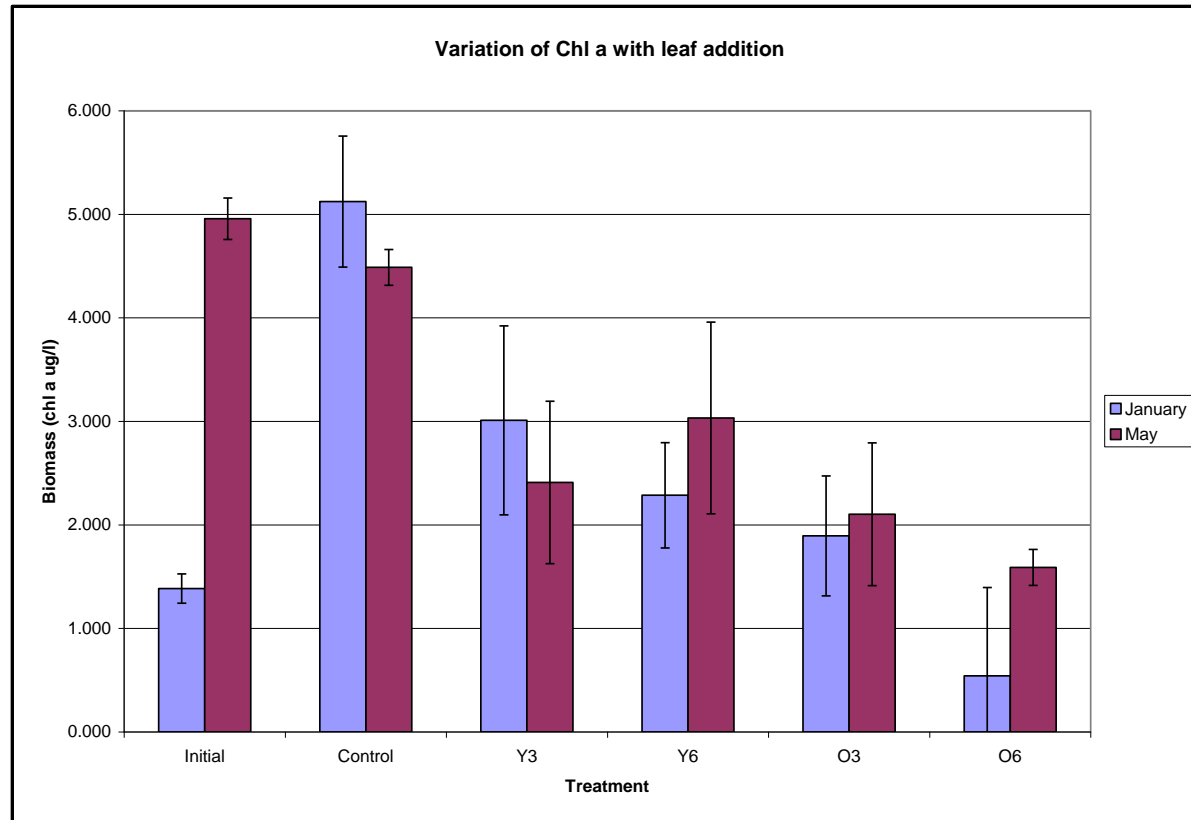


Figure 1.6. Chl a variation with treatment in January and May. Biomass was measured by chl a ($\mu\text{g/l}$). These values are for the 48 hour treatment, except time 0. Y3= 3 yellow leaves, Y6= 6 yellow leaves, O3= 3 orange leaves, O6= 6 orange leaves.

Table 1.6. Leaf addition bioassay results in January and May. Biomass was measured by chl a ($\mu\text{g/l}$) These values are for the 48 hour treatment, except ambient water. Y3= 3 yellow leaves, Y6= 6 yellow leaves, O3= 3 orange leaves, O6= 6 orange leaves.

Jan	Cyanobacteria	EuglenoChloroPrasino	Dinoflagellates	Haptophytes	Cryptophytes	DiatomsChryso	NonPeridDinos
Ambient	0.00 \pm 0.00	0.88 \pm 0.08	0.13 \pm 0.02	0.09 \pm 0.04	0.02 \pm 0.01	0.23 \pm 0.10	0.01 \pm 0.00
C	0.33 \pm 0.04	0.01 \pm 0.02	0.26 \pm 0.30	0.06 \pm 0.01	0.33 \pm 0.06	4.12 \pm 0.46	0.01 \pm 0.00
Y3	0.35 \pm 0.09	0.02 \pm 0.00	0.03 \pm 0.04	0.02 \pm 0.02	0.35 \pm 0.13	2.24 \pm 0.78	0.01 \pm 0.00
Y6	0.12 \pm 0.14	0.02 \pm 0.02	0.12 \pm 0.23	0.00 \pm 0.01	0.22 \pm 0.03	1.75 \pm 0.32	0.05 \pm 0.06
O3	0.05 \pm 0.02	0.00 \pm 0.00	1.01 \pm 0.59	0.01 \pm 0.01	0.11 \pm 0.05	0.60 \pm 0.08	0.11 \pm 0.07
O6	0.03 \pm 0.05	0.00 \pm 0.00	0.24 \pm 0.31	0.00 \pm 0.00	0.04 \pm 0.06	0.19 \pm 0.37	0.05 \pm 0.07
May	Cyanobacteria	EuglenoChloroPrasino	Dinoflagellates	Haptophytes	Cryptophytes	DiatomsChryso	NonPeridDinos
Ambient	0.77 \pm 0.09	0.02 \pm 0.00	1.13 \pm 0.08	0.03 \pm 0.00	0.16 \pm 0.02	2.84 \pm 0.15	0.00 \pm 0.00
O	1.39 \pm 0.25	0.02 \pm 0.01	0.50 \pm 0.17	0.12 \pm 0.02	0.20 \pm 0.07	2.26 \pm 0.15	0.00 \pm 0.00
Y3	0.933 \pm 0.44	0.01 \pm 0.00	0.33 \pm 0.07	0.03 \pm 0.01	0.13 \pm 0.06	0.98 \pm 0.22	0.00 \pm 0.00
Y6	0.52 \pm 0.10	0.01 \pm 0.00	0.65 \pm 0.27	0.03 \pm 0.01	0.12 \pm 0.05	1.71 \pm 0.57	0.00 \pm 0.00
O3	0.59 \pm 0.10	0.01 \pm 0.00	0.43 \pm 0.23	0.02 \pm 0.02	0.06 \pm 0.03	1.00 \pm 0.36	0.00 \pm 0.00
O6	0.55 \pm 0.08	0.00 \pm 0.00	0.28 \pm 0.04	0.01 \pm 0.00	0.03 \pm 0.00	0.72 0.13	0.00 \pm 0.00

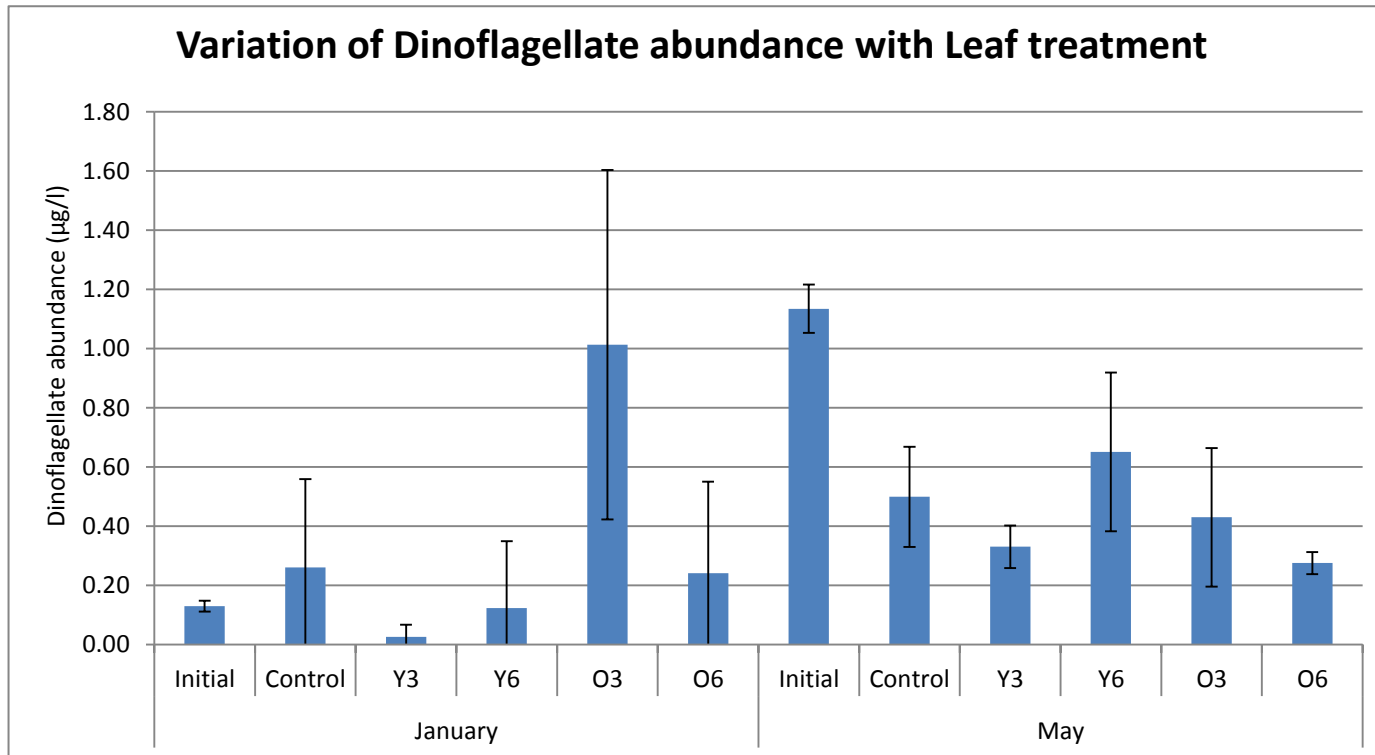


Figure 1.7. Dinoflagellate variation with treatment in January and May. Biomass was measured by chl a ($\mu\text{g/l}$). These values are for the 48 hour treatment, except the ambient/ initial. Y3= 3 yellow leaves, Y6= 6 yellow leaves, O3= 3 orange leaves, O6= 6 orange leaves.

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