

RELATIVE IMPORTANCE OF NUTRIENT ADDITION AND LIGHT REDUCTION
ON THE PRODUCTIVITY OF ALGAL COMMUNITIES

BIOS 569 - Practicum in Aquatic Biology

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1994

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ABSTRACT

In situ enclosure experiments were conducted over the summer of 1994 in Morris Lake, Gogebic County, Michigan to measure the effects of nutrient addition (nitrogen and phosphorus) versus reduction of light on the productivity of phytoplanktonic communities. Nutrient concentrations and chlorophyll *a* concentrations were determined daily to determine phytoplanktonic community productivity. The spectrophotometric method of pigment analysis was used with acetone as the solvent.

Nutrient enrichment was shown to stimulate increases in phytoplankton despite light reduction as was expected. Differences between treatments were minor. Chlorophyll *a* concentrations appeared to be negatively correlated with the duration of the experiment. This trend was supported by the soluble reactive phosphorus concentrations as well as the dissolved inorganic nitrogen concentrations. After an initial increase, the concentrations began their downward trend.

The results indicate that nutrient addition and light reduction do indeed affect the productivity of freshwater phytoplanktonic communities. However, due to lack of replication, more experiments should be forthcoming to provide further experimental evidence as to the relative impacts of light reduction versus nutrient addition on the productivity of phytoplankton communities.

INTRODUCTION

The mechanism of the "trophic cascade" has been repeatedly demonstrated by ecologists to explain the interaction between trophic levels in freshwater communities. The concept of light reduction and its effect on the trophic levels in the aquatic community has not been significantly tested.

It is commonly known that freshwater phytoplankton communities are strongly influenced by the concentration of nutrients in the system. The supply of nutrients is one of the most important factors which determines the species and quantity of plant material in lakes. Plant growth is regulated by light, nutrient supply, and temperature; controlling rates of photosynthesis and metabolism and by grazing and senescence, controlling rates of biomass removal (Harper 1992). After carbon (and excluding water - derived hydrogen and oxygen) nitrogen is one of the most abundant constituents of algal biomass (Thompson, 1989). As well as being most abundant in biomass, nitrogen along with phosphorus are known to be the primary limiting nutrients in most freshwater ecosystems. Several laboratory experiments have shown that the addition of phosphate and nitrate without any other additional nutrients was enough to increase phytoplankton growth (Harper 1992). As demonstrated in previous studies, freshwater phytoplankton communities may grow at rates that are measurably less than their maximum physiological capacity as a result of nutrient limitation (Lehman, 1985). The relative abundance of certain nutrients may also influence phytoplankton species composition because of a species' ability to fix nitrogen (Vanni, 1987). A specific example of this may be the abundance of blue - green algae at low N : P ratios.

Growth may be stimulated by increasing the input of phosphorus to a freshwater phytoplankton community (Vanni, 1987). Throughout periods of enrichment by different ratios of fertilization and of recovery, algal biomass always followed lake water phosphorus levels, but not nitrogen or carbon (Harper 1992). Einsele was able to detect, following a single dose of phosphate into the Schleinsee, that the phosphate quickly became part of the sediment in a combined organic form. There was no lasting increase of productivity beyond an initial surge (Schwoerbel 1987). Variability in the phytoplankton assemblage is also most likely at high nutrient supply (Carpenter and Hansson 1993). An increase in the supply and availability of nutrients in water bodies affect the rate of primary production of plants, the magnitude of standing crop biomass achieved, and the relative proportion of different species (Harper 1992).

The physiological control of photosynthesis by light intensity, varying through the day or with depth, is well understood. Gross photosynthesis initially shows a linear increase with irradiance, passing a threshold called the compensation point below which respiration matches photosynthesis. Thereafter gross

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photosynthesis exceeds respiration and net photosynthesis can be measured (Harper 1992). In any ecosystem, the transformation of solar energy into the production of organic matter will rarely be at the maximum physiological capacities of the individual plants living there. For each species it will be limited by the environmental resource in shortest supply at the time, even if others are present in excess of an individual organism's needs. The most usual environmental resources which limit production are light and nutrients (Harper 1992). Whichever falls below the minimum level to sustain growth will regulate the population of that species, in our case, light. Light conditions during growth are important in the determination of phytoplankton species composition.

A significant amount of data has accumulated from several hundreds of lakes that show mean phytoplankton biomass (usually measured as chlorophyll *a* concentrations) to be positively related to the phosphorus level (Sarnelle, 1992). Phosphorus - chlorophyll relationships generally use data averaged over the summer months (or approximately the algal bloom), so they are generally representative of an equilibrium density (Sarnelle, 1992). The light environment experienced by phytoplankton populations varies significantly during the bloom (Kanda, 1989). A nearly continuous photoperiod and moderate water temperature during the summer sampling period support high nutrient uptake rates by phytoplankton in lakes (Gu and Alexander 1993). The bloom may be initiated by a change of light environment caused either by stratification or local climatic fluctuations, and nitrate plays a major role in controlling bloom production (Kanda, 1989). Nitrate uptake in natural phytoplankton populations depends on light intensity (Kanda, 1989). According to seasonal fluctuations in lake productivity, there may also be similar fluctuations in the nitrate levels in the epilimnion (Schwoerbel 1987). Any changes made to this environment could possibly disturb normal community structure. Individual algal cells can show a range of adaptations to both high and low light levels. In low light conditions, pigment content and photosynthetic efficiency are shown to increase (demonstrating what is called 'shade' adaptation) (Harper 1992). Recent investigations have since emphasized the variabilities of loss rates in the phytoplankton communities, as opposed to growth rates, in governing the changes that occur within the communities (Lehman and Sandgren, 1985).

Determination of chlorophyll *a* has been, and still is, one of the most commonly used methods for estimation of algal biomass (Hansson, 1988). Measured chlorophyll *a* concentrations have been found to be limited by both nitrogen and phosphorus (Newman 1994). The accuracy of chlorophyll *a* measurements and subsequent estimates of algal biomass depends on the extraction efficiency of the solvent used, and the species of the phytoplankton present (Webb, 1992). Numerous organic solvents have been used to extract chlorophyll *a* from algae; however acetone is the most common (Webb, 1992). Recent studies have also reported that traditional nonseparative spectrophotometric

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and fluorometric techniques yield higher chlorophyll *a* values due to their erroneous measurement (and inclusion of pigments and degradation products other than chlorophyll *a*) (Webb, 1992).

The primary goal of my study was to determine the relative importance of nutrient addition versus light reduction on the productivity of algal communities. Both of these resources play a vital role in the regulation of phytoplankton community productivity. Even in the abundance of one, the phytoplankton species will be limited by the resource in shortest supply. The impact of shade, and whether it could overcome additional nutrients when determining the productivity of a phytoplankton community could perhaps be estimated by determining end chlorophyll *a* concentrations. Addition of nutrients made it easier to ascertain the effects of light reduction on the phytoplanktonic community because it was known that nitrogen and phosphorus were not limited as they may be in normal circumstances. In this type of scenario, phytoplankton growth or lack of growth could be a result of the shade variable. Spectrophotometry with acetone as the solvent was the method used for extraction and pigment analyses. By using an *in situ* mesocosm experiment, the experimental design allowed for the independent testing of nutrient and shade versus a control group as well as their combined interactions.

MATERIALS AND METHODS

Site Description

Morris Lake in Gogebic County, Michigan was the site of the *in situ* experiment conducted in the summer of 1994 at the University of Notre Dame Environmental Research Center. This fairly shallow lake had a Secchi depth of 1.44 meters, and a chlorophyll *a* level of 25.05 mg/L. It had an alkalinity of 10.8 mg/L, a pH of 7.5, and a conductivity of 95 umhos. (UNDERC unpublished and personal observation)

Experimental Design

The mesocosm experiment consisted of four treatment levels conducted in duplicate. These eight mesocosms were suspended from two rafts (previously constructed), at a water depth of approximately sixteen meters in Morris Lake. Treatments were assigned randomly to locations on the rafts. Mesocosms were constructed from sheets of translucent plastic formed into an open-ended cylinder using a heat gun. Each bag was approximately 2.2 m in length and 0.65 m in diameter with a volume of approximately 400 liters. Each mesocosm was open at the top and heat

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sealed over an inflated bicycle inner tube, and was then tied to the raft to stabilize it. A small weight was tied to the bottom of the bag to anchor the mesocosm and provide more stability (but not large enough to produce drag). The rafts were placed in approximately the center of the lake to prevent disturbances from macrophytes, the lake bottom, shore animals. Mesocosms were filled with epilimnetic lake water by manually pulling the bag through the water. They were topped off by manually filling them with buckets. Light was controlled by a square of black sheeting which reduced approximately 90% of the available light. Each square was stapled to the raft and covered the entirety of each individual mesocosm without disturbing its surface. The experiment ran from 12 June to 8 July.

The first treatment was labeled as the control group and no manipulations were made to this particular environment. The second treatment level contained nutrient additions of nitrogen and phosphorus. The third treatment level was shaded (with an approximate 90% reduction of light). The fourth and final treatment level was supplied with nutrient addition as well as a 90% reduction of light. As stated above, the treatments were randomly assigned to each raft.

Nutrient addition of phosphorus was in the form of K_2HPO_4 and nitrogen in the form of KNO_3 . Concentrations of 0.055g of K_2HPO_4 and 1.47g of KNO_3 were added once on 12 June to approximate a 16 : 1 (N : P) ratio, or the natural lake ratio. After addition of the nutrients we gently mixed the water columns in the mesocosms to more evenly distribute the nutrients. Water samples were taken using a Kemmer for five days consecutively following the nutrient addition. Water samples were then collected every three days for the remainder of the experiment beginning on 19 June.

Samples for nutrient analyses (soluble reactive phosphorus [SRP] and dissolved inorganic nitrogen [NO_3]) were measured daily using a HACH kit. Samples for pigment analyses were filtered onto Whatman GF/F filters and frozen. Before the analyses were done they were extracted with 90% acetone for twenty-four hours. Pigment analyses were completed using the HACH spectrophotometer. The trichromatic method was used to determine chlorophyll *a* levels.

RESULTS

The data which we collected was not replicated enough times to detect any statistical trends. When viewing the graphed results, there appeared to be a negative correlation between chlorophyll *a* concentrations and length of time of the experiment

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(Fig 1, Fig 2). An initial increase was then followed by a downward trend in chlorophyll *a* concentrations. In general, the phytoplankton chlorophyll *a* response was nearly the same in both the enriched and unenriched mesocosms. The light reduction treatment group remained quite close to the control group in final concentrations (Fig 1, Fig 2). As expected, their numbers were slightly higher than the control. The same appeared true for the nutrient enriched treatment groups. However, in both cases, the nutrient enriched treatment group produced the highest point of chlorophyll *a* concentrations (Fig 1, Fig 2). In the case of raft two, a significant decrease in concentration appeared in the following sample which may make this point less relevant to the graph (Fig 1). A drop-off in concentration also appeared in raft three but the chlorophyll *a* still remained positive (Fig 2). There appeared to be a positive correlation between the chlorophyll concentrations and the nutrient enriched - light reduction treatment group as compared to the control group (Fig 1, Fig 2)). The nutrient enriched - light reduction treatment groups had the second highest chlorophyll *a* concentrations at one significant point. There remained a downward trend for raft three (Fig 2), but disrupted this trend in raft two (Fig 1). In both of the replications, the chlorophyll *a* concentrations varied little between the nutrient enriched and nutrient enriched - light reduction treatment groups. The chlorophyll *a* content reached its peak approximately the fifteenth day of the experiment for each of the treatment groups (Fig 1, Fig 2). However, concentrations dropped significantly after this date.

The nutrient concentrations of soluble reactive phosphorus (SRP) showed different trends between rafts two and three (Fig 3, Fig 4). There appeared to be an oscillating phosphorus concentration in the nutrient enriched - light reduction treatment group for raft two which approached a downward trend (Fig 3). Phosphorus concentrations in the nutrient enriched - light reduction treatment group reached a low on approximately the fourth day and once again on about day twenty - seven (Fig 3). The oscillations leveled off somewhat after about day fifteen for the combined treatment group of raft two (Fig 3). For raft three, the nutrient enriched - light reduced treatment, phosphorus concentrations declined until approximately the eighteenth day where there appeared a substantial increase (Fig 4). The nutrient enriched treatment groups showed a similar trend in decreasing phosphorus concentrations (Fig 3, Fig 4). Concentrations reached a low after about the eighteenth day for raft two (Fig 3). However, concentrations remained steadily low for raft three (Fig 4). When comparing the different treatment groups, however, both treatments appeared to be negatively correlated to the length of the experiment (Fig 3, Fig 4).

The nutrient concentrations of dissolved inorganic nitrogen dropped significantly after the initial nutrient addition in approximately the first five days of the experiment (Fig 5, Fig 6). Both treatment groups remained close in their nitrogen concentrations. In both cases, the nutrient enriched - light reduction treatment group

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had slightly higher nitrogen concentrations (Fig 5, Fig 6). As demonstrated with SRP above, nitrogen concentrations appeared to be negatively correlated with duration of the experiment (Fig 5, Fig 6). After an initial oscillation, concentrations for raft two for the combined treatment group demonstrated an almost linear decrease (Fig 5). Disregarding the first point for raft three, this replication appeared similar (Fig 6). The nutrient enriched treatment group, on the contrary, showed a slight oscillation in nitrogen concentrations in the latter portion of the experiment for raft two (Fig 5). Nitrogen concentrations remained steady in raft three (Fig 6).

DISCUSSION

The relative importance of additional nutrients versus light reduction is the question being asked in this experiment. Whether or not nutrient addition can overcome the reduction of light to produce a significant chlorophyll response, or whether the impact of light reduction is too great of an obstacle for the phytoplankton community to overcome. As predicted from previous studies, nutrient addition should produce a marked increase in phytoplanktonic chlorophyll activity, which was demonstrated from the results of both raft two and raft three followed by a decrease as the experiment continued (Fig 1, Fig 2). Overall, there appeared to be a negative correlation between chlorophyll *a* concentrations and duration of the experiment. Chlorophyll *a* concentrations remained remarkably similar in both the enriched and unenriched treatments. The light reduction treatment group concentrations remained slightly higher than the control, as might be expected. This may be a result of the shade adaptation of phytoplanktonic communities in decreased light conditions. Pigment content and photosynthetic efficiency may have slightly increased relative to the control group. The nutrient enriched treatment groups demonstrate the highest chlorophyll *a* concentrations at one instance in both rafts two and three as would be expected (Fig 1, Fig 2). The substantial decrease to negative chlorophyll concentrations makes one question the accuracy of the initial point (Fig 1). This obvious error may have been due to inaccurate sampling techniques, or water chemistry. Raft three still retains the highest point, but it follows a general downward trend (Fig 2). The combined treatment group of both nutrient enrichment and light reduction demonstrated the second highest point of chlorophyll *a* concentrations for both rafts two and three (Fig 1, Fig 2). This point for raft three does not follow the general downward trend of the graph (Fig 2). Because of this isolated point, it may be disregarded as a sampling error. This treatment group demonstrated that even with the abundance of nutrients being provided to the system, the phytoplankton community was still being limited by light.

Nutrient effects on phytoplankton communities were independent of light reduction in this particular experiment. In the case of soluble reactive phosphorus,

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phosphorus concentrations appeared to be negatively correlated to the duration of the experiment (Fig 3, Fig 4). The nutrient enriched - light reduction treatment group showed slightly higher phosphorus concentrations for both rafts two and three (Fig 3, Fig 4). This may be due to an abundance of phosphorus remaining in the water column, not appearing as sediments. Phosphorus concentrations reached a low in the nutrient enriched treatment group in both rafts two and three on approximately day eighteen (Fig 3, Fig 4). The combined treatment group in raft two reached its peak slightly earlier on about day fourteen. These decreases may be a result of the time lag associated with nutrient additions. The unusual increase in phosphorus concentrations in raft three after day eighteen may be disregarded, due to their deviance from any statistical trend. Some unexpected occurrence must have taken place that was not seen by either sampler to cause this significant increase.

The concentrations of dissolved inorganic nitrogen was not significantly different in either the nutrient enriched or nutrient enriched - light reduction treatment groups. Similar to my previous graphs, nitrogen concentrations appeared to be negatively correlated to length of the experiment. Light reduction has apparently little to no effect on the degradation of nitrogen in aquatic systems. The treatment groups containing both nutrient addition and light reduction demonstrated slightly higher rates of dissolved inorganic nitrogen (Fig 5, Fig 6). Nitrogen (DIN) was taken up fairly rapidly when made available to the phytoplankton, but then appeared to level off (Fig 5, Fig 6). Within five days, approximately all of the excess nitrogen was taken up by the system, and nitrogen concentration levels remained fairly steady for the remainder of the experiment. This may in fact indicate a threshold for nutrient levels in the aquatic systems.

The results of our additions of nitrogen and phosphorus provide strong evidence that algae are limited by phosphorus. Light does not appear to be a primary limiting factor in this particular system. The phytoplanktonic community demonstrated evidence that in low light conditions, they could adapt to shade rather well. Through the course of the experiment, SRP and DIN were taken up almost as readily as it was made available. Thus, nutrient levels in the water column steadily decreased. This pattern of phosphorus limitation is consistent with conditions in many lake systems, as well as its adherence to the phytoplankton N : P ratio of 16 : 1.

In order to increase accuracy and decrease artifacts in this experiment more replications should be done. A longer time period may also reduce oscillation in the data as well. It would have been entirely more accurate if the samples collected could have been taken at the same time each day. This however was impossible provided the demands of our class schedule. An important factor which was not accounted for was the impact of zooplankton on the community as a result of nutrient additions and light reduction. Grazing losses and nutrient recycling are two very important effects of

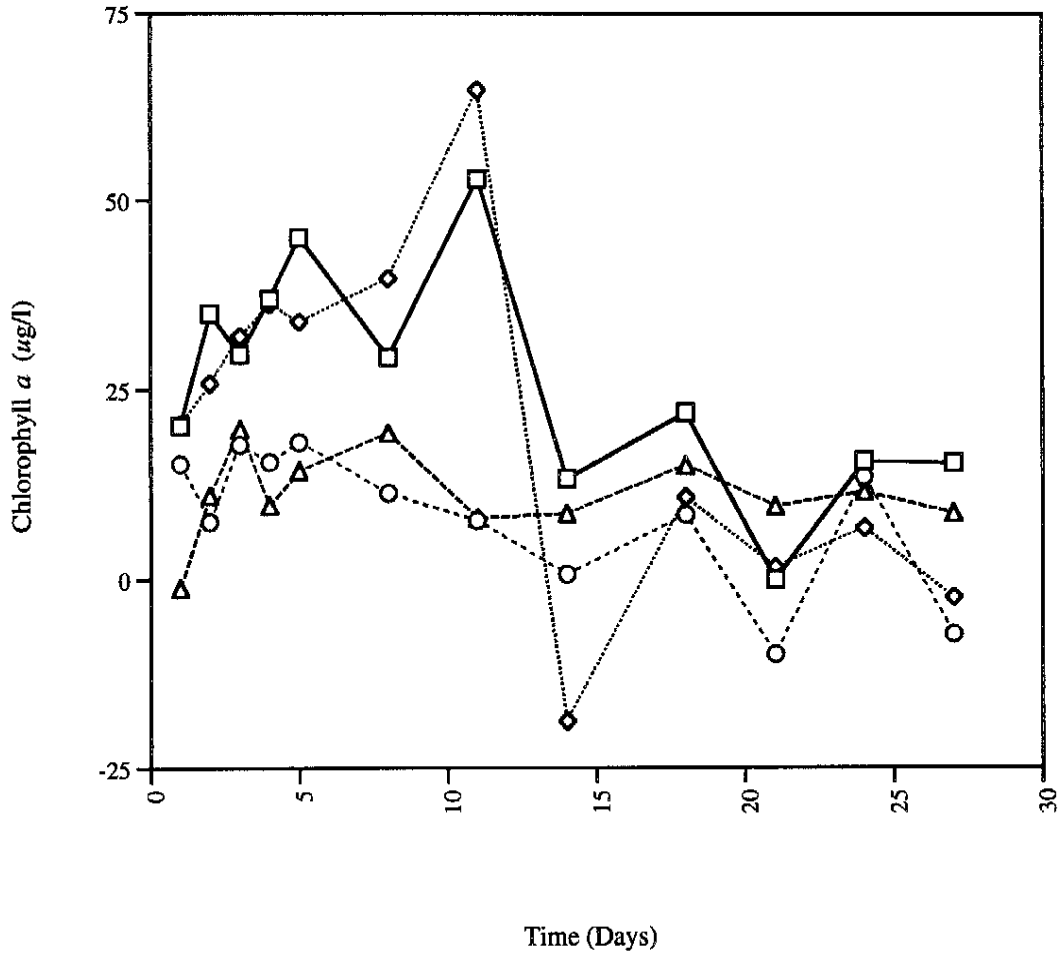
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zooplankton on phytoplanktonic communities.

Some related experiments which may generate more accurate results may be addition of nutrients once versus a continued nutrient enrichment. By looking at the structure of the phytoplankton community (what types of algae were present and where), and how the community changed could also be of interest. Also of interest may be the addition of zooplankton, or fish to the system. They may play an important role in the light/nutrient question. Determining the effects of ammonium as compared to nitrate may also be important, especially concerning light - limited growth.

Through my experiment, I attempted to determine the relative importance of nutrients and light on the dynamics of a freshwater phytoplankton community. My results corroborated previous studies by demonstrating that both light and nutrients are important factors when determining productivity in a phytoplankton community. Their combined effects still remain inconclusive. With an increased understanding of the dynamics of the freshwater ecosystem, more can be done to protect and preserve these systems in the future.

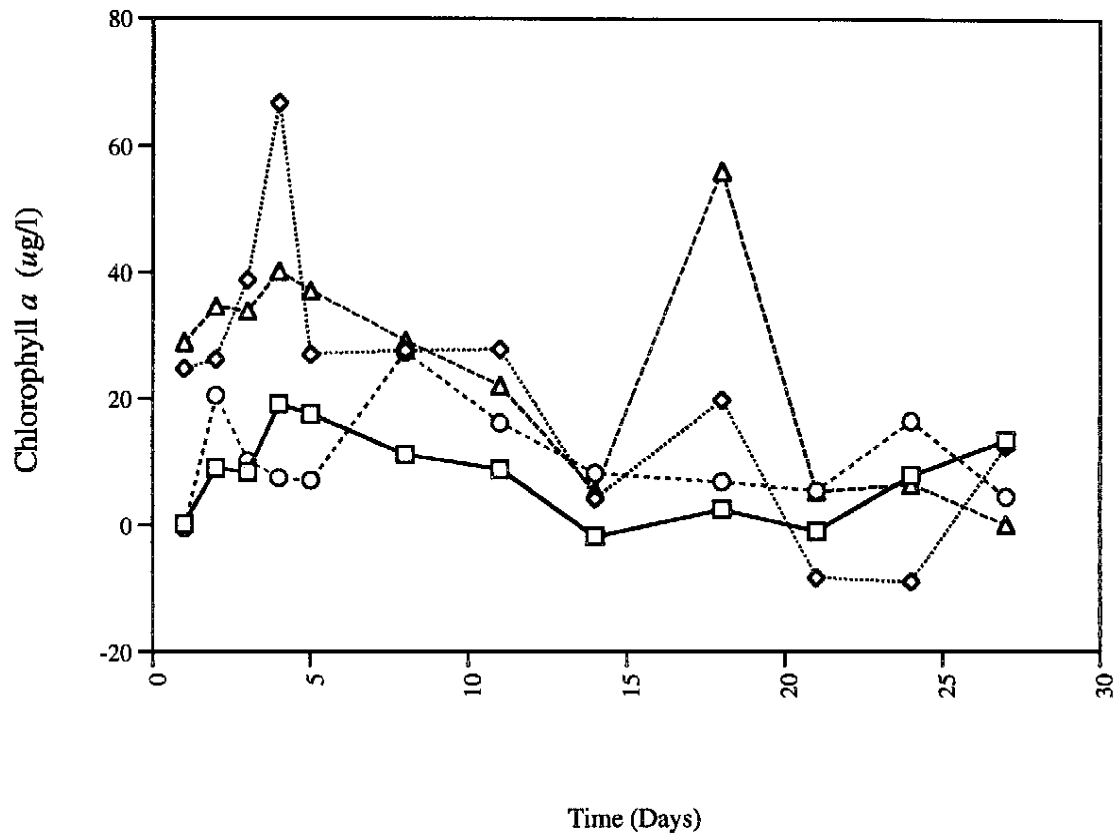
Chlorophyll *a* Concentrations



- Nutrient Enriched, Light Reduction
-◇..... Nutrient Enriched
- Control
- ▲--- Light Reduction

Fig 1. Chlorophyll *a* concentrations for raft 2 from 12 June to 8 July as affected by light and nutrients.

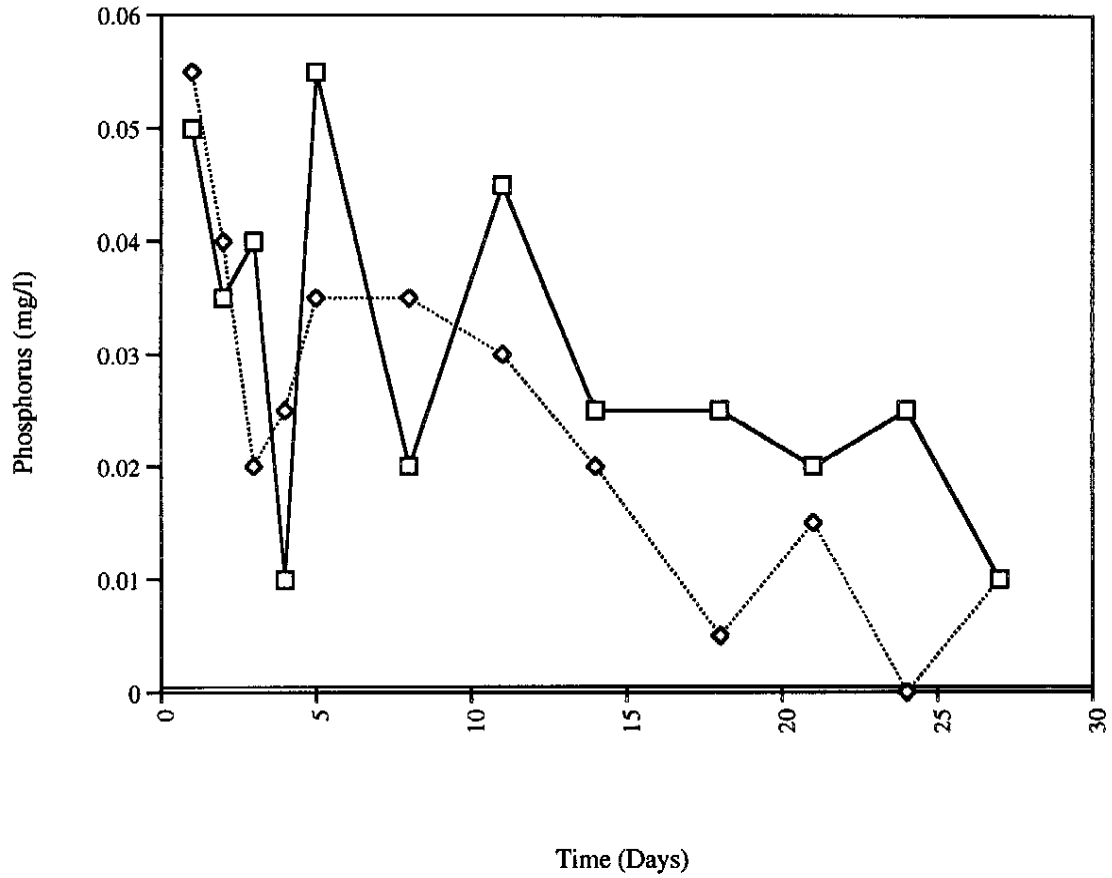
Chlorophyll a Concentrations



- Control
-◇..... Nutrient Enriched
- Light Reduction
- .-.-△-.-.- Nutrient Enriched, Light Reduction

Fig 2. Chlorophyll a concentrations for raft 3 from 12 June to 8 July as affected by light and nutrients.

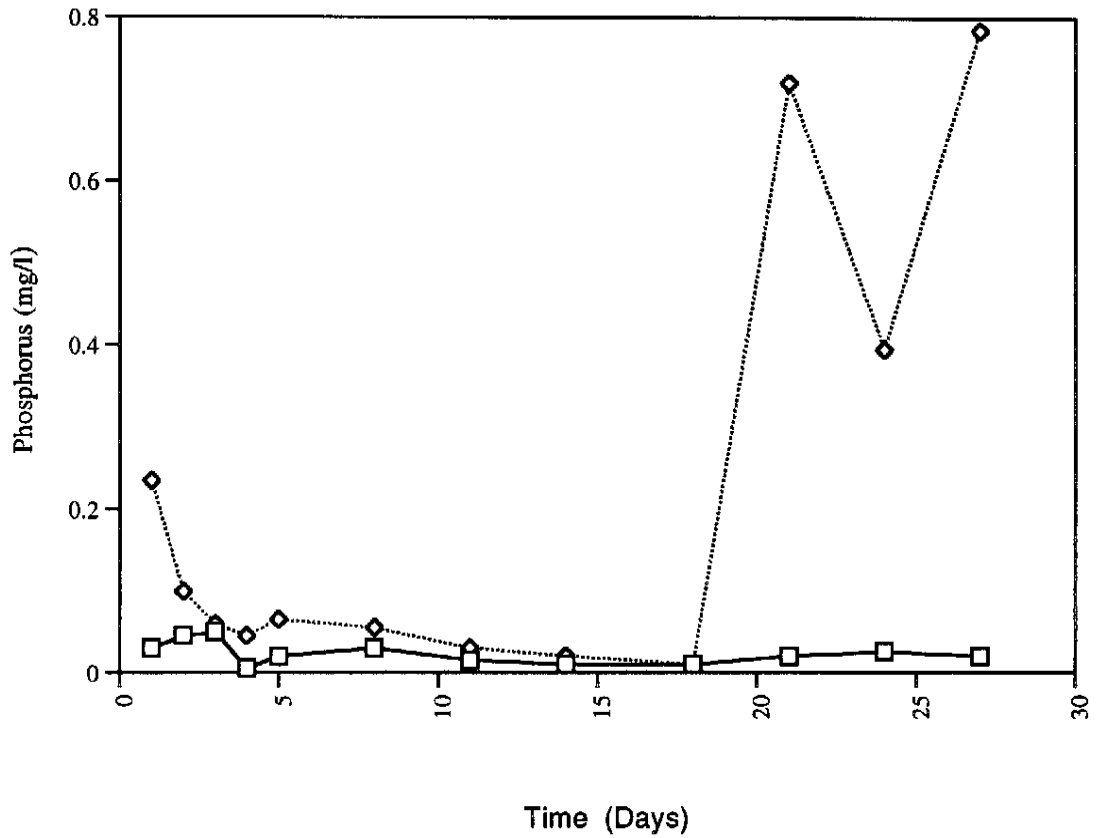
Soluble Reactive Phosphorus Concentrations



—□— Nutrient Enriched,
Light Reduction
.....◇..... Nutrient Enriched

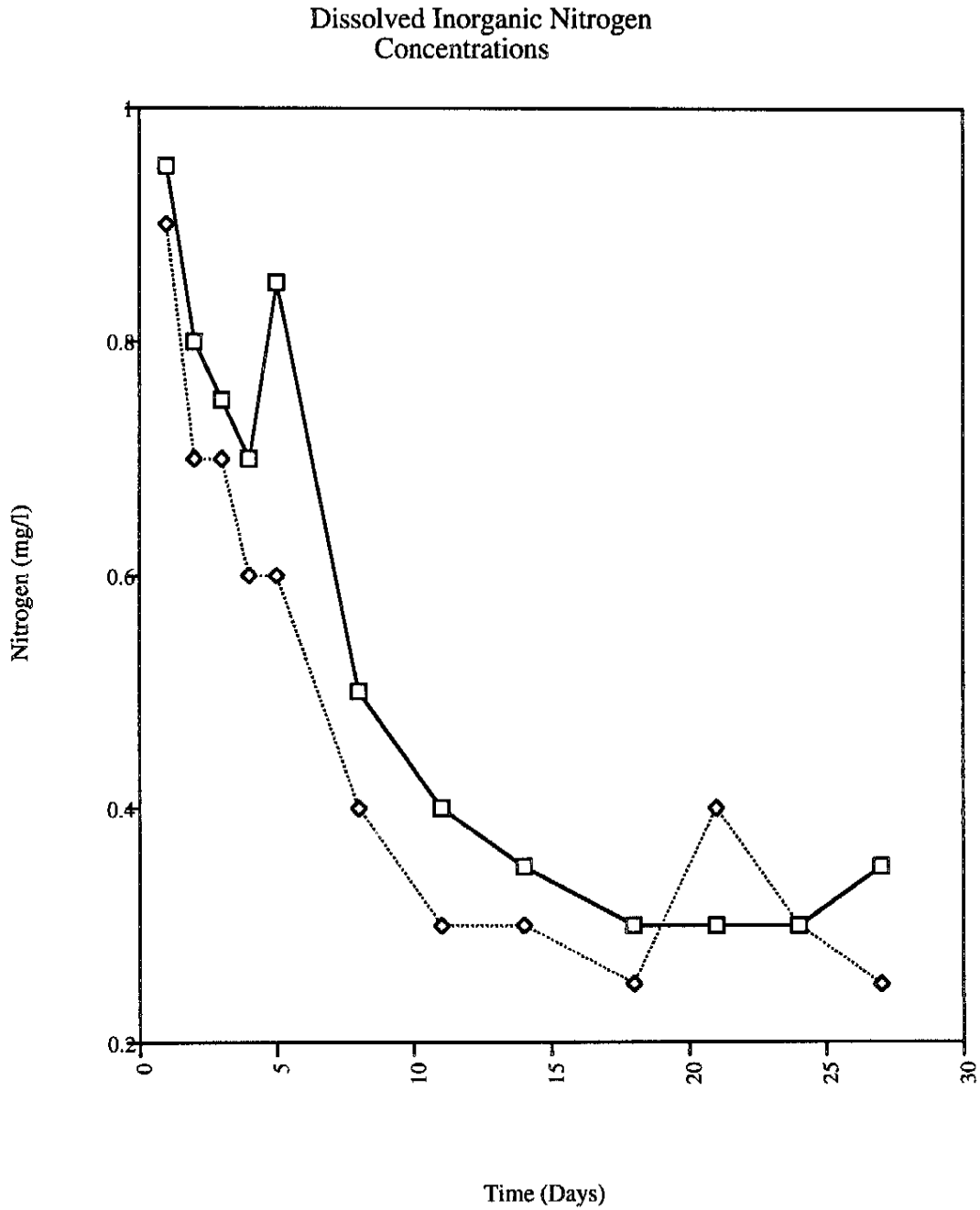
Fig 3. Soluble reactive phosphorus concentrations for raft 2 from 12 June to 8 July as affected by light and nutrients.

Soluble Reactive Phosphorus Concentrations



- Nutrient Enriched
-◇..... Nutrient Enriched, Light Reduction

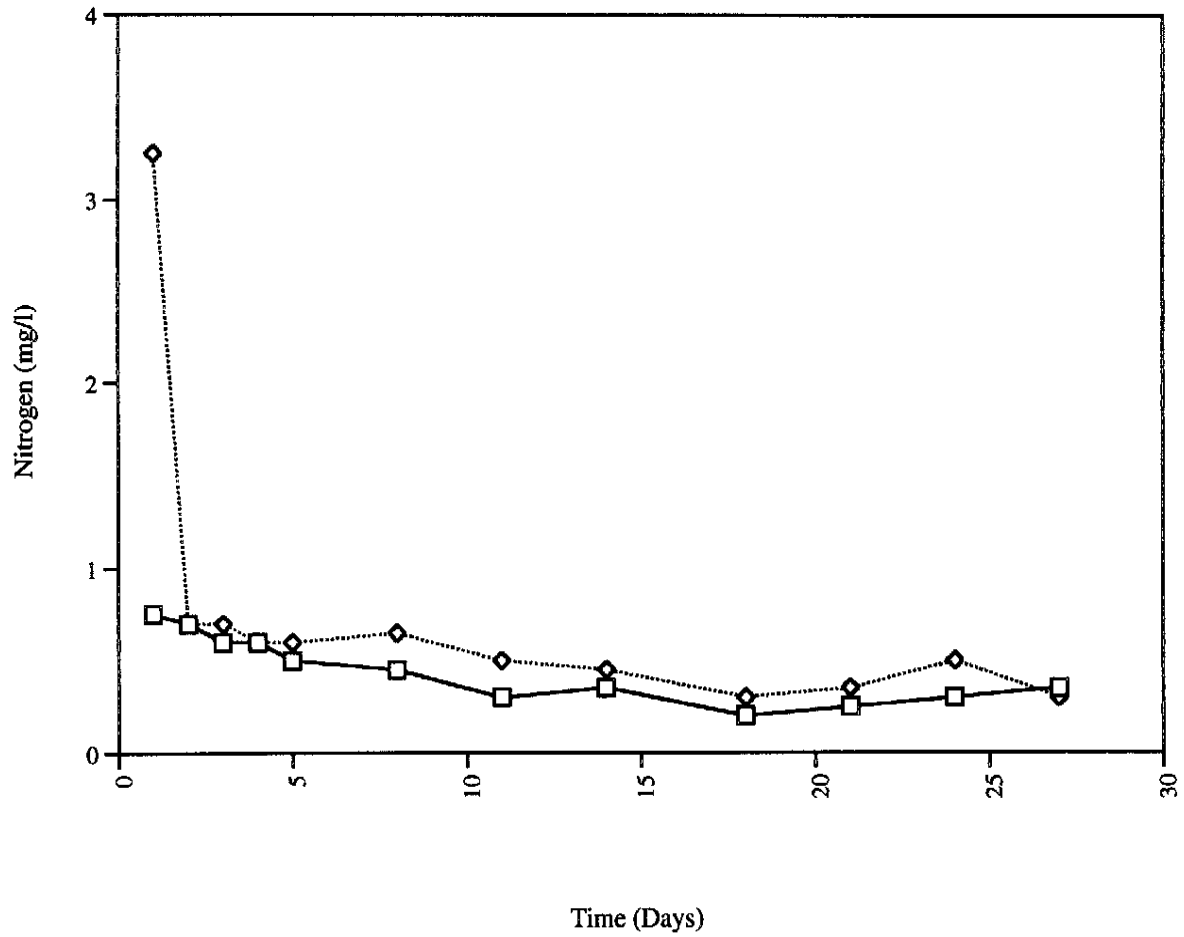
Fig 4. Soluble reactive phosphorus concentrations for raft 3 from 12 June to 8 July as affected by light and nutrients.



—□— Nutrient Enriched,
Light Reduction
.....◇..... Nutrient Enriched

Fig 5. Dissolved inorganic nitrogen concentrations for raft 2 from 12 June to 8 July as affected by light and nutrients.

Dissolved Inorganic Nitrogen Concentrations



—□— Nutrient Enriched
.....◇..... Nutrient Enriched,
Light Reduction

Fig 6. Dissolved inorganic nitrogen concentrations for raft 3 from 12 June to 8 July as affected by light and nutrients.

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Fig 7. Raft 2 as used from 12 June to 8 July.

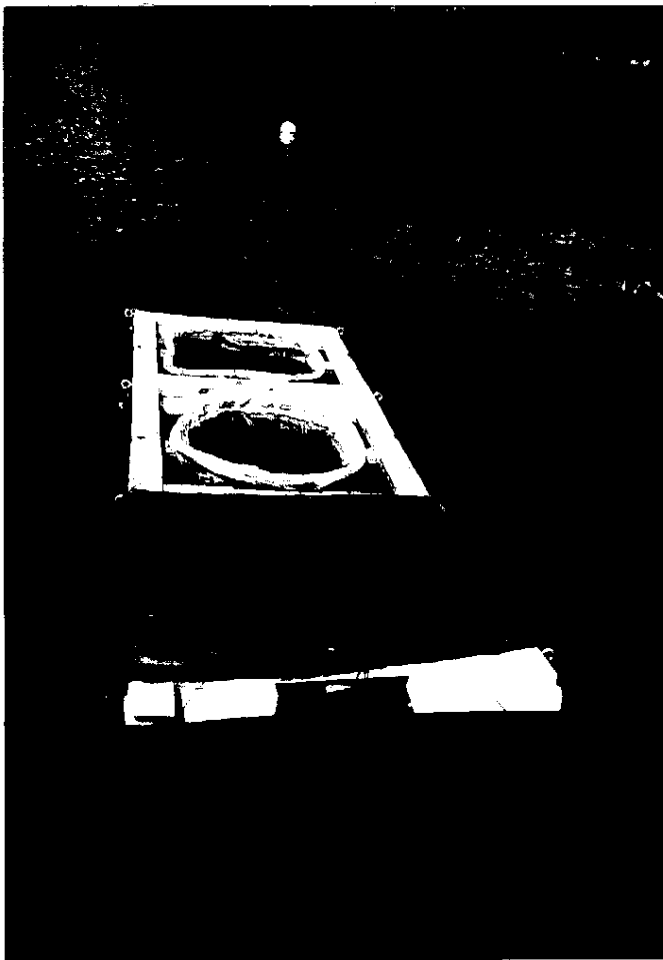
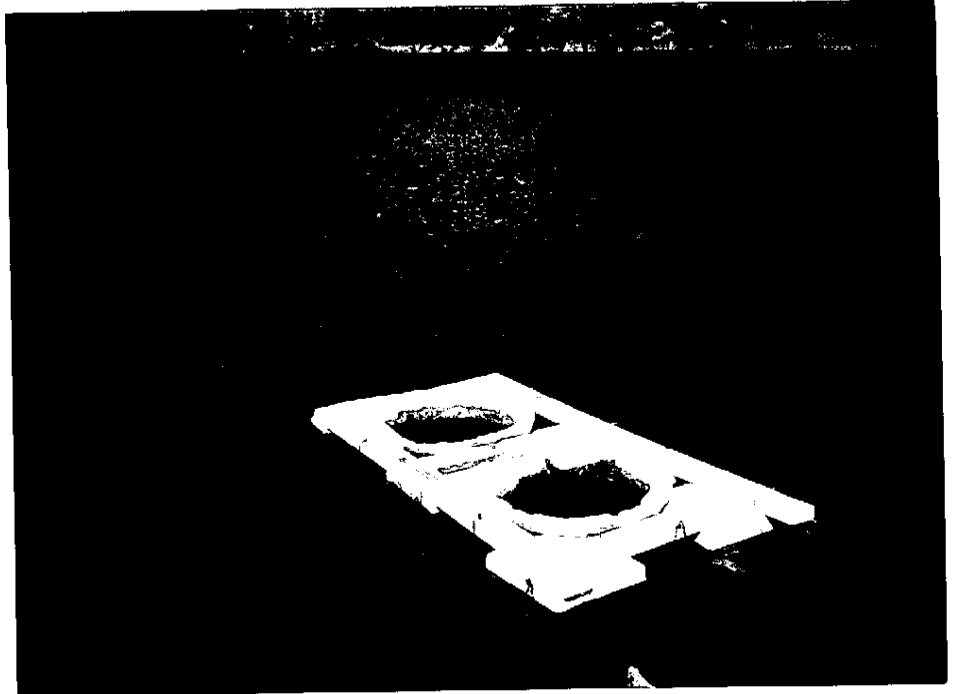


Fig 8. Raft 3 as used from 12 June to 8 July.

ACKNOWLEDGEMENTS

I would especially like to acknowledge field and laboratory assistance from Kathleen Daly. I would also like to thank Dr. Richard Carlton for providing me with the guidance throughout my entire project. I would like to thank Dr. Marty Berg for his advice and patience throughout the entire summer. I would also like to thank Dr. Ron Hellenthal for providing me with the opportunity of the UNDERC experience, and for all the students who spent long hours in the field with us. Most of all I would like to thank the Bernard J. Hank Family Endowment which has made all of this possible. This is a contribution from the University of Notre Dame Environmental Research Center.

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